Aquatic Health and Exposure Pathways of Trace Elements

By Aïda M. Farag, David A. Nimick, Briant A. Kimball, Stanley E. Church,
Don Skaar, William G. Brumbaugh, Christer Hogstrand, and Elizabeth MacConnell

Chapter D10 of

Integrated Investigations of Environmental Effects of Historical Mining in the Basin and Boulder Mining Districts, Boulder River Watershed, Jefferson County, Montana

Edited by David A. Nimick, Stanley E. Church, and Susan E. Finger

Professional Paper 1652–D10

U.S. Department of the Interior
U.S. Geological Survey

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Chapter D10
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Abstract

Historical mine adits, mill tailings, and waste-rock dumps in the Boulder River watershed, Montana, are sources of trace elements to streams. Biological studies were undertaken to characterize the aquatic health of fisheries and the exposure pathway of these trace elements in watershed streams. Aquatic health was assessed by measuring the survivability of hatchery fish, measuring the biomass and density of resident fish, and documenting the physiology of fish exposed to trace elements. Pathways of exposure were assessed by determining trace-element concentrations in water, sediment, biofilm, invertebrates, and fish tissues and by defining relations among these components.

Instream survivability experiments at sites that had no resident trout and were downstream from inactive mines indicated that elevated concentrations of filtered cadmium, copper, and zinc were associated with increased mortality as well as hypertrophy (swelling), degeneration (dying), and necrosis of epithelial cells in gills of hatchery trout. In lower Cataract Creek, a site farther downstream, the health of resident trout was impaired. A decrease in the number of trout per acre indicated population-level effects; and increased metallothionein, increased products of lipid peroxidation, and elevated concentrations of trace elements in fish tissues indicated individual-level effects.

The concentrations of cadmium, copper, and zinc in water and those of arsenic, cadmium, copper, lead, and zinc in sediment in some watershed streams were sufficient to affect aquatic life. Concentrations of arsenic, copper, cadmium, lead, and zinc in invertebrates from lower Cataract Creek (63, 339, 59, 34, and 2,410 µg/g (dry weight, respectively)) were greater than concentrations in invertebrates from the Clark Fork River watershed, Montana (19, 174, 2.3, 15, and 648, respectively), where these trace elements were associated with reduced survival, growth, and health of cutthroat trout fed diets composed of the Clark Fork River invertebrates. The concentrations of all trace elements, except cadmium, in colloids and biofilm were significantly correlated, which suggests that transfer of trace elements associated with colloids to biological portions of biofilm is an important pathway in which trace elements associated with abiotic components are first presented to biotic components. The interrelationship of trace elements accumulating in the abiotic and biotic components sampled suggests that copper, cadmium, and zinc concentrations increased in fish tissues as a result of direct exposure from water and sediment contact and indirect exposure through the food chain. Apparently, trace elements have made contact with biota through these two pathways and have thereby compromised the overall aquatic health of the Boulder River watershed.

Introduction

Environmental effects of historical mining in a watershed typically are manifested as impairment of the health of aquatic life in streams. The health of fisheries usually is considered an important aquatic life issue because fish represent the top of the food chain in streams and because fish are a resource of considerable interest to land managers and the public. Aquatic health of the fisheries in a watershed can be defined in multiple ways. For example, direct measures of the reduced survivability of fish or reduced levels of fish biomass or density may indicate the inability of a watershed to support an optimal population size. However, to explain possible mechanisms of this population-level impairment and to establish a cause-and-effect relationship between a stressor (for example, concentrations in tissues or "tissue dose" of trace elements) and the effects at the population level (for example, reduced biomass/density), the health of individual fish must be studied. Understanding the pathways of trace-element exposure can further establish the cause-and-effect relationship between stressors and the particular concentrations accumulated in the aquatic components (biofilm, invertebrates, and fish). Interpretation of trace-element concentrations in these components can help define the overall health of the aquatic life in a watershed and provide a baseline for comparisons following remediation activities.

In the Boulder River watershed, Basin, Cataract, and High Ore Creeks (fig. 1) have elevated trace-element

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[1] Montana Fish, Wildlife, and Parks, Helena, Montana.

[2] Kings College, London, England.

[3] U.S. Fish and Wildlife Service, Bozeman, Montana.

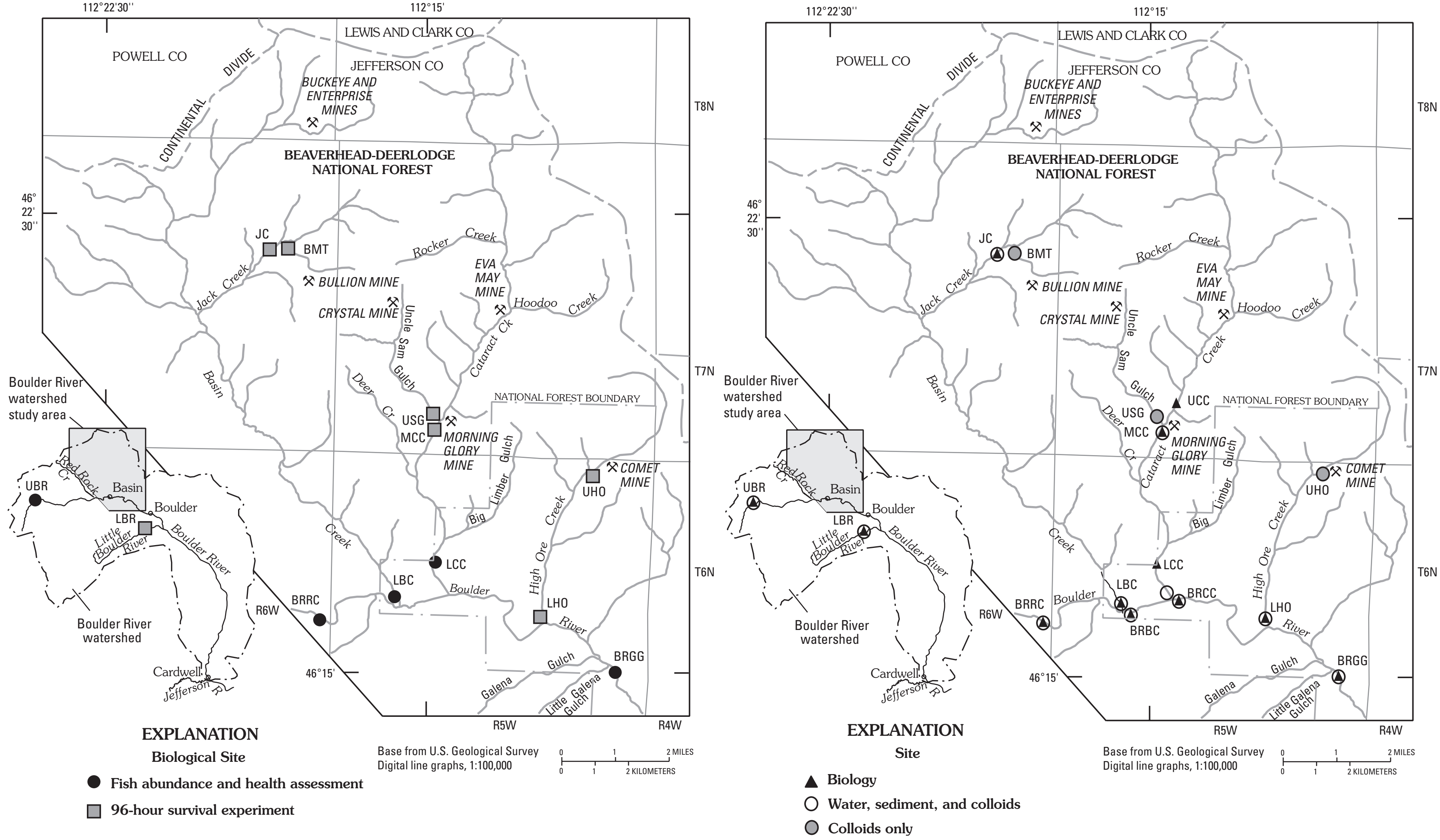

Figure 1. Biological sampling sites used for assessing aquatic health (left) and exposure pathways (right) in Boulder River watershed, Montana.

concentrations in water and streambed sediment, and trace-element loads from these streams affect trace-element concentrations in the Boulder River (Church, Unruh, and others, this volume, Chapter D8; Nimick and Cleasby, this volume, Chapter D5). All species of fish are absent from stream reaches downstream from a few draining mine adits in the watershed, but populations of brook trout (*Salvelinus fontinalis*), rainbow trout (*Oncorhynchus mykiss*), and cutthroat trout (*Oncorhynchus clarki*) reside farther downstream (Robert Wintergerst, United States Department of Agriculture (USDA) Forest Service, Missoula, Mont., oral commun., 1997). Additionally, viable populations of native, genetically pure, westslope cutthroat trout (*Oncorhynchus clarkii lewisi*) exist in High Ore Creek upstream from the Comet mine (fig. 1).

Purpose and Scope

This report examines the aquatic health of fisheries and the exposure pathways of trace elements in streams draining historical mining areas of the Boulder River watershed near Basin in southwestern Montana. Multiple investigations were conducted to fulfill the following biological objectives:

- Determine the survivability of westslope cutthroat trout in stream reaches that lacked fish
- Determine the biomass, density, and health of resident fish
- Characterize the pathway and partitioning of trace elements in water, sediment, biofilm, benthic macroinvertebrates, and fish
- Define the role of water, colloids, and sediment in the transfer of trace elements to aquatic life.

The first objective, to determine the survivability of westslope cutthroat trout in stream reaches that lacked fish, was important for documenting the maximum trace-element concentrations that would not be acutely toxic to fish. Some fishless reaches in Jack Creek, Uncle Sam Gulch, and High Ore Creek coincided with trace-element concentrations of filtered Cd (cadmium) greater than 50 µg/L (micrograms per liter) and as much as 5,000 µg/L of filtered Zn (zinc) in water (Nimick and Cleasby, this volume, figs. 11 and 14). Instream experiments to determine the survivability of cutthroat trout were conducted at seven sites (table 1; fig. 1). Additionally, the concentrations of whole-body ions (such as calcium, potassium, sodium) and histological changes in fish that occurred during the survival studies were investigated because they could provide insight about the mechanisms causing acute toxicity. Whole body calcium, potassium, and sodium could

Table 1. Biological sampling sites, Boulder River watershed.

Site ID (fig. 1)	Site name	Reference site	Assessments			
			Fish	Biofilm, invertebrates	Survival experiment	
					1998	1999
LBR	Little Boulder River	x		x	x	x
BMT	Bullion Mine tributary				x	x
JC	Jack Creek downstream from Bullion Mine tributary.			x	x	x
USG	Uncle Sam Gulch				x	x
UCC	Cataract Creek upstream from Uncle Sam Gulch.			x		
MCC	Cataract Creek downstream from Uncle Sam Gulch.			x	x	x
LCC	Cataract Creek at mouth		x	x		
UHO	High Ore Creek downstream from Comet mine.					x
LHO	High Ore Creek at mouth			x	x	x
LBC	Basin Creek near mouth		x	x		
UBR	Boulder River upstream from Trapper Creek.	x	x	x		
BRRC	Boulder River downstream from Red Rock Creek.	x	x	x		
BRBC	Boulder River downstream from Basin Creek.			x		
BRCC	Boulder River downstream from Cataract Creek.			x		
BRGG	Boulder River downstream from Galena Gulch.		x	x		

indicate upsets in ionoregulatory status as a result of metal exposure. Histological examinations could provide insight about acute changes in tissues, especially gills, which might result during the survivability experiments.

Our second objective was to determine the biomass, density, and health of fish in streams and in the mainstem of the study area. Trace-element exposure can affect aquatic biota and the overall ecological health of a river system (Farag and others, 1995). No assessments of individual fish health had been previously performed in the Boulder River. Surveys of fish biomass and density, measures of physiological function, and trace-element concentrations in tissues at five sites (table 1; fig. 1) were used to provide a picture of exposure related to physiological malfunction and any resulting decrease in fish populations. In addition, we used measures of metallothionein and products of lipid peroxidation (see results for definitions of these measurements) to define physiological malfunction. Then we interpreted these physiological malfunction data with data on trace-element concentrations in tissues and with data on fish biomass and density to determine the overall ecological health of sites in our study area within the Boulder River watershed.

Our third and fourth objectives were established to define the exposure pathways of trace elements. These objectives were to characterize the pathway and partitioning of trace elements in water, sediment, biofilm (also referred to *aufwuchs*, and consists of abiotic and biotic materials that form a surface layer on rocks in streams), invertebrates, and fish, and to define the influence of water, colloids, and sediment in the transfer of trace elements to aquatic life. To meet these objectives, we collected samples for trace-element analysis from both abiotic and biotic components throughout the watershed. Biofilm and benthic macroinvertebrates were collected from 12 sites; fish tissues were collected from 5 sites (table 1; fig. 1).

Previous Studies

Although aquatic health had not been previously rigorously studied in the Boulder River watershed, investigations of the effects of mining on aquatic life in the Boulder River began in 1975. Nelson (1976) found reductions in the survival of fish eggs during an egg bioassay and reductions in fish populations at sites on the Boulder River below Cataract Creek and High Ore Creek. Gardner (1977) found that the invertebrate community diversity in the Boulder River at a station downstream from High Ore Creek was reduced compared to an upstream station on the Boulder River located downstream from Red Rock Creek. In both studies, the differences between sites upstream and downstream of High Ore Creek were attributed to the greater concentrations of zinc in the water at the site below High Ore Creek.

In the 1990s, other investigations of the watershed were initiated to define the fitness of aquatic life in the Boulder River watershed. Gless (1990) designated Basin Creek a “stream of concern,” Cataract Creek as “degraded,” and High Ore Creek as “extremely degraded.” These classifications were based on elevated concentrations of arsenic in the water column and the rare presence of aquatic life in some stream reaches. Gless (1990) observed iron stains and dead vegetation as high as 4.5 ft above the stream banks of Cataract Creek. Martin (1992) documented elevated concentrations of cadmium, copper, and zinc in water, sediment, aquatic invertebrates, and fish in the Cataract Creek drainage and related these concentrations to the sources of trace elements in the drainage.

