

The effect of long-term metal exposure and mermithid parasitism on behavior and predation of nymphal *Baetis bicaudatus* by *Megarcys signata*

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Independent Research 2013

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Abstract

Abiotic stressors can affect organisms both directly and indirectly by causing behavioral changes and altering species interactions. Metal pollution caused by mining activities is a major stressor on freshwater ecosystems, particularly affecting macroinvertebrate communities. Historical mining has led to increased acid drainage resulting in high metal concentrations in streams that bioaccumulate from primary consumers to the predators and parasites that feed on those organisms. Our study aims to assess the prevalence of mermithid nematode parasites (*Gasteromermis* sp.) in mayfly hosts (*Baetis bicaudatus*), common stream insects, in metal-polluted compared to reference (unpolluted) streams. We also examined the direct effects of exposure to metals and parasites on mayfly behavior and the indirect effects on the predator-prey relationship between stoneflies and mayflies. To assess the response of *B. bicaudatus* to biotic and abiotic stressors, we conducted experiments in streamside microcosms and compared the foraging and drift dispersal behavior of parasitized and unparasitized individuals originating from metal-polluted and non-polluted streams. In addition, we experimentally assessed predation rates of *Baetis* by stoneflies (*Megarcys signata*) in response to exposure to metal pollution and mayfly parasitism. We found that prevalence of parasites in mayflies was uniformly high in metal-rich streams, but highly variable in reference streams. Parasitized mayflies from all streams more actively foraged for algal resources, and parasitized mayflies from a metal-rich stream were more susceptible to predation by stoneflies than parasitized mayflies from unpolluted streams and all unparasitized individuals. These results agree with previous studies on aquatic insect responses to environmental stressors. Furthermore, observed induced changes in mayfly behavior show that abiotic stressors and parasitic infections may mediate predator-prey interactions in aquatic insect communities.

Introduction

Environmental stressors have been shown to alter community structure by impacting the physiological, morphological, and behavioral traits of organisms (Menge and Sutherland 1987, Menge and Olson 1990). Abiotic stressors resulting from natural and human related activities may affect organisms directly, but also indirectly by altering interactions between species (Mogren and Trumble 2010, Pietrock and Marcogliese 2003). In addition, biotic stressors such as predation and parasitism, have been shown to regulate the impact of abiotic influences on organisms, by causing behavioral modifications as well as changes in survival, growth and fecundity of prey organisms (Peckarsky et al. 1993, Gerard et al. 2008).

Metal pollution is a major stressor on freshwater ecosystems, particularly those in the Colorado Rocky Mountains, due to various mining, smelting and industrial activities (Mogren and Trumble 2010). Throughout the Rocky Mountain region, metal-laden discharge coming from abandoned as well as active mines is a growing problem (Kiffney 1996). Aquatic macroinvertebrates, particularly insects, may be exposed to those contaminants at toxic levels (Clements et al. 1989) and may bioaccumulate already high levels of dissolved and particulate metals present in stream water (Mogren and Trumble 2010). When predators and parasites feed on insects containing heightened concentrations of metals, they further bioaccumulate those contaminants (Mogren and Trumble 2010).

Currently, there has been very little research investigating parasitism of aquatic insects in metal-contaminated, high-elevation streams (Peckarsky, B. L., personal communication). Our study aims to assess host-parasite interactions between the mayfly *Baetis bicaudatus* and a mermithid nematode parasite (*Gasteromermis* sp.) in metal-polluted streams in comparison to unpolluted streams of the region. Mermithid nematodes infect larval mayflies as early instars,

develop inside the hemocoel of their hosts until the late instar stage, and remain in the host during emergence to the winged adult stage (Vance and Peckarsky 1996). Previous studies have found that infection by the mermithid nematode parasite alters dispersal behavior in mayfly larvae (Vance 1996), as well as preference for parasitized larvae by certain aquatic insect predators (Vance and Peckarsky 1997).

When infected by parasites or exposed to pollution, aquatic invertebrates including mayfly larvae are faced with behavioral tradeoffs associated with foraging, which comes with physiological costs, and may increase the risk of predation (Cummins and Wilzbach 1988, Mullens and Luhring 1996, Kamiya and Poulin 2012, Hernandez and Peckarsky 2013). Mayflies drift in the water column to search for food (Kohler and McPeck 1989), and also as a predator-avoidance behavior that is effective against invertebrate predators (Peckarsky and McIntosh 1998). However, drifting mayflies are conspicuous prey for visually-feeding trout, which presents a tradeoff between food acquisition and predator-avoidance behaviors (Hernandez and Peckarsky 2013) that may be affected by parasitic infections.

Several studies have evaluated the independent effects of either metal exposure (Mogren and Trumble 2010) or parasitism (Vance 1996) and predation (Peckarsky and McIntosh 1998) on mayfly behavior; however, there has been no research that examines the interactive effects of both parasitism and metal pollution on the behavior and rates of predation by stoneflies on mayflies. However, the interactive effects of parasitism and metal pollution have been studied in terrestrial insects, and although metals have been shown to significantly modify host behavior, they have minimal effects on infectivity by parasites (Gintenreiter and Nopp 1993, Kazimirova et al. 1997). Those metal-induced changes in host behavior suggest that environmental stressors may increase rather than decrease prey susceptibility to predation. Furthermore, Clements et al.

(1989) found that heavy metal exposure increased susceptibility of both caddisflies and mayflies (*B. tricaudatus*) to predation by stoneflies.

The goals of this study are to implement a field survey comparing the prevalence of mermithid infections of mayflies in polluted and unpolluted streams, and to assess the responses of *B. bicaudatus* to biotic and abiotic stressors individually and interactively. To accomplish the second goal we performed experiments to compare the foraging and drift behavior of parasitized and unparasitized individuals originating from metal-polluted and non-polluted streams. We also measured predation rates of *Baetis* by stoneflies (*Megarcys signata*) in response to exposure to metal pollution and mayfly parasitism.

Methods

Study Organism

Mayflies are among the most metal-sensitive stream invertebrates (Kiffney 1996), making them a relevant organism for this study and are also useful for biomonitoring streams (Barbour et al. 1992). *Baetis bicaudatus* (Ephemeroptera: Baetidae) larvae are highly abundant algal grazers in high elevation Rocky Mountain streams, and are main prey items of large predatory stoneflies such as *Megarcys signata* (Allan 1983, Peckarsky and Penton 1989) and also trout predators (Allan 1981). *B. bicaudatus* early instar larvae can be infected by the mermithid nematode, an obligate endoparasite that completes its larval stage in a diversity of aquatic invertebrates and has only one host (Vance and Peckarsky 1996). Infection by the mermithid not only sterilizes hosts, but also causes morphological and behavioral sex reversal in *B. bicaudatus*, converting males to females, which then return to the aquatic habitat as if to oviposit and thereby disperse the parasite to locations with abundant vulnerable hosts (Vance 1996, Cromwell, K,

personal communication). Prevalence of the mermithid worm in *Baetis* varies across temporal and spatial scales in unpolluted streams (Vance and Peckarsky 1996, Robbins and Peckarsky unpublished manuscript). We examined the contribution of metal pollution to observed variation of parasite prevalence in mayflies by conducting a field survey to compare mermithid prevalence in streams with high and low metal concentrations.

