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Influence of metal contamination and sediment deposition on benthic invertebrate colonization at the North Fork Clear Creek superfund site, Colorado, USA

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Abstract

Assessing benthic invertebrate community responses to multiple stressors is necessary to improve the success of restoration and biomonitoring projects. Results of mesocosm and field experiments were integrated to predict how benthic macroinvertebrate communities would recover following the removal of acid mine drainage from the North Fork of Clear Creek (NFCC), a U.S. EPA Superfund site in Colorado, USA. We transferred reference and metal-contaminated sediment to an upstream reference site where colonization by benthic macroinvertebrates was measured over 30 days. Additionally, a mesocosm experiment was performed to test the hypothesis that patches of metal-contaminated substrate impede recolonization downstream. Abundance in all treatments increased over time during field experiments; however, colonization was slower in treatments with metal-contaminated fine sediment. Community assemblages in treatments with metal-contaminated fine substrate were significantly different from other treatments. Patterns in the mesocosm study were consistent with results of the field experiment and showed greater separation in community structure between streams with metal-contaminated sediments and reference-coarse habitats; however, biological traits also helped explain downstream colonization. This study suggests that after water quality improvements at NFCC, fine-sediment deposition will likely reduce recovery potential for some taxa; however highly mobile taxa that avoid patches of contaminated habitats can recover quickly.

TOC image

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Supporting Information

Supporting information includes figures of the study site (Figure S1) and composition of trait groups in mesocosm experiment (Figure S2), and tables showing results of metal concentrations (Table S1), sediment and organic matter concentrations (Table S2) and correlations to community composition (Table S3), ANOVA outputs for field (Table S4) and mesocosm (Table S5) experiments, pairwise-comparisons and SIMPER output for the mesocosm experiment (Table S6), and pairwise comparisons of communities by tray position (Table S7).

INTRODUCTION

Fine sediment and low amounts of trace metals occur naturally in aquatic ecosystems; however, human activities increase these inputs and result in low abundances of aquatic organisms at sites affected by mining activities.^{1–4} Mining can contribute to fine sediment deposition in aquatic ecosystems resulting in habitat loss, streambed homogenization, contaminant-loading and alterations of ecosystem functions, each of which impacts aquatic organisms.^{4–7} Independent of metal contamination, accumulation of sediment particles < 2 mm is often associated with the physical stress by abrasion and disruption of benthic invertebrate community structure.^{8–11} As the release of metals and sediment from historical and modern mining activities continues to degrade aquatic ecosystems, restoration managers require information on macroinvertebrate community responses if they hope to improve the likelihood of success in restoring mined watersheds.

Research on the combined effects of metals and fine sediment on aquatic macroinvertebrates has mostly focused on single-species laboratory tests and observational studies.

Observational studies show low benthic invertebrate abundances at sites with metal contamination.^{12,13} However, similar community responses to fine sediment accumulation have been reported from field experimental and observational studies.^{14,15} Laboratory studies show metal-contaminated fine-sediment can inhibit growth of invertebrates,¹⁶ reduce fertility,¹⁷ and that metals bioaccumulate in organisms.^{10,18,19} Exposure to metals in the sediment may also be higher compared to aqueous-only exposures depending on feeding strategy and behavioral avoidance.^{20–22} Benthic invertebrates may ingest metal-contaminated fine sediments, thereby increasing body burdens of metals.^{23,24}

Macroinvertebrates may also avoid metal-contaminated sediment, thus reducing likelihood of exposure.^{21,22,25} These species-specific factors may result in an over- or underestimation of the impacts of metal-contaminated fine sediment on recovery of mining-impacted streams. Field and mesocosm experiments can be useful in determining cause-and-effect relationships at sites with multiple stressors, and many authors have recognized the importance of incorporating experimentation in applied studies.^{7,13,26,27} However, field experiments that test the combined effects of both metal-contamination and fine-sediment deposition on benthic invertebrate community responses have not received much attention, even though these stressors often co-occur in mining-impacted watersheds.

