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Characterizing Aquatic Health Using Salmonid Mortality, Physiology, and Biomass Estimates in Streams with Elevated Concentrations of Arsenic, Cadmium, Copper, Lead, and Zinc in the Boulder River Watershed, Montana

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Abstract.—Abandoned tailings and mine adits are located throughout the Boulder River watershed in Montana. In this watershed, all species of fish are absent from some tributary reaches near mine sources; however, populations of brook trout *Salvelinus fontitalis,* rainbow trout *Oncorhynchus mykiss,* and cut-throat trout *O. clarki* are found further downstream. Multiple methods must be used to investigate the effects of metals released by past mining activity because the effects on aquatic life may range in severity, depending on the proximity of mine sources. Therefore, we used three types of effects—those on fish population levels (as measured by survival), those on biomass and density, and those at the level of the individual (as measured by increases in metallothionein, products of lipid peroxidation, and increases in concentrations of tissue metals)—to assess the aquatic health of the Boulder River watershed. Elevated concentrations of Cd, Cu, and Zn in the water column were associated with increased mortality of trout at sites located near mine waste sources. The hypertrophy (swelling), degeneration (dying), and necrosis of epithelial cells observed in the gills support our conclusion that the cause of death was related to metals in the water column. At a site further downstream (lower Cataract Creek), we observed impaired health of resident trout, as well as effects on biomass and density (measured as decreases in the kilograms of trout per hectare and the number per 300 m) and effects at the individual level, including increases in metallothionein, products of lipid peroxidation, and tissue concentrations of metals.

Measures of physiological function, tissue residues, and fish biomass or density can provide a clear picture of exposure related to physiological malfunction and a resulting decrease in biomass or density of fish. These data interpreted simultaneously can provide a more complete assessment of the ecological health of a river and its tributaries (Johnson 1968).

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Streams in the Boulder River watershed in southwestern Montana receive drainage from abandoned mine adits and runoff from old tailings piles. All species of fish are absent from some reaches below draining mine adits, but populations of brook trout *Salvelinus fontinalis*, rainbow trout *Oncorhynchus mykiss*, and cutthroat trout *Oncorhynchus clarki* are found further downstream (Montana Fish, Wildlife, and Parks 2002). Additionally, viable populations of native, genetically pure, westslope cutthroat trout *O. c. lewisi* exist in High Ore Creek above the Comet Mine (Figure 1).

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 biomass (density) at sites on the Boulder River below Cataract Creek and High Ore Creek. Gardner (1977) found that the diversity of the invertebrate community in the Boulder River below High Ore Creek was reduced compared with the upstream station below 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 (Zn) in the water at the site below High Ore Creek.

It was not until the 1990s that other investigations of the watershed were initiated. Gless (1990) designated Basin Creek as a ''stream of concern,'' Cataract Creek as ''degraded,'' and High Ore Creek as ''extremely degraded.'' These classifications were based on elevated concentrations of arsenic (As) in the water column and the rare presence of aquatic life in some stream reaches. Gless (1990) observed metal stains and dead vegetation as high as 1.5 m above the stream banks of Cataract Creek. Martin (1992) documented elevated concentrations of cadmium (Cd), copper (Cu), and Zn in the water, as well as in the sediment, aquatic invertebrates, and fish of Cataract Creek, and related these concentrations to the sources of metals in the drainage.

There are three tributaries of concern in the Boulder River watershed: Basin Creek, Cataract Creek, and High Ore Creek. Some of the major sources of mine waste in the watershed enter these three creeks. For example, wastes from the Bullion Mine are discharged into Jack Creek, which flows into Basin Creek. Six miles downstream, Basin Creek then flows into the Boulder River. The Crystal Mine in Uncle Sam Gulch provides almost all of the metal input into Cataract Creek, which flows into the Boulder River downstream of Basin Creek. The Comet mine is located along High Ore Creek, which flows into the Boulder River upstream of Galena Gulch.

One objective of this study was to determine the survivability of westslope cutthroat trout in sections of creeks that lack fish. Some reaches of Boulder River tributaries are devoid of fish. Fishless reaches in Jack Creek, Uncle Sam Gulch, and High Ore Creek coincide with metal concentrations in the ambient water exceeding 50 μg Cd/L and up to 5,000 μg Zn/L (Nimick and Cleasby 2000). We also examined the whole-body ion status and histological changes in fish during the survival studies to provide insight into the mechanisms of acute toxicity.

Our second objective was to estimate the biomass and density (measured as kilograms of trout per hectare) for all trout and health of rainbow trout in the Boulder River watershed. Metal exposures can affect aquatic biota and the overall ecological health of a river system (Farag et al. 1995). No assessments of individual fish health had been previously performed in the Boulder River, and changes in fish health have not been studied in conjunction with biomass surveys.

Physiological malfunction can be defined with measurements of metallothioneins and products of lipid peroxidation (Farag et al. 1994, 1995). In laboratory experiments, measures of metallothioneins have been associated with reduced fish growth and with metal exposure (Dixon and Sprague 1981; Roch and McCarter 1984; Marr et al. 1995). Measures of lipid peroxidation have been similarly associated with reduced growth (Woodward et al. 1995) and with metal exposure (Stern 1985; Wills 1985; DiGiulio et al. 1989).

Metallothioneins are proteins that bind metals (e.g., Cu, Cd, and Zn; Hogstrand and Haux 1991; Stegeman et al. 1992). Because metallothionein synthesis and the resulting concentrations increase when fish are exposed to metals, the induction of these proteins indicates metal exposure in fish. The induction of metallothioneins in trout has also been associated with slowed growth of trout that maintain induced metallothionein concentrations (Marr et al. 1995). Therefore, elevated metallothionein concentrations may indicate reduced fitness of trout.

Elevated concentrations of products of lipid peroxidation indicate cell death and tissue damage (Farag et al. 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

