Published in final edited form as:

Environ Sci Technol. 2016 July 19; 50(14): 7825–7833. doi:10.1021/acs.est.6b01911.

The Use of Field and Mesocosm Experiments to Quantify Effects of Physical and Chemical Stressors in Mining-Contaminated Streams

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Abstract

Identifying causal relationships between acid mine drainage (AMD) and ecological responses in the field is challenging. In addition to the direct toxicological effects of elevated metals and reduced pH, mining activities influence aquatic organisms indirectly through physical alterations of habitat. The primary goal of this research was to quantify the relative importance of physical (metal-oxide deposition) and chemical (elevated metal concentrations) stressors on benthic macroinvertebrate communities. Mesocosm experiments conducted with natural assemblages of benthic macroinvertebrates established concentration–response relationships between metals and community structure. Field experiments quantified effects of metal-oxide contaminated substrate and showed significant differences in sensitivity among taxa. To predict the recovery of dominant taxa in the field, we integrated our measures of metal tolerance and substrate tolerance with estimates of drift propensity obtained from the literature. Our estimates of recovery were consistent with patterns observed at downstream recovery sites in the NFCC, which were dominated by caddisflies and baetid mayflies. We conclude that mesocosm and small-scale field experiments, particularly those conducted with natural communities, provide an ecologically realistic complement to laboratory toxicity tests. These experiments also control for the confounding variables associated with field-based approaches, thereby supporting causal relationships between AMD stressors and responses.

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Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.6b01911. Water quality characteristics and metal concentrations in stream mesocosms, a map of sampling stations, and schematics of equipment used to measure community metabolism and respiration, and macroinvertebrate data showing responses in mesocosms and the field are provided (PDF)

Notes

The authors declare no competing financial interest.

Graphical abstract

INTRODUCTION

Responses of stream benthic communities to metals and other stressors associated with abandoned mines have been well characterized in the literature.^{1–5} Reduced abundance, loss of species and shifts in community composition from sensitive to tolerant taxa routinely occur downstream from historical mining operations. Although less commonly measured, alterations in ecosystem processes, including reduced primary productivity, lower rates of litter decomposition and altered trophic relationships, have also been reported.^{6,7} Because mining discharges are a complex mixture of physical and chemical stressors, establishing causal relationships between mining disturbance and community responses, and establishing water quality criteria for these stressors is challenging.^{8,9} In addition to the direct toxicological effects associated with elevated concentrations of metals and reduced pH, mining activity can influence aquatic organisms indirectly through physical alterations of habitat. At circumneutral pH, metals such as Fe and Mn precipitate and coat substrate, fill interstitial spaces and can smother periphyton and benthic macro-invertebrate consumers.¹⁰ Although water quality criteria have been developed for some metals associated with mining activities (e.g., Zn, Cu, Cd), effects of metal-oxide precipitates are generally not considered.¹¹ Furthermore, there is evidence that aquatic insects, particularly early instars which are underrepresented in the databases used to develop water quality criteria,¹² are considerably more sensitive to metals compared to traditional test species.¹³

Results of studies attempting to quantify the relative importance of chemical and physical stressors on benthic communities in streams have been somewhat equivocal. In an assessment of acid mine drainage (AMD) impacts on macroinvertebrates and periphyton, McKnight and Feder¹⁴ concluded that physical effects of metal oxides were greater than the direct toxic effects of low pH or high concentrations of dissolved metals. In contrast, Battaglia et al.⁹ reported relatively little effect of metal-oxide coated substrate on macroinvertebrates in the field, attributing most effects to poor water quality. Relatively low toxicity of metal-oxide precipitates to benthic communities has also been reported in field⁸ and laboratory¹⁵ experiments. However, investigators have cautioned that metals associated with the substrate could become a more significant source of contamination following remediation treatments that are designed to reduce metal loading at the source.⁸

A better understanding of the relative contributions of chemical and physical stressors in mining-impacted streams is crucial for designing stream restoration treatments and predicting effectiveness of these programs. Similar to the development of water quality

standards, restoration efforts in mining-polluted streams often focus on improving water quality, with the implicit assumption that reduced loading of dissolved metals will improve physical habitat. Although sophisticated mechanistic models have been developed to predict downstream improvements in water quality following treatment of mining discharges,¹⁶ it is unlikely these models can predict biological recovery in systems where metal-oxide deposition has occurred.⁶ These indirect effects on benthic habitat will likely delay biological recovery of streams following improvements in water quality.¹⁷

Predicting responses of benthic communities to chemical and physical stressors associated with AMD is also complicated by significant variation in sensitivity among taxa. Field experiments conducted in a mining-impacted Colorado stream demonstrated that some metal-sensitive taxa readily colonized metal-oxide contaminated substrate, whereas other known metal-tolerant taxa avoided these materials.¹⁸ Sasaki et al.¹⁹ hypothesized that the physical effects of metal-oxide deposition will be greater for erosional species (e.g., mayflies, stoneflies, and caddisflies) than for depositional species (e.g., chironomids), and showed that the sequence of recolonization of a restored coal mining stream was consistent with these predictions.¹⁷ A better understanding of differences in sensitivity to physical and chemical stressors among taxa may help explain the observed variation in ecological effects of mine discharges and improve our ability to predict responses to restoration treatments.²⁰

In addition to variation in susceptibility of macroinvertebrates to dissolved metals and contaminated substrate, recovery of AMD streams following improvements in habitat and water quality is highly dependent on recolonization from upstream. Because macroinvertebrate colonization in streams occurs primarily by downstream drift,²¹ predicting recovery requires an assessment of drift rates of individual taxa. The primary objective of this research was to quantify the relative importance of physical and chemical stressors on benthic communities. To predict colonization rate and recovery potential for dominant macroinvertebrate taxa, we integrated experimentally derived estimates of metal tolerance with measures of drift propensity obtained from the literature. Our estimates of recolonization potential were then compared to benthic macroinvertebrate data collected at recovery sites downstream from a source of metal inputs.