Several studies have found that high sediment inputs at mining sites exceed the input produced from natural landscapes.^{28–30} Metal contaminated sediments can remain in aquatic

ecosystems long after the source of contamination has been eliminated,³¹ thereby increasing the duration of metal exposure, with long-term implications on stream health. This can be especially detrimental in the Rocky Mountain streams where most species are adapted to cobble and gravel-bed habitats that provide enough interstitial space for refuge. A loss of macroinvertebrate habitat due to clogging of interstitial spaces can have impacts on species abundance and distributions in streams.^{4,32}

The ability of macroinvertebrates to recolonize previously disturbed areas has been demonstrated,³³ but field experiments to determine cause-and-effect relationships are lacking. Understanding how macroinvertebrates respond following a disturbance is especially important in stream restoration projects and estimating recovery potential.³⁴ Additionally, behavioral avoidance of contaminated sediments, which has been understudied in macroinvertebrate communities, can provide a more environmentally realistic assessment of ecological responses to stressors.³⁵ This study used an experimental approach to quantify the combined effects of metal contamination and fine sediment deposition on benthic invertebrate communities. We performed a field experiment with the objective of predicting responses of benthic invertebrate communities after remediation of the North Fork Clear Creek (NFCC), a U.S. EPA Superfund site impacted by both metal contamination and fine sediment deposition. Environmental stressors may increase the patchiness of benthic invertebrate populations in lotic environments and influence populations colonizing downstream reaches.^{36,37} There is also evidence that benthic invertebrates exhibit avoidance behavior when exposed to metals and that avoidance is a highly sensitive indicator of environmental stress.³⁵ Therefore, a mesocosm experiment was conducted to test the hypothesis that contaminated habitats influence downstream colonization, and the likelihood of benthic invertebrate movement beyond patches of contaminated sediment. Since previous research has shown that feeding strategies and mobility traits may influence sensitivity or exposure to metals,^{20,38} we also examined whether trait responses influenced downstream colonization in our mesocosm experiment. These research objectives were designed to help predict recovery at the NFCC following improvements in water quality, but also to answer broader ecological questions about the effects of multiple stressors on the distribution and recruitment of macroinvertebrates in restored streams.

MATERIALS AND METHODS

Study Site

The colonization experiment was performed at a reference site upstream of metal contamination in the North Fork of Clear Creek (NFCC; N39.81271, W105.49821) in Blackhawk, Colorado, USA in (Figure S1). NFCC is a tributary to the Clear Creek watershed and located approximately 50 km west of Denver, Colorado, USA. The downstream reach on NFCC was designated a U.S. Environmental Protection Agency (EPA) Superfund site in 1983 due to elevated levels of metals. High concentrations of zinc, cadmium, copper, iron and aluminum³⁹ have resulted in low benthic invertebrate abundances and there are no fish populations present. Clear Creek is used for drinking water, local industry, and recreational purposes, making the water quality issues on NFCC a serious human health concern. Construction of a water treatment plant on NFCC was initiated and

became operational in early 2017. Due to previous mining activities, NFCC has been severely degraded by both acid mine drainage from a point source and fine sediment accumulation from various non-point sources. Steep incline of the streambanks, tailings piles in the riparian areas, and the close proximity to a road makes NFCC highly susceptible to sediment accumulation from mining and other anthropogenic activities.

Field Experiment

The colonization experiment was performed upstream of the source of mining contamination at a reference site from August to September 2014. Physicochemical characteristics of the reference site were monitored throughout the project with YSI meters (models 550A and 63; YSI Incorporated, Yellow Springs, OH), with an average $\pm$ standard deviation (SD) of water temperature of 8.16 ± 3.06 °C, dissolved oxygen of 9.18 ± 0.78 mg/L, and pH of 7.86 ± 0.15 . Unlike sites downstream of the contamination, habitat at the reference site is a heterogeneous mixture of riffles and pools. The high diversity of benthic invertebrates and presence of fish populations at the reference site are the targeted restoration goals for the downstream reaches. Because recolonization of the downstream reaches will occur predominantly from macroinvertebrate drift, it is important to understand how this community will respond to stressors.