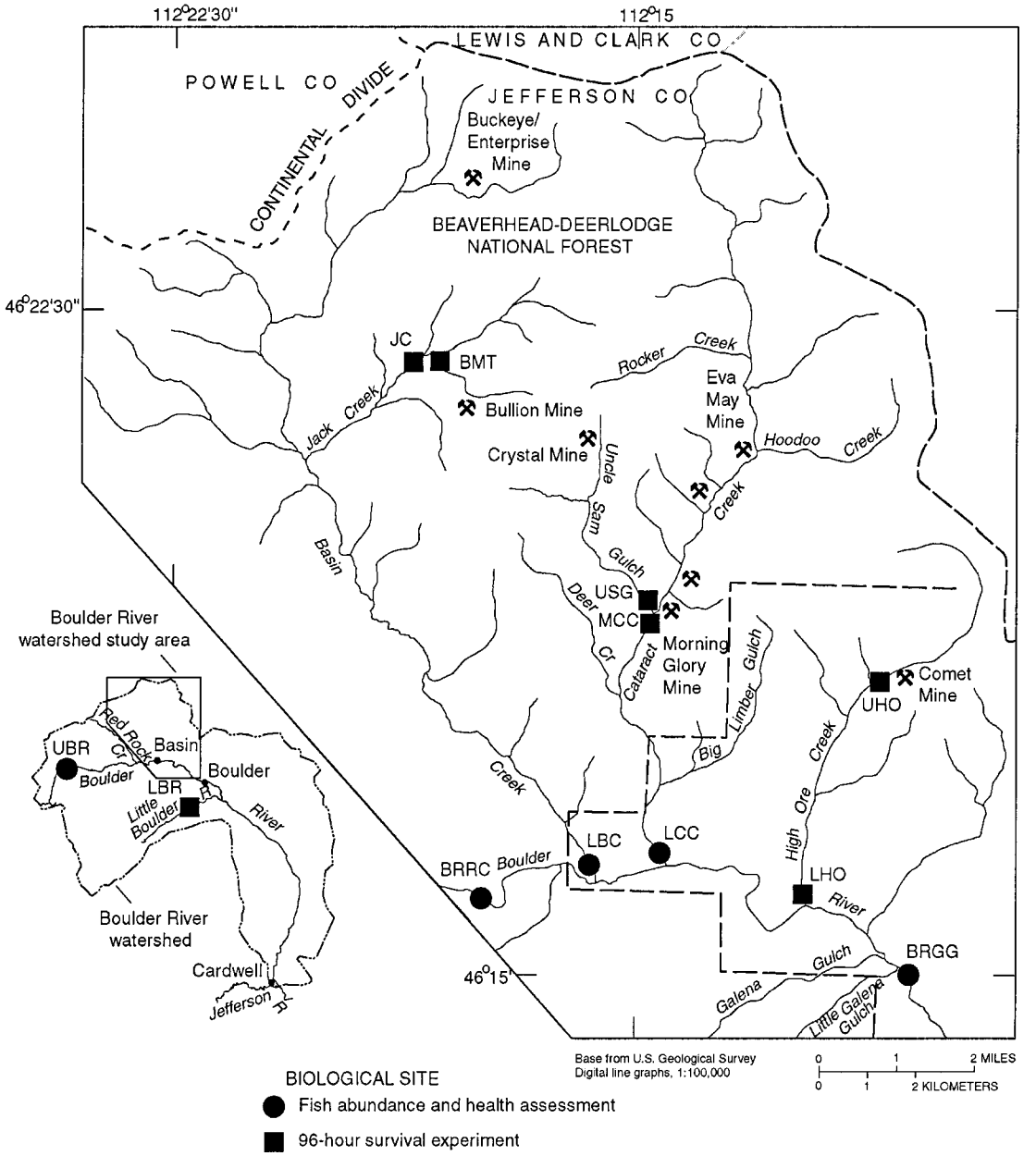


FIGURE 1.—Map of the Boulder River watershed and designated sites where survival until 96 h was studied (LBR = Little Boulder River, BMT = Bullion Mine Tributary, JC = Jack Creek, USG = Uncle Sam Gulch, MCC = Middle Cataract Creek, LHO = Lower High Ore, and UHO = Upper High Ore) and sites where fish abundance and health assessments were investigated (UBR = Upper Boulder River, LBC = Lower Basin Creek, LCC = Lower Cataract Creek, BRRC = Boulder River below Red Rock Creek, BRGG = Boulder River Below Galena Gulch).

membrane and may ultimately result in cell death and tissue damage (Halliwell and Gutteridge 1985; Wills 1985). Metals that exist in more than one valence state, such as Cu, may directly initiate lipid peroxidation (Wills 1985). Metals may also initiate lipid peroxidation because they inhibit antioxidant enzymes such as glutathione peroxidase and transferase (Reddy et al. 1981).

To meet our second objective, we used measures of metallothionein and the products of lipid per-

oxidation to define physiological malfunction. These physiological malfunction data were then interpreted with tissue residue and biomass and density data to determine the overall ecological health of sites within the Boulder River watershed.

Methods

The survival of westslope cutthroat trout from the Washoe Park State Fish Hatchery, Anaconda, Montana, was determined with 96-h in situ experiments at six sites located in the upper reaches of Basin and Cataract creeks and in lower High Ore Creek during low-flow conditions in 1998 (all sites used in our study are presented in Figure 1 and have been identified with two- to four-letter abbreviations).

The experiment was repeated in 1999 with the addition of a seventh site located in upper High Ore Creek. This site was added because of its close proximity to the Comet Mine and remediation efforts that were initiated during 1999. This site was also in close proximity to a native population of westslope cutthroat trout that resides upstream of the Comet Mine. A site on the Little Boulder River, which lacked historical mine activity, was designated as the reference site.

We used age-1 trout; in 1998, they were about 150 mm (total length) and weighed 30 g, whereas in 1999 a subsample of 23 age-1 trout measured 103± 16 mm (mean ± SD) and weighed 11 ± 5 g. During both years, the fish were transported to each site in Little Boulder River water maintained at 12.5 ± 2°C and greater than 6.4 mg/L dissolved oxygen (DO). At each site, fish were tempered for approximately 30 min in mixtures of site water and water from the Little Boulder River. Piper et al. (1982) suggested that fish be tempered with site water if a temperature difference of more than 2.5°C exists between site and transport water. We believed that 30 min to temper was acceptable, and no hyperactivity was observed in the site water (100%). Site water temperatures ranged from 13°C to 17°C among sites at the beginning of the experiment. The highest temperature was recorded at the reference site because fish were tempered at this site late in the day. The 4-L polyethylene enclosures were rinsed three times with site water before fish were placed in them. We placed 20 fish at each site, separated into groups of 5 fish in each of four enclosures. Fish that were put in enclosures deployed at the reference site had been first transported to other sites; therefore, handling stress was introduced to all experimental fish. During the experiments, fish were considered dead when gill action ceased.

We used two approaches to investigate the mechanisms of acute toxicity that might occur. We investigated the whole-body ion status of experimental fish during 1998 to determine if ionoregulatory failure due to elevated metals in the water column was the cause of death. During 1998, three to five experimental fish held at each site were collected, frozen, and shipped to the U.S. Geological Survey (USGS) Columbia Environmental Research Center (Columbia, Missouri) for whole-body analyses of (Ca), potassium (K), and sodium (Na). In 1999, we studied changes in histology to determine whether such changes would be consistent with those generally found as a result of metal stress. We also held five additional experimental fish at each site, and these whole fish were subsequently fixed in Davidson's solution and transferred to the Fish Technology Center, Bozeman, Montana, for histological analyses. Tissues were processed by standard histological methods and examined by light microscopy (Humason 1979).

Water quality was monitored at each site during the 96-h survival experiments. Frequency of sampling varied among sites and between years. Specific conductance, pH, and dissolved oxygen concentration were measured daily in 1998 at all sites, and electronic instruments with data-loggers were deployed to collect measurements every 15 min at JC, LHO, and LBR. In 1999, electronic instruments were deployed to collect measurements every 15 min in each tributary. Water samples for metal analyses were collected daily, except in 1999, when dissolved Zn samples were collected hourly at some sites. The sample design was altered in 1999 to define whether the time of day that a sample was collected affected the concentration of metals in that sample.

Water samples for metal analyses were collected using depth- and width-integrated methods (Edwards and Glysson 1988). Sample filtration and preservation were performed according to procedures described by Horowitz et al. (1994) and Ward and Harr (1990). Samples were filtered through 0.45-μm filters and were analyzed by the USGS National Water Quality Laboratory, Lakewood, Colorado. These data are reported as dissolved constituents, although we acknowledge the potential presence of particulates less than 0.45 μm (e.g., colloids) in this fraction.

Five sites inhabited by fish were selected to estimate biomass and density and study physiology. Two of these sites with little or no known historical

mining activity were designated as the reference sites. The three tributary sites included UBR in the upper Boulder River (reference), LCR in lower Cataract Creek, and LBC in lower Basin Creek (Figure 1). Two other sites were studied on the Boulder River below Galena Gulch (BRGG) and below Red Rock Creek (BRRC, reference). The differences in discharge between the tributary and main-stem sites required that different methods be used to study these two types of sites. Multiple-pass depletion was used to estimate biomass and density in the smaller tributary sites: two-step removal estimates (Seber and Le Cren 1967) and three- or four-step removals (Zippen 1958). For the main stems, we used modified Peterson mark–recapture techniques (Chapman 1951). Fish were collected by electrofishing and, except those sampled for physiological measurements, were anesthetized with tricaine methanesulfonate (MS-222). Total lengths and weights were recorded, and scales were collected to age fish. Scales were analyzed with the Fraser-Lee method that uses a linear regression between scale and body length to back-calculate lengths at age (DeVries and Frie 1996).