Study Sites

We collected organisms from streams in two drainage basins: the upper East River near the Rocky Mountain Biological Laboratory (RMBL) and Coal Creek near Crested Butte, in Gunnison County, Colorado (Figure 1). While there has been some historic mining in the Upper East River drainage basin, levels of heavy metals are low (Bothwell et al. 2012) relative to those in Coal Creek, which is a highly mineralized drainage basin (Brown, W. S., unpublished data, Peckarsky and Cook 1981). Available data on heavy metal concentrations shows that streams in the Coal Creek drainage basin have considerably higher levels of heavy metals, such as lead, zinc, cadmium and aluminum than those in the Upper East River drainage basin (Peckarsky and Cook 1981, <http://www.coalcreek.org/filesandpublications>).

The Coal Creek watershed was actively mined from 1874-1974, and is currently under consideration for future mining because it contains both natural metal deposits, which have resulted in acidic metal-laden drainage from waste rock and mill tailings upstream (Shanklin and Ryan 2006). Peckarsky and Cook (1981) found that the stream reach downstream of the mine effluent after a catastrophic break in a tailings dam from the Keystone Mine was unsuitable for benthic communities, and percent mortality of *B. bicaudatus* was significantly higher downstream of the effluent compared to an upstream reference reach. A recent biomonitoring study reported that most stream sites along Coal Creek are ranked as ‘poor’ with respect to heavy

metal pollution, despite cleanup efforts by the USEPA at sites downstream of the Standard Mine on Elk Creek, a tributary of Coal Creek (Brown, W.S., unpublished data).

Surveys of parasite prevalence in metal-rich and unpolluted reference streams

To compare parasite prevalence in metal-polluted and unpolluted reference streams, we took benthic invertebrate samples with a D-frame net with 500 μ m mesh at stream sites in both drainage basins in mid-July, 2013 when mermithids typically achieve sufficient size in their mayfly hosts to be easily detected (Cromwell, K., unpublished data). We collected random samples of 100 *Baetis bicaudatus* from five sites in the Coal Creek drainage basin (Coal Creek 05, Coal Creek 11, Coal Creek 15, Elk Creek 00 and Evans Creek), and eight sites along the East River to serve as non-polluted reference streams (Emerald Lake Outlet, Upper Bradley, Upper Rock, Oregon Mine Road Crossing, Marmot, Upper Benthette 3, East River at Hunter riffle, and Copper Creek in the RMBL townsite). The mayflies were preserved in 70% ethanol and taken to a laboratory to be dissected to estimate the prevalence of the mermithid parasite at each site.

At each site we installed Onset HOBO Pendant temperature loggers (Pocasset, MA, USA) took GPS coordinates with a GeoXT (Trimble 2005 series, Terrasync software), measured stream width and average water depth, pH (Hannah Instruments meter) and conductivity (YSI Model 85 meter). We also took qualitative samples of benthic algae to compare the composition of the algal assemblages in polluted and unpolluted streams. Oviposition habitat for *Baetis* at each site was scored as good or poor based on the number of large exposed rocks in places with high flow. Oviposition substrates are important because parasitized adult *Baetis* return to streams and crawl under the same rocks on which healthy females oviposit (Encalada and Peckarsky 2006, Cromwell, K, unpublished data). Therefore, streams with high levels of prevalence may be those with the best places for worm transmission to the next generation of *Baetis* (Cromwell, K,

personal communication). Those stream attributes were measured with the intention of pairing sites between metal-polluted streams and unpolluted streams to maximize the probability that the differences in parasite prevalence between the streams in the two drainages are due to metal pollution as opposed to other factors. To facilitate those pairings, we did a preliminary screening of sites in early July to select sites with abundant populations of *B. bicaudatus* that could be matched with well-studied locations in the East River drainage (Cromwell, K, unpublished data).

The original survey design consisted of comparing 10 stream sites (Figure 1), five with low metals and five metal-rich, and to match sites along the East River to sites in Coal Creek with similar attributes that are known or suspected to influence parasite prevalence, such as hydrology (discharge), water temperature (Robbins and Peckarsky unpublished manuscript), and *Baetis* oviposition habitat (Cromwell, K., unpublished data). However, due to unexpectedly accelerated phenology of *B. bicaudatus* at metal-rich sites, most of the mayflies had already emerged from the aquatic larval stage to the terrestrial adult stage by the time collections were made. Consequently, only two sites (Elk 00 and Evans, Figure 1) had sufficient numbers of *B. bicaudatus* to compare to levels of parasitism in the unpolluted sites. Therefore, we were unable to do formal statistical comparisons of levels of prevalence between matched sites in each drainage basin, and instead, have presented a graphically comparison of the infection rates between the two metal-polluted sites and all eight non-polluted sites.

Experiments testing pollution and parasitism effects on Baetis behavior and predation

Experiments were carried out in streamside microcosms used in previous studies at the RMBL. The system of microcosms is comprised of 60 circular flow-through chambers, each 15 cm in diameter, powered by natural streamwater from a nearby, unpolluted fishless stream and housed in a weatherport (white portable greenhouse, Hansen Weatherport, Delta, CO) for shade

(see Peckarsky and Cowan 1991, Vance 1997). Clements (1999) used a similar set-up to examine the effects of heavy metal exposure on macroinvertebrate communities and species interactions. *Baetis* behavior, including drift dispersal and foraging activity, were compared between individuals originating from polluted (Coal Creek) and unpolluted (East River) sites. Our experimental design included four treatments: six parasitized *Baetis* originating from high metal streams, six parasitized *Baetis* originating from low metal streams, six healthy *Baetis* from high metal streams, and six healthy *Baetis* from low metal streams. This experiment was completed in mid July using late instar *Baetis* because parasitism can be identified without dissection (mermithid worms can be observed through the cuticle at this stage). Due to scarcity of *B. bicaudatus* in the metal-rich sites of Coal Creek, the experimental treatments had unequal replication. We stocked 14 microcosms with parasitized and 14 with unparasitized *Baetis* from East River sites (low metals), and four microcosms with parasitized and four with unparasitized *Baetis* from Coal Creek (high metals). The floor of each chamber was lined with six unglazed ceramic tiles as substrates to be colonized by algae as a food source for *Baetis* because they allow behavior to be more easily observed than natural rock substrates. We prepared tiles by placing them in flow-through cattle watering tanks near the weatherport with fishless water from the same fishless, unpolluted source as the microcosms, and allowing them to acclimate in the sun for one week adding plant fertilizer containing NH_4 and PO_4 (Continuous Release Bloom Booster, Scotts Miracle-Gro Company, Marysville, OH, USA) to facilitate growth of algae. This method standardized the amount of algae growth on the tiles, to avoid effects of uneven food levels on *Baetis* drift and foraging behavior (Alvarez and Peckarsky 2005, Hernandez and Peckarsky 2013). Onset HOBO Pendant temperature loggers (Pocasset, MA, USA) installed in the drains of the microcosm channels showed that the mean, maximum and minimum water

temperatures (degrees C) during the experiment were 6.6 ± 0.02 , 11.8 ± 0.19 , and 4.7 ± 0.06 , respectively, which mimics water temperature fluctuations in the stream water delivered to the microcosms.

