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Evaluation of *Cinygmula* (Ephemeroptera: Heptageniidae) Drift Behavior as an Indicator of Aqueous Copper Contamination

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ABSTRACT

We designed an *in situ* assay for investigating macroinvertebrate drift behavior in response to a point source of environmental pollution. As a model, we used *Cinygmula* nymphs to assess both the presence and level of copper contamination in a stream that was polluted with effluent from an abandoned copper mine. The study showed that *Cinygmula* exhibited increased tendency to drift with increased exposure to aqueous copper. *Cinygmula* drift behavior exhibited graded responses to increasing concentrations of aqueous copper up to and including 78 ppb. The results indicated that a simple yet sensitive *in situ* bioassay could be used to detect environmentally important levels of copper contamination in Haggarty Creek.

KEY WORDS: aqueous copper, *Cinygmula,* in situ bioassay, pollution, drift behavior

INTRODUCTION

Standard laboratory-based, dose-response studies for acute toxicity are often used to determine how some of the aquatic biota respond to specific pollutants (e.g., Dobbs et al. 1994). However, dose-response studies may not take into account sub-lethal effects of some pollutants, especially in a field setting. Drift behavior of benthic macroinvertebrates may be a more sensitive indicator of how pollution affects the aquatic biota than standard laboratory-based, dose-response tests for acute toxicity because benthic macroinvertebrates may release their grip on the substrate and drift downstream when they detect pollutants that are present in relatively low concentrations (Clements 1999). When that occurs, the populations are reduced or are not present in habitats that otherwise would be suitable.

Benthic fauna have been used as indicators of anthropogenic disturbance in some lotic systems. Ormerod et al. (1987) found a drift response of some dipterans, plecopterans, and ephemeropterans, as well as a crustacean, to acidification and acidification plus aluminum. Hopkins et al. (1989) found a substantial drift response of the ephemeroptean, *Baetis,* to acid conditions. Gerhardt et al. (1998) studied the drift response of a crustacean to toxic discharges of copper when developing an online biomonitoring system. Courtney and Clements (1998) found that percent invertebrate drift in most acidic streams was nine times that of the control and also noted that Ephemeroptera was the only insect order to exhibit a significant drift response. Clements (2004) found concentration–response relationships between macroinvertebrate drift and heavy metal concentrations and indicated these were generally more sensitive to metal concentrations than were structural measures such as abundance and richness. Given these studies, we investigated developing an *in situ* bioassay to assess the effects of copper through the drift behavior of Ephemeroptera.

The study system was Haggarty Creek, which is a high-elevation montane stream in the Sierra Madre Range of southeast Wyoming. Portions of this stream are adversely affected by copper-laden effluent from the Ferris–Haggarty copper mine. The effluent has

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reduced the diversity of benthic macroinvertebrates to just a few copper-tolerant species where it flows into the creek, as well as in downstream reaches (Rockwell 2001).

As the effluent-laden water flows downstream, the diversity of benthic macroinvertebrates gradually increases because water entering Haggarty Creek from uncontaminated tributaries dilutes the copper-laden water, making it increasingly less toxic to benthic fauna (Rockwell 2001). As a result, Haggarty Creek exhibits a longitudinal gradient of aqueous copper concentrations, providing a series of sites at which the influence of varying copper concentrations on benthic macroinvertebrate drift could be evaluated.

Individuals in the ephemeropteran family Heptageniidae were chosen as test subjects because of their sensitivity to aqueous metals (Hickey and Clements 1998; Clements 1999). Further, individuals of this family are usually present in high altitude streams of southeast Wyoming as they have morphological adaptations that allow them to cling to the substrate during spring flows. Our surveys (unpublished data) and Rockwell (2001) indicated that there was an abundance of heptageniid nymphs in the genus, *Cinygmula,* in streams that surround Haggarty Creek; so we used this genus in our *in situ* drift study on Haggarty Creek as well as on Bachelor Creek, an uncontaminated tributary. Given the similarities in elevation, substrate, shade, and flow regime between Haggarty Creek and Bachelor Creek, and given that *Cinygmula* were collected in streams all around the affected reaches of Haggarty Creek, we would have expected *Cinygmula* to be in Haggarty Creek were it not for copper contamination.