Using methods described by Platts et al. (1983), depth, velocity, and substrate were measured at sites sampled for fish abundance (data not presented). Microhabitat features were also measured in accordance with guidelines described by Bovee (1986) for inclusion in the PHABSIM models for simulating amount of weighted usable area, which could be used to differentiate available habitat among sites. Suitability indices for all life stages of brook trout were from Chapman (1995), while those for rainbow trout fry were from Raleigh et al. (1984) and those for rainbow trout juveniles and adults from Ken Bovee (USGS, personal communication) for the South Platte River, Colorado.

Water quality conditions were monitored periodically between October 1996 and September 1999 at the five fish health assessment sites. Data for these samples were summarized to characterize metal concentrations typical of high-flow and low-flow conditions at these sites.

Thirteen to 25 rainbow trout (203 $\pm$ 43 mm total length, 89 $\pm$ 62 g) were collected from each site for physiological analyses during low-flow conditions in 1997. The species composition available at the lower Cataract Creek site dictated that we collect rainbow trout as a surrogate species to determine the physiological health of trout in the watershed. Each fish was pithed and a necropsy was performed immediately to identify any gross abnormalities (e.g., nodules on internal organs, discolored or frayed gills; Goede 1989). Samples of gill and liver were dissected from each fish, frozen immediately with liquid nitrogen, and transported to the USGS, Jackson Field Research Station, Jackson, Wyoming, where they were stored at -90°C to adequately preserve tissues for biochemical analyses. Five additional rainbow trout (same size as above) were collected from each site and frozen for measurements of whole-body metal concentrations.

At least five composite samples of each tissue from each site were prepared in the laboratory by combining tissues from two to five rainbow trout. The tissues were removed from the -90°C freezer and ground in mortars cooled with liquid nitrogen. To define the physiological condition of fish from the sites, concentrations of tissue metals, products of lipid peroxidation, and metallothionein were measured in aliquants of the composite samples.

Arsenic, Cd, Cu, lead (Pb), and Zn were measured in gills, livers, and whole fish ($N \geq 5$ for each tissue type). All tissue samples were lyophilized to a constant dry weight. Approximately 100 mg of each sample was digested in 1 mL of nitric acid and 1 mL 30% hydrogen peroxide. Concentrations of the elements were determined by inductively coupled plasma–mass spectroscopy (PE/SCIEX Elan 6000 ICP-MS). Quality control included measurements of predigestion spikes, postdigestion spikes, digestion replicates, and reference tissue samples.

Quality control tissues for metal analyses included whole striped bass *Morone saxatilis,* International Atomic Energy Agency (IAEA) fish fillet, National Institute of Standards and Technology RM-50 tuna fillet, and National Research Council of Canada Dorm-1 dogfish muscle and bovine liver. Recoveries for the 25 reference samples measured between 87% and 121% with two exceptions. One bovine liver sample measured 132% recovery for As, and a Pb result for one IAEA fish fillet measured 53% recovery. The relative percent difference among triplicate analyses were generally less than 4% for liver, 15% for gill, and 16.5% for whole body. Deviations from these were noted in a few measurements of Pb. The analyses of predigestion and postdigestion spikes ranged from 85% to 122% for all tissues.

A competitive double-antibody radioimmuoassay (RIA) developed by Hogstrand and Haux (1990) and later modified by Hogstrand et al. (1994) was used to measure metallothionein (MT) in groundfish gills and livers. The MT assay used

rabbit antiserum raised against MT for yellow perch *Perca flavescens* as the first antibody, ^{125}I-labeled rainbow trout MT as a tracer, and goat anti-rabbit IgG as a second antibody. A 10,000 × gravity supernatant prepared from livers of Cd-injected rainbow trout was used as a MT standard. The MT content of the standard was calibrated against a standard curve prepared from rainbow trout MT (Hogstrand et al. 1994). The working range of the RIA was 10–100 ng rainbow trout MT per assay tube, which corresponds to 0.6–6.0 μg/g liver wet weight.

A fluorometric assay that measures the relative intensity of fluorophores formed during lipid peroxidation (Dillard and Tappel 1984; Fletcher et al. 1973; Farag et al. 1995) was used to measure products of lipid peroxidation. Ground tissue (200 mg frozen weight) was combined with a 2:1 mixture of high-performance-liquid-chromatography-grade chloroform:methanol (7 mL for a 200-mg sample) in a glass homogenizer. The tissue was homogenized with a glass pestle, diluted with an equal volume of water, homogenized again, and vortexed for 1.5 min. The mixture was centrifuged at 1,200 × gravity for 1.5 min in a Corex tube, and the chloroform layer was removed. Fluorescence was measured (Hitachi f-2000) at a wavelength of 435 nm emission during excitation at 340 and 360 nm.

Data for tissue concentrations of metals, metallothionein, products of lipid peroxidation, length at age, and biomass and density estimates were analyzed with one-way analysis of variance (ANOVA; SAS Institute 1989). The data were tested for equality of variances with the Levene's test for homogeneity of variances (Toxstat 1994) and were transformed if they did not pass the test. Means for tissue metal concentrations were compared by a Fischer's least-significant-difference test, those for metallothionein and products of lipid peroxidation by Tukey's test, and those for biomass and density by Dunnett's test (Zar 1984). A statistical criterion of $\alpha = 0.05$ was used for all comparisons. The percent survival data were not statistically analyzed.

Results

Survival Experiments

Compared with the LBR reference site, the survival of caged westslope cutthroat trout at 96 h was less at all experimental sites in 1998 and 1999 (BMT, JC, USG, MCC, LHO, and UHO; Figure 2). Survival at 96 h was 0% at all experimental sites during both years, except survival at LHO

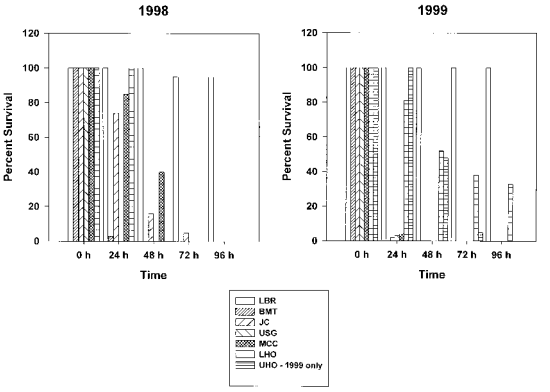
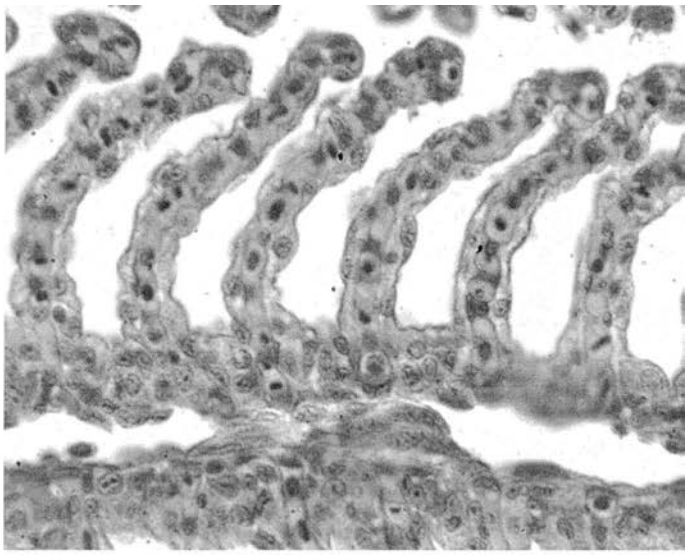


FIGURE 2.—Survival of hatchery cutthroat trout placed in various tributaries of the Boulder River for up to 96 h during 1998 and 1999. See Figure 1 for site abbreviations.

was 33% during 1999. In most cases, survival was affected in 24–48 h during 1999. Fish died more quickly in JC and MCC during 1999 compared with 1998. In the two most extreme cases, cutthroat trout placed in USG and BMT died in 5 and 8 h, respectively, during 1999. Survival was reduced to 5% at UHO at 72 h, and the experiment at this site was ended at that time.

