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# Dietary Effects of Metals-Contaminated Invertebrates from the Coeur d'Alene River, Idaho, on Cutthroat Trout

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*Abstract.*—Benthic macroinvertebrates with elevated concentrations of metals were collected from the Coeur d'Alene (CDA) River, Idaho, pasteurized, and fed to cutthroat trout *Oncorhynchus clarki* in the laboratory from start of feeding until 90 d posthatch. Invertebrates were collected from two sites known to contain elevated concentrations of metals: near Pinehurst in the South Fork of the CDA River and at Cataldo, approximately 5 km below the confluence of the South Fork and the North Fork. Invertebrates collected from a relatively clean site in the North Fork were used as a reference diet. We performed measurements of fish health that indicate reduced fitness of fish fed the South Fork and Cataldo diets. Effects measured were reduced feeding activity, increased number of macrophage aggregates and hyperplasia of cells in the kidney, degeneration of mucosal epithelium in the pyloric caecae, and metallothionein induction. These effects would likely reduce growth and survival of fish in the wild. Vacuolization of glial cells were also observed in fish fed the Cataldo diet. Metals in the water often exacerbated the histological effects observed. Although the invertebrates collected near Cataldo had lower concentrations of arsenic (As), cadmium (Cd), copper (Cu), lead (Pb), and zinc (Zn) than the invertebrates from the South Fork, fish fed the Cataldo diet had equally high or higher concentrations of all metals except As by day 44. The Cataldo diet also caused the most deleterious effects on survival and growth. These findings are especially important for early life stage fish, whose diet consists wholly of benthic macroinvertebrates. Therefore, fish feeding on invertebrates in the CDA River below the Bunker Hill smelting complex are at risk of reduced fitness.

Westslope cutthroat trout *Oncorhynchus clarki lewisi* are native to Lake Coeur d'Alene and the Coeur d'Alene (CDA) River system located in northern Idaho. Historically, the cutthroat trout was the dominant species present in this basin and the Coeur d'Alene Tribe depended on this fishery resource for food (Graves et al. 1990). However, numbers of cutthroat trout in the CDA River began to decrease in the early 1900s, and managers from

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the Idaho Fish and Game Department reported that the subspecies made up only 4% of the catch in 1967 (Mallet 1969).

Mining and milling operations have been present in the watershed of the South Fork of the CDA River since metallic ores were discovered in the 1880s (Reece et al. 1978; Blus et al. 1991; Harenberg et al. 1994; Brennan et al. 1995). Smelting operations in the basin were centralized near Kellogg at the Bunker Hill smelting complex, which processed ores and concentrates from the entire region. From 1881 until the construction of the Bunker Hill tailings pond in 1928, all liquid and solid wastes from the milling and smelting operations were discharged into either the South Fork of the CDA River or its tributaries. Additionally, tailings discharged from milling operations and solids eroded from mine dumps covered much of the floodplain of the South Fork.

Metals most frequently elevated in water, sediment, and biota of the CDA River basin are arsenic (As), cadmium (Cd), copper (Cu), lead (Pb), mercury (Hg), and zinc (Zn). Recent studies have demonstrated that fish fed diets contaminated with metals exhibit reduced survival, growth, and health (Farag et al. 1994; Woodward et al. 1994, 1995). During these experiments, trout were fed diets composed of metal-contaminated benthic macroinvertebrates collected from the Clark Fork River, Montana. The authors reported that metals were apparently taken across the gut lining during digestion and accumulated in the tissues, where damage can result.

Westslope cutthroat trout spawn in tributaries of the CDA Basin, and survival of emergent fry is critical for the maintenance of the subspecies' populations. From the time they emerge from gravels to the time they reach a length of 100 mm, westslope cutthroat trout feed exclusively on aquatic invertebrates (Liknes and Graham 1988). Small invertebrates, likely to be the targeted prey of emergent fry, accumulate greater concentrations of metals than large invertebrates (Farag et al. 1998). Dependence of cutthroat trout on a metals-contaminated diet during this sensitive life stage could be a limiting factor for trout populations in the South Fork of the CDA River and for recruitment of these fish into Lake Coeur d'Alene.

Water quality criteria are formulated to protect sensitive species against individual contaminants. In the CDA basin, however, several trace metals in the water interact, and because metals are chronically present in water and sediments, food chains are contaminated (Farag et al. 1998). The objective of this study was to determine the potential for injury to the cutthroat trout fishery from a combination of trace metals interacting through both aqueous and dietary exposures. We investigated how metals in food and water in the CDA basin affected survival, growth, and other physiological functions of cutthroat trout.

## Methods

*Experimental diets*.—The four dietary treatments were the commercial fish feed ''Biodiet,'' a reference diet of aquatic invertebrates collected from the North Fork of the CDA River, an experimental diet of aquatic invertebrates collected from the South Fork of the CDA River near Pinehurst, and a second experimental diet of aquatic invertebrates collected from further downstream in the CDA River near Cataldo (Figure 1). Biodiet was adequate for fish nutritionally and formulated to achieve optimal growth. For this reason, Biodiet was used as a secondary reference only, and data from fish fed that diet were not included during the statistical analyses.

Benthic invertebrates were collected from the three field sites (Figure 1) by raking and kicking the river bottom in front of a 3-mm-mesh seine. Benthic invertebrates were removed from the net with acid-washed plastic forceps and placed into acid-washed plastic vials. The samples were frozen immediately after collection and transported under dry ice to the Fish Technology Center, Bozeman, Montana, where the diets were prepared. Diets were pasteurized to kill any disease organisms, and vitamins and minerals were added to ensure proper nutrition. Aluminum (Al), As, Cd, Cu, Pb, and Zn were measured in diet samples by graphite furnace or flame atomic absorption spectrophotometry (AAS). Proximate analyses were also performed to determine the percentages of protein (P), fat (F), carbohydrate (C), and ash in each diet. The energy content (EC) was calculated as kcal/100 g (1 kcal = 4.18 J) with the formula (Piper et al. 1982)

$$EC = 3.9 \cdot \%P + 8.0 \cdot \%F + 1.6 \cdot \%C.$$

The diets were not analyzed for contaminants other than metals. The history of the CDA basin suggests that metals are the contaminants of concern. Heinz et al. (in press) measured sediments in the basin and reported that organochlorines and polychlorinated biphenyls (PCBs) were below detection limits (detection limits were 0.01 μg/g dry weight for all except toxaphene and PCBs, for which limits were 0.05 μg/g).

