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APPLIED ISSUES

Assessing the influence of water and substratum quality on benthic macroinvertebrate communities in a metal-polluted stream: an experimental approach

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SUMMARY

1. Field and laboratory experiments were conducted to assess the relative influence of water quality and substratum quality on benthic macroinvertebrate communities in the Animas River, a metal-polluted stream in south-western Colorado (U.S.A.).
2. A community-level *in situ* toxicity test measured direct effects of Animas River water on benthic invertebrates collected from a reference stream (Elk Creek). The effects of metal-contaminated biofilm were examined by comparing macroinvertebrate colonisation of clean and contaminated substrata placed in Elk Creek. A feeding experiment with the mayfly *Baetis tricaudatus* Dodds (Ephemeroptera: Baetidae) examined metal bioaccumulation and effects of metal-contaminated biofilm on growth and survival.
3. Animas River water was acutely toxic to most taxa, with greatest effects observed on mayflies (Heptageniidae, Ephemerellidae) and stoneflies (Taeniopterygidae and Capniidae).
4. Although Animas River biofilm was characterised by high concentrations of metals and low algal biomass, most taxa colonised substratum from the reference stream and the Animas River equally. The exceptions were Ephemerellidae, Taeniopterygidae and Simuliidae, which were less abundant on Animas River substratum. Mayflies grazing Animas River biofilm accumulated significantly more metals and showed reduced growth compared with organisms feeding on Elk Creek biofilm.
5. Results of our experiments demonstrated that effects of heavy metals on benthic community structure in the Animas River were complex, and that responses to metals in water and contaminated substratum were species-specific. Predicting recovery of benthic communities following remediation requires an understanding of these species-specific responses.

Keywords: aquatic insects, heavy metals, mining pollution, substratum quality, water quality

Introduction

Links between mining activity, metal pollution and degradation of aquatic communities in streams are well established (Luoma & Carter, 1991; Hare, 1992; Clements *et al*., 2000). However, identifying specific characteristics of mining pollution which have the greatest effects on aquatic systems is often difficult. Benthic organisms may be directly and/or indirectly impacted by metals in water (Kiffney & Clements, 1996), substratum (Chapman *et al*., 1998), and food resources (Hare, 1992; Kiffney & Clements, 1993; Farag *et al*., 1998). Direct toxic effects of metals reduce diversity and abundance of benthic invertebrates and

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result in loss of sensitive species (Clements, 1994). Indirect effects include modifications of species interactions (Clements, 1999) and reductions in food quality (Carlisle, 2000). Mining activity can also influence aquatic organisms through physical alterations of habitat, including sedimentation and increased substratum embeddedness (Church *et al.*, 1997). Because different taxa respond differently to toxic effects of metals and habitat alterations, community-level responses to metals observed in the field are complex. Although biomonitoring and water quality data are important for characterising metal impacts, experimental methods can provide insight into cause-and-effect relationships between specific characteristics of metal pollution and community responses.

Impacts of heavy metals and associated stressors on aquatic systems often persist long after cessation of mining activity. The Animas River, located in south-western Colorado, is one of the many streams in the Rocky Mountain region severely impacted by historic mining operations (Caruso, 1999; Church *et al.*, 1999). Although most mining in the Animas River subsided by the 1940s, metals from natural sources and from numerous abandoned mines continue to degrade water and substratum quality. Previous research has shown that benthic communities in the Animas River are degraded by heavy metals (Besser *et al.*, 2001). However, it is uncertain if these impacts are a result of toxic effects of metals in water and sediment or physical alteration of the substratum.

Current and proposed restoration efforts of the Animas River have focused primarily on improving water quality. Because benthic communities are affected by both degraded water quality and substratum quality, understanding the relative importance of these two stressors will improve our ability to assess recovery. This research integrated field and laboratory experiments to quantify the relative influence of water quality and substratum quality on benthic community composition. Community level *in situ* toxicity tests measured the direct effects of Animas River water on benthic invertebrates. We compared invertebrate colonisation of clean and metal-contaminated substrata to assess the effects of metals in biofilm on benthic community structure. We measured metal concentrations in biofilm (Newman & McIntosh, 1989; Farag *et al.*, 1998), transfer of metals to a grazing mayfly (*Baetis tricaudatus*) and effects on invertebrate survival and growth.

Methods

Study sites

The Animas River flows southward from the San Juan Mountains of south-western Colorado to the confluence with the San Juan River, New Mexico (Fig. 1). The upper Animas River Basin is rich in minerals, and was mined intensively between 1872 and the 1940s. After the discovery of gold in 1871 near Silverton, 1000–1500 mining claims were staked in this section of the watershed (Church *et al.*, 1999). Numerous abandoned mines are scattered throughout the upper Animas River Basin, and drainage from these mines

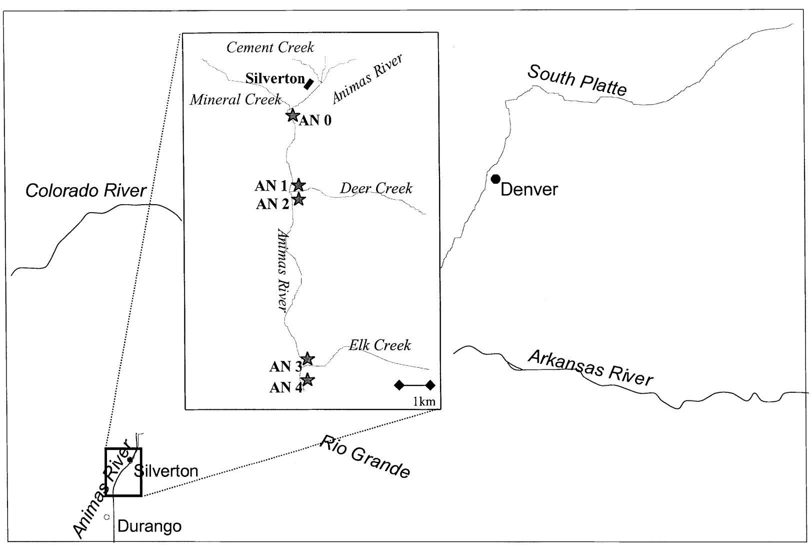

Fig. 1 Map of sampling locations in Elk Creek and the Animas River, Colorado.

