Selection of the Mayfly *Rithrogena hageni* as an Indicator of Metal Pollution in the Upper Arkansas River

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ABSTRACT

Macroinvertebrates from the upper Arkansas River were studied to identify taxa sensitive to heavy metal (zinc) impacts. Field collections and introduced substrates indicated that Rithrogena hageni was intolerant of conditions caused by mine drainage into the Arkansas River. Numbers of R. hageni at the impacted site increased significantly after treatment of mine drainage water was initiated. Data collected from this study suggest that the use of colonized substrates may be useful in studying metal impacts on macroinvertebrate communities.

INTRODUCTION

The upper Arkansas River flows from northwest to southeast, roughly parallel to the Continental Divide in central Colorado. The upper basin lies in a valley between the Sawatch and Mosquito mountain ranges at an elevation of approximately 2900 meters. Gravel-rubble is the dominant substrate in this section of the Arkansas River. Heavy metals, associated with historical mining of this mineral rich area, drain into portions of this upper basin resulting in water quality degradation (LaBounty et al. 1975). The most significant sites contributing heavy metals to the river are the Leadville Mine Drainage Tunnel (LMDT) and the California Gulch Drainage Basin (Fig. 1). These conduits were originally built to drain off abundant groundwater which impeded mining in the area. The LMDT was acquired by the U. S. Bureau of Reclamation in 1959 for water rights associated with the drain. It was later discovered that water flowing from the tunnel contained high concentrations of heavy metals, especially zinc, consequently, a chemical precipitation water treatment plant was constructed and began operation in March 1992.

As part of the monitoring plan for the clean-up of the LMDT, macroinvertebrates above and below the LMDT were monitored since 1988. Macroinvertebrates are considered a useful part of a biomonitoring program because they integrate water quality fluctuations between sampling periods (Plafkin et al. 1989). The objective of this study was to distinguish indicator organisms useful in identifying zones of zinc impact and recovery in the upper Arkansas River and to validate their use in monitoring the clean-up of the LMDT.

METHODS AND MATERIALS

Three different methodologies were used to identify indicator organisms: (1) observational longitudinal data were collected in 1991 to compare abundance of macroinvertebrates at sites with a gradient of zinc concentrations, (2) also in 1991, substrates that had been colonized at reference stations were transferred to downstream sites to study metal impact on macroinvertebrates while eliminating substrate variability as a factor, and (3) organism response to improved water

quality at specific sites was studied by comparison of organism abundance before and after initiation of water treatment.

Sampling at the various stations (Fig.1) took place in July and October of each year. Stations 1a-3 were sampled from 1988-1992, while stations 4 and 5 were added for studies performed in 1991. Three discrete samples were collected from riffle-type habitat at each site using a Surber sampler (0.09 m^2 and ca. 5 cm depth, 728 micron mesh size). In addition, qualitative samples were collected from a variety of habitats within each site using a D-frame net. All samples were placed in individual containers, labeled, and preserved in alcohol or formalin solution for identification and enumeration in the laboratory.

Substrate filled trays were also used at some sites during 1991. Thirty trays measuring approximately 10 X 10 X 6 cm were filled with substrate from a dry gravel bar at the reference station (Site 1b) and allowed to colonize for 30 days at the reference station. After colonization, six trays were left at Site 1b and six each were caged and placed at Site 1b (removed and then returned to the same station), Site 4, and Site 5 on 20 September 1991. Site 2 and Site 3 were not used because of the high public use at these areas and the susceptibility of substrates to vandalism. The use of these rock filled trays was described by Clements et al. (1989a). Colonized trays were placed in enclosures made of PVC pipe with 750 micron mesh netting on both ends to inhibit loss of organisms during transfer and during site exposure. A current meter was used to measure water velocity to ensure that trays were relocated to areas with similar velocity. After transfer, enclosed trays were exposed for 12 days and then recovered, and the contents were preserved. This technique was used to allow for a more objective approach to the determination of metal effects on macroinvertebrate communities than can generally be obtained with Surber or qualitative sampling.

