THESIS

THE IMPACTS OF METAL-CONTAMINATION AND FINE SEDIMENT DEPOSITION ON BENTHIC MACROINVERTEBRATE COMMUNITIES

Submitted by

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ABSTRACT

ASSESSMENTS OF THE IMPACTS OF METAL-CONTAMINATION AND FINE SEDIMENT DEPOSITION

Understanding how to assess benthic invertebrate community responses to multiple stressors before and after remediation is necessary to improve the success of restoration and biomonitoring projects. Currently, there is no consensus on whether communities at restored sites should mirror pre-disturbance communities or reflect a "new-normal". In most cases, scientists do not know what the community looked like pre-disturbance; however, they may be able to understand what it should look like by incorporating ecological theory in ecotoxicological-related bioassessments. My thesis project examines the influence of metal-contamination and fine sediment deposition on benthic invertebrate community colonization. I used field colonization experiments to examine changes in communities over time to compare responses to a single stressor (metal-contamination) and multiple stressors (metal-contamination and fine sediment). This study further attempts to incorporate ecological concepts on metacommunities and ecosystem function, and goes beyond taxonomic comparisons to study the usefulness of species traits.

Chapter one is focused on the combined effects of fine sediment deposition and metal contamination and their risks to aquatic ecosystems, particularly in areas of historic mining. This study uses both mesocosm and field experiments to determine how both stressors impact benthic invertebrate colonization after the source of acid mine drainage has been removed at the North Fork of Clear Creek (NFCC), a U.S. EPA Superfund site in Black Hawk, Colorado, USA. In

August 2014 metal-contaminated coarse (>2360 µm) and fine (<2360 µm) sediment was transported to an upstream reference site where the rate of colonization by benthic macroinvertebrates was measured over 30 days. In addition, a mesocosm experiment was performed to test the hypothesis that patches of metal-contaminated habitat impede movement downstream. Data were analyzed by comparing the effect of treatment over time, and using the factorial design to compare the effect of metal-contamination (reference-site coarse versus metal-contaminated coarse sediment) and fine sediment (treatments with no fine, reference-site fine, and metal-contaminated fine sediments) over time. Abundance and species diversity in all treatments increased over time for all insect orders; however, these changes were much slower in treatments with metal-contaminated fine sediment. The results indicate the need to consider community composition along with total abundance and dominant taxa (*Baetis* sp., *Taenionema pallidum*, and *Rhyacophila* sp.). All dominant taxa were significantly affected by metal-contamination ($p < 0.05$); however only *Taenionema pallidum* and *Rhyacophila* sp. populations were affected by fine sediment. Overall community response showed an effect of metal-contamination throughout the experiment, and an effect of fine sediment on day 30. This study suggests that even after water quality improvement at NFCC, fine sediment deposition will likely reduce recovery potential. Remediation and management of sediment inputs will be necessary for recovery of aquatic ecosystems with both metal-contamination and fine sediment deposition.

Chapter two investigated colonization of benthic invertebrates on metal-contaminated sediments at an EPA superfund site on the Upper Arkansas River near Leadville, Colorado, USA. The goal of this study was to determine if responses to metal-contamination varied between sites with different histories of metal-contamination due to mining activities. I transferred reference-site and metal-contaminated coarse sediments from NFCC to a reference

and impaired site on the Upper Arkansas River to observe colonization over time. The reference-site (AR1) has a diverse community of metal-sensitive EPT taxa, whereas the impaired site (AR5) has fewer EPT taxa and is dominated by metal-tolerant taxa (e.g., Hydropsychidae and Chironomidae). There is limited information on invertebrate community composition at both sites pre-disturbance; however, I predicted that AR1 communities would show a greater response to metal-contamination compared to AR5. Although I saw differences at the end of the experiment, I did not see a significant response to metal-contamination. Measures of diversity, richness, and number of taxa varied over time and differed between sites, but there were no differences in community colonization of reference and metal-contaminated substrates between sites. This study highlights a significant issue regarding how scientists determine community tolerance and restoration success. In contrast to results of Chapter 1, I did not see large responses in benthic invertebrates at AR1. However, more work needs to be completed to determine differences in community tolerance between sites and the possibility of sensitive taxa adapting to metals over time.

The final chapter discusses the usefulness of species traits in assessing responses to multiple stressors and a single stressor (metal-contamination) at sites with different taxonomic compositions. Because trait responses may not be easily comparable to taxonomic responses, it is unclear which traits are most important to predict species occurrence. This study compared trait abundances to taxonomic responses traditionally used in bioassessments. My goals were to identify individual traits that may be more useful in studying effects of physical and chemical stressors and to distinguish effects of single and multiple stressors. When comparing the impact of both metal-contamination and fine sediment deposition on community traits, I observed a greater effect of fine sediment deposition compared to responses in taxonomic composition.

Specifically, mobility, morphological and life history traits were most useful for distinguishing the impacts of fine sediment quality. I observed that dominant taxa were less relevant in my experiment when observing trait responses over time. Moreover, individual traits had a stronger relationship with fine sediment and organic matter data than dominant taxa. When measuring trait responses to a single-stressor across three sites, I saw that proportional differences of invertebrates utilizing sprawler and clinger habitats varied most between reference and metal treatments. However, unlike responses to multiple stressors, single-stressor comparisons of traits did not show strong responses to metal treatments. Continued experimental approaches will be needed if researchers are to move forward in developing a universal traits-based approach to aid in stream restoration and management.

What remains clear throughout my thesis project is the importance of community-based and multiple-stressor studies. Although, community approaches based on taxonomic composition are helpful, I observed greater responses when considering the physical effects of fine sediment on community trait response. When assessing only the effects of metal-contamination, it was more difficult to determine which traits would be most helpful, both within and between sites. This could simply be because metal-contaminated coarse sediment without additional stressors in the sediment or water column are not harmful. However, scientists still lack an understanding of the influence of disturbance history of the site and varying within-species tolerance to metal contamination.

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CHAPTER 1: INFLUENCE OF METAL CONTAMINATION AND SEDIMENT DEPOSITION ON BENTHIC INVERTEBRATE COLONIZATION AT NORTH FORK CLEAR CREEK SUPERFUND SITE, COLORADO, USA

Introduction

Metal contamination and fine sediment deposition are global threats to aquatic ecosystems, particularly in mountainous regions with a legacy of mining activities. Low amounts of trace metals and fine sediment are natural in streams and rivers; however, human activities increase these inputs and result in low abundances of benthic invertebrate and fish populations at sites affected by mining activities (Clements et al. 1988, Daniel et al. 2015). Anthropogenic disturbances, such as surface-mining, contributes to fine sediment accumulation in aquatic ecosystems (Balamurugan 1991, Bobrovitskaya 1996, Nelson and Booth 2002, Walling and Fang 2003) resulting in habitat loss, streambed homogenization, contaminant-loading and alterations of ecosystem functions, each of which impacts fish and macroinvertebrate populations (Waters 1995, Wood and Armitage 1997, Boulton et al. 1998, Jones et al. 2011). Fine sediment particles < 2mm are typically of concern at mining sites and have been associated with heavy metal loading and disrupting benthic invertebrate community structure (Chutter 1969, Erman and Erman 1984, Moore et al. 1989). Benthic invertebrates are sensitive to contaminants and, because of their relatively short lifecycles, are early indicators of disturbance and stream recovery. 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.

Studies on the combined effects of metals and fine sediment on aquatic macroinvertebrates have been limited and focused on single-species laboratory tests and observational studies. Laboratory studies have shown metal-contaminated sediment can inhibit growth of invertebrates (Wentsel et al. 1977), bioaccumulate in organisms (Goodyear and McNeill 1999) and be more toxic compared to aqueous exposure to metals (Milani et al. 2003, Hill et al. 2009). Alternatively, observational studies have shown low benthic invertebrate abundances at sites with metal contamination (Hickey and Clements 1998, Giddings et al. 2001, Courtney and Clements 2002, DeNicola and Stapleton 2002, Milani et al. 2003, Clements 2004). Similar community responses to fine sediment deposition have been reported from field experimental and observational studies (Richards and Bacon 1994, Larsen et al. 2011, Mathers and Wood 2016), including field experiments that have demonstrated metal-contaminated sediments can cause benthic invertebrate declines (Courtney and Clements 2002, Burton et al. 2005). Field 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 (Clements 2004, Townsend et al. 2008, Palmer et al. 2010, Burton and Johnston 2010). However, field experiments that test the effects of both metal-contamination and fine sediment deposition on benthic community colonization have not received much attention even though these stressors routinely co-occur in mining-impacted watersheds.

Mining activities have long contributed to sediment loading at mining sites. Several studies have found that high sediment inputs at mining sites far exceeds the input produced from natural landscapes (Bobrovitskaya 1996, Nelson and Booth 2002, Chalov 2014). Metals bind to sediment and other particulates in the water column, which deposit on streambeds and clog interstitial spaces that serve as refugia for aquatic insects (Boulton et al. 1998). Metal-contaminated sediments can remain well after the source of contamination has been eliminated, thereby increasing metal exposure, with long-term implications on stream health. This can be especially detrimental in the Rocky Mountain streams where many species of macroinvertebrates are adapted for cobble and gravel-bed habitats that provide enough interstitial space for refuge. A loss macroinvertebrate habitat due to clogging of interstitial spaces can have impacts on species abundance and distributions in streams (Richards and Bacon 1994, Wood and Armitage 1997), ultimately impacting recovery potential.

The ability of macroinvertebrates to recolonize previously disturbed areas is documented yet scientific investigation of recolonization lacks field rigorous experiments to determine causal-and-effect relationships (Wallace 1990). Understanding how macroinvertebrates respond following a disturbance is especially important in stream restoration projects. Although the main source of metal contamination may be removed in many mining-disturbed streams, other anthropogenic stressors may impede community recovery. However, it would be difficult to justify allocation of restoration resources without applied studies to determine causal relationships.

This study presents experimental approaches to understand the combined effects of metal contamination and fine sediment deposition on benthic invertebrate communities. I performed a field experiment that was designed to predict benthic invertebrate recovery after restoration of metal contamination in the North Fork Clear Creek (NFCC), a U.S. EPA Superfund site impacted by both metal-contamination and fine sediment accumulation. There is evidence to suggest that physical and chemical stressors can increase the patchiness of benthic invertebrate populations in lotic environments and influence populations colonizing downstream reaches

(Lake 2000, Malmqvist 2002, Kiffney et al. 2006). Therefore, I conducted a mesocosm experiment to determine if contaminated habitats influence downstream colonization and the likelihood of benthic invertebrate movement beyond patches of contaminated sediment.

My research tested the following four hypotheses:

1. Effects of sediment deposition will be greater than that of metal-contamination;
2. Caddisflies (Trichoptera) would be most sensitive to treatments with fine sediment, whereas more mobile taxa, would only be impacted by treatments with metal-contaminated fine sediment;
3. Patches of habitat with sediment deposition will be more detrimental to benthic invertebrate colonization compared to patches with metal-contaminated substrate;
4. Patches of both metal-contaminated coarse and fine sediment will have greater effects on less mobile species and those with preferences for habitats where fine sediment quality is most important.

Although sediment deposition and metal-contamination often co-occur, the focus of most research conducted in mining polluted streams has been on metal contamination. In general, I believe this experimental approach will help predict recovery at the NFCC following improvements in water quality, but also answer broader ecological questions about the role of multiple stressors in the distribution and recruitment of macroinvertebrates in restored streams.

Methods

Study Site

The colonization experiment began in August 2014 at the North Fork Clear Creek (NFCC; N39.81271, W105.49821) in Blackhawk, Colorado, USA in (Figure 1.1). NFCC is Operable Unit #4 of Clear Creek, and declared a U.S. Environmental Protection Agency (EPA)

Superfund site in 1983 due to elevated levels of metals. High concentrations of metals such as zinc, cadmium, copper, iron and aluminum (Butler et al. 2009) have resulted in low benthic invertebrate abundances and elimination of fish populations (Clements, unpublished data). NFCC is a tributary to the Clear Creek watershed and located approximately 50 km west of Denver, Colorado. Clear Creek is used for drinking water, local industry, and recreational purposes, making the water quality issues on NFCC a serious concern. Construction of a water treatment plant on NFCC was initiated and will become operational in early 2017. Due to previous mining activities, NFCC, a naturally gravel-bed stream, 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 and close proximity to a road makes NFCC highly susceptible to sediment accumulation from other anthropogenic activities. Tailings piles in Blackhawk, Colorado and at sites downstream along the contaminated areas of the NFCC also contribute to sediment and metal inputs.

Field Experiment

The field experiment was performed upstream of the source of mining contamination at a reference site. Physiochemical characteristics of the reference site remained relatively constant during the study, with an average water temperature of 8.16°C, dissolved oxygen of 9.18 mg/L, and pH of 7.86. The benthic community at the reference site was dominated by *Baetis* sp., *Taenionema pallidum*, *Rhyacophila* sp., and Chironomidae. High abundances of sensitive EPT taxa also were present, including *Rhithrogena* sp., *Sweltsa* sp., *Zapada* sp., *Capnia* sp., and *Drunella doddsii*.

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 that managers would like to reproduce in the downstream reaches. Because the reference-site community will predominantly be repopulating downstream sections of NFCC, it is important to understand how this community will respond to stressors.

Metal-contaminated sediments were collected in Blackhawk, Colorado near the source of contamination (N39.79867, W105.48174) and moved 2.6 km upstream to the reference site, which has no history of metal contamination (Fig. 1.1). At both the reference and metal-contaminated sites, areas of sediment deposition were located and fine sediment was collected from the stream. This experiment used six treatments in a full factorial design to discern between the impacts or metal-contamination and sediment deposition (Fig. 1.2a). Each treatment had three replicates and, to get a better representation of the benthic community within the stream, two trays were combined for each replicate (Fig. 1.2b). The composition of the fine sediment mixture was not manipulated, but included silt, sand and small gravel to maintain the composition that invertebrates are exposed to at the reference and contaminated sites. Large cobble and gravel for the coarse sediment treatments were collected randomly from metal-contaminated or reference sites.

Treatments were created by placing coarse sediment from the metal-contaminated or reference site in colonization trays. To assess effects of fine sediment, trays were either filled with sediment from the reference site, filled with sediment from the metal-contaminated site or received no fine sediment. A total of 144 colonization trays (25 x 25 x 10-cm) with ½ inch diameter holes were attached to racks and then placed in the stream (Clements et al. 1988). Table 2.1 shows the experimental design with trays from the six treatments were collected on days 5, 10, 20, and 30 (36 per day, one replicate from the F-treatment was lost on day 30). Replicates consisted of 2 trays from a single treatment which were combined in a bucket. 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 m^2 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 samples were sorted using a dissecting microscope under 0.7X magnification. All insects were identified to the genus level except for chironomids and early instars, which were identified to order or family.

Sediment and organic matter was measured in every sample to account for material entering and leaving the trays throughout the experiment. After removing insects from each tray sample, sediment was sieved though 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 and combusted at 550°C for 3 h to obtain organic matter and raw sediment content. Additional trays for metals analysis were placed into the stream and collected on days 15 and 30 to measure metal concentrations during the experiment. Whole trays were digested using modified EPA 3050b method (U.S. EPA 1996) at Colorado School of Mines, Golden, Colorado, USA. Sediment in the trays were weighed, digested in HNO3 and analyzed using ICP-MS.

Mesocosm Experiment

The mesocosm experiment was designed to measure the ability of benthic invertebrate communities to colonize patches of suitable habitat located downstream of contaminated patches. I also wanted to compare results of the mesocosm experiment to those of the field experiment. Colonization trays containing coarse substrate collected from the reference site were placed at the reference site on NFCC for 30 days and then transported to the Streams Laboratory at

Colorado State University (Fort Collins, Colorado, USA). Two colonized trays from NFCC were placed in stream mesocosms, directly upstream of un-colonized trays containing one of the six substrate treatments (barriers) and trays with only reference-site sediment (Sink 1 and Sink 2; Fig. 1.2c). There were three streams per treatment and each stream contained four categories of colonization trays (source population, treatment, sink 1, and sink 2). Sink 1 trays were immediately downstream of the barrier tray and sink 2 was further downstream to test assumptions about mobile taxa. In this study, the source is defined as source populations on colonization trays, and the sinks are habitats that will be colonized by the source population. Water-quality conditions did not fluctuate throughout the experiment and flow rate in the stream was maintained at 1-L/min. After ten days, trays were collected from each stream, rinsed through a 355µm sieve, and benthic invertebrate communities were assessed for each category.

Statistical Analysis

For the field experiment, I tested effects of the six treatments and time (days 5, 10, 20, and 30), and analyzed three-way comparisons between day, metal-contamination (RC vs. MC), and fine sediment deposition (NF vs. RF vs. MF). All univariate and multivariate analyses, except diversity, evenness, and richness, were performed on log-transformed data. Univariate analysis was performed on Shannon-diversity, species richness, abundance of dominant taxa (*Baetis* sp., *Taenionema pallidum*, and *Rhyacophila* sp.), total abundance and Ephemeroptera, Plecoptera, and Trichoptera (EPT) metrics. Multivariate analysis was used to test changes in benthic invertebrate community composition between treatments. Major trends in the data were then visually represented using nonmetric-multidimensional scaling (NMDS) plots. A Bray-Curtis similarity matrix was created using log-transformed community abundance data. A three-way permutational multivariate analysis of variance (PERMANOVA) was run at 999 permutations to examine the effects of factors coarse, fine sediment, and time. Pairwise-comparisons for each analysis were performed to examine more complex trends in the data.

Sediment and organic-matter (sizes 355µm and 63µm) data and were compared to biotic data using the BEST procedure run with 999 permutations with the distance-based linear model (DISTLM) package in Primer-e. BEST is similar to stepwise procedures, but uses all possible combinations of environmental variables and lists the best explanatory variables. DISTLM was performed separately for each collection day with corrected-Akaike Information Criterion (AICc) selection criteria being used to determine the best environmental variables that explained trends in community composition. Additionally, SIMPER analysis was performed on log-transformed data to determine the invertebrates that account for >50% of the dissimilarity between response variables. SIMPER analysis was also used to determine similarity of communities in the Hess samples and colonization trays (Clarke and Warwick 2001).

Macroinvertebrate community and trait composition were the primary the focus of the mesocosm study. Mobility and ecology traits were assessed based on available trait data (Poff et al. 2006). Using a factorial design, relationships among fine sediment, metal-contamination, and macroinvertebrate communities were examined. Both univariate and multivariate invertebrate data were analyzed the same way as the field experiment. Comparisons of communities on trays were made both within (e.g. reference coarse vs. metal coarse) and between factors (coarse vs. fine sediment). My interest was both how communities change within treatments and if significant differences exist on trays between treatments.

Univariate data were analyzed using the statistical package, SAS v9.4 (SAS Inc., Cary, NC, U.S.A.). Multivariate data analyzing differences in community composition was analyzed using PRIMER-e v7 (Quest Research Limited; Cambridge, United Kingdom) with the

+PERMANOVA package (Anderson 2001). Significance for all tests was determined based on a $p < 0.05$ with Monte Carlo p-values being used for tests with 10 permutations (Anderson 2005).

Results

For all variables, there was a significant effect of day and treatment ($p < 0.05$), with more taxa colonizing trays with reference-site coarse sediment (RC) than trays with both metal-contaminated coarse and fine sediment (MC + MF; Table 1.1). Results were mainly summarized by comparing the effect of metal-contamination (RC vs. MC) and fine sediment (NF vs. RF vs. MF). Iron, zinc, manganese, copper and nickel were the dominant metals measured on NFCC substrate and were combined to estimate threshold effect concentrations. Metal concentration in the trays were not significantly different throughout the experiment; however, metal concentration in treatments with reference-site coarse substrate (RC) were significantly lower than in treatments with metal-contaminated coarse and fine sediment ($F = 10.1$, $p = 0.0002$; Fig. 1.3). Additionally, the amount of sediment in the trays showed little variation over time, with NF trays having significantly less fine sediment than RF and MF trays during the entire experiment ($p < 0.01$; Appendix 1A). Organic matter in the trays was constant except on day 30 with more in RC and MF treatments.

Community Structure in Field Colonization Experiment

Over 24,000 insects (7,183 Ephemeroptera, 4,794 Plecoptera, 1,033 Trichoptera, and 11,492 Diptera) comprised of 37 genera were collected and identified during this experiment. Mixed-model (PROC GLM) results showed varying responses of total abundance, taxa, and diversity metrics to metal-contamination and sediment deposition (Table 1.1). Total benthic invertebrate abundance increased over time in all treatments. The effect of metal contamination on total abundance was reduced over time, with significantly higher abundances on trays with

RC only on days 5 and10 ($p < 0.05$; Fig. 1.4a). However, the impact of sediment deposition appeared greater over time between treatments with NF and those with MF, particularly on day 30 (although these differences were not statistically significant; $p = 0.1708$; Fig. 1.4b).

Of the 37 taxa collected during this experiment, the *Baetis* sp. (Ephemeroptera), *Taenionema pallidum* (Plecoptera), and *Rhyacophila* sp. (Trichoptera) were among the most dominant in their corresponding families. 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 (Fig. 1.5). For example, *Baetis* sp. did not respond significantly to sediment deposition ($p = 0.1906$); however, *T. pallidum*. showed strong preferences for treatments without fine sediment throughout the experiment and were most abundant in treatments with only coarse sediment on day 30 ($p < 0.05$). *Rhyacophila* sp. abundance was lower on metal-contaminated fine sediment compared to coarse sediment or reference fines, particularly on day 30, although this difference was not statistically different ($p > 0.05$). For all dominant taxa, the different responses to fine sediment treatments became more distinct either by day 20 or 30.

Maximum EPT abundance was achieved by day 20 in treatments with reference-coarse (RC) sediment. Diptera and Ephemeroptera were early colonizers of treatments with RC sediment, making up 90.4% of the total abundance on day 5. Yet fewer Diptera comprised the total community on day 30, with an increase of EPT taxa, colonizing into treatments with RC substrate (Fig. 1.6a). When comparing the effect of metal-coarse (MC), Diptera dominated the samples, comprising up 76.7% of the total community on day 5. On day 30, abundances of EPT taxa were similar to that of treatments with RC, except a lower portion of Trichoptera in the MC treatments (Fig. 1.6b). Trays with and without fine sediment showed an increase in all EPT and a decrease in Diptera over time (Fig. 1.6c-e). However, in treatments with metal-contaminated fine sediment (MF), there was a slower colonization of Plecoptera and Trichoptera compared to Ephemeroptera and Diptera. Proportions of EPT and Diptera taxa were similar in treatments with no fine sediment (NF) and those with reference-site fine sediment (RF; Fig. 1.6c, 1.6d). Trichoptera comprised a small portion of the samples; however, they made up a higher portion in the treatments with RF (Fig. 1.6c-e). Overall the trends observed in EPT abundance in RC treatments appeared to be driven by responses to RF; and trends of MC resulted primarily from colonization of treatments with no-fines.

The amount of organic matter and sediment in trays influenced colonization, but the importance of these variables varied among taxa and over time. Output from the DISTLM analysis showed that sediment dry weight and organic matter (355 µm) were the best predictors of trends in abundance on day 5, except for *Rhyacophila* sp. which was only influenced by sediment 355 µm ($r^2 = 0.337$; Table 1.2). The impact of fine sediment on *T. pallidum* abundance was generally consistent, with an $r^2 > 0.45$ on days 5, 10, and 30; and a low r^2 of 0.046 on day 20. The predictors of the best model shifted throughout the experiment for all response variables, with most variation explained by either fine sediment or organic matter >355 µm. DISTLM analysis showed that correlations between community composition and abiotic data in the treatments was reduced over time compared to day 5 where fine sediment and organic matter >355 µm were the best predictors ($r^2 = 0.314$).

Results from multivariate analysis showed community assemblages significantly responded to fine sediment and metal contamination and that these patterns varied over time (Table 1.3). Complete results from the pairwise comparisons over time (Appendix 1C) showed differences in community composition between NF and MF treatments only on days 5 (p =

0.003) and 30 ($p = 0.035$). Although there were effects of metal-contamination on community composition throughout the experiment, differences between treatments with and without metal-contaminated fine sediment were greatest on day 30 (Fig.1.7). Greater differences between RC and MC sediment were observed early in the experiment. Pairwise comparisons of individual treatments showed that all treatments were statistically different ($p < 0.05$) from trays with both metal-coarse and metal fines (MC + MF), except for trays with metal-coarse and reference-fines (MC + RF; $t = 1.1633$; $p = 0.329$). I also observed consistent separation between communities on treatment-A (RC + NF), which was representative of the reference site habitat, and treatment-F (MC + MF), which was representative of habitat downstream of the acid mine drainage, on each day (Fig. 1.7).

SIMPER analysis was used to compare the natural benthic community in Hess samples at NFCC to the community in the trays (Appendix 1D). SIMPER analysis showed that communities on RC sediment were more similar to the benthic community collected at NFCC on day 5. However, there was little difference on day 30 between Hess samples and RC treatments (similarity = 54.36) or MC treatments (similarity = 53.56). Lower similarity values are likely due to greater abundances of *Drunella doddsii* and *Heterlimnius* sp. in Hess samples than in colonization trays. This trend of increasing similarity over time was also consistent with fine sediment and individual treatments.

Using SIMPER analysis, I was also able to identify taxa most responsible for the differences between treatments. On day 5 Simuliidae, Chironomidae, and the 3 dominant taxa (*Baetis* sp., *T. pallidum*, and *Rhyacophila* sp.) contributed to the difference between treatments with and without fine sediment (Table 1.4). However, as the experiment progressed more EPT taxa made up the differences between fine sediment treatments. Dominant taxa contributed less to the differences on day 30, with late colonizers such as, *Sweltsa* sp., *Zapada* sp., *Lepidostoma* sp., *Capnia* sp., and *Rhithrogena* sp. comprising most of the differences between the treatments with no fine sediment and those with either reference-site or metal-contaminated fine sediment. On day 5, treatments with reference site and metal-contaminated fines were the most dissimilar (average dissimilarity = 54.64), compared to day 30 treatments (average dissimilarity = 25.97).

Community Structure in Mesocosm Barrier Experiment

The goal of the mesocosm experiment was to determine if benthic invertebrates were immigrating downstream of patches with contaminated sediments and, if so, does species composition and diversity change. All community metrics were significant by tray position, however only Shannon-Weiner diversity was significantly affected by the main effects and interactions of fine sediment and tray position (Appendix 1F). For univariate community metrics, significant differences did not occur between fine sediment treatments on source and barrier trays, although total abundance was higher on barrier trays in treatments with no-fines (Fig. 1.8). I did observe differences in abundances within treatments. Specifically, mayfly abundance in NF treatments were not statistically significant between trays; however, in MF treatments sink trays were significantly higher than in the barrier trays. Similar trends were observed in other community metrics such as diversity and number of taxa, where a gradual decrease was observed from source to sink 2 trays in NF treatments, whereas there was a significant increase downstream of the barrier tray ($p < 0.05$; Fig. 1.8).

The results of PERMANOVA showed an effect of fine sediment and tray position on community colonization. There was also a significant interaction of metal-contamination and tray position ($p < 0.05$; Appendix 1F). Pairwise comparisons also indicated a significant difference between streams with and without metal-contaminated fine sediment ($p = 0.022$).

Community colonization between the different sediment treatments (NF, RF, and MF) can be visualized in nMDS plots (Fig.1.9). In each treatment, communities on the source population were significantly different from all downstream trays. However, differences in community colonization among downstream trays did occur. In the streams with NF treatments, community composition downstream of the source population was not statistically different ($p > 0.05$; Table 1.5); however, *Zapada* sp., *Capnia* sp., *Sweltsa* sp., *Lepidostoma* sp., and *Rhyacophila* sp. were least likely to colonize in sink 2 (Appendix 1G). Streams with RF and MF treatments showed greater separation in the barrier trays with the additional stressor of MC substrate (Fig. 1.9). The difference between the source and downstream trays in streams with RF and MF treatments were largely due to EPT taxa, *Capnia* sp., *Rhithrogena* sp., *Rhyacophila* sp., *Zapada* sp., and *Taenionema* sp., remaining on source trays (Appendix 1G). Alternatively, in those treatments, trays downstream of the barrier trays did have higher abundances of chironomids (Diamesinae and Orthocladiinae) compared to the source trays.

One question I considered was how community composition may change if there are small patches with both metal-contaminated coarse and fine sediments (MC + MF). To address this question, I compared the composition of Ephemeroptera, Plecoptera, Trichoptera, and Diptera (EPTD) in streams with treatments A (RC) and F (MC + MF) as barriers (Fig. 1.10). Treatment-A represents the habitat type typical at the reference-sites in NFCC, whereas treatment-F is representative of contaminated sites downstream of the acid mine drainage. In general, significantly lower abundance of invertebrates colonized the barrier trays with both MC + MF treatments compared to the streams with only RC sediment (ANOVA; $p < 0.0041$). Of the taxa colonizing the barrier trays, the largest difference in composition was observed in Diptera where only 17.4% made up the composition in treatment-F and 36.1% colonized the barrier trays in treatment-A (Fig. 1.10). The trend of EPT composition was similar in both treatments; however, a higher abundance of Trichoptera and Plecoptera remained on the source trays in treatment-F (although this difference was not significant, $p = 0.05$). As expected, significantly fewer taxa in treatment-A colonized sink 2 trays compared to those remaining on the source trays ($p < 0.0121$), while the abundance of taxa that moved further downstream in treatment F was not significantly less ($p = 0.1050$).

One of my hypotheses was that analysis of traits may provide better insight into movement past patches of metal contamination and fine sediment. I categorized taxa by 4 species traits (drift frequency, swimming ability, habitat use, trophic level) to quantify the influence of barriers on macroinvertebrate communities. Species that are uncommon in drift (*Pericoma* sp., *Drunella doddsii*, *Micrasema bactro, Rhyacophila* sp. and *Lepidostoma* sp.) generally remained on the source trays in both treatments A (RC) and F (MC + MF; Fig. 1.11b). However, there was a significantly higher portion of those invertebrates that remained on the source trays compared to downstream trays in treatment F ($p < 0.01$). Invertebrates that were typically abundant in the drift samples had higher abundances downstream of the barrier trays in treatment F, and abundances on sink trays were not significantly different from the source trays. Swimming ability also influence downstream immigration of aquatic insects. I found that of the species that do not swim (e.g., *Lepidostoma* sp. and all dipterans), only 4.3% colonized the barrier with metal-contaminated sediment, which was significantly lower from all other trays in treatment F ($p < 0.05$; Fig. 1.11a). In contrast, there was no significant difference in abundance of non-swimmers between trays in treatment A. Weak swimmers responded similarly to in both treatments, with significantly higher abundances remaining on the source trays; however, only in treatment F were abundances in sink trays significantly higher ($p < 0.01$) compared to the barrier.

Strong swimmers responded similarly in both treatments; however, they made up a larger higher portion of the community in the sink trays in treatment F.

When comparing the impact of habitat preferences downstream of patches with treatment F (MC + MF), lower proportions of invertebrate habitat preferences were observed on barrier trays than those in treatment-A (RC; Fig. 1.12a). Swimmers were least impacted by metal-contaminated substrate; however, burrowers, sprawlers, and clingers were significantly less on the barrier trays in treatment F than treatment A. Clingers, which comprise a diverse group of EPT taxa including *Rhithrogena* sp., *Drunella* sp., *Serratella* sp., *Sweltsa* sp., *Rhyacophila* sp., and *Isoperla* sp., remained on the source trays in both treatments ($p < 0.05$). Swimmers made up a higher portion of the community on all sink trays, and a significantly higher abundance of clingers were present on sink 2 trays in treatment F compared to treatment A. Across functional feeding groups, abundances of collector-gatherers and predators were significantly lower in treatment F than treatment A ($p < 0.05$). However, the response of predators, and shredders was not significantly different when comparing source and sink trays (Fig. 1.12b).

Discussion

The current study presents an experimental approach to answer the questions of how benthic invertebrates will respond to the combined effects of metal-contamination and sediment deposition after water quality improvements. Although previous research has investigated the adverse effects of metal contamination on benthic communities (Hickey and Clements 1998, Clements et al. 2000, Landers 2016), few studies have examined the additional effects of fine sediment deposition. An important approach commonly used by regulatory agencies is to quantify how macroinvertebrate metrics such as abundance of dominant taxa, total abundance, and species richness respond to disturbance. These data are often used to extrapolate responses of the entire community; however, these community responses were complicated by the effect of sediment deposition. Although these univariate metrics are important, community composition was more helpful in predicting the impacts of fine sediment deposition. The results of my study have broader implications for understanding what recovery may look like at sites with multiple stressors, as well as understanding the ecological effects of contaminants on benthic invertebrate colonization and distribution.

Predicting Community Response After Point-Source Removal

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 improvements in water quality, metal-contaminated sediments, both coarse and fine, will likely impede benthic invertebrate colonization downstream of the reference site. Metal-contamination in the early time periods would likely impact early colonizers and dominant taxa such as, *Baetis* sp., Chironomidae (Orthocladiinae and Diamesinae), and Simuliidae. In contrast, late colonizers such as *Rithrogena* sp., *Zapada* sp., *T. pallidum*, and *Capnia* sp., would be affected by fine sediment deposition. Although the impact of metal-contamination on macroinvertebrate communities was reduced over time, this could have largely been due to either reduced concentration of metals or accumulation of organic matter and clean sediment.

The impact of sediment deposition was generally greater in treatments with metal-contamination. SIMPER analysis compared community colonization to the actual NFCC benthic community and supported the findings that early colonizers have different community composition on trays with metal-contaminated coarse and either reference or metal-fines. These results suggest that regardless of whether fine sediment was metal-contaminated or not, there will be a negative impact on benthic communities at NFCC. Therefore, the rate of sediment inputs and residence time will likely determine the rate of recovery potential after restoration.