We collected approximately 200 *B. bicaudatus* using D-frame nets at East River sites (one with high prevalence: Marmot and one with low prevalence: Upper Benthette 3), and used dissecting microscopes to sort parasitized and unparasitized individuals. Alka-Seltzer tablets dissolved in water were added to water to suppress larval movement, allowing observers to detect mermithid worms more efficiently. We were only able to collect 75 *B. bicaudatus* from Coal Creek (Elk 00), resulting in smaller replication of parasitized and unparasitized individuals for high-metal treatments.

Baetis were acclimated in the microcosms for seven hours before the first behavioral observations were made. Drift behavior in response to pollution and parasitism was measured by comparing estimates of per capita drift rates of *Baetis* in channels subjected to the four treatments. Foraging behavior was quantified by counting the number of *Baetis* feeding on exposed algal-rich top and side surfaces of tiles. Although *Baetis* originating from fish streams are mostly nocturnal, *Baetis* from fishless streams may be more day active (Peckarsky and McIntosh 1998); therefore, behavioral observations took place at two times of day: 9 PM (23 July 2013) and 9 AM (24 July 2013) MDT to test whether parasitism, exposure to heavy metals, or their interaction affect the risks taken by *Baetis* while foraging. Foraging during the daytime is more risky because brook trout are visual feeders on drifting prey (McIntosh et al. 2002). For each observation, four observers each scanned one quarter of the microcosms and counted the number of individuals visibly foraging on exposed surfaces, and subsequently counted the numbers drifting past a transect of each microcosm for one minute. Treatments were randomized

among the microcosms to avoid position effects, and independent observers recorded behaviors blind to treatments to avoid bias.

Statistical Analysis

Mayfly foraging behavior was compared among parasitized and unparasitized individuals originating from low-metal and high-metal streams using a Type 3 Sums of Squares 2-factor ANOVA with an unbalanced design (R Development Core Team 2008). For statistical tests, we combined the numbers of individuals exposed or drifting (see below) during both day and night observations to obtain total foraging activity and total drift.

Drift frequencies were very low, containing many zero values. Therefore, we used a contingency table analysis to compare the total number of individuals drifting in all channels with parasitized and unparasitized mayflies from high (Coal Creek) and low metal (East River) sites. Because of the unequal replication across high (N=4) and low-metal (N=14) treatments, we used a bootstrap technique to randomly sample 20,000 sets of 4 replicates (without replacement) from each of the 14 low-metal treatments for parasitized and unparasitized mayflies. Total drift counts were summed for each of the 20,000 replicates, and the median value was used in the contingency table analysis (Fisher's exact probability test) to compare frequency of drift among high and low metals and parasitized and unparasitized treatments.

Preliminary Predation Trials

In early July, pilot predation trials were conducted on healthy *Baetis* to estimate feeding rates of female and male *Megarcys* stoneflies over different time periods, to determine a reasonable duration for experimental trials during which exploitation of prey by predators would be approximately 50% of those available (3 of the 6 *Baetis* in each microcosm). Approximately

300 *B. bicaudatus* were collected from Copper Creek, a low-metal East River site, and six individuals were added to each of 48 microcosms in the weatherport. We also collected 12 female and 4 male *Megarcys* from Copper Creek and immediately added them to 4 flow-through holding microcosms lined with larger rocks to starve for 24 hours (note: five individuals were only starved for 5 hours).

Microcosms containing *B. bicaudatus* were exposed to predators for four different time durations: 1 h, 2 h, 4 h, and 16 h and controls with no *Megarcys* were used to correct for non-predatory losses of *Baetis*. Predation trials began at 5 PM MDT because these stoneflies have previously been shown to be nocturnal predators (Peckarsky and Penton 1989). Based on the results of this preliminary trial and previous studies, we chose to use female *Megarcys* for the full experiment because males were less abundant, and generally eat fewer *Baetis* than females (Peckarsky et al. 1993). Furthermore, since the 16 h (overnight) treatment resulted in an average feeding rate of 1.5 *B. bicaudatus*, to increase exploitation, we starved *Megarcys* in the full-design experiment for twice as long (48 h) and conducted the full experiment for 16h.

Predation Trial

The full design predation trial was implemented using one predatory stonefly in half of the chambers of each treatment, resulting in eight treatments (fully-crossed design with two levels of each treatment: predator x parasite x pollution). Chambers containing no stoneflies served as controls to correct for non-predatory losses of *Baetis*. Two days before the predation trial (22 July 2013) stoneflies (female *Megarcys signata*) were collected from the Emerald Lake Outlet (the headwaters of the East River), where this species is very abundant. *Baetis* used for predation trials were the same individuals that were observed on 23 and 24 July and stoneflies were added at 5 PM 24 July and allowed to forage until 9 AM 25 July.

Predatory losses were calculated for each chamber by subtracting the mean number of *Baetis* missing from control channels with no predators (note: only one *Baetis* was missing from all controls combined) from those missing from channels with corresponding parasite and metal-exposure treatments where *Megarcys* had been foraging. All *Baetis* remaining in the channels were preserved and dissected to determine if parasitized and unparasitized individuals were accurately identified prior to the experiment. *Megarcys* retrieved from microcosms with missing prey were also preserved and dissected to check whether *Baetis* observed in their guts were parasitized. However, gut contents were too well digested to provide a definitive test of the infection status of prey consumed. Replicates containing more than two errors would have been omitted from the analyses; however most replicates had one or no errors in identification. Subsamples of organisms were preserved at the end of the experiment and average ($\pm$ 1 SE) head capsule widths of *Baetis* ($\mu=1.726$ mm, $SE=0.07453$) and *Megarcys* ($\mu=4.328$ mm, $SE=0.2244$) were calculated.