MATERIALS AND METHODS

Study Site

The field study was conducted in the North Fork of Clear Creek (NFCC), a third-order tributary to the Clear Creek watershed located near Blackhawk, Colorado (Figure S1, Supporting Information (SI)). NFCC was placed on the National Priorities (“Superfund”) List in September 1983 because of elevated concentrations of metals in Clear Creek, which supplies drinking water to over 500 000 residents in metropolitan Denver. NFCC is characterized as a high-gradient stream with steep canyon walls and strong seasonal variability in stream discharge. Two point sources of metals (National Tunnel and the Gregory Incline Tunnel) account for most of the metal loadings to NFCC.²² Water quality in NFCC downstream from these inputs is characterized as circumneutral (pH 6.5–7.5), with low alkalinity (<15 mg/L as CaCO₃), moderate conductivity (300–600 μ S/cm), low concentrations of dissolved organic carbon (DOC; 1–3 mg C/L) and high concentrations of

Fe (2–20 mg/L), Zn (0.5–1.5 mg/L), and copper (0.01–0.05 mg/L). Water hardness is low upstream of the AMD inputs (<50 mg/L as CaCO₃), but increases downstream to 200–300 mg/L as CaCO₃ as a result of those inputs.

Field Biomonitoring

To assess effects of mining discharges in NFCC, quantitative benthic samples were collected in spring 2012 and 2013 along a longitudinal gradient of metal contamination (Table S1, SI). Reference, impacted and recovery reaches (n = 2 sites per reach) were located upstream, immediately downstream and several km downstream from the sources of metal inputs in the NFCC, respectively. Replicate (n = 5) samples were collected at each site from riffle areas using a 0.1 m² Hess sampler. Samples were washed through a 350 µm sieve in the field and organisms were preserved in 80% ethanol. In the laboratory, all organisms were identified to the lowest practical level of taxonomic resolution (genus or species for most aquatic insects; subfamily for chironomids) and enumerated.²³

Mesocosm Experiments

To quantify direct effects of metals on benthic communities, 2 mesocosm experiments were conducted using natural communities collected from an upstream reference site on the NFCC. Details of the experimental facility and the process used to obtain benthic communities have been described previously.^{13,18} Briefly, 10 × 10 × 6 cm plastic trays filled with natural pebble and cobble substrate were placed at a NFCC reference site for 30 days. Three holes (2.5 cm diameter) drilled in each side of the trays increased water flow and facilitated macroinvertebrate colonization. Trays were colonized by a diverse assemblage of aquatic insects that are very similar to communities in the natural substrate.¹⁸ After colonization, trays were removed from the stream, placed in 4 L insulated containers filled with streamwater (4 trays per container) and transported to the mesocosm facility. The contents of each container were randomly assigned to one of 18 stream mesocosms. Diluent water for the mesocosms originates from a deep mesotrophic reservoir and was delivered to each 20 L stream at a rate of 1.0 L/min. Water quality characteristics (pH, conductivity, temperature, dissolved oxygen) were typical of mountain streams in Colorado. Paddlewheels maintained a constant current velocity of 0.35 m/s in the mesocosms.

Mesocosm experiment I (July 2012) exposed benthic communities to a mixture of metals (Cu, Fe, Mn, and Zn) at concentrations representative of those measured at the reference, impacted and recovery reaches in NFCC (described below). Stock solutions of metals for this experiment were prepared using analytical grade ZnSO₄·7H₂O, CuSO₄·5H₂O, FeCl₃·6H₂O and MnCl₂·4H₂O dissolved in streamwater that was collected from the reference reach. Streams (n = 2 per treatment) were dosed for 10 d with a mixture of metals at nine concentrations: 0X (reference), 0.015X, 0.030X, 0.060X, 0.125X, 0.25X, 0.375X, 0.50X, and 1.0X, where X = the mean concentration previously measured at impacted sites in the NFCC (13 µg/L Cu; 6,580 µg/L Fe; 2,326 µg/L Mn; and 776 µg/L Zn). Stock solutions were brought to pH 7 using sodium hydroxide and were aerated to ensure that Fe precipitates remained in suspension. Peristaltic pumps delivered stock solutions to treated streams from 20 L carboys at a rate of 10 mL/min. At the end of the 10 days exposure period the four trays from each stream mesocosm were removed, rinsed through a 350 µm sieve and

organisms were preserved in 80% ethanol. Samples were processed in the laboratory as described above. Mesh nets covering the standpipes prevented emigration of organisms out of the flow-through mesocosms. Therefore, differences among treatments at the end of the experiment were attributed to mortality.