Metal contaminated sediments were collected in NFCC near the source of contamination (N39.79867, W105.48174) and moved 2.6 km upstream to the reference site (Figure S1). At both the reference and metal contaminated sites, areas of sediment deposition were located, and fine sediment was collected from the stream. The experiment used six treatments in a full factorial design to discern between the impacts of metal contamination and sediment deposition (Figure 1a). Treatments were created by placing coarse sediment (i.e. cobble > 2360 μm) from the metal contaminated or reference site in colonization trays ($25 \times 25 \times 10$ cm). In addition to the coarse sediment, these colonization trays were either filled with fine-sediment (i.e. sand/silt < 2360 μm) from the reference or metal-contaminated site, or received no fine sediment. Each of the six treatments (A–F) had three replicates and each replicate was a composite of two colonization trays (Figure 1b). A total of 144 colonization trays were attached to racks and then placed in the stream.⁴⁰ Small holes (1.25 cm diameter) in each side of the trays allowed water to flow through and facilitated colonization by macroinvertebrates. Trays were collected on days 5, 10, 20, and 30 (36 per day; however, one replicate from the F-treatment, was lost on day 30). Large substrate was scrubbed to remove insects and the remaining contents of the trays were transferred to a container and preserved in 80% ethanol. Additionally, on each collection day, three benthic samples were collected using a 0.1 m² Hess sampler for comparisons with the invertebrate community in the trays. Benthic samples were rinsed through a 355 μm sieve and preserved in 80% ethanol. All insects were identified to genus except for chironomids and early instars, which were identified to order or family.^{41,42}

Concentrations of metals are often higher in fine-grain sand and silt (< 355 μm),⁴³ whereas large sand particles (> 355 μm) can be associated with physical stress and abrasions to macroinvertebrates.¹¹ Therefore, sediment and organic matter at two size fractions (355–2360 μm and 63–355 μm) were measured in every sample to account for material entering

and leaving the trays throughout the experiment. After removing insects from each tray, sediment was sieved through a series of 2360 μm , 355 μm , and 63 μm sieves. Sediment captured in the 355 μm and 63 μm was then dried at 65 °C for 24 h and combusted at 550 °C for 3 h to estimate organic matter and sediment weight (g) for days 5, 10, 20, and 30.

Additional trays of each treatment were placed at the reference site and collected on days 15 and 30 to provide a measurement of metal concentrations during the experiment. The samples were transferred to a pre-weighed HDPE digestion container, weighed, and were acid extracted using a modification of U.S. EPA Method 3050B at Colorado School of Mines, Golden, Colorado, USA. A 1:1 mix of nanopore water and concentrated HNO_3 was added and the extraction was performed in a water bath at 90 °C for 20 minutes. An additional 50 mL of concentrated HNO_3 was added and the digestion process was continued for 2 hours. Concentrated hydrogen peroxide (30%) was added to the digestion chamber and reheated in the water bath at 90 °C for 15 minutes. The final mass was recorded, and a 50 mL sample was removed and filtered (0.45 μm) into a falcon tube and taken for metal analysis by inductively coupled plasma-optical emission spectrometry (ICP-OES) using a PerkinElmer Optima 5300 DV (PerkinElmer, Waltham, Massachusetts). Quality assurance/quality control (QA/QC) measures included use of In as an internal standard (introduced continuously). Approximately every 15 samples an analysis of a 2% trace-metal-grade concentrated HNO_3 (Thermo Fisher Scientific) blank and two certified continuing calibration verification (CCV) standards was performed. All analyses were performed in triplicate. Metal concentrations for each treatment were then converted to total threshold effect concentrations (TEC). Total metals TEC values for each treatment is the sum of the ratio of measured metal concentrations divided by the predicted threshold concentration. Predicted threshold effect values for each metal are derived from species sensitivity values and indicate whether or not metal concentrations are harmful to aquatic organisms.⁴⁴