Acknowledgments

The authors thank Thomas E. Cleasby for assistance with water-sample and fish-tissue collections; Jack Goldstein, Dave Harper, and Brad Mueller for assistance during collections of biology samples and performance of toxicity experiments; Michèle Williams for assistance with measurements of lipid peroxidation; Ron Spoon and Kurt Hill for assistance during biomass/density and habitat assessments; and Mark Ellesieck and Darren Rhea for statistical assistance and graphics.

Methods

Aquatic Health

Assessing aquatic health of fisheries involved investigations that measured survivability of hatchery trout in instream experiments, measured fish biomass and density, assessed fish physiology, and statistical analysis of the data. Farag and others (2003) have provided more complete details of the methods described herein.

Survival Experiments

The survival of westslope cutthroat trout from the Washoe Park State Fish Hatchery, Anaconda, Mont., was determined with 96-hr in-situ experiments at five sites downstream from large inactive mine sites in Basin and Cataract Creek basins and in lower High Ore Creek (fig. 1) during low-flow conditions in 1998. We repeated the experiment in 1999 and added a sixth site in upper High Ore Creek. This site was added because of its close proximity to the Comet mine site, where remediation efforts were underway (Gelinas and Tupling, this volume, Chapter E2). This site also was in close proximity to a native population of westslope cutthroat trout living upstream of the Comet mine. A site on the Little Boulder River (fig. 1, LBR), which lacked significant historical mine activity, was selected as a reference site.

Twenty Age-1 fish were placed at each site, with five fish in each of four 4-L polyethylene enclosures. We investigated the whole-body ion status of sampled fish during 1998 to determine if ionoregulatory failure due to elevated concentrations of trace elements in the water column was the cause of death. During 1999, additional experimental fish were held at each site, and the tissues were processed by standard histological methods and examined by light microscopy.

Water quality was monitored at each site during the 96-hr survival experiments. Specific conductance, pH, and dissolved oxygen were measured onsite, and water samples were analyzed for total hardness and filtered and total-recoverable trace elements (Lambing and others, this volume, Chapter D7; Nimick and Cleasby, this volume).

Fish Biomass, Density, and Physiology

Five sites were selected to study resident fish population and physiology. The three tributary sites were upper Boulder River (UBR, reference site), lower Cataract Creek (LCC), and lower Basin Creek (LBC) (fig. 1). The two mainstem sites were the Boulder River downstream from Red Rock Creek (BRRC, reference) and downstream from Galena Gulch (BRGG). The differences in flow between the tributary and mainstem sites required that different methods be used to study populations in these two types of sites. Multiple-pass depletion was used to estimate populations in the smaller, tributary sites, and mark-recapture techniques were used to estimate populations in the mainstem sites (Zippen, 1958). Fish were collected by electrofishing. Total lengths and weights were recorded, and scales were collected to define the length at age.

Using methods described by Platts and others (1983), depth, velocity, substrate, and microhabitat features were measured at sites sampled for fish abundance. Weighted usable area was calculated from these parameters using the PHABSIM models (Platts and others, 1983). Calculated weighted usable area was used to differentiate the available habitat among sites. Suitability indices (SI) for all brook trout life stages were from Chapman (1995), rainbow trout fry SI values were from Raleigh and others (1984), and rainbow trout juvenile and adult SI values came from Ken Bovee (oral commun., 1997) for the South Platte River, Colo. Water-quality conditions were monitored periodically between October 1996 and September 1999 at the five fish-health-assessment sites (Nimick and Cleasby, this volume).

Thirteen to twenty-five rainbow trout of 8 ± 1.7 inches (mean ± 1 σ) and 0.20 ± 0.14 pounds were collected from each site for physiological analyses during low flow in 1997. Each fish was pithed, and a necropsy was performed immediately to define any gross abnormalities (for example, nodules on internal organs, discolored or frayed gills; Goede, 1989). Samples of gill and liver were dissected from each fish and immediately frozen with liquid nitrogen. Five additional rainbow trout (same size as preceding) were collected from each site and frozen for measurements of whole-body trace-element concentrations. At least five composite samples of each tissue from each site were prepared in the laboratory by combining tissues from two to five fish. To define the physiological condition of fish from the sites, concentrations of tissue trace elements, products of lipid peroxidation, and metallothionein were measured in aliquants of the composite samples. Arsenic, cadmium, copper, lead, and zinc were measured in gills, livers, and whole fish ($n \geq 5$ for each tissue type). Quality control included measurements of predigestion spikes, postdigestion spikes, digestion replicates, and reference tissue samples.

Statistical Analyses

Data for tissue trace-element concentrations, metallothionein, products of lipid peroxidation, length at age, and fish biomass/density estimates were analyzed with one-way analysis of variance (SAS Institute, 1989). We tested the data for equality of variances with the Levene test after transforming the data when necessary. Means were compared using a Fischer least significant difference test with a statistical criterion of $\alpha = 0.05$. We did not statistically analyze the survival data.

Exposure Pathways

We collected water, sediment, biofilm, and invertebrates from 12 sites within the Boulder River watershed (table 1) to examine the pathway of metals in abiotic and biotic components of streams. Five sites were on the mainstem Boulder River (UBR, BRRC, BRBC, BRCC, and BRGG) and seven sites were on tributaries (UBR, LHO, LCC, MCC, UCC, LBC, and JC; fig. 1). BRRC was a reference for the mainstem sites. Data from UBR (characteristics were similar to tributaries) and LBR were combined as a reference for the tributary sites. Once the boundaries of a site were established, we collected samples of biofilm and invertebrates at four riffle localities at the site ($n = 4$). Methods of sample collection and data analysis are summarized herein. A more complete description of these methods is in Farag and others (work in progress).

Water samples were collected periodically during 1996–99 at all sites. Water temperature, specific conductance, pH, and streamflow were measured at the time of sampling (Nimick and Cleasby, this volume). Water samples were analyzed for total-recoverable (unfiltered) and filtered (0.45-µm pore-size) trace-element concentrations. We determined colloidal concentrations of trace elements in the water column indirectly by subtracting an ultrafiltered trace-element concentration (0.1-µm, or 10,000 Dalton, pore size) from a total-recoverable trace-element concentration. A composite sediment sample was collected at each site over a 100–150 ft reach by means of a plastic scoop (Church, Unruh, and others, this volume). We collected biofilm by gently scraping the surface of rocks collected from the near-shore streambed. Macroinvertebrates were sampled with a 3-mm mesh net attached to a 2 × 4-ft frame. The substrate in approximately 65 ft^2 of riffle upstream from the net was overturned, and the dislodged macroinvertebrates were collected in the net. Fish were collected as described in the preceding section. (See "Fish Biomass, Density, and Physiology.")

Statistics

Concentrations of trace elements measured in water, sediment, biofilm, invertebrates, gill, liver, and whole fish collected from the test sites were compared to concentrations in samples collected from the reference sites. Data from multiple reference sites were combined into one group and are referred to as the pooled reference (REF). Multiple reference sites were not sampled for gill, liver, and whole fish. Data for all sites were tested for homogeneity of variance and transformed when necessary. Under the assumption of equal variances, an Analysis of Variance (ANOVA) was performed to test for differences between means. If any difference was detected, Dunnett's one-tailed t-test (Dunnett, 1955) was used to make comparisons between each experimental site and the reference site for each trace element. Regressions also were performed to define correlations of trace-element accumulation among water, sediment, colloids, biofilm, and fish.

Results

Aquatic Health

Survival Experiments

Survival of caged westslope cutthroat trout at 96 hours was less at all test sites (table 1) than at the Little Boulder River reference site during 1998 and 1999 (fig. 2). Except survival at the lower High Ore Creek site, which was 33 percent during 1999, survival at 96 hr was 0 percent at all experimental sites during both years. In most cases, survival was affected in 24 to 48 hr during 1999. Fish died more quickly at Jack Creek and middle Cataract Creek during 1999 as compared to 1998. In the two most extreme cases, cutthroat trout placed in Uncle Sam Gulch and the Bullion Mine tributary died in 5 and 8 hr, respectively, during 1999. Survival was reduced to 5 percent at upper High Ore Creek at 72 hr, and the experiment at this site was ended at that time.

The concentrations of whole-body ions in fish in 1998 did not differ significantly among sites (reference site included).

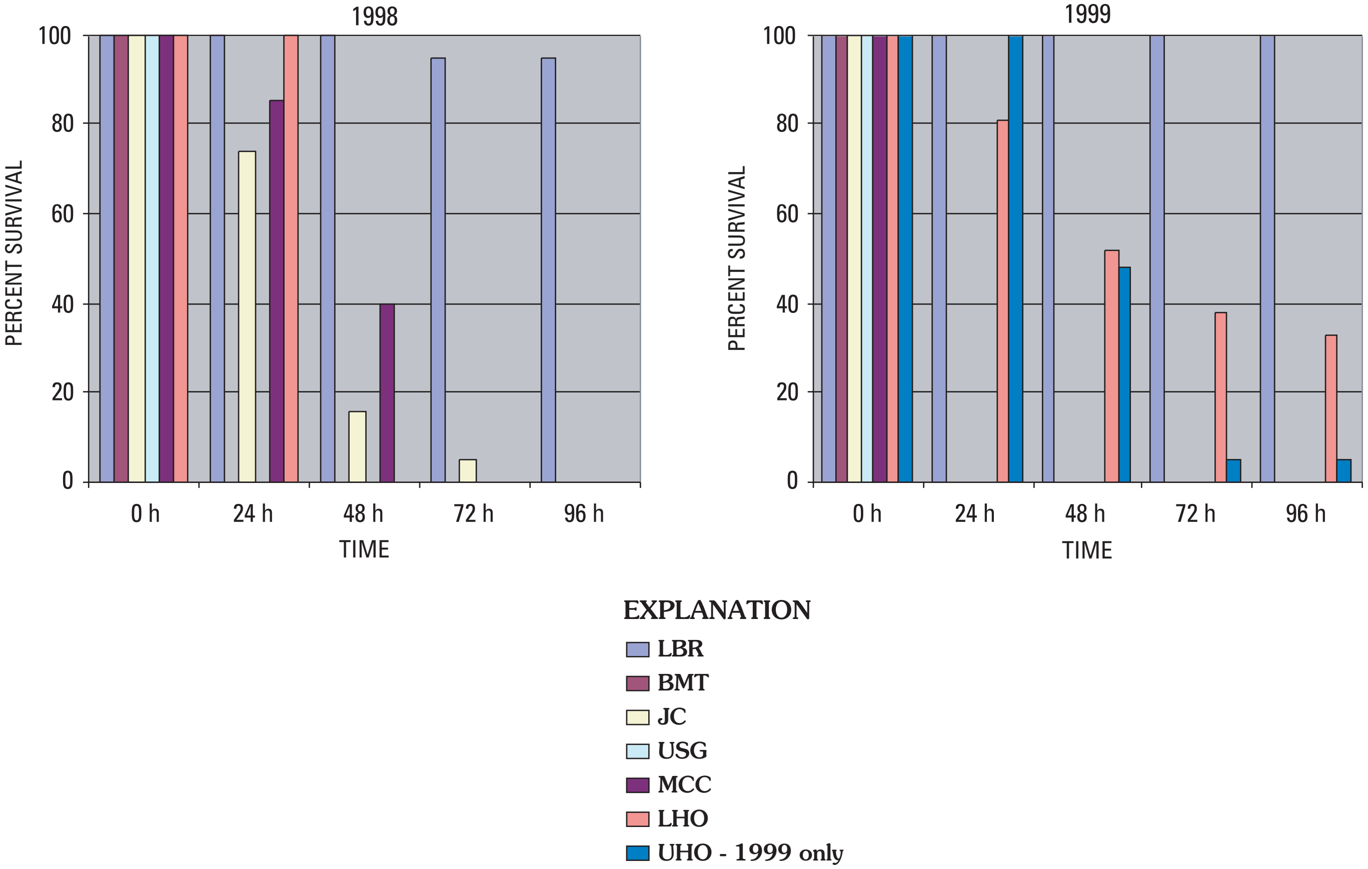

Figure 2. Survival of hatchery cutthroat trout placed in various tributaries of Boulder River for as long as 96 hr during 1998 and 1999.

Concentrations of ions ranged from 22,500 to 24,900 µg Ca/g, 12,000 to 15,100 µg K/g, and 2,050 to 3,180 µg Na/g, with the highest concentrations noted in fish from Uncle Sam Gulch. The histological analyses during 1999 indicated that degeneration (dying) and necrosis (death) of gill epithelia was the most significant tissue lesion. Excessive mucus production and hypertrophy (swelling) also were noted in gill epithelia of fish from the test sites (fig. 3). Spongiosis, a condition of edema and necrosis, was also observed in the nares (nasal sensory organ) of fish held in the test sites. Additionally, excessive mucus was noted in the skin of fish from all test sites. There was some hypertrophy in the gill lamellae and abundant mucus in the nares of fish held at the reference site, but these changes were less severe than those at the test sites. The proliferation of epithelial cells noted in gills of fish from both the upper and lower High Ore Creek sites indicates that toxicity was less acute at these sites, consistent with the longer times to death at these sites in 1999. Additionally, the pseudobranch of fish from lower High Ore Creek contained cystic (fluid-filled) areas, an abnormal condition not noted at other sites. We noted dark longitudinal coloration in the skin at death for fish at the High Ore Creek sites. This discoloration was about 0.5 cm wide and was observed across the length of the fish. Correspondingly, an increased accumulation of melanocytes was noted in the skin of fish collected from lower High Ore Creek.