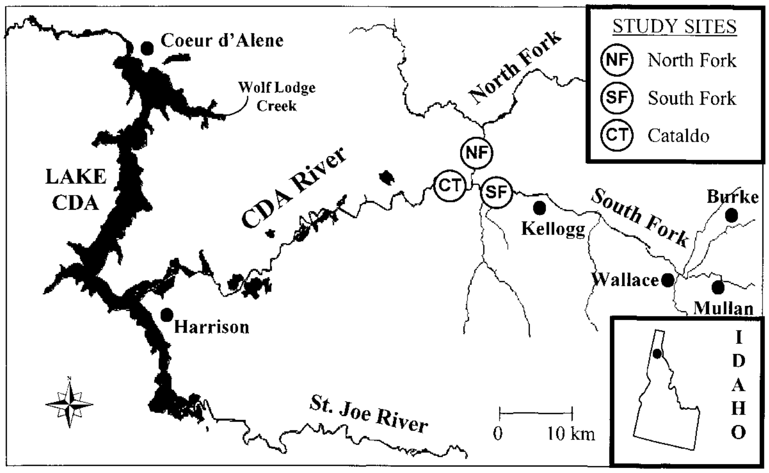

FIGURE 1.—Map of the Coeur d'Alene (CDA) River basin, Idaho, where invertebrates were collected. Diets were prepared from these invertebrates and fed to Snake River cutthroat trout from onset of feeding until 90 d posthatch.

*Fish culture and experimental matrix.*—Snake River cutthroat trout *Oncorhynchus clarki* sp., were used for this study because a disease-free source of westslope cutthroat trout eggs could not be obtained. Snake River cutthroat trout is an undefined subspecies native to the intermountain western United States. Like the westslope cutthroat trout, the Snake River subspecies feeds exclusively on invertebrates during early life (Kiefling 1978), and we considered them acceptable surrogates for westslope cutthroat trout for our experiment. We refer to them as ''cutthroat trout'' henceforth in this paper.

Eyed embryos of cutthroat trout were obtained from the Jackson National Fish Hatchery, Wyoming. Eggs were held in Heath incubators until they hatched. The water was formulated to simulate water in the CDA River (hardness, 50 mg/L as $CaCO_3$; alkalinity, 50 mg/L as $CaCO_3$; pH, 7.0–7.4). Temperature was maintained at 10°C while fish were held and tested. Conductivity and dissolved oxygen were maintained at 115 μS/cm and 8.0 mg/L, respectively. Test waters were analyzed daily to ensure that water quality stayed within 10% of nominal hardness, alkalinity, conductivity, dissolved oxygen, pH, and temperature.

Newly hatched alevins were exposed for 90 d to an aqueous mixture of Cd, Pb, and Zn. Each metal's concentration was four times (4×) its chronic water quality criterion established by the U.S. Environmental Protection Agency (EPA 1987) for the protection of aquatic life. The 4× concentrations were 2.5 μg Cd/L ($CdCl_2$), 5.2 μg Pb/L ($PbCl_2$), and 200 μg Zn/L ($ZnCl_2$). These concentrations of Pb and Zn were below concentrations measured in the CDA River at Cataldo, where means ± SE ($N = 22$) were13 ± 3 μg Pb/L and 426 ± 28 μg Zn/L (Harenberg et al. 1994; Brennan et al. 1995). The 4× concentration of Cd was similar to that measured near Cataldo (3 ± 0 μg/L). Hereafter, the experimental water will be referred to as 4×, and the reference water with no metals added will be referred to as 0×.

Each of the 0× and 4× waters was combined with the four dietary treatments described above to produce a total of eight water × diet treatments. Each treatment was replicated four times, producing 32 experimental units. Exposures were conducted in a flow-through proportional diluter system designed to deliver 250 mL of the appropriate water to each of the 32 experimental units. Each unit contained 4.2 L of water and received 10 volume additions per day. The appropriate concentration of metals was maintained by an automated pipetting system (Micromedic). Each dietary treatment began at exogenous feeding.

*Measurements.*—The experiment began (day 0) when 100 newly hatched alevins were placed in each experimental unit. On day 19, the number of fish in each experimental unit was reduced to 40, and fish removed from each exposure were weighed and analyzed for tissue As, Cd, Cu, Pb, and Zn. Exogenous feeding began on day 22, and fish were fed 6.45% of their body weights per day (calculated as dry weight of food per wet weight of fish). Fish samples were collected for weight, total length, and tissue metal measurements on day 44, when the number of fish in each chamber was reduced to 25, and on day 90, when the experiment was terminated. Tissue metals were measured with graphite furnace or flame AAS and reported as μg/g dry weight ± SE. Dead fish in each experimental unit were removed and counted daily.

Feeding behavior was monitored weekly by video (Woodward et al. 1994). Five fish were separated from the others in each chamber. Food was added to the isolation area, and the number of feeding strikes recorded during 2 min was tabulated.

Fish health measurements were made on survivors at the end of exposures. Externally, the conditions of eyes, skin, and fins were noted (Goede 1989). From each treatment, eight whole fish (two from each experimental unit) were collected for histological analyses. These fish were fixed immediately in Davidson's solution (Humason 1979) and stored in 10% ethanol until they were embedded in paraffin. Sections were cut 4 μm thick and stained with hematoxylin and eosin for light microscopy. Sections were observed and scored by a histopathologist who had no knowledge of any fish's exposure history. Livers were removed from additional fish for measurements of metallothionein with a radioimmunioassay described by Hogstrand and Haux (1990). The livers were placed in vials, frozen immediately in liquid nitrogen, and stored at −90°C until analyses were performed. Due to heavy mortality among fish fed the Cataldo diet, only one sample was collected for metallothionien measurements from the Cataldo–0× and Cataldo–4× experimental groups.

Water was collected weekly to verify the concentrations of metals. Samples were filtered through a 0.45-μm filter in an acid-washed Nalgene 300-mL filter holder, 100 mL were transferred to a precleaned, 125-mL I-Chem polyethylene bottle with 1 mL Ultrex-II nitric acid. Dissolved Cd, Pb, and Zn, were measured in the water samples with graphite furnace or flame AAS.

*Quality assurance.*—The limits of detection (μg/g) for tissue analyses were: Al,1.4; As, 0.7; Cd, 0.01; Cr, 0.3; Cu, 0.3; Hg, 0.08; Pb, 0.3; and Zn, 7.2. Water analyses had detection limits (μg/L) of: Cd, 0.036; Pb, 0.513; and Zn, 11. Analysis spikes run as matrix suppression or enhancement checks ranged from 88 to 110% recovery. Spike recoveries of digested samples ranged from 80 to 120%. Instrument precision, measured as the relative standard deviation of triplicate analyses, was between 0.7 and 4.3. Method precision was generally below 30 relative percent difference (RPD), but one measurement of Pb in tissue was 35 RPD.