Community composition on trays with fine sediment was generally more variable both between and within treatments. These results suggest that the ability to detect benthic invertebrate recovery may be difficult post-restoration. With the likelihood of increased patchiness of benthic invertebrates at contaminated sites (Burton and Johnston 2010), there needs to be careful consideration of sampling methods and necessary sample sizes to detect effects (Carter and Resh 2001).

The hypothesis that motivated my mesocosm study was to consider patches of contaminated substrate as a barrier to colonization to downstream habitats. Although this idea is not new, the application of patch dynamics within the context of chemical stressors in lotic ecosystems has not been investigated. In aquatic systems, patches of chemically or physically degraded habitats may impede colonization to less contaminated areas. The higher abundance of invertebrates located downstream of contaminated patches, as well as varying diversity and richness downstream, may support the idea that chemical and physical stressors will create population patchiness post-recovery at NFCC.

My hypothesis that fewer insects will move downstream of the contaminated patches was not fully supported in the mesocosm study. Even though most organisms avoided contaminated barriers, I observed relatively high movement of insects to the furthest downstream sinks. Some species of Trichoptera and Plecoptera may be less likely to move further away from a contaminated habitat. However, the ability of some insects to colonize patches furthest downstream may suggest that more mobile species are also more resilient to the presence of both chemical and physical stressors. Although this observation was not consistent with my predictions, it may have implications for insect energy budgets and the role of insects in certain ecosystem functions (e.g., detrital breakdown) in metal-contaminated systems.

My study suggests that responses of insects following improvements in water quality will likely depend on the distribution of fine sediments and metal-contamination in the stream, in addition to how far insects must drift to locate suitable habitat. The high rate of avoidance of contaminated habitats, is likely due to the insects continuing to drift to the mainstem of Clear Creek or experience significant mortality due to prolonged metals exposure in the sediments. Results of my mesocosm experiment have important implications for predicting recovery of NFCC. Overall, I predict low population abundances and increased patchiness of benthic communities downstream of the reference site following improvements in water quality. If metal-contaminated fine sediment remains in the NFCC, I predict a much slower rate of recovery and that recovery would be highly dependent on hydrologic characteristics and the input of sediment to the watershed.

Ecological Implications of Metal-Contamination and Sediment Deposition

The results of the field and mesocosm experiments provide insights into how metal-contamination and sediment deposition interact to influence macroinvertebrate communities. The likelihood of increased patchiness of benthic populations post-recovery can have implications on ecosystem processes. In my mesocosm study, a higher abundance of mobile species and collector-gatherers were present on the trays furthest away from the contamination. These groups also comprise a large proportion of the benthic community, which may affect total abundances and restoration sites. Due to their high abundances in lotic ecosystems, collector-gatherers play an important role in processing detritus and fine-particulate organic matter (Wallace and Webster 1996). It is important to note this study uses one dataset (Poff et al. 2006) to categorize trophic levels, but these classifications may vary based on life stage, geographic area, and habitat conditions (Rempel et al. 2000, Yule et al. 2010). Regardless of trait classifications, several studies have shown a relationship between decreases in collector-gatherers with changes algal growth and community structure (Hoiland et al. 1994, Schultheis et al. 1997, Nelson 2000).

Similar observations were observed with other trophic levels and habitat groups; however, climbers and clingers, which are mostly comprised of Trichoptera, were the least likely of all groups to move downstream from the barriers. Despite not knowing, this is likely due to their specific niche requirements, possible reliance on fine-grain sediments for case building caddisflies, and low drift abundances. The most sensitive group of taxa to fine sediment deposition appeared to be Plecoptera, which were characterized primarily as predators and sprawlers that utilize surface habitat of streambeds. It is still unclear as to why certain traits and taxa appear sensitive to either metal-contamination or fine sediment inputs, but more research analyzing trait differences may provide insight into the impact of these stressors on ecosystem structure.

Differences Between Single-Contaminant Exposures and the Current Study

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 emergence, predator-prey interactions, or behavioral avoidance. What makes this study unique is that natural responses to contaminants can be observed in the field. Using a combination of behavioral and toxicity studies, I may be able to improve predictions of community responses to contaminants and other anthropogenic stressors. My results suggest that behavioral avoidance of contaminated patches of substrate complicates 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 (Milani et al. 2003, Wright and Burgin 2009); however, in the current study Chironomidae (primarily Orthocladiinae) showed similar avoidance of metal-contaminated and fine sediments in the mesocosm and field exposures. Although the assumption of Chironomidae being tolerant to metal-contamination appears valid, their perceived tolerance in contaminated environments may largely be a function of rapid colonization, which was shown during the field and mesocosm experiments. In fact, much of the variation in abundance of EPT and dipteran taxa could be explained by the amount of fine sediment in trays. In particular, fine sediment >355 µm explained most of the variation of community metrics. This may support the idea that fine sediment has an integral role in determining macroinvertebrate abundance and distribution, particularly at sites, such as NFCC with a high amount of anthropogenic fine sediment inputs. Future work should investigate the role of depositional sediments versus suspended sediments, and how this may impact distributions of contaminants in mountain ecosystems.

Numerous field studies and mesocosm experiments have shown the following sequence of relative tolerance to metals: Chironomidae > Trichoptera > Plecoptera > Ephemeroptera (Clements et al. 1992, Hickey and Clements 1998, Courtney and Clements 2002, Qu et al. 2010). Based on field colonization and mesocosm experiments, results of the present study indicate a different sequence of response to metal-contaminated fine sediments (Chironomidae > Ephemeroptera > Trichoptera > Plecoptera), indicating the importance of accounting for colonization ability and behavioral avoidance. Invertebrate populations may experience mortality to particular metals; however, avoidance of patches of metal-contaminated substrate may be a more important factor determining the success of stream restoration projects.

Previous investigators have measured effects of contaminated substrate on colonization dynamics and recovery potential of benthic macroinvertebrates (Courtney and Clements 2002; Cadmus et al. 2016). For example, Cadmus et al. (2016) estimated recovery potential based on tolerance to aqueous metals, avoidance of metal-contaminated substrate and natural drift propensity of aquatic macroinvertebrates. Differences between this study and the present research were likely related the different experimental designs and my focus on fine sediments. Although natural drift propensity will 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. Because recovery of mining-disturbed streams may largely depend on avoidance of contaminated patches, these findings demonstrate the need to develop better information about the importance of species traits in response to metal contamination and sediment deposition. My results also demonstrate the importance of accounting for multiple stressors assessing recovery potential of disturbed watersheds.

Conclusion

I used a relatively simple experimental design to test the importance of fine sediments and metal-contaminated sediments on benthic macroinvertebrates. My study suggests that sediments in metal-contaminated streams may continue to affect benthic invertebrate communities even after water quality is improved. My field and mesocosm experiments provided insights into recovery potential that could not be obtained using traditional laboratory procedures. Relatively simple field experiments are important to consider because of the growing emphasis on more complex metal-mixtures studies, which do not improve the ability to make ecologically relevant inferences. Although field experiments have been criticized because they are generally more variable than single species laboratory tests, some of this variability can be attributed to context-dependent responses, such as differences in benthic community composition, ecoregions, or history of contamination (Clements et al. 2012). I suggest that the benefits of field experiments outweigh the limitations and can provide direct answers needed for restoration managers, regulatory agencies, and industry.

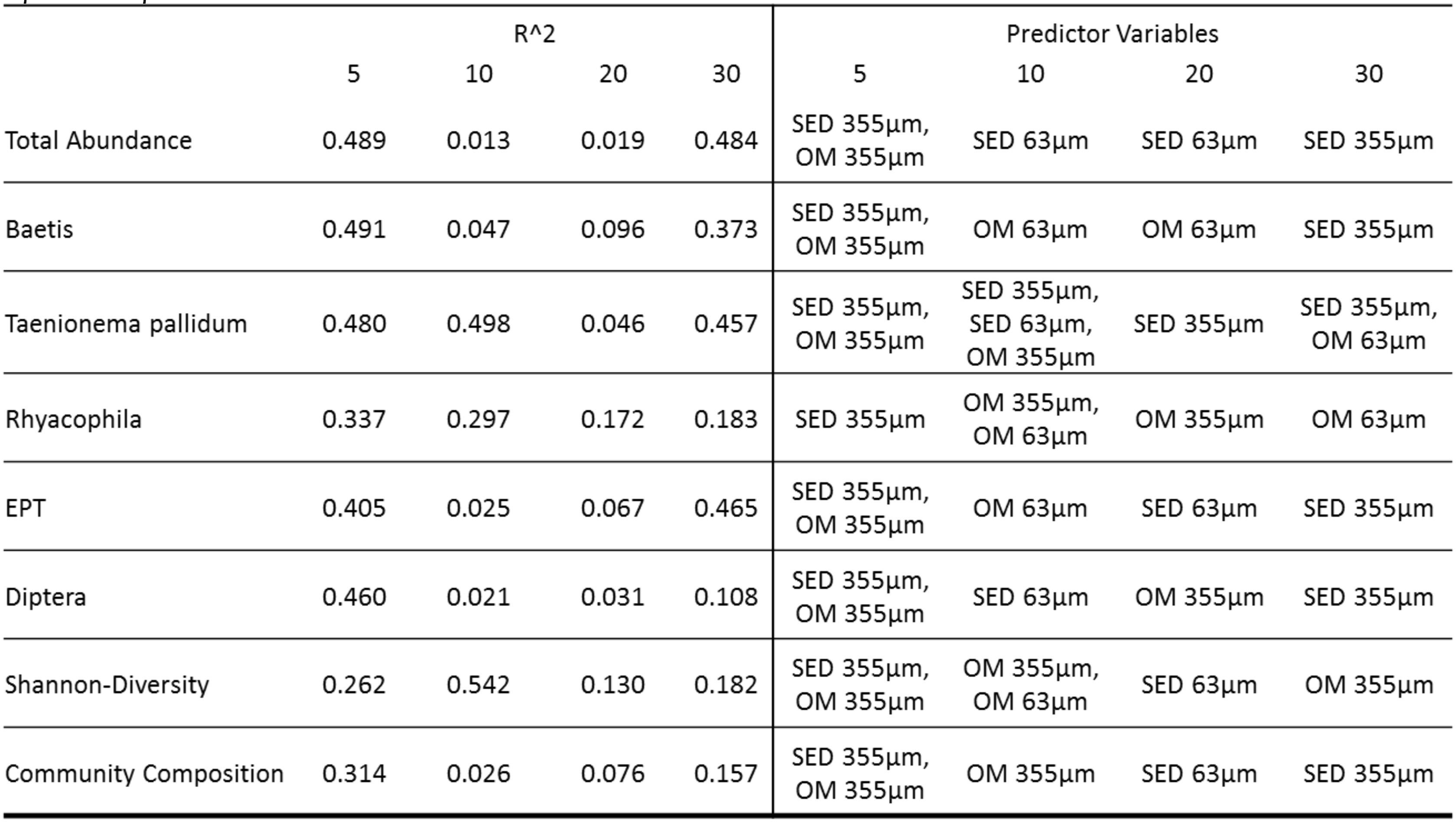
In this study, I observed changes in community composition over time in treatments with metal-contamination and sediment deposition. Although my experiments were relatively short-term, I was able to determine how several dominant taxa responded to contamination and predict how benthic communities would respond during the early and late stages of recovery. I also predict that the high variability and movement of aquatic insects downstream of metal-contaminated fine sediment may increase population patchiness. In most cases, species used in laboratory toxicity tests are characterized as being sensitive (e.g., Baetidae) or tolerant (e.g., Chironomidae) to metal-contamination. In the present study *Baetis* sp. avoided metal-contaminated substrate and chironomids were relatively sensitive. Such disparities 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. For example, the application of species traits for estimating recovery potential and quantification of community patchiness are important ecological concepts that could help us understand effects of contaminants. Integrating basic ecological principles into the field of applied ecotoxicology could improve the likelihood of successful restorations projects and reduce the likelihood of underestimating effects of contaminants.

Tables and Figures

Table 1.1: Results of the two-way ANOVA (treatment x day) and three-way ANOVA (fines x metals x day) of univariate community metrics. P-values and F-values in parentheses are listed, with values representing significance ($p < 0.05$) in bold. H' = Shannon-Weiner diversity; J' = Pielou's evenness; d = Margalef richness (d).

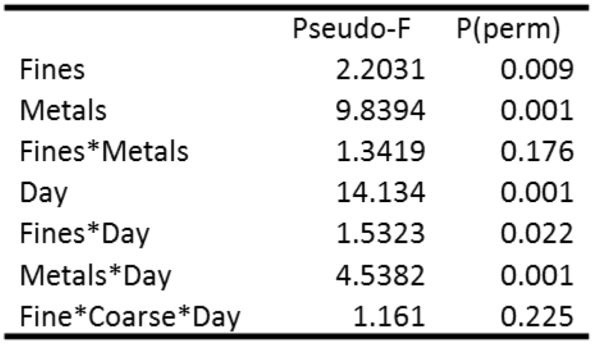
	df	Total Abundance	Shannon-Diversity	Evenness	Species Richness	# of Taxa	EPT	Diptera	Baetis	Taenionema	Rhyacophila
Treatment	5	**0.0005** **(5.51)**	**0.0189** **(3.03)**	0.7443 (0.54)	**0.0009** **(5.05)**	**<.0001** **(6.99)**	**<.0001** **(12.36)**	**0.005** **(3.89)**	**<.0001** **(8.98)**	**<.0001** **(9.46)**	**0.0001** **(6.57)**
Day	3	**<.0001** **(17.73)**	0.0905 (2.29)	0.6228 (0.59)	**<.0001** **(18.04)**	**<.0001** **(29.52)**	**<.0001** **(56.38)**	**0.0004** **(7.39)**	**<.0001** **(36.96)**	**<.0001** **(39.93)**	**<.0001** **(46.20)**
Treatment*Day	15	**0.0227** **(2.16)**	0.2402 (1.30)	0.8259 (0.64)	0.0513 (1.88)	**0.0058** **(2.63)**	**0.0002** **(3.78)**	**0.109** **(1.60)**	**0.0062** **(2.61)**	**0.0367** **(1.99)**	0.4246 (1.05)
Fines	2	0.1451 (2.01)	0.6651 (0.41)	0.6028 (0.51)	**0.003** **(6.59)**	**0.0037** **(6.33)**	**0.0038** **(6.28)**	0.4698 (0.77)	0.1906 (1.72)	**0.0006** **(8.69)**	**0.0003** **(9.79)**
Metals	1	**<.0001** **(22.84)**	**0.0005** **(14.13)**	0.2005 (1.69)	**0.0128** **(6.69)**	**0.0001** **(17.28)**	**<.0001** **(46.1)**	**0.0002** **(16.86)**	**<.0001** **(40.1)**	**<.0001** **(27.69)**	**0.0007** **(13.08)**
Fines*Metals	2	0.7138 (0.34)	0.9109 (0.09)	0.9705 (0.03)	0.4615 (0.79)	0.712 (0.34)	0.2198 (1.57)	0.6014 (0.51)	0.5119 (0.68)	0.3348 (1.12)	0.9057 (0.1)
Day	3	**<.0001** **(17.73)**	0.0905 (2.29)	0.622 (0.59)	**<.0001** **(19.08)**	**<.0001** **(30.84)**	**<.0001** **(56.38)**	**0.0004** **(7.39)**	**<.0001** **(36.96)**	**<.0001** **(39.93)**	**<.0001** **(46.2)**
Fines*Day	6	0.4229 (1.02)	0.3804 (1.09)	0.6674 (0.68)	0.6614 (0.69)	0.3963 (1.07)	0.2544 (1.35)	0.5501 (0.83)	0.6466 (0.71)	**0.0443** **(2.37)**	0.3554 (1.14)
Metals*Day	3	**0.0009** **(6.52)**	**0.0325** **(3.18)**	0.3112 (1.22)	**0.0087** **(4.35)**	**0.0004** **(7.29)**	**<.0001** **(10.78)**	**0.0048** **(4.9)**	**0.0002** **(8.1)**	**0.0348** **(3.12)**	0.2206 (1.52)
Fine*Metals*Day	6	0.3653 (1.12)	0.7541 (0.57)	0.9342 (0.3)	0.0938 (1.94)	0.0945 (1.94)	**0.0239** **(2.72)**	0.6294 (0.73)	0.1253 (1.77)	0.4017 (1.06)	0.6301 (0.73)

Table 1.2: DISTLM output of the BEST procedure using AICc selection criterion and associated r^2 and predictor variables for response variables over time. Predictor variables are fine sediment (SED) and organic matter (OM) that were captured in sieves of 355µm and 63µm.



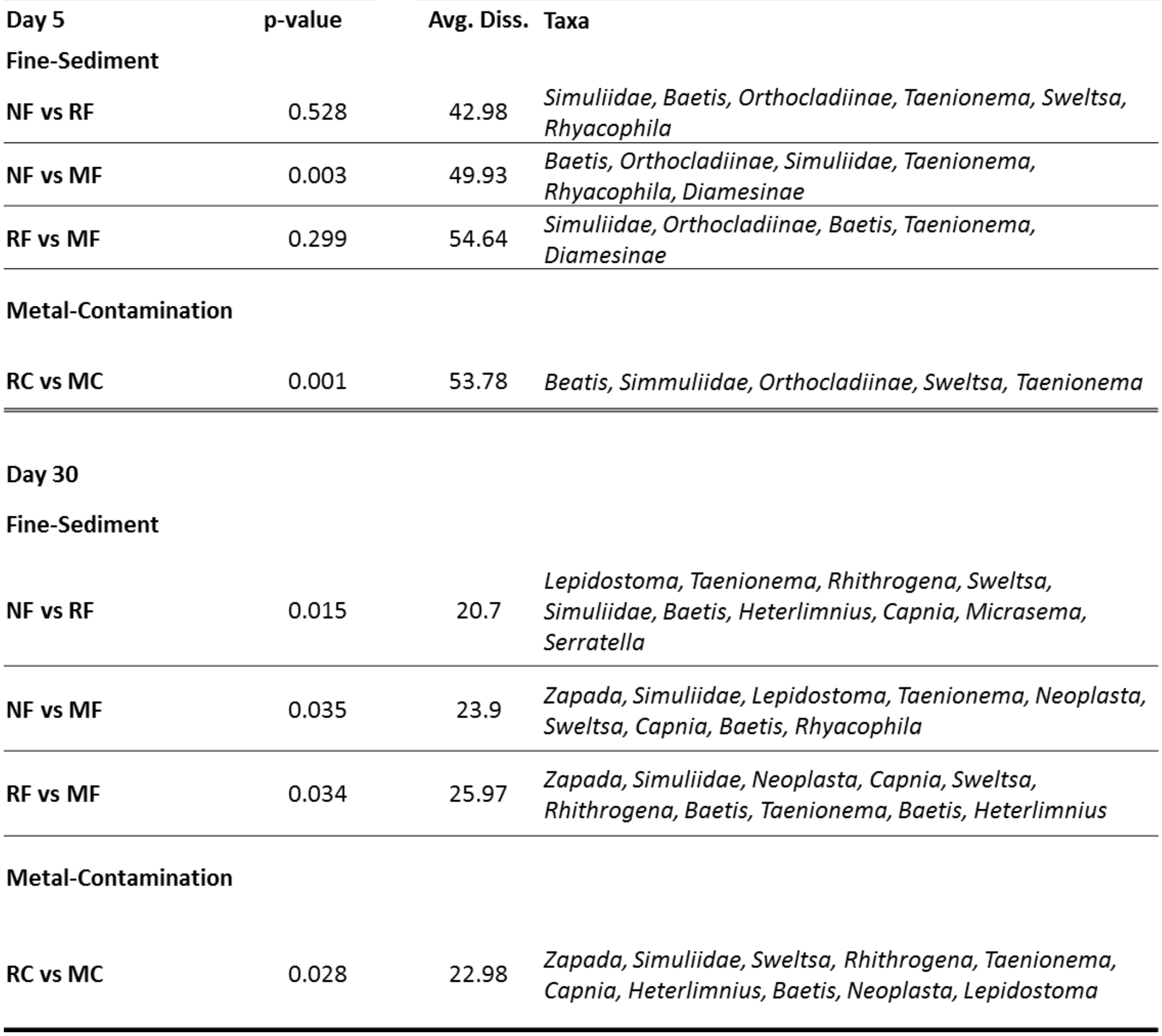
	R^2				Predictor Variables			
	5	10	20	30	5	10	20	30
Total Abundance	0.489	0.013	0.019	0.484	SED 355µm, OM 355µm	SED 63µm	SED 63µm	SED 355µm
Baetis	0.491	0.047	0.096	0.373	SED 355µm, OM 355µm	OM 63µm	OM 63µm	SED 355µm
Taenionema pallidum	0.480	0.498	0.046	0.457	SED 355µm, OM 355µm	SED 355µm, SED 63µm, OM 355µm	SED 355µm	SED 355µm, OM 63µm
Rhyacophila	0.337	0.297	0.172	0.183	SED 355µm	OM 355µm, OM 63µm	OM 355µm	OM 63µm
EPT	0.405	0.025	0.067	0.465	SED 355µm, OM 355µm	OM 63µm	SED 63µm	SED 355µm
Diptera	0.460	0.021	0.031	0.108	SED 355µm, OM 355µm	SED 63µm	OM 355µm	SED 355µm
Shannon-Diversity	0.262	0.542	0.130	0.182	SED 355µm, OM 355µm	OM 355µm, OM 63µm	SED 63µm	OM 355µm
Community Composition	0.314	0.026	0.076	0.157	SED 355µm, OM 355µm	OM 355µm	SED 63µm	SED 355µm

Table 1.3: Results of PERMANOVA tests comparing community composition over time to the effects of fine sediment and metal-contamination. p-values <0.05 represent comparisons that were significant.



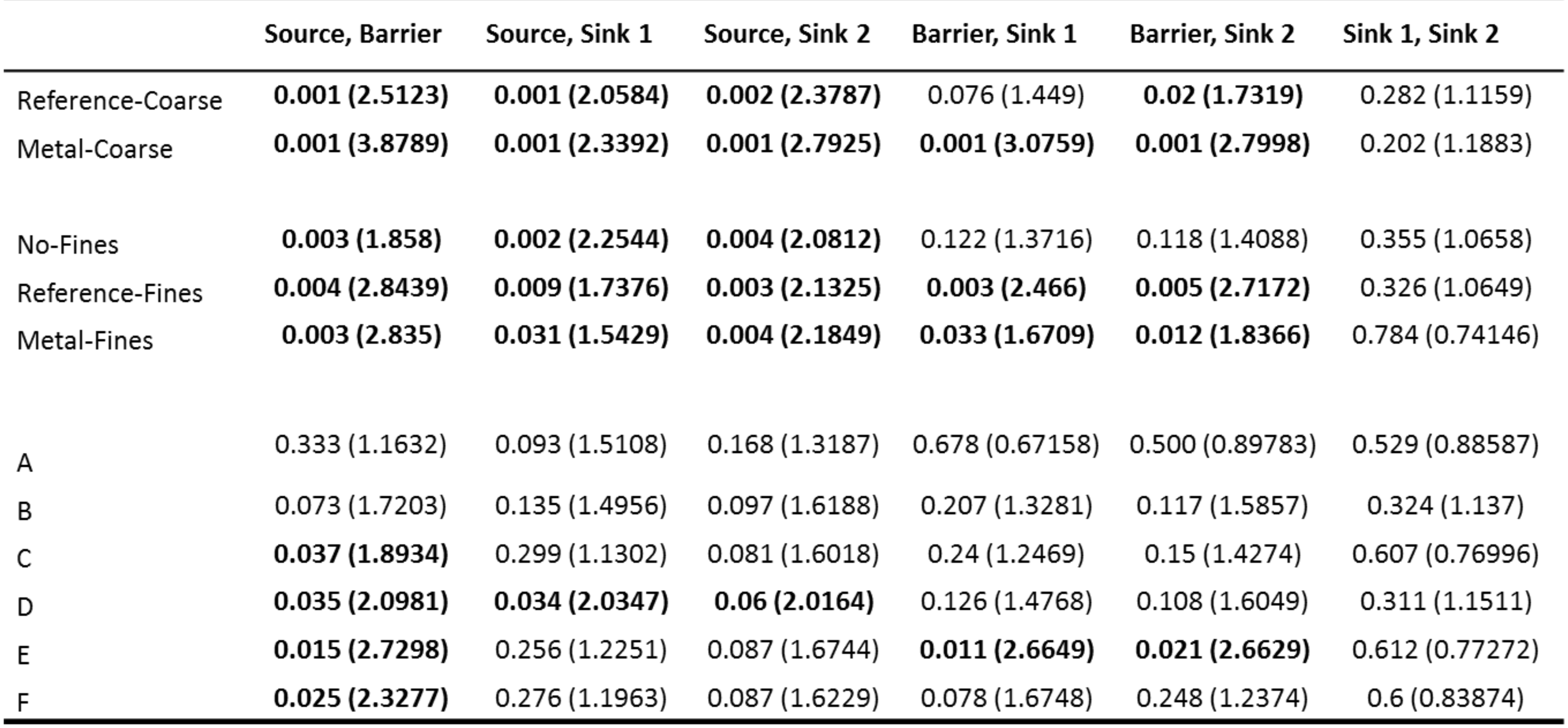
	Pseudo-F	P(perm)
Fines	2.2031	0.009
Metals	9.8394	0.001
Fines*Metals	1.3419	0.176
Day	14.134	0.001
Fines*Day	1.5323	0.022
Metals*Day	4.5382	0.001
Fine*Coarse*Day	1.161	0.225

Table 1.4: Pairwise-comparisons from three-way PERMANOVA (metals*fines*day) and SIMPER output for days 5 and 30. SIMPER output shows average dissimilarity (Avg. Diss.) between comparisons and species that contributed to the top 50% of the differences between factors. Taxa are listed from the highest to lowest contribution.



Pairwise-Comparisons		SIMPER Analysis	
Day 5	**p-value**	**Avg. Diss.**	**Taxa**
Fine-Sediment			
NF vs RF	0.528	42.98	*Simuliidae, Baetis, Orthocladiinae, Taenionema, Sweltsa, Rhyacophila*
NF vs MF	0.003	49.93	*Baetis, Orthocladiinae, Simuliidae, Taenionema, Rhyacophila, Diamesinae*
RF vs MF	0.299	54.64	*Simuliidae, Orthocladiinae, Baetis, Taenionema, Diamesinae*
Metal-Contamination			
RC vs MC	0.001	53.78	*Beatis, Simmuliidae, Orthocladiinae, Sweltsa, Taenionema*
Day 30			
Fine-Sediment			
NF vs RF	0.015	20.7	*Lepidostoma, Taenionema, Rhithrogena, Sweltsa, Simuliidae, Baetis, Heterlimnius, Capnia, Micrasema, Serratella*
NF vs MF	0.035	23.9	*Zapada, Simuliidae, Lepidostoma, Taenionema, Neoplasta, Sweltsa, Capnia, Baetis, Rhyacophila*
RF vs MF	0.034	25.97	*Zapada, Simuliidae, Neoplasta, Capnia, Sweltsa, Rhithrogena, Baetis, Taenionema, Baetis, Heterlimnius*
Metal-Contamination			
RC vs MC	0.028	22.98	*Zapada, Simuliidae, Sweltsa, Rhithrogena, Taenionema, Capnia, Heterlimnius, Baetis, Neoplasta, Lepidostoma*

Table 1.5: Pairwise-comparisons of community composition based on tray position of fine sediment, metal-contamination, and treatments. Significant results with a $p < 0.05$ are in bold with t-values in parentheses.



	Source, Barrier	**Source, Sink 1**	**Source, Sink 2**	**Barrier, Sink 1**	**Barrier, Sink 2**	**Sink 1, Sink 2**
Reference-Coarse	**0.001 (2.5123)**	**0.001 (2.0584)**	**0.002 (2.3787)**	0.076 (1.449)	**0.02 (1.7319)**	0.282 (1.1159)
Metal-Coarse	**0.001 (3.8789)**	**0.001 (2.3392)**	**0.001 (2.7925)**	**0.001 (3.0759)**	**0.001 (2.7998)**	0.202 (1.1883)
No-Fines	**0.003 (1.858)**	**0.002 (2.2544)**	**0.004 (2.0812)**	0.122 (1.3716)	0.118 (1.4088)	0.355 (1.0658)
Reference-Fines	**0.004 (2.8439)**	**0.009 (1.7376)**	**0.003 (2.1325)**	**0.003 (2.466)**	**0.005 (2.7172)**	0.326 (1.0649)
Metal-Fines	**0.003 (2.835)**	**0.031 (1.5429)**	**0.004 (2.1849)**	**0.033 (1.6709)**	**0.012 (1.8366)**	0.784 (0.74146)
A	0.333 (1.1632)	0.093 (1.5108)	0.168 (1.3187)	0.678 (0.67158)	0.500 (0.89783)	0.529 (0.88587)
B	0.073 (1.7203)	0.135 (1.4956)	0.097 (1.6188)	0.207 (1.3281)	0.117 (1.5857)	0.324 (1.137)
C	**0.037 (1.8934)**	0.299 (1.1302)	0.081 (1.6018)	0.24 (1.2469)	0.15 (1.4274)	0.607 (0.76996)
D	**0.035 (2.0981)**	**0.034 (2.0347)**	**0.06 (2.0164)**	0.126 (1.4768)	0.108 (1.6049)	0.311 (1.1511)
E	**0.015 (2.7298)**	0.256 (1.2251)	0.087 (1.6744)	**0.011 (2.6649)**	**0.021 (2.6629)**	0.612 (0.77272)
F	**0.025 (2.3277)**	0.276 (1.1963)	0.087 (1.6229)	0.078 (1.6748)	0.248 (1.2374)	0.6 (0.83874)

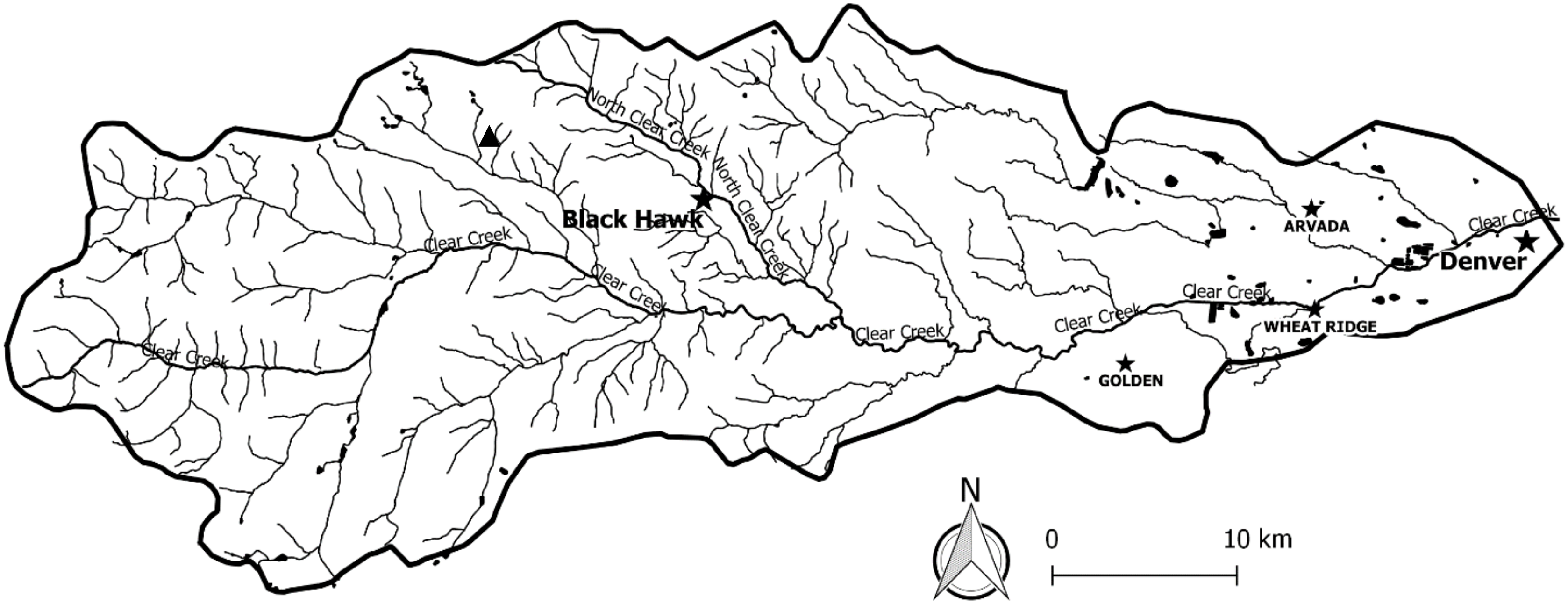


Figure 1.1: Map of North Clear Creek in Colorado, USA. Reference site (triangle) where the colonization experiment and un-contaminated sediment for the study was collected and the site where metal contaminated sediment was collected for the experiment (star).