Statistical Analysis

Data analyses for predation effects consisted of a two-way completely cross-classified two-way Type 3 Sums of Squares analysis of variance (ANOVA) with an unbalanced design ($N = 4, 4, 6, 7$ for high metals parasitized, high metals unparasitized, low metals parasitized and low metals unparasitized) comparing consumption (predatory losses) of parasitized and non-parasitized *Baetis* collected from metal polluted and non-polluted streams. The replication of the high metals treatments was limited by availability of *Baetis* in Elk Creek, and one stonefly emerged to the adult stage in a low metals parasitized treatment, reducing replication from 7 to 6. This analysis tested whether parasitism, exposure to metals, or their interaction affected the vulnerability of *Baetis* individuals to stonefly predation. Data were tested for normality and

homogeneity of variance, and transformed if necessary to conform to the assumptions of the ANOVA (R Development Core Team 2008).

Results

Surveys of parasite prevalence in metal-rich and reference streams

Given that we were unable to complete the matched site design of the survey, and studies of the East River sites are ongoing (data collection by Kara Cromwell is not yet complete), attributes of only the Coal Creek sites, which are new to studies of parasite prevalence, are presented here. The sites within the Coal Creek drainage basin had variable physical characteristics, including attributes that could influence the prevalence of mermithid parasites (Robbins and Peckarsky unpublished manuscript) (Table 1a). However, qualitative samples of benthic algae (taken by M. A. Penton) showed the algal assemblages were similar among Coal Creek and East River sites, despite the differences in heavy metal concentrations (Table 1b). All sites contained predominantly *Oscillatoriales*, *Hydruras*, and *Diatoma*.

Mermithid prevalence in *B. bicaudatus* was uniformly high in the two high metal sites (44% and 79%), but was highly variable in the low metal sites ranging from 2 – 60% (Figure 2). This study will be repeated earlier in summer 2014 before *B. bicaudatus* emerges from the Coal Creek sites to obtain more definitive data that can be subjected to formal statistical comparison of mermithid prevalence between high and low metal sites.

Experiments testing pollution and parasitism effects on Baetis behavior and predation

Behavior

Mayfly foraging behavior was significantly influenced by parasitism. Parasitized individuals were observed foraging on exposed surfaces of tiles in significantly greater numbers than unparasitized individuals ($P = 0.0368$, $F = 4.75$, $DF = 1$) (Figure 3). However, metal exposure did not influence foraging behavior on exposed surfaces of tiles ($P = 0.6172$, $F = 0.2548$, $DF = 1$).

The distribution of drift frequencies among treatments was not significantly different from that expected by chance alone (Fisher's Exact $P = 0.07$), suggesting that drift dispersal was not affected by parasitism or metals (Table 2). However, this result could be a consequence of low power to detect significant differences among treatments. Nonetheless, preliminary drift data suggest a trend showing that parasitized individuals from high metal streams drifted at least four times more frequently than individuals from unparasitized individuals and parasitized individuals from the low-metal stream (Figure 4).

Predation Trials

The two-way Type 3 Sums of Squares ANOVA showed significant independent effects of parasitism ($P = 0.0055$, $F = 9.07$, $DF = 1$) and metal exposure ($P = 0.0078$, $F = 10.1$, $DF = 1$) on the number of live mayflies remaining per channel after exposure to stoneflies (Figure 5). Specifically, parasitized individuals were more susceptible to predation by stoneflies than unparasitized individuals (Figure 5a) and mayflies originating from metal-rich streams were consumed in greater numbers than those originating from non-polluted streams (Figure 5b). There was also a significant interaction between the effects of parasitism and metal exposure on mayfly susceptibility to predation, indicating that the impact of parasitism on mayfly survival depends on the level of metal exposure ($P = 0.03509$, $F = 5.24$, $DF = 1$), and the treatment most

susceptible to predation by stoneflies was parasitized mayflies originating from metal-rich streams (Figure 6).

Discussion

The goals of this study were to compare mermithid infection rates between *Baetis* originating from metal-rich and non-polluted streams, and to examine the extent to which long-term metal exposure and mermithid parasitism directly influence *B. bicaudatus* foraging and dispersal, and indirectly affect the susceptibility of mayflies to predation by predatory stoneflies, *Megarcys signata*. While the results of the prevalence survey are preliminary, they suggest that prevalence is consistently higher in metal-rich streams, while a range of infection rates, most sites containing lower mermithid prevalence, were observed in the non-polluted reference streams. In addition, we found that parasitized individuals originating from both metal-rich and unpolluted streams more actively searched for food on algal-rich exposed surfaces of substrates in microcosms, but drift dispersal behavior was not significantly influenced by metals or parasitism. While both exposure to metal pollution and parasitism independently increased the susceptibility of *Baetis* to predation by stoneflies, parasitized *Baetis* from the metal-rich stream in Coal Creek were consumed by stonefly larvae in the highest numbers as indicated by a significant interaction between parasitism and metal exposure.

Preliminary survey data suggest that mayfly hosts are more negatively affected by metal exposure than the mermithid parasite, based on the uniformly high infection rates observed in the Coal Creek sites and lower, but highly variable prevalence found in most of the East River sites. The direction of effects of abiotic stressors on species interactions is potentially related to the

predator and prey stress models proposed by Menge and Olson (1990). The relationship between parasites and hosts has been described as a manifestation of the general predator-prey interaction (Anderson and May 1978), and we can therefore expect similar outcomes predicted by predator-prey environmental stress models proposed by Menge and Olson (1990).

Prey stress models assume that prey are more sensitive to environmental stress than predators (or parasites), resulting in greater susceptibility to predation; thus, higher levels of predation are predicted under stressful environmental conditions. In contrast, predator stress models predict that predators (or parasites) are affected to a greater extent than prey in high stress environments, reducing predation rates, thereby releasing prey from predation and potentially leading to increased competition among prey (Menge and Olson 1990). Our preliminary results agree with the latter prediction, which suggests that parasite prevalence is higher in polluted streams because host immunity is compromised resulting from metal toxicity.

Little research provides support for the first scenario, in which parasites are influenced to a larger extent than their hosts. However, in one example, Pietrock and Marcogliese (2003) found that toxicants reduced survival of free-living nematodes. In contrast, host populations have been widely shown to be affected by abiotic stressors through behavioral changes that decrease both their fitness and survival. When infected by parasites or exposed to pollution, mayfly larvae (Peckarsky and McIntosh 1998) and other aquatic invertebrates (Cummins and Wilzbach 1988, Kamiya and Poulin 2012, Mullens and Luhring 1996) exhibit fitness costs associated with behavioral tradeoffs between foraging and predator-avoidance, potentially caused by the physiological costs of parasitic infections and abiotic stressors.

