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Relative sensitivity of brown and rainbow trout to pulsed exposures of an acutely lethal mixture of metals typical of the Clark Fork River, Montana[1]

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Abstract: Brown trout (*Salmo trutta*) and rainbow trout (*Oncorhynchus mykiss*) fry and juveniles were episodically or continuously exposed to a metals mixture (Zn, Cu, Pb, Cd): the concentrations and ratios of the metals, and variations in water quality (pH, hardness), were selected to represent conditions measured during episodic storm events in the Clark Fork River, Montana. Brown trout fry were more sensitive (lower LC_{50}) than rainbow trout fry to the metals in 8-h exposures with constant hardness and pH, but less sensitive to elevated metal concentrations in conjunction with depressed hardness and pH. Fry were more sensitive than juveniles when exposure was continuous, but neither life stage was clearly more sensitive when exposure was pulsed. Whole-body concentrations of K^+ and Ca^{2+} but not Na^+ were significantly depressed in fry exposed to metals. Results support the hypotheses that changes in water quality during thunderstorms are lethal to fry and juvenile life stages of brown and rainbow trouts and that the relative sensitivity of the species to the metals mixture may explain their distributions in the Clark Fork River. Low-frequency extreme conditions may effectively act as a bottleneck on the viability of populations whose relative sensitivities to such extremes may control distributions of species in a system.

Résumé : Des alevins et des juvéniles de truite brune (*Salmo trutta*) et de truite arc-en-ciel (*Oncorhynchus mykiss*) ont été exposés de façon épisodique ou continue à un mélange de métaux (Zn, Cu, Pb, Cd) : les concentrations et les proportions relatives des métaux, ainsi que les variations de la qualité de l'eau (pH, dureté), ont été sélectionnées de façon à reproduire les conditions mesurées pendant des épisodes orageux dans la rivière Clark Fork, au Montana. Les alevins de truite brune étaient plus sensibles (CL_{50} plus faible) aux métaux que les alevins de truite arc-en-ciel pendant des expositions de 8 h, la dureté et le pH étant constants, mais moins sensibles à des concentrations élevées de métaux coïncidant avec une baisse de la dureté et du pH. Les alevins étaient plus sensibles que les juvéniles quand l'exposition était continue, mais ni aucun de ces stades n'était nettement plus sensible que l'autre quand l'exposition était épisodique. Les concentrations corporelles de K^+ et de Ca^{2+}, mais non de Na^+, étaient nettement abaissées chez les alevins exposés aux métaux. Les résultats confirment l'hypothèse selon laquelle la qualité de l'eau pendant les orages est mortelle pour les stades de l'alevin et du juvénile chez les truites brune et arc-en-ciel, et la sensibilité relative des espèces au mélange de métaux peut expliquer leur répartition dans la rivière Clark Fork. Les conditions extrêmes, de faible fréquence, peuvent jouer efficacement un rôle de goulot d'étranglement sur la viabilité des populations dont les sensibilités relatives à de tels extrêmes peut ainsi régir la répartition des espèces dans un système.
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(Sprague 1969). For the continuous-exposure experiment involving juveniles and fry, water was delivered to 340-L tanks (total volume) at 1.5 L/min to provide an average flow rate of 90 L/h to each tank and a 90% volume replacement time of 8 h (Sprague 1969). For experiments using both life stages combined in the 340-L tanks, fry were placed in suspended enclosures (20.3 × 9.2 × 9.2 cm) made of nitex polypropylene mesh (4 mm diameter mesh) to prevent interactions with juveniles.

Stock solutions of combined metals were prepared from reagent-grade chloride salts ($ZnCl_2$, $CuCl_2$, $PbCl_2$, $CdCl_2$) dissolved and continuously mixed in deionized water. Exposure concentrations of the metals were achieved by metering the stock solution via a Mariotte bottle into continuous-flow diluters. Exposure and control waters were delivered by proportional diluters that provided replicates for each exposure dilution and control.