Water Chemistry—Survival Experiments

Water chemistry was variable in stream reaches where 96-hr survival experiments were performed in 1998 and 1999. The onsite experiments were performed between mid-July and early August, but streamflow conditions were different each year. During the 1999 experiments, streams had low-flow conditions typical of late summer, whereas the 1998 experiments were conducted near the end of spring runoff. Streamflow was higher during the 1998 experiments, and trace-element concentrations generally were lower in 1998 than in 1999. In both years, almost all the cadmium and zinc was in the filtered (0.45 µm) fraction, and copper was divided about equally between the filtered and particulate phases. Therefore, filtered concentrations of cadmium, copper, and zinc are presented (table 2). Concentrations of arsenic and lead generally were low relative to concentrations of the other trace elements (<3 µg As/L except LHO and UHO with 22–33 µg As/L; <1 µg/L for Pb/L except BMT with 3.1 µg Pb/L), and the data are not presented. The pH values were neutral to slightly basic (7.0–8.3) except for the site on Bullion Mine tributary, where pH was 5.2–5.4. The temperature range was 11°–21°C for all sites.

The relationship between trace-element concentrations and mortality followed a consistent pattern; higher concentrations resulted in greater and more rapid mortality. At the reference site on the Little Boulder River (LBR), hardness (48–56 mg/L) and trace-element concentrations (<0.3 µg Cd/L, 2 µg Cu/L, 2–5 µg Zn/L) were low. Water-quality conditions at this reference site were similar in 1998 and 1999. However, streamflow was 4–8 times higher during the experiments in 1998 than in 1999 in the Jack and Cataract Creek drainages. Consequently, constituent concentrations were higher in 1999 at the four sites in these two drainages (BMT, JC, USG, MCC). In 1999, concentrations of cadmium, copper, and zinc were about 100 percent higher and hardness values were about 50 percent higher than the corresponding 1998 levels at each site. Hardness measured generally higher at the two High Ore Creek sites (LHO and UHO 130–140 mg/L) compared to all other sites. Concentrations of trace elements were similar at the two sites on High Ore Creek in 1999 and somewhat higher at lower High Ore Creek in 1998 compared to 1999, apparently as a result of streamflow (table 2).

Results of hourly sampling in 1999 indicated that filtered zinc concentrations typically varied at many sites (Lambing and others, this volume). These variations resulted from normal diel concentration cycles and from the effect of storm runoff, which occurred during the toxicity experiments in Jack Creek in 1999. Diel cycles resulting in 2- to 3-fold changes in dissolved cadmium and zinc concentrations have been documented at several sites in and near the study area and are thought to be caused by the effect of water temperature and pH on the partitioning of cadmium and zinc between dissolved and sorbed phases (Fuller and Davis, 1989; Lambing and others, this volume). Therefore, a wide range of concentrations may occur daily at each site.

Fish Biomass and Density Estimates

Of the three tributaries studied, the combined biomass of all species of trout observed (on either an areal or a lineal basis) was the smallest at the lower Cataract Creek site (table 3). There were 12 ± 3.5 lb/acre at lower Cataract Creek versus 60±4.9 lb/acre at the upper Boulder River site and 37±1.9 lb/acre at the lower Basin Creek site. Brook and rainbow trout were the two trout species found in the tributaries; brook trout were the most common species of fish at the upper Boulder River site, whereas rainbow trout were the most common species of fish present at the lower Cataract Creek site. However, both species were present in about equal numbers in lower Basin Creek. The species composition in lower Cataract Creek and lower Basin Creek seemed to generally reflect the composition of the nearest mainstem sites; the Boulder River site near Red Rock Creek had a similar composition to that in lower Basin Creek, and the Boulder River site near Galena Gulch was similar to that in lower Cataract Creek. Although there was a trend of less biomass at the Boulder River site near Galena Gulch (BRGG) compared to the reference site upstream from the affected study reach (BRRC), the difference was not significant (table 3). There were no significant differences among lengths at age calculated from scales of rainbow trout samples from the three tributary sites (fig. 4), nor was there a difference in length at age between fish sampled from the mainstem sites (data are not presented). The observations for density (number per 1,000 ft) have the same pattern as biomass results for all sites (table 3).

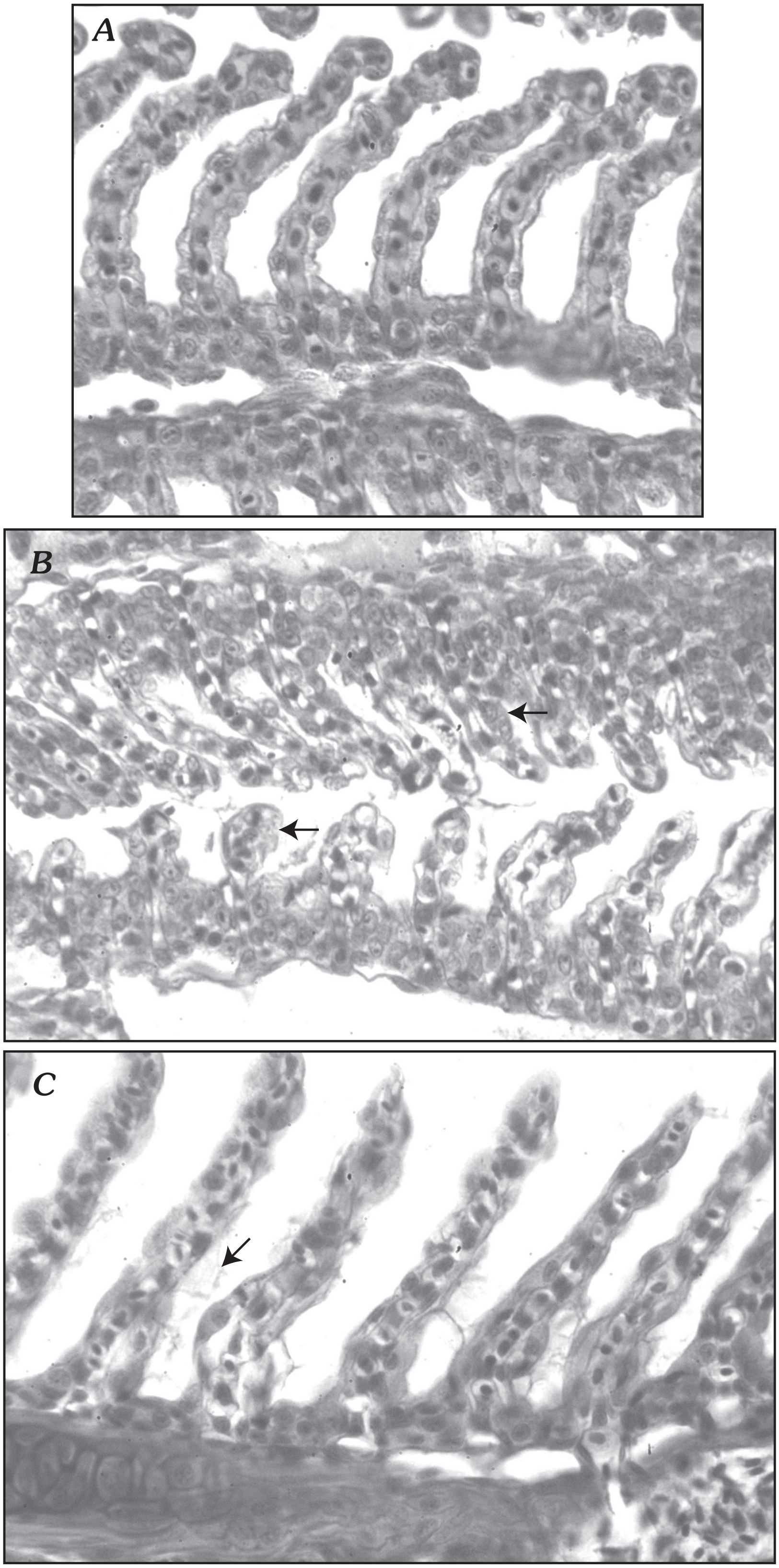

Figure 3. Gill tissue of trout held during the 1999, 96-hr survival experiment. *A*, Gill section of a cutthroat trout held at Little Boulder River site (reference). Section is 5 µm thick, stained with hematoxylin and eosin, × 250. *B*, Gill section of a trout held at Middle Cataract Creek site. Top arrow points to hypertrophied cells; bottom arrow points to degenerate epithelial cell. Section is 5 µm thick, stained with hematoxylin and eosin, x 250. *C*, Gill section of a trout held at upper High Ore Creek site. Arrow points to excessive mucus. Section is 5 µm thick, stained with hematoxylin and eosin, x 250.

Table 2. Physical and chemical data for stream sites during the 96-hour survival experiments.

[Trace-element concentrations are for filtered samples; median values are listed when multiple samples were collected during an experiment]

Site (fig. 1)	Sample date	Cadmium (µg/L)	Copper (µg/L)	Zinc (µg/L)	pH (standard units)	Hardness (mg $CaCO_3$/L)	Streamflow (ft^3/s)
LBR – Reference	7/21/98	<0.3	2.0	2.0	7.9-8.1	48	6.4
	8/2/99	<0.3	2.4	2.5	8.0-8.2	56	2.5
BMT	7/21/98	20	113	2,160	7.4	39	1.1
	8/2/99	38	557	3,970	5.2-5.4	66	0.02
	8/3/99	35	314	3,920	5.9	--	--
JC	7/21/98	3.4	33	377	7.7-7.8	29	32.5
	8/2/99	8.0	51	656	7.0-7.4	44	1.1
USG	7/21/98	22	84	1,830	7.6	41	4.9
	8/2/99	75	377	5,730	7.3	--	--
	8/4/99	59	206	4,840	7.5	63	0.71
MCC	7/21/98	4.4	34	391	7.8-8.2	36	20.5
	8/2/99	9.6	48	714	7.9	--	--
	8/4/99	9.3	48	651	7.8-7.9	48	4.6
UHO	8/3/99	2.5	3.7	550	8.2	140	0.35
LHO	7/21/98	3.7	5.0	987	8.1-8.3	130	1.8
	8/2/99	2.0	3.6	459	8.1-8.3	140	0.71

Fish species observed at the five fish-abundance sites during 1997–99 included three native species (longnose sucker *Catostomus catostomus*, mottled sculpin *Cottus bairdi*, and mountain whitefish *Prosopium williamsoni*) and four non-native species (Yellowstone cutthroat trout, rainbow trout *Salmo gairderi*, eastern brook trout *Salvelinus fontinalis*, and brown trout *Salmo trutta*). The mainstem sites had a greater number of species than did the tributary sites. Most of the brown trout and mountain whitefish captured in the mainstem (in 1998 and 1999) were in spawning condition and may have migrated from sites farther downstream. Mottled sculpin were found at the upper Boulder and the lower Basin Creek tributaries, and mountain whitefish were found at lower Basin Creek. Neither mottled sculpin nor whitefish were found at lower Cataract Creek. None of the rainbow, cutthroat, or brook trout collected from the tributary sites appeared to be spawning.

Habitat Characterization of Fish Abundance Sites

The habitat of the tributary sites differed in many ways. The lower Cataract Creek site had a higher stream gradient (2.7 percent) than the other tributary sites (2.0 and 1.1 percent for UBR and LBC, respectively) as well as a higher percent of habitat described as riffles (94 percent as compared to 70 percent for UBR and 60 percent for LBC). Lower Cataract Creek had the widest bankfull channel width (80 ft) and a corresponding lower mean depth and velocity than the other sites. All sites were similar in having a fair amount of canopy cover (13.4–19.8 percent), little woody debris (0.1–1.4 percent), low levels of embeddedness (1.3–1.4 percent), and small amounts of undercut bank (1–4 percent) or overhanging vegetation (1–4 percent). For the mainstem sites, the Boulder River near Galena Gulch had a much higher percentage of riffles (84 percent) than the Boulder River near Red Rock Creek (38 percent), even though the Boulder River near Galena Gulch site had a slightly lower gradient (0.8 percent vs. 1.2 percent). Both sites were characterized as having no pool habitat, and the sites were also similar in the amount of canopy cover, woody debris, embeddedness, bank cover, undercut banks, and overhanging vegetation. Mean velocity at the Boulder River near Red Rock Creek site was slightly higher (1.04 ft/s) than that at the Boulder River near Galena Gulch (0.99 ft/s). Boulder River near Galena Gulch is a larger stream, having a bankfull channel width of 114 ft, compared to only 68.6 ft for the Boulder River near Red Rock Creek. Microhabitat was quantified in terms of weighted usable area from one set of measurements taken along 10 or 11 transects at each site in late September–early October 1998. Large amounts of available habitat are reflected by large amounts of weighted usable area. Therefore, observed excessive differences in weighted usable area among sites would suggest that habitat is responsible for differences in biomass/density estimates. For both brook and rainbow trout, the weighted usable area in the tributaries was greatest in lower Basin Creek for fry and

Table 3. Size ranges and biomass/density estimates of brook, rainbow, and cutthroat trout in tributaries and mainstem of the Boulder River.

[The estimates were performed during late July (1997) and early October (1998 and 1999), and numbers are reported with standard error in parentheses; "*" indicates significant difference from reference at $p = 0.05$]

Site (fig. 1)	Size range (inches)	No./1,000 ft	No./acre	Pounds/acre
		Tributaries		
UBR – Reference				
1997	2.7-12.4	214 (53)	551 (137)	57 (13)
1998	2.0-9.6	212.4 (19)	547 (49)	53 (4.6)
1999	2.4-10.2	325 (19)	837 (50)	70 (4.0)
Mean			645 (96)	60.0 (4.9)
LBC				
1997	1.6-13.2	144 (23)	404 (63)	55 (7.7)
1998	2.0-10.5	210 (8)	591 (22)	55 (2.8)
1999	1.7-11.1	381 (13)	1,070 (35)	61 (1.9)
Mean			688 (198)	56.8 (1.9)
LCC				
1997	2.7-8.3	41 (14)	74 (25)	5.2 (1.8)
1998	1.4-8.6	142 (12)	257 (21)	14 (1.2)
1999	3.3-8.6	94 (3)	171 (5)	17 (0.5)
Mean			167 (152)*	12 (3.5)*
		Boulder River		
BRRC – Reference				
1998	4.2-11.8	148 (30)	275 (18) (37)	31 (6.1)
1999	3.9-12.5	219 (15)	180 (37)	50 (5.4)
Mean			227 (48)	40 (9.3)
BRGG				
1998	4.2-12.6	177 (10)	200 (15)	19 (1.1)
1999	4.3-13.8	227 (17)	156 (9)	27 (2.5)
Mean			178 (22)	225 (4.2)

juveniles, and greatest for adults in upper Boulder River. Lower Cataract Creek had the lowest weighted usable area for each life stage (table 4). In the mainstem, weighted usable area for juveniles and adults was greatest for both species in Boulder River near Galena Gulch, but the weighted usable area for fry was greatest for both species at Boulder River near Red Rock Creek.