MATERIALS AND METHODS

Drift Chamber Development

The presence of bedrock and boulders in the upper reaches of Haggarty Creek prevented us from using the techniques of Kiffney et al. (1997), Clements (1999), or even drift nets to study the drift behavior of *Cinygmula* because the streambed could not be penetrated; these sampling techniques all require that sampling tools be deeply inserted into the streambed. Such conditions are common in western montane streams. As such, we designed and used drift chambers that would lie flat on the streambed.

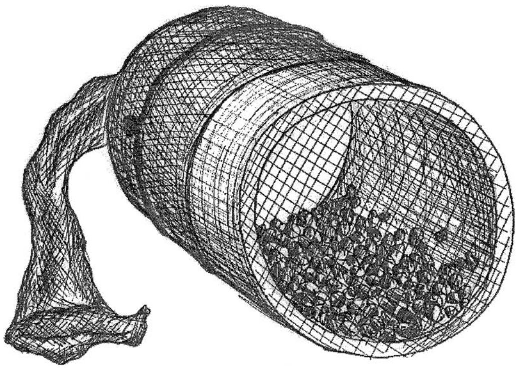

Figure 1. A drift chamber.

Drift chambers (Figure 1) were constructed from 12 cm long and 7.6 cm inside diameter PVC pipes. Chambers were fitted on the downstream end with white nylon leggings, creating catch-bags that extended 10–14 cm when placed in the water. Catch-bags allowed us to easily and unobtrusively count *Cinygmula* that had drifted from the chambers. Plastic screens (2 mm pore size) were permanently affixed to the upstream end of each chamber. Stream substrate particles 2–8 mm in diameter were collected from Haggarty Creek, acid-washed, and secured in a single layer to the inside bottom of each chamber using silicone caulk. This created a substrate of small stones to which *Cinygmula* could cling.

Site Descriptions

Sites on Haggarty and Bachelor Creeks (Figure 2) were chosen for their uniformity of flows, similarity of shady conditions, and differences in aqueous copper concentrations. Specifically, all sites were situated on straight-sided riffles whose flows were within 10 percent of one another; flows were determined using the methods of Gore (1996). We also made sure the sites were situated in the shade to prevent sun-warmed water from causing *Cinygmula* drift. Sites on Haggarty Creek were established so the uppermost (#1) had a high aqueous copper concentration (229 ppb), the middlemost (#2) an intermediate copper concentration (78 ppb) and the lowermost (#3) a low copper concentration (3 ppb)