Exposure waters were identical to the control water, but contained one of four or five geometric dilutions of an 8P or 5P reference mixture during the exposures (P units represent the notation for observed metals concentrations during a documented fish kill in the Clark Fork River), where nominal concentrations for the 1P mixture were 230 μg/L Zn, 120 μg/L Cu, 3.2 μg/L Pb, and 2.0 μg/L Cd. The pulsed exposures used four dilutions (8P, 4P, 2P, and 1P) and the continuous exposure used five dilutions (5P, 2.5P, 1.2P, 0.6P, and 0.3P). The metals concentrations (in μg/L) and ratios of Zn, Cu, and Cd in the 1P reference mixture represent dissolved concentrations measured in the Clark Fork River at Deer Lodge, Montana, during a fish kill on July 12, 1989 (Lambing 1991); because measured concentrations of dissolved Pb at the Deer Lodge site were below detection limits on that date, the Pb concentration for the 1P reference mixture was set at 3.2 μg/L, which is the U.S. Environmental Protection Agency's chronic water quality criterion at a water hardness of 100 mg/L $CaCO_3$ (U.S. Environmental Protection Agency 1987).

Experimental procedures

Fish were acclimated for a minimum of 1 month to the control water used during a given experiment (e.g., hardness, 100 mg/L as $CaCO_3$; alkalinity, 80–110 mg/L as $CaCO_3$; pH, 7.2–8.0; temperature, 10°C). Positions were randomly assigned for the species or life stage to the exposure and control chambers. Individuals of each species and life stage were sequentially transferred in groups of two from holding chambers to exposure or control chambers, thus randomizing the allocation of individuals to the chambers. Ten or 15 individuals were used per exposure dilution and control within each replicate. The initial loading of fish in exposure or control chambers was less than 5.5 g/L.

Mortality, defined as cessation of opercular movement, was monitored every 20–40 min during the pulsed exposure tests and twice daily for 96 h after the pulse; during continuous exposure tests, mortality was monitored every 2 h for the first 12 h and every 6 h subsequently. Exposure and control waters were sampled for metals, temperature, dissolved oxygen, pH, alkalinity, and hardness every 20–40 min before and during pulsed exposures and twice each day after the pulse, or once each day during continuous exposures. Water samples (25 mL) for metals analysis were taken directly from each exposure or control chamber and immediately acidified with 250 μL of 70% HNO_3 to preserve the sample until atomic absorption spectroscopy (AAS) analysis was performed. Water samples (50 mL) for pH, alkalinity, and hardness were also taken directly from the exposure or control chamber and analyzed immediately.

For fry receiving pulsed exposure (tests I–V), metals were increased linearly over a 1-h period from control to maximum pulse concentrations, held constant for 6 h, and decreased linearly over a 1-h period to control concentrations (1,6,1-h pulse); the water chemistry was then held constant at control concentrations for the remainder of the experiment for monitoring postpulse mortality. For juveniles and fry receiving pulsed exposure (tests VI and VII), metals were increased linearly over a 2-h period from control to maximum pulse concentrations, held constant for 4 h, and decreased linearly over a 2-h period to control concentrations (2,4,2-h pulse); again, the water chemistry was then held constant at control concentrations for postpulse monitoring. Additionally, during each pulse, the hardness, alkalinity, and pH either remained constant, were depressed, or were increased; changes were linear and occurred over the same time as did changes in metals concentrations. In the text that follows, we use the following abbreviations for exposure conditions according to directions of change and parameters changed: constant hardness and constant pH (CHCp), depressed hardness and constant pH (DHCp), depressed hardness plus depressed pH (DHDp), depressed high hardness plus depressed pH (DHHDp), and elevated hardness and depressed pH (EHDp). Water chemistry was held constant during the continuous exposure. The experimental design for this sequence of tests is shown in Table 1, which includes nominal hardness, alkalinity, and pH during baseline (pre- and post-pulse) and during pulse conditions.