Water Chemistry—Resident Fish Biomass/Density and Health

Data from water samples collected in 1996–97 at the five biomass/density and fish-health sites indicated that filtered and total-recoverable concentrations of Al, Sb, As, Ba, Be, Cr, Co, Fe, Mn, Hg, Mo, Ni, U, Ag, and U (Nimick and Cleasby, this volume) were either less than method-detection levels or less than concentrations established by the U.S. Environmental Protection Agency (1999, 2001) to protect aquatic life. In contrast, concentrations of Cd, Cu, Pb, and Zn (table 4) frequently were elevated at sites downstream of the historical mining activity (LBC, LCC, and BRGG).

Trace-element concentrations generally were low (table 5) during all flow conditions at the sites (UBR and BRRC) upstream of historical mining activities. At Boulder River near Red Rock Creek, concentrations of copper exceeded the acute and chronic aquatic-life standard in 2 of 4 samples measured for filtered and 6 of 10 samples measured for total-recoverable copper during high flow. Some total-recoverable lead concentrations also exceeded the chronic but not acute aquatic-life standard at Boulder River near Red Rock Creek. Trace-element concentrations were higher at the other three sites. The highest concentrations of trace elements occurred at the lower Cataract Creek site, where

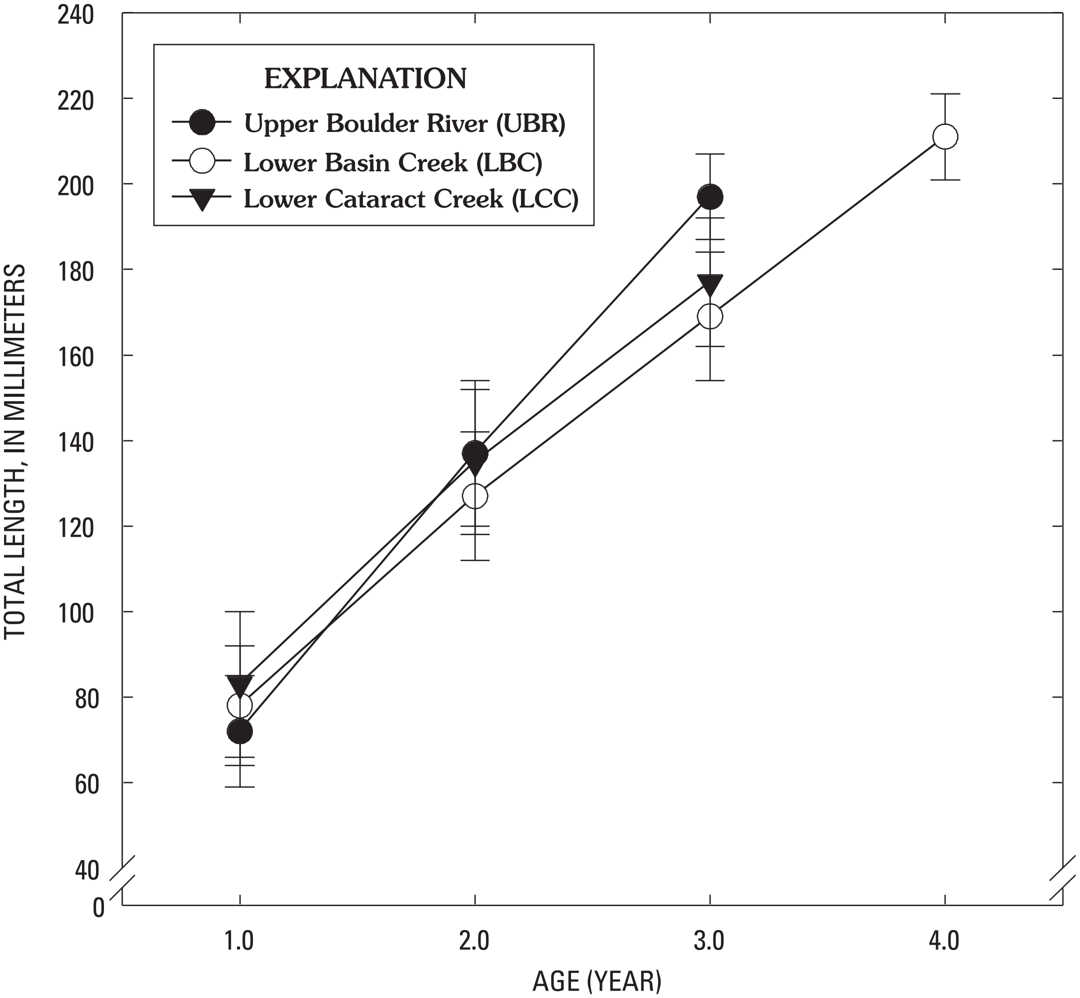

Figure 4. Comparisons of growth of trout in three tributaries in Boulder River watershed. Means are presented as symbols and ± standard deviations are represented with bars.

filtered cadmium, copper, and zinc concentrations exceeded acute standards in every sample. Filtered zinc concentrations also exceeded acute standards in all samples from lower Basin Creek and Boulder River near Galena Gulch. Concentrations of cadmium and copper frequently exceeded standards during high flow at these two sites and at Boulder River near Galena Gulch during low flow as well.

Concentrations of filtered cadmium and zinc at the lower Cataract Creek site were similar to concentrations associated with mortality in the 96-hr survival experiments. Median concentrations (table 5) at this site were higher than at middle

Table 4. Weighted usable area (ft^2 WUA) per 1,000 ft^2 of stream for brook and rainbow trout fry, juveniles, and adults.

[WUA based on the product of suitability index values for depth, velocity, and substrate; Fry (<2 inches), Juveniles (2–8 inches), and Adults (>8 inches)]

Site (fig. 1)	Brook trout			Rainbow trout		
	Fry	Juvenile	Adult	Fry	Juvenile	Adult
UBR	4.6	5.6	27.2	20.6	9.6	3.7
LBC	5.2	5.6	25.7	23.6	15.6	3.1
LCC	4.5	5.6	19.7	17.8	8.6	2.1
BRRC	4.1	2.5	14.5	12.4	16.0	5.3
BRGG	2.2	2.5	21.3	18.1	23.3	6.0

Table 5. Physical and chemical data for stream sites where fish health was assessed, Boulder River watershed, Montana, 1996–99.

[Median filtered (filt.) and total-recoverable (total) trace-element concentrations are listed, and *n* represents the number of samples collected on different dates throughout the flow condition]

Site (fig. 1)	*n*	Streamflow (ft^3/s)	pH	Hardness (mg/L as $CaCO_3$)	Filtered trace-element concentration, median (µg/L)									
					Arsenic		Cadmium		Copper		Lead		Zinc	
					Total	Filt.	Total	Filt.	Total	Filt.	Total	Filt.	Total	Filt.
High-flow conditions														
UBR	2	14-81	7.6-8.1	21-38	4.0	2.5	<1	<0.3	3	1.5	<1	<1	<10	1
BRRC	10	106-724	7.6-8.2	22-50	5.5	3.3	<1	<0.3	5	3.3	1.1	<1	11	3
LBC	10	14-251	7.0-8.0	12-35	11	4.0	<1	0.4	12	8.7	2.6	<1	81	61
LCC	11	14-170	6.8-8.0	18-54	17	3.4	1.8	1.3	46	33	6.7	<1	177	139
BRGG	11	177-1,049	7.5-8.2	19-48	12	4.0	<1	0.3	20	11	5.9	<1	73	46
Low-flow conditions														
UBR	2	4-4	8.0	52-53	2.5	2.0	<1	<0.3	1.5	<1	<1	<1	<10	<1
BRRC	8	7-25	7.4-8.3	54-61	3.0	2.4	<1	<0.3	1.4	1.7	<1	<1	<10	2.3
LBC	7	4-7	7.5-8.2	39-42	7.0	4.0	<1	0.4	4.0	3.0	<1	<1	66	63
LCC	7	4-7	7.5-8.3	59-69	4.8	3.0	5.0	4.9	24	22	<1	<1	419	397
BRGG	8	18-35	7.7-8.5	61-68	6.0	5.0	<1	0.8	10	9.0	<1	<1	180	160

Cataract Creek and Jack Creek during the 1998 experiments in which 100 percent mortality was observed at 96 hr. Filtered trace-element concentrations at the other four fish-assessment sites during low flow were considerably less than the concentrations associated with mortality in the survival experiments (tables 2 and 5).

Tissue Trace Elements

Concentrations of most trace elements in the livers of resident rainbow trout were greatest in fish from lower Cataract Creek (table 6). Concentrations in the livers of rainbow trout from lower Cataract Creek were higher than 1,000 µg Cu/g, 60 µg Cd/g, and 13 µg As/g (dry wt). Concentrations of trace elements in the livers of fish from lower Basin Creek and Boulder River near Galena Gulch also were elevated above those measured at the reference sites (UBR and BRRC). In general, the pattern of metal accumulation in livers was LCC > LBC ≥ BRGG > BRRC ≥ UBR (table 6). In an exception to this pattern, the greatest concentration of lead was in fish from Boulder River near Galena Gulch.

The pattern of trace-element concentrations in livers was similar to the pattern in gills (table 6). The greatest concentrations of copper, cadmium, and zinc were in the gills of fish from lower Cataract Creek (>20 µg Cu/g, >60 µg Cd/g, and >700 µg Zn/g) (table 6). Whereas lesser concentrations of copper and greater amounts of zinc occurred in the gill compared to the liver, the concentrations of cadmium in livers and gills of fish from lower Cataract Creek were nearly identical. As was noted for liver, the greatest concentrations of lead were in gills from fish at Boulder River near Galena Gulch. However, unlike liver, there was no significant accumulation of arsenic in the gills.

The pattern of trace-element accumulation described for livers and gills was similar in whole fish. Again, the greatest concentrations of most trace elements in whole fish were in samples from lower Cataract Creek, where 22 µg Cu/g, 4.9 µg Cd/g, and 8 µg As/g were measured (table 6). Although less cadmium was observed in the whole body versus liver and gill, the mean cadmium concentration in whole fish from lower Cataract Creek was 4.9 µg/g. There was no significant accumulation of lead in whole fish.

Metallothionein

Physiological malfunction can be defined with measurements of metallothioneins and products of lipid peroxidation (Farag and others, 1994, 1995). These measurements have been associated with reduced growth in laboratory experiments (metallothioneins: Dixon and Sprague, 1981; Roch and McCarter, 1984; Marr and others, 1995; lipid peroxidation: Woodward and others, 1995) and with exposure to trace elements (metallothioneins: Dixon and Sprague, 1981; Roch and

Table 6. Mean trace-element concentrations in fish, 1998.

[Means followed by the same letter (within a tissue) are not significantly different at $p = 0.05$; standard error of the mean is in parentheses]

Site (fig. 1)	Trace-element concentration, mean (µg/g dry weight)[1]				
	Arsenic	Cadmium	Copper	Lead	Zinc
			Gill		
UBR	2.7 (0.4)^{a}	0.79 (0.04)^{a}	5.6 (1.9)^{ab}	0.3 (0.9)^{a}	667 (162)^{a}
LBC	3.1 (0.5)^{a}	30.1 (0.2)^{b}	7.7 (0.1)^{b}	1.2 (0.1)^{b}	845 (148)^{abc}
LCC	2.8 (0.4)^{a}	71.4 (3.9)^{c}	21.5 (1.9)^{c}	1.3 (0.1)^{bc}	744 (103)^{abc}
BRRC	1.3 (0.4)^{b}	3.2 (1.6)^{a}	4.3 (0.1)^{a}	0.4 (0.2)^{a}	404 (89)^{b}
BRGG	1.3 (0.04)^{b}	25.7 (1.2)^{b}	5.6 (0.2)^{ab}	1.7 (0.2)^{c}	1,070 (179)^{c}
			Liver		
UBR	2.7 (0.3)^{a}	1.5 (0.2)^{a}	125 (22)^{a}	0.60 (0.01)^{a}	110 (3)
LBC	7.2 (1.0)^{b}	29.2 (2.3)^{b}	488 (83)^{b}	0.29 (0.03)^{b}	174 (3)
LCC	13.8 (0.5)^{c}	70.6 (4.9)^{c}	1,010 (100)^{c}	0.37 (0.03)^{b}	262 (13)
BRRC	2.0 (0.4)^{a}	7.1 (2.4)^{a}	319 (106)^{a}	0.09 (0.03)^{a}	126 (8)
BRGG	4.8 (0.4)^{d}	20.7 (2.7)^{d}	548 (74)^{d}	0.54 (0.08)^{c}	204 (14)
			Whole fish		
UBR	1.7 (0.6)^{ac}	0.15 (0.01)^{a}	5.2 (0.2)	<0.25	104 (4.4)
LBC	6.0 (1.7)^{ab}	2.7 (0.4)^{b}	13 (1.1)	2.1 (1.1)	318 (24)
LCC	8.0 (1.2)^{b}	4.9 (0.3)^{c}	22 (2.1)	1.2 (0.28)	353 (9.8)
BRRC	0.9 (0.1)^{c}	0.16 (0.02)^{a}	7.7 (0.8)	0.26 (0.01)	105 (8.1)
BRGG	3.5 (1.0)^{abc}	2.4 (0.3)^{b}	14.3 (0.7)	0.88 (0.05)	309 (28)

[1] Different letters designate a significant difference among sites at $p<0.05$ within a trace element for mainstem and tributaries.

McCarter, 1984; Marr and others, 1995; lipid peroxidation: Wills, 1985; Stern, 1985; DiGiulio and others, 1989).