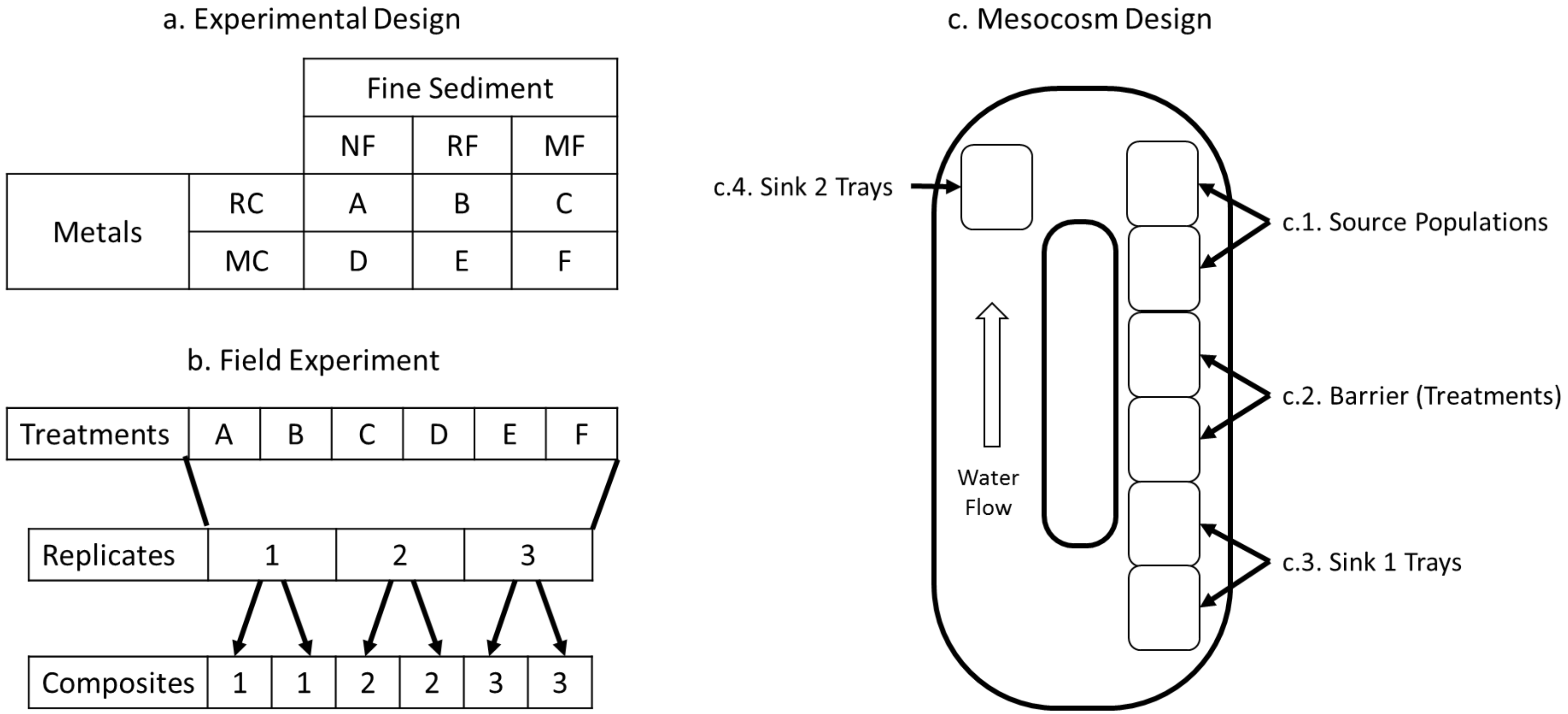


Figure 1.2: Study Design of showing the 2 x 3 factorial design and all six treatment (a); field design where each treatment has three replicates and each replicate is a composite of two colonization tray (b); and the design of the streams during the 10-day mesocosm.

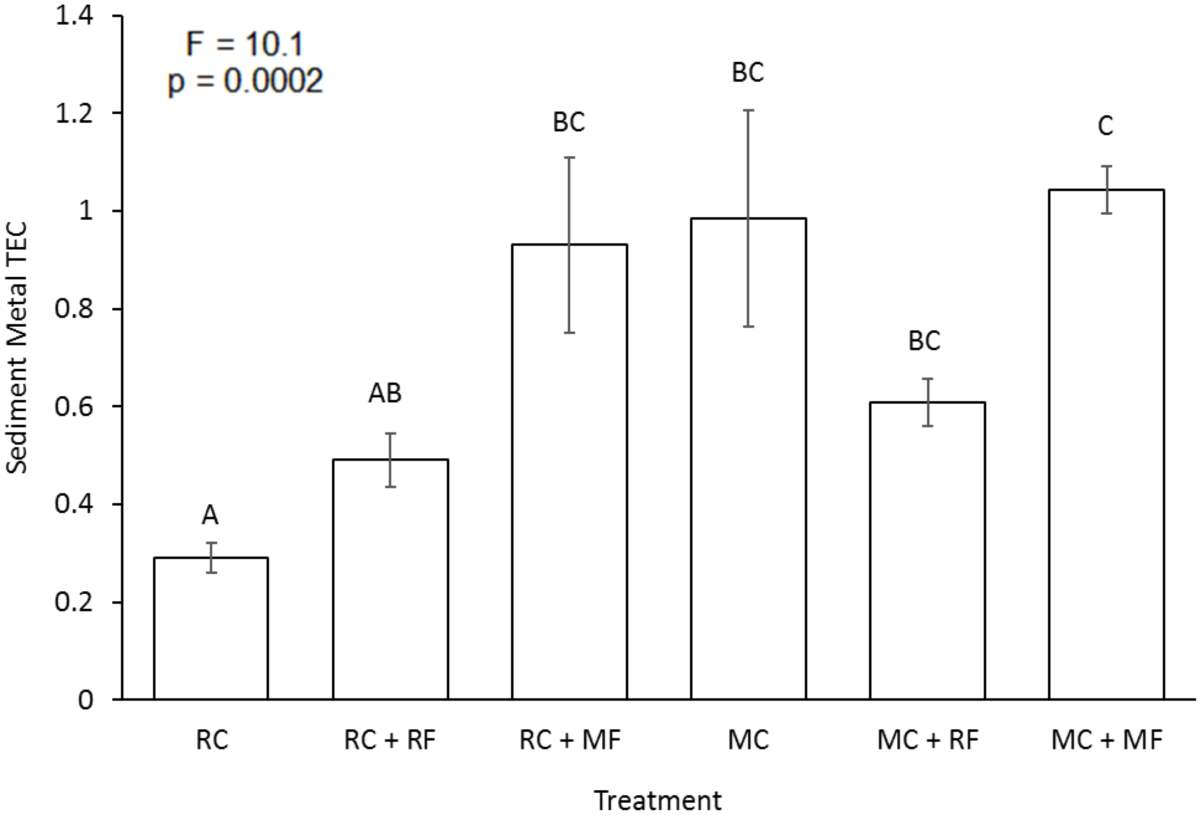


Figure 1.3: Metal concentrations in the trays during the experiment expressed as threshold-effect concentrations. Data is a combination of metal concentrations on days 15 and 30. Letters indicate the differences in significance between all six treatments (ANOVA; $p < 0.05$). Bars represent standard error of the mean.

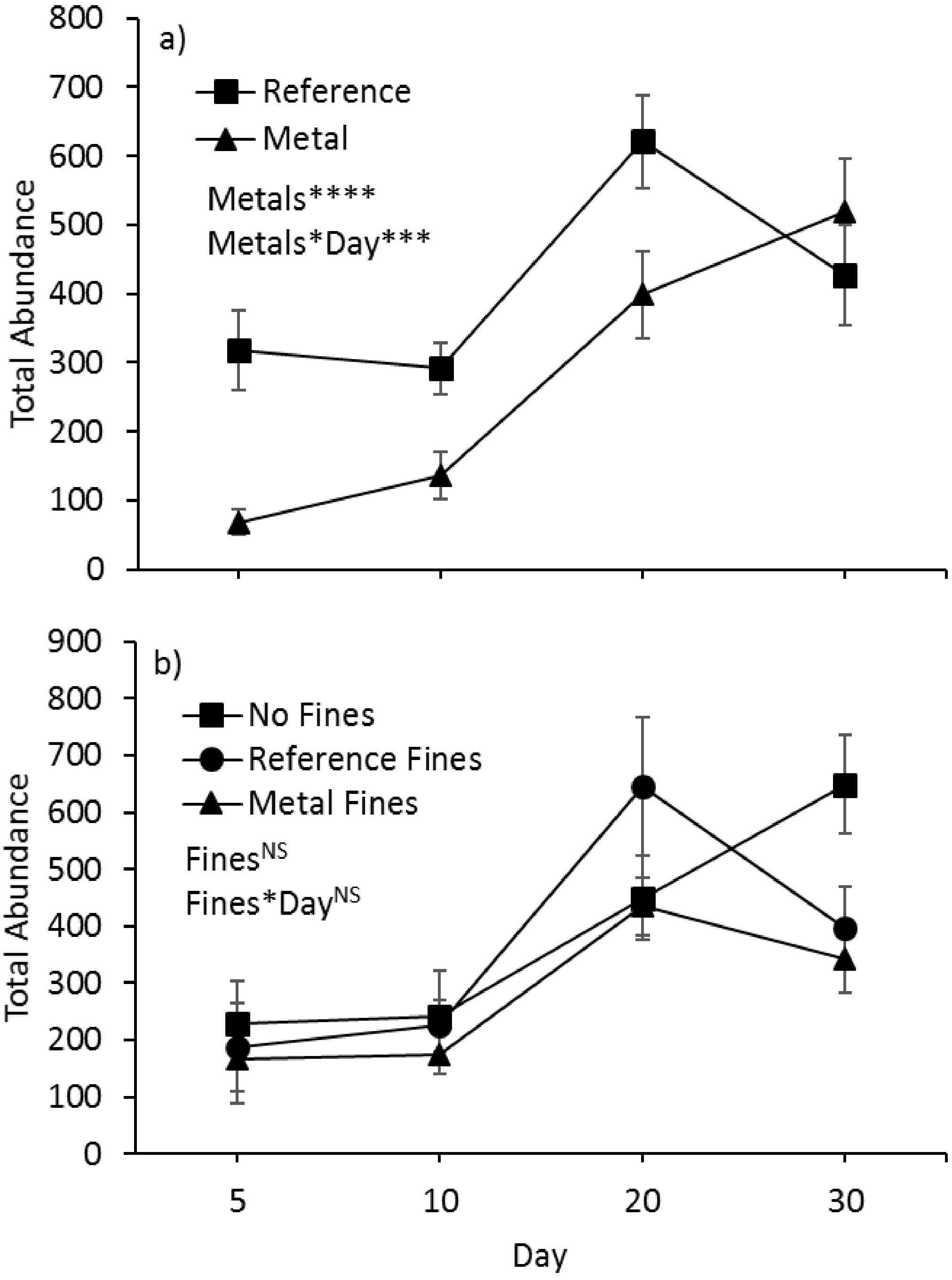


Figure 1.4: The effect of metal-contamination (a) and fine-sediment deposition (b) on mean total Abundance over time. The impact of metal contamination compared the factors of reference-site coarse substrate (treatments A, B, and C) and metal-contaminated coarse sediment (treatments B, C, and D). Trays with no fine sediment (treatments A and D), reference-site fine sediment (treatments B and E), with metal-contaminated fine sediment (treatments C and F). Bars represent standard error of the mean. Superscripts after the factorial comparisons show ANOVA output of either, not significant (NS), $p < 0.001$ (***), and $p < 0.0001$ (****).

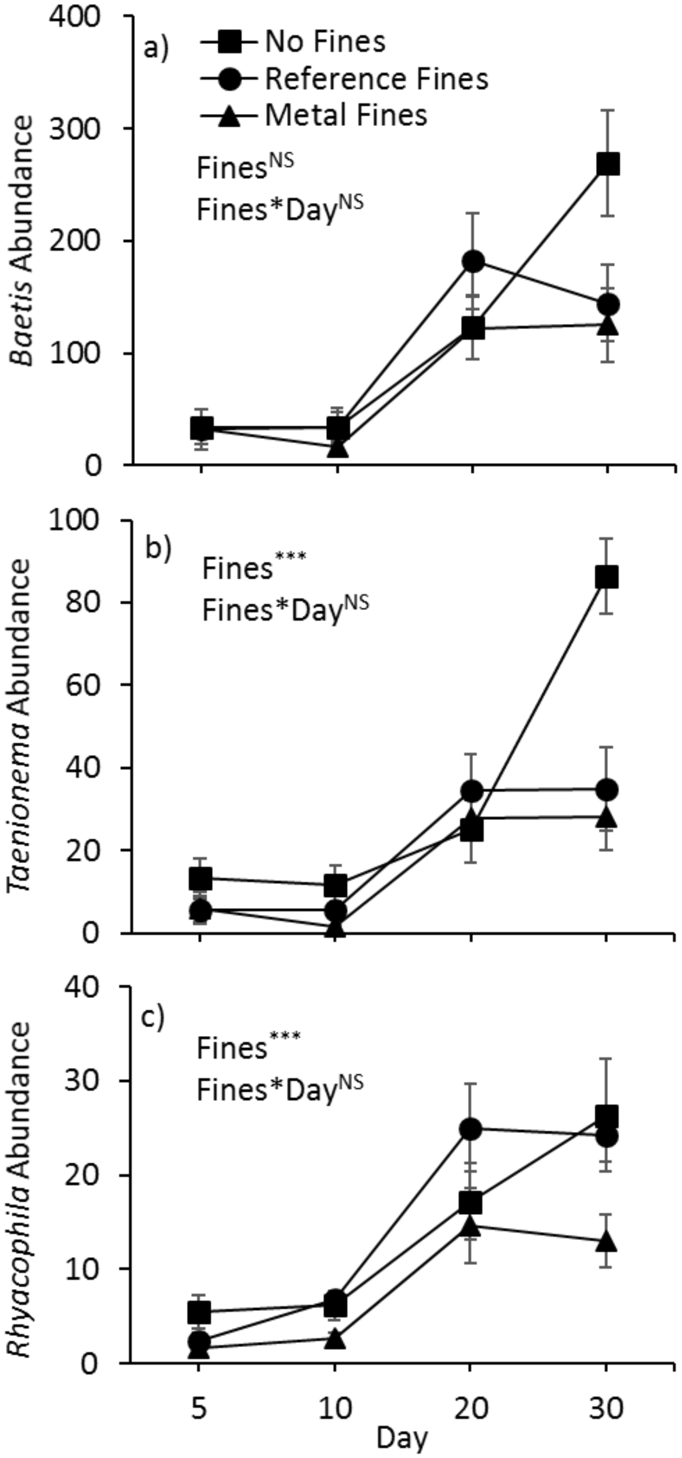


Figure 1.5: Mean abundances of dominant taxa *Baetis* sp. (a), *Taenionema pallidum* (b) and *Rhyacophila* sp. (c) over time. Comparisons of each taxa response to fine sediment treatments. Bars represent standard error of the mean. Significance values displayed from the effects of fine sediment as either not significant (NS) or p-value < 0.001 (***).

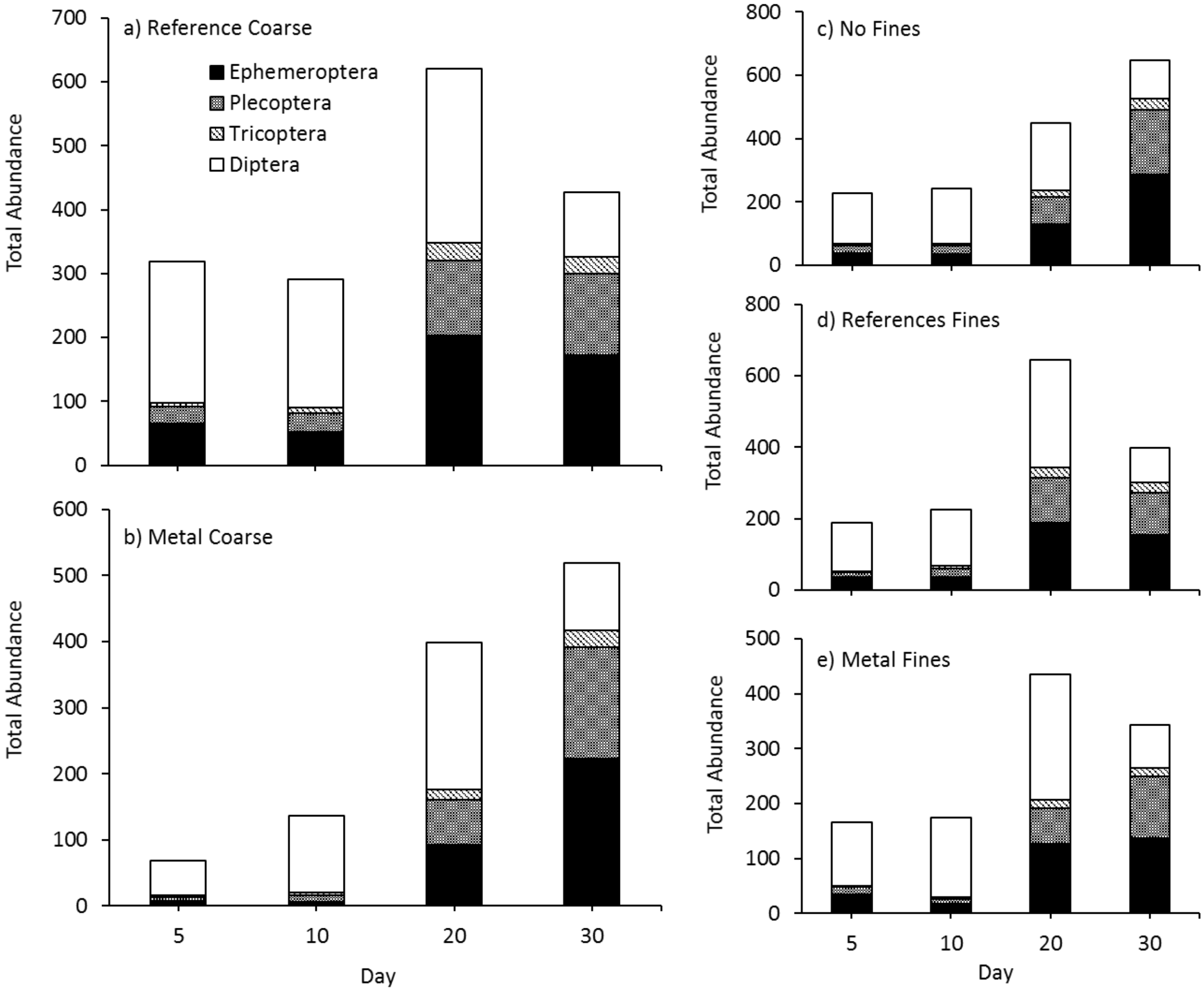


Figure 1.6: Proportions of Ephemeroptera, Plecoptera, Trichoptera, and Diptera over time in trays with reference-site coarse sediment (a), metal-contaminated coarse sediment (b), no fine sediment (c), reference-site fine sediment (d), and metal-contaminated fine sediment (e).

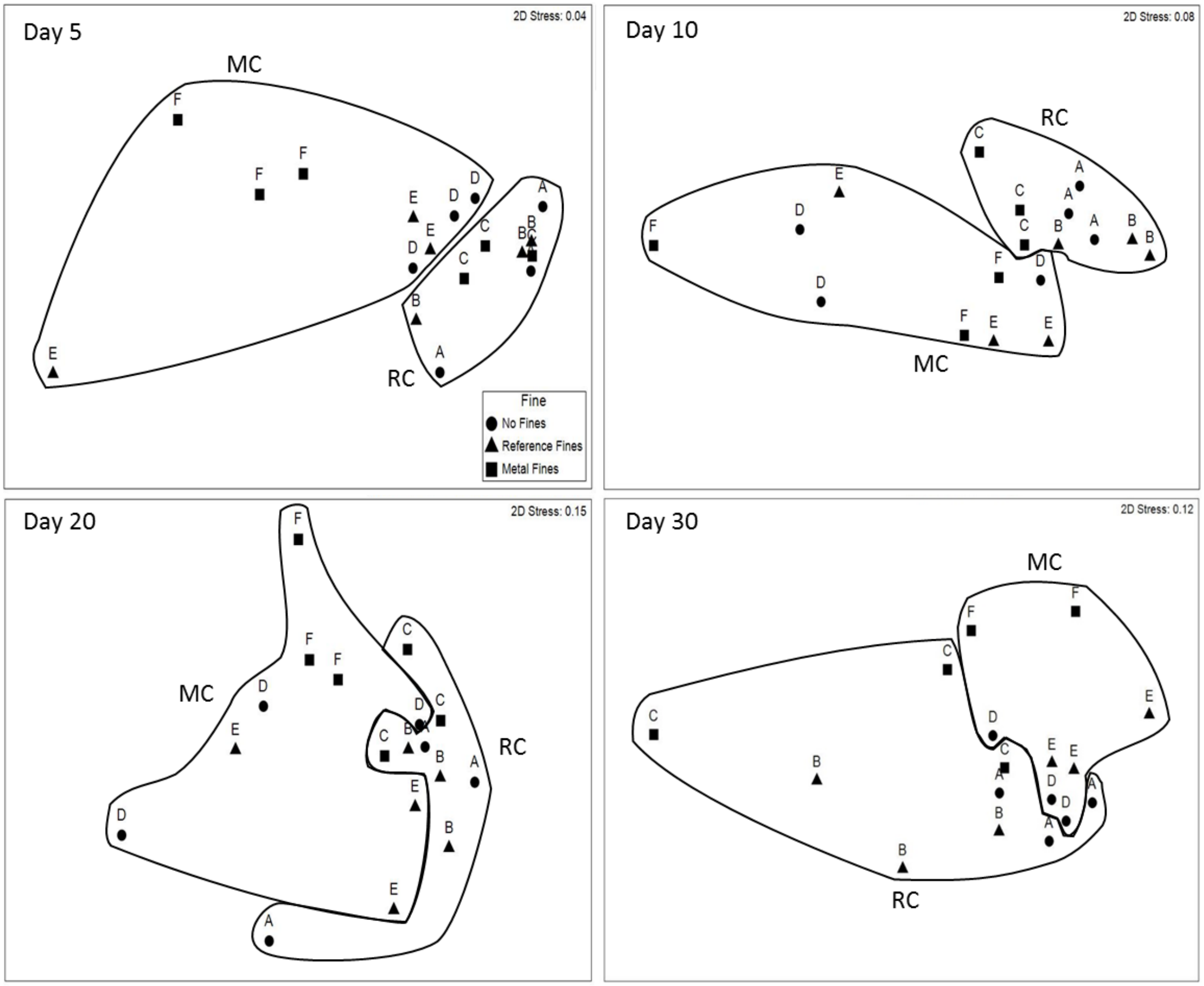


Figure 1.7: nMDS plot showing comparisons of community composition based by fine sediment (symbols), metal-contamination (black outline; RC = reference coarse; MC = metal-coarse), and individual treatments (letters). Treatments A (reference-coarse), B (reference-coarse and fines), C (reference-coarse and metal-fines), D (metal-coarse), E (metal-coarse and reference-fines), and F (metal-coarse and fines; refer to Figure 1.2).

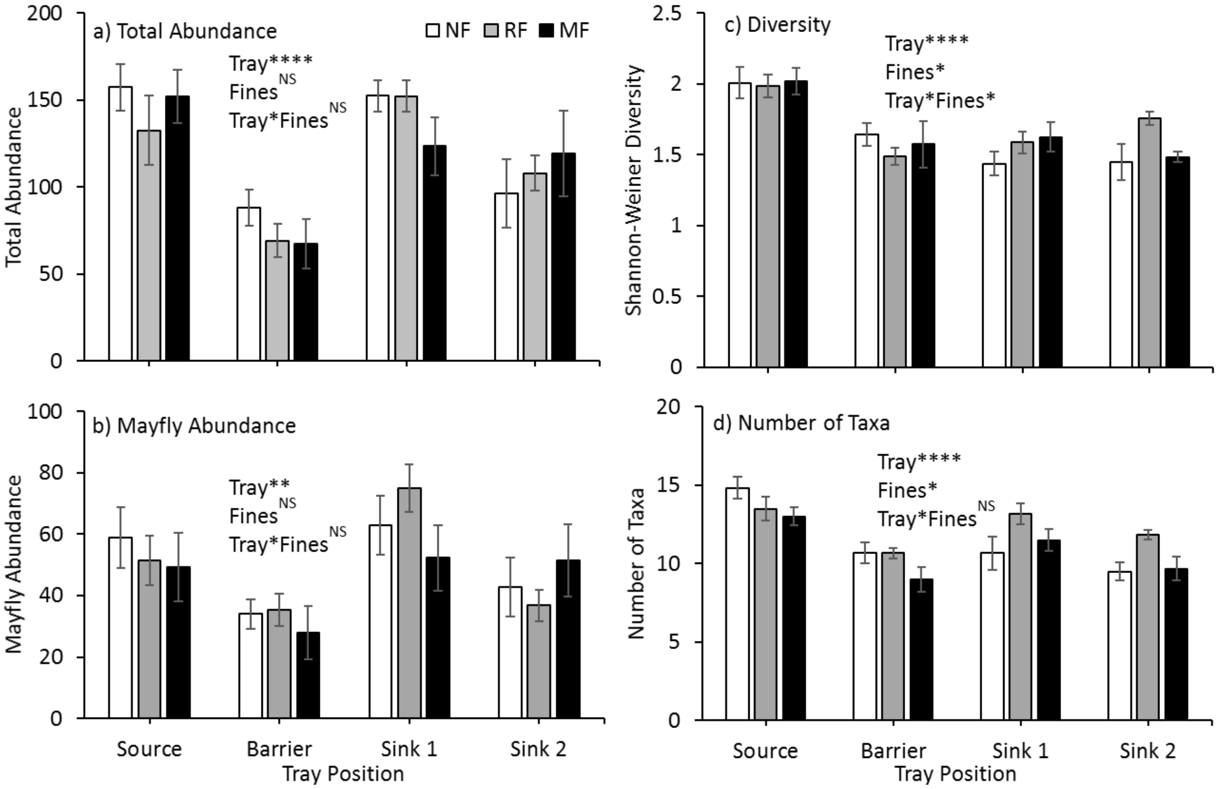


Figure 1.8: Mean total abundance (a), mayfly abundance (b), Shannon-Weiner diversity (c), and number of taxa (d) by tray position in treatments with no-fines (NF), reference-fines (RF), and metal-fines (MF). Error bars represent standard error of the mean and factors are shown for tray position and fine sediment with level of significance as, not significant (NS), $p < 0.05$ (*), $p < 0.01$ (**), and $p < 0.0001$ (****).

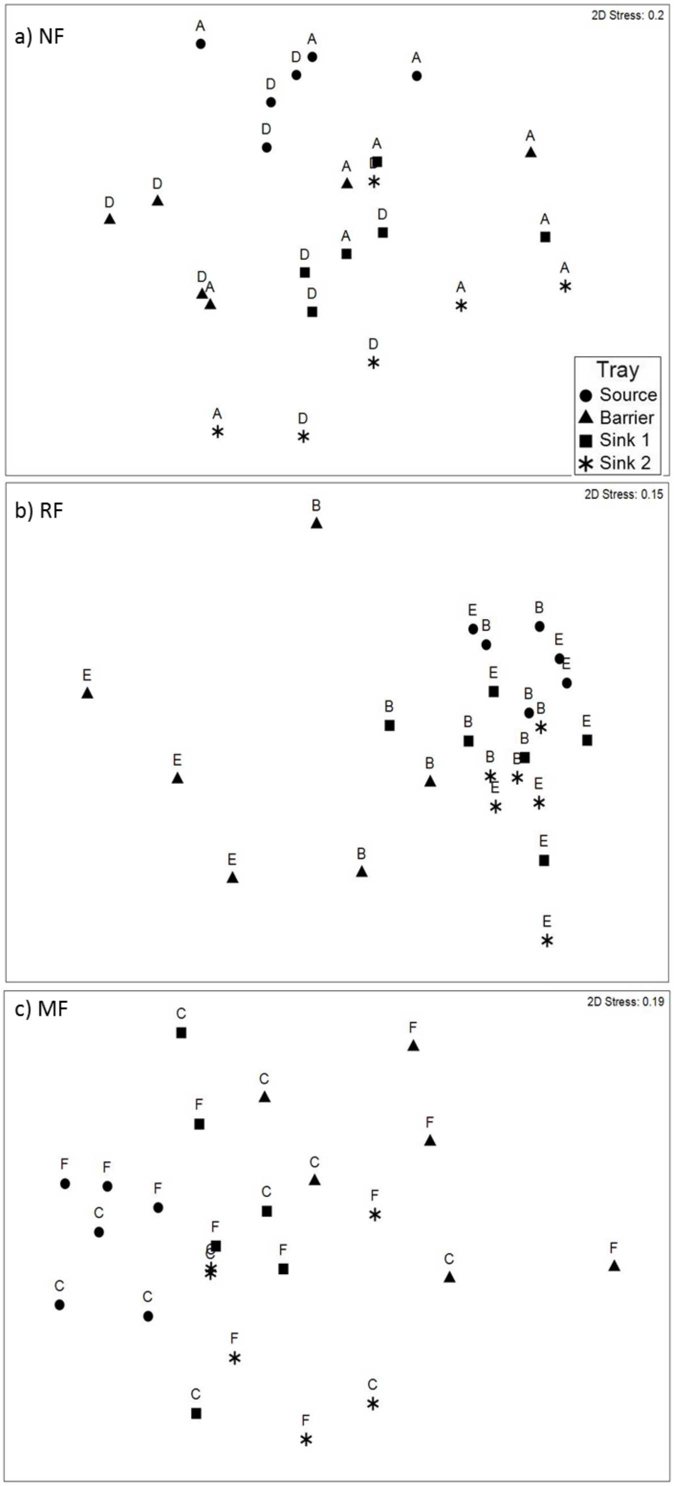


Figure 1.9: nMDS plots showing the separation of trays based on community composition in treatments with no-fines (a), reference-fines (b), and metal-contaminated fines (c). Refer to Figure 1.2 for treatment definitions (A-F); and Appendix 1G for SIMPER output with p-values and taxa contributions.

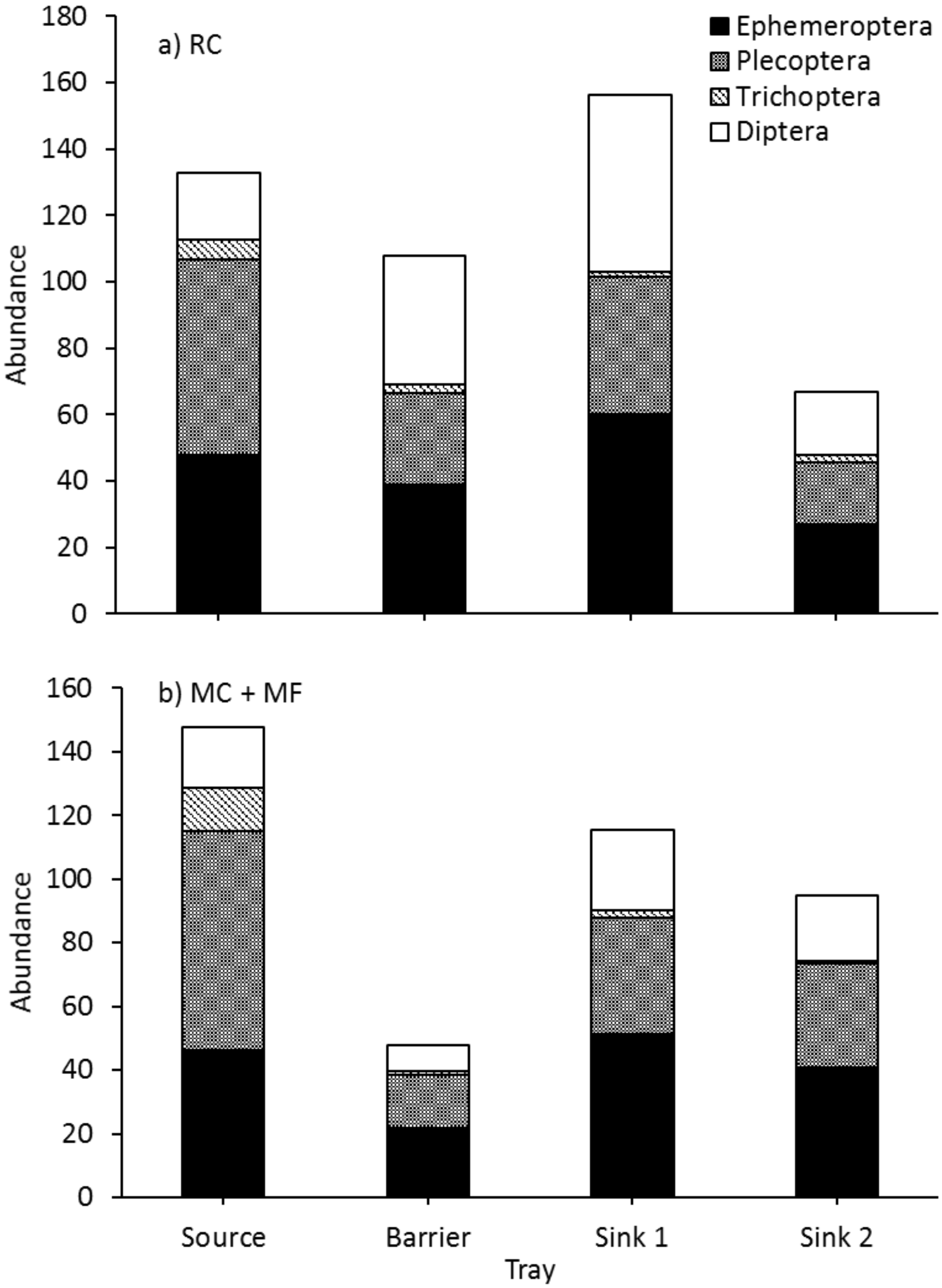


Figure 1.10: Mean abundance of Ephemeroptera, Plecoptera, Trichoptera and Diptera taxa in streams with treatment-A (a: Reference coarse sediment; RC) and treatment-F (b: metal contaminated coarse and fine sediments; MC + MF) in each of the trays.