Fish sampled for whole-body Na^+, K^+, and Ca^{2+} concentrations were frozen in acid-cleaned vials until they were processed for analysis. Individuals were collected at the time of death or at the end of the experiment.

Analysis of water and tissue chemistry

Concentrations of Zn, Cu, Pb, and Cd dissolved in water were determined by AAS using a graphite furnace or flame (Perkin-Elmer models 2380 and 372). Whole-body ion concentrations (Na^+, K^+, and Ca^{2+}) in fish were determined on a wet-weight basis (Shearer 1984) with AAS using techniques described by Woodward et al. (1989). Blank, spike, standard, and replicate analyses of the same samples used to evaluate quality control were also used to verify instrument calibration and accuracy. Blanks always had element concentrations below instrument detection limits. Spikes introduced at the beginning of sample preparation and spikes added to digestates at the instrument showed an average recovery of >90%, standards generally within 10% of the theoretical values, and values for the second replicate analysis generally within 10% of the first value. Analyses were rejected and samples rerun if values for standards were not within 20% of the theoretical values.

Table 2. Measured metals concentrations in exposure dilution (P units) and control (C) waters during the pulsed and continuous exposures.

Test	Dilution (P units)	Maximum concentration (μg/L)			
		Zn[a]	Cu[b]	Pb[b]	Cd[b]
I	8	2537.6±48.7	1245.0±7.1	58.12±0.53	11.25±0.35
	4	1294.9±68.6	601.0[c]	23.30[c]	5.80[c]
	2	628.1±35.0	285.2±4.0	11.05±0.14	2.63±0.25
	1	321.6±21.8	141.0±2.4	5.30±0.21	1.28±0.04
	C	22.2±15.8	nd[c]	nd[c]	nd[c]
II	8	2294.5±149.1	1323.0±72.1	58.88±0.53	11.63±0.53
	4	1216.3±39.4	680.5±23.3	23.85±0.92	6.80±0.07
	2	577.0±24.2	314.7±14.1	10.60±0.78	3.48±0.04
	1	283.2±17.8	149.2±17.2	4.50±0.42	1.78±0.00
	C	23.6±15.0	nd[c]	nd[c]	nd[c]
III	8	2290.8±178.3	1478.8±158.4	60.63±10.08	12.00[c]
	4	1151.0±89.8	674.8±19.6	22.70±0.78	6.13±0.04
	2	566.0±20.6	404.0±22.6	12.68±0.60	3.75±0.14
	1	270.7±20.6	186.0[c]	7.00±0.42	1.90±0.55
	C	14.3±13.3	nd[c]	nd[c]	nd[c]
IV	8	2170.3±179.4	1286.6±132.1	55.50±3.54	10.75±0.35
	4	1186.3±120.2	708.2±6.4	25.05±2.62	6.35±0.07
	2	569.4±8.8	360.8±16.3	14.50±2.19	3.77±0.25
	1	300.4±10.5	200.7[c]	7.00±0.28	1.68±0.39
	C	8.6±7.5	nd[c]	nd[c]	nd[c]
V	8	2042.3±251.1	1473.2±9.2	63.62±2.30	12.63±0.18
	4	1040.4±50.2	789.0±142.2	25.38±3.50	6.73±0.18
	2	545.7±8.2	399.7±31.5	15.00±0.64	3.80±0.07
	1	302.6±27.4	213.0±8.6	8.30±0.00	2.18±0.04
	C	nd[c]	7.2±5.5	nd[c]	nd[c]
VI	8	1814.0±212.7	1146.7±23.8	31.50±0.71	8.80±0.14
	4	947.4±31.8	557.6±18.2	13.48±0.18	3.83±0.04
	2	468.1±42.9	291.3±8.1	7.25±0.64	2.05±0.07
	1	251.1±47.3	190.5±39.6	3.53±0.04	1.10±0.00
	C	12.7±20.6	6.0±0.3	nd[c]	nd[c]
VII	8	1898.8±75.3	1146.9±40.1	31.75±5.66	8.23±0.67
	4	958.3±21.8	578.2±16.3	14.30±0.07	4.08±0.11
	2	466.4±47.7	286.6±3.3	7.00±0.00	1.97±0.18
	1	231.2±5.0	145.7±0.1	2.98±0.32	0.95±0.00
	C	nd[c]	11.2±0.0	nd[c]	nd[c]
Continuous[d]	5	1187.7±78.4	753.0±3.6	16.68±0.35	13.05±0.35
	2.5	590.8±45.4	369.2±11.3	8.18±0.11	5.60±0.49
	1.2	297.7±19.6	180.4±3.9	4.05[c]	3.06±0.06
	0.6	146.5±9.9	92.0±3.2	nd[c]	1.57±0.04
	0.3	68.6±8.6	44.6±0.0	nd[c]	0.70±0.00
	C	nd[c]	nd[c]	nd[c]	nd[c]