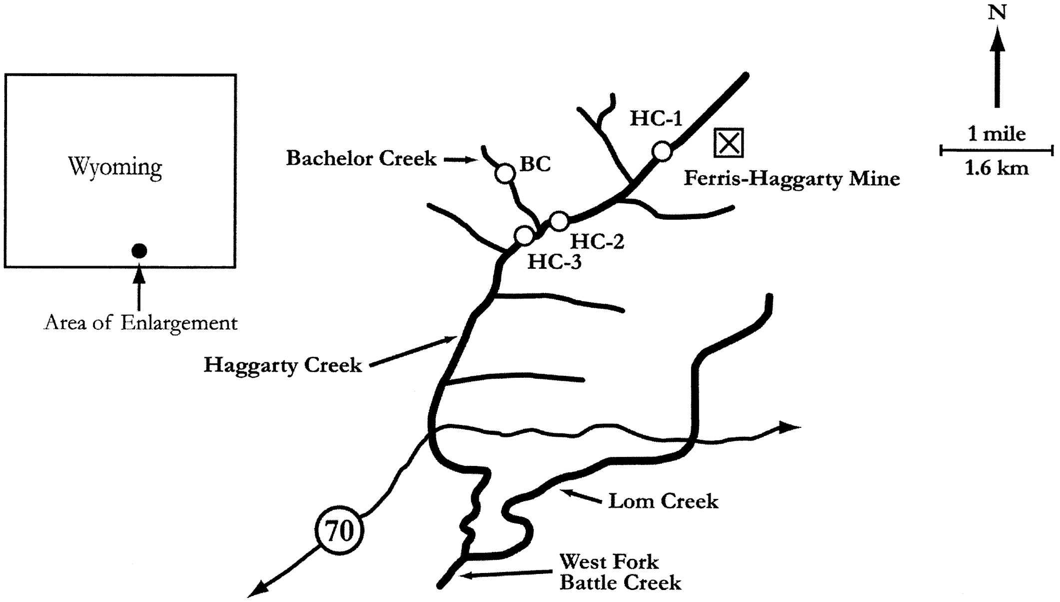

Figure 2. Location of test sites on Haggarty (polluted) and Bachelor (unpolluted) Creeks.

(Rockwell 2001). In contrast, the copper concentration at Bachelor Creek was less than detection limits (<1 ppb) (Rockwell 2001). Sites #1 and 2 on Haggarty Creek and the Bachelor Creek site were on first order streams while site #3 on Haggarty Creek was on a second order stream. All sites had cobble/gravel substrates with moderate slope. Elevations of Haggarty Creek sites #1, 2, and 3 were at 2957, 2865, and 2743 m while elevation of the site on Bachelor Creek was at 2774 m a.s.l.

Cinygmula Collection

Cinygmula were collected from Bachelor Creek, via kick sampling, using a D-net (1 mm pore size). *Cinygmula* were transferred with a glass pipette to a 1-L Nalgene® bottle filled with water from Bachelor Creek; specimens were identified to genus because dichotomous keys to species were unavailable (Ward et al. 2002).

Chamber Loading and Submersion

Chambers were loaded with *Cinygmula* by first placing a PVC cap over their upstream ends. Water from a given site was then added by dipping the chambers into the stream. Next, 10 *Cinygmula* were transferred from the Nalgene® bottle to each water-filled chamber using a glass pipette; no more than 10 *Cinygmula* were placed in a chamber to prevent crowding. After loading, nylon catch-bags were placed over the downstream ends and secured with cable ties. Three chambers were loaded at each site, providing a total of 30 *Cinygmula* per replicate per site.

Before submersion, a second PVC cap was placed over each downstream (i.e., catch-bag) end. Chambers were then submerged, placed on the stream bottom, and anchored with large rocks; chambers were covered by 5 cm of water. After submersion, the *Cinygmula* were allowed to settle on the chamber's substrate for 10 min. The downstream cap then was carefully removed so that a backwash was not created and the catch-bag extended slowly downstream. Finally, the upstream cap was slowly removed to prevent an initial sudden flow through the chamber.

Chamber Monitoring

After submersion, each catch-bag was assessed at three intervals. The first occurred 3 min after the upstream cap was removed (post-settling). Additional assessments were made at 10 and 60 min post-settling (exposure times). At each exposure time, *Cinygmula* found in all catch-bags at a site were counted (drifting *Cinygmula*). The study was first conducted on 30 June 2000, and then repeated

Table 1. Estimated logistic parameters for drift curves developed at each of four stream sites; the Bachelor Creek site was not contaminated with aqueous copper, but all Haggarty Creek sites were, with Haggarty Creek site #1 being the most contaminated and Haggarty Creek site #3 being the least: Sierra Madre Range, southeast Wyoming.