Mesocosm Experiment

This experiment was designed to measure benthic invertebrate colonization to patches of reference substrate located downstream of contaminated patches. Each stream ($n = 18$) contained source, treatment, and sink trays which were defined by the tray's position in the mesocosm. Colonization trays (25 $\times$ 25 $\times$ 10 cm with 1.25 cm holes) containing clean substrate were placed at the reference site on NFCC for 30 days and then transported to the Stream Research Laboratory at Colorado State University (Fort Collins, Colorado, USA). Two colonized trays (defined as source populations on reference-coarse sediment) were placed in stream mesocosms (Figure 1c), directly upstream of two un-colonized trays containing one of the six substrate treatments described above (Figure 1a). Three un-colonized trays containing clean reference substrate (defined as sink trays) were placed downstream from the treatment trays. Individual trays from each of the 3 categories were composited to represent a single sample and the unit of replication was an individual stream mesocosm. Since community composition was not significantly different among the two sink trays adjacent to the treatment-trays and the one sink tray further downstream, based on results of pairwise PERMANOVA of tray position ($Df = 24$; $t = 1.1841$; $p = 0.196$), all three trays were combined for analysis. Dissolved oxygen (8.61 ± 0.0274 SD mg/L), water temperature (10.55 ± 0.0211 SD °C), and conductivity (113.98 ± 4.13 SD) were measured

throughout the experiment and flow rates were maintained at 1 L/min. After ten days, trays were collected from each stream, rinsed through a 355 µm sieve, and benthic invertebrate samples were processed as described above.

Statistical Analysis

For the field experiment, we used three-way ANOVA to measure effects of colonization time (days 5, 10, 20, and 30), metal treatment (reference coarse, RC; metal contaminated coarse, MC) and fine sediment deposition (no fines, NF; reference fines, RF; metals fines, MF). This design allowed us to test for the effects of metals associated with coarse (RC vs. MC) and fine (NF vs. RF vs. MF) substrate. For univariate analysis, we performed three-way ANOVAs using the generalized linear models (PROC GLM; SAS v9.4, SAS Inc., Cary, NC, U.S.A.) procedure to measure the effects of day, metals, and fine-sediment deposition on several macroinvertebrate metrics, including Shannon-diversity, Pielou's evenness, Margalef's richness, number of taxa, abundance of dominant taxa (*Baetis* sp., *Taenionema pallidum*, *Rhyacophila* sp., and Chironomidae), total abundance and abundance of the three major macroinvertebrate orders (Ephemeroptera, Plecoptera, and Trichoptera). All data were log-transformed except for Shannon-diversity, Pielou's evenness, Margalef's richness, and number of taxa.

Multivariate analysis (PRIMER-e v7; Quest Research Limited, Cambridge, United Kingdom) with the +PERMANOVA package⁴⁵ was used to assess changes in benthic invertebrate community composition among treatments. A Bray-Curtis similarity matrix was created using log-transformed community abundance data.⁴⁶ A three-way permutational multivariate analysis of variance (PERMANOVA) was run at 999 permutations to examine the effects of metals, fine sediment, and day. Pairwise-comparisons for each analysis were performed to examine more complex trends in the data. Significance for all tests was determined based on a $p < 0.05$, with Monte Carlo p-values being used for pairwise-comparisons tests with 10 permutations.⁴⁷ Major trends in the data were then visually represented using nonmetric-multidimensional scaling (NMDS) plots and similarity ellipses were added to plots. Similarity ellipses between 60% and 80% were superimposed onto NMDS plots based on output of hierarchical cluster analysis of group averages.⁴⁸ We selected 60–80% because this was the broadest estimate of similarity values between treatments where statistical differences occurred.

Sediment and organic-matter data and were compared to biotic data using the stepwise procedure run with 999 permutations with the distance-based linear model (DISTLM) package in Primer-e. DISTLM procedure determines the relationship between multivariate datasets and predictor variables.⁴⁵ DISTLM was performed separately for each treatment with corrected-Akaike Information Criterion (AICc) selection criteria to determine the best environmental variables that explained trends in community composition.⁴⁷

For the mesocosm study, macroinvertebrate community structure and trait composition were the primary response variables measured. Univariate data were analyzed as described in the field study. A three-way PERMANOVA (multivariate data) was performed to measure effects of metal treatment (RC vs. MC; Figure 1a), fine sediment deposition (NF vs. RF vs. MF; Figure 1a) and tray position (source, treatment, and sink; Figure 1c) on community

composition. Pairwise-comparisons for differences between treatments were performed to examine trends in the data. SIMPER analysis was also performed on log-transformed data to determine which taxa accounted for > 50% of the dissimilarity between treatments and tray positions in the PERMANOVA tests.⁴⁸