Mesocosm experiment II (August 2013) used this same experimental design except that streams were dosed with effluent collected directly from Gregory Incline, the primary source of metals to the NFCC. Effluent (~1200 L) was collected every 2 days for these experiments and transferred to the mesocosm facility. To achieve a range of metal concentrations similar to that in mesocosm experiment I, peristaltic pumps delivered effluent to each treated stream at a rate of 1.3–86.9 mL/min. At the end of the 10 day experiment whole community metabolism was estimated in each stream by measuring changes in dissolved oxygen (DO) concentrations of trays placed in clear, sealed polycarbonate chambers (Figure S2, SI). Peristaltic pumps circulated water in the chambers over a DO probe (YSI Incorporated, Yellow Springs, OH) and concentrations (mg O₂/L) were measured every 1–4 min for 30 min in light and dark conditions. Gross primary productivity (mg O₂ per hour) was estimated by summing oxygen production during light conditions (net community production) and oxygen consumption during dark conditions (community respiration). Trays were then removed from the chambers and benthic communities were processed as described in mesocosm experiment I.

To measure the effects of metals on periphyton, we estimated net primary productivity on 6.25 cm² unglazed porcelain tiles placed in each mesocosm at the start the experiment. After 8 days 2 colonized tiles were removed and placed in a 24 mL respiration chamber (Figure S3, SI). Assessments were conducted under uniform light in a chilled water bath that matched the temperature of mesocosm streams. Changes in DO were calculated as described above.

Field Experiments

To assess the effect of metal-contaminated substrate on macroinvertebrates in the field, we conducted a substrate colonization experiment in NFCC during May 2013. Trays (n = 32) filled with small cobble substrate (2–5 cm) were placed at an impacted site for 31 d. During this period the substrate in the trays accumulated a coating of metal oxides. These metal-contaminated trays and an additional 32 control trays containing clean substrate were then deployed at an upstream reference site. To determine the influence of reduced water flow on macroinvertebrate colonization and metal loss, 16 trays of each type were nested within a second tray that lacked holes. After 31 d colonization at the reference site, 4 trays within the same treatment were randomly selected, composited and treated as a single experimental unit. The final experimental design was a 2 × 2 factorial (n = 4), with 2 levels of substrate quality (control versus metal-contaminated) and 2 levels of water flow (low versus high). Benthic macro-invertebrates in the trays were processed as described above. Additional trays of each type were sampled before and after deployment at the reference site to measure metal deposition and loss during the colonization period.

Estimating Colonization and Recovery Potential

To estimate recolonization and potential for recovery in NFCC, we integrated our estimates of tolerance to dissolved metals and metal-oxide coated substrate with published values of macro-invertebrate drift propensity. We defined tolerance to dissolved metals in the mesocosm experiments as the percent survival at the two highest metal treatments (relative to controls), averaged across both experiments. Tolerance to metal-oxide deposition was defined as the percent of organisms on contaminated substrate relative to control substrate. To estimate colonization potential for dominant macroinvertebrates in NFCC we used Rader's²⁴ assessment of drift propensity for 95 species based on 6 traits (intentional drift, habitat preferences, flow exposure, mobility, an index of drag and drift distance). Recovery potential for dominant NFCC macroinvertebrates was estimated based on their tolerance to dissolved metals, tolerance to contaminated substrate and relative drift propensity.

Chemical Analyses

Routine water quality characteristics (dissolved oxygen, pH, conductivity, temperature) were measured on days 2, 4, and 7 of each mesocosm experiment using hand-held meters (models 550A and 63; YSI Incorporated, Yellow Springs, OH). Water samples (0.5 L) were collected to determine hardness and alkalinity in the laboratory using standard titration procedures. Concentrations of Cu, Fe, Mn, and Zn, the four primary metals of concern in NFCC, were measured in the field and in stream mesocosms. Water samples (15 mL) for analysis of dissolved metals (Cu and Zn) were filtered through a 0.45 μm prerinsed glass fiber filter and acidified to a pH of <2.0 with analytical grade nitric acid. We report total concentrations of Fe and Mn because they are more relevant to the physical effects of these metals. Metal concentrations were analyzed using inductively coupled plasma-optical emission spectrometry (ICP-OES) with a PerkinElmer Optima 5300 DV (PerkinElmer, Waltham, Massachusetts). Quality assurance/quality control (QA/QC) samples included deionized (DI) water blanks (Barnstead Nanopure system, Thermo Fisher Scientific) that contained trace-metal-grade concentrated HNO_3 (Thermo Fisher Scientific) and certified continuing calibration verification (CCV) standards.

We determined metal concentrations on metal-oxide contaminated substrate in the field experiment using a modification of EPA Method 3050B. Sediment was transferred from exposure trays to a 2 L polypropylene container, 100 mL of 50% trace-metal-grade nitric acid (diluted with Milli-Q water) was added to the container and the opening was covered with a Teflon watch-glass. The container was placed in a hot-water bath ($\sim 90^\circ\text{C}$) for ~ 15 min, then removed from the bath and allowed to cool. Concentrated trace-metal-grade nitric acid (50 mL) was added to the digestion container and this mixture was allowed to sit at room temperature for 30 min. The container was returned to the hot-water bath ($\sim 90^\circ\text{C}$) for 2 h, then removed and allowed to cool. Milli-Q water (50 mL) and 75 mL of hydrogen peroxide solution (Macron, 30% reagent grade) were added to the mixture. The container was returned to the hot water bath ($\sim 90^\circ\text{C}$) for 15 min, allowed to cool and the total mass was measured. The acid solution remaining in the container was sampled with a disposable polyethylene pipet into a conical tube (Falcon, 50 mL polypropylene), diluted 20 $\times$ with Milli-Q water, and analyzed for dissolved metal concentrations as described above.