The concentrations of whole-body ions in 1998 did not differ significantly among sites (reference site included). Concentrations of ions ranged from 22,533–24,860 μg Ca/g, 12,000–15,140 μg K/g, and 2,046–3,178 μg Na/g, the highest concentrations noted in fish from USG. The histological analyses during 1999 indicated that degeneration (dying) and necrosis (death) of gill epithelia was the most significant tissue lesion. Excessive mucous production and hypertrophy (swelling) were also noted in gill epithelia of fish from experimental sites (Figure 3). Spongiosis, a condition of edema and necrosis, was also observed in the nares of fish held in the test sites. Additionally, excessive mucous was noted in the skin of fish from all test sites. There was some hypertrophy in the gill lamellae and abundant mucous in the nares of fish held at the reference site, but these changes were less severe than those at the experimental sites. The proliferation of epithelial cells noted in gills of fish from UHO and LHO indicate that toxicity was less acute at these sites, which is consistent with the longer times to death at these sites in 1999. Additionally, the pseudobranchs of fish from LHO contained cystic (fluid filled) areas, an abnormal condition not noted at other sites. We noted dark longitudinal coloration in the skin at death



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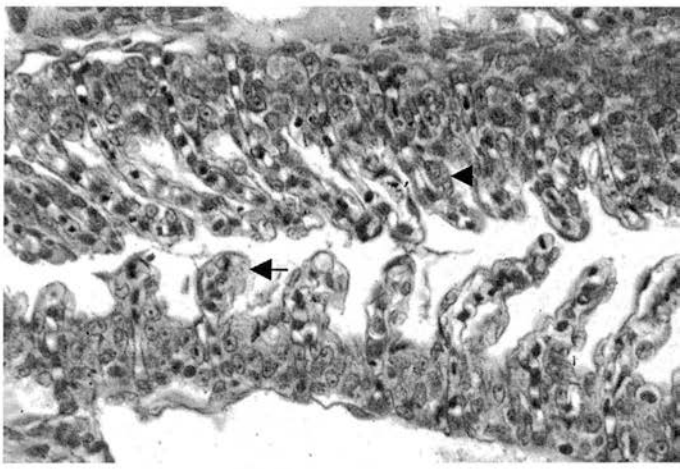

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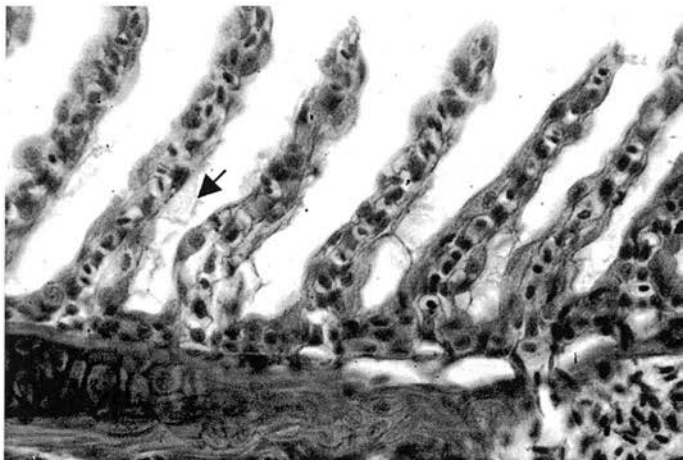

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for UHO and LHO fish. This discoloration was about 0.5 cm wide and was observed across the length of the fish. Correspondingly, an increased accumulation of melanocytes was noted in the skin of fish collected from LHO.

Water Chemistry of Survival Experiments

Water chemistry was variable in stream reaches where 96-h survival experiments were performed in 1998 and 1999. The on-site experiments were conducted between mid-July and early August during both years, but stream-flow conditions were different each year. During the 1999 experiments, streams had base-flow conditions typical of late summer, whereas the 1998 experiments were conducted about the end of spring runoff. Therefore, stream flow was higher during the 1998 experiments, and trace-element concentrations generally were lower in 1998 than in 1999. In both years, Cd and Zn were almost entirely dissolved and Cu was divided about equally between the dissolved and particulate phases (the dissolved concentrations are presented in Table 1). Arsenic and lead generally measured in low concentrations compared with the other metals (<3 μg As/L, except at LHO and UHO [22–33 μg As/L] and <1 μg Pb/L except at BMT [3.1 μg Pb/L]; data not otherwise presented). The pH values were neutral to slightly basic: 7.0–8.3, except for BMT where pH was 5.2–5.4. Temperature appeared to fluctuate more with time of day rather than among sites (Table 1). As expected, the manual temperatures recorded during 1998 did not capture the temperature range defined during 1999 when electronic measurements were recorded every 15 min. The maximum daily temperature recorded was 21°C (High Ore Creek), which is below the lethal limits for trout.

The relationship between metal concentrations and mortality followed a consistent pattern, with higher concentrations resulting in greater and more rapid mortality. At the reference site (LBR) hardness (48–56 mg/L) and metal concentrations were low (<0.3 μg Cd/L, 2 μg Cu/L, 2–5 μg Zn/L). Water quality at this site was similar in 1998 and 1999. Stream flow was 4–8 times higher during the experiments in 1998 than in 1999 in the Jack Creek and Cataract Creek drainages. Consequently, constituent concentrations were higher in 1999 at all four sites (BMT, JC, USG, and MCC) in these two drainages. In 1999, concentrations of Cd, Cu, and Zn were about 100% higher and hardness values were about 50% higher than the corresponding 1998 levels at each site. Hardness was generally higher at the two High Ore Creek sites (LHO and UHO 130–140 mg/L) compared with all other sites. Concentrations of metals were similar between LHO and UHO in 1999 and somewhat higher at LHO in 1999 compared with 1998, apparently as a result of stream flow (Table 1).

Results of hourly sampling in 1999 indicated that dissolved Zn concentrations typically varied at many sites during the 1999 experiments (D.A. Nimick, USGS, unpublished data). These variations resulted from normal diel concentration cycles and from the effect of storm runoff, which occurred during the toxicity experiments at JC in 1999. Diel cycles resulting in 2–3-fold changes in dissolved Cd and Zn concentrations have been documented at several sites in and near the study area and are thought to be caused by the effects of water temperature and pH on the partitioning of Cd and Zn between dissolved and sorbed phases (Brick and Moore 1996; D.A. Nimick, unpublished data). Therefore, a wide range of concentrations may occur daily at each site.

Biomass and Density Estimates

Of the three tributaries studied, the combined biomass of all brook and rainbow trout observed (on either an area or linear basis) was the smallest at LCC (Table 2): 13.5 ± 3.9 kg/ha versus 63.6 ± 2.1 kg/ha at UBR (reference) and 63.7 ± 2.1 kg/ha at LBC. Brook and rainbow trout were the two trout species found in the tributaries; brook trout predominated at UBR, whereas rainbow trout predominated at LCC. Our decision to compare combined biomass estimates was based on the assumption that this combination provides the best estimate of the biomass-producing capacity of a

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FIGURE 3.—**(a)** Gill section of a cutthroat trout held in the Little Boulder River reference site during the 96-h survival experiment in 1999; **(b)** gill section of a cutthroat trout held at the Middle Cataract Creek site during the same experiment, with the upper arrowhead pointing to hypertrophied cells and the lower one to a degenerative epithelial cell; and **(c)** gill section of a cutthroat trout held in High Ore Creek during the experiment, with an arrow pointing to a degenerate epithelial cell. The sections were 5 μm thick, stained with hematoxylin and eosin, and magnified 250 times.

TABLE 1.—Physical and chemical data for stream sites during 96-h survival experiments performed on-site (see Figure 1 for site abbreviations) in the Boulder River watershed of Montana. Dissolved metal concentrations are median values when multiple samples were collected during an experiment.