Metallothioneins are proteins that bind metals, such as copper, cadmium, and zinc (Hogstrand and Haux, 1991; Stegeman and others, 1992). Because metallothionein synthesis and concentrations increase when fish are exposed to metals, the induction of these proteins indicates metal exposure in fish. The induction of metallothioneins in trout also has been associated with slowed growth of trout that maintain induced metallothionein concentrations (Marr and others, 1995). Therefore, elevated metallothionein concentrations also may indicate reduced fitness of trout.

Concentrations of metallothionein were greatest in the livers of rainbow trout from lower Cataract Creek (921 µg/g). The mean concentration of metallothionein in livers of fish from lower Cataract Creek was significantly greater than that in livers of fish from the reference site on the upper Boulder River (UBR, table 7). A trend toward greater metallothionein appeared in livers of fish from lower Basin Creek compared to the reference site, but this finding was not significant. The gills generally had small concentrations of metallothionein, and concentrations in gills measured at lower Basin Creek were less than those collected from lower Cataract Creek and Boulder River near Galena Gulch. Concentrations of metallothionein in livers or gills of fish from the mainstem sites (BRRC and BRRG) showed no significant differences.

Table 7. Metallothionein concentrations in gill and liver samples of resident rainbow trout, 1997.

[Values are reported as mean with 2σ error in parentheses; means followed by the same letter (within a tissue) are not significantly different at $p = 0.05$; $n = 5$ for all sites except BRGG, where $n = 6$; metallothionein concentration in µg/g wet weight]

Site (fig. 1)	Metallothionein (µg/g) Gill	Metallothionein (µg/g) Liver
Tributaries		
UBR – Reference	9.37^{ab} (0.63)	31.2^{a} (7.70)
LBC	7.61^{a} (1.00)	597^{ab} (289)
LCC	12.0^{b} (1.18)	921^{b} (216)
Boulder River		
BRGG – Reference	12.4^{b} (0.52)	271^{ab} (195)
BRGG	11.1^{ab} (1.15)	293^{ab} (60.5)

Lipid Peroxidation

Elevated concentrations of products of lipid peroxidation indicate cell death and tissue damage (Farag and others, 1995). Cell membranes are composed of polyunsaturated fatty-acid side chains and generally have a fluid composition. However, these side chains are targets of lipid peroxidation, a process that changes the structural integrity of the cell membrane and may ultimately result in cell death and tissue damage (Halliwell and Gutteridge, 1985; Wills, 1985). Trace elements that exist in more than one valence state, such as copper, may initiate lipid peroxidation (Wills, 1985). Trace elements may also initiate lipid peroxidation because they inhibit antioxidant enzymes such as glutathione peroxidase and transferase (Reddy and others, 1981). The amounts of products of lipid peroxidation measured with 340-nm excitation in the livers of rainbow trout from lower Cataract Creek were significant compared to amounts measured in livers of rainbow trout from upper Boulder River (table 8). No difference in the mean amount of products of lipid peroxidation was found in livers of fish from the two mainstem sites (BRRC and BRGG). There was no significant difference in lipid peroxidation in the gills of fish among tributary or mainstem sites. Products of lipid peroxidation also were measured with 360-nm excitation. Although a trend appeared of increased products of lipid peroxidation measured with 360-nm excitation, the difference was not significant (data not presented).

Exposure Pathways

Water

In stream water, concentrations of arsenic and lead were greatest at lower High Ore Creek (table 9, total recoverable of 64 µg/L and 54 µg/L respectively) during high flow. Copper concentrations also were greater at most sites during high flow, although the highest total-recoverable concentrations at any site occurred during low flow at Jack Creek (140 µg/L). Unlike arsenic and lead, the concentrations of cadmium and zinc were greater during low rather than high

Table 8. Lipid peroxidation of tissues sampled from resident rainbow trout, 1997.

[Values are reported as mean with 2σ error SEM in parentheses; means followed by the same letter (within a tissue) are not significantly different at $p = 0.05$; $n = 5$ for all sites except site BRGG, where $n = 6$]

Site (fig. 1)	Relative intensity[1] Gill	Relative intensity[1] Liver
Tributaries		
UBR – Reference	73.1^{a} (8.19)	114^{a} (5.31)
LBC	66.4^{a} (6.91)	115^{a} (11.7)
LCC	73.8^{a} (3.77)	141^{b} (7.28)
Boulder River		
BRRC – Reference	79.2^{a} (6.57)	125^{ab} (11.9)
BRGG	73.1^{a} (4.09)	127^{ab} (4.63)

[1] Note: Lipid peroxidation is expressed as the fluorometric measurement (relative intensity) of a chloroform extract of tissue. The relative intensity was measured at 340-nm excitation and 435-nm emission when 0.05 µg/mL of quinine sulfate measured 322 with settings to measure gill and 136 with settings to measure liver (Farag and others, 2003). Different letters designate a significant difference among sites at $p<0.05$ within a trace element for mainstem and tributaries.

Table 9. Median trace-element concentrations in water, 1996–99.

[*n* = number of samples collected on different dates throughout the flow condition; Tot = total recoverable concentration; Filt = concentration in 0.45-µm filtrate; REF 1 = pooled value for LBR and UBR site and REF 2 = BRRC]

Site (fig. 1)		*n*	Streamflow (ft³/s)	Hardness (mg/L $CaCO_3$)	Trace-element concentration, median (µg/L)									
					Arsenic		Cadmium		Copper		Lead		Zinc	
					Tot	Filt	Tot	Filt	Tot	Filt	Tot	Filt	Tot	Filt
High-flow conditions														
Tributaries														
	LBR	2	69-94	19	6.0	3.0	<1	<0.1	6.5	2.0	3.0	<1	<10	3.5
Ref 1	UBR	2	15-83	21-38	4.0	2.5	<1	<0.3	3	1.5	<1	<1	<10	1
	LHO	15	1.8-6.8	74-150	64	13	4.9	2.4	17	5.8	54	<1	177	139
	LCC	11	13-168	18-54	17	3.4	1.8	1.3	46	33	6.7	<1	1,290	678
	MCC	7	17-186	18-37	15	3.0	3.3	2.3	62	33	13	<1	322	231
	UCC	6	15-121	17-37	3.5	2.3	<1	<0.3	8.9	7.2	3.0	<1	58	43
	LBC	10	15-251	12-35	12	5.2	5.2	.5	14	10	4.7	4.7	93	66
	JC	6	4.4-35	19-38	32	3.0	3.5	3.2	59	32	9.6	<1	397	352
Mainstem														
Ref 2	BRRC	10	106-725	22-50	5.4	3.3	<1	<0.3	5.0	3.3	1.2	<1	11	3.4
	BRBC	2	94-436	24-47	7.0	4.4	<1	<0.3	9.2	7.2	1.9	<1	39	29
	BRCC	2	106-513	24-47	8.2	4.1	<1	.4	15	11	2.5	<1	66	94
	BRGG	11	175-1,050	19-48	12	4.0	<1	.3	23	12	8.5	<1	100	51
Low-flow conditions														
Tributaries														
	LBR	6	2.4-6.3	37-56	2.0	2.0	<1	<0.1	5.0	2.0	<1	<1	<10	2.0
REF 1	UBR	2	2.3-3.8	52-53	2.5	2.0	<1	<0.3	1.5	<1	<1	<1	<10	<1
	LHO	8	0.52-1.3	140-170	33	17	4.5	3.9	4.6	3.1	6.8	<1	1,970	1,800
	LCC	8	1.9-5.4	59-69	4.0	3.1	5.0	4.7	24	20	<1	<1	420	402
	MCC	5	3.6-4.6	48-57	3.5	2.0	9.4	8.9	82	53	2.0	<1	765	727
	UCC	4	2.8-5.0	43-52	2.7	2.4	<1	.3	4.6	4.5	<1	<1	64	60
	LBC	8	3.1-5.8	39-42	7.1	4.6	<1	.4	4.1	3.0	<1	<1	73	62
	JC	4	.86-1.2	44-51	18	1.9	12	11	140	69	4.1	<1	1,270	1,200
Mainstem														
REF 2	BRRC	9	8.6-25	54-61	3.0	2.5	<1	<0.3	1.4	1.6	<1	<1	<10	2.5
	BRBC	2	25-28	55-59	4.0	3.5	<1	<0.3	9.4	7.9	<1	<1	44	41
	BRCC	2	26-33	57-60	4.2	3.7	<1	.6	12	9.2	<1	<1	86	80
	BRGG	9	17-36	61-68	6.0	5.0	<1	.8	10	8.9	<1	<1	149	140

flow. Zinc concentrations were greatest at lower High Ore Creek during low flow (1,970 µg/L) (table 9). The greatest concentrations of cadmium were measured at Jack Creek (12 µg Cd/L) and middle Cataract Creek (9.4 µg Cd/L) during low flow. The pH ranged from 7.0 to 8.5 at all sites regardless of flow (Nimick and Cleasby, this volume).

Colloids

The presence of colloids in the water column is indicated most clearly by the high concentration of colloidal iron (table 10) and, to a lesser extent, aluminum (data not presented) at most sites including the reference sites. These data support the conclusion of Church, Unruh, and others (this volume) that, based on an analysis of suspended sediment collected during high flow in 1997, much of the sediment in watershed streams is iron oxyhydroxide colloidal material. This colloidal material has a large capacity for sorbing trace elements, and the degree of metal enrichment varies among sites.

The general pattern of partitioning of trace elements between dissolved (0.001 µm) and colloidal fractions in the water column depended primarily on the trace element and, to a lesser extent, on the proximity of the sampling site to a source of trace elements. Almost all iron and lead were present in the colloidal fraction, whereas cadmium and zinc were primarily dissolved. Large amounts of arsenic and copper also partitioned to the colloidal phase, but unlike lead, arsenic and copper also existed in the dissolved phase.

The spatial pattern of colloidal trace-element concentrations generally was the same as for trace-element concentrations in water and sediment in that the highest colloidal concentrations were directly downstream from inactive mines. Trace-element-rich acidic water draining from mines in the Boulder watershed study area is neutralized in mixing zones in streams, producing substantial loads of metal-rich colloids rich in trace elements. These colloids then can be transported long distances downstream (Kimball and others, this volume, Chapter D6). Substantial colloid loads occurred in the Bullion Mine tributary (BMT) and Jack Creek (JC) downstream from the Bullion mine, in Uncle Sam Gulch (USG) and Cataract Creek (MCC) downstream from the Crystal mine, and in High Ore Creek (UHO and LHO) downstream from the Comet mine (table 10). For the 12 main sampling sites, the highest concentrations of colloidal trace elements were at lower High Ore Creek (0.5 µg/L Cd, 8.2 µg/L Pb, and 322 µg/L Zn) and Jack Creek (24 µg/L As and 74 µg/L Cu). Concentrations at sites nearer inactive mines (BMT, USG, and UHO) were even higher, and often exceeded 1 mg/L for iron and zinc (table 10). Colloidal concentrations of arsenic, copper, lead, and zinc increased in the Boulder River in a step-wise downstream pattern as each of the three mining-affected tributaries entered the mainstem.

Table 10. Mean trace-element concentrations in colloids and ultrafiltrates during low-flow conditions, 1996–98.

[Dis = dissolved concentration in ultrafiltrate; Col = concentration in colloidal fraction; REF 1 = pooled value for LBR and UBR site; REF 2 = BBRC; *, ancillary sampling site near an inactive mine]

Site (fig. 1)		*n*	Trace-element concentration, mean (µg/L)											
			Arsenic		Cadmium		Copper		Iron		Lead		Zinc	
			Dis	Col	Dis	Col	Dis	Col	Dis	Col	Dis	Col	Dis	Col
Tributaries														
	LBR	2	<3	1.0	0.1	0.4	2.5	<3	<50	225	0.4	<1	<2	<10
REF 1	UBR	3	2.6	1.4	<0.1	<0.3	<1	1.2	<50	193	0.4	<1	<2	<10
	LHO	3	14	14	4.2	0.5	2.5	3.2	<50	197	0.5	8.2	1,750	322
	LCC	3	3.2	2.2	4.0	<0.3	16	15	<50	95	0.3	1.2	329	22
	MCC	4	<3	3.5	6.5	<0.3	27.5	38	<50	204	0.5	1.7	546	73
	UCC	3	<3	1.5	0.3	0.2	5.3	<1	<50	135	0.3	<1	56	5
	LBC	3	5.6	4.6	0.4	0.1	5.1	2.6	<50	107	0.1	1.8	54	11
	JC	3	<3	24	9.0	0.3	41	74	33	857	0.5	4.5	942	88
	BMT*	3	<1	27	45	1	469	87	748	1,940	2	13	5,000	270
	USG*	3	<1	6	43	1	141	256	<10	196	0.5	8	3,520	350
	UHO*	1	8	102	6	<0.3	1	9	<1	1,600	<1	44	4,390	710
Mainstem														
REF 2	BRRC	2	3.6	1.5	<0.1	0.4	2.5	<2	48	490	0.3	0.4	2	3
	BRBC	3	4.4	1.2	0.2	0.4	8.4	1.2	39	316	0.3	0.2	35	5
	BRCC	3	4.5	1.5	0.5	<0.3	10	2.8	<50	270	0.4	0.1	65	15
	BRGG	3	5.0	7.0	0.7	0.3	8.0	8.7	<50	283	0.5	0.6	112	41

Sediment

In streambed sediment, the concentrations of all trace elements except copper were highest at lower High Ore Creek (740 µg As/g, 14 µg Cd/g, 1,100 µg Pb/g, and 3,400 µg Zn/g) (table 11). The concentrations of arsenic, cadmium, and zinc in Cataract and Basin Creeks were 2 to 5 times less than concentrations in lower High Ore Creek but were still much higher than the concentrations at the reference site on the upper Boulder River (UBR). The concentrations of cadmium were similar in Cataract Creek (9.3 µg/g at MCC and 11 µg/g at LCC) compared to lower High Ore Creek. The highest copper concentrations were in Cataract Creek (450 µg/g at MCC, 440 µg/g at LCC) followed by Jack Creek (180 µg/g at JC) and High Ore Creek (140 µg/g at LHO).