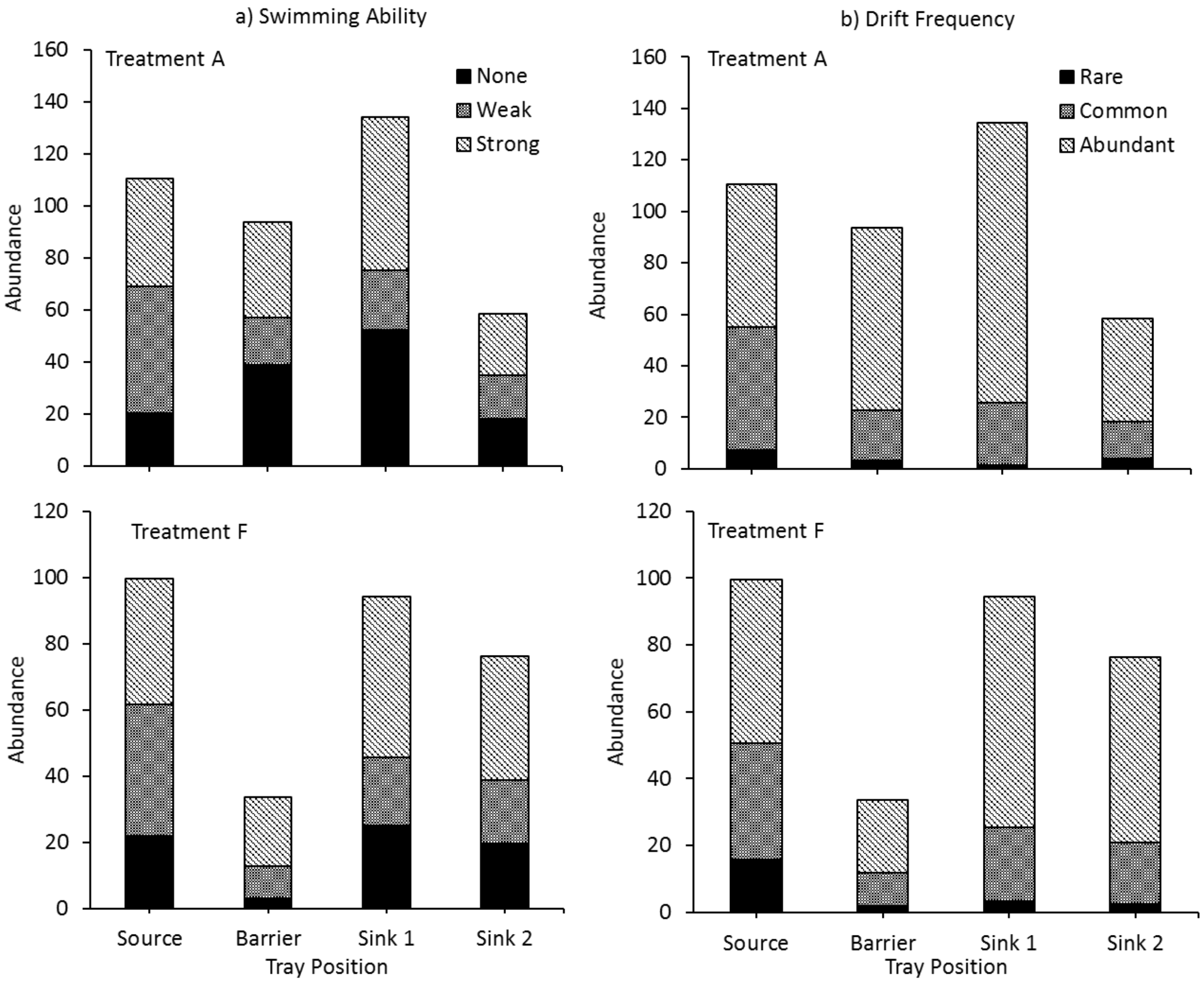


Figure 1.11: Comparisons of mobility trait abundances on each tray in treatments A (reference-coarse) and F (Metal coarse and fines). Swimming ability of invertebrates are classified into three categories (no swimming ability or weak or strong swimming ability). Drift frequency of invertebrates are separeated based on their occurance in drift samples (rare, common or abundant in drift samples).

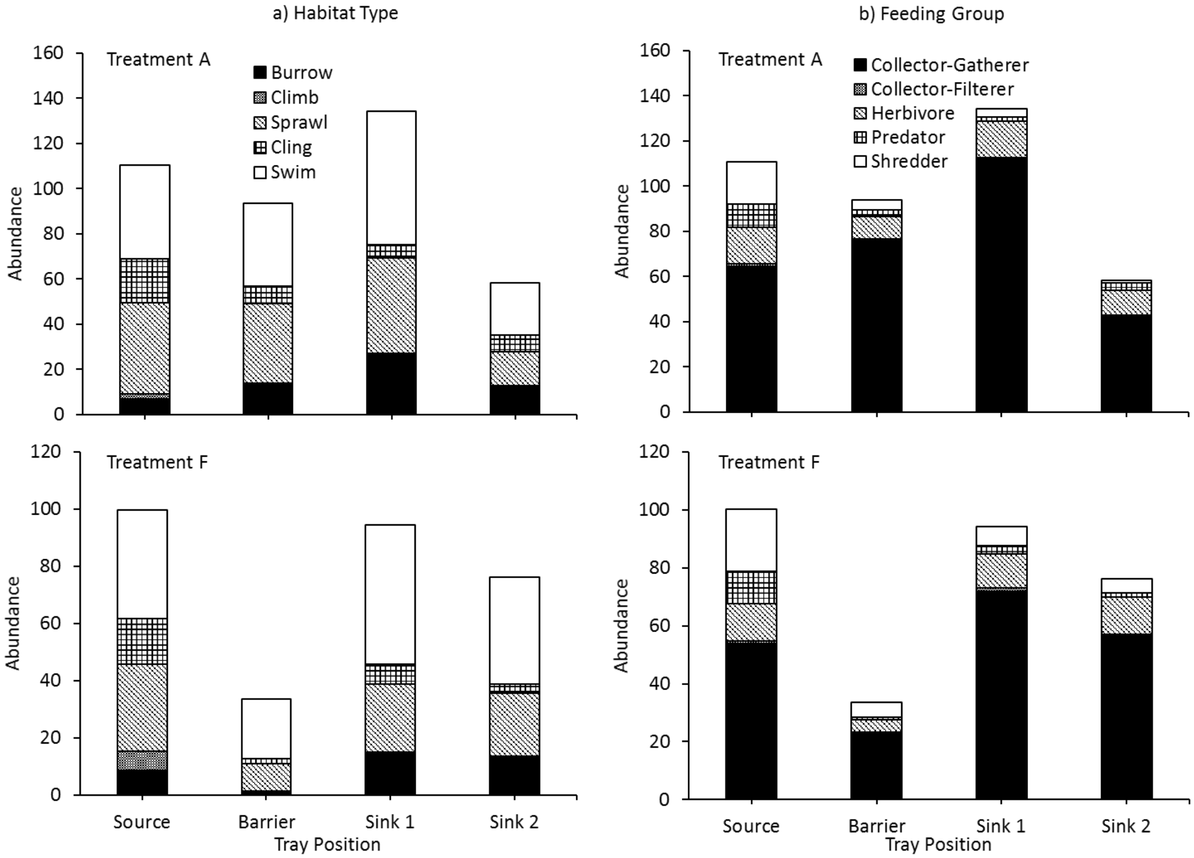


Figure 1.12: Comparisons of ecological trait abundances on trays in treatments A (reference-coarse) and F (Metal coarse and fines). Habitat type of invertebrates are classified into five categories (burrow, climb, sprawl, cling, and swim). Functional feeding groups are also separated into five categories (collector-gatherers, collector-filterers, herbivores, predators, and shredders).

CHAPTER 2: EFFECTS OF METAL-CONTAMINATED SEDIMENT ON COLONIZATION DYNAMICS OF BENTHIC INVERTEBRATES IN THE UPPER ARKANSAS RIVER, COLORADO, USA

Introduction

Interspecific variation in the tolerance of benthic invertebrates to contaminants is an important concept that is useful for understanding recovery of aquatic ecosystems. Although the most direct impact of exposure to contaminants is often mortality, aquatic organisms can develop tolerance to contaminants due to natural environmental conditions or prolonged exposure (Weis and Weis 1989, Clements 1999, Seguin et al. 2002). Community tolerance to contaminants is difficult to assess and often a confounding factor when trying to extrapolate the results of laboratory and field experiments to actual responses in the field. One approach to assess to community tolerance is the Pollution-Induced Community Tolerance (PICT) concept, where contaminants remove the most sensitive species, thus increasing community tolerance to contaminants (Boivin et al. 2002). The PICT method involves exposing communities, collected along a gradient of contamination, to a certain stressor to obtain EC50 values. These EC50 values for each community are then compared to predict how communities will respond to contaminants in the field (Boivin et al. 2002). PICT is an accepted way of linking laboratory data to actual responses in the field, and researchers have used this method to determine tolerances of phytoplankton communities to atrazine (Seguin et al. 2002), benthic communities to metals (Clements 1999), and bacterial communities to copper and temperature (Boivin et al. 2005). Although, the PICT method is helpful for understanding community tolerance, scientists have a limited understanding of how long it takes for communities to become tolerant or lose tolerance to contaminants. Because sensitive species are generally eliminated from communities exposed to contaminants, I would expect these communities to be less diverse. However, if sensitive species are simply replaced by tolerant species after restoration, whether or not those communities become less tolerant may be difficult to determine in regions with a long history of contamination. This is the situation is likely to occur in systems with a long history of metal inputs due to mining activities.

Determining how to assess both community tolerance and the effectiveness of restoration may be difficult in streams that receive low levels of metal contamination after remediation. This is especially true since in situations where pre-disturbance data are lacking. Macroinvertebrates communities may vary among locations throughout a stream reach due to physical habitat characteristics and habitat requirements rather than the presence of contaminants (Rogers et al. 2002, Rasmussen et al. 2012). Additionally, the presence of species at contaminated sites may not be simply be due to tolerance, but a result of life history characteristics such as generations per year and fecundity (Niemi et al. 1990, Marchand et al. 2004). Many factors can influence the presence or absence of species at restored sites and therefore mask the ability to determine responses to water quality or habitat improvements. The majority of stream restoration projects are managed for aesthetics and do not consider ecological theory, and many of these projects do not assess improvements in benthic communities (Lake et al. 2007, Thompson and Lake 2010). Using ecological theory in relation to contaminant exposure may provide insight on how to measure restoration success.

Several ecological theories are potentially influenced by tolerance of organisms to contaminants. Benthic communities at contaminated sites are influenced by the removal of more sensitive taxa and the ability of these tolerant species to outcompete sensitive taxa, a concept that relates to interspecific competition and resource availability. Tolerance to contaminants has also been linked to indirect effects on resource availability and impacts on other levels of organization in aquatic ecosystems (Fleeger et al. 2003). In turn, the energy spent avoiding predation or locating resources may also come at a cost in the form of increased mortality, reduced fecundity, or changes in development (Clements 1999, Lefcort et al. 2000, Marchand et al. 2004, Xie and Klerks 2004). This concept also supports the intermediate disturbance hypothesis, which states that areas of low and extreme disturbances have lower diversity compared to areas with an intermediate level of disturbances (Connell 1978). Results of several studies examining diversity along contaminant gradients may be explained by the intermediate disturbance hypothesis (Gerhardt et al. 2004, Piscart et al. 2005, Fields et al. 2006, Żmudzki and Laskowski 2012). However, few studies attempt to characterize the link between the intermediate disturbance hypothesis and the low diversity at restored sites (Louhi et al. 2011, Stranko et al. 2012). Finally, because of the diverse number of microhabitats along a river continuum, the distribution of macroinvertebrates and contaminants may influence the perceived tolerance observed in benthic communities. This idea is nested in the concept of patch dynamics, where benthic community composition varies along a river continuum with changes in microhabitat (Pringle et al. 1988). Yet, certain microhabitats, such as those with more fine sediments, may have higher concentrations of contaminants than other patches, thus influencing actual contaminant exposure. The distribution of contaminants in these patches will likely play a role in determining how macroinvertebrate populations differ in resilience and resistance to contaminants.

All of these concepts are important for understanding how benthic communities recover from anthropogenic disturbance and the success of stream restoration projects. Most importantly, it is imperative to develop objective approaches to determine if stream restoration projects are successful. Typically, stream restoration managers conduct benthic surveys to assess if benthic invertebrate communities are recovering, and these results are used to extrapolate how all taxa in the community are responding. One way to improve assessments of stream restoration success is to quantify behavioral responses, such as colonization or avoidance of contaminated sediments. Several studies have used macroinvertebrate colonization as a useful tool to understand avoidance and sensitivity to contaminated sediments (Courtney and Clements 2002, Cadmus et al. 2016, Dabney - Chapter 1). For this study, I used a similar experimental approach to determine community tolerance to metal-contaminated substrate at two sites affected by mining activities in the Upper Arkansas River. The Upper Arkansas River, a metal-contaminated stream in central Colorado, has been the subject of a long-term (1980-2016) monitoring project and numerous experiments designed to assess restoration effectiveness (Clements 1994, 1999, 2004, Kashian et al. 2007). Historical mining operations near Leadville, Colorado, USA have caused benthic invertebrate population declines in the Upper Arkansas River; however, the primary source of contamination has been eliminated for about 15 years, with only low concentrations of metals leaching from the mine shafts (Clements et al. 2010). In this study, I tested the hypothesis that avoidance of contaminated substrates by macroinvertebrates will differ between reference and historically contaminated locations. Specifically, I hypothesized that taxa from communities collected from a reference site will show a greater level of substrate avoidance compared to those from a previously metal-contaminated site.   Since metal contamination can remain in the streambed long after the source of contamination has been eliminated, it is important to study responses to contaminated sediments. Several experiments have been performed using communities from the Upper Arkansas River;

therefore, this study provides a unique opportunity for comparisons of species responses in the laboratory and field.

Methods

Study Site

The Upper Arkansas River near Leadville, Lake County, Colorado, USA was once heavily contaminated with metals due to mining activities in the Leadville Mining District. In 1998 the Yak Tunnel, the primary source of metals to the system, collapsed causing acid mine drainage to flow into the California Gulch, a tributary of the Upper Arkansas River. Acid mine drainage in the Arkansas River has impaired water quality for over 100 km downstream (Clements 1994). For this study, a metal-contaminated and reference-site on the Upper Arkansas River was chosen (Figure 1). The metal-contaminated site (AR5; N39.12816, E253.68822) is located 11.1 km downstream of California Gulch and the reference-site (AR1; N39.25481, E253.65434) is located 3.8 km upstream of this metals source. The reference-site (AR1) historically received low concentrations of metals from Leadville Mine Drainage Tunnel (LMDT), but this site was remediated in 1992 and now supports a diverse benthic community with high abundances of metal-sensitive taxa. Metal concentrations at the impaired site (AR5) have also been greatly reduced, but seasonal releases during spring runoff continue to impact benthic communities, which are dominated by metal-tolerant species (Clements et al. 2002, 2010)

Colonization experiment

A colonization experiment was conducted in October 2014 to measure the effects of metal-contaminated substrate on benthic invertebrate communities at AR1 and AR5. Metal-contaminated coarse substrate (cobble and gravel > 2360 µm) from reference-site and metal-contaminated sites on North Fork Clear Creek (Blackhawk, Gilpin County, Colorado, USA) was transported to the two Arkansas River sites. Based on previous data using sediment from North Fork Clear Creek (Dabney – Chapter 1), I expected metal-concentrations to remain significantly higher on the metal-contaminated substrate compared to reference substrate throughout the experiment.

Reference and metal-contaminated substrate were placed in colonization trays and left in the stream to be collected on days 5, 12, and 28 (Clements et al. 1988). The experiment has 2 treatments, two locations and three collection days in a full factorial design (site x treatment x day) to quantify the impacts of metal-contaminated sediment at the reference and impaired sites. Each treatment had three replicates and to obtain a more accurate representation of the benthic community, two trays were combined for each replicate. Colonization trays (25cm x 25cm x 10cm) with 1.25 cm diameter holes (to facilitate flow) were attached to PVC racks and placed in the stream. On each collection day, three benthic samples were collected with a 0.1 m^2 Hess sampler to assess natural community composition. Both Hess and tray samples were rinsed through a 355µm sieve and preserved in 80% ethanol. Samples were transported back to the lab where the entire sample was sorted and identified using a dissecting microscope. Insects in all samples were identified to genus or family.

Statistical Analysis

For the colonization experiment, I tested effect of contaminated substrate (Reference vs Metals), site (AR1 and AR5) and day (5, 12, and 28). All analyses were performed on log-transformed data, except diversity, richness, and evenness metrics. Univariate analysis was performed on Shannon-diversity, species richness, number of taxa, total abundance and Ephemeroptera, Plecoptera, and Trichoptera (EPT) metrics using the PROC GLM procedure with statistical package, SAS v9.4 (SAS Inc., Cary, NC, U.S.A.). Multivariate analysis was used to test changes in community structure using PRIMER-e v7 (Quest Research Limited; Cambridge, UK) with the +PERMANOVA package (Anderson 2001). Major trends in the data were then visually represented by using nonmetric-multidimensional scaling (NMDS) plots. A Bray-Curtis similarity matrix was created using log-transformed community data. A three-way permutational multivariate analysis of variance (PERMANOVA) was run at 999 permutations under the reduced model using a Bray-Curtis resemblance matrix (dummy variable = 1). Pairwise-comparisons were performed on univariate and multivariate data to examine more complex trends in the data. Additionally, SIMPER analysis was performed on log-transformed data to identify the invertebrates that account for 50% of the differences between response variables (Clarke and Warwick 2001). SIMPER analysis was also used to determine the taxa most responsible for the dissimilarity between reference and metal-contaminated substrate, and AR1 and AR5. Significance for all tests was determined based on a $p < 0.05$ with Monte Carlo p-values being used for tests with 10 permutations (Anderson 2005).

Results

Univariate Metrics of Community Structure

During this study, I collected a total of 7,010 invertebrates and 34 genera that colonized the trays. Throughout the experiment, the mayfly *Baetis* sp. and chironomids (Orthocladiinae and Diamesinae) were the most dominant taxa in the community, with an increase in number of EPT taxa on day 28. Community composition differed over time and between sites (Table 2.1), with an increase in abundance of *Drunella* sp. and *Rithrogena* sp. at AR1 and *Arctopsyche grandis* at AR5 (Fig. 2.2). In general, the AR5 community had a higher abundance of caddisflies, especially on day 28, whereas AR1 had a higher abundance of EPT taxa and dipterans. Total macroinvertebrate abundance increased in reference and metal-contaminated treatments at both sites (Fig. 2.2a). Although these differences were not significant, by the end of the experiment there were more invertebrates on reference trays compared to metal-contaminated trays at AR5. Similar trends of EPT abundance were observed at both sites, with *Baetis* sp. driving the trends in abundance (Fig. 2.2e). Abundance of Diptera showed greater separation between treatments at AR5 on day 28 (Fig. 2.2d), which accounted for differences observed in total abundance. However, none of the metrics I examined was statistically different when comparing the interaction between site and treatment effect ($p > 0.05$; Table 2.1).

With the exception of evenness, I did not observe significant differences in the community indices I examined. However, a slight increase over time in Shannon-Weiner diversity and species richness was observed at AR1, which either decreased or remained steady at AR5. Unlike the other community indices, number of taxa changed significantly over time ($p = 0.007$; Table 2.1; Fig. 2.3c). The total number of taxa significantly increased over time at AR1; however, there were no significant differences at AR5 ($p > 0.05$; Fig. 2.3). Despite AR5 having a lower number of taxa, species richness and diversity, I did not see an effect of metal-contamination and no significant site x treatment interaction (Table 2.1).

Multivariate Analysis of Community Structure

Communities colonizing reference and metal-contaminated trays at AR1 and AR5 became more similar over time. NMDS plots show separation of samples for the entire experiment, with MDS1 accounting primarily for separation over time and MDS2 showing sites converging over time separation between sites. Although there was no significant difference between communities colonizing metal-contaminated and reference substrate, I did observe modest separation on day 28 at AR1 (Fig. 2.4). In addition, communities colonizing AR5 showed greater separation and variability between samples on day 5 and 12. In contrast to AR5, AR1 shows not only temporal clustering, but also an increase in similarity over time. Although communities at AR5 also clustered by collection day, there were greater differences between treatments on days 5 and 12. SIMPER analysis showed less similarity between colonization trays with metal contamination (day 5 = 55.43; day 12 = 55.96), than treatments with reference substrate (day 5 = 68.43; day 12 = 70.80). These differences within treatments were not observed at AR1 or on day 28 at AR5.

NMDS plots also showed that the two dominant taxa, *Baetis* sp. and Chironomidae, were positively correlated with temporal effects (MDS1; r > 0.80). Additionally, late colonizers, such as *Rhithrogena* sp., *Drunella grandis*, and *Brachycentrus* sp., were positively correlated with community colonization over time (Fig. 2.4; Appendix 2A). Although most taxa had a positive relationship with time, taxa such as the cased caddisfly, *Micrasema bactro*, were more abundant on days 5 and 12 than on day 28.

The relative contribution of dominant taxa to separation between sites also varied among taxa. *Arctopsyche grandis* was highly correlated with community colonization at AR5 (r = 0.877; Appendix 2A), whereas other dominant taxa showed weaker responses (Simuliidae r = 0.51; *Brachycentrus* sp. r = 0.51). Although 13 taxa showed a trend towards greater colonization at AR1, most of these were relatively weak relationships. Comparisons of insect orders by site (MDS2 axis) showed that Diptera and mayflies made up most of these weaker trends at AR1, whereas stoneflies and caddisflies showed strong relationships at AR5.

Most of the species throughout the experiment had higher abundances on reference substrate compared to metal-contaminated substrate (Appendix 2B). SIMPER analysis showed that Chironomidae (Orthocladiinae and Diamesinae), *A. grandis*, and *Baetis sp*. contributed to the differences between reference and metal-contaminated substrate on day 5 at both sites.

However, on day 28 different species were responsible for observed differences between treatments at AR1 and AR5.

Discussion

My findings did not fully support the hypothesis that reference communities were more susceptible to metal-contaminated substrate than historically metal-impacted communities. Throughout this experiment, there were no statistically significant effects of metal-contamination on community composition at the reference or impaired sites. This finding was consistent with previous field and mesocosm experiments that showed no significant differences in community colonization in trays without fine sediment, although larger differences were observed than in the current study (Dabney - Chapter 1). Additionally, in the previous experiment a much larger difference was observed among early colonizers, whereas in the current experiment separation between treatments increased over time at AR1. This trend suggests that if the experiment had continued longer, I may have seen greater differences between treatments. Additionally, the stronger correlations of species to community trends at AR5 are likely due the site being dominated by caddisflies, which are relatively uncommon at AR1. The weak correlation of mayflies and dipterans to overall community response at AR1 is likely due to the slow colonization of mayflies, which has been observed in previous experiments (Dabney - Chapter 1). Additionally, based on individual taxa correlations from nMDS analysis, the benthic invertebrates that were highly correlated with community composition at AR1, were either of low abundances and not highly exclusive to the AR1. These correlations may indicate if trends of single species can be used to make inference on the overall community trends.

Usefulness of Colonization Experiments in Restoration Studies

Measuring colonization of benthic invertebrate communities allows researchers to better quantify sensitivity to metal-contaminated sediments while accounting for natural variation in habitats. In this study, I used colonization experiments to determine if benthic populations from reference sites were more sensitive to metal-contaminated sediments than those from metal-impacted sites. I observed few differences between sites, suggesting that either both communities have developed tolerance to metal-contamination or that other factors may influence the distribution of invertebrates at the reference and impaired sites.

In addition to mortality, benthic invertebrates can exhibit behavioral characteristics that allow them to either avoid metal contamination or survive in contaminated environments. Laboratory and mesocosm studies have shown behavioral responses such avoiding metal-contaminated substrate (McMurtry 1984, Clements 1999, Kashian et al. 2007, Mogren and Trumble 2010), shifts in oviposition behavior (Mogren and Trumble 2010) and reductions in both predatory (Vuori 1994) and anti-predatory (Clements et al. 1989, Clements 1999, Lefcort et al. 2000) responses. An improved understanding of behavioral responses to contaminated substrate can provide insight into the potential effects on stream invertebrates and the likelihood of restoration success. Other studies have shown that freshwater organisms can develop resistance to contaminants over time (Wentsel et al. 1978, Klerks and Weis 1987, Klerks and Levinton 1989), which may improve the likelihood of rapid recolonization of contaminated habitats. Research on both behavioral characteristics and development of metal tolerance is lacking, particularly in the context of river restoration. Future research effort on these impacts will allow scientists to predict the likelihood of restoration success in metal-contaminated systems.

Diversity and Tolerance as a Measure of Restoration Success

Assumptions of restorations success are based on ecological theory, and one important goal of river and stream restoration is to improve biodiversity. Although I assumed an increase of biodiversity would result from improvements in habitat heterogeneity and water quality, long-term studies suggest this is often not the case (Lepori et al. 2005, Palmer et al. 2010, Louhi et al. 2011, Stranko et al. 2012, Rios-Touma et al. 2015, Paillex et al. 2017). Some researchers have hypothesized that habitat improvements at local scales may not improve biodiversity at larger spatial scales because of the negative effects of surrounding land use (Walsh et al. 2004, Palmer et al. 2014, Kail et al. 2015). Other investigators have suggested changes in how to analyze restoration success and recovery potential, such as the use of species traits (Tullos et al. 2009, Menezes et al. 2010) or the use of restoration trajectories instead of simple comparisons to reference sites (Hughes et al. 2005). These different strategies for assessing biological recovery may prove to be more useful than the traditional approach used in this study for areas with a long history of contamination. Moreover, different strategies of analyzing restorations success may also help to set reasonable goals for increasing biodiversity at restored sites.

In this study, I used the concept of species tolerance to determine if a reference site community was more sensitive to metals than an impacted community. Studying community tolerance to contaminants can provide important insight into long-term recovery potential of degraded ecosystems. Although I did not observe consistent differences in colonization dynamics between treatments at each site, previous studies on the Upper Arkansas river have demonstrated that reference communities are more sensitive to metal exposure in the water column compared to those from historically contaminated sites (Clements 1999, Kashian et al. 2007). Differences between these studies suggest that macroinvertebrates from reference sites were relatively tolerant of metal-contaminated substrate, but remain sensitive to aqueous exposure.

The use of tolerance data could also be helpful in setting the goals of restoration success if researchers had a better understanding of the impact contaminants on dispersal. This may be especially difficult in watersheds with a long history of disturbance. Cadmus et al. (2016) integrated measures of dispersal (drift propensity) of aquatic insects with estimates of metal tolerance to predict restoration success in a metal-contaminated watershed. Previous investigators have suggested measuring the success of stream restoration projects by comparing disturbed communities to those at reference sites (Rohr et al. 2015). Similarly, it has also been suggested that in order for more complex methods such as PICT to be effective, there needs to be an adequate reference site for comparison (Boivin et al. 2002). However, without a good understanding of factors that influence dispersal ability, making inferences about species recovery is difficult, especially when comparing responses to reference site conditions (White and Walker 1997).

Conclusion

This study identifies an important issue regarding how to assess stream recovery at sites with a legacy of contamination. The expectations of a high diversity of sensitive EPT taxa may not always be an important restoration goal. However, achieving these goals for benthic communities may difficult to achieve without considering habitat quality and dispersal mechanisms. Studies of community tolerance and comparisons to reference sites are limited because baseline (e.g., pre-impact) community composition is often unknown. Additionally, the predictive potential of restoration success could be hampered by selecting the wrong reference site conditions. Although station AR1 has been consistently used as a reference site for comparison with sites downstream of contamination, it also has some history of contamination. White and Walker (1997) identified several limitations of using reference sites in bioassessments, two of which involve the ability to make inferences about populations: (1) the presence of taxa can suggest species tolerance to contaminants, but not the extent to which they tolerate other conditions; and (2) the absence of taxa does not tell us whether that absence is due failure of dispersal or inability to tolerate site conditions. Unfortunately, very few studies have attempted to test the usefulness of reference sites in studying stream restoration success, although this may be difficult in areas with a legacy of mining history. In this study, I compared sites separately to measure responses to a single stressor, metal-contaminated substrate. Although it has been previously reported that communities from reference sites are more sensitive to aqueous metal exposure (Clements 1999; Kashian et al. 2007), there was little evidence of differences in tolerance to metal-contaminated substrate between sites. These findings further demonstrate the need for improving understanding of ecological theory in setting priorities and goals for stream restoration. Moreover, the lack of field experiments examining tolerance and dispersal ability of aquatic insects limits the ability to measure restoration success and to test different strategies for predicting success.

Tables and Figures

Table 2.1: Results of three-way ANOVA (Treatment x Day x Site) testing for differences in univariate metrics. The table shows p-values and F-values (in parentheses). Results in bold are statistically significant ($p < 0.05$).

	df	**Community Composition**	**Total Abundance**	**H'**	**J'**	**d**	**# of Taxa**	**EPT**	**Diptera**	**Baetis**	**Rhithrogena**	**Arctopsyche**
Treatment	1	0.488 (0.94958)	**0.0243** **(5.77)**	0.4643 (0.55)	0.1268 (2.50)	0.6849 (0.17)	0.2794 (1.22)	0.1176 (2.64)	0.0899 (3.12)	0.1199 (2.60)	0.2533 (1.37)	0.7535 (0.100)
Day	2	**0.001** **(12.974)**	**<.0001** **(144.10)**	0.2073 (1.68)	0.2316 (1.56)	0.1521 (2.04)	**<.0001** **(31.4)**	**<.0001** **(76.39)**	**<.0001** **(38.00)**	**<.0001** **(48.84)**	**<.0001** **(17.92)**	**0.0164** **(4.90)**
Treatment*Day	2	0.711 (0.76309)	0.4053 (0.94)	0.9224 (0.08)	0.7125 (0.34)	0.9663 (0.03)	0.9928 (0.01)	0.2513 (1.46)	0.7466 (0.30)	0.2889 (1.31)	0.2579 (1.43)	0.3387 (1.13)
Site	1	**0.001** **(12.925)**	**0.0008** **(14.66)**	0.0518 (4.19)	0.551 (0.37)	**0.0047** **(9.74)**	**<.0001** **(23.54)**	0.501 (0.47)	**0.0003** **(18.04)**	0.651 (0.21)	**<.0001** **(57.05)**	**<.0001** **(29.63)**
Treatment*Site	1	0.873 (0.42298)	0.1921 (1.80)	0.9249 (0.01)	0.4747 (0.53)	0.2732 (1.26)	0.2139 (1.63)	0.3307 (0.99)	0.3092 (1.08)	0.3658 (0.85)	0.7184 (0.13)	0.1219 (2.57)
Day*Site	2	**0.001** **(2.6054)**	0.2864 (1.32)	0.6564 (0.43)	0.9613 (0.04)	0.0673 (3.03)	**0.007** **(6.15)**	0.5723 (0.57)	0.5208 (0.67)	0.7935 (0.23)	**<.0001** **(25.12)**	0.762 (0.27)
Treatment*Day*Site	2	0.652 (0.82783)	0.8627 (0.15)	0.659 (0.42)	0.7768 (0.26)	0.1497 (2.06)	0.2529 (1.46)	0.9504 (0.05)	0.8529 (0.16)	0.9792 (0.02)	0.7921 (0.24)	0.5084 (0.700)

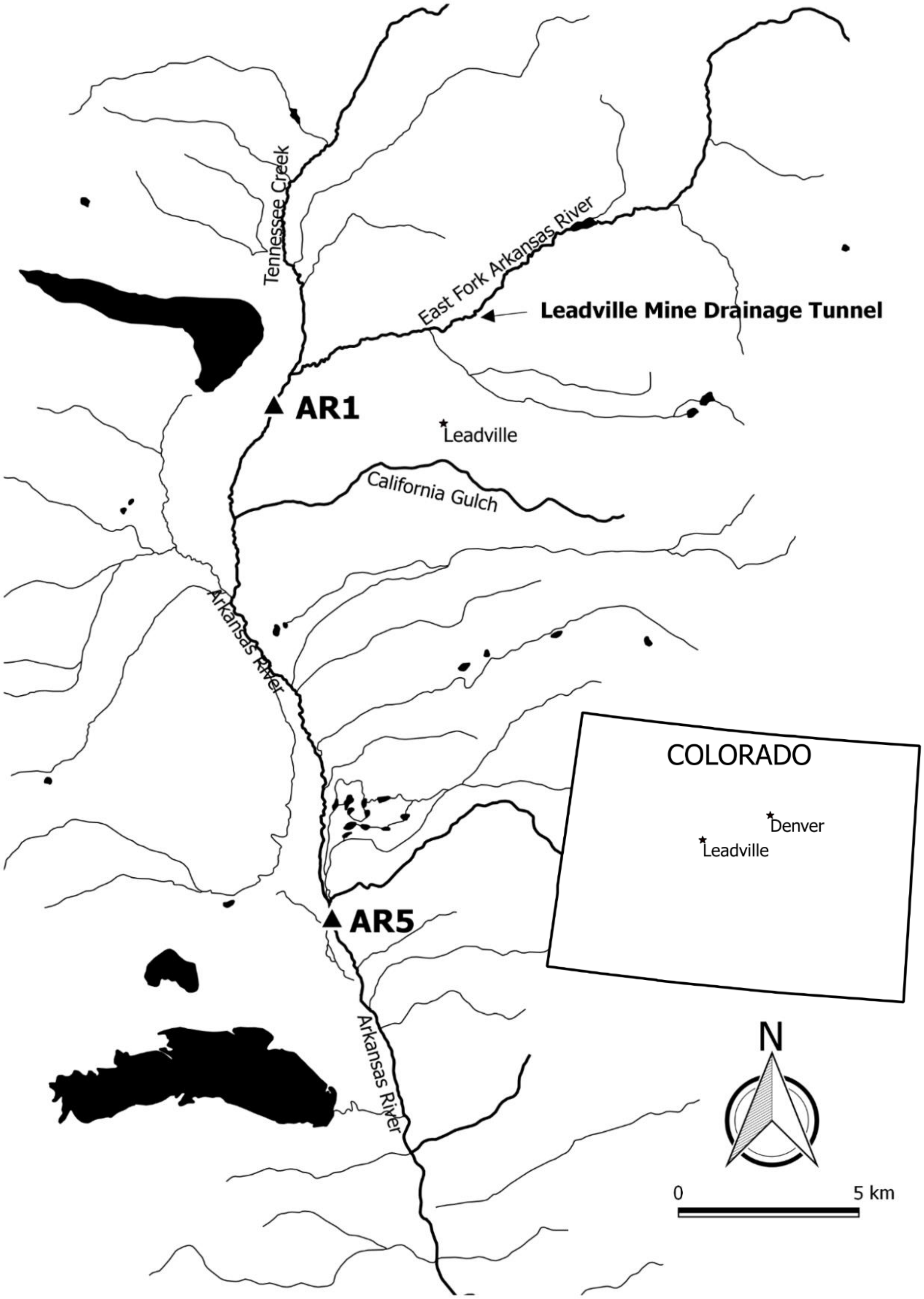


Figure 2.1: Map of the Upper Arkansas River near Leadville, Colorado, USA. Experiments were conducted at two sites (▲), AR1 (reference site) and AR5 (impaired site).