Statistical Analyses

Differences in macroinvertebrate abundance and number of taxa among reaches (reference, impacted and recovery) in the NFCC were analyzed using one-way ANOVA (PROC ANOVA; SAS Institute, Version 9.3, Cary, NC). If the overall effects were significant, we used multiple comparisons (REGWQ) to test for differences among means. We developed concentration–response relationships for several community metrics (total macroinvertebrate abundance, taxonomic richness, abundance of major macroinvertebrate orders) and abundance of dominant taxa using regression (PROC REG) on $\log_{10}(x+1)$ transformed values. We also used general linear models (PROC GLM) to test for differences in responses to metals between the 2 experiments (year $\times$ treatment interaction). We used 2-way ANOVA (PROC ANOVA) to determine effects of substrate quality, water flow and the substrate $\times$ flow interaction in the field experiment. Where necessary, abundance measures were $\log_{10}(x+1)$ transformed to satisfy assumptions of parametric statistics.

RESULTS

Field Biomonitoring

Metal concentrations in the NFCC were generally low or below detection at the reference reach, increased 20X–120X below the mine adits and then decreased downstream at the recovery reach (Table S1, SI). Benthic macroinvertebrate communities were effectively eliminated at the metal-impacted sites, but showed significant improvement at the downstream recovery sites (Table S1, SI). Total number of species per sample was reduced by approximately 90% at the impacted sites and mayflies (Ephemeroptera) were rarely collected at these downstream stations. Although communities at the reference sites consisted of a diverse assemblage of between 20 and 23 species of mayflies, stoneflies, caddisflies and dipterans, 3 dominant taxa (*Baetis* sp., *Hydropsyche* sp., and orthoclad chironomids) accounted for 74% of total macro-invertebrate abundance at the recovery sites.

Mesocosm Experiments

With the exception of variables that were directly affected by metal treatments (e.g., conductivity), routine water quality characteristics in mesocosms were generally similar among treatments and between the two experiments (Table S2, SI). Conductivity and water hardness increased as a function of metal treatment in both experiments, but this trend was more apparent in 2013. Circumneutral pH values were observed across all treatments in both experiments, although pH decreased with metal treatment in 2013. Measured concentrations of Cu, Fe, Mn, and Zn increased with treatment levels (Table S3, SI) and were generally consistent between the two experiments.

After 10 days of exposure to metals, we observed highly significant concentration–response relationships in both mesocosm experiments (Figure 1). Eight macroinvertebrate families (Baetidae, Ephemerellidae, Heptageniidae, Nemouridae, Chloroperlidae, Rhyacophilidae, Chironomidae, and Elmidae) were dominant in stream mesocosms and accounted for over 97% of total macroinvertebrate abundance in the two experiments. As indicated by the slopes and strength (r^2 values) of the concentration–response relationships, we observed considerable variation in sensitivity to metals among these groups. Some taxa were reduced

at low to moderate metal concentrations, whereas other groups were unaffected even at the highest concentrations. Effects of metals were greatest on the three mayfly families (Figure 1a–c). Although we observed significant concentration–response relationships for other taxa (chloroperlid stoneflies in mesocosm experiment II; chironomids in both experiments), these relationships were relatively weak. Rhyacophilidae, the dominant caddisflies in our mesocosms, and elmids beetles were unaffected by metals in either experiment.

Compared to control mesocosms, total macroinvertebrate abundance was reduced by 53–58% and number of taxa was reduced by 25–30% at the highest treatment concentrations (Figure S4, SI). Total abundance of mayflies (Ephemeroptera), stoneflies (Plecoptera) and dipterans were also significantly reduced by metals in both mesocosm experiments (Figure S5, SI). Again, the greatest effects were observed on abundance of mayflies, which was reduced by >90% at metal concentrations similar to those measured at impacted sites in the NFCC. In contrast, caddisflies (Trichoptera) were highly tolerant to metals and their abundance was not significantly related to metal concentration in either experiment.

The responses of macroinvertebrate communities to metals in the two mesocosm experiments were quite similar, despite using very different dosing procedures. Results of general linear models analysis showed no significant year $\times$ treatment interaction for most macroinvertebrate groups. These results indicate that regardless of whether metals were delivered as a mixture of laboratory salts or as whole effluent collected from Gregory Incline, effects were similar across macroinvertebrate taxa. The only exception to this observation was for abundance of chloroperlid stoneflies (Figure 1e), which showed significantly greater effects in the whole effluent experiment (mesocosm experiment II) compared to the experiment using metal salts (year $\times$ treatment interaction, $p < 0.0001$).

Effects of effluent from Gregory Incline significantly reduced whole community metabolism in colonization trays and net primary productivity on colonized tiles (Figure S6, SI) in mesocosm experiment II (these end points were not measured in mesocosm experiment I). Both end points showed large decreases at the lowest treatment level compared to controls. Although our estimates of community metabolism measured in whole trays included both biofilm (e.g., microbial and periphyton communities) and macroinvertebrates, the similarity of this response to that observed on colonized tiles suggests that effects were primarily on microbial and periphyton communities. Whole community respiration in trays exceeded photosynthesis in all treatments above 0.125X. Effects of metals were somewhat greater on biofilm than colonized tiles during the experiment. All treatment groups >0.03X produced little or no oxygen, suggesting no periphyton colonization occurred on these tiles.