Although drift behavior was not significantly influenced by metal exposure or parasitism, limited data were suggestive of a trend that parasitized *Baetis* originating from metal-rich

streams drifted more frequently than unparasitized *Baetis* and individuals or both healthy and infected individuals from non-polluted streams. Many aquatic insects use drift as a food search and predator-avoidance behavior (Hernandez and Peckarsky 2013). Metal pollution has been shown to significantly increase drift in some mayfly species (Clements 1999), while *Baetis tricaudatus* (of close relation to *B. bicaudatus*) drifted less when exposed to metals in a recirculating stream system (Mogren and Trumble 2010). Similarly, although mermithid infection of *B. bicaudatus* caused a decrease in drift frequencies, parasitized individuals drifted higher in the water column, and consequently increased their susceptibility to predation (Vance 1996). Furthermore, many studies have reported increased exposure to pollution in streams results in an overall increase drifting invertebrates (e.g. Hall et al. 1980), which illustrates that abiotic stressors can have indirect effects on predator-prey interactions, thereby influencing stream communities.

In our experiment, parasitism caused a significant increase in foraging activity, in which parasitized *Baetis* foraged on algal-rich exposed on tiles more frequently than unparasitized individuals. This increased activity suggests that the physiological costs of feeding the parasite cause mayfly larvae to spend more time foraging and less time hiding, potentially leading to an increased risk of encountering predators. Other studies have found that parasitism in mayflies (Vance 1996, Cromwell, K, unpublished data) and other stream invertebrates (Mogren and Trumble 2010) results in increased foraging activity, which could compromise anti-predatory behavior. Specifically, freshwater crustaceans have consistently displayed more active foraging and less time hiding from predators when infected by parasites (Maynard et al. 1998, Seppala et al. 2008).

We did not detect significant effects of metal exposure on mayfly foraging or anti-predatory behavior, possibly due to very low replication within metals treatments. However, several other studies on aquatic insects have investigated the mechanistic causes of long-term metal exposure impacts of on anti-predatory behavior. Not only does metal exposure alter insect behavior and life-history traits (Hall et al. 1980), but it may also directly affect morphology and physiology. Heavy metals in stream water can lead to increased acidity, which has also been shown to have physiological costs in arthropods, such as reduced oxygen consumption and respiration efficiency (Thurberg, Dawson, and Collier 1973). Furthermore, reduced mayfly growth has also been documented in metal-polluted streams, possibly resulting from the metabolic costs involved in regulating ion exchange under these conditions (Hermann and Anderson 1987).

The weakening of prey health and predator avoidance behavior caused from parasitism and metal exposure can impact prey susceptibility to predation. Parasite-induced behavioral modifications affecting host susceptibility to predation has been documented previously in *Baetis bicaudatus* (Vance and Peckarsky 1997) as well as in other host organisms, including larval amphibians (Han, Searle, and Blaustein 2011) and snails (Kamiya and Poulin 2012). In our experiment, mermithid infected mayflies from metal-rich streams were the most susceptible to predation, showing a combined effect of parasitism and metal exposure. One possible mechanism of this effect could be because greater foraging activity of parasitized individuals and higher drift rates of parasitized individuals exposed to metal pollution could increase the susceptibility of parasitized individuals to stonefly predation.

In summary, this study shows that both abiotic and biotic stressors, and their interactions, can affect insect behavior, which can lead to changes in predator-prey interactions. Due to these

changes in species interactions, metal pollution and parasitism can thereby alter the structure of aquatic macroinvertebrate communities. These results further highlight the important implications of considering the impact of multiple stressors on species interactions.

Acknowledgments

I would like to thank my project mentor, Barbara Peckarsky, for continued guidance and support in the designing, implementing of this study, as well as preparation of the manuscript. I give special thanks to the 2013 Benthettes: Kara Cromwell, Marge Penton, Aaron Koning, Wendy Brown, Steve Horn, Maruxa Alvarez, Andrea Landiera, Bobby Pogoloff, Jeff Mold, Joanna Hard, Jose Sanchez, and Emily Mooney for various contributions in data collection, analysis, and manuscript revisions. I also thank the NSF REU program, Rocky Mountain Biological Laboratory coordinators, staff, and project mentors for encouragement and funding for this project.

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Tables

Table 1a. Physical attributes of the Coal Creek and East River stream sites, including heavy metal content, stream size, *B. bicaudatus* oviposition habitat quality, water acidity, conductivity and temperature.

Site	Date	Heavy Metals	width (m)	avg depth (cm)	Oviposition Habitat	pH	Conductivity (µs/cm)	Water Temperature (deg C) (7 July - 6 Aug 2013)		
								Average	Maximum	Minimum
Coal Creek Drainage										
Coal 05	28-Jun-13	High	5.8	24.4	Good	7.82	147	13.1	19.1	9.0
Coal 11	28-Jun-13	High	5.8	24.0	Good	7.76	88	12.8	21.3	8.6
Evans	28-Jun-13	High	1.3	8.3	Good	7.65	69	9.2	18.9	7.0
Elk 00	28-Jun-13	High	2.8	17.8	Good	7.89	135	9.0	15.4	6.6
Coal 15	28-Jun-13	High	4.6	21.8	Good	7.98	80	11.9	17.9	8.2
East River Drainage*										
Emerald Lake Outlet	2-Jul-13	Low	3.8	7.3	Good	8.14	266			
Oregon Mine Rd Crossing	2-Jul-13	Low	2.6	20.6	Good	8.19	234			
Upper Bradley	2-Jul-13	Low	5.0	13.7	Good	8.32	215			
Upper Rock	2-Jul-13	Low	3.65	13.4	Good	8.14	149			
Marmot	2-Jul-13	Low	0.9	8.0	Good	8.51	266			

* Temperature data from the East River sites not yet downloaded

Table 1b – Algal taxa collected from Coal Creek and East River stream sites.