significantly degrades water and sediment quality (Caruso & Ward, 1998; Church *et al*., 1999; Caruso, 1999). Although several metals have been detected in water and sediment collected from the Animas River, concentrations of Zn are especially high within the catchment (Caruso & Ward, 1998; Church *et al*., 1999; Caruso, 1999).

Studies were conducted along a 11-km reach (altitude range = 2707–2825 m a.s.l.) of the Animas River from Silverton to Elk Creek (Fig. 1) during summer 1998 and 1999. Mean depth, bankfull width, current velocity (determined using a Marsh-McBirney Flow Mate, Fredrick, MD, U.S.A.) and slope (estimated from topographic maps) of Animas River sites were 0.6 m, 27 m, 0.50 m s^{-1}, 1.3%, respectively. Elk Creek is a tributary of the Animas River, with metal concentrations generally low or below detection. Depth, bankfull width, velocity and slope at the reference site on Elk Creek were 0.26 m, 9.2 m, 0.54 m s^{-1} and 4.2%, respectively. Substratum size was similar at the Animas River and Elk Creek sites, and consisted primarily of medium gravel (12–16 mm) and large cobble (128–192 mm). However, most substratum in the Animas River was coated with an iron oxide precipitate as a result of metal deposition and sedimentation.

Colonisation of clean substratum in the Animas River

A colonisation study using clean substratum examined benthic invertebrate community structure in the Animas River and Elk Creek. Substratum-filled trays (10 × 10 × 6-cm plastic trays filled with pebble and small cobble) were placed at four sites in the Animas River (AN1, AN2, AN3, AN4) and one site in Elk Creek in July 1998. Wooden racks were secured to the stream bottom in riffle areas and 18 trays were attached to the racks with plastic ties. Previous studies have shown that benthic macroinvertebrates rapidly colonise these trays, and that communities in the trays are similar to those in the natural substratum (Kiffney & Clements, 1996; Clements, 1999; Courtney & Clements, 2000). After 30 days colonisation, three trays were selected arbitrarily and combined to comprise one sample, and six samples were collected from each station. The three trays were combined to facilitate comparison with exposure chambers (which contained three trays) used in subsequent experiments. Samples were rinsed using a 350-μm mesh sieve and organisms were preserved in 70% ethanol in the field. Organisms were sorted and identified to genus or species for most invertebrates and to tribe for chironomids.

Effects of water quality on benthic invertebrate communities

A community-level *in situ* toxicity experiment measured the toxic effects of Animas River water on benthic macroinvertebrates collected from Elk Creek. Trays filled with substratum were placed in Elk Creek in August 1998 and removed after 30 days colonisation as described above. Twenty-four of the colonised trays were arbitrarily assigned to eight exposure chambers (three trays per chamber). The plastic exposure chambers (25 × 25 × 10-cm) were completely enclosed except for three 5-cm holes drilled in each side and the top to allow water flow. The holes were covered with a 350-μm mesh to limit immigration and emigration of organisms. Four chambers were returned to Elk Creek (controls) and the remaining chambers were placed in the Animas River, immediately upstream from Elk Creek (station AN3). After 96 h, each chamber was removed and the remaining organisms were processed as described above. To estimate control mortality and/or loss of organisms during transfer of trays, communities in chambers at the end of the 96-h experiment were compared with those in samples ($n = 2$) collected from Elk Creek at the start of the experiment (day 0 trays).

Effects of substratum quality on benthic invertebrate communities

Colonisation of clean and metal-contaminated substratum. These experiments tested the hypothesis that benthic macroinvertebrates in Elk Creek avoided metal-contaminated substratum from the Animas River. In July 1998, cobble substratum (9–12 cm) was collected from either the Animas River (station AN3) or Elk Creek, brushed lightly to remove attached invertebrates and placed into 25 × 25 × 10-cm containers ($n = 3$). The containers were placed in shallow (25 cm) riffle areas of Elk Creek. After 30 days colonisation the containers were removed and the samples were processed as described above. This experiment was repeated in September 1999 with increased replication ($n = 6$) and included a block design to assess the influence of location in the stream on invertebrate colonisation. Containers with Elk Creek or Animas River substratum were placed in Elk Creek in pairs and removed after 30 days colonisation.

Effects of contaminated biofilm on Baetis tricaudatus. A feeding experiment with the mayfly *Baetis tricaudatus* (Ephemeroptera: Baetidae) examined the effects of metal-contaminated biofilm on invertebrate grazers. We measured bioaccumulation of metals and growth and survival of *B. tricaudatus* exposed to biofilm collected from the Animas River (stations AN0 and AN3) and two locations in Elk Creek. Ceramic tiles (23-cm^2) were placed directly on the streambed in shallow riffle areas of both streams in August 1998. After 30 days, the tiles with their associated biofilm were transferred to the Colorado State University Stream Research Laboratory (Fort Collins, Colorado). Tiles were placed in fifteen $12 \times 12 \times 15$-cm rectangular chambers (four tiles per chamber) immersed in 13-L, flow-through stream microcosms (see Courtney & Clements, 2000 for a description of the microcosms). Water to the microcosms was supplied from a nearby reservoir and paddlewheels provided current through mesh-covered (250 μm) openings in each chamber. Five mayflies collected from the Cache la Poudre River, an unpolluted stream located north-west of Fort Collins, were placed into each replicate chamber ($n = 5$). To determine initial body length, mayflies were briefly anesthetised with carbonated water, quickly measured under a dissecting scope with an ocular micrometer and returned to stream water. Only organisms which immediately recovered from this procedure were used in the experiment.