Note: Values are given as the average ± SD, as determined by atomic absorption spectroscopy.
nd, values below the method detection limit of 4.9 μg/L Zn, 4.6 μg/L Cu, 1.7 μg/L Pb, and 0.4 μg/L cd.
[a] $7 \leq n \leq 16$.
[b] $n = 2$.
[c] $n = 1$.
[d] Average during continuous test.

or Ca^{2+}. Tukey's multiple means comparison test was used to detect differences between the mean ionic concentrations in fish that died during exposure and the mean concentrations in both fish that survived exposure and control fish. A type-I error of 0.05 (p) was used to judge significance in statistical tests.

Results

Water chemistry

Measured hardness, alkalinity, and pH did not deviate from the nominal values by more than 26% in all tests for all characteristics (Table 1). Dissolved oxygen was greater

Table 4. Mean whole-body Na^+, K^+, and Ca^{2+} concentrations (μequiv./g, wet weight) for brown and rainbow trout fry determined from pulsed exposure (test I) using dilutions of the metals mixture of Zn, Cu, Pb, and Cd. Control and exposure survivor groups were sampled at the end of the 8-h pulse + 96-h post-pulse observation and exposure mortality groups were sampled at the time of death.

	Whole-body ion concentration (μequiv./g)					
	Na^+		K^+		Ca^{2+}	
Species and group	Mean ± SE	*n*	Mean ± SE	*n*	Mean ± SE	*n*
Brown trout						
Control	108.3±10.9	38	159.9±8.8	36	570.8±51.3	36
Survivor	103.6±7.5	88	165.1±7.2	87	554.8±23.2	87
Mortality	85.5±4.3	112	115.9±3.5[a,b]	110	385.1±10.4[a,b]	110
Rainbow trout						
Control	53.8±10.2	38	141.2±15.3	38	484.9±35.0	38
Survivor	88.1±10.2	41	127.0±7.5	49	443.1±35.1	49
Mortality	55.5±3.7	42	96.1±5.1[a,b]	41	293.0±13.1[a,b]	41

[a]Significantly different ($p < 0.005$) from control.
[b]Significantly different ($p \leq 0.01$) from survivor.

rainbow trout appeared to be more sensitive to pulsed exposures than either life stage of the brown trout.

Juvenile and fry continuous exposure: survival response

Survival was 100% for all control fish during the continuous exposure. In the highest exposure concentration, mortalities occurred as early as 8 h and survival was at or near 0% at 12 h for all species and life stages. At 96 h, LC_{50} values for both fry species were significantly lower than those for juveniles (Table 3). Whereas the values of LC_{50} and LOEC for brown and rainbow trout fry were nearly identical, the LC_{50} values for juveniles were not significantly different from one another but brown trout juveniles had notably lower LC_{50} and LOEC values than rainbow trout (Table 3).