Mobility and ecology traits were assessed based on a comprehensive trait dataset,⁴⁹ which was obtained from the literature. We analyzed four groups of functional traits: drift frequency (abundance, common, and rare drift frequency); swimming ability (none, weak, and strong swimmers); habitat preference (burrow, climb, crawl, sprawl, and swim habitat) and feeding guild (collector-gatherer, collector-filterer, herbivore, predator, and shredder). Using a factorial design, relationships among fine sediment, metal contamination, tray (source, treatment, and sink trays) and macroinvertebrate communities were examined. For trait comparisons, only treatments A (reference-coarse sediment) and F (metal-contaminated coarse and fine sediment) were compared to determine if patches of metal-contaminated sediment present at NFCC could affect the type of invertebrates that colonize downstream suitable habitats. A two-way PERMANOVA was performed to test effects of treatment (A vs. F; Figure 1a) and tray position (source, treatment, and sink; Figure 1c).

RESULTS

Field Colonization Experiment

Iron, zinc, manganese, copper and nickel were the dominant metals measured on NFCC substrate, and concentrations of these metals were combined to estimate threshold effect concentrations. Metal concentrations in the trays were not significantly different throughout the experiment and concentrations in the metal treatments approximated values measured at our metal-contaminated collection site.^{38,50} Total metal concentrations in treatments with reference-site coarse substrate (RC) remained significantly lower than in treatments with metal-contaminated coarse and fine sediment ($F = 10.1$, $p = 0.0002$; Table S1). Additionally, the amount of fine sediment in each treatment did not significantly change over time ($p > 0.05$), and NF (no fines) trays had significantly less fine sediment than RF (reference fines) and MF (metal fines) trays ($p < 0.01$; Table S2). Organic matter in treatments was relatively constant throughout the experiment (Table S2). DISTLM analysis showed that organic matter was the most important variable influencing macroinvertebrate trends in all treatments expect for trays with metal coarse and fines (Table S3).

Over 24,000 insects distributed among 37 genera were collected and identified during this experiment. PROC GLM results showed varying responses of total abundance, number of taxa, and diversity to metal contamination and sediment deposition with no three-way interaction between fines, metals, and day (Figure 2; Table S4). Total benthic invertebrate abundance increased over time in all treatments but was significantly lower in metal treatments compared to reference sediment. The effect of metal contamination on total abundance decreased over time, as indicated by the significant metals $\times$ day interaction term. In contrast, the impact of fine sediment appeared to increase over time between treatments with NF and MF. On day 30, treatments with reference coarse-sediment and reference-fines declined.

Of the 37 taxa collected during this experiment, *Baetis* sp. (Ephemeroptera), *Taenionema pallidum* (Plecoptera), *Rhyacophila* sp. (Trichoptera), and Chironomidae (Diptera) were the most dominant in their corresponding insect orders. The responses of these dominant taxa to metal contamination were similar to those observed for total abundance; however, each taxon had a varying response to fine sediment deposition (Figure 2). The dominant mayfly (*Baetis* sp.) was not significantly affected by fine sediment ($p = 0.1906$), whereas abundance of the stonefly *T. pallidum* was greatest in treatments with only coarse sediment. Abundance of the caddisfly *Rhyacophila* sp. was also significantly lower on metal contaminated fine sediment compared to the other treatments. Chironomidae responded negatively to fine-sediment deposition; however, specific responses to metal-contaminated fines were variable throughout the experiment.

Results of multivariate analysis showed that community assemblages significantly responded to fine sediment and metal contamination, and that these results varied over time (Figure 3; Table 1). Similar to responses of dominant taxa, community assemblages showed significant responses to fine-sediment ($p = 0.009$) and metal contamination ($p = 0.001$); however, there were no significant interaction effect. There was also no interaction among fines, metals, and day ($p = 0.225$).