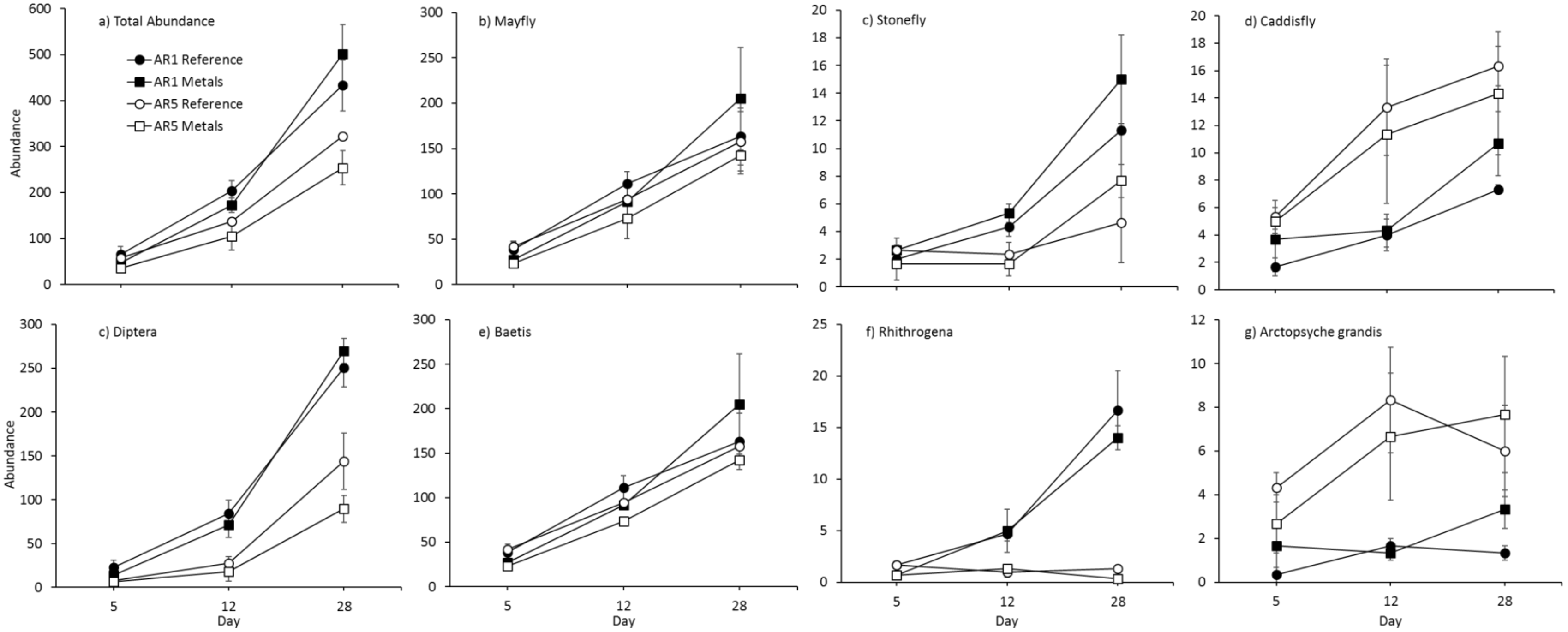


Figure 2.2: Total macroinvertebrate abundance and abundance of the 4 major orders (Ephemeroptera, Plecoptera, Trichoptera, and Diptera) that colonized clean and metal-contaminated substrate in the Arkansas River, Colorado.

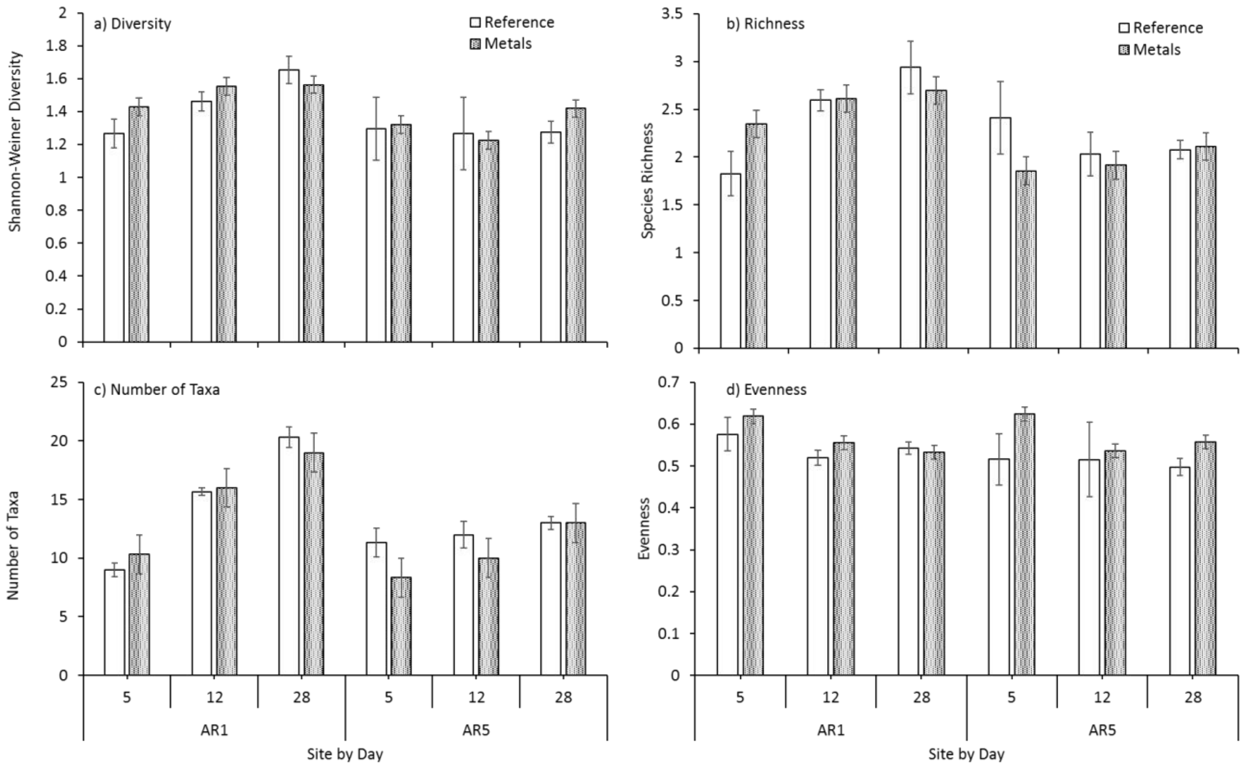


Figure 2.3: Plot of mayfly diversity based on Shannon-Wiener index (a), species richness (b), total number taxa in the communities (c), and species evenness (d). Bars based on standard error.

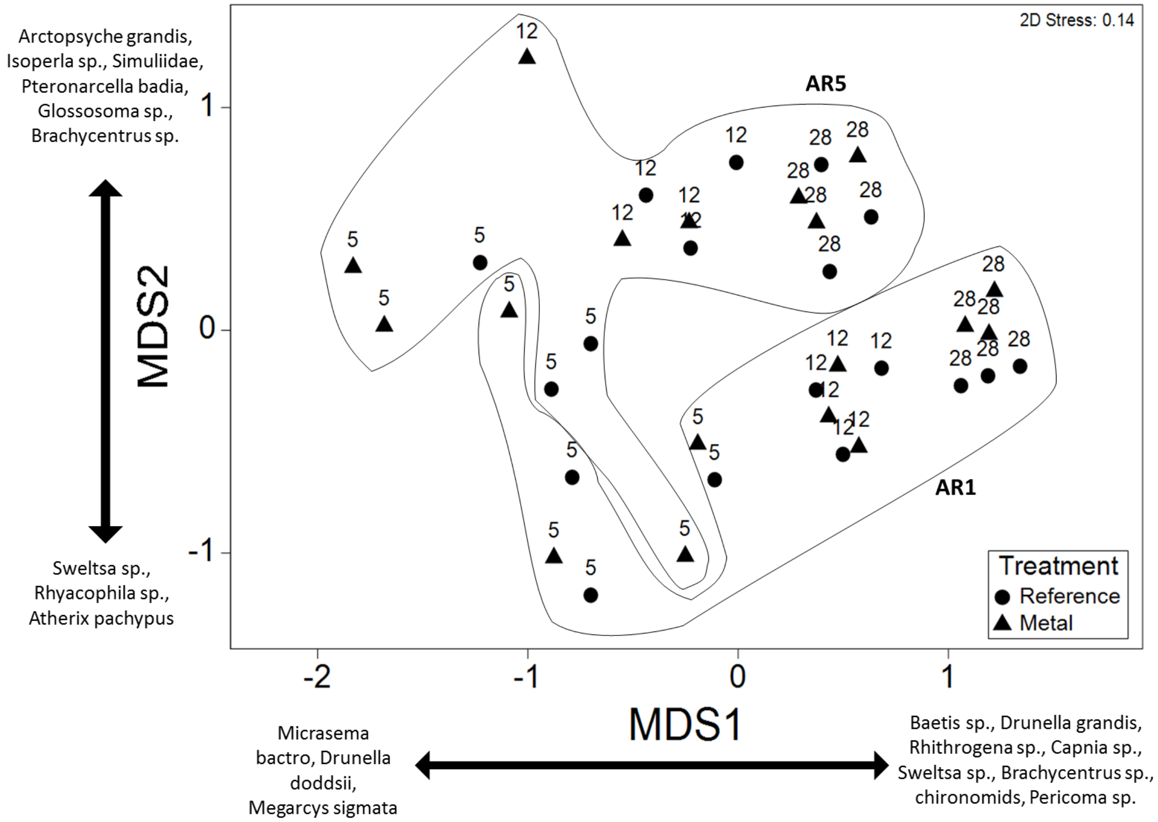


Figure 2.4: Results of non-metric dimensional scaling analyses showing separation of days (5, 12, 28), communities (sites AR1 and AR5) and treatments (metals vs. reference sediment). Taxa list show the species contributing to the differences among samples.

CHAPTER 3: THE USEFULNESS OF SPECIES TRAITS TO ASSESS BENTHIC COMMUNITY RESPONSES TO METAL CONTAMINATION AND SEDIMENT DEPOSITION: TEMPORAL AND SPATIAL APPROACHES

Introduction

Metal contamination and sediment deposition are global threats to aquatic ecosystems and human health. Metal contamination has been linked to declines in aquatic biota, particularly in areas with a long history of mining activity. Additionally, fine-sediment deposition is of increasing concern for aquatic ecosystem health as the physical effects of fine-sediment loading can lead to a decrease in water volume, habitat loss for benthic invertebrates and fish populations, and is strongly associated with contaminant loading (Waters 1995, Wood and Armitage 1997, Boulton et al. 1998, Jones et al. 2011). Both anthropogenic stressors are typically associated with mining activity through physical disturbance of land surfaces (Balamurugan 1991, Bobrovitskaya 1996, Nelson and Booth 2002, Walling and Fang 2003). Fine sediment may be of greater concern in areas where flows are reduced by human modifications such as dams and water diversions. The inputs of metal-contamination and fine-sediment deposition in mountainous regions also impact downstream water resources which have recreational, industrial, and municipal uses. Although the first step to restoration of mined watersheds is to eliminate the source of metal-contamination, metals can remain in the sediments and have significant effects on stream health. Metals have a high affinity for fine-sediment (< 63µm), and have been associated with high toxicity (Giusti 2001, Zhang et al. 2002, Campana et al. 2013). Interstitial spaces in streambeds also provide refugia and high quality habitat for benthic invertebrates to avoid metal-contamination (Lancaster and Hildrew 1993, Townsend et al. 1997b, Fuller and del Moral 2003). However, the settling of metal-contaminated fine-sediment on the streambed simultaneously results in chemical exposure to metals and physical stresses of habitat loss.

Many restoration programs at mining sites have been successful in removing metal-contamination (Clements et al. 2010, Dean et al. 2013); however, few large-scale studies conducted at restoration sites that have attempted to monitor the accumulation of fine-sediment after contaminant removal. Studies that have used experimental approaches to understand the impacts of fine sediment on recruitment of biota showed that fine-sediment infiltration occurs quickly after simulating removal (Lisle and Lewis 1992, Ramezani et al. 2014). These studies have also demonstrated that fine-sediment alone is linked with the decrease in benthic invertebrates. However, not all species recolonize at the same rate, and some species, such as Hydropsychidae and Chironomidae, are more successful at recolonizing sites recovering from metal-contamination (Cain et al. 2004, Sasaki et al. 2005). Although there is a lack of comprehensive studies that have investigated the effectiveness of fine-sediment remediation at contaminated sites, instream structures such as rock sills and digger logs used to improve fish habitat by way of sediment reductions been shown to be very short-lived solutions for decreasing sediment loading (Line et al. 2000, Kreutzweiser and Capell 2001, Thompson 2005). Since long-term solutions on how to reduce sediment loading in aquatic ecosystems are lacking, this makes it difficult to assess how to determine restoration success and predict recovery potential of sites.

Many authors have called for the inclusion of ecological theory in stream restoration projects when determining how to measure restoration success and how to remediate sites (Choi 2004, Lake et al. 2007, Palmer et al. 2010). One way to incorporate ecological theory is using species traits, and several authors have noted the potential benefits of using species traits rather than traditional taxonomic assessments for stream restoration and biomonitoring (Poff et al. 2006, Tullos et al. 2009, Statzner and Bêche 2010, Van den Brink et al. 2011, Verberk et al. 2013). These benefits include comparisons of sites with different taxonomic composition, predicting environmental stress, and providing insight into the underlying mechanisms for species occurrence (Bonada et al. 2007, Tullos et al. 2009, Berg et al. 2010, Verberk et al. 2013). One of the current challenges in stream ecology is to compare responses to stressors across large geographic regions, which is severely hampered because sites vary greatly in community composition. Moreover, specific stressors may be responsible for selection of biological traits that facilitate recolonization after remediation (Tullos et al. 2009). Understanding species trait responses can improve the ability to make inferences about species dispersal and aid in predicting effects of environmental stress (Statzner et al. 2001, Berg et al. 2010).

Currently, the drawbacks and limitations of species traits analysis are availability of trait information and consensus on how scientists analyze trait responses. For example, trait responses can be analyzed traditionally, like taxonomic data that is transformed logistically and analyzed using a linear based model. Other researchers have opted to use “fuzzy” coding and trait affinities to interpret trait data (Chevenet et al. 1994, Poff et al. 2006, Tonkin et al. 2014), and many other methods of analyzing traits exist (Verberk et al. 2013). However, it remains unclear which method should be used and whether these more recent methods are any better than traditional taxonomic assessments (Monaghan and Soares 2014). Additionally, the lack of trait information may complicate the ability to compare studies, as species may shift in trait affinities depending on life stage or may belong to more than one ecological niche. However, if there was a consensus on trait information, researchers may be able to resolve ecological problems and obtain more accurate abundance information at larger spatial scales (Dolédec et al. 2000).

In this study, I used taxonomic data obtained from field experiments at three sites in Colorado and transformed it to species traits data to determine responses to metal-contaminated and fine sediment. Two research objectives were addressed in this study about the usefulness of species traits:

1) Distinguishing between the effects of multiple stressors (metal-contaminated and fine sediment.
2) Assessing the impacts of a single stressor (metal-contaminated sediment) across spatial scales.

I hypothesized that traits within life history, mobility, morphological, and ecology groups will respond to stressors. Since there is a lack of information on which traits are most appropriate when assessing chemical and physical stressors, traits were analyzed by community composition and individually.

It remains unclear how a traits-based assessment will perform in the context of multiple stressors, particularly for applied studies with the aim of predicting responses after remediation and recovery. However, I hypothesized that fine-sediment will have a greater effect on community responses than metal-contamination. This is because fine-sediment may both be a detriment to colonization of benthic invertebrates, but also provides critical habitat for other taxa (Jones et al. 2011). While many species may be sensitive to the loss of interstitial habitat, those invertebrates that utilize patches of fine-sediment habitat may be greatly impacted by fine-sediment quality. Avoidance and re-colonization after a disturbance are important indicators of community resilience and resistance (Hershkovitz and Gasith 2013). Understanding trait dynamics is likely to provide more insight into community response than traditional taxonomic assessments.

Because few studies have attempted to use traits to quantify colonization of metal-contaminated sediments, I derived hypotheses based on preexisting knowledge of how traits respond to degraded habitats (Statzner and Bêche 2010, Van Der Linden et al. 2016). Specifically, I hypothesized that multivoltine and highly mobile invertebrates will show the greatest response to metal contamination and fine sediment deposition. Moreover, because I suspected mobility will be more related to avoidance of stressors, insects with traits related to high drift propensity, high crawl rate, and strong swimmers will be less likely to colonize patches of metal-contaminated and fine sediment habitats. Morphological traits may provide insight into potential mechanisms of sensitivity or tolerance to chemical and physical stressors; therefore, I measured the impacts on five morphological traits relating to size, shape, respiration type, body armoring, and attachment ability. Lastly, ecological traits such as habitat type and functional feeding groups are important for understanding the potential ecological consequences of anthropogenic disturbances. I hypothesize that sprawlers and clingers will be most affected by the degradation of habitat quality. In contrast, shredders, herbivores and collector-gatherers may respond negatively to metal-contamination and collector-filterers to fine-sediment deposition.

Previous experiments have shown benthic invertebrates are less likely to colonize metal-contaminated compared to clean substrates (Courtney and Clements 2002, Cadmus et al. 2016). Therefore, I expected to see similar responses when comparing trait response to a single stress (i.e. metal-contamination) at different sites. The degree to which traits will respond and whether it will be possible to identify the more useful traits is still unclear. Many studies have observed traits across broad spatial scales in connected systems (Townsend et al. 1997a, Statzner et al.

2001, Statzner and Bêche 2010); however, in this study, not all the sites are in the same catchment nor connected longitudinally.

Exploring the effects of multiple stressors and single stressors across spatial scales is crucial to understanding how scientists can improve biomonitoring and assessment of stream restoration projects. In turn, the promise of using a traits-based approach may inform expectations of recovery and understanding of disturbances post-restoration. In theory, after a natural disturbance occurs, the community may be resilient (Lake 2000); however, it is unclear in the face of anthropogenic disturbances whether communities will retain this resilience over time. For both objectives, a colonization experiment was performed to quantify colonization and succession of benthic communities on contaminated treatments. Studying colonization in the context of contaminants is a useful application for biomonitoring and aquatic restoration projects. Using this experimental technique to predict responses to contaminants that remain in the streambed after the source has been removed may provide insight into the likelihood of restoration success. Although researchers know that contaminants impose selection pressures on particular traits (Resh et al. 1994, Poff 1997), this study is attempting to understand how traits would respond to contaminated sediments after the source of contamination is removed.

Methods

This study used taxonomic data from previous studies (Chapters 1 and 2) and transformed these results to trait abundance data. Using trait classifications from Poff et. al. 2006, we treated every trait state as a variable. Each trait variable was essentially treated like species abundance data. The first site is North Fork Clear Creek (NFCC), which was declared a Superfund site by the Environmental Protection Agency in 1983.

The first colonization experiment was performed at a reference site on NFCC which is a predominantly gravel and cobble-bed stream (Dabney – Chapter 1). Two sites on the Upper Arkansas River were used for spatial comparisons to NFCC to measure the effects of only metal-contamination (Dabney – Chapter 2). The sites are characterized by gravel-cobble streambeds and fast flow, and the threat of fine-sediment loading in riffle areas are generally lacking. Although a single reference and impaired site was chosen, both sites have a history of metal contamination. AR1 has been restored since 1993, and is considered a reference because of the high diversity of metal-sensitive EPT taxa (Clements et al. 2010). In contrast, AR5 was restored more recently in 2000 and is a typical impaired site dominated by metal-tolerant species, such as the net-spinning caddisfly *Arctopsyche grandis*. NFCC and AR sites are approximately 91.6 km apart and have very different macroinvertebrate community compositions and physical characteristics. NFCC is a narrow tributary that connects to the mainstem of Clear Creek, whereas AR is a wide river and located further from Denver, Colorado, USA.

Colonization experiment

North Fork Clear Creek, Black Hawk, Colorado, USA

Metal-contaminated sediments were collected in Blackhawk, Colorado immediately downstream from the source of mining discharges (N39.79867, W105.48174) and moved 2.6 km upstream to the reference site with no history of metal contamination (Fig. 3.1). At both the reference and metal-contaminated sites, areas of sediment deposition were located and fine-sediment was collected from the stream. The composition of the fine sediment mixture was not manipulated, but included silt, sand and small gravel. Large cobble and gravel for the coarse sediment was collected randomly from the metal-contaminated and reference site. Treatments were created by first placing coarse sediment from the metal-contaminated and reference site in colonization trays (described in Chapter 1). Trays were then left without fine sediment, or filled with fine-sediment from the reference or metal-contaminated site. The experiment used six treatments in a fully factorial to discern between the impacts of metal-contamination and sediment deposition. Each treatment had three replicates and, to account for variability within the stream, two trays were combined for each replicate. A total of 144 colonization trays (25 x 25 x 10-cm) with 1.25 cm diameter holes were attached to racks and then placed in the stream (Clements et al. 1988). Trays (36 per day, 6 per treatment) were collected on days 5, 10, 20, and 30. Two trays for each sample were combined in a bucket and large substrate was scrubbed to remove insects. The remaining contents of the trays were transferred to a container and immediately preserved in 80% ethanol for further processing. Additionally, on each collection day three benthic samples were collected using a 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 benthic invertebrates were picked from colonization tray and Hess samples using a dissecting microscope. All insects were identified to the genus level except chironomids and early instars, which were identified to order or family.

Sediment and organic matter were measured in every sample to account for material entering and leaving the trays throughout the experiment. Benthic invertebrates were removed from the whole sample and the remaining tray contents were sieved though a 2360 µm, 355 µm, and 63 µm sieves. Sediment captured in the 355 µm and 63 µm was dried at 65°C and combusted at 550°C for 3hrs to obtain organic matter and raw sediment content. Additional trays were placed in the stream and collected on days 15 and 30 to measure metal concentrations during the experiment. Substrate in trays was digested using modified EPA 3050b method (U.S. EPA 1996) at Colorado School of Mines, Golden, Colorado, USA. Sediment in the trays were weighed, digested in HNO_3 and analyzed using ICP-MS. Detailed results of sediment and organic matter content, and metal concentrations were reported in chapter one.

Upper Arkansas River, Leadville, Colorado, USA

A colonization experiment was conducted in October 2014 to observe the effects of metal-contaminated substrate on benthic invertebrate communities at AR1 and AR5 sites. Metal-contaminated coarse substrate (cobble and gravel > 2360 µm) from a reference-site and metal-contaminated site on North Fork Clear Creek in Blackhawk, Colorado, USA was transported to two sites on the Arkansas River (AR1 – reference-site and AR5 – impaired-site). Based on previous data using sediment from North Fork Clear Creek, I expected the metal-concentrations to remain significantly elevated in the metal-contaminated substrate (Dabney – Chapter 1).

Treatments were place in colonization trays and collected on days 5, 12, and 28 (Clements et al. 1988). This experiment has four treatments in a fully factorial to discern the impacts of metal-contaminated and reference sediment at the reference and impaired sites. Each treatment had three replicates and to account for variability within the stream and two trays were combined for each replicate. Both benthic and tray samples were rinsed through a 355um sieve and persevered in 80% ethanol. Samples were then transported back to the lab where the entire sample sorted and identified using a dissecting microscope. Insects in all samples were identified down to genus or family.

Statistical Analysis

Traits abundances are used to provide clear distinction between tolerant and intolerant traits without making assumptions about trait importance. For comparisons of multiple stressors at NFCC, all univariate and multivariate analyses were performed on log-transformed data.

Univariate analysis was performed on Shannon-diversity, total abundance and abundance of Ephemeroptera, Plecoptera, and Trichoptera (EPT) metrics using the PROC GLM procedure with statistical package, SAS v9.4 (SAS Inc., Cary, North Carolina, U.S.A.). Multivariate analysis was used to test changes in community structure using PRIMER-e v7 (Quest Research Limited; Cambridge, UK) with the +PERMANOVA package (Anderson 2001). Major trends in the data were then visually represented using nonmetric-multidimensional scaling (NMDS) plots. A Bray-Curtis similarity matrix was created using log-transformed community data. A three-way permutational multivariate analysis of variance (PERMANOVA) was run at 999 permutations under the reduced model using a Bray-Curtis resemblance matrix (dummy variable = 1). Pairwise-comparisons for each were performed to examine more complex trends in the data. Additionally, SIMPER analysis was performed on log-transformed data to determine the invertebrates that account for 50% of the differences between response variables (Clarke and Warwick 2001). SIMPER analysis was also used to determine the taxa most responsible for the dissimilarity between treatments. Significance for all tests was determined based on a $p < 0.05$ with Monte Carlo p-values being used for tests with 10 permutations (Anderson 2005).

For assessing the effectiveness of species traits across spatial scales, I tested effect of treatments (Reference vs Metals), sites (NFCC, AR1, and AR5) and day ($5_{AR/NFCC}$, $12_{AR}/10_{NFCC}$, and $28_{AR}/30_{NFCC}$; Fig. 3.1a). Trait and taxonomic data was analyzed similar to the multiple stressor study except data was converted to proportions and arcsine square root transformed instead of log-transformed for site comparisons.

Results

Multiple Stressors: Metal-Contamination and Sediment Deposition

Previously, when comparing the effects of metal-contamination and fine-sediment deposition on overall community taxonomic composition, I observed a significant effect of both metal-contamination (F = 9.8394; p = 0.001) and fine-sediment deposition (F = 2.2031; p = 0.009). However, pairwise comparisons showed that the effects of fine-sediment deposition were greater at the end of the experiment (Dabney – Chapter 1). I observed a slightly different response based on community trait composition, with the main effects of metal-contamination being highly significant (F = 13.806; p = 0.001) and fine-sediment deposition as marginally significant (F = 2.1676; p = 0.05; Table 3.1). The pairwise comparisons showed that by the end of the experiment, community trait responses were not affected by metal-contamination (F = 0.75586; p = 0.607), but significantly affected by fine-sediment, particularly metal-contaminated fines (F = 2.9985; p = 0.013; Appendix 3A). Further, on day 30, community response based on taxonomic description was best able to distinguish between reference and contaminated fines (Dabney – Chapter 1), whereas no difference was observed by traits. Although, taxonomic response was significant across all levels of fine-sediment pairwise comparisons, traits had a significantly larger response to metal-fines (MF) compared to the community colonizing the trays with no-fines (NF; F = 2.9985; p = 0.013).

Non-metric dimensional scaling (NMDS) plots showing community traits over time decreased in separation based on metal-contamination. On day 5 separation between treatments was largely due to the differences on trays with metal-contaminated coarse and fine-sediment (MC + MF). As the experiment progressed, observed differences in colonization were largely due to the presence of fine-sediment (Fig. 3.2). Furthermore, SIMPER analysis showed a shift in traits contributing to differences between treatments over time. On day 5, when metal-contamination had the greatest effect, species that were strong swimmers, have a streamlined body shape, were abundant in drift, and multivoltine were more abundant on reference coarse trays (Table 3.2); these same trends in trait colonization were consistent in NF and MF treatments. By day 30, I saw similar trait contributions with the increased influence of the ecological traits collector-filterers, shredders, and predators contributing to the small difference between reference and metal-coarse trays. Moreover, fewer taxa contributed to these differences on day 30 compare to day 5, with less influence of dominant taxa.

A second important objective of my study was to identify which specific traits were most closely associated with anthropogenic stress. I analyzed each trait separately to determine which traits were significantly affected by fine-sediment and metal-contamination (Appendix 3B), as well as which traits were strongly associated with sediment and organic matter content. Appendix 3C shows the output from DISTLM analysis using the BEST procedure, using AICc to select the best predictor variables and associated r^2 over time for all traits. Generally, trait responses had a higher correlation to sediment trends compared to taxonomic responses; however, the best predictor variables were the same. As the experiment progressed, sediment 355um and organic matter 63um were the best predictors of community traits.

Many specific traits showed a significant response to metal-contamination, and differences between treatments were most obvious when comparing the additional stressor of fine-sediment deposition (Appendix 3B). Voltinism, swimming ability, respiration, body shape, and trophic level were generally the most prevalent and consistently important traits throughout the multiple stressors study. Both semivoltine and univoltine invertebrates were significantly reduced by fine-sediment deposition ($p < 0.01$; Fig. 3.3), with fewer invertebrates colonizing the treatments with both metal-contaminated coarse and fine sediments (MC + MF) trays. Although fewer benthic invertebrates colonized MF treatments for all swimming ability traits, there was only a significant effect of fine-sediment on invertebrates with weak swimming abilities ($p < 0.0001$; Fig. 3.3). Morphological traits were important for distinguishing between the physical effects of fine-sediment. I found that insects with gills and a non-streamline body shape (i.e. round) were most likely to avoid patches of fine-sediment, particularly those with metal-fines ($p < 0.01$; Fig. 3.3).

I hypothesized that ecological traits may provide insight into the effects of metal-contamination and sediment deposition on community structure. I found that all trophic levels, except collector-filterers, were significantly impacted by both metal-contamination and sediment deposition, whereas collector-filterers were only affected by metal-contamination (Fig. 3.4). Collector-gatherers comprised much of the community and were generally the first colonizers; however, they were more impacted by the effects of metal-contamination until day 30. Collector-filterers were also early colonizers, but I observed a sharp decline by the end of the experiment, which coincided with the increase of predators, herbivores, and shredders in treatments without fine sediment.

Spatial Comparisons of Metal-Contamination

All sites (AR1, AR5, and NFCC) were used for spatial comparisons, but only reference and metal coarse treatments without fine-sediment were compared. I observed larger differences between sites than between treatments. However, when I evaluated trait composition, separation between sites increased over time (Fig. 3.6). Significant differences were observed between day and sites ($p = 0.001$), but not when considering the main effect of metals ($F = 0.90215$; $p =$

0.466; Table 3.3). The differences between sites were dependent on day; however, this was only due to sites AR1 and AR5 being significantly different on day 30 ($p < 0.05$).

I wanted to determine how effective traits were for spatial analysis compared to taxonomic descriptions. I used SIMPER analysis to identify the traits and taxa on days 5 and 30 that contributed to the differences between reference and metal contaminated sediment among all sites. These traits were also present as every site. Table 3.4 shows the correlations between traits and select taxa chosen based on their occurrence across all sites. I compared trait responses with those of several dominant taxa (*Baetis* sp., Orthocladiinae, Diamesinae, and Simuliidae, groups which were shown to vary significantly during my experiments (Chapter 1 and 2). I also included responses of a known metal-tolerant species (*A. grandis*) and metal-sensitive taxa (*Rhithrogena sp*.) present at all sites. Mobility and respiration traits consistently contributed to the differences between treatments over time; however, the correlation between species and respiration traits was reduced for most taxa over time. Mayflies (*Baetis sp*. and *Rhithrogena sp*.) and Simuliidae were less likely to explain the trends in respiration trait occurrence over time compared to *A. grandis* and chironomids (Orthocladiinae and Diamesinae). Many traits could be explained by abundance trends of taxa. For example, Baetis sp. correlated highly within with their trait category (VOLT3 and SWIM3); however, by the end of the experiment, *Baetis* sp. had little influence on drift traits (Table 3.4). When further separating the taxa and trait correlations by treatments, I found that by the end of the experiment relationships between all taxa except Simuliidae remained similar for most traits. Simuliidae in reference trays were highly correlated with traits; however, those in metal contaminated trays, particularly the mobility traits, showed a near random trend.