In the Boulder River, trace-element concentrations in sediment generally were less than in the tributaries. However, concentrations of all trace elements, except cadmium at BRBC, were greater at the three downstream mainstem sites compared to the reference site (BRRC). For instance, arsenic concentrations were 99 µg/g at Boulder River near Galena Gulch, far higher than the 8.3 µg/g measured at Boulder River near Red Rock Creek upstream from the area of historical mining.

Biofilm

Trace-element concentrations in biofilm decreased at sites farthest downstream from historical mining areas (table 12). The greatest concentrations of cadmium, copper, and lead were in Jack Creek (all data presented as µg/g±σ; 70±5; 4,620±860; 885±84 respectively) followed by middle Cataract Creek (60±22; 1,940±850; 660±150 respectively), then lower High Ore Creek (41±3; 321±9; 1,100±30 respectively). The concentrations of these trace elements decreased substantially downstream in Basin Creek (LBC) but were still 18–23 times greater than the concentration for the combined reference (REF 1) used for the tributary sites. Concentrations of these trace elements in biofilm were significantly less at lower Cataract Creek compared to the middle Cataract Creek site. However, the downstream site in Cataract Creek (LCC) yielded elevated concentrations of trace elements compared to the combined reference. Biofilm from upper Cataract Creek (UCC) had concentrations of trace elements less than those of lower Cataract Creek but greater (though not significantly) than the combined reference. The concentrations of cadmium and lead but not copper were significantly elevated in biofilm from lower High Ore Creek compared to the combined reference.

Table 11. Leachable trace-element concentrations in composite streambed sediment, 1998.

[REF 1 = pooled value for LBR and UBR site and REF 2 = BRRC]

Site (fig. 1)		Arsenic (µg/g)	Cadmium (µg/g)	Copper (µg/g)	Lead (µg/g)	Zinc (µg/g)
Tributaries	LBR	20	<1	13	26	100
	UBR	13	<1	7.8	10	40
	REF 1	17	<1	10	18	70
	LHO	740	14	140	1,100	3,400
	LCC	580	11	440	390	1,300
	MCC	250	9.3	450	280	930
	UCC	96	3.0	110	220	440
	LBC	140	3.9	98	150	640
	JC	330	4.2	180	190	490
Mainstem	BRRC REF 2	8.3	<1	16	13	74
	BRBC	20	<1	38	27	180
	BRCC	55	3.2	110	80	430
	BRGG	99	2.8	84	99	490

Biofilm from lower High Ore Creek had the greatest concentrations of arsenic and zinc (all data presented as µg/g±σ, 3,300±400 and 18,100±1,700 respectively) followed by Jack Creek (2,600±170 and 6,210±170 respectively) and middle Cataract Creek (1,700±700 and 6,000±2,240 respectively). Concentrations of arsenic and zinc decreased farther downstream at lower Cataract Creek and lower Basin Creek but were still greater than 26 times the concentrations at the reference site.

Elevated concentrations of trace elements persisted in the mainstem of the Boulder River. The arsenic concentration in biofilm at Boulder River near Galena Gulch was 262 µg/g. The zinc concentrations at Boulder River near Galena Gulch were similar to the concentrations measured at lower Cataract Creek (3,200±250 versus 3,870±420), although the concentrations of arsenic in biofilm from the mainstem downstream from Cataract Creek (BRCC) were less than that from lower Cataract Creek (67±3 versus 731±67). The concentrations of trace elements in biofilm from the Boulder River downstream from Basin Creek and downstream from Cataract Creek also were elevated compared to concentrations at the reference site (BRRC, REF 2).

Benthic Macroinvertebrates

The concentrations of trace elements were generally less in benthic macroinvertebrates compared to the biofilm, but invertebrates from many of the test sites had concentrations greater than at the reference site (tables 12 and 13). This trend was apparent at sites throughout the watershed even in the absence of extensive statistical significance. Furthermore, some of the greatest concentrations of cadmium, copper, and lead were observed in the lower portion of Cataract Creek (all data presented as µg/g± σ, 59±5; 340±130; 34±16 respectively). In general, though concentrations of trace elements persisted in invertebrates from sites on the Boulder River, they were less than concentrations of invertebrates from the tributaries. One exception to this observation was lead; invertebrates from Boulder River near Galena Gulch had the greatest mean concentration of lead measured in the watershed. These results demonstrate that trace elements were being transported downstream and were transferred to the food chain.

Fish Tissues

As stated in a preceding section (see “Fish Biomass, Density, and Physiology”), mean concentrations of trace elements, especially arsenic, cadmium, and copper, were greatest in gill, liver, and whole fish from lower Cataract Creek (table 6). The concentrations of cadmium in the gill, liver, and whole fish from lower Cataract Creek were magnitudes greater than that found in the reference, upper Boulder River (90 times for gill, 47 times for liver, 33 times for whole fish). Likewise, the concentrations of copper in fish from lower Cataract Creek ranged from 4 to 8 times the concentrations in fish from the reference. Although concentrations of trace elements were less in fish from the mainstem, elevated arsenic, cadmium, and zinc were measured in fish as far downstream as Boulder River near Galena Gulch.

Table 12. Mean trace-element concentrations in biofilm, 1998.

[Standard error of the mean is in parentheses, and *n* = sample size; REF 1 = pooled value for LBR and UBR sites, REF 2 = BRRC]

Site (fig. 1)		*n*	Trace-element concentration, mean (µg/g dry weight)[1]				
			Arsenic	**Cadmium**	**Copper**	**Lead**	**Zinc**
Tributaries	LBR	4	17.7 (1.4)	1.0 (0.1)	25 (4)	12 (3)	99 (15)
	UBR	4	17.8 (2.0)	0.7 (0.1)	15 (2)	12 (1)	62 (8)
	REF 1	8	17.7 (1.1)^{a}	0.8 (0.1)^{a}	20 (3)^{a}	12 (1)^{a}	81 (11)^{a}
	LHO	4	3,300 (400)^{d}	41.2 (2.7)^{bcd}	321 (9)^{ab*}	1,100 (30)^{e}	18,100 (1,700)^{c}
	LCC	4	731 (67)^{ab*}	30.6 (4.5)^{abcd*}	853 (96)^{ab*}	389 (46)^{bc}	3,870 (420)^{ab*}
	MCC	4	1,700 (700)^{bc}	60 (22)^{cd}	1,940 (850)^{b}	660 (150)^{cd}	6,000 (2,240)^{b}
	UCC	4	130 (13)^{a}	23 (12)^{abc*}	260 (56)^{ab*}	256 (89)^{ab*}	2,360 (660)^{ab*}
	LBC	4	475 (77)^{ab*}	16.6 (2.7)^{ab*}	369 (62)^{ab*}	281 (29)^{ab*}	2,320 (350)^{ab*}
	JC	4	2,600 (170)^{cd}	70.3 (4.5)^{d}	4,620 (860)^{c}	885 (84)^{de}	6,210 (170)^{b}
Mainstem	REF 2	4	15.3 (1.2)^{a}	0.54 (0.06)^{a}	23 (2)^{a}	12.2 (0.9)^{a}	141 (34)^{a}
	BRBC	4	37.6 (4.1)^{a*}	3.3 (0.5)^{a*}	89 (14)^{b}	32.3 (2.3)^{ab*}	619 (95)^{ab*}
	BRCC	4	67.1 (2.9)^{b}	10.5 (2.4)^{b}	170 (18)^{c}	50.0 (7.5)^{b}	1,240 (180)^{b}
	BRGG	4	262 (12)^{c}	17.4 (1.5)^{c}	270 (16)^{d}	278 (9)^{c}	3,200 (250)^{c}

[1] Different letters designate a significant difference at $p<0.05$ within a trace element for mainstem and tributary sites; * indicates a significant difference at $p<0.05$ between specified site and reference *only*.

Table 13. Mean trace-element concentrations in invertebrates, 1998.

[Standard error of the mean (2 σ) is in parentheses; *n* = sample size; REF 1 = pooled value for LBR and UBR sites; and REF 2 = BRRC]

Site (fig. 1)			Trace-element concentration, mean (µg/g dry weight)[1]				
		n	Arsenic	Cadmium	Copper	Lead	Zinc
Tributaries	LBR	4	2.3 (0.3)	3.7 (0.4)**	38 (1)**	1.2 (0.2)	340 (27)**
	UBR	4	3.7 (1.0)	0.9 (0.2)**	30 (1)**	1.0 (0.3)	235 (18)**
	REF 1	8	3.0 (0.5)^{a}	2.3 (0.6)^{a}	34 (2)^{a}	1.2 (0.2)^{a}	288 (25)^{a}
	LHO	4	60 (11)^{bc}	16.7 (0.8)^{b}	74 (4)^{ab*}	36 (6)^{b}	3,090 (75)^{d}
	LCC	4	63 (27)^{bc}	59.3 (5.4)^{d}	340 (130)^{c}	34 (16)^{b}	2,410 (420)^{cd}
	MCC	4	80.1 (7.3)^{c}	35.0 (5.4)^{c}	268 (35)^{bc}	24.1 (2.0)^{ab*}	2,070 (290)^{bc}
	UCC	4	7.5 (2.9)^{ab}	15.9 (3.2)^{b}	77 (4)^{ab*}	11.2 (4.4)^{ab*}	1,050 (200)^{a*}
	LBC	4	21.5 (1.4)^{abc*}	18.1 (1.3)^{b}	92 (6)^{abc*}	12.4 (0.7)^{ab*}	929 (80)^{a*}
	JC	4	77 (29)^{c}	10.0 (2.8)^{ab*}	319 (89)^{bc}	12.6 (9.9)^{ab*}	580 (144)^{a}
Mainstem	REF 2	4	4.6 (0.3)^{a}	1.1 (0.3)^{a}	29 (1)^{a}	1.6 (0.1)^{a}	237 (11)^{a}
	BRBC	4	5.3 (0.3)^{a}	10.6 (5.7)^{ab*}	85 (5)^{b}	3.3 (0.4)^{a*}	584 (81)^{b}
	BRCC	4	13.1 (2.0)^{ab*}	16.2 (2.1)^{b}	111 (7)^{b}	8.5 (1.5)^{a*}	977 (127)^{c}
	BRGG	4	26.7 (8.8)^{b}	11.7 (3.0)^{ab*}	98 (16)^{b}	38 (18)^{a*}	669 (66)^{bc}

[1] Different letters designate a significant difference at $p<0.05$ within a trace element for mainstem and tributary sites.

* indicates a significant difference at $p<0.05$ between specified site and reference *only*.

**indicates a significant difference at $p<0.05$ between pooled reference sites.

Relationships among Components

Significant correlations were observed among the metal concentrations measured in the various abiotic and biotic components sampled in the Boulder River watershed. The concentrations of all trace elements, except lead, measured in low-flow total, filtered, or dissolved water correlated with concentrations in biological components (table 14). Significant correlations between water and biology were more frequent for cadmium, copper, and zinc than for arsenic. Copper and zinc measured in total, filtered, or dissolved water correlated with colloids, biofilm, and macroinvertebrates. However, arsenic in total water rather than filtered or dissolved correlated more significantly with colloids and biofilm. Because $r \leq 0.884$ for biofilm and total arsenic, the amount of variation explained by the corresponding r^2 values (not listed) is less for arsenic than was generally observed for cadmium, copper, and zinc. No significant correlations were observed between arsenic in water and arsenic in fish, although cadmium, copper, and zinc in water were significantly correlated to fish tissues. Copper in the water was directly correlated to gill, liver, and whole fish; cadmium was directly correlated to gill and liver; and zinc was directly correlated to liver. Therefore, cadmium, copper, and zinc accumulated in various levels of the food chain and directly in fish from the water column.

Some significant correlations were found between trace elements in water and sediment, but r values ranged from 0.537 to 0.877 and correspond to generally smaller r^2 values. These small r^2 values indicate that these correlations explain less variation than was generally observed between trace elements in water and biology. Some of the strongest correlations between trace elements in water and sediment were observed for zinc and cadmium in low-flow dissolved water ($r = 0.877$ and 0.857, respectively).

Concentrations in sediment correlated directly with biology for all trace elements. Trace elements in sediment correlated with biofilm and (or) macroinvertebrates for all trace elements, and the r ranged from 0.667 to 0.952. The strongest correlations were for zinc in biofilm ($r = 0.952$) and macroinvertebrates ($r = 0.899$). Additionally, cadmium and copper concentrations in sediment were correlated directly to gill, liver, and whole fish ($r \geq 0.922$). Arsenic concentrations in sediment were correlated directly to concentrations in liver and whole fish ($r = 0.978$ and $r = 0.882$, respectively). Zinc concentrations in sediment were correlated to zinc concentrations in liver ($r = 0.953$). These results indicate that sediment, in addition to water, appears to provide a source of trace elements to aquatic resources in the Boulder River watershed.

Concentrations of trace elements in colloids were correlated to concentrations in biofilm for all trace elements, except cadmium, at $p \geq 0.01$ with $r \geq 0.900$ for copper, lead, and zinc and $r = 0.819$ for arsenic. The corresponding r^2 values for most of the correlations indicate that much of the variation in the data is explained. Concentrations of arsenic, copper, and zinc in colloids also were correlated to macroinvertebrates but at $p \geq 0.05$ rather than $p \geq 0.01$, and the corresponding r values were ≤ 0.760. Colloids also correlated directly with liver and whole fish for copper and with whole fish for lead.

Biofilm and macroinvertebrates were correlated to one another for arsenic, copper, lead, and zinc. However, all r were <0.828 and indicated small corresponding values for r^2; as a result, small amounts of variation were explained. Biofilm

Table 14. Correlation coefficients for trace-element concentrations measured in water, sediment, colloids, biofilm, benthic macroinvertebrates, fish gill, fish liver, and whole fish, 1996–99.