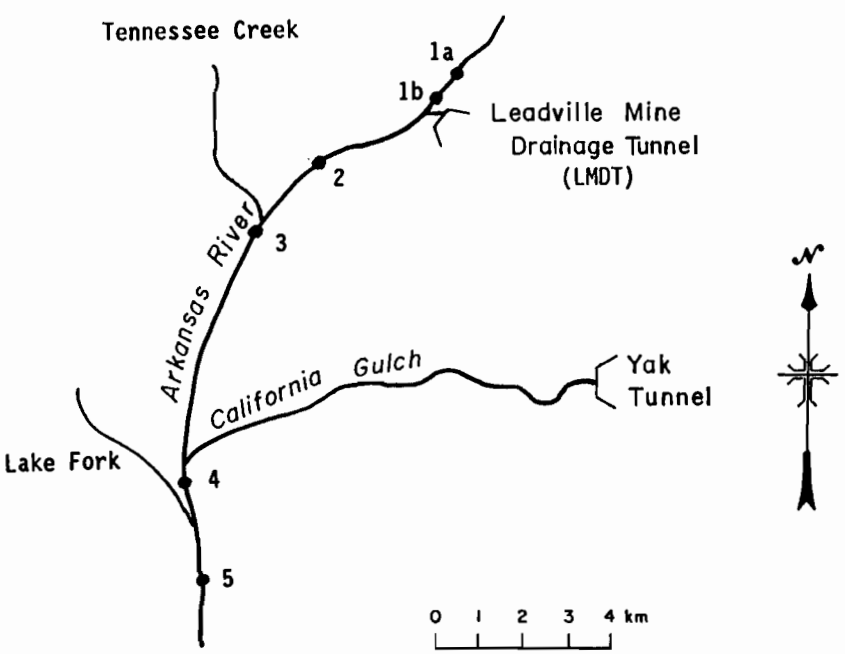

Figure 1. General location of sampling sites within the study area on the upper Arkansas River.

All macroinvertebrate samples were completely sorted from debris in the laboratory and then identified and counted, most with the aid of a dissecting microscope. Chironomids were cleared in 10% KOH overnight. Cleared specimens were then transferred to 70% alcohol and examined under a dissecting microscope. Tanytarsini were identified and removed from the sample at this point. The rest of the sample was then prepared as slides if it numbered less than twenty. If it numbered greater than twenty, a subsample was prepared as slides. Slides were then examined using a compound microscope. When only part of the total sample of Chironomidae larvae was mounted and identified, the results were projected from those mounted to the total number of specimens minus the Tanytarsini that were initially removed. Samples from enclosures were exceptions; in this case, the entire chironomid sample was prepared for identification.

Sorted sample material was rechecked in 68% of the picked samples. The mean number of additional specimens recovered was 6.7% of the sample. Taxonomic quality assurance was maintained by using a variety of literature for identifications and verification of some specimens by outside experts (Dave Ruiter, EPA Region VIII and Boris Kondratieff, Colorado State University).

During 1991 and 1992 sampling, water was collected in polyethylene containers for metal analyses (total and dissolved) and for major ions. Samples were chilled during transport to the laboratory. Total carbonate hardness was estimated from measurements of calcium and magnesium ions. Total alkalinity was determined by titration within 24 hours of sample collection. Samples collected for metal analyses were brought to pH $\leq$ 2 with concentrated HNO_3. Water collected for dissolved zinc analysis was filtered in the field through a 0.45-micron filter prior to preservation. Inductively coupled plasma/emission spectroscopy and an atomic absorption spectrophotometer with a carbon furnace were used for laboratory analyses of zinc samples (APHA 1989, EPA 1983). Other physico-chemical analyses (i.e, pH, dissolved oxygen, temperature, and specific conductance) were performed in situ using a multiparameter probe.

Differences among numbers of macroinvertebrates were analyzed using one-way analysis of variance (ANOVA). If ANOVA detected a difference, the multiple comparison Tukey's test was used to compare all treatments to each other. If data failed normality or homogeneity of variance tests, it was transformed using 1/square root (Y+0.5) in order to meet these assumptions for ANOVA.

RESULTS AND DISCUSSION

Water Chemistry

Upper Arkansas River waters were, in general, poorly buffered (total alkalinity ranged from 35 to 105 mg/L), low in dissolved solids (60-150 mg/L) and hardness (20.6-58.6 mg/L), and high in dissolved oxygen (7.2-9.8 mg/L). Values for pH ranged from 7.5 to 8.7. All values generally showed increases in October relative to July.