To further understand the role of dominant taxa in driving trends in trait abundances, I observed how relationships between voltinism and swimming ability. These traits were chosen because on day 30 they had high correlations with the most dominant taxa in the community, *Baetis* sp. (Table 3.4) and I also hypothesized that these traits have an important role in dispersal post-disturbance. Pearson correlations showed a negative relationship between all taxa; however, correlations were reduced when comparing Orthocladiinae and Simuliidae (Fig. 3.6). Relationships between swimming ability and voltinism traits had a strong negative relationship within each trait category; however, when swimming ability and voltinism traits were compared, I observed different responses. The trend between univoltine (VOLT2) and strong swimming (SWIM3) invertebrates (R^2 = - 0.416) followed closely that of *Baetis* sp. and Orthocladiinae (R^2 = -0.430). Alternatively, when observing the relationship between univoltine and non-swimming invertebrates (SWIM1), there was a positive relationship (R^2 = 0.477). It is worth noting this trend was also observed when I compared strong swimmers and multivoltine insects (R^2 = 0.527).

I further examined trait proportions over time among traits that have been noted in the literature as possibly important traits when observing the effects of metal contamination (Fig. 3.7). As noted previously, although trait abundances and composition were most different at NFCC, I observed little change over time in proportion of univoltine and multivoltine invertebrates in metal-contaminated treatments. I also observed fewer streamlined invertebrates on days 5 and 10 in metal-contaminated treatments. Sites AR1 and AR5 had similar trends over time; however, I only observed slight differences of trait proportions between sites and treatments.

When studying the effects of metal-contamination on habitat and trophic traits, proportional differences were most apparent when observing insect habitat preferences. Changes in sprawlers and clingers were characterized as the traits most responsive (Fig. 3.8). AR1 had a consistent proportion of clingers in reference trays; however, there was a decrease in metal-contaminated trays that coincided with the slight increase in swimmers. Sprawlers at AR5 made up a smaller proportion of the community in metal-contaminated treatments, which also allowed clingers and swimmers to make up a greater portion of the community, particularly on day 30. Generalists and collector-gatherers (CG) were the most dominant traits at AR1 and AR5; however, this was only observed at NFCC on day 30. This reduction of CF at NFCC has been attributed to the emergence of Simuliidae towards the end of the experiment (Dabney – Chapter 1). Unlike habitat preferences, I did not observe any notable changes between treatments at any of the sites (Fig. 3.8).

Discussion

This study shows that the application of trait assessments can be useful in applied ecotoxicological studies. As previously discussed in chapters 1 and 2, my initial motivations for the research were to predict community response and recovery at North Fork Clear Creek and to determine benthic invertebrate tolerance to metal-contaminated sediment at Arkansas River sites previously impacted by metal-contamination. After transforming the taxonomic data to trait data, I obtained more information about species occurrences and how best to approach these research questions. This study demonstrated that the use of species traits can be beneficial to determining the impacts of multiple stressors on benthic invertebrate communities and detecting effects of a physical stressor (e.g., sediment deposition). However, it is still unclear if traits are more effective when assessing the impacts of a single chemical stressor, especially at varying spatial scales. Although, the applicability of traits may be limited as researches have used different statistical techniques and there is a lack of trait information available, I believe a trait based approach should be incorporated in community analysis and applied ecotoxicological studies.

Influence of Multiple Stressors on Community Traits

Traits were useful in characterizing the impacts of multiple stressors at a single site (NFCC). I was mainly interested in predicting community responses to post-restoration of a contaminated site; however, traits may help us understand the mechanisms that allow certain species to re-colonize disturbed sites. This may provide promise for the development of descriptive and applied studies. When comparing taxonomic and trait data, I showed that the effects of fine sediment were greater over time. However, trait data had a higher correlation with environmental the stress of fine sediment grain size. Although individual traits supported my conclusions, testing responses on individual dominant taxa only obscured my ability to draw conclusions about the impacts of fine-sediment.

The impact of fine sediments (<2mm) on benthic invertebrates has been widely studied and linked to invertebrate habitat preferences (Lamouroux et al. 2004, Rabení et al. 2005, Townsend et al. 2008, Buendia et al. 2013). Yet, inference on species occurrence in fine sediments cannot be made solely based on habitat preferences and may not provide as much information on species occurrence as mobility, and morphological traits. Also, the correlations with environment data did not mean that each trait was significantly impacted by fine-sediment deposition. For example, invertebrates that were common or abundant in the drift were correlated with grain size (r2 > 0.40); however, only those insects that are abundant in drift were significantly impacted by fine-sediment deposition. Invertebrates characterized as swimmers were not significantly impacted by fine-sediment and were also comprised of invertebrates that are common in drift samples. Fine sediment contributes to streambed instability and causes species to become more mobile (Gibbins et al. 2010); however, the morphological traits have been shown to be affected by metal-contamination (Tullos et al. 2009). This is further support for the idea that traits respond to environmental stress in combination, rather than one trait driving invertebrate response (Verberk et al. 2013, Boersma et al. 2016).

In my multiple stressor study, analysis of dominant taxa response appeared to be less helpful in predicting community response. The use of dominant taxa in ecotoxicology is common in bioassessments and is an attempt to increase the ecological relevancy of studies. A large focus on dominant taxa may not be the best approach as population studies do not account for species interactions and context-dependent responses (Clements et al. 2016). For example, the dominant taxa in this community, *Baetis* sp., were not affected by fine-sediment deposition. However, trait groups which included *Baetis* sp., such as collector-gatherer, gill respiration, and abundance in drift were significantly impacted by fine-sediment. In contrast, other traits belonging to *Baetis* sp. (e.g., life history traits, streamlined shaped and strong swimming ability) were not significantly affected. This was further verified by the lack of importance of indicator traits belonging to *Baetis* sp. and other dominant taxa as the experiment progressed.

Typically, scientists assume populations respond to multiple stressors simultaneously and that combined stressors impose selection pressure on communities (Vinebrooke et al. 2004, Townsend et al. 2008). Previous studies have suggested that anthropogenic stressors have different modes of action that influences sensitivity to contaminants, thus resulting in additive effects (Wagenhoff et al. 2011, 2013, Schmitt-Jansen et al. 2016). My study indicated that the effects of metals and fine-sediment were independent for trait composition and individual traits, suggesting that the stressors simply have an additive effect. In contrast, the effect of metal-contamination was dependent on time. Therefore, the impact of metal-contamination in coarse substrates cannot be used to assume that fine-sediment will have the same impact on benthic invertebrates. Fine sediment was not dependent on time during my experiment and I believe the physical effect of fine sediment deposition that results in habitat loss has the greatest impact on community colonization. As others have reported (Townsend et al. 1997a, Tullos et al. 2009), highly mobile, small, and generalist traits were most likely to be found in metal-contaminated and/or fine-sediment treatments.

Spatial Comparisons of Traits to Metal Contamination

Previously, I determined that fine-sediment deposition has the greatest impact on benthic communities; however, at some sites impacted by mining activity, sediment deposition is not a major stressor. In this study, I examined the impacts of a single-stressor (metal-contaminated coarse sediment) across three sites. Compared to trait composition all three sites had different taxonomic composition largely due to the differences in abundances of Plecoptera and Trichoptera (Table 3.5). These data suggest that traits reduced the complexity involved in comparing sites with very different species composition. Although I could identify traits that contribute most to the differences between treatments, the overall benefits of using traits for single-stressor studies is uncertain.

While several authors have used traits to compare sites (Statzner et al. 1997, 2012, Dolédec et al. 1999, Mokany and Roxburgh 2010, Statzner and Bêche 2010), very few studies have attempted to use a field experiment to determine sensitivity to contaminated sediments. The distribution of traits among sites may be linked to similarities of land use disturbances in the watershed (Sponseller et al. 2001, Larsen and Ormerod 2010) and abundance of dominance taxa

(Verberk et al. 2010). In my study, trends in trait composition also appeared to follow that of overall site quality. Ideally, the most productive sites would have a broad diversity of traits (e.g., for fewer generalists and more specialists). Based on contamination history I would expect that NFCC would be the highest quality site and AR5 as would be the most disturbed. As predicted, univoltine invertebrates decreased with site quality; however, these trends in species traits were less obvious when comparing ecological traits (habitat and feeding preference).

One factor that could be contributing to the ability to make inference about species traits across larger spatial scales is that previously disturbed sites are already occupied by species that could become established at contaminated sites. Although AR1 is considered a reference site due to high diversity, there is little known about ecosystem function at this site. In my traits analysis, the dominance of generalist, highly mobile taxa, and those occupying swim habitats at AR1 suggests that taxonomic descriptions may be misleading at sites with a complicated disturbance history. Disturbance history is a key factor when identifying a reference site, particularly for spatial studies (White and Walker 1997). As it is still unclear how to should determine the success of restoration projects, using a traits approach along with other concepts in ecological theory may provide a key link for determining how to improve restoration success (Choi 2004, Lake et al. 2007, Palmer et al. 2010).

Analysis and Interpretation of Traits Interactions

Many methods have been used to analyze to community traits, such as fuzzy coding (Chevenet et al. 1994, Menezes et al. 2010, Verberk et al. 2013); however, these approaches may fail to link environmental conditions to traits (Verberk et al. 2013). In the current study, I analyzed colonization of all traits together and individually to facilitate better comparison to species data. Using this approach, I was able to quantify individual responses of traits to specific stressors and understand which traits may be most closely related to stressor gradients. The idea behind my approach is that species and traits are thought to co-evolve in response to environmental gradients. Therefore, only analyzing individual responses of traits without consideration of overall trait composition is unlikely to be helpful in trait-based ecological assessments (Townsend and Hildrew 1994). Moreover, the improvement of trait-based approaches depends on the understanding of which traits are most important. To further this understanding scientists should determine which combination of traits interact, which should be a priority in risk assessments (Berg et al. 2010, Verberk et al. 2013).

Selection of traits may depend on scientists understanding of trait combinations that give insight into population resistance and resilience. For example, drift behavior may increase population resilience to drought; however, it also increases likelihood of predation and relies on habitat connectivity and flow (Robson et al. 2011). In this study, I identified traits that responded to the individual and combined effects of fine-sediment and metal-contamination. Although most traits were significantly affected by metal-contamination, assessing the effects of fine-sediment was more useful for making inferences on which traits should be observed.

Measuring trait responses was not as useful in studying effects of metals alone, at least not when using the same method of trait selection as in the multiple-stressor study. Whether I would observe a similar response if insects were exposed to other chemical contaminants is unclear. Unfortunately, few comparable studies exist to help make inferences on the impacts of chemical stressors on trait responses. Since traits are thought to interact, chemical stressors may cause selection pressures to the point where all traits are impacted simultaneously. Thus, a trait-based approach may be less helpful when considering a single chemical stressor at individual sites.

Use of Traits and Temporal Analysis in Ecotoxicological Assessments

The use of trait based approaches in environmental toxicology is a relatively new approach. Most toxicology studies are still performed on single species, and taxa are usually selected based on the ability to culture a species rather than ecological relevancy. Also, the assignment of traits to species is not well developed, particularly for invertebrates at early life stages. However, the use of the traits in toxicity and applied studies has been mentioned by several authors as a promising tool for bioassessments (Baird et al. 2008, Violle and Jiang 2009, Rubach et al. 2011). To improve the relevancy of these studies, scientists have tried to create ways of selecting test species based on traits that are related to modes of action (Usseglio-Polatera et al. 2000, Ducrot et al. 2005, Baird and Van den Brink 2007, Beketov et al. 2013b). However, these methods are disadvantaged because of the lack of trait data and therefore a robust research effort to derive a priori hypotheses about the impacts of various stressors on traits is required.

Determining how to quantify spatial and temporal variation among traits is widely studied; however, it is still uncertain how to apply this in ecotoxicological studies. One of the drawbacks of community data is that the high natural variation may make it difficult to detect responses to stream restoration (Brooks et al. 2002, Heino et al. 2002, Beketov et al. 2013a, Fischer et al. 2013). High variability is also an issue in studies of multiple stressors (Downes 2010). In my study, temporal trends had a significant role in interpreting trait response for both the multiple stressor and spatial analysis. Although my study was for 30 days, previous studies have found that large shifts in trait abundances can be observed for longer periods (Griswold et al. 2008, Walters 2011, Veríssimo et al. 2012) and temporal analysis of functional traits has been suggested as an ecological indicator (Hewitt et al. 2014).

Another area that needs additional research is the impact of stressors on trait combinations and how these combinations of traits may change temporally and spatially. I observed that abundances of one dominant taxa cannot fully explain abundances of traits. However, species may be dominant based on the number of opportunistic traits they possess. For example, *Ameletus sp*. and *Baetis* sp. have many traits in common, but being highly abundant in the drift may allow *Baetis* sp. to dominate a shared niche. Even though traits may help us understand species occurrence in nature, researchers still lack fundamental knowledge on phylogenetic relationships and how to determine species tolerance (Poff et al. 2006, Baird and Van den Brink 2007). Additionally, how to decide which traits are important when predicting responses to contaminants may be one the more important questions when determining how to use traits in biomonitoring. For example, it is unclear if insignificant responses of strong swimmers are due to their avoidance of fine-sediments or simply because it is more difficult to predict responses of highly mobile taxa in the field. The insignificance of the non-swimmer trait could simply be due to the difficulty of reaching fine-sediment patches during the experiment and not due to intolerance of fines.

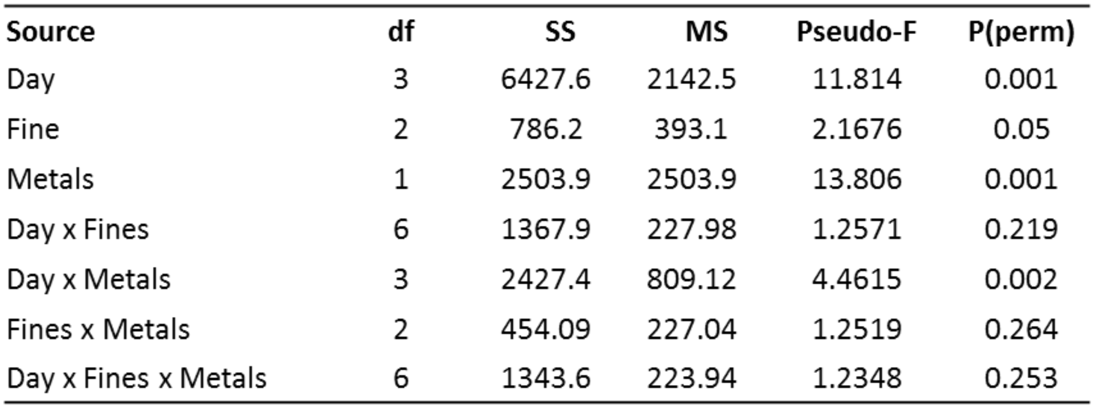
Conclusion

Using a trait-based approach can be useful for investigating effects of multiple stressors; however, it is still unclear how best to analyze species traits across broad spatial studies. I have demonstrated that although traits were helpful in understanding the impacts of multiple stressors, different stressors may have different modes of action that determine how they affect benthic invertebrates. For the spatial analysis, I learned that while there was a small difference in response to metal contamination, I was able to use an approach to understand the influence of dominant taxa. Dominant taxa were not highly correlated with all traits groups that were comprised of a large number of less dominant taxa, suggesting a need to better understand the influence that traits have on driving population dynamics in the field. Although there fewer rare species in the community, together those species can be driving most of the trends observed, further supporting the notion that biomonitoring studies should incorporate more community analysis.

In applied studies, it would be useful to include more field experimental studies for the determination of causal relationships between stressors and community response. The similarity in trait outcomes of my field experiment to other observational studies demonstrates that not only can field experiments be used to understand trait responses, they can also help answer specific questions and determine causal relationships. Moreover, the incorporation of experiments that account for changes over time and include multiple stressors can help predict the success of restoration projects. Thus, a traits-based approach provides much promise for the field of applied ecotoxicology and warrants further study with the use of experimental approaches.

Tables and Figures

Table 3.1: Three way PERMANOVA output for the multiple stressor experiment to test the effect of metal-contamination and fine sediment deposition on species trait colonization at NFCC over time. P < 0.05 represents significance. MS = means square, SS = sums of squares, and df = degrees of freedom.



Source	df	SS	MS	Pseudo-F	P(perm)
Day	3	6427.6	2142.5	11.814	0.001
Fine	2	786.2	393.1	2.1676	0.05
Metals	1	2503.9	2503.9	13.806	0.001
Day x Fines	6	1367.9	227.98	1.2571	0.219
Day x Metals	3	2427.4	809.12	4.4615	0.002
Fines x Metals	2	454.09	227.04	1.2519	0.264
Day x Fines x Metals	6	1343.6	223.94	1.2348	0.253

Table 3.2: Outcome of SIMPER analysis on days 5 and 30 in the multiple-stressor study between reference and metal coarse substrate. Additional information is given on the number of taxa belonging to each trait and the main dominant taxa previously discussed in Chapter 1. Av. Abund = Average Abundance on log scale. Trait assignments are based on Poff et al. 2006.

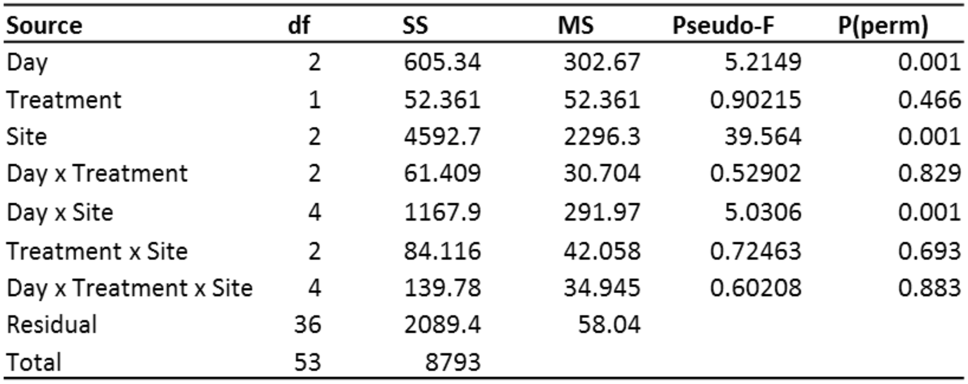
Day 5
Groups Reference & Metal
Average dissimilarity = 34.33

Trait Category	Trait	Group Reference Av.Abund (Log)	Group Metal Av.Abund (Log)	Number of Taxa	Dominant Taxa
Morphology	Streamlined Shaped	3.68	1.45	8	*Baetis sp.*
Mobility	Strong Swimming Ability	3.63	1.42	3	*Baetis sp.*
Ecology	Swim Habitat	3.63	1.43	3	*Baetis sp.*
Mobility	Abundant in Drift	4.63	2.44	3	*Baetis sp., Orthocladiinae*
Life History	Bi- or multivoltine	4.68	3.05	3	*Baetis sp., Simuliidae sp.*
Morphology	No Armoring	5.43	3.44	25	*Baetis sp., Taenionema pallidum, Orthocladiinae, Simuliidae sp.*
Life History	Fast Seasonal Development	5.34	3.41	15	*Baetis sp., Taenionema pallidum, Orthocladiinae, Simuliidae sp.*
Mobility	Very Low Crawl Rate	4.64	2.54	10	*Baetis sp., Orthocladiinae*
Morphology	No Attachment Ability	4.93	2.84	28	*Baetis sp., Taenionema pallidum, Orthocladiinae*
Morphology	Gills	4.69	2.62	20	*Baetis sp., Orthocladiinae*
Ecology	Collector-filterers	4.23	2.91	3	*Simuliidae sp.*
Ecology	Collector-gatherers	4.75	2.71	12	*Baetis sp., Orthocladiinae*
Morphology	Some Attachment Ability	4.34	2.95	3	*Simuliidae sp.*
Morphology	Small	5.35	3.53	13	*Baetis sp., Orthocladiinae, Simuliidae sp.*
Mobility	Weak Swimming Ability	3.41	1.58	15	*Taenionema pallidum, Rhyacophila sp.*

Day 30
Groups Reference & Metal
Average dissimilarity = 7.75

Trait Category	Trait	Group Reference Av.Abund (Log)	Group Metal Av.Abund (Log)	Number of Taxa	Dominant Taxa
Ecology	Collector-filterers	2.62	2.58	3	*Simuliidae sp.*
Ecology	Shredder	3.02	3.46	5	N/A
Ecology	Herbivore	3.77	3.8	4	*Taenionema pallidum*
Life History	Nonseasonal Development	2.76	2.1	1	N/A
Ecology	Swim Habitat	4.85	5.19	3	*Baetis sp.*
Mobility	Strong Swimming Ability	4.85	5.2	3	*Baetis sp.*
Morphology	Streamlined Shaped	4.88	5.26	8	*Baetis sp.*
Morphology	Some Attachment Ability	3.5	3.51	3	*Rhyacophila sp., Simuliidae sp.*
Ecology	Climb Habitat	0.62	0.53	1	N/A
Life History	Bi- or multivoltine	4.99	5.25	3	*Baetis sp., Simuliidae sp.*
Morphology	Both Attachment Abilities	0.7	0.93	2	N/A
Morphology	Good Armoring	0.7	0.93	2	N/A
Morphology	Gills	5.29	5.62	20	*Baetis sp., Orthocladiinae*
Mobility	Abundant in Drift	5.17	5.45	3	*Baetis sp., Orthocladiinae*
Mobility	Very Low Crawl Rate	5.29	5.56	10	*Baetis sp., Orthocladiinae*
Mobility	High Crawl Rate	3.01	3.12	2	*Rhyacophila sp.*

Table 3.3: Results of PERMANOVA tests comparing trait composition over time to the effects of metal-contamination at sites (NFCC, AR1 and AR5) over time. p-values <0.05 represent comparisons that were significant.



Source	df	SS	MS	Pseudo-F	P(perm)
Day	2	605.34	302.67	5.2149	0.001
Treatment	1	52.361	52.361	0.90215	0.466
Site	2	4592.7	2296.3	39.564	0.001
Day x Treatment	2	61.409	30.704	0.52902	0.829
Day x Site	4	1167.9	291.97	5.0306	0.001
Treatment x Site	2	84.116	42.058	0.72463	0.693
Day x Treatment x Site	4	139.78	34.945	0.60208	0.883
Residual	36	2089.4	58.04		
Total	53	8793			

Table 3.4: Correlation (r^2) values for the proportions of select taxa to the traits that contributed to the difference between treatments on days 5 and 30. VOLT2 = semivoltine, VOLT3 = multivoltine, DRFT2 = common in drift, DRFT3 = abundant in drift, CRWL1 = very low crawl rate, CRWL2 = low crawl rate, SWIM1 = no swimming ability, SWIM2 = weak swimming ability, SWIM3 = strong swimming ability, SHPE1 = streamlined shape, SHPE2 = non-streamlined shape, RESP1 = tegument respiration, RESP2 = gill respiration, HABI5 = swim habitat, TROP1 = collector-gatherers. Dashed line means the trait did not contribute to the top 50% of the difference between treatments on that day. Trait assignments are based on Poff et al. (2006).

	Baetis sp.		*Rhithrogena sp.*		*Arctopsyche grandis*		Simuliidae		Orthocladiinae		Diamesinae	
Trait	**Day 5**	**Day 30**	**Day 5**	**Day 30**	**Day 5**	**Day 30**	**Day 5**	**Day 30**	**Day 5**	**Day 30**	**Day 5**	**Day 30**
VOLT2	-	-0.770	-	0.407	-	-0.041	-	-0.614	-	0.666	-	0.943
VOLT3	-	0.920	-	-0.557	-	0.248	-	0.645	-	-0.464	-	-0.901
DRFT2	-0.854	-0.031	-0.421	0.286	-0.461	-0.557	0.936	0.292	0.127	-0.831	0.010	-0.561
DRFT3	0.749	-0.021	0.448	-0.203	0.197	0.473	-0.926	-0.356	0.100	0.835	0.097	0.612
CRWL1	0.781	-	0.432	-	0.266	-	-0.939	-	0.037	-	0.078	-
CRWL2	-	-0.022	-	0.288	-	-0.500	-	0.295	-	-0.799	-	-0.583
SWIM1	-0.978	-0.641	-0.616	0.225	-0.645	0.116	0.749	-0.514	0.584	0.756	0.296	0.915
SWIM2	-	-0.047	-	0.302	-	-0.586	-	0.230	-	-0.846	-	-0.529
SWIM3	0.999	0.999	0.532	-0.678	0.655	0.501	-0.711	0.494	-0.577	-0.146	-0.355	-0.743
SHPE1	0.958	-	0.569	-	0.635	-	-0.529	-	-0.705	-	-0.436	-
SHPE2	-0.958	-	-0.569	-	-0.635	-	0.529	-	0.705	-	0.436	-
RESP1	-0.855	0.048	-0.503	0.157	-0.441	-0.509	0.950	0.335	0.143	-0.845	-0.026	-0.621
RESP2	0.855	-0.048	0.503	-0.157	0.441	0.509	-0.950	-0.335	-0.143	0.845	0.026	0.621
HABI5	0.996	-	0.553	-	0.630	-	-0.723	-	-0.571	-	-0.325	-
TROP1	0.672	-0.185	0.465	-0.008	0.107	0.364	-0.953	-0.483	0.164	0.858	0.219	0.708

Table 3.5: Similarities of taxonomic and trait composition at sites throughout the experiment.

Site Comparisons	Taxonomic	Traits
AR1 & AR5	52.38	82.22
AR1 & NFCC	43.91	77.6
AR5 & NFCC	39.15	75.33

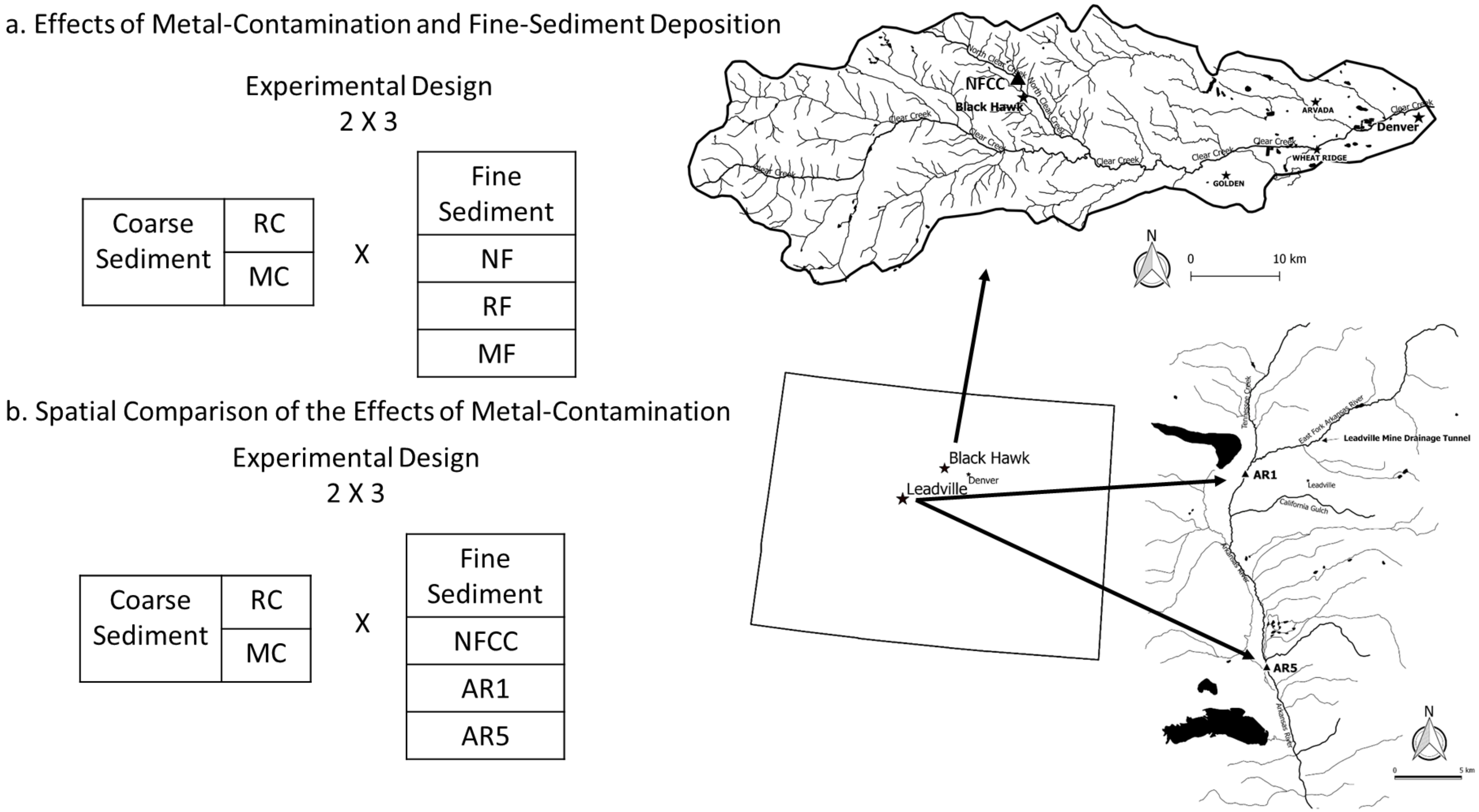


Figure 3.1: Map of study sites on North Fork Clear Creek (NFCC) and the Upper Arkansas River (AR1 and AR5) in Colorado, USA. Factorial designs for (a) multiple stressor (metal-contamination and fine-sediment deposition) and (b) spatial comparisons used for trait responses to a single-stressor (metal-contamination). RC = reference coarse, MC = metal coarse, NF = no fines, RF = reference fine, MF = metal fines, NFCC = North Fork Clear Creek, AR = Arkansas River.

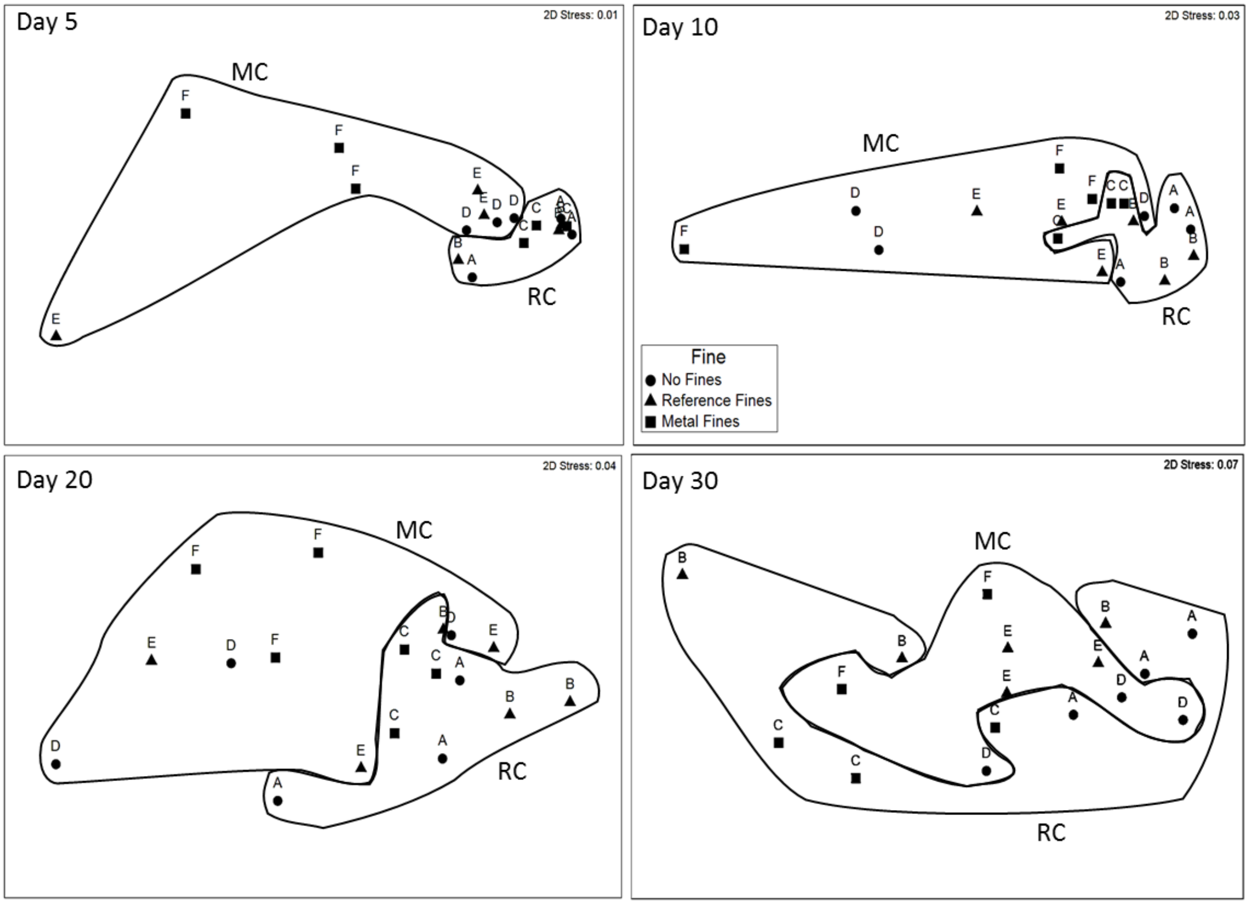


Figure 3.2: Multiple Stressor Data: nMDS plot showing comparisons of trait composition based by fine-sediment (symbols), metal-contamination (black outline; RC = reference coarse; MC = metal-coarse), and individual treatments (letters). Treatments A (reference-coarse), B (reference-coarse and fines), C (reference-coarse and metal-fines), D (metal-coarse), E (metal-coarse and reference-fines), and F (metal-coarse and fines).