Finally, LC_{50} and LOEC values from the continuous exposures were lower than for the pulsed exposures for both species and life stages, reflecting the larger time-integrated exposure of metals to fish.

Whole-body ions

Data for whole-body ion concentrations are restricted to the individual fish from the pulsed exposure of test I. Brown and rainbow trout fry that died during the pulsed exposures had significantly decreased concentrations of whole-body K^+ and Ca^{2+}, but not Na^+ (Table 4). Relative to controls, both brown and rainbow trout fry that died exhibited mean losses of K^+ and Ca^{2+} of ≥27 and ≥33%, respectively. When data from fish that survived exposure to metals were compared with data from control fish, no significant differences were found in whole-body concentrations for any of the measured ions.

Discussion

Episodic fish kills have been observed in the Clark Fork River coincident with spring and summer thunderstorms that release elevated concentrations of Zn, Cu, Pb, and Cd from contaminated floodplain, bank, and, potentially, bed sediments (Nimick and Moore 1991; Lipton et al. 1995). The set of experiments described in this study demonstrates that pulsed exposure to elevated Zn, Cu, Pb, and Cd concentrations, within the range of concentrations and ratios observed during fish kills in the Clark Fork River, adversely and significantly affected survival in both fry and juveniles of brown and rainbow trout. For experiments during which maximum concentrations of metals were present for 6 h or less (8-h exposures), metals concentrations as low as 1P for rainbow trout and 2P for brown trout adversely affected fry survival (see Table 2 for measured metals concentrations). Thus, results from both this laboratory simulation study and from the field support the conclusion that short-term pulsed exposures to metals cause mortality in brown and rainbow trout in the Clark Fork River, Montana.

Additional data emphasize the toxicity of the metals mixture and support the conclusion that episodic events in the Clark Fork produce toxic conditions; significant mortality occurred during or after pulsed exposures in all species and life stages tested. For example, although rainbow trout fry survived the 8-h pulse, none survived the 96-h postpulse observation in the 2P exposure concentration (test II). Pascoe and Shazili (1986) made similar observations; whereas there was no mortality in rainbow trout fry during brief pulsed exposures to Cd (1.0 mg/L for 32 min), mortality after transfer to control water was 50% by 8 d.

As described in Lipton et al. (1995), rainbow trout are uncommon in the more contaminated, upstream reaches of the Clark Fork River. This study and additional studies (Marr et al. 1995; Woodward et al. 1995) examined whether this observed species distribution is consistent with the relative sensitivities of the two trout species to metals. To evaluate the relative sensitivity of the trout species to a metals mixture typical of Clark Fork River

found to be reduced by more than one pH unit, whereas at ambient pH in the range of 4–5, the gill surface pH was increased slightly by 0.5 of a pH unit (Playle and Wood 1989). Further, over the pH range 5.5–8 of ambient water, net H^+ excretion increased linearly at the gill surface in response to increased ambient pH (Lin and Randall 1993). It is possible that, in our study, the observed increase in metals toxicity for brown trout as ambient pH increased may be a function of both H^+ competition and decreased pH at the gill surface.

Alkalinity moderates copper toxicity externally; it influences copper speciation by controlling the equilibrium of toxic forms, Cu^{2+}, $CuOH^+$, and $CuOH_2^0$, and the less toxic carbonate forms, $CuHCO_3^+$, $CuCO_3^0$, and $Cu(CO_3)_3^{2-}$. The carbonate or less toxic form generally predominates in high-alkaline conditions, which is why alkalinity is considered to afford protection for fishes (Chakoumakos et al. 1979; Laurén and McDonald 1986). However, in some cases copper toxicity to rainbow trout depends on the total concentration of copper (that is, the concentration of cupric copper, Cu^{2+}, and copper carbonate, $CuCO_3^0$) rather than the concentration of either cupric copper or copper carbonate alone (Shaw and Brown 1974).