*Statistical analyses.*—The experiment was a completely randomized, two-factor, split-plot design with four replicates (Steel and Torrie 1980), and all data were analyzed with analysis of variance (ANOVA) provided by an SAS statistical software package (SAS Institute 1989). The statistical model included aqueous metal concentration as the main-plot treatment effect and dietary metal concentration and the interaction of aqueous and dietary metals as subplot effects. Percent survival data were arcsine-transformed prior to analyses. All other data were tested for equality of variances with the Brown–Forsythe modification of Levenes's test and transformed when necessary. Means were compared via Fischer's least significant difference test with a statistical criterion of $\alpha = 0.05$. If there was no interaction of the aqueous and dietary exposure effects, the overall mean of each was presented. When interactions existed, the mean of each subplot (diet and water exposure) was presented to highlight the interactions more clearly.

## Results

### Invertebrate Composition

The dominant invertebrate genera collected from the North Fork site were *Arctopsyche* (caddisflies), *Acroneuria* (stoneflies), *Hydropsyche* (caddisflies), and *Pterenarcella* and *Pteronarcys* (stoneflies). Species of *Arctophsyche*, *Hydropsyche*, *Pterenarcella*, *Pteronarcys*, and *Tipula* (true flies) were dominant at Cataldo. Species of *Arctopsyche*, *Ephemerella* (mayflies), *Hydropsyche*, and *Tipula* were dominant at South Fork.

### Chemical Analyses of Diets

The energy available from the four diets ranged from 332 kcal/100 g in Biodiet to 267 kcal/100 g in the South Fork diet (Table 1). There was a larger percentage of fat (17.7%) and a lower percentage of carbohydrates (5.7%), in Biodiet than in the invertebrate diets (5.6–9.9% fat, 18.2–29.4% carbohydrate). All three invertebrate diets had more than the minimum recommended percentage of protein (40%) for trout starter diets (Piper et al. 1982), but the invertebrate diets had less than the minimum recommended amount of fat (15%). Both the South Fork and Cataldo diets had more than the maximum recommended concentrations of carbohydrates (20%). The possible effects of the dietary differences, except for carbohydrate, were minimized because all fish were overfed by 25% (6.25%/d versus the 5% body weight/d recommended by Piper et al. 1982).

The concentrations of metals varied among the

TABLE 1.—Proximate analyses and calculated energy contents of commercial Biodiet and invertebrate diets collected from the Coeur d'Alene River and fed to young cutthroat trout; $N = 3$ for protein and $N = 2$ for fat, moisture, and ash. Percent carbohydrate was calculated as the remainder from the total of the other components measured.

| | % of wet weight | | | | | | Energy content (kcal/100 g) | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Diet | Protein | Fat | Moisture | Ash | Carbohydrate | Total | Protein | Fat | Carbohydrate | Total |
| Biodiet | 46.5 | 17.7 | 20.5 | 9.6 | 5.7 | 100 | 181 | 142 | 9 | 332 |
| North Fork | 54.4 | 9.9 | 7.0 | 10.5 | 18.2 | 100 | 212 | 79 | 29 | 320 |
| South Fork | 42.7 | 6.7 | 7.9 | 13.3 | 29.4 | 100 | 167 | 54 | 47 | 267 |
| Cataldo | 47.6 | 5.6 | 9.0 | 12.1 | 25.7 | 100 | 186 | 45 | 41 | 272 |

three invertebrate diets, and the South Fork diet contained the largest concentrations of metals (Table 2). Except for Al and Cd, the pattern for concentrations of metals in the invertebrate diets was South Fork $>$ Cataldo $>$ North Fork. The concentrations of Zn in Cataldo and South Fork were at least 5× the amount in North Fork, and Pb concentrations in the Cataldo and South Fork diets were 61 and 107 times the amount in the North Fork diet, respectively. The mean concentrations of Al were similar between North Fork and Cataldo and greatest in the South Fork diet. Cadmium concentrations were similar between Cataldo and South Fork diets and 30 times greater than in the North Fork diet. Except for As and Hg, Biodiet had smaller concentrations of metals than any of the invertebrate diets.

### *Chemical Analyses of Water*

Cadmium, Pb, and Zn concentrations were trivial in the 0× water; mean concentrations (μg/L ± SD; $N = 48$) in 0× water were Cd, 0.05 ± 0.03; Pb, 0.55 ± 0.40; and Zn, 12 ± 3. In 4× water, concentrations were Cd, 2.18 ± 0.12; Pb, 3.63 ± 0.71; and Zn, 218 ± 10. The mean, measured concentrations of Cd were 13% below the nominal concentration of 2.5, Pb was 30% below the nominal concentration of 5.2, and Zn was 9% above the nominal concentration of 200.

### *Survival, Weight, and Necropsy Assessments*

Diet type, but not water treatment, had a significant effect on survival (Table 3). The survival of fish fed the Cataldo diet was less than that of fish fed the North Fork and South Fork diets. This decrease was first observed on day 44 and continued to day 90, when fish fed the Cataldo diet had only 68% survival. The South Fork diet and the 4× water treatment did not affect survival.

The South Fork and Cataldo diets had different effects on growth (Table 4). Fish fed the South Fork diet weighed more than fish fed the North Fork diet on day 44 but, there was no significant difference between these two groups on day 90. On the other hand, fish fed the Cataldo diet weighed less on days 44 and 90 than fish fed either the North Fork or South Fork diet. The effects of diet were independent of effects attributed to water treatment. The 4× water caused a reduction in weight on day 44, but not on day 90. All external necropsy assessments were unremarkable.

### *Feeding Behavior*

Diet type affected feeding behavior independent of the water treatment. Relative to fish on the North Fork diet, fish on the South Fork diet made 18–40% fewer strikes per minute on seven of nine observation dates, and fish on the Cataldo diet made 40–60% fewer strikes per minute on all nine

TABLE 2.—Mean (SE) concentrations of metals in commercial Biodiet and invertebrate diets collected from the Coeur d'Alene River. Statistical comparisons do not include data from the Biodiet feed; otherwise, means in a column without a letter in common are significantly different ($P > 0.05$).

| | | Metal concentration (μg/g dry weight) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Diet | *N* | Al | As | Cd | Cu | Cr | Pb | Hg | Zn |
| Biodiet | 3 | 44(3) | 3.5(0.2) | 0.21(0.01) | 9.9(0.5) | 0.74(0.01) | 0.20(0.01) | 0.17 (0.02) | 135(3) |
| North Fork | 3 | 1,122(155)z | 2.6(0.2)z | 0.97(0.01)z | 32.9(0.8)z | 3.70(0.17)z | 7.4 (0.26)z | 0.04(0.01)z | 384(9)z |
| South Fork | 3 | 1,993(135)y | 50.8(3.2)y | 29.9(0.27)y | 61.5(1.3)y | 8.52(0.22)y | 792 (18.2)y | 0.51(0.01)y | 2,336(35)y |
| Cataldo | 3 | 1,144(63)z | 13.5(1.0)x | 29.1(0.43)y | 43.8(1.9)x | 5.35(0.19)x | 452 (5.2)x | 0.41(0.01)x | 2,119(41)x |