Field Experiments

Trays deployed at the AMD-impacted site in the NFCC accumulated high concentrations of metals directly on the cobble substrate (Table S4, SI). Although metal concentrations decreased when trays were transferred to the reference site, they remained elevated compared to control substrate. Concentrations of Cu, Fe, and Zn were 1.1–4.0 times greater on metal-oxide contaminated substrate relative to control substrate at the end of the field experiment. Differences between control and metal-contaminated treatments were more apparent under low-flow conditions.

Eight macroinvertebrate families (Baetidae, Heptageniidae, Nemouridae, Chloroperlidae, Lepidostomatidae, Psychodidae, Chironomidae, and Elmidae) accounted for over 88% of total macroinvertebrate abundance in the field experiment (Figure 2). Although abundance of most taxa was reduced in trays containing contaminated substrate, baetid mayflies and the stoneflies Nemouridae and Chloroperlidae were unaffected by substrate quality. However, abundance of Baetidae, Heptageniidae, and Nemouridae was significantly lower in trays with reduced water flow. Heptageniidae was the only group to show a significant substrate $\times$ flow interaction, indicating that effects of contaminated substrate on colonization were greater at low flow conditions.

Total macroinvertebrate abundance was 47% lower on trays containing metal-contaminated substrate compared to controls (Figure S7, SI). Effects of metal-contaminated substrate on macroinvertebrate colonization were highly significant, but there was no effect of water flow on total abundance. Total number of taxa was also significantly lower in trays with contaminated substrate; however, this effect was limited to trays under low flow conditions, as indicated by the significant substrate $\times$ flow interaction term.

Recolonization Potential of Benthic Macroinvertebrates

Estimates of recolonization potential based on tolerance to dissolved metals, avoidance of metal-contaminated substrate and drift propensity showed considerable variation among taxa (Figure 3). Because of their tolerance to metals and moderate drift propensity, we predict that some caddisflies (Hydropsychidae, Rhyacophilidae) and stoneflies (Chloroperlidae) should recover rapidly following improvements in water quality. Despite their extreme sensitivity to dissolved metals in mesocosm experiments, baetid mayflies are also expected to recover quickly because of their high drift propensity and tolerance of contaminated substrate. In contrast, the mayflies Ephemerellidae and Heptageniidae have moderate drift propensity, but were sensitive to both dissolved metals and contaminated substrate. These groups would be expected to recover slowly. Finally, the dipteran Psychodidae was highly tolerant to dissolved metals in stream mesocosms experiments; however, these organisms are expected to recover very slowly because of their low tolerance to contaminated substrate and very low drift propensity.

DISCUSSION

Although the focus of most restoration efforts in AMD-contaminated streams is on improving water quality, removing this single stressor may not be sufficient to restore structural and functional integrity of these systems.^{6,25} The frequently observed lag in recovery of benthic communities following improvements in water quality has been attributed to either the residual impacts of Fe hydroxide deposition¹⁷ or the contribution of AMD precipitates to aqueous metal loading.⁸ Our mesocosm and field experiments provided insights into the relative importance of physical and chemical stressors. We observed that several macroinvertebrate groups, particularly the mayflies Baetidae, Ephemerellidae and Heptageniidae, were highly sensitive to dissolved metal exposure. We believe these effects were primarily a result of aqueous metal exposure; however, some metal-oxide deposition was observed in the stream mesocosms at the end of the experiment which may have limited

resources for grazing insects. These findings are consistent with previous field^{1,26} and mesocosm¹³ studies that reported low tolerance of mayflies to metals. In the present study, exposure to only 12.5% of the metal concentrations measured in the impacted reach reduced total mayfly abundance by 31–35% after only 10 days. These results suggest that mayflies in NFCC would respond to improvements in water quality, but large reductions in dissolved metal concentrations would be necessary. Other macroinvertebrate groups (e.g., chironomids, chloroperlid stoneflies) were also affected by metals in our mesocosm experiments; however, these taxa were considerably more tolerant of AMD stressors and would likely recover following modest improvements in water quality.

Although macroinvertebrate abundance was approximately 50% lower on metal-oxide contaminated substrate compared to controls in the field experiment, it is possible that our field experiment underestimated these effects. Because contaminated substrate was deployed at a reference site with clean substrate, organisms could easily avoid these small patches of poor habitat. Our field experiment did not allow us to distinguish between avoidance and direct toxic effects, but we do not believe that exposure to metal oxides resulted in significant mortality. Our previous mesocosm experiments have shown little evidence of direct toxic effects of dissolved Fe or metal-oxide coated substrates (Cadmus, unpublished results). Furthermore, measured concentrations of Cd, Cu, and Zn on NFCC substrate were typically an order of magnitude less than the probable effect concentrations (PECs) for these metals.²⁷ Similar sediment quality guidelines have not been developed for Fe; however, it is well established that ferric Fe is relatively nontoxic to macroinvertebrates²⁸ and that effects in Fe-contaminated streams result primarily from deposition of Fe hydroxides either directly on organisms^{28,29} or in benthic habitats.^{10,11} In addition, results of toxicity tests and geochemical modeling suggest that the presence of Fe and Mn oxides in surface sediments can reduce the bioavailability of some metals.³⁰ We hypothesize that reduced colonization of contaminated substrate in our experiments was most likely a result of behavioral avoidance by macroinvertebrates. The direct mechanism responsible for avoidance is uncertain, but it was likely a result of poor habitat quality and/or limited food abundance. Given the low periphyton biomass observed at NFCC and the extreme sensitivity of periphyton to metals in the mesocosm experiment, avoidance of contaminated substrate may be partially attributed to reduced food quality for grazing insects.