Site	Sample date	Temperature (°C)	Cd (μg/L)	Cu (μg/L)	Zn (μg/L)	pH	Hardness as $CaCo_3$ (mg/L)	Discharge (m^3/s)
LBR (reference)	Jul 21, 1998	13–19	<0.3	2.0	2.0	7.9–8.1	48	0.18
	Aug 2, 1999	13–18	<0.3	2.4	2.5	8.0–8.2	56	0.07
BMT	Jul 21, 1998	15–16[a]	20	113	2,160	7.4	39	0.03
	Aug 2, 1999	12–16	38	557	3,970	5.2–5.4	66	0.00067
	Aug 3, 1999	9–15	35	314	3,920	5.9		
JC	Jul 21, 1998	10–14[a]	3.4	33	377	7.7–7.8	29	0.92
	Aug 2, 1999	9–16	8.0	51	656	7.0–7.4	44	0.03
USG	Jul 21, 1998	16[a]	22	84	1,830	7.6	41	0.14
	Aug 2, 1999	14–17	75	377	5,730	7.3		
	Aug 4, 1999	12–14	59	206	4,840	7.5	63	0.02
MCC	Jul 21, 1998	14–16[a]	4.4	34	391	7.8–8.2	36	0.58
	Aug 2, 1999	11–18	9.6	48	714	7.9		
	Aug 4, 1999	11–18	9.3	48	651	7.8–7.9	48	0.13
UHO	Aug 3, 1999	12–21[a]	2.5	3.7	550	8.2	140	0.01
LHO	Jul 21, 1998	11–21	3.7	5.0	987	8.1–8.3	130	0.05
	Aug 2, 1999	12–21	2.0	3.6	459	8.1–8.3	140	0.02

[a] Range of measurements recorded manually, where $N = 2–6$.

stream, and that the biomass of one species is not independent of (and is in fact affected by) the occurrence of another salmonid species. This interdependency among trout species in stream environments was demonstrated by Shepard et al. (in press) in White's Gulch, Montana, where cutthroat trout biomass increased after brook trout were physically removed. Therefore, measurements of biomass that do not include all trout species would probably be inadequate for comparative purposes.

The species were evenly represented at LBC. The species composition in LCC and LBC seemed to generally reflect the composition of the nearest mainstem sites; BRRC had a similar composition to LBC, and BRGG was similar to LCC. Although there was a trend of less biomass at BRGG compared with the reference, BRRC, the difference was not significant. Differences among lengths at age calculated from scales of rainbow trout samples from the three tributary sites (Figure 4) were not significant, nor were differences in length at age between fish sampled from the main-stem sites (data not presented). The observations for density (number/300 m) have the same pattern as biomass results for all sites (Table 2).

Fish species observed at the five fish abundance sites between 1997 and 1999 included three native species (longnose sucker *Catostomus catostomus*, mottled sculpin *Cottus bairdi*, and mountain whitefish *Prosopium williamsoni*) and four nonnative species (Yellowstone cutthroat trout *Oncorhynchus clarki bouvieri*, rainbow trout, brook trout, and brown trout *Salmo trutta*). The main-stem sites had a greater number of species than did the tributary sites and contained all of the species listed, except that cutthroat trout were not found at either mainstem site and brown trout were not found at BRRC (reference). Most of the brown trout and mountain whitefish captured in the main stem (in 1998 and 1999) were in spawning condition and may have migrated from sites farther downstream. Mottled sculpin were found at UBR (reference) and LBC and mountain whitefish were found at LBC. Neither mottled sculpin nor mountain whitefish were found at LCC. None of the rainbow, cutthroat, or brook trout collected from the tributary sites appeared to be spawning.

Habitat Characterization of Fish-Abundance Sites

Microhabitat was quantified in terms of weighted usable area and was based on one set of measurements taken along 10 or 11 transects at each site between late September and early October. For both brook and rainbow trout, weighted usable area in the tributaries was greatest in LBC for fry and juveniles and greatest for adults in UBR. LCC had the least weighted usable area for all three life stages (Table 3). In the main stem, juvenile and adult weighted usable area was greatest for both species in BRGG, but fry weighted usable area was greatest for both species at BRRC.

Water Chemistry of Resident Fish Biomass and Density and Health

Data from water samples collected in 1996–1997 at the five biomass and fish health sites in-

TABLE 2.—Size ranges and biomass and density estimates of brook and rainbow trout in the tributaries and main stem of the Boulder River, Montana. The estimates were obtained during late July 1997 and early October 1998 and 1999. Asterisks indicate significant differences from the reference site ($P \leq 0.05$). Fish species include rainbow trout (RBT) and brook trout (EBT). Because few brook trout were located each year in lower Cataract Creek and Galena Gulch, they are not represented separately in the table. Similarly, few cutthroat trout were observed in the tributaries, and they are included in the numbers for rainbow trout. See Figure 1 for site abbreviations.

Site and year	Species	Size range (cm)	Number/300 m ± SE	Biomass ± SE (kg/ha)
		Tributaries		
UBR (reference)				
1997	RBT	8.1–25.9	22 ± 1	10.8 ± 0.7
	EBT	6.9–31.5	189 ± 52	53.0 ± 14.7
	RBT and EBT	6.9–31.5	211 ± 52	63.8 ± 14.7
1998	RBT	5.1–24.4	42 ± 3	14.9 ± 1.2
	EBT	6.4–22.6	167 ± 20	45.0 ± 5.0
	RBT and EBT	5.1–24.4	209 ± 19	59.9 ± 5.2
1999	RBT	6.4–25.9	57 ± 2	16.4 ± 0.7
	EBT	6.1–22.9	263 ± 19	61.7 ± 4.5
	RBT and EBT	6.1–25.9	320 ± 19	78.0 ± 4.5
Mean	RBT and EBT		247 ± 37	63.6 ± 2.1
LBC				
1997	RBT	4.1–33.5	75 ± 10	38.0 ± 5.0
	EBT	9.7–24.4	67 ± 20	23.4 ± 7.0
	RBT and EBT	4.1–33.5	142 ± 22	61.4 ± 8.6
1998	RBT	5.1–26.2	154 ± 4	37.6 ± 0.9
	EBT	7.1–26.7	53 ± 7	24.2 ± 3.0
	RBT and EBT	5.1–26.7	207 ± 8	61.8 ± 3.1
1999	RBT	4.3–28.2	328 ± 12	56.7 ± 2.1
	EBT	5.3–25.1	47 ± 0.3	11.1 ± 0.1
	RBT and EBT	4.3–28.2	375 ± 12	67.8 ± 2.1
Mean	RBT and EBT		241 ± 70	63.7 ± 2.1
LCC				
1997	RBT and EBT	6.9–21.1	40 ± 14	5.8 ± 2.0
1998	RBT and EBT	3.6–21.8	139 ± 12	16.1 ± 1.3
1999	RBT and EBT	8.4–21.8	92 ± 3	18.6 ± 0.6
Mean	RBT and EBT		90 ± 29*	13.5 ± 3.9*
		Boulder River		
BBRC (reference)				
1997	RBT	11.2–27.7	44 ± 13	9.7 ± 3.0
	EBT	11.9–30.0	24 ± 10	5.3 ± 2.1
	RBT and EBT	11.2–30.0	68 ± 13	15.0 ± 3.7
1998	RBT	10.7–30.0	73 ± 5	17.9 ± 1.8
	EBT	11.9 ± 29.0	72 ± 30	16.7 ± 6.8
	RBT and EBT	10.7–30.0	146 ± 30	34.6 ± 7.1
1999	RBT	9.9–30.2	135 ± 7	28.1 ± 1.9
	EBT	14.2–31.8	86 ± 14	27.4 ± 6.1
	RBT and EBT	9.0–31.8	221 ± 14	55.5 ± 6.1
Mean	RBT and EBT		146 ± 45	35.0 ± 11.7
BRGG				
1997	RBT and EBT	10.4–31.0	79 ± 19	10.9 ± 2.6
1998	RBT and EBT	10.2–32.0	179 ± 12	21.5 ± 1.5
1999	RBT and EBT	10.9–35.1	236 ± 25	32.4 ± 3.4
Mean	RBT and EBT		165 ± 44	21.6 ± 6.2

dicated that dissolved and total recoverable concentrations of aluminum (Al), antimony (Sb), As, barium (Ba), beryllium (Be), chromium (Cr), cobalt (Co), iron (Fe), manganese (Mn), mercury (Hg), molybdenum (Mo), nickel (Ni), silver (Ag), and uranium (U) were either less than method detection levels or less than concentrations established by the U.S. Environmental Protection Agency to protect aquatic life (EPA 1987). In contrast, concentrations of Cd, Cu, Pb, and Zn frequently were elevated at sites downstream of mining activity (LBC, LCC, and BRGG).