TABLE 3.—Mean (SE) survival of cutthroat trout fed Biodiet or diets collected from the Coeur d'Alene River and exposed to reference water (0×) or to water with 2.5 µg Cd/L, 5.2 µg Pb/L, and 200 µg Zn/L (4×) until 90 d posthatch; ND = no data because the dietary treatments began on day 19. Statistical comparisons do not include water treatments or Biodiet treatments. For other dietary treatments, means in a column without a letter in common are significantly different ($P > 0.05$).

| Treatment | N | % survival on day: 19 | 44 | 90 |
|---|---|---|---|---|
| Water | | | | |
| 0× | 4 | 99.0(0.3) | 97.1(0.7) | 88.8(0.7) |
| 4× | 4 | 98.8(0.3) | 96.8(0.6) | 87.1(1.9) |
| Diet | | | | |
| Biodiet | 8 | ND | 98.5(0.5) | 98.0(0.5) |
| North Fork | 8 | ND | 97.9(0.4)z | 97.9(0.4)z |
| South Fork | 8 | ND | 98.2(0.3)z | 97.7(0.6)z |
| Cataldo | 8 | ND | 94.8(1.1)y | 68.2(2.6)y |

observation dates (Table 5). Cataldo fish had 33–38% lower strike rates than South Fork fish on three observation dates. The water exposure to metals did not affect feeding activity.

### *Tissue Metals*

*General*.—Concentrations of metals were greater in fish fed the South Fork and Cataldo diets than in fish fed the North Fork diet (Tables 6–9; Figure 2). Although dietary concentrations of metals were generally greatest in the South Fork diet, fish fed the Cataldo diet had greater concentrations of Cu and Zn in their tissues than fish fed the South Fork diet. A 4× water exposure resulted in greater tissue concentrations of Cu and As, although neither was added to the water. Micronutrients such as Zn accumulate over time from diet and water. However, Pb accumulation from the South Fork diet seemed to have reached steady state by day 44. Fish in 4× water accumulated more Pb with the Cataldo diet than with the South Fork diet; thus, there was a significant interaction of diet and water for Pb.

TABLE 4.—Mean (SE) weights of cutthroat trout in experimental treatments. Conventions are those of Table 3, except asterisks denote a significant difference between water treatments on a given day ($P \leq 0.05$).

| Treatment | N | Weight (mg) on day: 19 | 44 | 90 |
|---|---|---|---|---|
| Water | | | | |
| 0× | 4 | 85(1) | 132(1)* | 459(15) |
| 4× | 4 | 83(2) | 125(2)* | 421(15) |
| Diet | | | | |
| Biodiet | 8 | ND | 253(5) | 1,294(20) |
| North Fork | 8 | ND | 144(4)z | 587(13)z |
| South Fork | 8 | ND | 156(5)y | 570(23)z |
| Cataldo | 8 | ND | 86(1)x | 163(6)y |

*Zinc*.—Tissue concentrations of Zn did not appear to reach steady state during this experiment (Table 6). Both dietary (South Fork and Cataldo) and water (4×) exposures contributed to continued tissue accumulation from day 44 to day 90. Although Zn concentrations were the greatest in the South Fork diet, fish fed South Fork and Cataldo diets had similar amounts of tissue Zn on day 44 and the Cataldo diet caused the greater tissue Zn accumulations by day 90.

*Copper*.—As was the case for Zn, both diet and water contributed to elevated concentrations of Cu in the tissues (Table 6). Also like Zn, Cu accumulated in greater concentrations in fish fed the Cataldo diet than in fish fed the South Fork diet. A steady state appears to have been reached by day 44 for the South Fork diet, but the Cataldo diet continued to promote increased uptake of tissue Cu by day 90. The 4× water caused elevated tissue Cu even though Cu was not added to the water.

*Lead*.—On day 90, fish from South Fork–0× and Cataldo–0× treatments had elevated tissue Pb, but fish from Cataldo–4× had the greatest concentrations of Pb (Table 7). Also by day 90, fish in the North Fork–4× treatment had significantly

TABLE 5.—Mean (SE) numbers of strikes per minute during feeding activities of cutthroat trout in experimental treatments. Conventions are those of Table 3.

| Treatment | N | Number of strikes/min on day: 29 | 36 | 43 | 50 | 57 | 64 | 71 | 78 | 85 |
|---|---|---|---|---|---|---|---|---|---|---|
| Water | | | | | | | | | | |
| 0× | 4 | 5(1.1) | 7(0.6) | 10(0.6) | 9(0.7) | 8(0.6) | 7(0.7) | 10(0.9) | 8(0.9) | 9(1.3) |
| 4× | 4 | 3(0.6) | 7(0.8) | 10(0.9) | 8(0.5) | 8(1.2) | 7(0.7) | 10(0.4) | 6(0.4) | 8(0.7) |
| Diet | | | | | | | | | | |
| Biodiet | 8 | 4(1.4) | 8(1.0) | 9(0.6) | 10(1.1) | 10(1.3) | 11(0.6) | 10(1.5) | 9(0.7) | 11(1.0) |
| North Fork | 8 | 5(1.3)z | 10(0.8)z | 13(1.0)z | 12(0.9)z | 12(1.0)z | 10(0.9)z | 13(1.1)z | 9(1.0)z | 11(1.2)z |
| South Fork | 8 | 4(0.7)zy | 6(0.6)y | 8(0.6)y | 8(0.5)y | 8(0.9)y | 6(0.8)y | 10(0.8)y | 7(0.9)zy | 9(1.1)y |
| Cataldo | 8 | 3(0.4)y | 4(0.5)x | 8(0.4)y | 6(1.0)y | 5(0.5)x | 6(0.5)y | 8(0.9)y | 5(0.7)y | 6(0.3)x |

TABLE 6.—Mean (SE) zinc and copper concentrations in whole bodies of cutthroat trout in experimental treatments. Conventions are those of Tables 3 and 4.[a]

| | | Zinc concentration (μg/g dry weight) on day: | | | Copper concentration (μg/g dry weight) on day: | | |
|---|---|---|---|---|---|---|---|
| Treatment | *N* | 19 | 44 | 90 | 19 | 44 | 90 |
| Water | | | | | | | |
| 0× | 4 | 78(1)* | 227(6) | 289(8)* | 5.2(0.1) | 7.6(0.4) | 7.1(0.1)* |
| 4× | 4 | 105(1)* | 322(4) | 389(3)* | 5.3(0.2) | 8.6(1.2) | 8.3(0.2)* |
| Diet | | | | | | | |
| Biodiet | 8 | ND | 156(9) | 130(9) | ND | 6.5(2.2) | 3.5(0.1) |
| North Fork | 8 | ND | 209(19)z | 190(12)z | ND | 7.4(0.3)z | 6.1(0.2)z |
| South Fork | 8 | ND | 319(19)y | 417(26)y | ND | 8.1(0.3)z | 9.0(0.5)y |
| Cataldo | 8 | ND | 413(29)y | 621(54)x | ND | 10.2(0.4)y | 12.3(0.9)x |