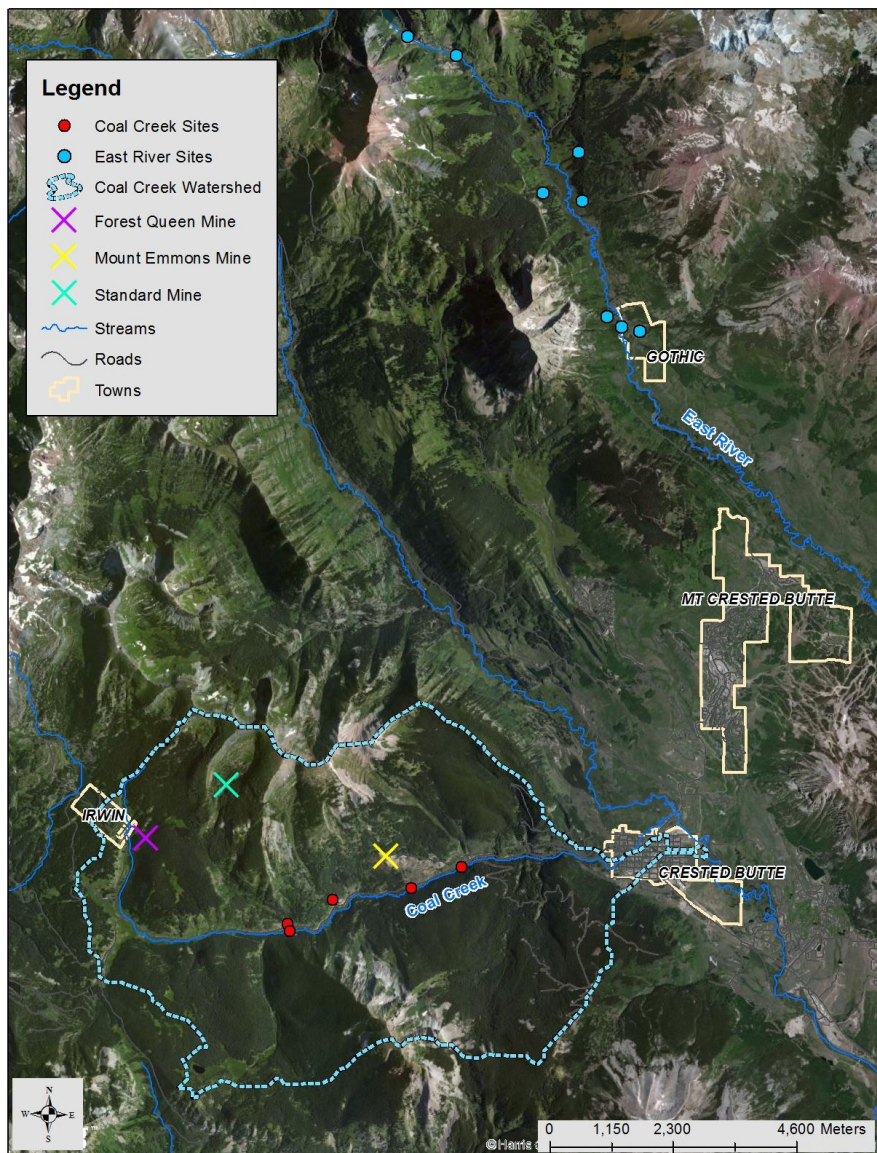
Site	Date	Dominant algal taxa present
All samples were taken from rocks (epilithic) and in flowing water		
Coal Creek Drainage		
Coal Creek 05	7/7/2013	Diatoms (Bacillariophyta) are dominant (Order Fragilariales, cymbelloid diatoms, <i>Hannaea arcus</i>) . Also present: unbranched filamentous green algae, a few desmids (green alga), filamentous cyanobacteria and coccoid chrysophyte algae.
Coal Creek 11	7/7/2013	A filamentous Cyanobacteria (Order Oscillatoriales) was dominant. Also present: unbranched filamentous green algae and a few desmids, diatoms (Order Fragilariales, cymbelloid diatoms, <i>Hannaea arcus</i>), golden palmelloid cells (chrysophytes).
Evans Creek	7/7/2013	The Chrysophyte <i>Hydruras</i> was dominant. Also present: diatoms (<i>Hannaea arcus</i> and <i>Diatoma</i>), a few desmids (green algae).
Elk Creek 00	7/7/2013	Cyanobacteria dominant- mainly <i>Chamaesiphon</i> and a filamentous blue-green (Order Oscillatoriales). Also present: diatoms, mainly <i>Diatoma</i>
Coal Creek 15	7/7/2013	Diatoms are dominant. The most abundant diatoms were Order Fragilariales, but also present <i>Hannaea arcus</i> , cymbelloid and naviculoid diatoms, <i>Rhopalodia</i> , <i>Diatoma</i> , a centric diatom, and <i>Didymosphenia</i> . A few green algae (desmids) were present. Note: on July 16 2013 the blue-green bacteria <i>Nostoc</i> was present.
East River Drainage		
B-3 Stream	7/15/2013	The diatom <i>Diatoma</i> was abundant. Other diatoms present <i>Meridion circulare</i> , <i>Hannaea arcus</i> , cymbelloid diatoms and other araphid diatoms. The blue-green bacteria <i>Phormidium</i> was also present.
Marmot Creek	7/15/2013	Mixed algal assemblage. The blue-green bacteria <i>Phormidium</i> formed small mats on some of the rocks. A very small filamentous blue-green in the Order Oscillatoriales was present. A variety of diatoms were present. Also present was <i>Hydruras</i> and a parenchymatous chrysophyte.
Oregon Mine	7/15/2013	Mixed algal assemblage. The green unbranched filamentous alga <i>Ulothrix</i> was present. Also present were: <i>Hydruras</i> , blue-green <i>Phormidium</i> , diatoms <i>Meridion</i> , <i>Hannaea arcus</i> , <i>Diatoma</i> . Gompho-cymbelloid diatoms and diatoms in the Order Fragilariales were found.
Upper Rock Creek	7/15/2013	Algae in low abundance. There was a parenchymatous golden brown alga and a few diatoms such as <i>Diatoma</i> , <i>Hannaea arcus</i> , and diatoms in the Order Fragilariales
Upper Bradley Creek	7/15/2013	Mixed algal assemblage. The green algae <i>Ulothrix</i> spp., the chrysophyte <i>Hydruras</i> , blue-green alga <i>Phormidium</i> were present. The following diatoms were present: cymbelloid diatoms, diatoms in the Order Fragilariales, and <i>Meridion</i> , <i>Diatoma</i> , and <i>Hannaea arcus</i> .

Table 2 – The total number of *B. bicaudatus* drifting in channels during a 2 minute period (counts are pooled for day and night observations) for each of the treatments.

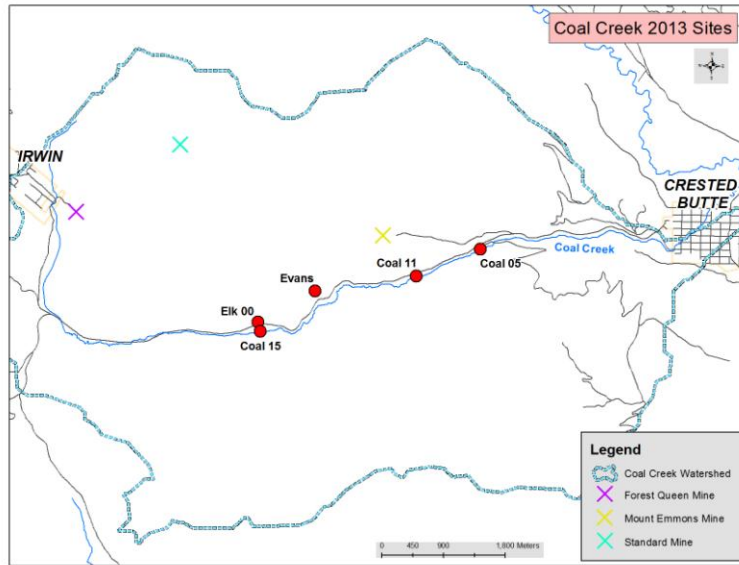
	Parasitized	Unparasitized	Totals
High Metals (Coal Creek)	12	0	12
Low Metals (East River)	3	2	5
Totals	15	2	17

Figures

A.