Calcium, which typically contributes to hardness, may moderate copper toxicity internally by controlling the permeability of branchial epithelium in gills. Thus, the presence of metals when hardness and alkalinity are low should increase electrolyte loss and water uptake (Eddy 1975; Laurén and McDonald 1986; Everall et al. 1989). Eddy (1975) found that, in the absence of calcium, the potentials of brown trout gill epithelia were more negative than in other species (e.g., goldfish), and that calcium additions immediately restored the normal potential. This suggests that in brown trout, calcium greatly reduces ion loss by reducing membrane permeability to Na^+. Everall et al. (1989) also found that high Ca^{2+} (i.e., high hardness) altered the membrane permeability of branchial epithelial cells to zinc in brown trout, so that influx was lowered and efflux was enhanced.

Our results showing loss of whole-body ions suggest that the mode by which metals caused toxicity involved ionoregulatory disturbance. For both brown and rainbow trout fry, significant losses of K^+ and Ca^{2+} occurred only in individuals that died from the pulsed exposure (Table 4). Acute toxicity of metals to fish often is attributed to cytological damage to gill epithelia (e.g., Mueller et al. 1991). This in turn may disrupt ionoregulation or specific gill functions (e.g., chloride cell function) that are particularly important to freshwater fishes (Eddy 1981, 1982). For example, sticklebacks exposed for 16 h to approximately 1.0 mg Zn/L demonstrated extensive gill damage 5 d after replacement in control water (Matthiessen and Brafield 1973). Laurén and McDonald (1985) showed that loss of K^+, Na^+, and Cl^- in rainbow trout was strongly dependent on the exposure concentration of Cu and was due to disruptions of branchial ionoregulatory mechanisms within 2 h of the Cu exposure.

Although significant losses of K^+ and Ca^{2+} but not Na^+ were attributed to the metals toxicity in the present study, some previous work suggests this might not be expected. For instance, Cu may cause loss of both K^+ and Na^+ by increasing the permeability of (*i*) apical cell membranes, thereby stimulating passive loss of intracellular K^+, and (*ii*) branchial tight junctions controlled by bound Ca^{2+}, thereby stimulating loss of Na^+ (Laurén and McDonald 1985). In the present experiments, the effects of metals on branchial tight junctions may have been repressed by high concentrations of Ca^{2+} in exposure waters. These waters may have supplied sufficient Ca^{2+} to binding sites and thus prevented passive Na^+ efflux.

Calcium protects against respiratory impairment from metal toxicity in several fish species. For example, the presence of calcium significantly reduced Zn toxicity in fathead minnows (Judy and Davies 1979). Conversely, lack of Ca^{2+} (low hardness) increased the acute toxicity of Cd to rainbow trout fry (Calamari et al. 1980). Calcium reduced the permeability of branchial epithelia to water in carp but did not reduce Cu uptake, so here apparently calcium provided protection by stabilizing cell membranes rather than reducing toxicant (Cu) uptake (Gregory and Macfarlane 1981). The effect of Ca^{2+} may involve increased stability of gill-epithelial membranes and cell junctions owing to maintenance of cross links between proteins and other cell-wall structures (McWilliams 1983).

In summary, our results support the conclusion that episodic pulses of metals in the Clark Fork River are lethal to both early and juvenile life stages of brown and rainbow trout. This suggests that episodic acute exposures reduce survival in the wild and lower recruitment of subadults into resident trout populations, thus affecting overall population viability.