After 7 days exposure to biofilm, surviving mayflies were collected to determine final length and metal bioaccumulation. Because of the small size of these organisms it was necessary to combine some replicates to obtain enough biomass for metal analyses (generally four or five individuals per sample). To measure metal accumulation, *B. tricaudatus* were dried at 55 °C and weighed. Samples were digested in 1 mL HNO_3, heated to complete digestion and diluted to a final volume of 14 mL with distilled water. Zinc concentrations were analysed using flame AA spectrophotometry (Video 22 dual-channel spectrophotometer, Instrumentation Laboratory, Franklin, MA, U.S.A.), with detection limits of 10 μg L^{-1}.

Physicochemical analyses of water and biofilm

Water quality. We measured water quality at all Animas River stations and in Elk Creek during laboratory and field experiments in 1998 and 1999. Temperature, pH and conductivity were measured in the field with digital meters. Water samples (1 L) were collected for hardness determination in the laboratory (U.S. Environmental Protection Agency, 1983). Water samples for analysis of Zn (total recoverable) were acidified with HNO_3 in the field to a pH of 2.0. Metal concentrations were analysed using flame AA spectrophotometry, with detection limits of 10 μg L^{-1}. To calculate mean concentrations, measurements below detection limits were assigned values of 5 μg L^{-1} (e.g. 1/2 of the detection limit).

Zinc in biofilm. Concentrations of Zn in biofilm from Elk Creek and the Animas River were measured on natural cobble substrata and on tiles used in the mayfly grazing experiment. Biofilm was scraped from the substrata, dried at 55 °C and weighed. Biofilm was scraped from all sides of the substratum using a stiff bristle toothbrush. To quantify metal exposure to grazing mayflies, biofilm was removed only from the upper surface of the ceramic tiles. Samples were digested in 10 mL HNO_3, heated to complete digestion and diluted to a final volume of 50 mL with distilled water. Samples were analysed for Zn as described above. We also measured Zn concentrations in biofilm removed from Animas River substratum ($n = 3$) at the beginning and end of the substratum colonisation experiment conducted in 1999. These analyses were conducted to determine if metal concentrations in Animas River biofilm decreased over the 30-day period in Elk Creek. Samples were collected in the field and processed as described above.

Chlorophyll a *concentrations.* Additional tiles (2–4) were collected from Elk Creek and the Animas River in 1998 for determination of chlorophyll *a*, an indirect measure of algal biomass and food quality. Biofilm was scraped from ceramic tiles and collected on glass fibre filters (0.45-μm, 47-mm). Biofilm and filters were macerated with a tissue grinder in 90% acetone. Samples were steeped in acetone for complete chlorophyll *a* extraction, the extract was collected by centrifugation (20 min, 500 *g*) and chlorophyll *a* concentrations were measured using the fluorometric method (APHA, 1998).

Statistical analyses

Although benthic invertebrates were generally identified to genus or species, most families were represented by only one or two species. In addition, patterns observed for most species within a family were similar. Therefore, to simplify presentation of these results, we focused our statistical analyses on family level identifications. However, measures of richness (e.g. number of taxa) were based on species- or genus-level identifications and significant variation in responses among species are noted in the text. One-way analysis of variance (ANOVA) was used for most statistical analyses (SAS Institute Inc., 1996). The exception was the colonisation experiment in 1999, where two-way ANOVA tested the influence of stream location and substratum source (Elk Creek versus Animas River) on colonisation. Invertebrate data for all analyses were $\log(x + 1)$ transformed to meet assumptions of ANOVA. If ANOVA indicated significant main effects ($P < 0.05$), Ryan's Q multiple range test was used to test for differences among groups. Ryan's Q is a conservative test that has been recommended because of its rigorous control of experimentwise error rates (Day & Quinn, 1989).

Results

Water quality

Water hardness, conductivity and temperature were lower in Elk Creek compared with the Animas River, whereas pH was similar between the two streams (Table 1). Concentrations of Zn in water were generally low or below detection in Elk Creek, but exceeded the hardness-based chronic criterion value (140 μg L^{-1}; U.S. Environmental Protection Agency, 1986) at most Animas River stations. The only exception was AN4, immediately downstream from Elk Creek, where metal concentrations were considerably lower. Elevated levels of Zn in biofilm from the Animas River reflected higher concentrations in water (Table 1). The mean concentration of Zn in biofilm was approximately 10× greater at station AN3 compared with Elk Creek.

Colonisation of clean substratum in the Animas River and Elk Creek

Total number of individuals, number of taxa and abundance of most macroinvertebrate groups on clean substratum were greater in Elk Creek than the Animas River (Figs 2a & 3). Although abundance of some organisms and some measures of community composition (e.g. number of taxa) recovered at station AN4 (immediately downstream from Elk Creek), Animas River communities were severely degraded. Ephemeroptera, including the families Ephemerellidae (*Drunella doddsi* Needham, *D. grandis* Eaton) and Heptageniidae (*Rhithrogena hageni* Eaton, *Cinygmula* sp.), dominated Elk Creek communities, but were generally absent from the Animas River. Similarly, abundance of most stoneflies (Plecoptera) was lower in the Animas River. An important exception was the nemourid stonefly *Zapada* sp., which was common in both Elk Creek and Animas River samples. The two dominant groups in the Animas River were hydropsychid caddisflies (Trichoptera) and orthoclad chironomids (Diptera).