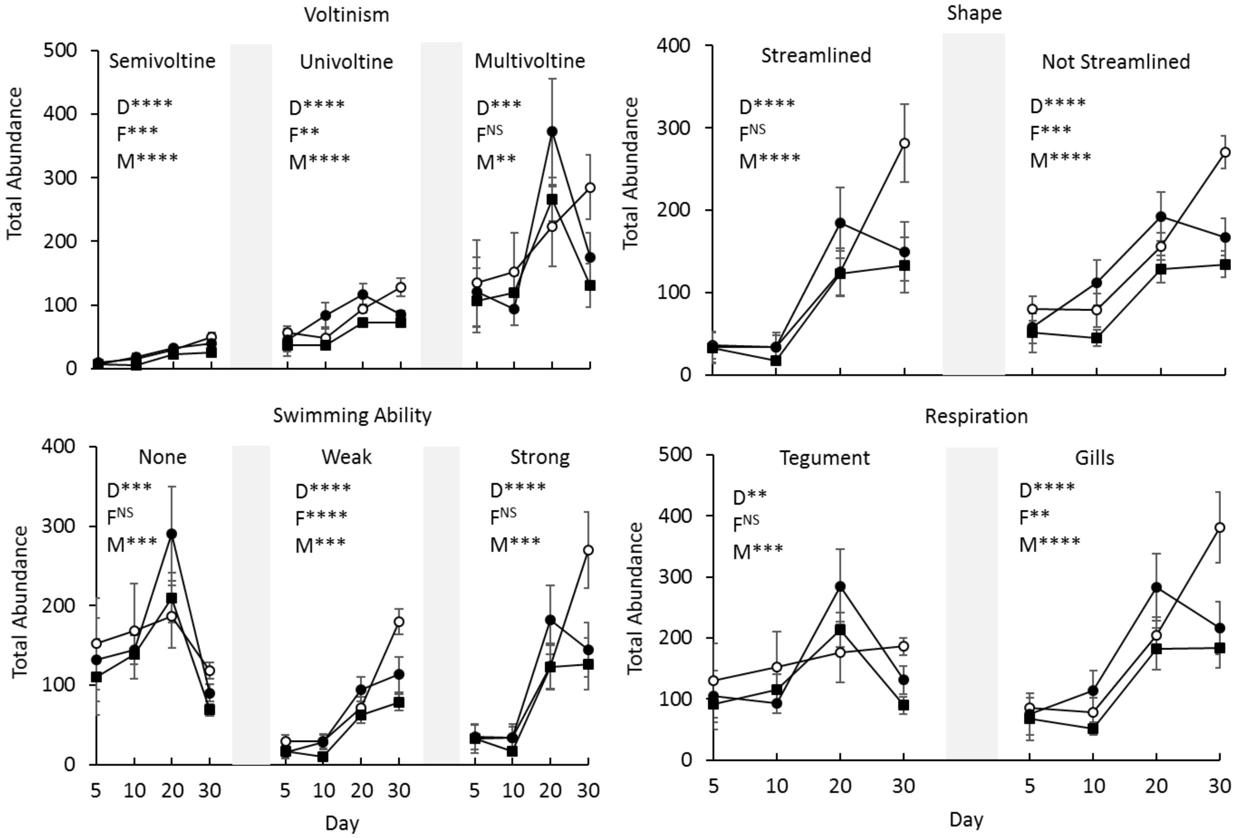


Figure 3.3: Multiple Stressor Data: Abundance of traits (voltinism, swimming ability, shape, and respiration) over time comparison treatments with no-fines (open-circle), reference-fines (closed-circle), and metal-fines (square). PERMANOVA output for the main effects of day (D), metal-contamination (M), and fine-sediment (F); NS = Not Significant, ** = <0.01, *** <0.001, **** <0.0001. Error bars represent standard error of the mean. Detailed PERMANOVA output for all traits in Appendix 3B.

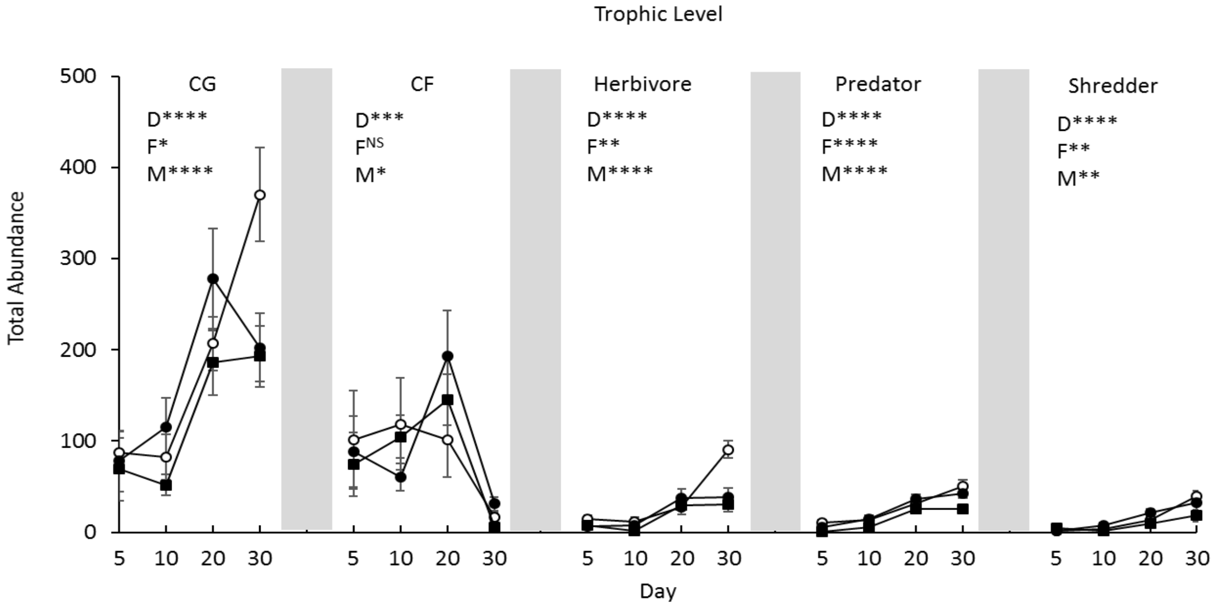


Figure 3.4: Multiple Stressor Data: Abundance of benthic invertebrate trophic position over time comparison treatments with no-fines (open-circle), reference-fines (closed-circle), and metal-fines (square). PERMANOVA output for the main effects of day (D), metal-contamination (M), and fine-sediment (F); NS = Not Significant, ** = <0.01, *** <0.001, **** <0.0001. CG = Collector-Gatherer; CF = Collector-Filterer. Error bars represent standard error of the mean. Detailed PERMANOVA output for all traits in Appendix 3B.

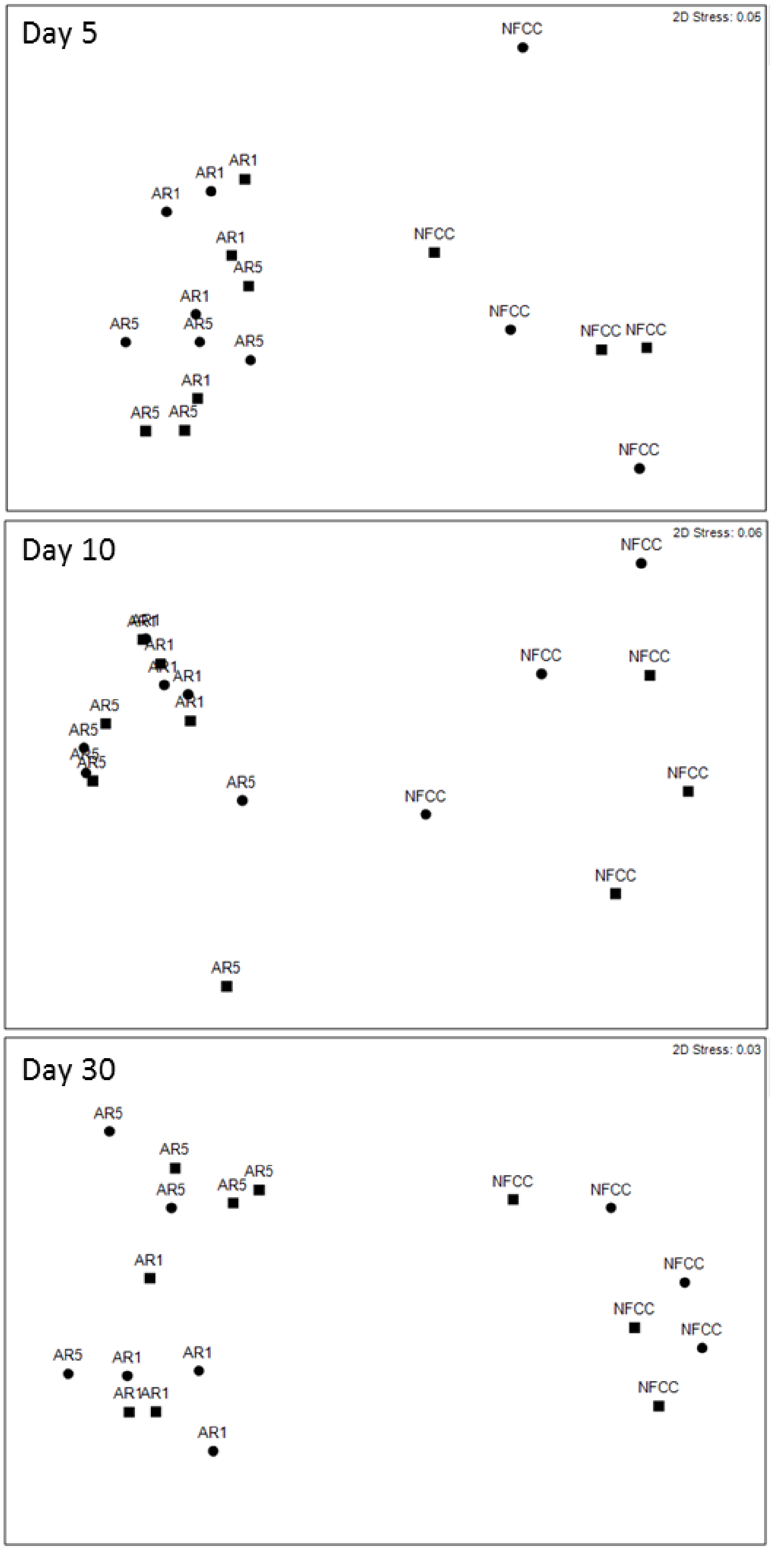
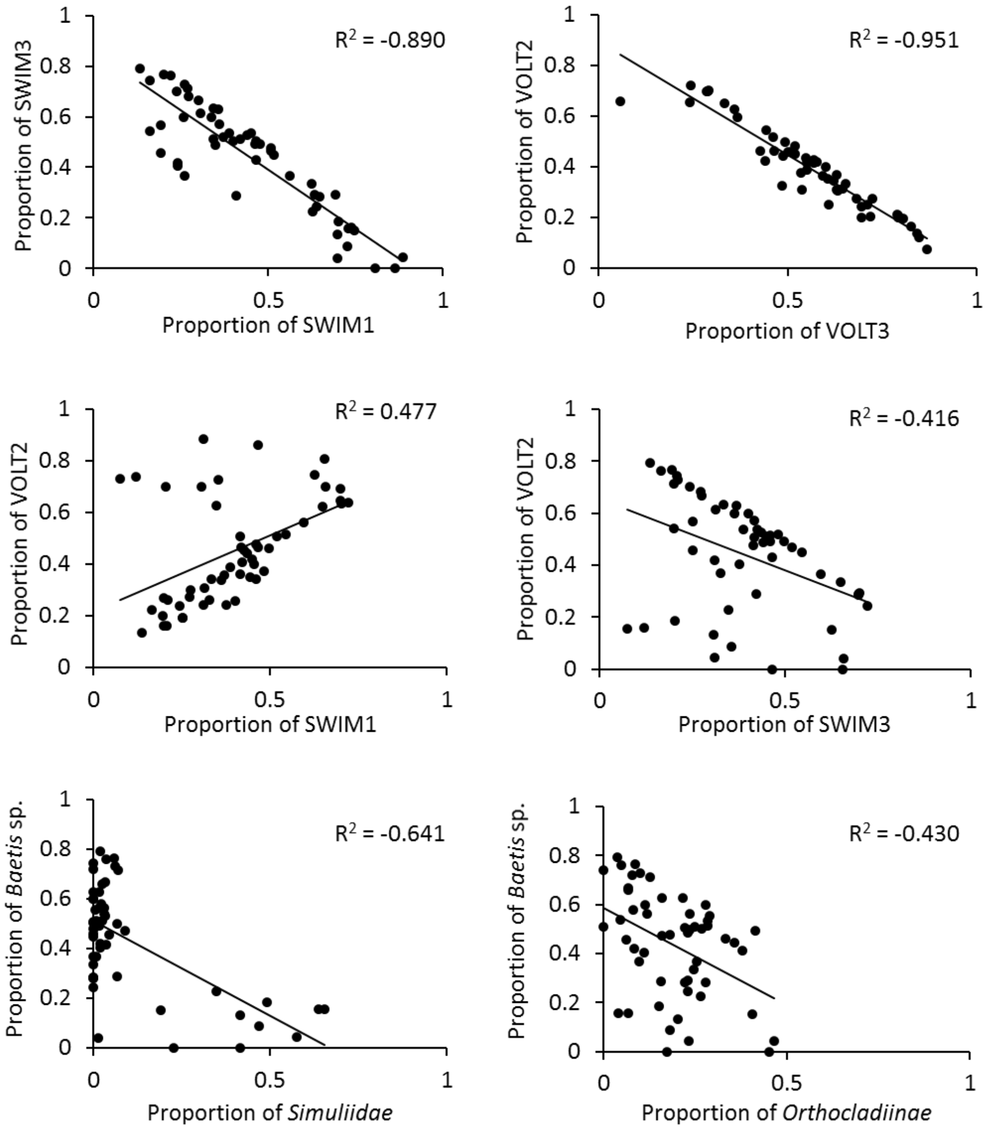
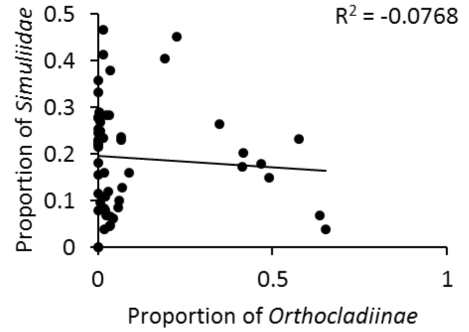


Figure 3.5: Spatial Study: nMDS plots showing the separation of treatments and sites based on trait composition in treatments with reference (circles), and metal-contaminated (triangles) substrate.





Trait Code	Trait Name	# of Taxa	Dominant Taxa
VOLT2	Univoltine	22	Orthocladiinae
VOLT3	Multivoltine	3	*Baetis* sp., Simuliidae
SWIM1	Non-Swimmer	13	Simuliidae, Orthocladiinae
SWIM3	Strong Swimmer	3	*Baetis* sp.

Figure 3.6: Relationship between proportion of voltinism (VOLT) and swimming ability (SWIM) traits and dominant taxa across all sites and collection days for the spatial analysis study. Table shows definition of species code along with the number of taxa and corresponding dominant taxa. R2 represent Pearson correlation coefficients. Trait assignments are based on Poff et al. 2006.

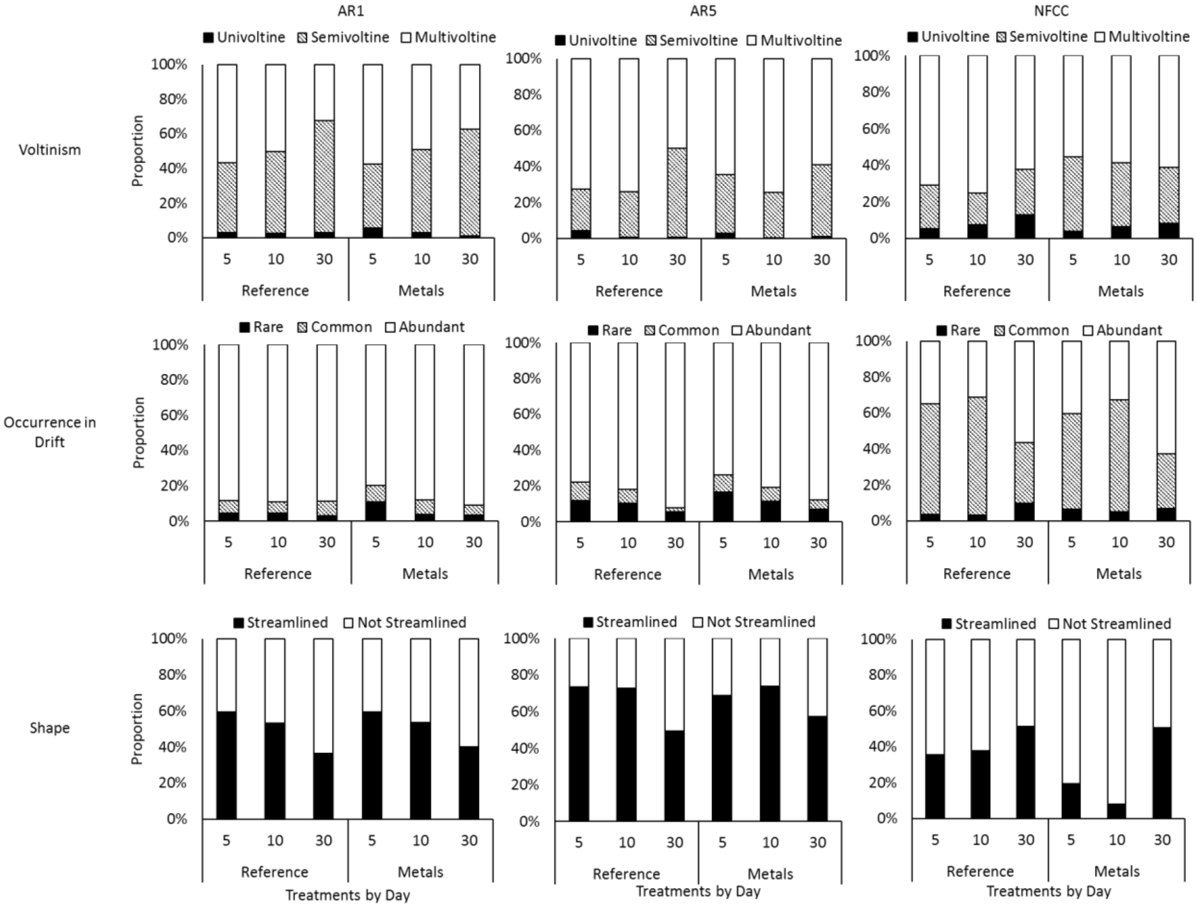


Figure 3.7: Proportions of voltinism, drift, and shape traits at each site over time in reference and metal-contaminated trays for the spatial study.

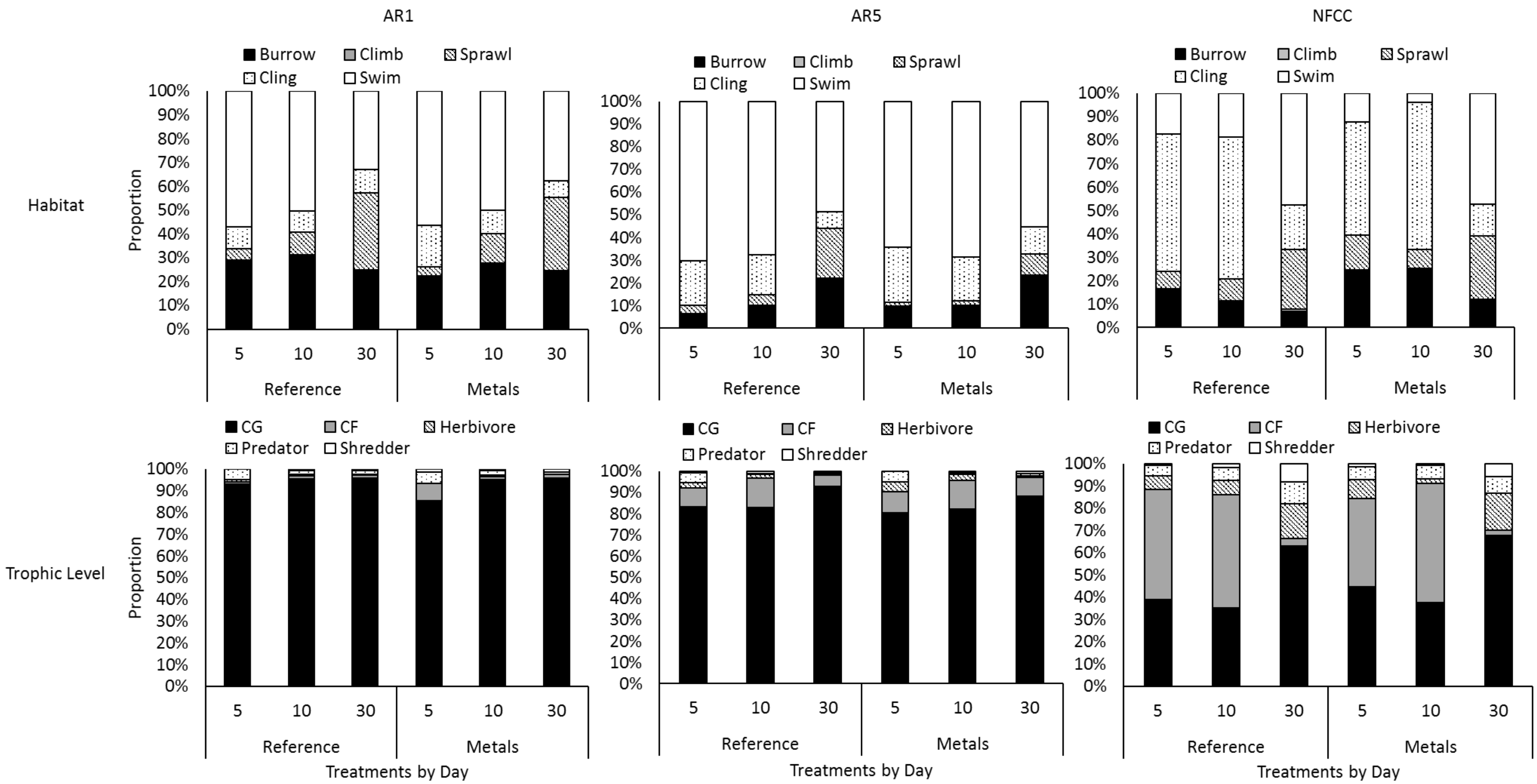


Figure 3.8: Proportions of habitat and trophic traits at each site over time in reference and metal-contaminated trays for the spatial study.

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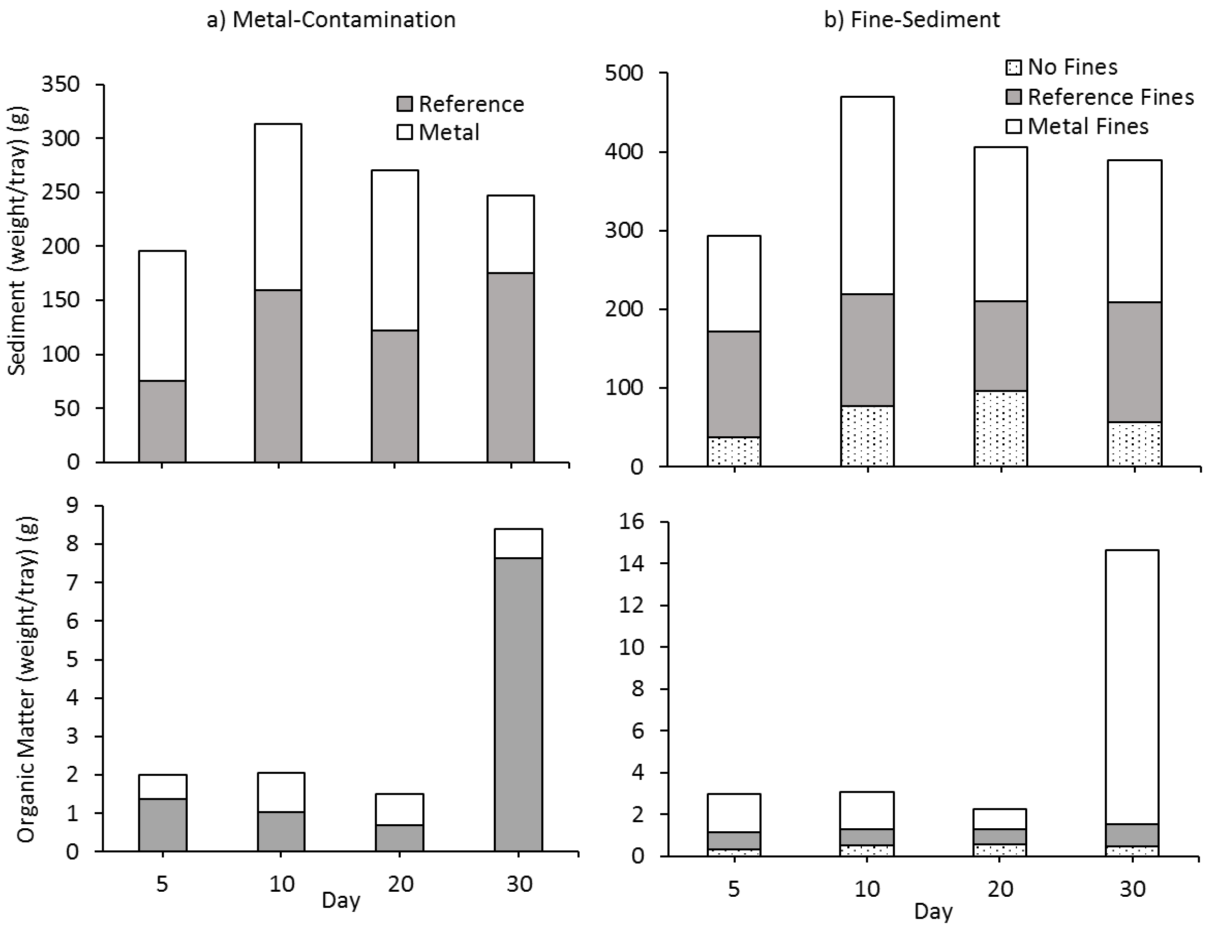
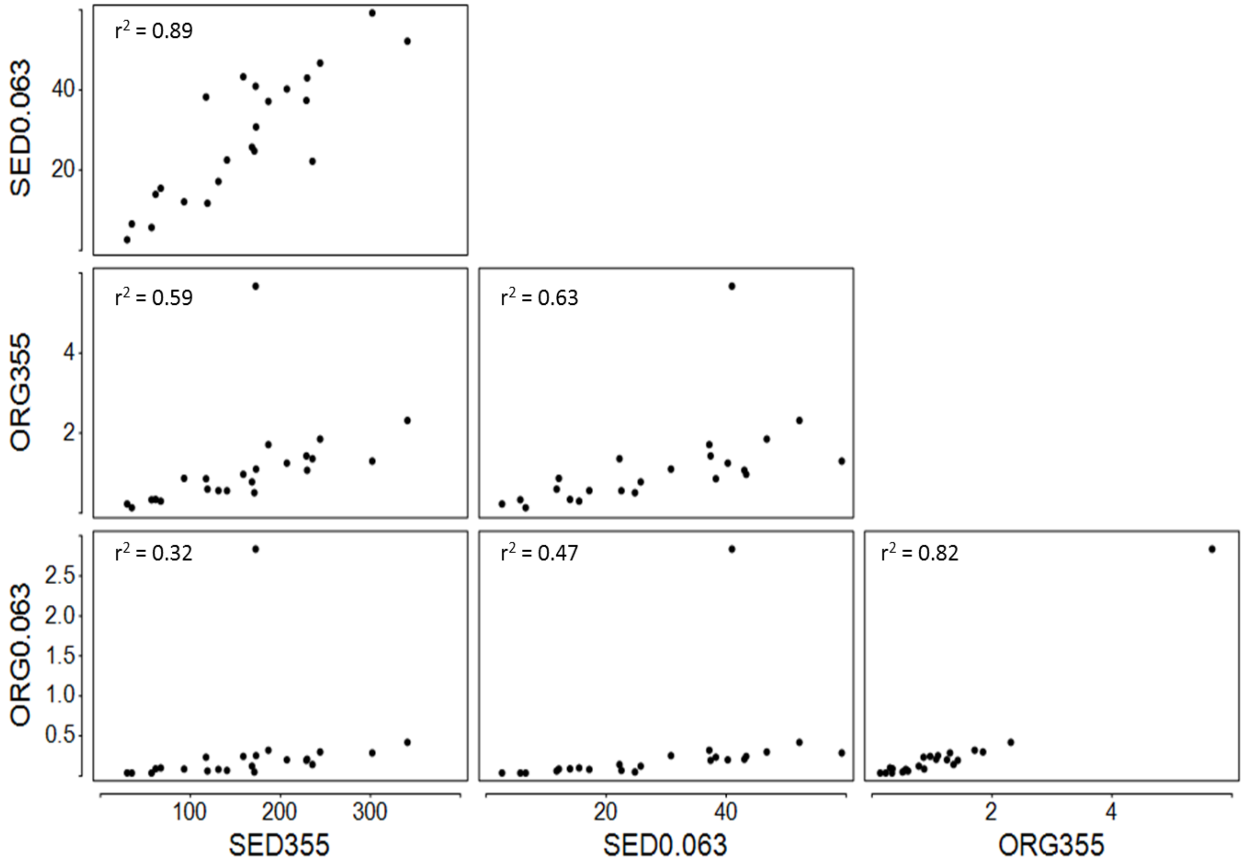


Appendix 1A: Comparisons of sediment and organic matter in treatments over time. Data is visualized based on the impact of metal-contamination (a. factors reference and metal coarse) and the impact of fine-sediment deposition (b. factors no fines, reference fines, and metal fines)

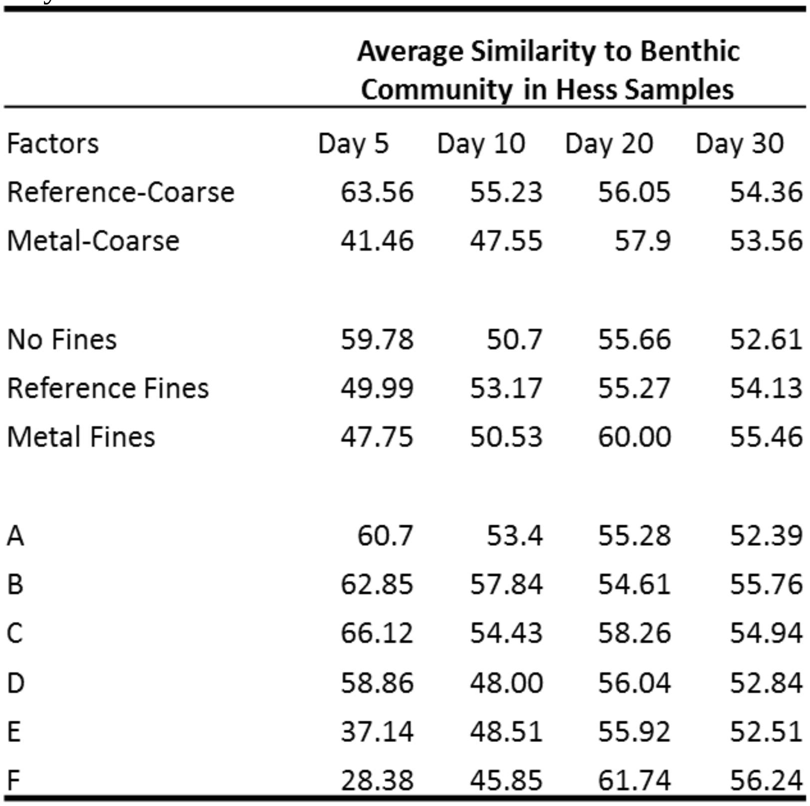


Appendix 1B: Draftsman plots showing the correlations among sediment (SED) and organic matter (ORG) variables at sizes 63µm and 355µm.

Appendix 1C: Pairwise-comparisons of community composition comparing factors of fine-sediment, metal-contamination, and treatments. Significant results with a $p < 0.05$ are in bold.

	Day 5		**Day 10**		**Day 20**		**Day 30**	
	t-statistic	**P(perm)**	**t-statistic**	**P(perm)**	**t-statistic**	**P(perm)**	**t-statistic**	**P(perm)**
Fine-Sediment								
NF vs RF	0.92037	0.528	1.1718	0.213	0.99277	0.45	**1.6218**	**0.015**
NF vs MF	**2.278**	**0.003**	0.98999	0.393	1.2664	0.135	**1.6233**	**0.035**
RF vs MF	1.1088	0.299	1.2703	0.19	**1.723**	**0.019**	**1.4561**	**0.034**
Metal-Contamination								
RC vs MC	**2.6693**	**0.001**	**2.6168**	**0.005**	**1.7588**	**0.003**	**1.5727**	**0.028**
Treatments								
A, B	0.55368	0.782	1.3899	0.17	0.99007	0.433	1.6411	0.094
A, C	0.88693	0.527	1.4883	0.123	0.87431	0.522	1.3179	0.218
A, D	1.5356	0.131	2.0814	0.069	1.1297	0.307	0.75524	0.673
A, E	1.2562	0.253	1.9161	0.073	1.0958	0.319	1.4067	0.144
A, F	**2.9721**	**0.009**	1.7235	0.118	1.6584	0.09	1.5259	0.133
B, C	0.72474	0.658	**1.893**	**0.047**	1.0286	0.408	0.99521	0.415
B, D	1.3381	0.196	**2.1592**	**0.048**	1.3678	0.188	1.714	0.067
B, E	1.159	0.288	1.8802	0.055	1.0971	0.351	**1.9456**	**0.037**
B, F	**2.9516**	**0.013**	1.8404	0.088	2.1503	0.036	1.5868	0.124
C, D	1.7287	0.072	1.5155	0.147	1.1595	0.29	1.2375	0.265
C, E	1.3099	0.221	1.1865	0.293	1.2118	0.245	1.6137	0.122
C, F	**3.4538**	**0.006**	1.2061	0.268	1.4333	0.134	0.97599	0.431
D, E	1.0415	0.369	0.93348	0.445	0.7346	0.683	1.4488	0.137
D, F	**3.2308**	**0.01**	0.65816	0.689	1.2279	0.243	1.5071	0.157
E, F	1.1633	0.329	0.73314	0.654	1.5478	0.112	1.6324	0.119

Appendix 1D: SIMPER analysis showing the similarity between the benthic community at NFCC and in the trays.