The majority of metal mine drainage enters the Arkansas River at the LMDT (just above Site 2) and at California Gulch (just above Site 4), and concentrations of metals in the water increase below these points. Zinc has been considered the major metal contaminant and toxicant in the upper Arkansas River (Roline 1988), and the pattern of zinc occurrence

during the year prior to treatment plant implementation is presented in Table 1. This is the general pattern for metals in the river; however, temporal and seasonal variation does occur (see Wetherbee et al. 1991). Dissolved zinc concentrations did not differ greatly from total zinc values, and therefore the dissolved data are not presented.

Table 1. Total zinc concentrations (ug/L) at sites on the upper Arkansas River before (1991) and after (1992) treatment plant operation.

Station No.	Zinc concentrations			
	1991		1992	
	July	October	July	October
Site 1a	7.1	4.4	11.1	8
Site 1b	5.4	8.3	11.1	6
Site 2	86.7	282	15.8	45
Site 3	64.4	157	19.5	29
Site 4	333	1160	--[a]	--[a]
Site 5	307	346	--[a]	--[a]

[a]Not sampled.

Macroinvertebrates

Initially, a descriptive approach was used to identify organisms that seemed to decline in impacted areas. Taxa which were distributed throughout the study reach and relatively common at reference and less impacted sites and uncommon at metal-impacted sites were categorized as being sensitive to metals. Data from July and October, 1991 from sites with different gradations of zinc concentrations indicated that four out of the 70 identified taxa responded this way (Fig. 2). Other organisms were either uncommon or associated only with upstream or downstream locations.

Colonized substrate-filled trays which were placed in enclosures and transferred to sites with differing heavy metal impacts were used to identify organisms sensitive to these effects. To check for enclosure effects, some substrates were left unenclosed at Site 1b. Average number of organisms present on substrates left at Site 1b was higher (165 $\pm$ SE 25/substrate) than enclosed substrates (100 $\pm$ SE 12/substrate) at the same station. Differences between caged and uncaged substrates may have been caused by mortality and handling loss in enclosed substrates, and further colonization of unenclosed substrates. Water velocities measured in front of enclosures averaged 0.57 m/S $\pm$ SE 0.01 during initial placement. Data obtained from enclosures were analyzed for the six numerically dominant organisms present at the reference site (1b) and these data are presented in Table 2. There was not a statistically significant ($P<0.05$) difference in numbers of Pericoma sp. and Heterlimnius sp. between Site 1b and impacted sites (4 and 5). However, numbers were significantly different for Chironomidae (mostly Orthocladiinae), Simulium sp., and Capnia sp., with all three of these taxa showing declines at the impacted sites. Data for Chironomidae was discounted since it would be relatively easy for midges to escape from the enclosures. This is not meant to imply that this group would not exhibit differences, but merely that this experimental design may not be appropriate for showing differences caused by mortality since these organisms could easily drift from the enclosures. The decline in

Simulium sp. might have been caused by metal toxicity at Site 4; however, Simulium sp. numbers at Site 5 may have been negatively affected by decreased water velocities (mean velocity from two measurements was 0.31 m/S) caused by partial blockage of enclosure screens by filamentous algae coming in from the Lake Fork tributary to the Arkansas River.

Enclosure data for R. hageni (Table 2) indicated no statistical difference between Site 1b and the most downstream site, while there was a significant difference between Site 1b and Site 4 with the mean number of organisms decreasing from 7.3 at Site 1b to 1.8 at Site 4. Mean number of R. hageni at Site 5 was 4.0. Differences in R. hageni numbers between the stations are believed to have been caused by the difference in metals concentrations. Analysis of water samples collected at the same time that enclosures were retrieved, showed zinc concentrations of 8.3, 1160, and 346 ug/L for Site 1b, Site 4, and Site 5 respectively. It is possible that a longer exposure time would have caused even greater decreases in numbers at Site 4 and possibly Site 5. In laboratory studies with another heptageniid mayfly, Epeorus latifolium, Hatakeyama (1989) found that major effects, caused by exposure to 100 and 300 ug/L zinc, occurred after two weeks. R. hageni was not found in Surber samples collected from Site 4 and Site 5 at the time that enclosures were retrieved. Qualitative sampling, however, resulted in the recovery of two and four individuals from the two sites, confirming their distribution throughout the study reach.