Table 1 Physical and chemical characteristics of Elk Creek and the Animas River during summer 1998 and 1999. The table shows mean values (±SD) or results of a single measurement

Location	Hardness (mg L^{-1} $CaCO_3$)	pH	Conductivity	Temperature (°C)	Total Zn in water (μg L^{-1})	Zn in biofilm (μg g^{-1})
Elk Creek	17.5 (2.5)	6.9 (0.4)	43 (7)	8.2 (2.0)	6.5 (2.6)	419 (352)
AN1	160	6.6 (0.2)	310 (90)	11.5	304.7 (57.8)	–*
AN2	130	7.4 (0.2)	293 (98)	11.7	230.5 (44.5)	–*
AN3	156.5 (12.5)	6.6 (0.3)	310 (81)	11.9 (3.2)	319.0 (66.6)	4630 (691)
AN4	64	6.6 (0.4)	102 (13)	9.4	64.0 (1.0)	–*

*No data collected.

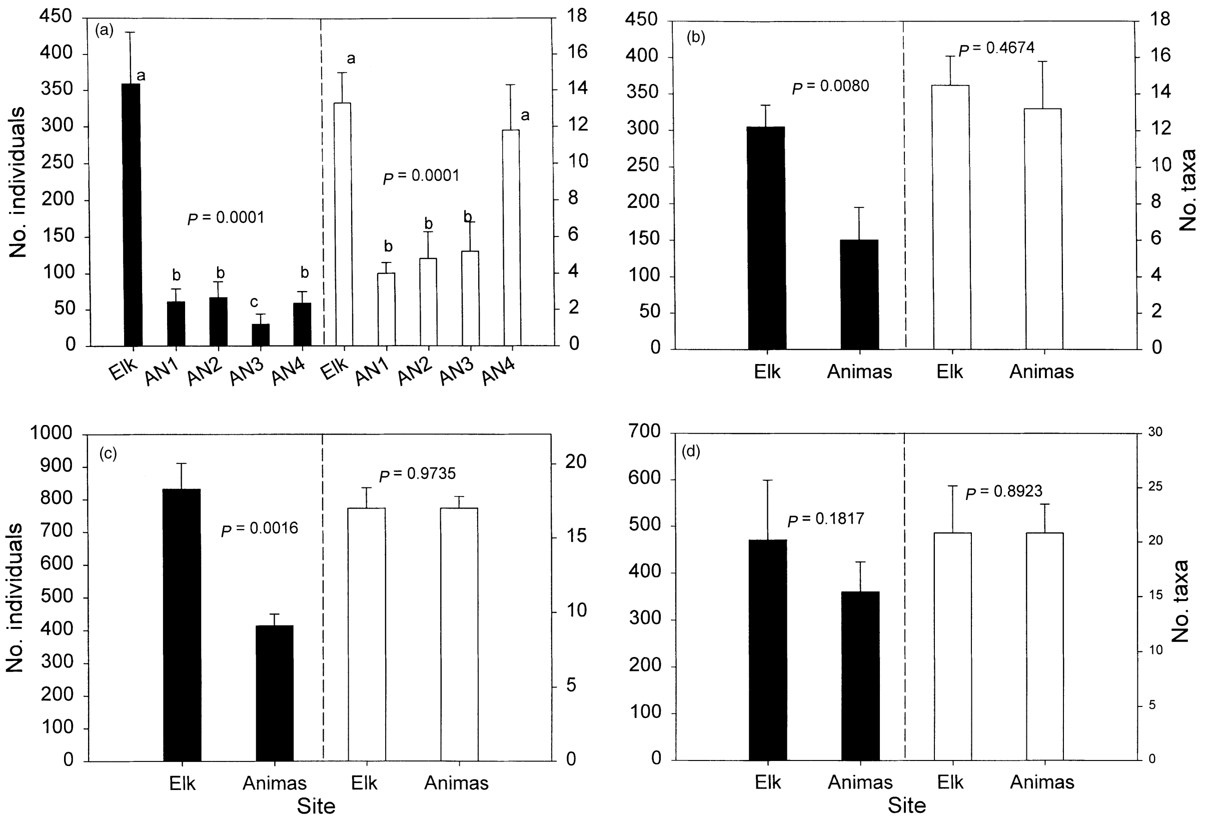

Fig. 2 Mean (+SD) macroinvertebrate abundance (solid bars) and number of taxa (open bars) in each of the four field experiments. (a) Colonisation of clean substratum in the Animas River and Elk Creek. (b) Results of the *in situ* toxicity test showing effects of Animas River water. (c) Colonisation of clean and metal-contaminated substratum placed in Elk Creek in 1998. (d) Colonisation of clean and metal-contaminated substratum placed in Elk Creek in 1999. Each panel shows *P*-values from one-way ANOVA. Means with the same letters are not significantly different based on Ryan's *Q* multiple comparison test.

Effects of water quality on benthic invertebrate communities

Comparison of benthic communities in day 0 trays with those in experimental chambers collected from Elk Creek indicated relatively little control mortality and/or loss of organisms for most taxa (Table 2). Although total macroinvertebrate abundance was approximately 25% lower in experimental chambers from Elk Creek compared with day 0 trays, most of this difference was attributed to the stonefly Taeniopterygidae.