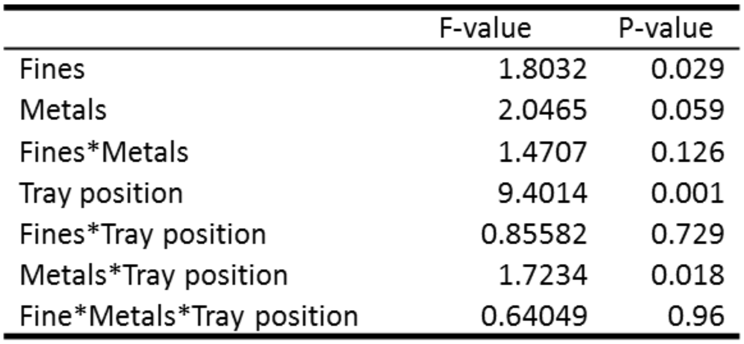


	Average Similarity to Benthic Community in Hess Samples			
Factors	Day 5	Day 10	Day 20	Day 30
Reference-Coarse	63.56	55.23	56.05	54.36
Metal-Coarse	41.46	47.55	57.9	53.56
No Fines	59.78	50.7	55.66	52.61
Reference Fines	49.99	53.17	55.27	54.13
Metal Fines	47.75	50.53	60.00	55.46
A	60.7	53.4	55.28	52.39
B	62.85	57.84	54.61	55.76
C	66.12	54.43	58.26	54.94
D	58.86	48.00	56.04	52.84
E	37.14	48.51	55.92	52.51
F	28.38	45.85	61.74	56.24

Appendix 1E: Results of the two-way ANOVA (treatment x tray) and three-way ANOVA (fines x metals x tray) of univariate community metrics. P-values and F-values are listed, with values representing significance (p < 0.05) in bold.

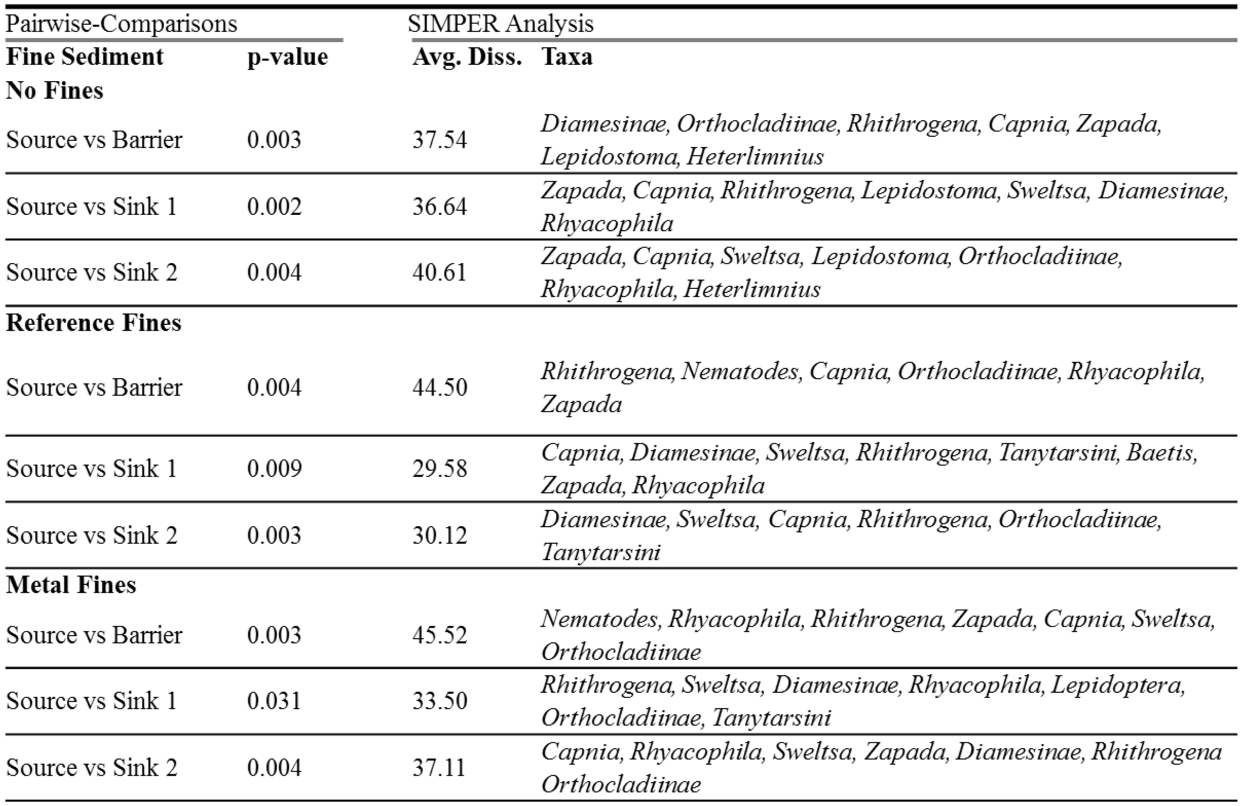
		Shannon-Weiner Diversity		**Margalef Richness**		**Pielou's Evenness**		**# of Taxa**		**Total Abundance**	
	df	F	p	F	p	F	p	F	p	F	p
Treatment	5	2.61	0.0365	1.76	0.1385	0.44	0.8215	2.35	0.0546	1.06	0.3933
Tray	3	21.19	<.0001	8.90	<.0001	5.94	0.0016	18.12	<.0001	18.44	<.0001
Treatment*Tray	15	1.67	0.0902	1.34	0.2153	0.55	0.8944	1.43	0.1732	1.21	0.297
Tray	3	21.19	<.0001	8.90	<.0001	5.94	0.0016	18.12	<.0001	18.44	<.0001
Fine	2	4.81	0.0125	3.96	0.0255	0.80	0.4537	4.81	0.0125	0.69	0.5089
Tray*Fine	6	2.48	0.0358	1.40	0.2336	0.72	0.6364	2.02	0.0805	1.16	0.3421
Metals	1	2.41	0.1268	0.73	0.3979	0.37	0.5447	1.96	0.1679	0.79	0.3789
Tray*Metals	3	1.38	0.2607	1.38	0.2612	0.59	0.6239	1.28	0.2915	1.87	0.1475
Fine*Metals	2	0.50	0.6098	0.08	0.9214	0.10	0.9054	0.09	0.9112	1.57	0.2176
Tray*Fine*Metals	6	1.00	0.4346	1.27	0.2905	0.37	0.8936	0.90	0.5003	0.93	0.4825

Appendix 1F: Three-way PERMANOVA output for comparisons of fine-sediment deposition, metal-contamination, and tray position in the mesocosm experiment.



	F-value	P-value
Fines	1.8032	0.029
Metals	2.0465	0.059
Fines*Metals	1.4707	0.126
Tray position	9.4014	0.001
Fines*Tray position	0.85582	0.729
Metals*Tray position	1.7234	0.018
Fine*Metals*Tray position	0.64049	0.96

Appendix 1F: Pairwise-comparisons from PERMANOVA and SIMPER output for the mesocosm experiment. SIMPER output shows average dissimilarity (Avg. Diss.) between comparisons and species that contributed to the top 50% of the differences between factors. Taxa are listed from the highest to lowest contribution.



Pairwise-Comparisons		SIMPER Analysis	
Fine Sediment	**p-value**	**Avg. Diss.**	**Taxa**
No Fines			
Source vs Barrier	0.003	37.54	*Diamesinae, Orthocladiinae, Rhithrogena, Capnia, Zapada, Lepidostoma, Heterlimnius*
Source vs Sink 1	0.002	36.64	*Zapada, Capnia, Rhithrogena, Lepidostoma, Sweltsa, Diamesinae, Rhyacophila*
Source vs Sink 2	0.004	40.61	*Zapada, Capnia, Sweltsa, Lepidostoma, Orthocladiinae, Rhyacophila, Heterlimnius*
Reference Fines			
Source vs Barrier	0.004	44.50	*Rhithrogena, Nematodes, Capnia, Orthocladiinae, Rhyacophila, Zapada*
Source vs Sink 1	0.009	29.58	*Capnia, Diamesinae, Sweltsa, Rhithrogena, Tanytarsini, Baetis, Zapada, Rhyacophila*
Source vs Sink 2	0.003	30.12	*Diamesinae, Sweltsa, Capnia, Rhithrogena, Orthocladiinae, Tanytarsini*
Metal Fines			
Source vs Barrier	0.003	45.52	*Nematodes, Rhyacophila, Rhithrogena, Zapada, Capnia, Sweltsa, Orthocladiinae*
Source vs Sink 1	0.031	33.50	*Rhithrogena, Sweltsa, Diamesinae, Rhyacophila, Lepidoptera, Orthocladiinae, Tanytarsini*
Source vs Sink 2	0.004	37.11	*Capnia, Rhyacophila, Sweltsa, Zapada, Diamesinae, Rhithrogena Orthocladiinae*

CHAPTER 2: APPENDICES

Appendix 2A: List of genera along with corresponding order and correlations with community patterns on the NMDS plot. MDS1 refers to separation over time and MDS2 by site.

Order	Genus	MDS1	Order	Genus	MDS2
Diptera	**Diamesinae**	**0.943**	Caddisfly	**Arctopsyche grandis**	**0.877**
Diptera	**Orthocladiinae**	**0.934**	Diptera	**Simuliidae**	**0.513**
Mayfly	**Baetis**	**0.799**	Caddisfly	**Brachycentrus**	**0.510**
Mayfly	**Drunella grandis**	**0.678**	Stonefly	**Pteronarcella**	**0.503**
Mayfly	**Rhithrogena**	**0.671**	Stonefly	**Isoperla**	**0.419**
Stonefly	**Capnia**	**0.640**	Caddisfly	**Glossoma**	**0.396**
Diptera	**Pericoma**	**0.635**	Mayfly	Baetis	0.389
Caddisfly	**Brachycentrus**	**0.613**	Stonefly	Skwala	0.379
Mayfly	Paraleptophlebia	0.558	Caddisfly	Oligophlebodes	0.375
Mayfly	Ameletus	0.536	Caddisfly	Lepidostoma	0.374
Mayfly	Ephemerella	0.477	Stonefly	Taenionema	0.229
Diptera	Tanytarsini	0.466	Mayfly	Acentrella	0.113
Stonefly	Sweltsa	0.429	Diptera	Diamesinae	0.099
Diptera	Dicranota	0.416	Diptera	Dicranota	0.073
Caddisfly	Rhyacophila	0.413	Mayfly	Drunella grandis	0.069
Stonefly	Skwala	0.407	Stonefly	Megarcys	0.066
Caddisfly	Oligophlebodes	0.343	Mayfly	Drunella doddsi	0.053
Mayfly	Serratella	0.320	Caddisfly	Micrasema	0.027
Diptera	Tanypodinae	0.294	Stonefly	Capnia	-0.040
Diptera	Ceratopogonidae	0.287	Mayfly	Ameletus	-0.051
Stonefly	Pteronarcella	0.231	Diptera	Tipula	-0.076
Diptera	Tipula	0.215	Diptera	Orthocladiinae	-0.084
Mayfly	Acentrella	0.210	Diptera	Clinocera	-0.103
Stonefly	Taenionema	0.090	Diptera	Pericoma	-0.133
Diptera	Clinocera	0.044	Mayfly	Ephemerella	-0.157
Caddisfly	Glossoma	0.031	Mayfly	Rhithrogena	-0.188
Diptera	Atherix	0.022	Mayfly	Serratella	-0.217
Caddisfly	Lepidostoma	-0.042	Diptera	Ceratopogonidae	-0.237
Diptera	Simuliidae	-0.090	Diptera	Tanytarsini	-0.270
Caddisfly	Arctopsyche grandis	-0.090	Diptera	Tanypodinae	-0.330
Stonefly	Isoperla	-0.117	Diptera	**Atherix**	**-0.364**
Mayfly	Drunella doddsi	-0.172	Mayfly	**Paraleptophlebia**	**-0.380**
Caddisfly	**Micrasema**	**-0.272**	Caddisfly	**Rhyacophila**	**-0.426**
Stonefly	**Megarcys**	**-0.511**	Stonefly	**Sweltsa**	**-0.437**

Appendix 2B: Output of SIMPER analysis on days 5, 12 and 28 between reference and metal treatments. Av. Abund = Average Abundance on log scale; Av. Diss = Average Dissimilarity; SD = Standard Deviation; Contrib% = Percent Contribution.

AR1 - Day 5
Groups Reference & Metal
Average dissimilarity = 35.94

Species	RC Av.Abund	MC Av.Abund	Av.Diss	Diss/SD	Contrib%
Orthocladiinae	2.72	2.21	4.16	1.64	11.59
Diamesinae	1	0.69	3.34	1.31	9.3
Arctopsyche grandis	0.23	0.77	2.94	1.09	8.19
Brachycentrus	0	0.6	2.46	1.19	6.85
Baetis	3.52	3.19	2.43	1.23	6.76
Simuliidae	0.23	0.69	1.97	1.31	5.47
Rhithrogena	0.6	0.46	1.96	1.13	5.45

AR5 - Day 5
Groups Reference & Metal
Average dissimilarity = 38.70

Species	RC Av.Abund	MC Av.Abund	Av.Diss	Diss/SD	Contrib%
Orthocladiinae	1.5	1.1	3.99	1.37	10.32
Arctopsyche grandis	1.66	1.07	2.75	1.03	7.1
Baetis	3.67	3.1	2.62	2.34	6.78
Diamesinae	0.6	0.37	2.51	1.17	6.48
Micrasema	0.46	0.96	2.35	1.14	6.07
Sweltsa	0.6	0.23	2.31	1.14	5.96
Megarcys	0	0.46	2.28	1.32	5.89
Tanytarsini	0.23	0.46	2.18	0.94	5.64

AR1 - Day 12
Groups Reference & Metal
Average dissimilarity = 24.45

Species	RC Av.Abund	MC Av.Abund	Av.Diss	Diss/SD	Contrib%
Sweltsa	0.69	1.23	1.55	1.22	6.34
Drunella Grandis	1.13	0.6	1.42	1.18	5.82
Tanytarsini	0.23	0.69	1.38	1.14	5.65
Micrasema	0.23	0.83	1.32	1.51	5.42
Rhyacophila	0.73	0.46	1.3	1.58	5.3
Capnia	0.6	0.37	1.27	1.17	5.19
Brachycentrus	0.37	0.6	1.26	1.16	5.16
Paraleptophlebia	0.46	0.69	1.2	1.17	4.89
Serratella	0.6	0.23	1.16	1.17	4.74
Ameletus	1.25	0.83	1.12	1.01	4.58

AR5 - Day 12
Groups Reference & Metal
Average dissimilarity = 31.13

Species	RC Av.Abund	MC Av.Abund	Av.Diss	Diss/SD	Contrib%
Orthocladiinae	2.56	1.81	3.47	0.99	11.14
Diamesinae	1.7	0.83	3.19	1.53	10.26
Simuliidae	2.16	1.48	2.85	0.96	9.15
Brachycentrus	1.01	0.83	2.39	1.25	7.67
Micrasema	0.37	0.77	2.22	1.15	7.12
Glossoma	0.46	0.46	1.81	1.46	5.82

AR1 - Day 28
Groups Reference & Metal
Average dissimilarity = 20.71

Species	RC Av.Abund	MC Av.Abund	Av.Diss	Diss/SD	Contrib%
Sweltsa	1.69	0.69	1.89	1.79	9.12
Skwala	0.92	0.69	1.49	1.36	7.2
Capnia	0.92	1.79	1.31	1.74	6.35
Ameletus	0.23	1.1	1.31	2.41	6.32
Paraleptophlebia	1.27	1.23	1.2	1.75	5.79
Ephemerella	0.83	0.37	1.04	1.15	5.01
Arctopsyche grandis	0.83	1.43	0.89	1.65	4.3
Tanypodinae	0.6	0.23	0.77	1.17	3.7
Baetis	4.87	5.1	0.72	1.4	3.47

AR5 - Day 28
Groups Reference & Metal
Average dissimilarity = 24.02

Species	RC Av.Abund	MC Av.Abund	Av.Diss	Diss/SD	Contrib%
Simuliidae	0.83	2.15	3.26	1.65	13.56
Drunella grandis	0.23	1.16	1.9	1.85	7.91
Glossoma	0.83	0	1.68	4.47	6.99
Diamesinae	3.9	3.1	1.65	0.94	6.86
Oligophlebodes	0.83	0.46	1.52	2.94	6.31
Skwala	0.69	0.69	1.27	1.09	5.3
Sweltsa	0.37	0.46	1.15	0.92	4.8

Appendix 3A: Pairwise-comparisons of trait composition comparing factors of fine-sediment, metal-contamination, and treatments. Significant results with a $p < 0.05$ are in bold.

Pairwise-Comparison	5	10	20	30
Reference Coarse vs Metal Coarse	**0.003 (2.724)**	**0.015 (2.6964)**	**0.015 (2.5074)**	0.607 (0.75586)
No Fines, Reference Fines	0.447 (1.0000)	0.331 (1.0453)	0.262 (1.1734)	**0.047 (1.9408)**
No Fines, Metal Fines	**0.015 (2.5823)**	0.628 (0.65932)	0.406 (0.97825)	**0.013 (2.9985)**
Reference Fines, Metal Fines	0.523 (0.85584)	0.199 (1.3025)	0.111 (1.5584)	0.329 (1.0892)
A, B	0.845 (0.32001)	0.481 (0.86435)	0.146 (1.577)	0.12 (1.788)
A, C	0.79 (0.45074)	0.067 (1.805)	0.48 (0.87509)	**0.021 (2.8299)**
A, D	0.327 (1.1453)	0.102 (1.9704)	0.319 (1.1926)	0.493 (0.86525)
A, E	0.276 (1.2057)	0.052 (2.364)	0.739 (0.57134)	0.135 (1.5585)
A, F	**0.015 (3.2647)**	0.164 (1.6107)	**0.039 (2.1523)**	**0.049 (2.169)**
B, C	0.751 (0.47219)	**0.04 (2.184)**	0.132 (1.6325)	0.485 (0.87283)
B, D	0.396 (0.9718)	0.086 (2.0816)	0.095 (2.0614)	0.167 (1.5392)
B, E	0.303 (1.1503)	**0.047 (2.4871)**	0.202 (1.5129)	0.26 (1.2556)
B, F	**0.019 (3.1355)**	0.132 (1.6718)	**0.011 (3.5759)**	0.887 (0.36994)
C, D	0.059 (2.1299)	0.231 (1.4436)	0.246 (1.2813)	0.063 (2.153)
C, E	0.279 (1.296)	0.163 (1.4082)	0.518 (0.81239)	0.059 (2.0604)
C, F	**0.004 (3.8159)**	0.295 (1.1681)	**0.024 (2.5155)**	0.68 (0.70724)
D, E	0.336 (1.0454)	0.413 (0.91478)	0.655 (0.63957)	0.275 (1.1831)
D, F	**0.016 (3.2111)**	0.878 (0.31906)	0.554 (0.77349)	0.158 (1.699)
E, F	0.414 (0.96195)	0.477 (0.85725)	0.379 (1.0247)	0.169 (1.5157)

Appendix 3B: Output of p-values associated with overall traits composition (3-way PERMANOVA) and each individual trait (3-way ANOVA). NS = Not Significant, * = <0.05, ** <0.01, *** <0.001, **** <0.0001

	Day F-value	Fine F-value	Metals F-value	Day x Fines F-value	Day x Metals F-value	Fine x Metals F-value	Day x Fine x Metals F-value
All Traits	11.814**	2.1676^{NS}	13.806**	1.2571^{NS}	4.4615**	1.2519^{NS}	1.2348^{NS}
Voltinism							
Semivoltine	35.7****	8.51***	40.41****	0.67^{NS}	5.64**	0.5^{NS}	1.4^{NS}
Univoltine	21.15****	7.56**	23.8****	2.27^{NS}	10.14****	2.26^{NS}	1.66^{NS}
Bi- and Multivoltine	6.95***	0.05^{NS}	9.58**	0.51^{NS}	2.11^{NS}	0.12^{NS}	0.7^{NS}
Development							
Fast Seasonal	11.07****	0.99^{NS}	15.58***	0.82^{NS}	4.48***	0.29^{NS}	0.84^{NS}
Slow Seasonal	62.19****	13.59****	51.28****	1.69^{NS}	12.62****	1.97^{NS}	2.23^{NS}
None Seasonal	8.29***	1.08^{NS}	24.95****	1.08^{NS}	0.21^{NS}	0.23^{NS}	0.72^{NS}
Occurrence in Drift							
Rare	52.02****	8.14***	16.06***	1.08^{NS}	2.03^{NS}	0.14^{NS}	0.89^{NS}
Common	5.43***	0.41^{NS}	12.49***	0.69^{NS}	3.13*	0.14^{NS}	0.54^{NS}
Abundant	29.63****	4.49*	33.09****	1.59^{NS}	9.51****	1.18^{NS}	1.72^{NS}
Maximum crawling rate							
Very Low	34.98****	5.64***	35.96****	1.67^{NS}	9.97****	1.34^{NS}	2.02^{NS}
Low	5.12***	0.35^{NS}	12.66***	0.66^{NS}	3.04*	0.18^{NS}	0.54^{NS}
High	48.65****	9.21***	11.41***	1.05^{NS}	1.76^{NS}	0.25^{NS}	0.82^{NS}
Swimming Ability							
None	5.87***	0.86^{NS}	14.81***	0.7^{NS}	3.92*	0.48^{NS}	0.71^{NS}
Weak	67.77****	11.66****	44.83***	2.03^{NS}	11.46****	0.99^{NS}	1.84^{NS}
Strong	37.95****	1.31^{NS}	37.96***	0.68^{NS}	8.23***	0.27^{NS}	1.77^{NS}
Attachment							
None	36.88****	5.87***	37.98****	1.6^{NS}	9.93****	1.06^{NS}	1.68^{NS}
Some	4.77**	0.23^{NS}	5.72*	0.72^{NS}	1.06^{NS}	0.01^{NS}	0.69^{NS}
Both	7.36***	4.75*	0.35^{NS}	1.47^{NS}	1.63^{NS}	1.57^{NS}	0.63^{NS}
Armoring							
None	11.46****	1.18^{NS}	17.03	0.85^{NS}	5.08***	0.29^{NS}	0.87^{NS}
Poor	38.31****	6.75**	25.81	0.56^{NS}	2.17^{NS}	0.02^{NS}	0.76^{NS}
Good	7.36***	4.75*	0.35^{NS}	1.47^{NS}	1.63^{NS}	1.57^{NS}	0.63^{NS}
Shape							
Streamlined	38.75****	1.25^{NS}	37.67****	0.7^{NS}	8.6***	0.24^{NS}	1.89^{NS}
Not Streamlined	33.68****	9.8***	35.48****	2.07^{NS}	9.58****	1.72^{NS}	1.73^{NS}
Respiration							
Tegument	6.32**	0.46^{NS}	13.48***	0.64^{NS}	2.74^{NS}	0.15^{NS}	0.57^{NS}
Gills	33.38****	5.29**	34.65****	1.68^{NS}	10.18****	1.19^{NS}	1.88^{NS}
Size at Maturity							
Small	11.22****	1.09^{NS}	18.01***	0.77^{NS}	4.89***	0.26^{NS}	0.87^{NS}
Medium	64.98****	13.03****	50.43****	2.55*	11.09****	1.69^{NS}	1.83^{NS}
Large	0.27^{NS}	0.47^{NS}	0.27^{NS}	0.74^{NS}	0.78^{NS}	1.23^{NS}	0.4^{NS}
Habitat							
Burrow	6.03**	3.6*	18.9****	2.39*	7.71***	2.58^{NS}	1.1^{NS}
Climb	12.94****	7.55**	0.43^{NS}	6.03****	0.17^{NS}	0.55^{NS}	0.38^{NS}
Sprawl	69.9****	15.27****	29.59****	1.54^{NS}	6.79***	2.32^{NS}	1.77^{NS}
Cling	4.70**	0.34^{NS}	12.16**	0.58^{NS}	2.92*	0.06^{NS}	0.63^{NS}
Swim	37.96****	1.33^{NS}	38.13****	0.69^{NS}	8.25***	0.3^{NS}	1.78^{NS}
Trophic Level							
Collector-Gatherer	31.51****	4.79*	35.62****	1.59^{NS}	9.42****	0.95^{NS}	1.54^{NS}
Collector-Filterer	7.79***	0.51^{NS}	4.54*	0.96^{NS}	0.71^{NS}	0.02^{NS}	0.6^{NS}
Herbivore	43.34****	7.64**	28.4****	2.46*	3.65*	0.84^{NS}	1.11^{NS}
Predator	79.76****	14.5****	54.98****	1.55^{NS}	13.73****	1.42^{NS}	2.58*
Shredder	64.17****	7.73**	10.6**	0.87^{NS}	6.33**	0.14^{NS}	1.83^{NS}

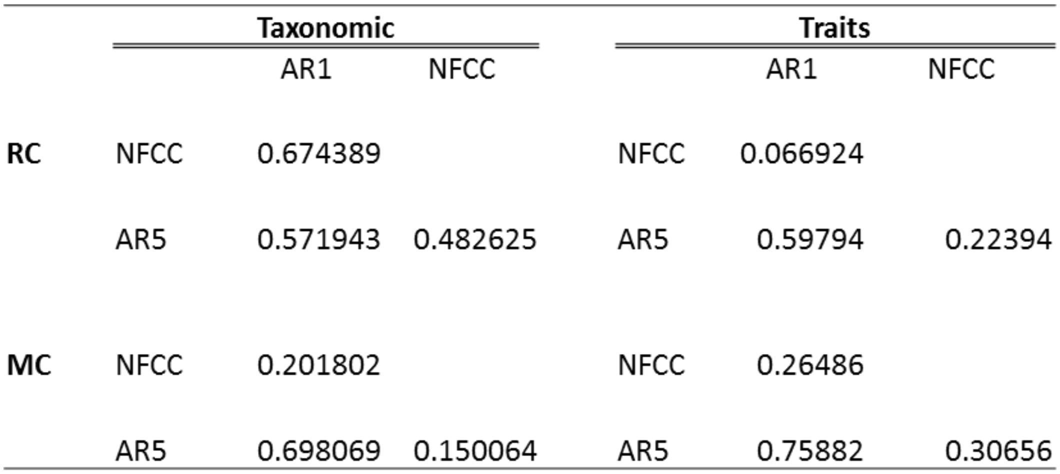
Appendix 3C: DISTLM output of trait associations to sediment and organic matter weights. The BEST procedure with AICc selection criteria was used to determine the best predictor variables and associated r^2.

	R^2				Predictor Variables			
	5	10	20	30	5	10	20	30
Taxonomic Composition*	0.314	0.026	0.076	0.157	SED 355, OM 355	OM 355	SED 63	SED 355
All Traits	0.38335	0.02	0.062114	0.31613	SED 355, OM 355	OM 355	SED 63	SED 355
Voltinism								
Semivoltine	0.26652	0.11236	0.14679	0.22332	SED 355, OM 63	OM 355	SED 63	SED 355, OM 355
Univoltine	0.48162	0.2716	0.18169	0.43454	SED 355, OM 355	SED 355, OM 63	OM 355	SED 355
Bi- and Multivoltine	0.38992	0.040618	0.048187	0.36356	SED 355, OM 355	OM 355	OM 355	SED 355
Development								
Fast Seasonal	0.42855	0.029757	0.017695	0.45468	SED 355, OM 355	OM 355	OM 63	SED 355
Slow Seasonal	0.36146	0.32731	0.1882	0.23539	SED 355, OM 63	OM 355, OM 63	OM 355	SED 355
None Seasonal	0.04241	0.081646	0.074572	0.080173	OM 63	OM 355	SED 63	OM 63
Occurrence in Drift								
Rare	0.33094	0.2788	0.17468	0.16487	SED 355	OM 355, OM 63	OM 355	SED 63
Common	0.41914	0.056493	0.032954	0.4257	SED 355, OM 355	OM 355	OM 355	SED 355, OM 63
Abundant	0.47037	0.22086	0.064451	0.46327	SED 355, OM 355	OM 355, OM 63	OM 63	SED 355
Maximum crawling rate								
Very Low	0.49391	0.21826	0.072918	0.47949	SED 355, OM 355	OM 355, OM 63	OM 355	SED 355
Low	0.42004	0.054378	0.037045	0.45962	SED 355, OM 355	OM 63	OM 355	SED 355, OM 63
High	0.33633	0.2972	0.15995	0.21232	SED 355	OM 355, OM 63	OM 355	OM 355
Swimming Ability								
None	0.47532	0.036311	0.030801	0.18953	SED 355, OM 355	SED 63	OM 355	SED 355
Weak	0.42309	0.31875	0.075931	0.50419	SED 355, OM 355	OM 355, OM 63	OM 63	SED 355, OM 63
Strong	0.49076	0.04688	0.095456	0.37756	SED 355, OM 355	OM 63	OM 63	SED 355
Attachment								
None	0.49147	0.24025	0.068713	0.58833	SED 355, OM 355	OM 355, OM 63	OM 355	SED 355, OM 63
Some	0.35453	0.2078	0.054881	0.14199	SED 355, OM 355	SED 63, OM 63	OM 355	OM 63
Both	0.04121	0.36236	0.30088	0.052971	OM 355	OM 355, OM 63	SED 63	SED 355

Appendix 3C continued: DISTLM output of trait associations to sediment and organic matter weights.

	R^2				Predictor Variables			
	5	10	20	30	5	10	20	30
Armoring								
None	0.42733	0.028014	0.020736	0.54927	SED 355, OM 355	SED 63	OM 63	SED 355, OM 63
Poor	0.12699	0.087972	0.15288	0.056811	SED 355	OM 355	SED 63	SED 63
Good	0.04121	0.36236	0.30088	0.052971	OM 355	OM 355, OM 63	SED 63	SED 355
Shape								
Streamlined	0.47603	0.042093	0.094184	0.38215	SED 355, OM 355	OM 63	OM 63	SED 355
Not Streamlined	0.46872	0.2908	0.14258	0.47415	SED 355, OM 355	OM 355, OM 63	SED 63	SED 355, OM 63
Respiration								
Tegument	0.41716	0.056394	0.024423	0.24585	SED 355, OM 355	SED 63	OM 355	SED 355
Gills	0.50568	0.21725	0.073191	0.4852	SED 355, OM 355	OM 355, OM 63	SED 63	SED 355
Size at Maturity								
Small	0.4879	0.028894	0.017126	0.46607	SED 355, OM 355	SED 63	OM 355	SED 355
Medium	0.43847	0.49727	0.09482	0.52697	SED 355, OM 355	SED 63, OM 355, OM 63	OM 63	SED 355, OM 63
Large	0.01421	0.18166	0.063035	0.20934	SED 63	SED 355	OM 63	SED 355
Habitat								
Burrow	0.47795	0.19061	0.047134	0.25865	SED 355, OM 355	OM 355, OM 63	OM 355	OM 63
Climb	-	-	0.16301	0.34343	-	-	SED 63	SED 63, OM 63
Sprawl	0.41572	0.31333	0.15522	0.51072	SED 355, OM 355	OM 355, OM 63	SED 63	SED 355, OM 63
Cling	0.40936	0.053983	0.034391	0.17059	SED 355, OM 355	SED 63	OM 355	SED 355
Swim	0.49288	0.047483	0.096978	0.3733	SED 355, OM 355	OM 63	OM 63	SED 355
Trophic Level								
Collector-Gatherer	0.48982	0.20784	0.072578	0.48033	SED 355, OM 355	OM 355, OM 63	SED 63	SED 355
Collector-Filterer	0.32126	0.21123	0.079919	0.11854	SED 355, OM 355	SED 63, OM 63	OM 355	SED 63
Herbivore	0.36198	0.57442	0.061136	0.4794	SED 355	SED 63, OM 355, OM 63	OM 63	SED 355, OM 63
Predator	0.34274	0.37517	0.15132	0.18447	SED 355, OM 63	OM 355, OM 63	SED 63	SED 355
Shredder	0.4172	0.12164	0.038313	0.14453	SED 63	OM 355	OM 355	SED 355

Appendix 3D: Correlation values showing relationship of taxonomic and trait composition between sites in reference and metal treatments throughout the experiment



		Taxonomic			Traits	
		AR1	NFCC		AR1	NFCC
RC	NFCC	0.674389		NFCC	0.066924	
	AR5	0.571943	0.482625	AR5	0.59794	0.22394
MC	NFCC	0.201802		NFCC	0.26486	
	AR5	0.698069	0.150064	AR5	0.75882	0.30656