Trace element concentrations generally were low (Table 4) during all flow conditions in sites upstream of historical mining activities (i.e., UBR and BRRC). In BRRC, concentrations of Cu during

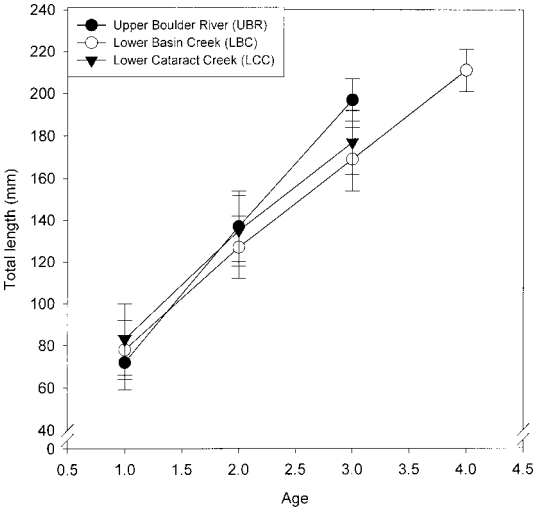

FIGURE 4.—Comparisons of trout growth at three sites in tributaries of the Boulder River watershed.

high-flow periods exceeded the acute and chronic water quality criteria in 2 of 4 samples measured for dissolved Cu and 6 of the 10 samples measured for total recoverable Cu. Some total recoverable Pb concentrations also exceeded the chronic but not acute water quality criteria at BRRC. Trace element concentrations were higher at the other three sites. The highest concentrations of metals occurred at LCC, where dissolved Cd, Cu, and Zn concentrations exceeded acute standards in every sample. Dissolved Zn concentrations also exceeded acute standards in all samples from LBC and BRGG. Water at these two sites frequently exceeded Cd and Cu standards during high flows, and BRGG exceeded the standards during low-flow conditions as well.

The concentrations of dissolved Cd and Zn at LCC were similar to concentrations associated with mortality in the 96-h survival experiments. Median concentrations (Table 4) at this site were higher than at MCC and JC during the 1998 experiments when 100% mortality was observed at 96 h. Dissolved metal concentrations at the other four fish-assessment sites during low-flow conditions were considerably less than the concentrations associated with mortality in the survival experiments.

Tissue Metals

The concentrations of most metals measured in the livers of resident rainbow trout were greatest in fish from LCC (dry weights: >1,000 μg Cu/g, 60 μg Cd/g, and 13 μg As/g; Figure 5). Concentrations of metals in the livers of fish from LBC and BRGG were also elevated above those measured at the reference sites (UBR and BRRC). In general, the pattern of metal accumulation in livers was greatest at LCC, followed by LBC, BRGG, BRRC, and UBR, an exception to this pattern being that the greatest concentration of Pb was observed in fish from BRGG.

TABLE 3.—Weighted usable area (m^2; WUA) per 1,000 m^2 of stream (Boulder River watershed, Montana) for brook and rainbow trout fry (<5 cm), juveniles (5–20 cm), and adults (>20 cm). The WUA is based on the product of suitability index values for depth, velocity, and substrate. Site abbreviations are provided in Figure 1.

	Brook trout			Rainbow trout		
Site	Fry	Juvenile	Adult	Fry	Juvenile	Adult
UBR	49.3	59.8	293	222	103	39.4
LBC	55.7	60.5	277	254	168	33.8
LCC	48.9	59.8	212	192	92.2	22.7
BRRC	44.6	27.2	156	133	172	57.3
BRGG	24.1	27.4	229	195	251	64.9

The pattern of metal concentrations observed in livers was similar in gills (Figure 6). The greatest concentrations of Cu and Cd were observed in the gills of fish from LCC (20 μg Cu/g and >60 μg Cd/g). Although lesser amounts of Cu and greater amounts of Zn were observed in gill than in liver tissue, the amounts of Cd in livers and gills of fish from LCC were nearly identical. Concentrations of Zn in the gills (>600 μg/g) of rainbow trout from the reference site (UBR), however, were greater than we have measured in other reference streams in the intermountain west (Farag et al. 1998). As was noted for liver, the greatest concentrations of Pb were observed in gills of BRGG fish. However, unlike liver, there was no significant accumulation of As in the gills.

The pattern of metal accumulation described for livers was similar in whole fish. Again, the greatest concentrations of most metals in whole fish were observed in samples collected from LCC (>20 μg Cu/g, 5 μg Cd/g, and 8 μg As/g were measured; Figure 7). Although less Cd was observed in the whole body versus liver and gill, a mean of 5 μg Cd/g was measured in the whole fish from LCC. There was no significant accumulation of Pb in whole fish.

Metallothionein

Concentrations of metallothionein were greatest in the livers of rainbow trout collected from LCC (921 μg/g). The mean concentration of metallothionein in livers of fish from LCC was signifi-

TABLE 4.—Physical and chemical data for sites (see Figure 1 for abbreviations) where fish health was assessed in the Boulder River watershed of Montana. Data were collected between October 1996 and September 1999. Median total recoverable (TR) and dissolved (disv.) trace element concentrations are listed; *N* represents the number of samples collected on different dates throughout the flow condition.

Site	*N*	Discharge (m^3/s)	pH	Hardness (mg/L as $CaCO_3$)	Median trace element concentration (μg/L)									
					As		Cd		Cu		Pb		Zn	
					TR	Disv.	TR	Disv.	TR	Disv.	TR	Disv.	TR	Disv.
High flow conditions														
UBR	2	0.4–2.3	7.6–8.1	21–38	4.0	2.5	<1	<0.3	3	1.5	<1	<1	<10	1
BRRC	10	3.0–20.5	7.6–8.2	22–50	5.5	3.3	<1	<0.3	5	3.3	1.1	<1	11	3
LBC	10	0.4–7.1	7.0–8.0	12–35	11	4.0	<1	0.4	12	8.7	2.6	<1	81	61
LCC	11	0.4–4.8	6.8–8.0	18–54	17	3.4	1.8	1.3	46	33	6.7	<1	177	139
BRGG	11	5.0–29.7	7.5–8.2	19–48	12	4.0	<1	0.3	20	11	5.9	<1	73	46
Low flow conditions														
UBR	2	0.1–0.1	8.0	52–53	2.5	2.0	<1	<0.3	1.5	<1	<1	<1	<10	<1
BRRC	8	0.2–0.7	7.4–8.3	54–61	3.0	2.4	<1	<0.3	1.4	1.7	<1	<1	<10	2.3
LBC	7	0.1–0.2	7.5–8.2	39–42	7.0	4.0	<1	0.4	4.0	3.0	<1	<1	66	63
LCC	7	0.1–0.2	7.5–8.3	59–69	4.8	3.0	5.0	4.9	24	22	<1	<1	419	397
BRGG	8	0.5–1.0	7.7–8.5	61–68	6.0	5.0	<1	0.8	10	9.0	<1	<1	180	160

cantly greater than livers of fish from the reference site, UBR (Table 5). Although there was a trend of greater metallothionein in livers of fish from LBC, this finding was not significant. The gills generally had small concentrations of metallothionein, and concentrations in gills measured at LBC were less than those collected from LCC and BRGG. There were no significant differences between concentrations of metallothionein measured in livers or gills of fish collected from the mainstem sites, BRRC and BRRG.