Previous field studies have attributed reduced abundance of macroinvertebrates and alterations in ecosystem processes to the deposition of Fe hydroxides.^{6,14,18,19} In contrast, others have shown relatively modest responses to AMD precipitates, attributing most of the observed effects to either low pH or elevated concentrations of dissolved metals in water.^{8,9,15} Our stream mesocosm experiments were designed explicitly to quantify the direct effects of toxic metals. Because there were essentially no (or very limited) physical effects of AMD during these short-term exposures, the responses we observed were a direct result of metal exposure. In contrast, our field experiments were conducted at an uncontaminated site using substrate that was coated with metal oxides. Because direct toxicity of metal-oxides was unlikely, avoidance by macro-invertebrates observed in the field was attributable to physical effects. Although we are not the first to report that both physical and chemical effects limit macroinvertebrate communities in streams, we are the first to

quantify how specific taxa will respond to these individual stressors and then use this information to predict patterns of recovery in the field.

Our study used drift propensity to estimate recolonization potential for dominant macroinvertebrates in the NFCC. Species that are relatively tolerant to AMD discharges (e.g., caddisflies) and/or have high drift propensity (baetid mayflies) are expected to recover faster than sensitive species or those that are poorly represented in the drift. The dominance of hydropsychid caddisflies and baetid mayflies at the downstream recovery reach in the NFCC is consistent with this prediction. The extreme sensitivity of grazing mayflies (e.g., Heptageniidae) to both dissolved metals¹³ and Fe-contaminated substrate¹¹ is well established in the literature. Although these organisms have a relatively high drift propensity, we would expect slower recovery because of their sensitivity to AMD stressors. Heptagenid mayflies were not collected from the impacted reach and comprised a very small percentage (<3%) of organisms collected at the downstream recovery reach. Finally, despite their high tolerance of dissolved metals, the dipteran *Pericoma* (Psychodidae) is expected to recover very slowly because of their extremely low drift propensity. These organisms were never collected downstream from the source of metals in NFCC.

Because the dominant taxa in our study are widely distributed in many western streams, our estimates of recovery potential should be broadly applicable to other systems. These estimates generally agree with results of other spatially extensive^{1,26} and longitudinal³¹ surveys conducted along gradients of metal contamination; however, there were notable differences. In particular, orthoclad chironomids, organisms that are generally characterized as metal-tolerant based on field surveys,^{31,32} are expected to recolonize slowly because of their low drift propensity. Because our estimates of recovery potential only account for sensitivity to AMD stressors and drift propensity of dominant taxa, responses to stream restoration treatments may differ from those observed in field surveys. In addition, recovery will likely vary among streams and is dependent on availability of upstream colonists and the specific restoration treatment. For example, if metal concentrations in water decrease rapidly but remain elevated in substrate, species with high drift propensity that are also tolerant of metal-oxide contaminated substrate (e.g., Baetidae) should recover quickly. Because the downstream transport of metals and the deposition of metal oxides in benthic habitats are strongly influenced by streamflow,³³ hydrologic characteristics of a watershed must also be considered when estimating potential recovery rates. Benthic communities in high-gradient streams in which interstitial spaces are frequently scoured may recover faster from the physical effects of metal-oxide deposition compared to those in low-velocity streams.

The development of water quality criteria using traditional test species (e.g., *Ceriodaphnia dubia*, *Pimephales promelas*) has been criticized, largely because of the inability of these experiments to account for the indirect effects of contaminants.^{34,35} Because aquatic insects are poorly represented in the database used to establish these criteria,¹² there is the potential that these organisms will not be protected by current water quality standards.⁵ Additionally, laboratory toxicity tests conducted with aquatic insects, particularly those using later instars, show much greater tolerance to metals compared to patterns observed in the field.^{36,37} For contaminants that do not show significant acute toxicity (e.g., total dissolved solids,

suspended sediments, nutrients, and deposition of metal oxides), traditional laboratory-based toxicity tests may be inappropriate and therefore should be supported by alternative approaches.³⁸ For example, using a field-based assessment, Linton¹¹ concluded that a more stringent benchmark for Fe would be necessary to protect mayflies and other grazers from the indirect effects of metal-oxide deposition. Similarly, benchmarks for total dissolved solids based on field surveys of macroinvertebrate communities were much lower than those based on traditional laboratory toxicity tests.³⁹ A crucial challenge with field-based approaches is that other confounding factors will influence patterns of abundance and distribution and complicate the ability to determine causal relationships between stressors and responses.

Mesocosm experiments represent an important “middle ground” between laboratory toxicity tests and field-based assessments.⁴⁰ Although small-scale experiments have been criticized in the literature,^{41,42} we believe that mesocosm experiments, particularly those conducted with natural communities and an obvious connection to natural ecosystems, provide an ecologically realistic complement to laboratory toxicity tests.⁴³ These experiments also control for the confounding variables associated with field-based approaches, thereby supporting causal relationships between stressors and responses. The similarity of responses between our mesocosm experiments, which were conducted in 2 different years and using very different exposure regimes, demonstrates the reproducibility of this approach. Our study quantified the relative importance of chemical and physical stressors associated with AMD discharges and predicted recovery potential for dominant macroinvertebrate taxa. A better understanding of the mechanisms responsible for variation among macroinvertebrate taxa should further improve our ability to predict restoration effectiveness in AMD-contaminated streams.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Funding for this research was provided to J.R., W.C., and J.M. by the National Institute of Environmental Health Sciences (1R01ES020917-01) and the International Zinc Association. We are especially grateful to Sam Duggan, Henry McLaughlin, and Ramiro Pastorinho for assistance with the mesocosm and field experiments. Comments by Brian Wolff, Chris Kotalik, and three anonymous reviewers are greatly appreciated. Authorship contributions are as follows: PC, MG and WC conducted the field bioassessments and the mesocosm experiments; PC and JW conducted the field experiment. PC and WC were responsible for taxonomic identification and conducted the statistical analyses; metals analyses were conducted by J.W. and J.R.; P.C., W.C., and J.M. wrote the final manuscript.