[a] On day 44 for diet, $P = 0.001$ for Zn and $P = 0.0005$ for Cu. On day 90 for water, $P = 0.01$ for Zn, and $P = 0.0012$ for Cu. On day 90 for diet, $P = 0.0001$ for Zn and Cu.

more tissue Pb than fish in the North Fork–0× treatment. However, within both the 0× and 4× water series, Pb accumulation was significantly less in fish on the North Fork diet than for fish on South Fork and Cataldo diets. Therefore, the effects of diet and water on Pb accumulation were not simply additive by day 90, as was the case for Zn and Cu. However, as with Zn, the water exposure elevated concentrations of tissue Pb before the onset of feed administration on day 19. And diet type alone caused elevated tissue Pb on day 44, similar to the pattern for Zn and Cu.

*Cadmium*.—The water exposure increased tissue Cd by day 19 and both diet and water caused increased tissue Cd on days 44 and 90 (Table 8). Fish on the North Fork diet always had lower Cd concentrations than fish on the South Fork and Cataldo diets. In the 4× water series, Cataldo-diet fish had significantly greater loadings than South Fork-diet fish on days 44 and 90.

*Arsenic*.—Arsenic accumulation reached a steady state by day 44 (Table 9). By day 90, tissue As was significantly higher in fish on South Fork and Cataldo diets than in fish on North Fork diets. The 4× water exposure significantly added to tissue As in fish eating the South Fork diet, even though As was not added to the 4× water, but it did not affect tissue As of fish fed the North Fork or Cataldo diets.

### *Metallothionein and Histology*

The interaction of diet and water on metallothionein concentrations was additive, and exposures from both water and diet were necessary to induce metallothionein. Fish in the South Fork–4× treatment had 299 μgmetallothionein/g in the

TABLE 7.—Mean (SE) lead concentrations in whole bodies of cutthroat trout from experimental treatments. Conventions are those of Tables 3 and 4.[a]

| | | Lead (μg/g dry weight) on day: | | |
|---|---|---|---|---|
| Treatment | *N* | 19 | 44 | 90 |
| Water | | | | |
| 0× | 4 | 0.2(0.1)* | 20.8(1.3) | 22.6(1.7) |
| 4× | 4 | 2.3(0.1)* | 51.2(23.4) | 32.4(1.5) |
| Diet | | | | |
| Biodiet | 8 | ND | 48.4(45.7) | 2.3(0.8) |
| North Fork | 8 | ND | 4.2(1.2)z | 3.6(0.9) |
| South Fork | 8 | ND | 43.3(3.7)y | 44.0(3.4) |
| Cataldo | 8 | ND | 48.3(3.5)y | 60.1(5.6) |
| Combination | | | | |
| Biodiet–0× | 4 | ND | 1.1(0.7) | 0.2(0.1) |
| North Fork–0× | 4 | ND | 1.0(0.2) | 1.2(0.2)z |
| South Fork–0× | 4 | ND | 41.0(2.6) | 36.8(3.3)y |
| Cataldo–0× | 4 | ND | 40.3(3.2) | 52.3(8.5)yw |
| Biodiet–4× | 4 | ND | 95.8(90.8) | 4.4(0.4) |
| North Fork–4× | 4 | ND | 7.3(0.6) | 6.0(0.3)x |
| South Fork–4× | 4 | ND | 45.5(7.2) | 51.3(2.7)yw |
| Cataldo–4× | 4 | ND | 56.3(2.2) | 68.0(5.5)w |

[a] On day 44 for diet, $P = 0.0001$. On day 90 for water, $P = 0.01$; for diet, $P = 0.0001$.

TABLE 8.—Mean (SE) cadmium concentrations in whole bodies of cutthroat trout from experimental treatments. Conventions are those of Tables 3 and 4.

| Treatment | *N* | Cadmium (μg/g dry weight) on day: 19 | 44 | 90 |
|---|---|---|---|---|
| Water | | | | |
| 0× | 4 | 0.04(0.01)* | 1.24(0.08) | 1.83(0.13) |
| 4× | 4 | 0.70(0.02)* | 3.51(0.09) | 4.70(0.21) |
| Diet | | | | |
| Biodiet | 8 | ND | 0.98(0.34) | 0.92(0.35) |
| North Fork | 8 | ND | 1.28(0.42) | 1.16(0.40) |
| South Fork | 8 | ND | 3.11(0.37) | 4.06(0.52) |
| Cataldo | 8 | ND | 4.14(0.63) | 6.93(1.10) |
| Combination | | | | |
| Biodiet–0× | 4 | ND | 0.09(0.02) | 0.04(0.01) |
| North Fork–0× | 4 | ND | 0.18(0.03)z | 0.10(0.01)z |
| South Fork–0× | 4 | ND | 2.18(0.20)y | 2.88(0.51)yx |
| Cataldo–0× | 4 | ND | 2.53(0.15)y | 4.33(0.89)xw |
| Biodiet–4× | 4 | ND | 1.88(0.08) | 1.80(0.24) |
| North Fork–4× | 4 | ND | 2.38(0.08)y | 2.23(0.09)y |
| South Fork–4× | 4 | ND | 4.05(0.18)x | 5.25(0.24)w |
| Cataldo–4× | 4 | ND | 5.75(0.31)w | 9.53(0.60)v |

liver, which was 6 times the liver concentration in North Fork–0× fish (46 ± 8 μg/g) and 3.5 times the concentration in North Fork–4× fish (86 ± 7 μg/g). More liver metallothionein was produced in South Fork–4× than in South Fork–0× fish (99 ± 16 μg/g), but metallothionein induction did not differ significantly between South Fork–0× and North Fork–0× fish. Because survival was reduced, only one sample was taken from the Cataldo–0× and Cataldo–4× exposures for metallothionein analysis; the respective concentrations were 200 and 221 μg/g.