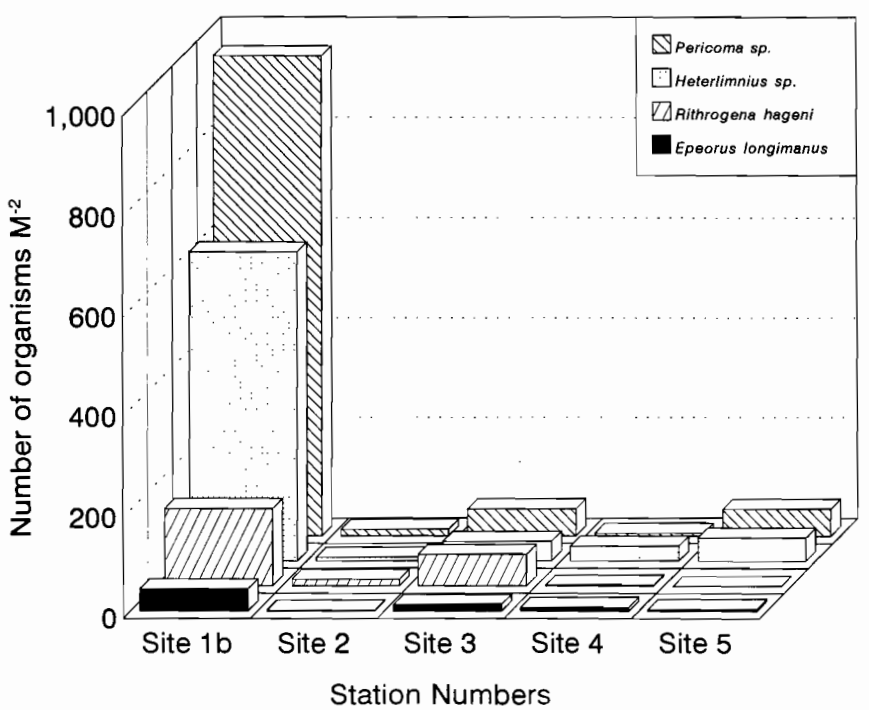

Figure 2. Variation in abundance of four common invertebrate taxa at stations with differing zinc concentrations in the Arkansas River.

Table 2. Mean (n=6) number of numerically dominate taxa in enclosures at Sites 1b, 4, and 5 after transfer studies in 1991. Standard error in parentheses.

TAXA	Mean number of organisms in enclosures at stations		
	Site 1b	Site 4	Site 5
Rithrogena hageni	7.33 (1.82)	1.83[a] (0.40)	4.00 (0.97)
Capnia sp.	8.83 (1.99)	1.50[a] (0.56)	2.00[a] (0.58)
Heterlimnius sp.	6.00 (0.82)	7.00 (0.93)	7.17 (1.08)
Chironomidae	19.83 (3.78)	3.33[a] (1.33)	3.50[a] (0.76)
Pericoma sp.	26.67 (8.53)	23.20 (10.50)	24.50 (6.25)
Simulium sp.	14.50 (3.28)	2.83[a] (0.70)	0.67[a] (0.49)

[a]Significantly different ($p < 0.05$) from mean number of the given taxa at the reference station (Site 1b).

Table 3. Mean number of R. hageni collected in October from 1988-1992 by Surber samplers. Standard error in parentheses.

Station no.	1988	1989	1990	1991	1992
Site 1a	11.66[a] (2.96)	15.66[a] (7.12)	29.33[a] (12.77)	14.67[a] (1.85)	30.67[a] (1.67)
Site 1b	24.00[a] (4.58)	14.67[a] (2.73)	25.33[a] (6.98)	20.00[a] (3.60)	53.00[a] (17.01)
Site 2	1.33[a] (0.88)	1.67[a] (1.20)	1.33[a] (1.33)	2.33[a] (1.86)	24.00[b] (2.08)
Site 3	0.33[c] (0.33)	2.67[a] (1.67)	6.67[a] (3.28)	11.33[a] (3.33)	27.67[a] (14.31)

[a]No significant difference between these years at the given site.
[b]Significant difference ($p<0.05$) between 1992 and years 1988-1991 at Site 2.
[c]Significant difference ($p<0.05$) between 1988 and 1990-1992, but not 1989 at Site 3.