Site	γ[a]	α[b]	β[c]	$\lvert t \rvert$	P_{slope}
Bachelor Creek	1.0	−2.9079	0.011792	1.32	0.1142
Haggarty Creek #1	−0.6	1.3635	−6.6626	7.12	0.0001
Haggarty Creek #2	−0.4	1.5781	−4.2962	5.75	0.0004
Haggarty Creek #3	−0.8	−0.81599	−2.3246	2.32	0.0267

[a] Curve fitting exponent.
[b] Logit *y*-intercept.
[c] Logit slope; values of the logit slope were subjected to *t* tests, for which one-tailed *P* values are provided.

on 1 and 2 July 2000, providing a total of three replicates for statistical analysis.

Statistical Analysis

Response of drifting *Cinygmula* to exposure times was determined through regression analysis. For each site, we regressed the number of drifting *Cinygmula* on exposure times. As the relation between drifting *Cinygmula* and exposure times was not linear, being bound by an asymptote of 30 *Cinygmula* at a site per replicate, we used logistic regression to develop the response curves (drift curves) (Haberman 1978). Specifically, we used the software program, LINLOGIT (Legg 2003), to fit drift curves of the following form:

$$\hat{Y}_i = T/\{1 + \exp(-1(\alpha + \beta X_i^{\gamma})]\} \qquad (1)$$

where $\hat{Y}_i$ is the *i*th fitted value on a curve, T is the total number of *Cinygmula* being tested at a site for a replication (30 in this study), exp is the base of the Napierian logarithm, α is the *y*-intercept expressed in logit scale, β is the slope also in logit scale, X_i is the *i*th exposure time (min), and γ is a curve-fitting exponent, the value of which is iteratively derived. The slopes of Equation 1 were then tested for significant departures from zero using *t* tests, testing the null hypothesis that the logit slope was equal to zero against the alternate that it was less than zero (type I error rate = 0.05) (Haberman 1978); logit slopes less than zero corresponded with positive drift curves. The PROBT function of the Statistical Analysis System (1989) was used to calculate *P* values. Once drift curves were developed, we compared them, in pair-wise fashion, using the *F* statistic (Weisberg 1980) to test the null hypothesis that drift curves were the same (equal drift rates) against the alternate that they were different.

RESULTS

Influence of Exposure Times on Drift

Results showed that, for the uncontaminated site on Bachelor Creek, the logit slope was essentially zero (Table 1). Graphically, this appeared as a ‘flat’ drift curve (Figure 3) and indicated that, in the absence of detectable aqueous copper contamination, the drift behavior of Cinygmula did not increase with increasing exposure times through 60 min.

At the most contaminated site of Haggarty Creek (#1), the logit slope was less than zero (Table 1). Graphically, this corresponded with a drift curve that rose distinctly through the 3 and 10 min assessments but less so between the 10 and 60 min assessments (Figure 3). At the next-most contaminated site of Haggarty Creek (#2), the logit slope was also less than zero (Table 1). Graphically, this corresponded with a drift curve that also rose distinctly through the 3 and 10 min assessments but less so between the 10 and 60 min assessments (Figure 3). At the least-contaminated site of Haggarty Creek (#3), the logit slope was again less than zero (Table 1). Graphically, this corresponded with a drift curve that rose distinctly through the 3 min assessment, but less so between the 3, 10, and 60 min assessments (Figure 3).

Influence of Aqueous Copper on Drift Rates

An overlay of the four drift curves (Figure 4) suggested that the drift rates of *Cinygmula* differed among some sites. Results from *F* tests indicated that the drift curve from the uncontaminated site of Bachelor Creek was different from that of the least contaminated site of Haggarty Creek (site #3) ($F_{2,14} = 5.12$, $P = 0.0214$). Further, the drift curve from Bachelor Creek was different from the curves