Histological changes were most common in fish fed the Cataldo diet, but changes were also caused as a result of the South Fork diet and the 4× water (Table 10). Vacuolation of neuroglia cells was observed in the brains of fish from Cataldo/0×, and this effect was more extensive in fish from Cataldo–4× (Figure 3). The 4× water exposure also caused vacuolization of glial cells in fish from North Fork–4× and South Fork–4×. As would be expected, diet type, but not water exposure, caused degeneration of the mucosal epithelium of pyloric caeca. This degeneration occurred in both South Fork–0× and Cataldo–0× treatments but was more severe in fish from Cataldo–0× (Figure 4). Water

TABLE 9.—Mean (SE) arsenic concentrations in whole bodies of cutthroat trout from experimental treatments. Conventions are those of Tables 3 and 4.[a]

| Treatment | *N* | Arsenic (μg/g dry weight) on day: 19 | 44[a] | 90 |
|---|---|---|---|---|
| Water | | | | |
| 0× | 4 | <0.76(0.0) | 2.1 (0.2) | 2.1(0.1) |
| 4× | 4 | <0.76(0.0) | 2.3(0.2) | 2.4(0.2) |
| Diet | | | | |
| Biodiet | 8 | ND | 1.6(0.1) | 2.0(0.1) |
| North Fork | 8 | ND | <0.8(0.1)z | 0.8(0.1) |
| South Fork | 8 | ND | 4.2(0.4)y | 3.8(0.4) |
| Cataldo | 8 | ND | 2.3(0.4)x | 2.1(0.2) |
| Combination | | | | |
| Biodiet–0× | 4 | ND | 1.6(0.2) | 1.8(0.1) |
| North Fork–0× | 4 | ND | 0.6(0.1) | 0.9(0.1)z |
| South Fork–0× | 4 | ND | 3.6(0.6) | 3.3(0.4)y |
| Cataldo–0× | 4 | ND | 2.8(0.7) | 2.4(0.3)yx |
| Biodiet–4× | 4 | ND | 1.5(0.2) | 2.3(0.2) |
| North Fork–4× | 4 | ND | 1.1(0.2) | 0.8(0.1)z |
| South Fork–4× | 4 | ND | 4.8(0.4) | 4.5(0.4)w |
| Cataldo–4× | 4 | ND | 1.9(0.4) | 1.9(0.1)x |

[a] On day 44 for diet, $P = 0.0001$.

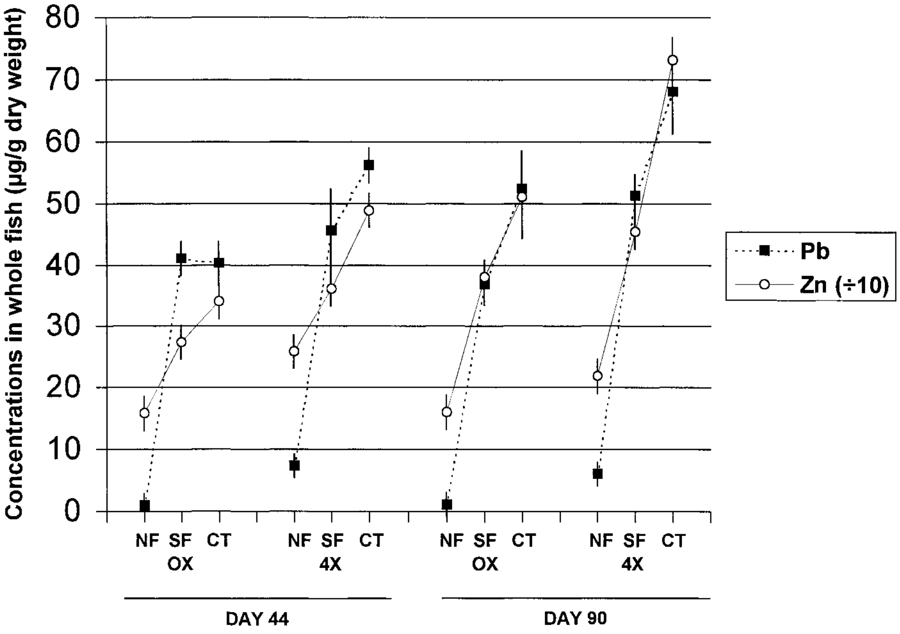

FIGURE 2.—Mean (±SE) concentrations of lead (Pb) and zinc (Zn) in whole cutthroat trout fed diets collected from the Coeur d'Alene (CDA) River and exposed to either reference water (no metals added; 0×) or water to which metals had been added to concentrations four times the EPA quality criteria (4×: 5.2 µg Pb/L and 200 µg Zn/L) until day 90 posthatch. Sources of invertebrate diets are North Fork of CDA River (NF), South Fork of CDA River near Pinehurst (SF), and CDA River near Cataldo (CT). For significant differences refer to Tables 6 and 7.

and diet exposures caused hyperplasia of hematopoietic cells in the kidney interstitium (Figure 5). This pathology occurred in half the examined fish from South Fork–0×, as well as in North and South Fork–4× fish.

Some liver and gill changes were observed in all four diet groups and could not be distinguished by exposure except that moderately severe necrosis of hepatocytes was observed in two of eight South Fork–4× fish. This necrosis was not observed in any other fish in the study. More extensive hypertrophy and hyperplasia of the gill epithelium was observed in fish exposed to 4× water, but these changes were also noted in fish fed the North Fork diet and Biodiet and not exposed to metals via the water.

## Discussion

Methods used to incorporate metals into fish diets influence the degree of toxicity caused by met-

TABLE 10.—Summary of histological changes observed in cutthroat trout from experimental treatments. Eight fish from each treatment were examined; data are numbers of those eight fish showing degradation. Intensity of degradation is coded M = minimal, D = moderate, and S = moderately severe.

| Treatment | Brain glial vacuolization | Degeneration of pyloric caeca mucosal epithelium | Kidney: Macrophage accumulation | Kidney: Hematopoietic hyperplasia |
|---|---|---|---|---|
| Biodiet–0× | 0 | 2 M | 0 | 0 |
| North Fork–0× | 0 | 0 | 0 | 0 |
| South Fork–0× | 0 | 5 M–D | 3 M–D | 4 M–S |
| Cataldo–0× | 2 M–D | 8 M–S | 4 M–D | 0 |
| Biodiet–4× | 0 | 0 | 0 | 0 |
| North Fork–4× | 3 D–S | 0 | 0 | 2 M–D |
| South Fork–4× | 3 D–S | 1 M–D | 0 | 3 M–D |
| Cataldo–4× | 6 D–S | 6 M–S | 3 M–D | 0 |