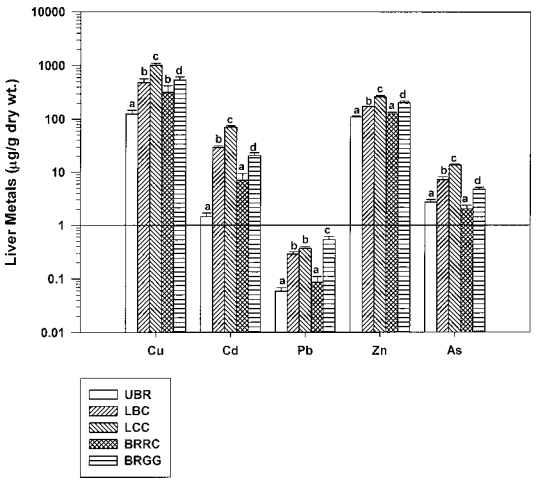

FIGURE 5.—Metal concentrations in the livers of resident rainbow trout collected from sites in the Boulder River watershed. Whiskers are SEs; columns with different lowercase letters are significantly ($P \leq 0.05$) different.

Lipid Peroxidation

We found a significant amount of products of lipid peroxidation (at 340 nm excitation) in the livers of rainbow trout from LCC relative to UBR fish(Table 6). The mean amounts of products of lipid peroxidation in livers of fish from the two mainstem sites, BRRC and BRGG, did not differ significantly, nor did differences in lipid peroxidation in the gills of fish among tributary or mainstem sites. Products of lipid peroxidation measured at 360 nm excitation showed an increasing trend, but the difference was not significant (data not presented).

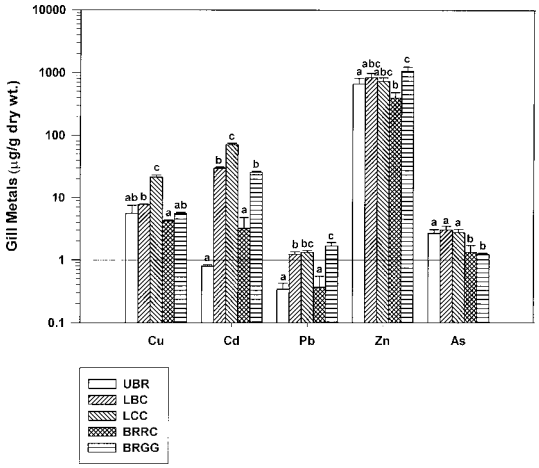

FIGURE 6.—Metal concentrations in the gills of resident rainbow trout collected from sites in the Boulder River watershed. See Figure 5 for additional details.

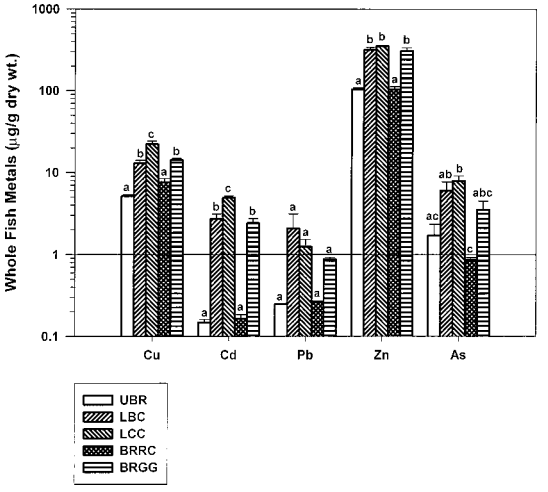

FIGURE 7.—Metal concentrations in whole resident rainbow trout collected from sites in the Boulder River watershed. See Figure 5 for additional details.

Discussion

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

The association between fish mortality and the elevated concentrations of metals in the water provides evidence that metals caused the observed mortalities. Although the water quality criteria are hardness dependent, we can calculate standards for the average hardness measured at various sites in this watershed based on water quality criteria established by the U.S. Environmental Protection Agency for the chronic and acute protection of aquatic life (USEPA 1987). These acute criteria are 12 μg Cu/L and 82 μg Zn/L in Basin and Cataract creeks at a hardness of 66 mg/L. Hardness was greater at lower High Ore Creek (140 mg/L) and this may explain why survival was slightly better in this creek.

To further investigate the cause–effect relationship of metals and fish mortality, we studied the ionoregulatory status and histopathology of fish near death. Metals in water have often been associated with ionoregualtory upset (Laurén and McDonald 1985). A disturbance of the ionoregulatory status in fish may result as metals compete for calicium-binding sites at the gill or inhibit Na^+, K^+-ATPase (Evans 1987; Reid and McDonald 1988). Previously, we demonstrated reductions in whole-body potassium in juvenile fish exposed to Cd, Cu, and Pb in the water and diet (Farag et al. 1994). However, whole-body concentrations of metals were not affected at the end of the on-site survival experiments in the Boulder River watershed during 1998. This lack of measured response may be due to the size of fish studied. Although fish examined during 1998 were juveniles, they were several times the size of the 1–2.5-g fish studied by Reid and McDonald (1988) and Farag et al. (1994). The greater size of fish used in this study may have limited the usefulness of whole-body ion measurements to define ionoregulatory upset.

The lack of a measurable response with whole-body ion measurements in 1998 led us to collect histological samples in 1999 to better characterize the cause of death. The hypertrophy noted in gills of fish in this study are consistent with edema noted previously in the secondary lamellae of rainbow trout exposed to 40 mg Zn/L for 3 h (Skidmore and Tovell 1972). Skidmore and Tovell (1972) also observed severe curling of the secondary lamellae,

TABLE 5.—Mean ± SE metallothionein concentrations in gill and liver samples of resident rainbow trout collected from selected sites (see Figure 1 for site abbreviations) in the Boulder River watershed of Montana. Within columns, means with different letters are significantly different ($P \leq 0.05$); sample size was five for all sites except Galena Gulch, where it was six.

Site	Metallothionein (μg/g wet weight)	
	Gill	Liver
Tributaries		
UBR (reference)	9.37 ± 0.63 zy	31.23 ± 7.70 z
LBC	7.61 ± 1.0 z	596.81 ± 288.89 zy
LCC	12.00 ± 1.18 y	921.05 ± 215.58 y
Boulder River		
BRRC (reference)	12.42 ± 0.52 y	271.39 ± 194.67 zy
BRGG	11.07 ± 1.15 zy	292.51 ± 60.47 zy

TABLE 6.—Mean ± SE lipid peroxidation of tissues sampled from resident rainbow trout collected from selectd sites (see Figure 1 for site abbreviations) in the Boulder River watershed of Montana. 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 tissue and 136 with settings to measure liver tissue. Within columns, means with different letters are significantly different ($P \leq 0.05$); sample size was five for all sites except Galena Gulch, where it was six.

Site	Relative intensity	
	Gill	Liver
Tributaries		
UBR (reference)	73.14 ± 8.19 z	113.98 ± 5.31 z
LBC	66.38 ± 6.91 z	115.34 ± 11.74 z
LCC	73.78 ± 3.77 z	141.00 ± 7.28 y
Boulder River		
BRRC (reference)	79.17 ± 6.57 z	125.11 ± 11.87 zy
BRGG	73.08 ± 4.09 z	127.15 ± 4.63 zy

a finding not unlike the twisting that was noted in the gills of fish collected from middle Cataract Creek, where concentrations of Zn ranged from 400 to 700 μg/L. Cutthroat trout near death experienced excess mucous production in this study. Handy and Eddy (1991) suggested that excess mucous production is part of a general stress response in rainbow trout. Therefore, it is likely that ionoregulatory upset (though we could not measure this upset directly) caused hypertrophy (swelling), degeneration (dying), and necrosis (death) of epithelial cells in the gills. Also, mucous production occurred simultaneously as a general response to stress.