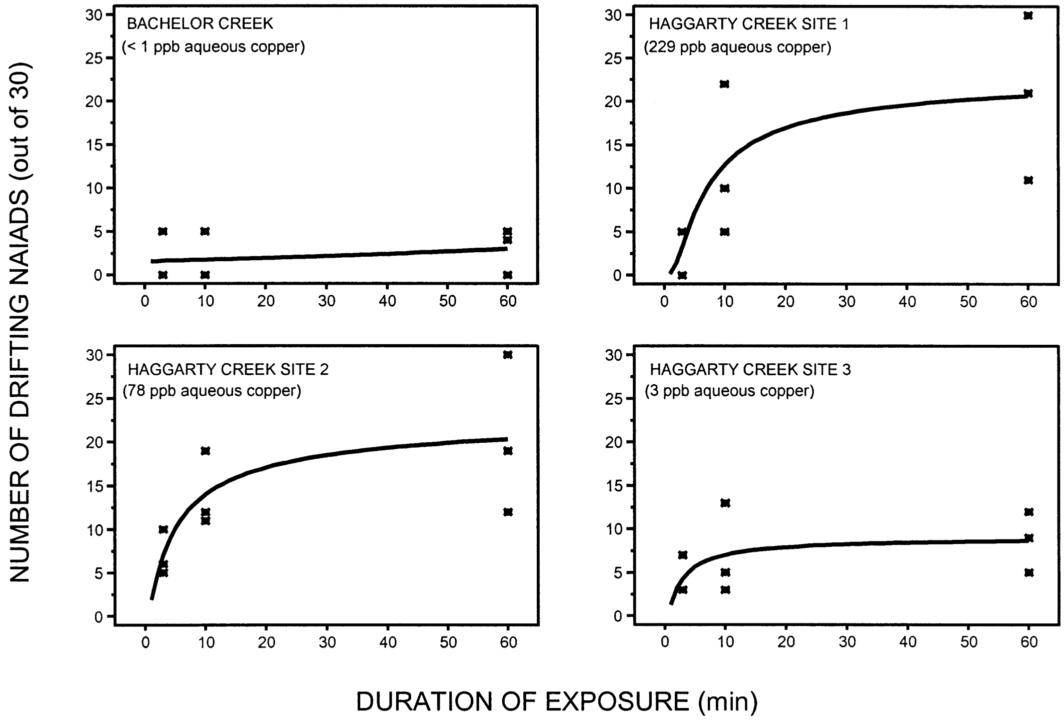

Figure 3. Logistic regression (i.e., drift) curves describing the relation between observed number of drifting *Cinygmula* and exposure times from a site that was uncontaminated by aqueous copper on Bachelor Creek, as well as from the most contaminated site on Haggarty Creek (#1), the next-most contaminated site on Haggarty Creek (#2), and the least contaminated site, also on Haggarty Creek (#3); Sierra Madre Range, southeast Wyoming.

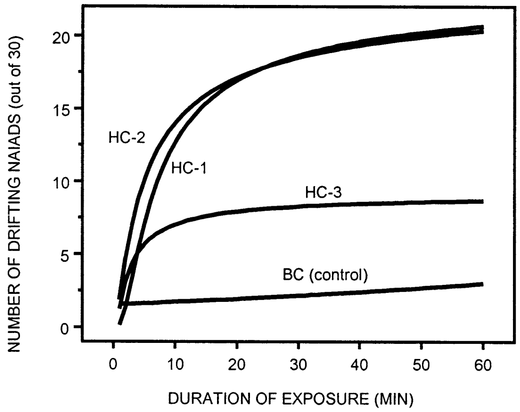

Figure 4. Superimposed drift curves developed from experiments conducted on the most contaminated site on Haggarty Creek (HC-1) (229 ppb aqueous copper), next-most contaminated site on Haggarty Creek (HC-2) (78 ppb aqueous copper), the least contaminated site on Haggarty Creek (HC-3) (3 ppb aqueous copper), and from an uncontaminated site that was located on Bachelor Creek (BC control) (<1 ppb aqueous copper); Sierra Madre Range, southeast Wyoming.

of the more heavily contaminated Haggarty Creek sites #2 ($F_{2,14}$ = 19.13, P = 0.0001) and #1 ($F_{2,14}$ = 11.64, P = 0.0011). In addition, the drift curve from the least contaminated Haggarty Creek site #3 was different from the curves of the more heavily contaminated sites #2 ($F_{2,14}$ = 6.55, P = 0.0098) and #1 ($F_{2,14}$ = 4.25, P = 0.0361). Drift curves from the two most heavily contaminated sites on Haggarty Creek were the same ($F_{2,14}$ = 0.2866 P = 0.7551) (Figure 4).