References

1. Clements WH, Carlisle DM, Lazorchak JM, Johnson PC. Heavy metals structure benthic communities in Colorado mountain streams. *Ecol Appl*. 2000; 10:626–638.
2. Niyogi DK, Lewis WM, McKnight DM. Effects of stress from mine drainage on diversity, biomass, and function of primary producers in mountain streams. *Ecosystems*. 2002; 5(6):554–567.
3. Maret TR, Cain DJ, Maccoy DE, Short TM. Response of benthic invertebrate assemblages to metal exposure and bioaccumulation associated with hard-rock mining in northwestern streams, USA. *J N Am Benthol Soc*. 2003; 22(4):598–620.

4. Hornberger MI, Luoma SN, Johnson ML, Holyoak M. Influence of remediation in a mine-impacted river: metal trends over large spatial and temporal scales. *Ecol Appl*. 2009; 19(6):1522–1535. [PubMed: 19769100]
5. Schmidt TS, Clements WH, Mitchell KA, Church SE, Wanty RB, Fey DL, Verplanck PL, San Juan CA. Development of a new toxic-unit model for the bioassessment of metals in streams. *Environ Toxicol Chem*. 2010; 29(11):2432–2442. [PubMed: 20853459]
6. Niyogi DK, Lewis WM, McKnight DM. Litter breakdown in mountain streams affected by mine drainage: Biotic mediation of abiotic controls. *Ecol Appl*. 2001; 11(2):506–516.
7. Carlisle DM, Clements WH. Growth and secondary production of aquatic insects along a gradient of Zn contamination in Rocky Mountain streams. *J N Am Benthol Soc*. 2003; 22(4):582–597.
8. DeNicola DM, Stapleton MG. Impact of acid mine drainage on benthic communities in streams: the relative roles of substratum vs. aqueous effects. *Environ Pollut*. 2002; 119(3):303–315. [PubMed: 12166664]
9. Battaglia M, Hose GC, Turak E, Warden B. Depauperate macroinvertebrates in a mine affected stream: Clean water may be the key to recovery. *Environ Pollut*. 2005; 138(1):132–141. [PubMed: 15894413]
10. Vuori KM. Direct and indirect effects of iron on river ecosystems. *Annales Zoologici Fennici*. 1995; 32(3):317–329.
11. Linton TK, Pacheco MAW, McIntyre DO, Clement WH, Goodrich-Mahoney J. Development of bioassessment-based benchmarks for iron. *Environ Toxicol Chem*. 2007; 26(6):1291–1298. [PubMed: 17571696]
12. Brix KV, DeForest DK, Burger M, Adams WJ. Assessing the relative sensitivity of aquatic organisms to divalent metals and their representation in toxicity datasets compared to natural aquatic communities. *Hum Ecol Risk Assess*. 2005; 11(6):1139–1156.
13. Clements W, Cadmus HP, Brinkman SF. Responses of aquatic Insects to Cu and Zn in stream microcosms: understanding differences between single species tests and field responses. *Environ Sci Technol*. 2013; 47(13):7506–7513. [PubMed: 23734565]
14. McKnight DM, Feder GL. The ecological effect of acid conditions and precipitation of hydrous metal-oxides in a rocky-mountain stream. *Hydrobiologia*. 1984; 119(2):129–138.
15. Dsa JV, Johnson KS, Lopez D, Kanuckel C, Tumlinson J. Residual toxicity of acid mine drainage-contaminated sediment to stream macroinvertebrates: relative contribution of acidity vs. metals. *Water, Air, Soil Pollut*. 2008; 194(1–4):185–197.
16. Walton-Day K, Runkel RL, Kimball BA. Using spatially detailed water-quality data and solute-transport modeling to support total maximum daily load development. *J Am Water Resour Assoc*. 2012; 48(5):949–969.
17. Walter CA, Nelson D, Earle JJ. Assessment of stream restoration: sources of variation in macroinvertebrate recovery throughout an 11-year study of coal mine drainage treatment. *Restor Ecol*. 2012; 20(4):431–440.
18. Courtney LA, Clements WH. Assessing the influence of water and substratum quality on benthic macroinvertebrate communities in a metal-polluted stream: an experimental approach. *Freshwater Biol*. 2002; 47(9):1766–1778.
19. Sasaki A, Ito A, Aizawa J, Umita T. Influence of water and sediment quality on benthic biota in an acidified river. *Water Res*. 2005; 39(12):2517–2526. [PubMed: 15978645]
20. Niyogi DK, McKnight DM, Lewis WM. Influences of water and substrate quality for periphyton in a montane stream affected by acid mine drainage. *Limnol Oceanogr*. 1999; 44(3):804–809.
21. Waters TF. The drift of stream insects. *Annu Rev Entomol*. 1972; 17(1):253–272.
22. Butler BA, Ranville JF, Ross PE. Spatial variations in the fate and transport of metals in a mining-influenced stream, North Fork Clear Creek, Colorado. *Sci Total Environ*. 2009; 407(24):6223–6234. [PubMed: 19801165]
23. Merritt, RW, Cummins, KW., Berg, MB., editors. *An Introduction to the Aquatic Insects of North America*. 4th. Kendall Hunt Publishing; Dubuque, IA: 2008. p. 1158
24. Rader RB. A functional classification of the drift: traits that influence invertebrate availability to salmonids. *Can J Fish Aquat Sci*. 1997; 54(6):1211–1234.