Additionally, the results of this laboratory study demonstrated that rainbow trout were more sensitive than brown trout to the metals mixture during pulse events when hardness and pH were depressed. This relative sensitivity appeared, in part, to be (*i*) a function of the relatively smaller protective effect of pH reductions on rainbow trout survival relative to brown trout, and (*ii*) a function of the greater adverse effects of hardness reductions on rainbow trout relative to brown trout. In a companion study (Marr et al. 1995), we demonstrated the apparent increased physiological resilience of brown trout to metals toxicity: enhanced capacity for metallothionein induction and sublethal metals acclimation. The results of our pulsed exposure study suggest that similar physiological distinctions between trout species may play a role in relative sensitivity to acute exposures. Given that metal speciation was similar for brown and rainbow trout for the various exposures, the differential responses to hardness, alkalinity, and pH changes support the hypothesis that physiological distinctions between species (e.g., branchial permeability and ion transport) control toxicity. Further, the concept of bioavailability should be viewed as an operational definition that is controlled by both chemical conditions (e.g., speciation) and physiological conditions.

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fontinalis). II. Gill histology. Can. J. Fish. Aquat. Sci. 48: 2016–2027.

Nimick, D.A., and J.N. Moore. 1991. Prediction of water-soluble metal concentrations in fluvially deposited tailings sediments, Upper Clark Fork Valley, Montana, U.S.A. Appl. Geochem. 6: 635–646.

Pascoe, D., and N.A.M. Shazili. 1986. Episodic pollution: a comparison of brief and continuous exposure of rainbow trout to cadmium. Ecotoxicol. Environ. Saf. 12: 189–198.

Playle, R.C., and C.M. Wood. 1989. Water chemistry changes in the gill micro-environment of rainbow trout: experimental observations and theory. J. Comp. Physiol. B, 159: 527–537.

Playle, R.C., R.W. Gensemer, and D.G. Dixon. 1992. Copper accumulation on gills of fathead minnows: influence of water hardness, complexation and pH of the gill micro-environment. Environ. Toxicol. Chem. 11: 381–391.

Ray, G.J. 1985. Effects of heavy metal enrichments on a riparian plant community in the upper Clark Fork River basin. M.S. thesis, University of Montana, Missoula, Mont.

Seim, W.K., L.R. Curtis, S.W. Glenn, and G.A. Chapman. 1984. Growth and survival of developing steelhead trout (*Salmo gairdneri*) continuously or intermittently exposed to copper. Can. J. Fish. Aquat. Sci. 41: 433–438.

Shaw, T.L., and V.M. Brown. 1974. The toxicity of some forms of copper to rainbow trout. Water Res. 8: 377–382.

Shearer, K.D. 1984. Changes in elemental composition of hatchery-reared rainbow trout, *Salmo gairdneri*, associated with growth and reproduction. Can. J. Fish. Aquat. Sci. 41: 1592–1600.

Siddens, L.K., W.K. Seim, L.R. Curtis, and G.A. Chapman. 1986. Comparison of continuous and episodic exposure to acidic, aluminum-contaminated waters of brook trout (*Salvelinus fontinalis*). Can. J. Fish. Aquat. Sci. 43: 2036–2040.

Sprague, J.B. 1969. Measurement of pollutant toxicity to fish. I. Bioassay methods for acute toxicity. Water Res. 3: 793–821.

U.S. Environmental Protection Agency. 1987. Quality criteria for water: 1986. U.S. Environ. Prot. Agency Rep. No. EPA 440/5-86-001.

U.S. Geological Survey. 1989. Water quality data and statistical summaries for the Clark Fork and selected tributaries from Galen to Missoula, Montana. Open File Rep. No. 89-229. U.S. Geological Survey, Helena, Mont.

Woodward, D.F., A.M. Farag, M.E. Mueller, E.E. Little, and F.A. Vertucci. 1989. Sensitivity of endemic Snake River cutthroat trout to acidity and elevated aluminum. Trans. Am. Fish. Soc. 118: 630–643.

Woodward, D.F., J.A. Hansen, H.L. Bergman, E.E. Little, and A.J. DeLonay. 1995. Brown trout avoidance of metals in water characteristic of the Clark Fork River, Montana. Can. J. Fish. Aquat. Sci. 52: 2031–2037.

Zar, J.H. 1984. Biostatistical analysis. Prentice-Hall, Inc., Englewood Cliffs, N.J.