[Concentrations in water: Total = total recoverable concentration, Filt = concentration in 0.45-µm filtrate, Dis = dissolved concentration in ultrafiltrate, LF = low flow; Inverts = benthic macroinvertebrates; probability values in parentheses; n_1 = number of water, sediment, colloid, biofilm, and invertebrate samples included in correlations with fish tissues; n_2 = number of gill, liver, or whole fish concentrations used in the same correlations. Bold type, significance at $p<0.01$; underline at $p<0.05$; standard type designates significance at $p<0.10$. Correlations not significant above $p<0.10$ not presented]

	n	Total LF	Filt LF	Dis	Sediment	Colloids	Biofilm	n_1	n_2	Gill	Liver	Whole fish
Arsenic												
Total LF	12							5	5	--	--	--
Filt LF	12	**0.852** (0.0004)						5	5	--	--	--
Dis	12	0.764 (0.0273)	**0.976** (<0.0001)					5	5	--	--	--
Sediment	12	0.754 (0.0047)	**0.677** (0.0156)	--				5	5	--	**0.978** (0.004)	0.882 (0.048)
Colloids	12	**0.771** (0.0033)	--	--	0.522 (0.0816)			5	5	--	--	--
Biofilm	12	**0.884** (0.0001)	0.619 (0.0318)	--	**0.809** (0.0962)	**0.819** (0.0011)		5	5	--	**0.971** (0.006)	**0.994** (0.001)
Inverts	12	0.522 (0.0817)	--	--	**0.769** (0.0035)	0.647 (0.0231)	**0.828** (0.0009)	5	5	--	**0.965** (0.008)	0.898 (0.039)
Gill	5										**--**	--
Liver	5									--		**0.960** (0.0090)
Whole fish	5									**--**	**--**	
Cadmium												
Total LF	4							1	5	0.888 (0.044)	0.916 (0.029)	--
Filt LF	9	**0.998** (<0.0001)						3	5	0.928 (0.023)	0.943 (0.016)	0.855 (0.065)
Dis	10	**0.986** (<0.0001)	**0.984** (<0.0001)					3	5	0.941 (0.017)	0.954 (0.012)	0.873 (0.053)
Sediment	8	0.537 (0.0717)	0.771 (0.0254)	0.857 (0.0032)				3	5	0.990 (0.001)	**0.996** (0.001)	0.952 (0.013)
Colloids	8	**--**	--	--	--			3	5	--	--	--
Biofilm	12	**0.943** (<0.0001)	**0.960** (<0.0001)	**0.932** (0.0003)	**0.852** (0.0072)	--	--	5	5	**0.971** (0.006)	0.942 (0.017)	**0.996** (0.001)
Inverts	12	--	0.733 (0.0246)	0.656 (0.0456)	0.687 (0.0596)	--	--	5	5	**0.983** (0.003)	**0.993** (0.001)	0.938 (0.019)
Gill	5										**0.994** (0.001)	**0.985** (0.002)
Liver	5									--		**0.964** (0.008)
Whole fish	5									--	--	
Copper												
Total LF	12							5	5	0.929 (0.023)	0.952 (0.013)	0.940 (0.018)
Filt LF	12	**0.986** (<0.0001)						5	5	0.908 (0.033)	0.957 (0.011)	0.943 (0.016)
Dis	11	**0.975** (<0.0001)	**0.987** (<0.0001)					4	5	0.897 (0.039)	**0.990** (0.001)	**0.984** (0.003)

Table 14. Correlation coefficients for trace-element concentrations measured in water, sediment, colloids, biofilm, benthic macroinvertebrates, fish gill, fish liver, and whole fish, 1996–99.—Continued

	n	Total	Filt LF	Dis	Sediment	Colloids	Biofilm	n_1	n_2	Gill	Liver	Whole fish
Sediment	12	--	0.611 (0.0350)	0.605 (0.0350)				5	5	**0.989** (0.002)	0.946 (0.015)	0.922 (0.026)
Colloids	9	**0.994** (<0.0001)	**0.974** (<0.0001)	**0.972** (<0.0001)	--			4	5	0.853 (0.066)	0.931 (0.021)	0.932 (0.021)
Biofilm	12	**0.983** (<0.0001)	**0.949** (<0.0001)	**0.951** (<0.0001)	--	**0.992** (<0.0001)		5	5	0.942 (0.017)	**0.966** (0.008)	0.968 (0.007)
Inverts	12	**0.770** (0.0034)	**0.830** (0.0008)	**0.867** (0.0003)	**0.859** (0.0003)	0.760 (0.0174)	**0.761** (0.0041)	5	5	**0.981** (0.003)	0.953 (0.012)	0.940 (0.020)
Gill	5										0.888 (0.045)	0.857 (0.063)
Liver	5									--		0.991 (0.001)
Whole fish	5									--	--	
						Lead						
Total LF	3							0	5			
Filt LF	0							0	5			
Dis	12	--						0	5			
Sediment	12	--		--				5	5	--	--	--
Colloids	9	--		0.591 (0.0941)	**0.875** (0.0020)			4	5	--	--	0.951 (0.013)
Biofilm	12	--		--	**0.808** (0.0015)	**0.920** (0.0004)		5	5	0.889 (0.044)	--	--
Inverts	12	--		--	0.667 (0.0178)	--	0.640 (0.0249)	5	5	0.921 (0.026)	--	--
Gill	5										--	--
Liver	5									--		--
Whole fish	5									--	--	
						Zinc						
Total LF	9							3	5	--	0.948 (0.014)	--
Filt LF	12	**1.000** (<0.0001)						5	5	--	0.946 (0.015)	--
Dis	10	**0.995** (<0.0001)	**0.922** (<0.0001)					4	5	--	0.945 (0.015)	--
Sediment	12	**0.835** (0.0007)	**0.827** (0.0009)	**0.877** (0.0009)				5	5	--	0.953 (0.012)	0.876 (0.051)
Colloids	10	**0.928** (<0.0001)	**0.918** (0.0002)	**0.957** (<0.0001)	**0.930** (<0.0001)		--	4	5	0.847 (0.070)	--	--
Biofilm	12	**0.946** (<0.0001)	**0.941** (<0.0001)	**0.969** (<0.0001)	**0.952** (<0.0001)	**0.983** (<0.0001)		5	5	--	**0.967** (0.007)	**0.970** (0.006)
Inverts	12	0.694 (0.0124)	0.691 (0.0128)	0.699 (0.0246)	**0.899** (<0.0001)	0.709 (0.0218)	**0.804** (0.0016)	5	5	--	0.911 (0.031)	--
Gill	5										--	--
Liver	5									--		0.904 (0.035)
Whole fish	5									--	--	

and invertebrates were directly correlated with gill, liver, and whole fish for cadmium, and copper; liver and whole fish for arsenic; liver for zinc; and gill for lead. Concentrations of all trace elements, except lead, in liver correlated with those measured in whole fish. Concentrations in gill were also correlated to liver concentrations for cadmium. However, concentrations between gill and whole fish were not correlated for any of the metals measured.

Discussion

This study provides evidence that populations of fish are unlikely to survive in close proximity to some inactive mine sites in Basin, Cataract, and High Ore Creeks. Limited survival in some stream reaches of these basins appears to be related to elevated concentrations of trace elements present in the water column. Furthermore, the health and biomass/density of resident fish in lower Cataract Creek may be compromised as a result of exposure routes that include water, sediment, and diet. In addition, one route in the dietary pathway may begin with colloids suspended in the water column.

Aquatic Health

The multiple tools we used to investigate the effects of historical mining in the Boulder River watershed allow us to define where trace elements affect the aquatic health of the watershed. In the most extreme cases, where concentrations of trace elements were greatest in the water column, population-level effects existed and fish survival was poor. The relation between trace-element concentrations and mortality was consistent, and greater trace-element concentrations were associated with greater and more rapid mortality.

The association between fish mortality and the elevated concentrations of trace elements in the water provides evidence that trace elements caused the observed mortalities. Water-quality criteria have been established by the U.S. Environmental Protection Agency (1999, 2001) for the chronic and acute protection of aquatic life. Although the water-quality criteria are hardness dependent, we can calculate standards for the average hardness measured at various sites in this watershed. The acute aquatic-life standards are 12 µg Cu/L and 82 µg Zn/L in Basin and Cataract Creeks at a hardness of 66 mg/L. The toxicity of some metals is alleviated by elevated hardness in the water. (See Finger, Farag, and others, this volume, Chapter C, for more complete discussion.) Therefore, the greater hardness values at lower High Ore Creek (LHO, 140 mg/L) may explain why survival was slightly better in this stream.

The hypertrophy noted in gills of fish in this study is consistent with the edema noted previously in the secondary lamellae of rainbow trout exposed to 40 mg Zn/L for 3 hr (Skidmore and Tovell, 1972). Skidmore and Tovell (1972) also observed severe curling of the secondary lamellae, a finding not unlike the “twisting” that was noted in the gills of fish collected from middle Cataract Creek (MCC), where concentrations of zinc ranged from 400 to 700 µg/L. Cutthroat trout near death experienced excess mucus production in this study. Handy and Eddy (1991) suggested that excess mucus production is part of a “general stress response” in rainbow trout. Therefore, ionoregulatory upset (though we could not measure this upset directly) was the likely cause of hypertrophy (swelling), degeneration (dying), and necrosis (death) of epithelial cells in the gills. And, mucus production occurred simultaneously as a general response to stress.

The concentrations of cadmium, copper, and zinc in water at lower Cataract Creek are near the concentrations reported at middle Cataract Creek site, where mortality was observed during in-place experiments. Therefore, resident fish in lower Cataract Creek may have acclimated to the elevated concentrations of trace elements. Simultaneously, a metabolic cost of acclimation may be expressed at lower Cataract Creek as acclimation coincides with a decreased mass (though not decreased growth) of trout in lower Cataract Creek.

Metallothioneins are proteins that bind trace elements such as copper, cadmium, and zinc and may play a role in the acclimation of fish to trace elements (Stegeman and others, 1992). Marr and others (1995) demonstrated that a physiological cost of acclimation exists for brown trout in the Clark Fork River, Mont., where trout acclimated to trace elements in the river had elevated concentrations of metallothionein in their livers and grew less than trout not acclimated to the trace elements. Furthermore, Dixon and Sprague (1981) concluded that decreased growth was a result of the metabolic costs associated with acclimation to copper in the laboratory. Trout from lower Cataract Creek had elevated metallothionein in their livers. Similar to Marr and others (1995), metallothionein was greatest in the livers of fish that also had the greatest concentrations of trace elements in the liver.

Further evidence of the compromised health of resident fish in lower Cataract Creek was the greater amounts of products of lipid peroxidation in the livers. Peroxidation of fatty-acid side chains in cell membranes can change the structural integrity of cell membranes and may lead to cell death and tissue damage (Halliwell and Gutteridge, 1985; Wills, 1985). These findings imply that the livers of trout in lower Cataract Creek were compromised and this state was associated with elevated concentrations of trace elements in the liver. The concentrations of arsenic, copper, cadmium, lead, and zinc in livers of trout from lower Cataract Creek also were elevated above the concentrations in the livers of fish from the reference site (UBR). In fact, the >1,000 µg Cu/g in the livers of fish from lower Cataract Creek was much greater than the upper limit of an effect concentration of 480 µg Cu/g suggested by Farag and others (1995) for the Clark Fork River, Mont.

The extent of the reduced fish biomass and density at lower Cataract Creek cannot be explained by habitat differences. The mass of trout per 1,000 ft in lower Cataract Creek was only 28 percent of the mass calculated for the reference site (UBR). This biomass difference between lower Cataract Creek and the reference site was much greater than the difference in weighted usable area. For all three lifestages (fry, juvenile, and adult), lower Cataract Creek had an average of 91 and 78 percent of the weighted usable area that the reference site had for brook and rainbow trout, respectively. That habitat differences are sufficient to explain the reduced biomass/density at lower Cataract Creek is thus unlikely.

We observed decreases in the density and mass per 1,000 ft at lower Cataract Creek but did not observe differences among sites in the lengths-at-age. However, the lower density of trout at lower Cataract Creek may have masked the growth-suppressing effects of trace elements. A situation of less competition for resources may result when fish are present in lower densities. Jenkins and others (1999) found that growth of individual brown trout increased as a result of lower densities. During electrofishing activities on Cataract Creek, we only collected fish from what we observed to be the most energy-efficient feeding locations, which may have allowed for good growth (Bachman, 1984).

We also documented elevated concentrations of arsenic, cadmium, copper, lead, and zinc in the tissues of trout from lower Basin Creek (LBC) and Boulder River at Galena Gulch (BRGG). Lowe and others (1985) and Schmitt and Brumbaugh (1990) reported the 85th percentile (values for which 85 percent of samples are below) of 1.1 µg As/g, 0.33 µg Cd/g, 5.0 µg Cu/g, 1.31 µg Pb/g, and 201 µg Zn/g of whole fish collected from more than 100 stations across the United States (80 percent moisture was assumed to calculate µg/g dry weight). The mean concentrations of trace elements in whole fish collected from lower Cataract Creek, lower Basin Creek, and Boulder River near Galena Gulch exceed these 85th percentiles. Therefore, cadmium was >121, >60, and >60 times, respectively, than the 85th percentiles for lower Cataract Creek, lower Basin Creek, and Boulder River near Galena Gulch, respectively. Also, the amount of arsenic in whole fish from lower Cataract Creek, lower Basin Creek, and Boulder River near Galena Gulch was 8, 6, and >3 times, respectively, greater than the 85th percentiles.

Although the concentrations of trace elements in fish from lower Basin Creek and Boulder River near Galena Gulch were greater than in fish sampled from across the country (Lowe and others, 1985; Schmitt and Brumbaugh, 1990), the concentrations were not as great as those noted in lower Cataract Creek. Moreover, we did not observe significant changes in mass of trout per acre, metallothionein, or lipid peroxidation at lower Basin Creek or Boulder River near Galena Gulch (tables 3, 7, and 8). Therefore, the impacts of trace elements at these two sites were less than the impacts at lower Cataract Creek. This study provides a baseline of data for future monitoring. Monitoring plans that include lower Basin Creek and Boulder River near Galena Gulch, as remediation proceeds in Basin and High Ore Creeks, could document improved conditions or else changes caused by inadvertent releases of trace elements downstream when the tailings are disturbed.