DISCUSSION

The Ferris–Haggarty copper mine was the most productive copper mine in North America at the turn of the 20th century (Rockwell 2001). Mine operations were such that untreated effluent was allowed to flow from the mine into Haggarty Creek. In the 1970s, the Wyoming Department of Environmental Quality instituted new environmental standards that required treating the effluent before it was allowed to flow from the mine. As the cost for supplying this treatment was not met, the mine became inactive and was abandoned, leaving the untreated effluent flowing into Haggarty Creek (Chervick and Harp 1977; Bell 1996). At present, the affected reaches begin where effluent joins Hagarty Creek and continue downstream about 18 km.

Plans for reclaiming the affected reaches of Haggarty Creek involve studying the bioaccumulation of metals in benthic fauna. This research has been conducted by Rockwell (2001). Plans for reclaiming the affected reaches of Haggarty Creek also have involved studying *in situ* responses of benthic fauna to effluent from the Ferris–Haggarty mine; this research has not yet been conducted.

Drift of some benthic macroinvertebrates is not always due to pollution, as some occurs naturally. For example, Peckarsky and Cowan (1995) found that most Ephemeroptera and Plecoptera exhibited a propensity for nocturnal drift. In another study, Peckarsky (1996) found that the drift of five ephemeropterans was sometimes induced by predators, but a lack of food also increased the drift of *Baetis.*

Given that a general drift response exists for Ephemeroptera to acids and metals, we used the ephemeropteran *Cinygmula* in this study as it was easy to find and was abundant in streams surrounding the affected reaches of Haggarty Creek. We designed drift chambers so *Cinygmula* would be easy to load and examine for drift; chambers also excluded predators. Chambers were used during the day to avoid a propensity for *Cinygmula* to drift at night, and we exposed *Cinygmula* for just 60 min to minimize drift due to a lack of food.

Results indicate that the drift behavior of *Cinygmula* is sensitive to the aqueous copper concentrations in Haggarty Creek. In particular, *Cinygmula* drift rate was significantly affected at all copper contaminated sites. Further, *Cinygmula* drift rate was sensitive enough to differ between the least contaminated Haggarty Creek site #3 and the more contaminated Haggarty Creek sites 1 and 2. We note that *Cinygmula* drift rate was not sensitive enough to differ between the two most heavily contaminated sites, suggesting that *Cinygmula* drift rate (60 min) may be at or near its maxima, for this system, at 78 ppb aqueous copper.

Currently, the USEPA standard for chronic continuous exposure to copper in freshwater systems is 9 ppb (USEPA 2005). That value is slightly less than the current value for acute exposure to copper in freshwater systems (13 ppb) (USEPA 2005). Given that *Cinygmula* exhibited a drift response to copper at 3 ppb, *Cinygmula* appears sensitive enough to indicate the presence of copper in Haggarty Creek at both the chronic and acute levels of contamination.

The results demonstrate that *Cinygmula* drift curves can be used in a simple *in situ* bioassay to determine whether aqueous copper concentrations in contaminated reaches of Haggarty Creek differ functionally from aqueous copper concentrations that occur in uncontaminated streams such as Bachelor Creek. Here we emphasize the functionality of such tests because aqueous copper concentrations are not being measured *per se*; rather, the effects of copper concentrations are being tested via drift behavior of *Cinygmula.* It may be possible to develop such tests for other point sources of pollution. Care must be taken, however, to both select an organism that is sensitive to specific types of pollution and will respond to pollution through drift behavior.

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