Although there were effects of metal contamination on community composition throughout the experiment, differences between treatments with (treatments C and F) and without (treatments A, B, D, and E) metal-contaminated fine sediment were greatest on day 30 (Figure 3). Treatments with metal-fines were only significantly different from treatments with no-fines on day 5 ($p < 0.01$); however, on day 30 all fine-sediment treatments were significantly different from one another ($p < 0.05$). Greater separation between communities on reference-coarse (treatments A–C) and metal-coarse (treatments D–F) trays were observed early in the experiment. Additionally, based on NMDS plots, benthic invertebrate abundances showed greater differences between reference-coarse trays over time, whereas trays with metal-coarse sediment became more similar (Figure 3).

Mesocosm Experiment

The goal of the mesocosm experiment was to determine if benthic invertebrates from reference communities could colonize reference substrate located downstream of contaminated substrate. All community metrics were significantly affected by tray position (source vs. treatment vs. sink trays; Table S5). The results of multivariate analysis showed that community composition was significantly affected by metal contamination, fine sediment and tray position. There was also a significant interaction of metals and tray position ($p < 0.05$; Table 2), with fewer taxa colonizing the metal-contaminated trays.

Differences in colonization between treatments and tray position were visualized in NMDS plots (Figure 4). In each treatment, communities from the source population were significantly different from all downstream trays. In the streams with no-fine sediment treatments (treatments A and D; Figure 1a), we observed significant differences ($p < 0.05$) in community composition between all trays, which were largely due to greater abundance of chironomids in the sink trays (Table S6). Trays with metal-coarse treatments (treatments D, E, and F; Figure 1a) showed greater separation between all trays, particularly between the

treatment trays and sinks trays (Figure 4). The difference between the source and downstream trays in streams with reference and metal fine-sediment treatments were largely due to several mayflies, stoneflies and caddisflies (e.g., *Capnia* sp., *Rhithrogena* sp., *Rhyacophila* sp., *Zapada* sp., and *Taenionema* sp.) that failed to colonize downstream trays (Table S6).

We analyzed four groups of functional traits (drift frequency, swimming ability, habitat preference, feeding guild) that could provide insight into the role of taxa mobility and ecological niche in downstream colonization of reference-coarse (treatment A) and metal-coarse + fines (treatment F) treatments. Species that were either rare or common in the drift (e.g., *Drunella* sp., *Micrasema* sp., *Rhyacophila* sp. and *Lepidostoma* sp.) generally remained on the source trays but decreased significantly in downstream treatment and sink trays (Figure 5). In contrast, species defined as abundant in the drift (e.g., *Baetis* sp. and Chironomidae) increased significantly in sink trays and were the only organisms reduced on metal-contaminated substrate. Although we observed significant differences between substrate treatments based on swimming ability, habitat preference and feeding guild, larger differences were associated with tray position, as organisms consistently avoided trays with contaminated substrate (Figure S3). Significant interactions between metal treatment and tray position resulted from greater separation among trays in streams with metal-contaminated substrate (treatment F) compared to reference substrate (treatment A; Table S7).

DISCUSSION

The most important finding of our research was that macroinvertebrate communities responded quite differently to the effects of metal contamination and sediment deposition in both the field and in stream mesocosms. Although previous research has investigated the adverse effects of metal contamination on benthic communities,^{1,33,38} few studies have examined the combined effects of metals and fine sediment deposition. Because these stressors often co-occur,^{28–30} understanding their combined and interactive effects is critical for predicting responses to restoration of mine-polluted watersheds.

Construction of a water treatment plant on the NFCC is expected to result in a rapid decrease in metals discharged to the system. Despite these predicted improvements in water quality, our results suggest metal-contaminated sediments, both coarse and fine, will likely impede benthic invertebrate colonization downstream. In particular, metal contamination had the greatest impact on early colonizing taxa, such as *Baetis* sp. and Chironomidae (Orthocladiinae and Diamesinae). These taxa are dominant at NFCC and very common in the drift⁴⁹, which may explain why they rapidly colonized trays in our field experiment. Both groups were also sensitive to metals in coarse and fine sediments, especially early in the study. This trend was also observed in our mesocosm experiment where baetids and chironomids avoided metal-contaminated coarse and fine treatments.