The target organ of arsenic toxicity is the skin (Goyer, 1986), and arsenic is elevated in sediment, invertebrates, and fish at Boulder River near Galena Gulch (tables 5, 10, and 11). The dark coloration and the increased accumulation of melanocytes observed in the skin of fish held at lower High Ore Creek (located upstream of BRGG) during the onsite 96-hr survival experiments may have been caused by the elevated arsenic present at this site. Obvious changes in coloration were observed during the necropsy evaluations, but histological evaluations would be necessary to observe more subtle changes such as increased numbers of melanocytes. Additionally, color changes during necropsies may not always be observed because melanocyte regulation and control are lost immediately at death. For these reasons, we suggest that histological analyses be added to future assessments of aquatic health in an effort to document any tissue pathology more completely.

Exposure Pathways

Another goal of this study was to characterize the pathway and partitioning of trace elements into water, sediment, biofilm, benthic macroinvertebrates, and fish. This pathway documents the routes of trace-element exposure to fish that appear to have caused the impaired health status that we observed. Not only were trace elements elevated in all three of the tributaries of concern (Basin, Cataract, and High Ore Creeks), but also, trace elements accumulated in all components and accumulated to the greatest extent at sites nearest inactive mines. Furthermore, the pathway has resulted in concentrations of trace elements in water and sediment that may adversely affect the aquatic health in the Boulder River watershed.

Median concentrations of cadmium, copper, and zinc in stream water at most sites exceeded acute and chronic aquatic-life standards (U.S. Environmental Protection Agency, 1999, 2001). In the Boulder River watershed, hardness generally was < 50 mg $CaCO_3$ /L at all sites except lower High Ore Creek. At 50 mg/L hardness, a conservative estimate for the Boulder River watershed, the standards are (acute/chronic) 1.0/0.15 µg Cd/L, 7.0/5.0 µg Cu/L, and 66/65 µg Zn/L. These general guidelines suggest that the water quality of most of the tributary sites and even some of the mainstem sites (zinc at BRCC and BRGG) was sufficiently degraded to cause detrimental effects to aquatic life.

As was observed in the water column, cadmium, copper, and zinc were elevated in sediment throughout the Boulder River watershed. Additionally, arsenic and lead persisted in the sediment though concentrations of these trace elements generally were not elevated in the filtered fraction of the water column. Although sediment-quality criteria have not been established, several researchers have suggested concentrations to be used as sediment-quality guidelines. MacDonald and others (2000) determined that guidelines of 33 µg As/g, 4.98 µg Cd/g, 149 µg Cu/g, 128 µg Pb/g, and 459 µg Zn/g would protect benthic macroinvertebrates from toxicity of these trace elements. These consensus-based guidelines for freshwater sediment correctly predicted no toxicity for invertebrates during the majority of laboratory investigations (correct prediction 74 percent for As, 80 percent for cadmium, 82 percent Cu, lead, and zinc). In the Boulder River watershed, sediment from all tributary sites exceeded the consensus-based guidelines for arsenic and lead, and the consensus-based guideline for arsenic was exceeded on the mainstem as far downstream as Boulder River near Galena Gulch. All tributary sites with the exception of upper Cataract Creek exceeded the consensus-based guideline for zinc. And several of the tributary sites exceeded the consensus-based guidelines for cadmium and copper. Therefore, this study suggests that the pathway of trace elements in the Boulder River watershed would lead to invertebrate toxicity in the watershed. This finding that invertebrate toxicity may be present in the Boulder River watershed is supported further by Boyle and Gustina (2000). These researchers defined the invertebrate community structure at some of the same sites investigated during this study and observed a depleted number of Ephemeroptera-Plecoptera-Tricoptera (EPT) taxa in High Ore Creek, lower Cataract Creek, and Jack Creek.

Colloids and biofilm appear to play a critical role in the pathway of trace elements to the food chain. Colloidal iron oxyhydroxides are formed immediately downstream of mine drainage mixing zones and are involved in the downstream transport of copper, lead, and zinc (Schemel and others, 2000). The association of colloids and trace elements is dynamic, and the trace elements may adsorb or desorb frequently as pH changes in mixing zones during transport. Therefore, the formation of iron colloids may play an important role in the transport of trace elements downstream and in the transfer of trace elements to biofilm.

During transport downstream, colloids commonly are trapped by biofilm on rock surfaces. Newman and McIntosh (1989) questioned the bioavailability of trace elements associated with iron oxyhydroxides. However, we suggest that the transfer of trace elements associated with iron colloids to biological components of biofilm is an important pathway where trace elements associated with abiotic components are first presented to biotic components. Significant accumulations may occur if only small portions of these trace elements are bioavailable. The significant correlations we observed between concentrations of arsenic, copper, lead, and zinc in colloids and biofilm support the hypothesis that colloids transport trace elements, at least in part, to biofilm. And, we have documented that arsenic, in addition to copper, lead, and zinc, as documented by Schemel and others (2000), likely is transported downstream by colloids. Furthermore, trace elements in biofilm are associated with both the abiotic and biotic components present in biofilm (Newman and others, 1983, 1985). This association suggests that biofilm is a critical link in the movement of trace elements directly into the food chain.

Biofilm also may accumulate trace elements by means other than the exposure received from colloids. This appears especially evident for cadmium and zinc. The concentrations of these trace elements were greater in filtered water in comparison to concentrations in colloids. We observed strong correlations between dissolved metal in water and biofilm for cadmium but not between colloids and biofilm. This suggests that water may be the primary source of the cadmium in biofilm. Dissolved zinc concentrations were greater than zinc concentrations in colloids, and the correlations were strong among water, colloids, sediment, and biofilm for zinc. This suggests that biofilm may receive zinc from water, colloids, and sediment.

Large concentrations of trace elements were observed in biofilm throughout the Boulder River watershed. In fact, arsenic and copper concentrations were greater in the Boulder River watershed than in the Coeur d'Alene River watershed in Idaho (Farag and others, 1998). The largest arsenic concentration in biofilm from the Coeur d'Alene River watershed was 155 µg/g at Cataldo, just downstream of the boundary for a Natural Resource Damage Assessment, while arsenic concentrations of 3,300 µg/g and 2,600 µg/g were found at High Ore Creek and Jack Creek, respectively. Zinc and cadmium concentrations were similar between some sites in the Coeur d'Alene and Boulder River watersheds. For example, there was slightly more zinc in the biofilm collected from High Ore Creek (18,100 µg/g) than was observed at Pinehurst (11,600 µg/g), a site within the boundary for the Natural Resource Damage Assessment of the Coeur d'Alene River watershed. Also, cadmium concentrations were similar at Jack Creek (70 µg/L) and at Nine Mile, a site defined as a significant source of trace elements for the Coeur d'Alene River watershed. We note that concentrations of lead found in the Coeur d'Alene River watershed are still among the greatest documented. The concentrations of lead in biofilm from many sites in the Boulder River watershed were higher than concentrations at the reference site (for example, 1,100 µg/g at High Ore compared to 12.1 µg/g at the reference site), but lead concentrations in biofilm from the Coeur d'Alene River watershed were magnitudes greater (for example, 27,200 µg/g at Nine Mile).

Not only were concentrations of trace elements in invertebrates elevated throughout the Boulder River watershed when they were compared to reference concentrations, but they were also elevated compared to composite invertebrate samples collected from other sites in the West where historical mining activities have occurred. In fact, the concentrations of arsenic, cadmium, copper, lead, and zinc in invertebrates from lower Cataract Creek (63, 59, 340, 34, and 2,410 µg/g, respectively) were greater than the concentrations in invertebrates from the Clark Fork River watershed (19, 2.3, 174, 15, and 648, respectively) that were associated with reduced survival, growth, and health of cutthroat trout fed diets composed of these invertebrates from the Clark Fork River watershed (Farag and others, 1994; Woodward and others, 1995).

Silver Bow Creek is a tributary of the Clark Fork River in western Montana. Historically, mine tailings were deposited along Silver Bow Creek, and some of the greatest concentrations of trace elements in invertebrates in the Clark Fork River watershed were recorded in this stream. Arsenic concentrations in invertebrates from Jack Creek (77 µg/g) were 2 times the concentrations measured in invertebrates from Silver Bow Creek (34 µg/g before remediation of the area). And, zinc concentrations in invertebrates at lower High Ore Creek and lower Cataract Creek (3,090 and 2,410 µg/g, respectively) were greater than the amount of zinc measured in invertebrates from Silver Bow Creek (1,660 µg/g; Poulton and others, 1995). However, copper concentrations at Silver Bow Creek were considerably greater (1,380 µg/g) than those observed in invertebrates from the Boulder River watershed (for example, 340 µg/g, LCC).

As we noted in the case of biofilm, the concentrations of some trace elements in macroinvertebrates from the Boulder River watershed exceeded the concentrations in the Coeur d'Alene River watershed. Concentrations of arsenic and copper in invertebrates from Jack Creek (77 and 319 µg/g, respectively) were greater than in invertebrates collected from the Coeur d'Alene River watershed near Pinehurst (42 and 34 µg/g, respectively). However, the mean concentration of lead in invertebrates from the Coeur d'Alene River watershed at Cataldo was 292 µg/g, and as was noted with biofilm, this concentration of lead is magnitudes greater than those measured in invertebrates collected in the Boulder River watershed. Some of the largest concentrations of lead in aquatic life in the intermountain western United States continue to be documented in the Coeur d'Alene River watershed.

The pattern of accumulation in the components measured during this study differed from patterns previously observed. During a pathway investigation of the Coeur d'Alene River watershed, Farag and others (1998) found that the pattern of trace-element concentrations (from greatest to least) to be as follows: biofilm and sediment > macroinvertebrates > whole fish. However, during this investigation of the Boulder River watershed, the order of concentrations was biofilm > macroinvertebrates ≥ sediment > fish tissues > water and colloids. In fact, the concentrations of trace elements in biofilm during this study were often greater than the concentrations in sediment. This could result from the entrapment of colloid-bound trace elements by the biofilm, and this entrapment may tend to integrate the water-column concentrations over time.

The final goal of this study was to define the influence of water, colloids, and sediment in the transfer of trace elements to aquatic life. A clear interdependency among the components measured during this study exists, and all components that were measured appear to be important in the movement of trace elements up the food chain. The numerous significant correlations of trace-element concentrations among the various components support this interpretation. However, the primary pathway varied with the trace element. For example, copper, cadmium, and zinc appear to have moved directly to biota (biofilm, invertebrates, and (or) fish tissues) from water and sediment. In fact, not only did trace-element concentrations in biofilm increase with concentrations in water and sediment, but concentrations of trace elements in fish tissues also increased directly with concentrations in water and sediment. Therefore, concentrations of these trace elements appear to have increased in fish tissues as a result of direct exposure from water and sediment contact and indirect exposure through the food chain. The pathway of arsenic was slightly different, because, although arsenic accumulated in colloids and biofilm directly from water, arsenic did not accumulate in fish tissues directly from the water. The movement of lead into biological components appeared to be the result of a pathway that began with sediment because there were no correlations for lead between water and the other components.

Correlations between water and sediment were less significant than expected; two possible explanations for this observation arise. First, colloids present in the water and on sediment particles may affect the concentrations and diminish the strength of correlations. Iron colloids also are likely to be present in biofilm, and concentrations of most trace elements in biofilm were correlated with water and (or) sediment. If colloids made up a significant portion of the biofilm, these correlations of water and sediment with biofilm may have resulted in part from the significant colloid presence. A second possibility is the spatial aspect of the process by which trace elements are transferred from the water column to sediment. The process occurs as colloids form in mixing zones and then trace elements react with the colloids; this all occurs during transport downstream. Thus, a sample at a single site may not reflect the whole process, and correlations may not be good. Where a sequence of samples was collected in Uncle Sam Gulch during a metal-loading study (Kimball and others, this volume), the process was evident, but any single sample would not have indicated the process.

In summary, the pathway of trace elements to fish in the Boulder River watershed clearly included water, sediment, biofilm, and benthic macroinvertebrates. Trace elements accumulated in all of these components and to the greatest extent at sites nearest historical mining activities. The concentrations of trace elements in water and sediment routinely were such that aquatic life was affected at several sites in the watershed. Finally, the interrelationship of the trace elements accumulating in the various components suggests that fish were exposed to trace elements both directly from water and sediment and indirectly through the food chain.

Summary

Water quality in Bullion Mine tributary, Jack Creek, middle Cataract Creek, Uncle Sam Gulch, and to a lesser degree lower and upper High Ore Creek may have rendered the survival of trout unlikely at these sites and may have acted as a barrier that limited fish migration to upstream reaches in the Boulder River watershed. Additionally, because biomass/density was decreased, and metallothionein, products of lipid peroxidation, and tissue trace-element concentrations were simultaneously increased in resident fish at lower Cataract

Creek, we conclude that the aquatic health of lower Cataract Creek was compromised. Furthermore, the association of elevated trace-element concentrations in tissue, water, and sediment has led us to conclude that trace elements were the cause of the compromised aquatic health in lower Cataract Creek. Therefore, resident fish populations would benefit greatly if remediation efforts were directed to minimize the concentrations of trace elements in the water, sediment, and biota of lower Cataract Creek downstream from Uncle Sam Gulch.

The concentrations of trace elements in water and sediment routinely were such that aquatic life was affected at several sites in the watershed. And, the concentrations of arsenic, cadmium, copper, lead, and zinc in invertebrates from lower Cataract Creek were greater than the concentrations in invertebrates from some sites in the Clark Fork River watershed, Montana, where high trace-element concentrations were associated with reduced survival, growth, and health of cutthroat trout fed diets composed of these Clark Fork River invertebrates (Farag and others, 1994; Woodward and others, 1995). Arsenic concentrations in invertebrates from Jack Creek and zinc concentrations in invertebrates from lower High Ore Creek and lower Cataract Creek were greater than the concentrations of these trace elements in invertebrates from Silver Bow Creek, a tributary with some of the greatest invertebrate trace-element concentrations in the Clark Fork River watershed.

Finally, the interrelationship of the trace elements accumulating in the components measured suggests that fish were exposed to trace elements both directly from water and sediment and indirectly through the food chain. It appears that trace elements have contacted biota through these two pathways to compromise the overall aquatic health of the Boulder River watershed. One first step in the food-chain pathway seems to be associated with trace elements transferring from colloids to biofilm.

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