Results of the community-level *in situ* toxicity test showed that Animas River water was highly toxic to some groups of benthic macroinvertebrates. While taxa richness did not differ significantly between Elk Creek and Animas River chambers (ANOVA, d.f. = 7, $P = 0.4674$), total invertebrate abundance was reduced by 50% after 96 h in the Animas River ($P = 0.0080$) (Fig. 2b). Exposure to Animas River water significantly reduced abundance of Heptageniidae ($P = 0.0007$) and Taeniopterygidae (Plecoptera) ($P = 0.0403$) compared with assemblages in Elk Creek (Fig. 4). Abundances of Ephemerellidae ($P = 0.0549$) and Capniidae (Plecoptera) ($P = 0.0675$) were also lower in the Animas River chambers. Although abundance of Chironomidae was significantly higher in Animas River chambers compared with those from Elk Creek ($P = 0.0094$), these organisms comprised a relatively small portion of the benthic community.

Effects of substratum quality on benthic invertebrate communities

Colonisation of clean and metal contaminated substrata. Aquatic insects colonised both clean (Elk Creek) and metal-contaminated (Animas River) substrata, but the substrata were not colonised equally by all taxa. In

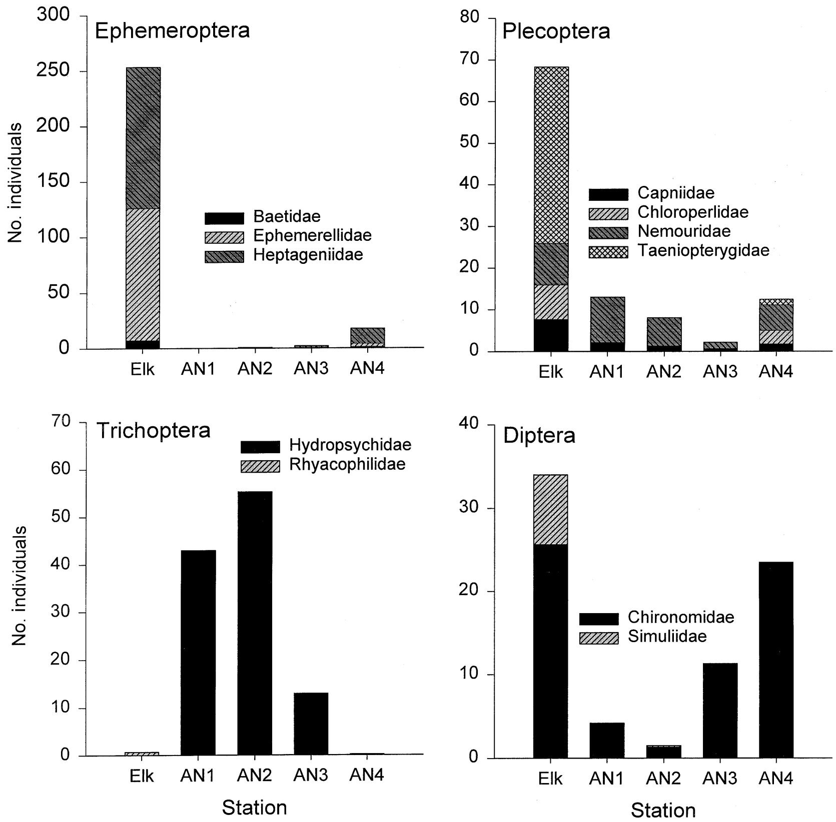

Fig. 3 Mean abundance of major macroinvertebrate groups that colonised clean substratum in Elk Creek and the Animas River.

Table 2 Total macroinvertebrate abundance, abundance of dominant groups and number of taxa (±SD) in day 0 trays and experimental chambers from Elk Creek. Day 0 trays were collected at the start of the *in situ* toxicity test

Benthic community measure	Day 0 trays	Elk Creek chambers
Baetidae	10.0 (1.4)	1.8 (1.3)
Ephemerellidae	129.0 (52.3)	106.5 (12.8)
Heptageniidae	85.0 (19.8)	104.3 (12.2)
Capniidae	29.5 (17.7)	22.5 (10.5)
Nemouridae	20.5 (4.9)	7.5 (5.8)
Taeniopterygidae	122.5 (70.0)	49.8 (10.8)
Hydropsychidae	3.5 (2.1)	2.5 (2.5)
Rhyacophilidae	3.5 (2.1)	1.5 (1.7)
Chironomidae	6.0 (2.8)	0.8 (0.5)
Simuliidae	2.0 (1.4)	0.8 (0.9)
Total abundance	415.5 (130.8)	304.8 (35.3)
Number of taxa	15.5 (0.7)	14.5 (1.9)

the 1998 experiment, total macroinvertebrate abundance was significantly greater on Elk Creek substratum, but taxa richness did not differ between substratum sources (Fig. 2c). The greatest differences in colonisation between substrata were observed for Ephemerellidae (primarily *D. doddsi*) (ANOVA, d.f. = 5, $P = 0.0019$), Taeniopterygidae ($P = 0.0472$) and Simuliidae ($P = 0.0194$) (Fig. 5a). Although abundance of heptageniid mayflies did not differ between substratum sources, there was variation among genera. Abundance of *Epeorus* sp. was significantly lower on Animas River substratum ($P = 0.0003$), whereas abundance of other heptageniids (*R. hageni* and *Cinygmula* sp.) did not differ between substratum sources.

The substratum quality experiment conducted in 1999 included greater replication and analyses of Zn levels associated with the biofilm. Mean (±SD) Zn concentration in Animas River biofilm collected at the end of the experiment (4365 ± 407 μg g^{-1}) was approximately 15× greater than in Elk Creek biofilm (288 ± 25 μg g^{-1}). Zinc concentrations in Animas River biofilm at the start of the experiment were similar to those measured on day 30 (4245 ± 1844 μg g^{-1}), indicating that potential exposure of invertebrates to metals remained high throughout the colonisation period.