25. Hogsden KL, Harding JS. Consequences of acid mine drainage for the structure and function of benthic stream communities: a review. *Freshw Sci.* 2012; 31(1):108–120.
26. Schmidt TS, Clements WH, Wanty RB, Verplanck PL, Church SE, San Juan CA, Fey DL, Rockwell BW, DeWitt EH, Klein TL. Geologic processes influence the effects of mining on aquatic ecosystems. *Ecol Appl.* 2012; 22(3):870–879. [PubMed: 22645817]
27. MacDonald DD, Ingersoll CG, Berger TA. Development and evaluation of consensus-based sediment quality guidelines for freshwater ecosystems. *Arch Environ Contam Toxicol.* 2000; 39(1):20–31. [PubMed: 10790498]
28. Gerhardt A. Effects of subacute doses of iron (Fe) on *Leptophlebia marginata* (Insecta, Ephemeroptera). *Freshwater Biol.* 1992; 27(1):79–84.
29. Soucek DJ, Cherry DS, Trent GC. Relative acute toxicity of acid mine drainage water column and sediments to *Daphnia magna* in the Puckett's Creek watershed, Virginia, USA. *Arch Environ Contam Toxicol.* 2000; 38(3):305–310. [PubMed: 10667927]
30. Costello DM, Burton GA, Hammerschmidt CR, Rogevich EC, Schlekot CE. Nickel phase partitioning and toxicity in field-deployed sediments. *Environ Sci Technol.* 2011; 45(13):5798–5805. [PubMed: 21648434]
31. Clements WH, Vieira NKM, Church SE. Quantifying restoration success and recovery in a metal-polluted stream: a 17-year assessment of physicochemical and biological responses. *J Appl Ecol.* 2010; 47(4):899–910.
32. Iwasaki Y, Kagaya T, Miyamoto K, Matsuda H. Effects of heavy metals on riverine benthic macroinvertebrate assemblages with reference to potential food availability for drift-feeding fishes. *Environ Toxicol Chem.* 2009; 28(2):354–363. [PubMed: 18754701]
33. Butler BA, Ranville JF, Ross PE. Observed and modeled seasonal trends in dissolved and particulate Cu, Fe, Mn, and Zn in a mining-impacted stream. *Water Res.* 2008; 42(12):3135–3145. [PubMed: 18433827]
34. Cairns JJ. The myth of the most sensitive species. *BioScience.* 1986; 36(10):670–672.
35. Clements WH, Rohr JR. Community responses to contaminants: using basic ecological principles to predict ecotoxicological effects. *Environ Toxicol Chem.* 2009; 28(9):1789–1800. [PubMed: 19358627]
36. Brinkman SF, Johnston WD. Acute toxicity of aqueous copper, cadmium, and zinc to the mayfly *Rhythrogena hageni*. *Arch Environ Contam Toxicol.* 2008; 54(3):466–472. [PubMed: 17917759]
37. Brinkman SF, Johnston WD. Acute toxicity of zinc to several aquatic species native to the Rocky Mountains. *Arch Environ Contam Toxicol.* 2012; 62(2):272–281. [PubMed: 21811884]
38. Pacheco MAW, McIntyre DO, Linton TK. Integrating chemical and biological criteria. *Environ Toxicol Chem.* 2005; 24(11):2983–2991. [PubMed: 16398137]
39. Cormier SM, Suter GW II. A method for deriving water-quality benchmarks using field data. *Environ Toxicol Chem.* 2013; 32(2):255–262. [PubMed: 23147651]
40. Clements WH, Kotalik C. Effects of major ions on natural benthic communities: an experimental assessment of the US Environmental Protection Agency aquatic life benchmark for conductivity. *Freshw Sci.* 2016; 35(1):126–138.
41. Carpenter SR. Microcosm experiments have limited relevance for community and ecosystem ecology. *Ecology.* 1996; 77(3):677–680.
42. Schindler DW. Replication versus realism: The need for ecosystem-scale experiments. *Ecosystems.* 1998; 1(4):323–334.
43. Clements WH. Small-scale experiments support causal relationships between metal contamination and macroinvertebrate community responses. *Ecol Appl.* 2004; 14(3):954–967.

Figure 1.

Mean (±s.e.) abundance of dominant macroinvertebrate taxa as a function of metal treatment in stream mesocosm experiments I (2012) and II (2013). Results of statistical analyses testing for significant concentration–response relationships are shown for each dominant taxa.

Figure 2.

Mean abundance of dominant macroinvertebrate taxa in colonization trays containing clean or metal-contaminated substrate placed at an upstream reference site. Results of 2-way ANOVA testing for effects of substrate (control versus metal-contaminated), flow (low versus high) and the substrate $\times$ flow ($S \times F$) interaction are also shown. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.0001$.

Figure 3. Estimated recovery potential for dominant macroinvertebrate taxa in the North Fork of Clear Creek, Colorado. Tolerances to metals and contaminated substrate were based on mesocosm and field experiments. Drift propensity was determined from Rader (1997).