The primary hypothesis that motivated our mesocosm study was that patches of contaminated substrate may act as barriers to downstream colonization. Although this idea is not new, to our knowledge the application of patch dynamics within the context of chemical

stressors in lotic ecosystems has not been investigated experimentally. In our study, this hypothesis was supported for caddisflies and some stoneflies, but generally communities on source and sink trays were very similar. However, inability of some less mobile taxa to colonize downstream of contaminated habitats, as well as lower diversity and richness downstream, supports the hypothesis that chemical and physical stressors will create habitat patches following improvements in water quality at NFCC. Although most organisms avoided contaminated substrates in our mesocosm study, some taxa were abundant on the downstream sink trays. This was especially true for highly mobile organisms that were abundant in the drift. Interestingly, these same organisms were also significantly reduced in mesocosms containing metal-contaminated substrate, suggesting greater mortality of these highly mobile species. The ability of some invertebrates (e.g., *Baetis* sp.) to rapidly colonize clean habitat creates significant patchiness in their abundance and distribution. Because of the increased patchiness of benthic invertebrates at contaminated sites,⁷ there needs to be careful consideration of sampling methods and necessary sample sizes to detect effects.⁵¹

One of the major criticisms of traditional laboratory toxicity tests is the lack of ecological realism and the inability to account for processes such as insect emergence, predator-prey interactions, or behavioral avoidance. Using a combination of field studies, community-level experiments, and laboratory toxicity tests, we may be able to improve predictions of community responses to contaminants and other anthropogenic stressors. This study suggests that behavioral avoidance and the inability of some taxa to colonize contaminated patches of substrate complicate the ability to predict responses to, and recovery from mining discharges.

One underlying question is whether the outcome of laboratory toxicity and single-contaminant experiments can be used to predict responses in the field. Several studies indicate that Chironomidae are generally more tolerant to metals than other taxa;^{33,52} however, in the current study chironomids (primarily Orthocladinae) generally avoided metal-contaminated substrate in mesocosm and field experiments. This may indicate that some chironomids are more sensitive to metals than previously thought, since the ecological consequences of avoidance and mortality are similar.³⁵ In contrast to the patterns for metal contamination, some of the variation in abundance of chironomids was explained by the amount of fine sediment in trays, which provided important habitat for these burrowing organisms. Field and mesocosm approaches play a critical role in addressing questions about the recovery of taxa after exposure to multiple anthropogenic disturbances. These experiments also demonstrate the importance of accounting for colonization ability, behavioral avoidance and patch dynamics when assessing impacts of mining on streams. Although our mesocosms cannot be completely scaled to the field, it is expected that patches of suitable habitat will be available for colonization downstream after restoration. While some invertebrates experience direct mortality due to metal exposure, avoidance of patches of metal-contaminated substrate may be a more important factor determining community composition following stream restoration.

Previous investigators have measured effects of contaminated substrate on colonization dynamics and recovery potential of benthic macroinvertebrates.^{22,38} For example, recovery potential based on tolerance to aqueous metals, avoidance of metal-contaminated coarse

substrate and natural drift propensity of benthic invertebrates have been previously estimated.³⁸ Although natural drift propensity may determine the movement of macroinvertebrates to downstream habitat patches, the present study suggests that recovery of some macroinvertebrates is also influenced by avoidance of fine sediments. Avoidance of fine sediment is likely due to the loss of habitat and interstitial spaces for macroinvertebrates.^{4,5,7} Since many taxa at NFCC are adapted to cobble and gravel bed habitats typical of high gradient streams, patches of fine sediment deposition may act as habitat filters for macroinvertebrates.⁵³ Because initial recovery of mining-disturbed streams may largely depend on avoidance of contaminated patches, these findings demonstrate the need to develop a better understanding of species traits in response to mining disturbance and the importance of accounting for multiple stressors when assessing recovery potential of disturbed watersheds.