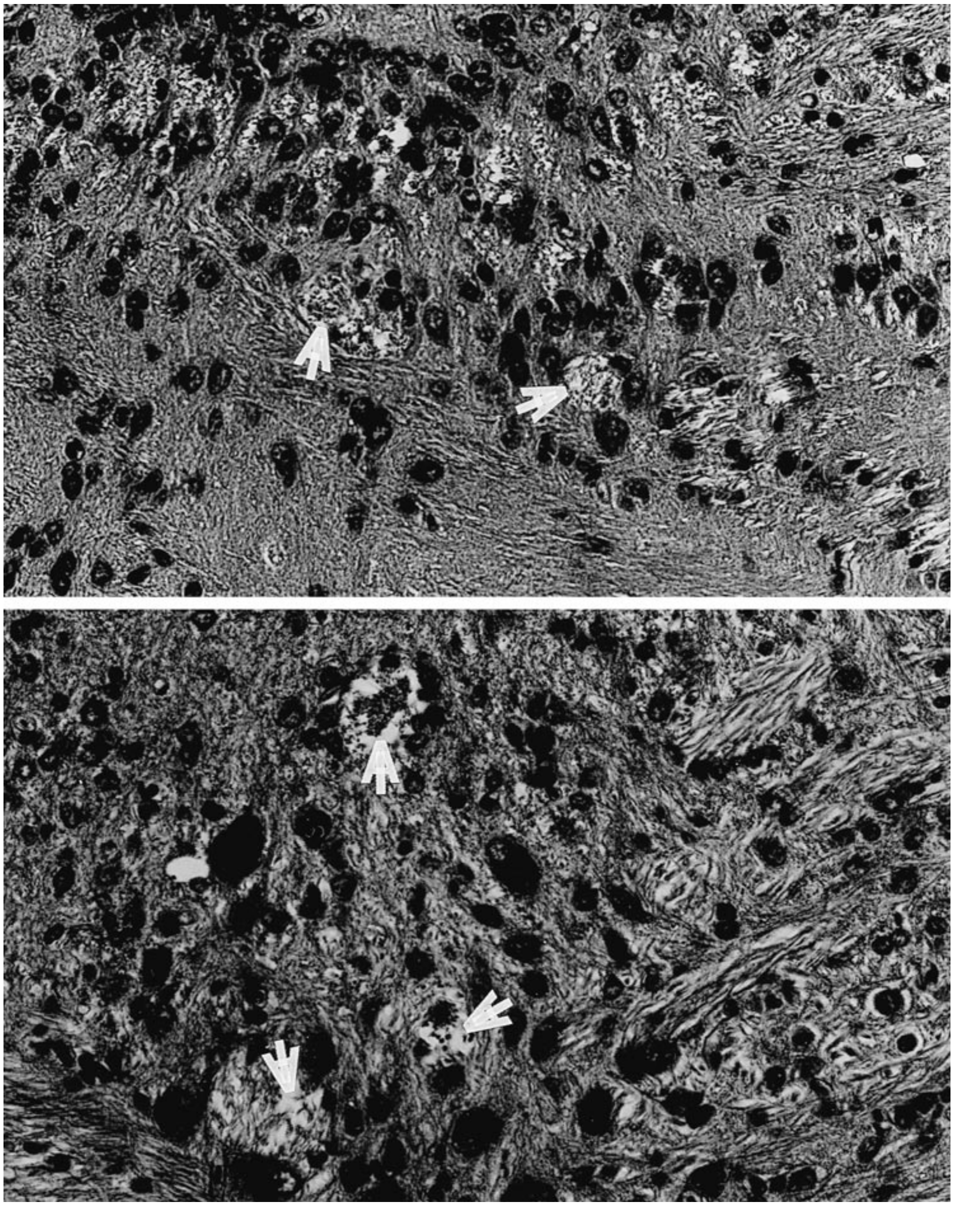

FIGURE 3.—Vacuolated neuroglia cells (arrows) in the brain (medulla) of cutthroat trout fed an invertebrate diet collected from the CDA River near Cataldo and held in water with 2.5 μg Cd/L, 5.2 μg Pb/L, and 200 μg Zn/L, concentrations of metals at or below those measured near Cataldo. Magnification = 400×.

als. Woodward et al. (1995) briefly reviewed these different methods. When metals were added superficially to commercial diets (Wekell et al. 1983; Lanno et al. 1985; Crespo et al. 1986), their toxicity to trout was less than when they occurred naturally in diets (Woodward et al. 1994, 1995). In some studies, invertebrates exposed to metals in the laboratory were used as a food source for trout. Although concentrations of metals in the diets were similar, the toxicity to trout reported by Mount et al. (1994) with *Artemia,* exposed for 24 h, differed from toxicities reported by Woodward et al. (1994, 1995) with chronically exposed wild invertebrates. Willis and Sunda (1984) suggested that when *Artemia* is used as food carriers, they should be exposed to metals from very young life

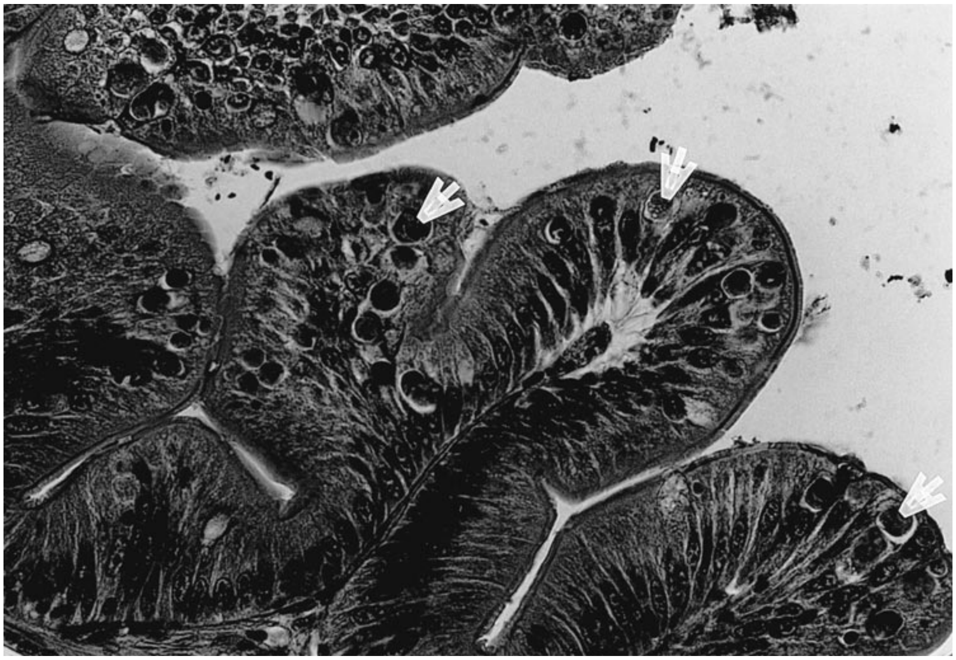

FIGURE 4.—Degeneration and necrosis of mucosal epithelial cells (arrows) in the pyloric caeca of a cutthroat trout fed an invertebrate diet collected from the CDA River near Cataldo. Magnification = 400×.