Other organisms initially identified as being sensitive to metals (Fig. 2) did not show a decline in numbers in caged substrates (i.e., Heterlimnius sp. and Pericoma sp.) when transferred to impacted sites or were not typically detected at the time of year that the enclosure studies took place (Epeorus longimanus). Organisms that were not identified from field collections as being sensitive to metals but responded negatively to increased metals concentrations on transferred substrates included Capnia sp. and Simulium sp. The response of Capnia sp. was of interest, but because Capnia sp. was rarely found in our Surber sampling, it would not be of use in a monitoring program based on Surber sampling. Simulium sp. results were likewise of interest, but variance in Surber samplers would seem to suggest difficulty in use of these data in a monitoring program. R. hageni, therefore, was selected for monitoring the LMDT cleanup, and it was predicted that numbers at the impacted station would increase once water treatment commenced.

Surber samples comparing numbers of R. hageni collected at each site, before and after treatment plant implementation, supported our selection of R. hageni for this purpose. Mean number of R. hageni increased significantly (Table 3) at the impacted Site 2 after water treatment commenced and zinc concentrations declined (Table 1). At reference stations 1a and 1b, there was no statistically significant difference in numbers of R. hageni between years 1988-1992 (Table 3). Relatively high numbers of R. hageni were maintained at these stations for the years sampled. Other potential indicator organisms (Fig. 2) showed no statistical improvement at Site 2 in 1992. It is possible that life history differences, or causes other than, or in addition to, direct metals toxicity were causing their declines at this station.

Before and after treatment data from Site 3 differed in pattern from the other stations; R. hageni data suggested lower water quality in 1988 and perhaps 1989, with improvement thereafter (Table 3). While we did not collect water samples during these years, data obtained from USGS (Wetherbee et al. 1991 and personal communication with the Pueblo, Colorado Water Resources Division) for this station suggests that water quality improved from 1988 to 1991. We only examined water quality data collected from July to October, since early instars, potentially the most sensitive life stage, would be present at this time. Degraded water quality in 1988 may have been caused by a reduced discharge which decreased metal dilution at this station. Mean discharge as measured by USGS on the Arkansas River at Buena Vista was 10 cubic meters/second in 1988 vs the 21 year average of 14 (Ugland et al. 1989).

Assessments utilizing a variety of community metrics have been developed by EPA (Plafkin et al. 1989) to evaluate habitat and water quality effects, and Barbour et al. (1992) determined that this approach is appropriate to evaluate impairment. Research, however, is needed which clarifies the relationships supporting these bioassessments, since a variety of causes may influence the makeup of a metal impacted community. Some taxa may be directly affected through acute or chronic toxicity (Warnick and Bell 1969, Anderson et al. 1980, Hatakeyama 1989) while others may experience increased vulnerability to predation (Clements et al. 1989b). Macroinvertebrates may also be affected indirectly through the loss of food items since some aquatic plants are affected by heavy metals (McLean and Jones 1975). Hildrew et al. (1984) noted that decreased numbers of fish, as may occur in metal-impacted areas, may lead to increased numbers of large bodied macroinvertebrate predators, which may have a profound effect on the macroinvertebrate community. It is difficult to predict responses of

organisms that are affected indirectly by metal impacts and these sorts of responses make bioassessment more difficult.

We believe that the use of enclosed substrates helped demonstrate a cause and effect relationship between numbers of R. hageni and zinc concentrations. This relationship is also supported by observational data showing that R. hageni responded negatively to an increased gradient of metal concentration and before and after treatment data that demonstrated increased numbers of R. hageni when zinc concentrations were reduced. The use of substrate-filled trays (caged and uncaged, colonized and transferred and colonized in situ) may serve as a useful tool to clarify relationships among macroinvertebrates that reside in metal-impacted streams.

ACKNOWLEDGEMENTS

We thank Thomas L. LaCasse and Rick Wydoski for assistance in the field, Henry Chambless and Andrew Montano for help in sorting samples, and William H. Clements for reviewing an earlier draft of this manuscript.

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Received: 24 December 1992 Accepted: 31 March 1993