Biomass, Density, and Health

The concentrations of Cd, Cu, and Zn in water at LCC are near the concentrations reported at MCC, where mortality was observed during in situ experiments. Therefore, resident fish at LCC may have acclimated to the elevated concentrations of metals. Simultaneously, a metabolic cost of acclimation may be expressed at LCC because acclimation coincides with a decreased mass (kg) of trout in LCC, though not decreased growth.

Metallothioneins are proteins that bind metals and may play a role in the acclimation of fish to metals (Stegeman et al. 1992). Marr et al. (1995) demonstrated that a physiological cost of acclimation exists for brown trout in the Clark Fork River, Montana, where trout acclimated to metals in the river had elevated concentrations of metallothionein in their livers and grew less than trout not acclimated to the metals. Furthermore, Dixon and Sprague (1981) concluded that decreased growth resulted from the metabolic costs associated with acclimation to Cu in the laboratory. Rainbow trout from LCC had elevated metallothionein in their livers. Similar to Marr et al. (1995), we found metallothionein to be greatest in the livers of fish that also had the greatest concentrations of metals in the liver.

The greater amounts of products of lipid peroxidation in the livers of LCC fish, further indicates their compromised health. 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 LCC are compromised, and this state is associated with elevated concentrations of metals in the liver (As, Cu, Cd, Pb, and Zn) that exceed the concentrations at the reference site (UBR). In fact, the copper concentraion (>1,000 μg/g) at LCC is much greater than the upper limit of an effect concentration of 480 μg Cu/g suggested by Farag et al. (1995) for the Clark Fork River, Montana.

The weighted usable area suggests that habitat differences explain some of the difference in the biomass and density between LCC and the reference site (UBR). For all three life stages, LCC had an average of 91% and 78% of the weighted usable area that the reference site had for brook and rainbow trout, respectively. However, the extent of the reduced biomass and density at LCC cannot be explained completely by habitat differences. The number of trout per 300 m of stream bed in LCC was only 36% of the number calculated for UBR, and on an area basis, the reduction in biomass in LCC was even greater, being 20% of the amount in UBR. This biomass difference between LCC and the UBR was much greater than the difference in weighted usable area. Thus, it is unlikely that habitat differences are sufficient to explain the reduced biomass at LCC.

We observed decreases in the number per 300 m and in kilograms per hectare of trout at LCC but did not observe differences among sites in the lengths –at age for rainbow trout. However, the lower density of trout at LCC may have masked the growth-suppressing effects of metals. Jenkins et al. (1999) found that growth of individual brown trout increased with 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).

These observations of compromised aquatic health coincide with elevated concentrations of metals in the water column of LCC (median dissolved concentrations under low-flow conditions: 4.9 μg Cd/L, 22 μg Cu/L, and 397 μg Zn/L) compared with the UBR reference site (<0.3 μg Cd/L, <1 μg Cu/L, and <1 μg Zn/L). Concentrations of metals in sediment, biofilm, and invertebrates were also elevated (Fey et al. 1999; Farag et al. unpublished data) at this site, which suggests that fish are accumulating metals through both water and dietary pathways.

Mottled sculpin were not found at LCC, where the concentrations of Zn in the water were 14 times greater than the concentrations found to cause 32% mortality in wild mottled sculpin (Woodling et al. 2002). Woodling et al. (2002) attributed the lack of sculpin at sites on the Eagle River, Colorado, to elevated Zn in the water column. They noted that sculpin were not present in the Eagle River, Colorado, when concentrations of Zn ranged from 315 to 711 μg/L, but they observed some sculpin at a site further downstream with less Zn (<166 μg/L). Our data support the findings of Woodling et al. (2002) because sculpin were not found at LCC but were observed at LBC (63 μg Zn/L) and the URB reference site (<1 μg Zn/L).

The water quality in BMT, JC, MCC, USG, and to a lesser degree LHO and UHO may render the survival of trout in these reaches unlikely and these areas may act as barriers to limit the colonization of upper reaches of the Boulder River watershed. Additionally, because biomass and density are decreased, and metallothionein, products of lipid peroxidation, and tissue metals are simultaneously increased in resident fish at LCC, we conclude that the aquatic health of LCC is compromised. Furthermore, the association of elevated metal concentrations in tissue, water, and sediment lead us to conclude that metals are the cause of the compromised aquatic health in LCC. Therefore, resident fish populations would benefit greatly if cleanup efforts were directed to minimize the concentrations of metals in the water, sediment, and biota of LCC downstream from USG.

There are also elevated metal concentrations (As, Cd, Cu, Pb, and Zn) in some tissues of trout from LBC and BRGG. Lowe et al. (1985) and Schmitt and Brumbaugh (1990) reported the 85th percentiles (values for which 85% of samples are below) were 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 for whole fish collected from over 100 stations across the United States (80% moisture was assumed to calculate μg/g dry weight). The mean concentrations of metals in whole fish collected from LCC, LBC, and BRGG exceeded these 85th percentiles (e.g., Cd was >121 times the 85th percentile at LCC, >60 times at LBC, and >60 times at BRGG and As in whole fish was 8 times at LCC, 6 times at LBC, and 3 times at BRGG).

Although the concentrations of metals in fish from LBC and BRGG are greater than fish sampled from across the country, the concentrations were not as great as those at LCC, nor did we observe significant changes in kilograms of trout per hectare, metallothionein, or lipid peroxidation at LBC or BRGG. Therefore, the impacts of metals at these two sites are less than at LCC. This study provides a baseline of data for monitoring efforts in the future. As remediation proceeds in Basin and High Ore creeks, monitoring efforts that include the LBC and BRGG sites could document improved conditions or changes due to inadvertent releases of metals downstream when the tailings are disturbed.

Fey et al. (1999; Farag, unpublished data) found that As is elevated in the sediment, invertebrates, and fish of BRGG. The target organ of As toxicity is the skin (Goyer 1986), so the dark coloration and increased accumulation of melanocytes observed in the skin of fish held at LHO (located upstream of BRGG) during the on-site 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 are necessary to observe more subtle changes, such as increased numbers of melanocytes. Additionally, color changes during necropsies may not always be observed because there is an immediate loss of melanocyte regulation and control at death. For these reasons, we suggest that histological analyses be added to future assessments of aquatic

health in an effort to more completely document any tissue pathology.

In this study, we used fish biomass and density estimates along with health assessments of individual fish to define the overall aquatic health of the Boulder River watershed. Combining toxicity studies, fish biomass and density estimates, and fish health assessments within a systematic design is an important accomplishment of this study. This study defines physiological changes that are linked to a biomass and density effect of elevated metals in the watershed. Although this concept is logical, it is unfortunately novel in application. We have performed health assessments of resident fish in three watersheds in the western United States: the Boulder River watershed (this study), the Coeur d'Alene River watershed (Woodward et al. 1999), and the Clark Fork River watershed (Farag et al. 1995). In each of these three watersheds, metallothionein measurements and tissue metal accumulations are related to decreased biomass and density estimates (Coeur d'Alene watershed and Boulder River watershed) or effects on growth measured as length at age (Clark Fork watershed). Lipid peroxidation is related to decreased biomass and density estimates or decreased growth in two of the three watersheds (lipid peroxidation was not measured by Woodward et al. 1999).

In summary, we studied population-level effects (survival) and biomass and density and individual-level effects to assess aquatic health in the Boulder River watershed, Montana. This study provides evidence that elevated concentrations of Cd, Cu, and Zn cause mortality in tributaries of Basin and Cataract creeks and in High Ore creek. Furthermore, the health of acclimated fish at these concentrations is impaired, as we observed at LCC. The aquatic health of trout at lower Cataract Creek is compromised, as indicated by the association of increased metallothionein, lipid peroxidation, and metal concentrations in tissues and a reduced number of trout per 300 m of streambed.

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