B.



C.

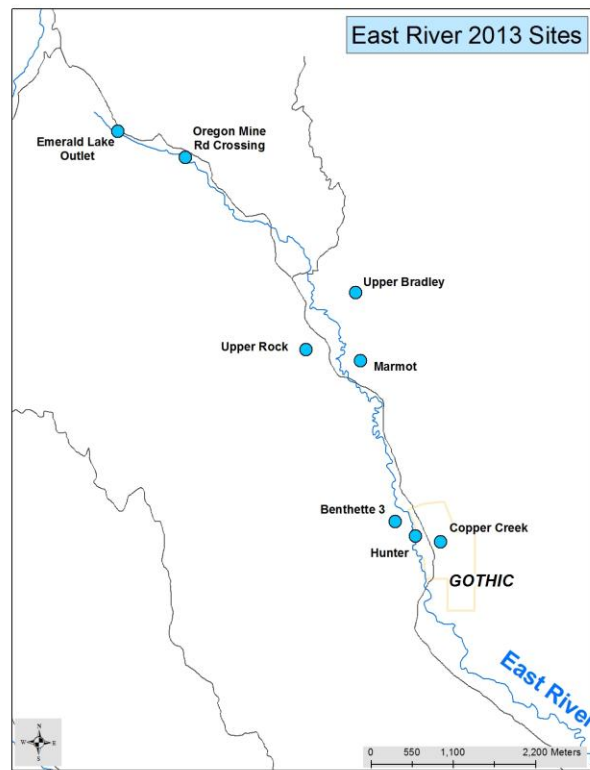


Figure 1. (A.) An aerial map depicting the locations of the Coal Creek and East River stream sites, nearby mining sites, towns, roads, and major streams. Coal Creek (B.) and East River (C.) site locations, including site names.

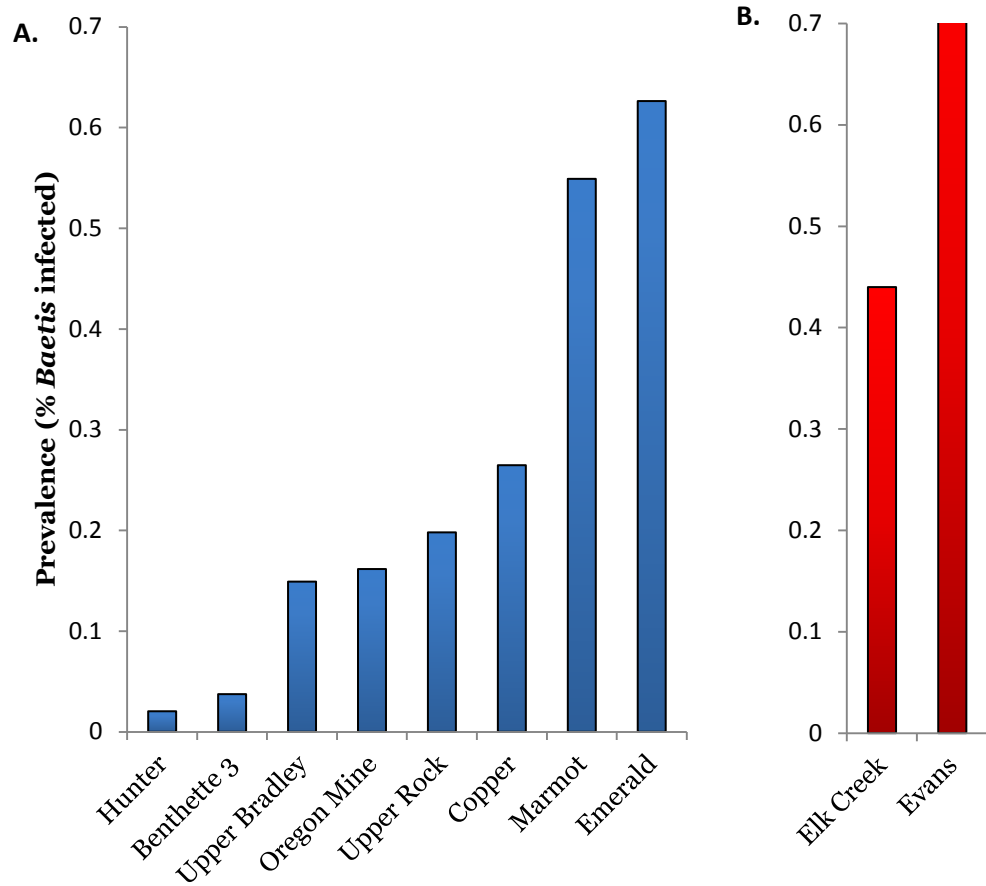


Figure 2. Infection rates of *B. bicaudatus* by the mermithid parasite in stream sites within the East River drainage basin (A.) and Coal Creek watershed (B.).

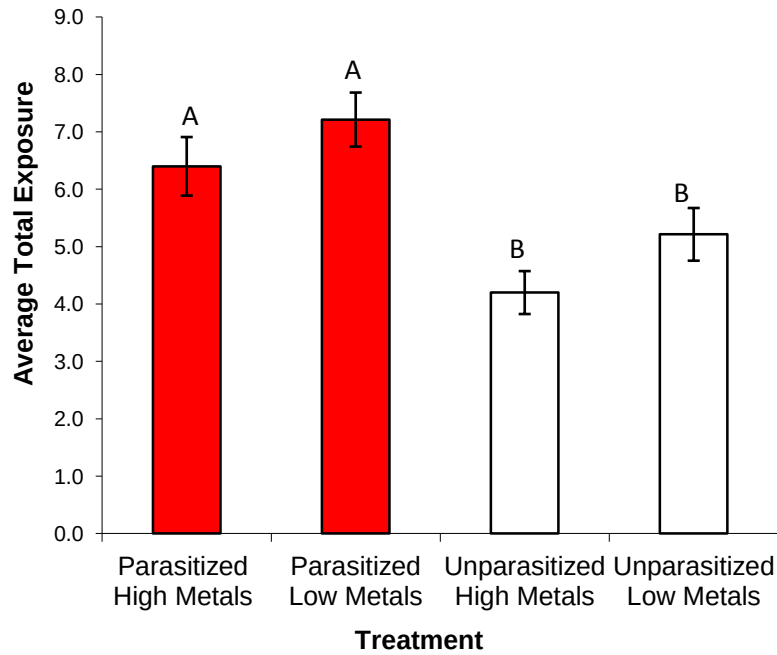


Figure 3. Mean ($\pm$ SE) number of *B. bicaudatus* observed foraging on exposed, algal-rich surfaces of tiles per channel (pooled for day and night observations) in each of the four treatments. Letters above each histogram indicate treatments that are significantly different (Tukey's Test of 2-way ANOVA with type 3 sums of squares).