In conclusion, our field and mesocosm experiments provided insights into recovery potential that could not be obtained using traditional laboratory or field bioassessment approaches. Although our experiments were relatively short-term, we determined how several dominant taxa were affected by mining disturbance and predicted how benthic communities would likely respond during the early and late stages of recovery following stream restoration. Our findings also suggest that the high variability and rapid recolonization of aquatic insects downstream from sources of metal contamination and fine sediment may increase population patchiness. We also identified important interspecific differences in the response to metals and sediment deposition. In the present study *Baetis* sp. avoided metal-contaminated coarse substrate, whereas chironomids were relatively sensitive to metal-contaminated fine sediment. These inconsistencies between traditional laboratory studies and responses in field and mesocosm experiments demonstrate the need to develop more creative approaches to quantify effects of multiple stressors. By accounting for ecological factors such as patch dynamics, biological traits and colonization we could improve our ability to predict success of stream restorations programs and reduce the likelihood of over- or underestimating effects of contaminants.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

(a) Schematic of the 2×3 factorial study design showing all six treatments used in the field and mesocosm experiments. Coarse sediment refers to all trays with reference-coarse (RC) and metal-contaminated coarse (MC) sediment. Fine sediment refers to the trays that were not filled with fine-sediment (NF), trays filled with reference-site fine sediment (RF), and trays with metal-contaminated fine sediment (MF); (b) Study design of the field experiment where each treatment has three replicates and each replicate is a composite of two randomly selected colonization trays. (c) Study design used in the 10-day mesocosm experiment. Numbers (c.1–3) refers to the tray's position in the experimental streams. Source populations (c.1) were trays containing reference-coarse sediment that were colonized with macroinvertebrates from NFCC. Treatments (c.2) refer to uncolonized trays from the six treatments A-F. Sink trays (c.3) are uncolonized trays containing reference-coarse sediment.

Figure 2.

The effect of metal-contamination and fine-sediment on colonization by dominant macroinvertebrates in the field study. The effect of metals compared reference-site coarse substrate and metal-contaminated coarse sediment (treatments in rows in figure 1a). The effect of fine-sediment compared trays with no fine sediment, reference-site fine sediment, and metal-contaminated fine sediment (treatments in columns figure 1a). Bars represent standard error of the mean. Superscripts after the factorial comparisons show ANOVA output of either, not significant (NS), $p < 0.05$ (*), $p < 0.001$ (***), and $p < 0.0001$ (****).

Figure 3. NMDS plot showing effects of fine sediment (symbols), and individual treatments (letters) from the field experiment. A = reference-coarse; B = reference-coarse + fines; C = reference-coarse + metal-fines; D = metal-coarse; E = metal-coarse + reference-fines; and F = metal-coarse + fines (see Fig. 1 for details). Ellipses show percent similarity between samples.

Figure 4.

NMDS plots showing the separation of trays based on community composition in treatments with reference and metal coarse sediments. A = reference-coarse; B = reference-coarse + fines; C = reference-coarse + metal-fines; D = metal-coarse; E = metal-coarse + reference-fines; and F = metal-coarse + fines (see Fig. 1 for details). Ellipses show percent similarity between treatments.

Figure 5.

Effects of metal-contamination on colonization of macroinvertebrates in stream mesocosms based on 3 categories of drift frequency (abundant, common, and rare). Treatment trays consisted of reference substrate (treatment A; upper panel) or metal-contaminated substrate (treatment F; lower panel) as described in Figure 1. Results of 2-way ANOVA testing for effects of metal treatment, tray position, and the treatment $\times$ position interaction on each drift category are also shown (* $p < 0.05$; ** $p < 0.001$; *** $p < 0.0001$).

Table 1

Results of PERMANOVA tests showing effects of metals, fine sediment and sampling day on community composition in the field experiment.

	Pseudo-F	P(perm)
Day	14.134	0.001
Fines	2.2031	0.009
Metals	9.8394	0.001
Fines*Metals	1.3419	0.176
Fines*Day	1.5323	0.022
Metals*Day	4.5382	0.001
Fine*Metals*Day	1.161	0.225

p-values <0.05 represent comparisons that were significant.

Table 2

Three-way PERMANOVA showing effects of fine-sediment deposition, metal-contamination, and tray position after a 10-day mesocosm experiment.

	Pseudo-F	P(permutation)
Tray position	16.722	0.001
Fines	1.7803	0.033
Metals	2.8355	0.006
Fines*Metals	1.195	0.271
Fines*Tray position	0.74422	0.841
Metals*Tray position	2.0042	0.009
Fine*Metals*Tray position	0.8979	0.617

p-values <0.05 represent comparisons that were significant.