Effects of substratum quality on total abundance and abundance of dominant groups were reduced when we repeated this experiment in 1999; however,

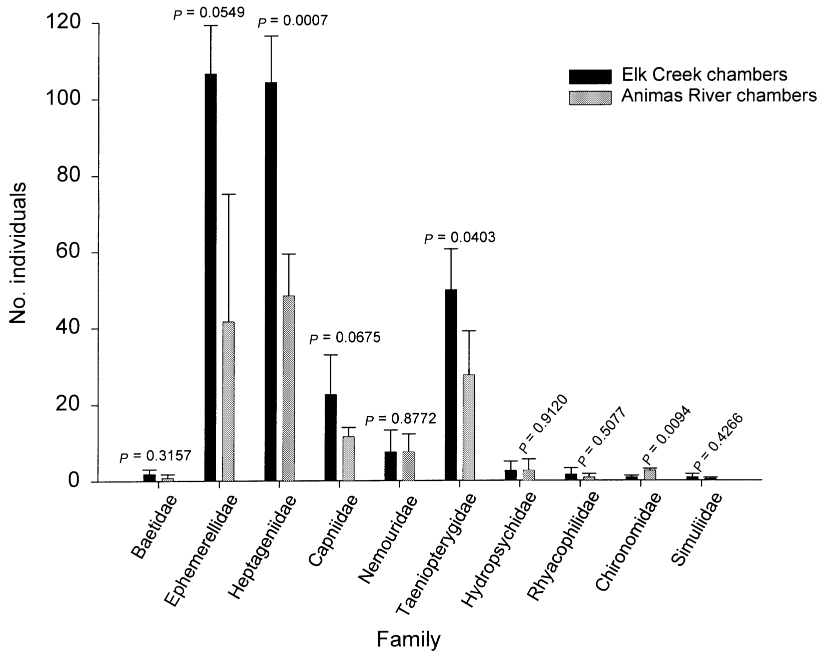

Fig. 4 Mean (+SD) abundance of dominant benthic invertebrates after 96 h in exposure chambers placed in Elk Creek and the Animas River. The figure shows *P*-values for each macroinvertebrate group based on one-way ANOVA.

the general patterns of colonisation were similar in the two studies (Figs 2d & 5b). As in 1998, abundances of Ephemerellidae (ANOVA, d.f. = 11, $P = 0.0068$) and Taeniopterygidae ($P = 0.0094$) were significantly greater on clean substratum, whereas Heptageniidae (all species) were relatively insensitive to substratum quality ($P = 0.2843$). Results of two-way ANOVA showed that location of substrata within the stream was insignificant ($P > 0.10$) for all taxa except Baetidae ($P = 0.0024$), a group which comprised a relatively small component of the benthic community. These results indicate that location in the stream generally did not influence patterns of colonisation.

Effects of contaminated substratum on Baetis tricaudatus. Metal concentrations in *B. tricaudatus* reflected concentrations measured in biofilm (Fig. 6a). Zinc levels in mayflies grazing Animas River biofilm were approximately two to four times greater than in organisms feeding on Elk Creek biofilm (ANOVA, d.f. = 13, $P = 0.0020$). Although mortality of *B. tricaudatus* was similar among treatments (mean = 16–20%), growth was lower for mayflies grazing on Animas River biofilm (Fig. 6b). The mean length of mayflies was similar among treatments at the start of the experiments (4.6–4.7 mm). After 7 days in the experimental chambers, mayflies grazing on Elk Creek biofilm grew 0.6–1.0 mm, whereas those grazing on Animas River biofilm grew 0.4 mm. The mean length of mayflies in the Elk Creek was 7–11% greater than in the Animas River treatments at the end of the experiment (ANOVA, d.f. = 19, $P = 0.0070$). Levels of chlorophyl *a* on tile substrata were also much greater in Elk Creek (23.8–103 μg cm^{-2}) than in the Animas River (0.13–0.48 μg cm^{-2}).

Discussion

While many studies in metal-polluted streams correlate metal concentrations with alterations in community structure (Winner, Boesel & Farrell, 1980; Nelson & Roline, 1996; Carlisle & Clements, 1999; Clements *et al.*, 2000), few have attempted to discern which aspects of the degraded habitat have the greatest effects on benthic communities. Our experimental approach measured the relative influence of water and substratum quality on benthic community structure in the Animas River. Although our study is limited to a single stream, the experimental results may have broad applicability to other metal-contaminated systems. The community-level toxicity experiment showed that Animas River water was acutely toxic to some macroinvertebrate groups, particularly Heptageniidae, Ephemerellidae and Taeniopterygidae. Results of the substratum colonisation experiments indicated that some groups