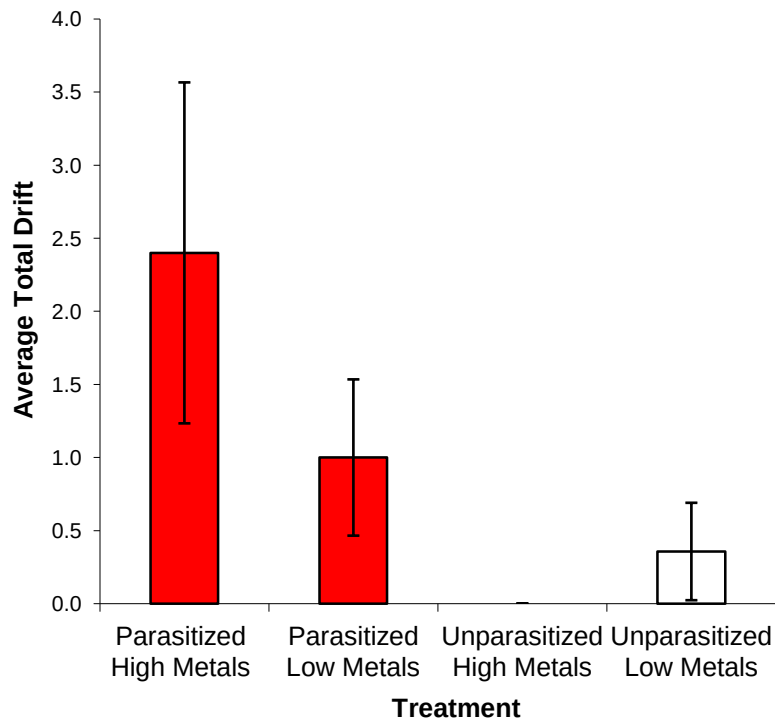


Figure 4. Mean ($\pm$ SE) number of *B. bicaudatus* observed drifting during two minutes per channel (pooled for day and night observations of one-minute each) in each of the four treatments.

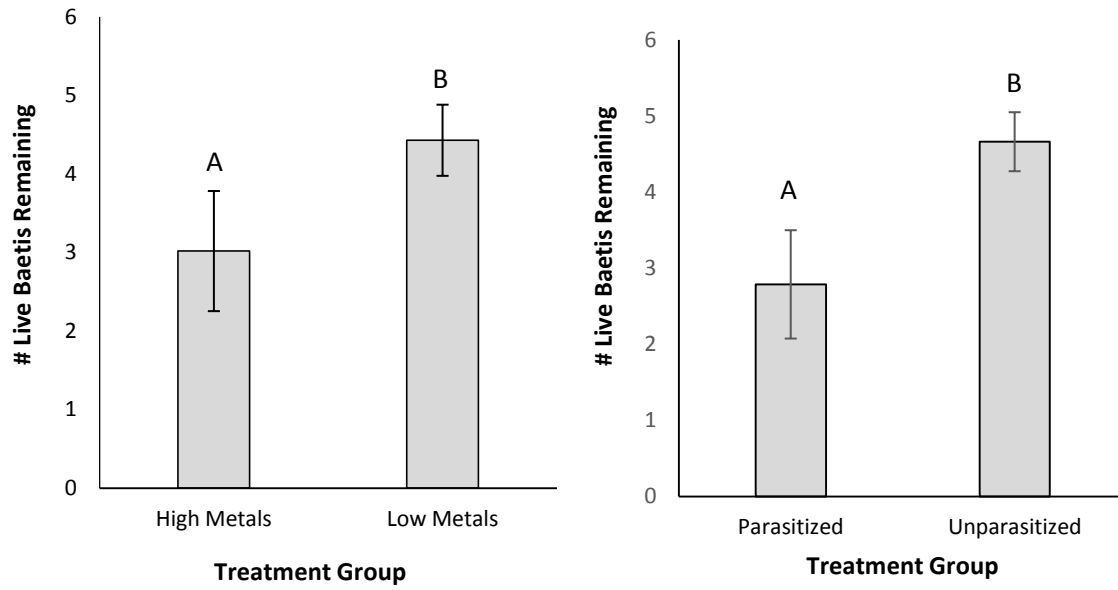


Figure 5. Mean ($\pm$ SE) number of live *B. bicaudatus* remaining per channel after 16 hours of exposure to stonefly predators (*Megarcys signata* females). Data were pooled separately based on the level of parasitism (parasitized vs. unparasitized) and metal exposure (high vs. low) to illustrate independent effects. Letters above each histogram indicate treatments that are significantly different (Tukey's Test of 2-way ANOVA with type 3 sums of squares).

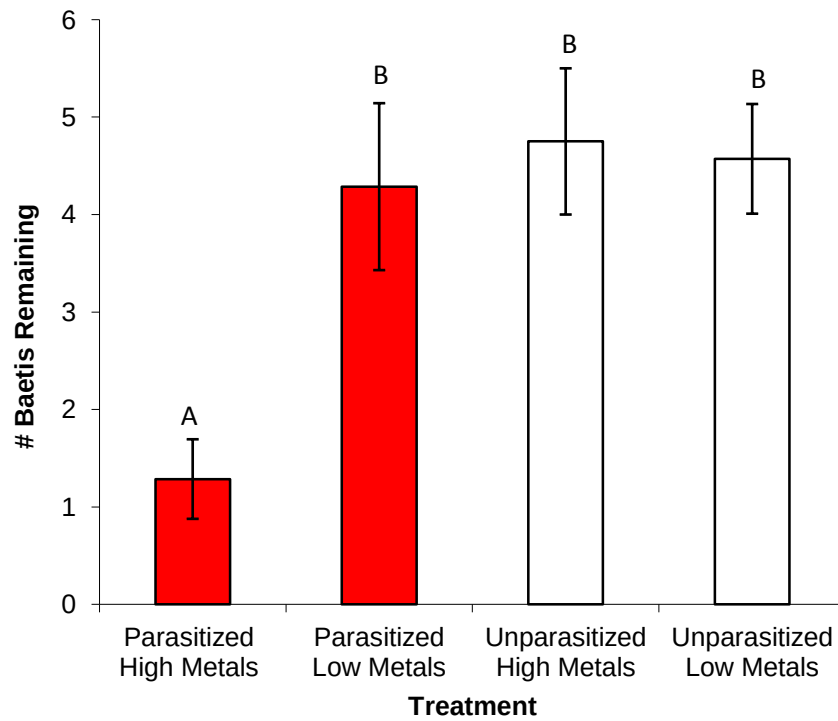


Figure 6. Mean ($\pm$ SE) number of live *B. bicaudatus* remaining per channel after 16 hours of exposure to stonefly predators (*Megarcys signata* females). Letters above each histogram indicate treatments that are significantly different (Tukey's Test of 2-way ANOVA with type 3 sums of squares).