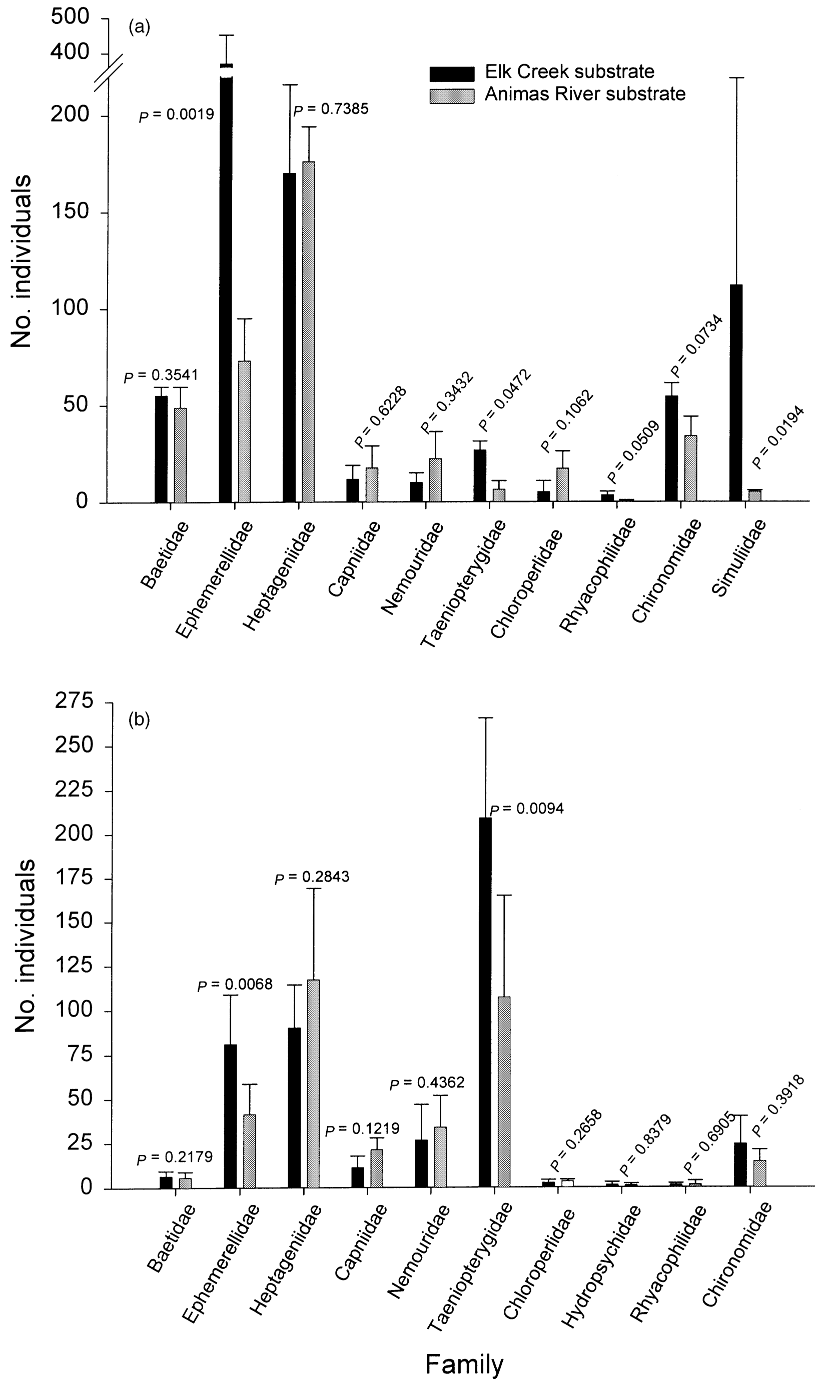

Fig. 5 Mean (+SD) abundance of dominant benthic invertebrates that colonised clean (Elk Creek) and metal-contaminated (Animas River) substrata placed in Elk Creek in 1998 (a) and 1999 (b). The figure shows *P*-values for each macroinvertebrate group based on one-way ANOVA.

(especially Ephemerellidae and Taeniopterygidae) were also highly sensitive to substratum quality.

Differences in metal sensitivity among taxa measured in the community-level toxicity experiment may explain patterns of benthic community structure observed in the Animas River. Although metal-sensitive groups were abundant in Elk Creek, they were generally absent in the Animas River where concentrations of Zn were two to three times greater than the U.S. Environmental Protection Agency chronic criterion value (U.S. Environmental Protection Agency, 1986). Benthic communities in the Animas River were dominated by taxa that exhibited little difference in survival between the Animas River and Elk Creek

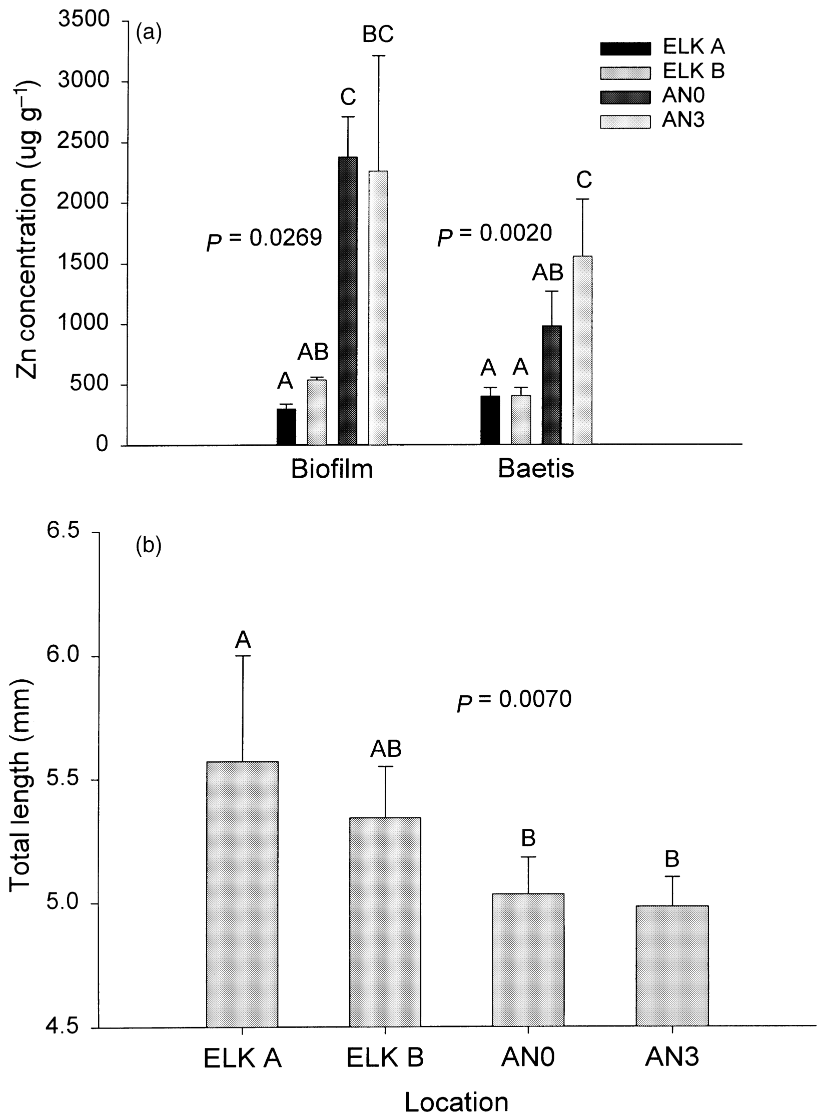

Fig. 6 (a) Mean (+SD) concentration of Zn in biofilm and *Baetis tricaudatus* after 7 days exposure to Elk Creek or Animas River tiles. (b) Mean length (mm) of *B. tricaudatus* after 7 days exposure to Elk Creek or Animas River tiles. Each panel shows *P*-values from one-way ANOVA. Means with the same letters were not significantly different based on the Ryan's *Q* multiple comparison test.

exposure chambers (Nemouridae, Hydropsychidae and Chironomidae). Some metal-sensitive taxa (Heptageniidae) were present in the Animas River immediately downstream from Elk Creek (station AN3) where Zn concentrations were considerably lower. It is likely that dilution from Elk Creek reduced metal concentrations and allowed these sensitive organisms to survive. These results suggest that Elk Creek and other unpolluted tributaries may be an important source for invertebrate colonisers following restoration of the Animas River. A similar result was reported for the Clark Fork River (Montana) where tributary inflows reduced exposure of metal-sensitive taxa (Axtmann, Cain & Luoma, 1997). These authors also suggest that tributaries serve as refugia from metal pollution and as potential sources of invertebrate colonists following remediation.

Results of the *in situ* toxicity test and patterns of benthic invertebrate community structure in the Animas River were similar to those observed in other western streams polluted by metals (Clements, 1994; Nelson & Roline, 1996; Farag *et al.*, 1998; Nimmo *et al.*, 1998). A spatially extensive study of 73 Rocky Mountain streams showed that heavy metals explained much of the variability in benthic invertebrate community structure between polluted and unpolluted sites (Clements *et al.*, 2000). In particular, mayfly abundance varied predictably along a gradient of metal pollution. Mayflies are generally more sensitive to metals than other aquatic insect taxa, and heptageniid mayflies have been suggested as indicators of metal pollution (Nelson & Roline, 1993, 1996; Clements, 1994). In the present study, Heptageniidae were very rare in the Animas River and greatly reduced (>50%) in experimental exposure chambers. These results support use of Heptageniidae as an indicator of metal contamination in western streams.

Poor substratum quality also influenced benthic invertebrate communities in the Animas River; however, the effects of substratum quality on macroinvertebrate colonisation varied among taxa. Despite high concentration of metals measured in biofilm, most taxa colonised Animas River substratum. The exceptions were Ephemerellidae, Taeniopterygidae (two groups that were also sensitive to Animas River water) and Simuliidae. Reduced abundance of Ephemerellidae on Animas River substratum resulted primarily from lower colonisation by *D. doddsi*, the dominant species. *D. doddsi* is found predominantly in clean, high-gradient streams with coarse substrata and a low degree of sedimentation (Mangum & Winget, 1991; Winget, 1993). We speculate that metal-contaminated biofilm associated with Animas River substratum inhibited colonisation by *Drunella* and other taxa.

Surprisingly, metal-sensitive heptageniids successfully colonised Animas River substratum placed in Elk Creek (with the exception of *Epeorus* in the first experiment). These results suggest that Heptageniidae tolerated short-term exposure to metal-contaminated biofilm. It is important to note that we were not able to simulate the high degree of sedimentation and embeddedness that occurred in some reaches of the Animas River. As a result, some taxa which colonised Animas River substratum in our experiment may be inhibited by other physical characteristics of the habitat. Furthermore, while some metal-sensitive taxa colonised Animas River substratum in our field experiments, laboratory experiments suggest that there may be chronic effects of exposure to metals in biofilm.

The grazing experiment conducted with *B. tricaudatus* demonstrated the potential for metal accumulation and reduced growth in invertebrates feeding on Animas River biofilm. Metal concentrations in Animas River biofilm were at least 10 times greater than in Elk Creek, and levels of Zn in *Baetis* reflected this pattern. However, because levels of chlorophyll *a* were much lower in the Animas River, it was not possible to discern the relative importance of metal contamination and food quality on *B. tricaudatus*. Although metal-sensitive taxa such as Heptageniidae colonised Animas River substratum, predicting chronic effects requires a more complete understanding of food quality and dietary exposure to metals in biofilm.

In summary, both substratum and water quality influenced benthic invertebrate community structure in the Animas River. Results of our field experiments indicated that toxicity of metals in water was probably the more important factor limiting benthic macroinvertebrates. Improved water quality in the Animas River may allow recolonisation by metal-sensitive taxa from uncontaminated tributaries if substratum conditions do not inhibit these organisms. Reduced instream metal concentrations as a result of planned restoration should eventually result in improved substratum quality and reduced concentrations of metals in biofilm. The potential for recovery of the Animas River is supported by results of a long-term study of the Arkansas River, a metal-polluted stream in central Colorado where remediation efforts have resulted in improved water quality (Nelson & Roline, 1996, 1999). Abundance of metal-sensitive organisms such as heptageniid mayflies have increased in the Arkansas River as a result of lower metal concentrations and improved substratum quality. Monitoring abundance of taxa such as Heptageniidae, which are sensitive to metals in water, and Ephemerellidae and Taeniopterygidae, groups sensitive to both water and substratum quality, would aid in assessing the progress of restoration in the Animas River and other metal-contaminated streams.

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