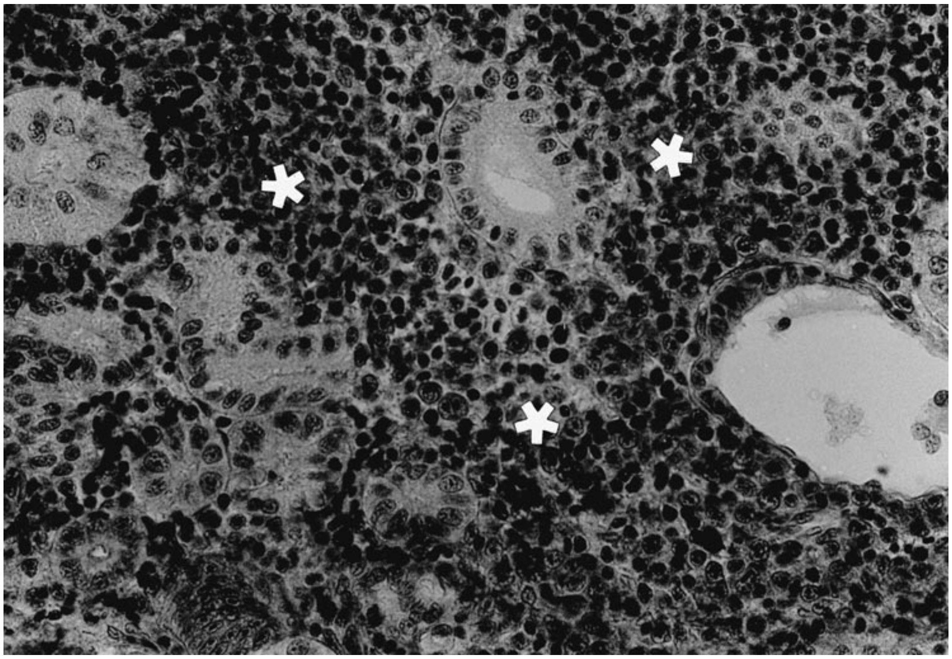

FIGURE 5.—Hyperplasia of hematopoietic cells (stars) in the kidney interstitium of a cutthroat trout fed an invertebrate diet collected from the South Fork of CDA River and held in water that simulated the CDA River without metals added. Magnification = 400×.

stages to enhance uniform labeling of the organisms. The *Artemia* prepared by Mount et al. (1994) probably had not been exposed long enough for metals to be organically bound within the organisms. Thus, although the concentrations of metals in diets may be similar, toxicological effects of those diets can differ.

Other researchers have documented the need for studies that mimic natural conditions more closely (Dallinger et al. 1987; Harrison and Curtis 1992), and invertebrates collected from the Coeur d'Alene River duplicated the diet sources available to fish in the river. These invertebrates have been exposed to metals throughout their aquatic life stages via their own diets, sediments, and water (Farag et al. 1998).

This is the latest in a series of laboratory experiments in which trout were fed invertebrate diets collected from the contaminated river system in question (Woodward et al. 1994, 1995). The results of this approach are reproducible over a series of different experiments with different trout species and between different contaminated rivers. Metals affected the health of trout in all cases. In none of these experiments were toxicological responses observed in fish fed diets collected from reference sites.

Similar toxicological endpoints were observed in these feeding studies: reduced survival (Woodward et al. 1994; this study), decreased growth (Woodward et al. 1994, 1995; this study), reduced feeding activity (Woodward et al. 1995; this study), and histopathological abnormalities (Woodward et al. 1994, 1995; this study). All of these toxicological endpoints have been associated with accumulations of metals in the tissues of fish. The concentrations of metals were measured in the whole bodies of the early life stage fish during these experiments. Data from Farag et al. (1994, 1995) indicate that metals from the invertebrates are accumulated in the liver, pyloric caeca, and large intestine in fish fed those invertebrates.

The Cataldo diet was responsible for the most deleterious effects on survival and growth and the greatest reductions in feeding activities. These results are counterintuitive because the South Fork diet had greater concentrations of all metals (except Cd) than did the Cataldo diet. At the end of this study, the concentrations of Cu and Zn in the fish fed the Cataldo diet were greater than fish fed the South Fork diet. There was 10% less Zn in the Cataldo diet, but by the end of the study, tissue Zn was 33% greater in fish fed the Cataldo diet than in fish fed the South Fork diet (Figure 2). Although there was 43% less Pb in the Cataldo diet, fish fed that diet had just as much Pb as fish fed the South Fork diet. On days 44 and 90, the mean concentrations of Cd, Cu, Pb, and Zn were greater in fish fed the Cataldo diet in 15 of the 16 possible comparisons with South Fork dietary treatments. Although the means were not always significantly greater for Cd and Pb, the trend was consistent. The increased availability of metals in the Cataldo diet could be attributed to the form of metals present in the diet. Vighi (1981) also suggested that organic forms of Pb in the food chain were responsible for greater accumulation of Pb via a natural diet than a water exposure. Vighi observed a slower rate of depuration of Pb from guppies *Poecilia reticulata* that accumulated the metal via a natural diet compared to aqueous exposure and suggested that this slower release of Pb was due to organic complexes of Pb present in the diets.

The amount of Al in the diets may help explain why metals in the Cataldo diet crossed the intestine more efficiently than metals in the South Fork diet. Aluminum is one of the most prevalent metals in the earth's crust, and the amount of aluminum in a sample is often associated with the amount of sediment present in that sample (Lobel et al. 1989). Metals in sediments are biologically relevant to fish because they are responsible for increases in metal concentrations in food-chain organisms (Smock 1983; Timmermans 1992). However, metals in sediments may not be absorbed by fish as efficiently as metals actually incorporated into invertebrate tissues. The 57% greater amount of Al in the South Fork diet may be due to the undigested material in the gut of the South Fork invertebrates. Thus, it is possible that metals in the South Fork diet were associated with inorganic particulates in the gut, whereas metals in the Cataldo diet were associated with invertebrate tissues.

Reduced feeding activity resulted from both the South Fork and Cataldo diets and may have serious implications for fish in the CDA River. With an 18–40% reduction in strike frequencies from the South Fork diet and a 40–60% reduction from the Cataldo diet, fish feeding in these two areas will likely consume less food than those at North Fork. This reduction in strike frequencies ultimately translates into reductions in caloric intake and reduced energy available for growth and maintenance.

Deleterious effects on survival and growth were noted in fish fed the Cataldo diet. Histological data support these findings and indicate that fish fed the

South Fork and Cataldo diets had impaired tissues. For example, histological changes were noted in the kidney and pyloric caeca of fish fed the South Fork and Cataldo diets. We noted increased macrophage aggregates and hyperplasia in the kidney. Norris et al. (1996) also reported hyperplasia and hypertrophy of interrenal cells of brown trout *Salmo trutta* collected from the Eagle River, Colorado, which has elevated concentrations of Cd and Zn in the water column and sediments. Changes in the interrenal section of the kidney were indicative of chronic stress, because the kidney releases cortisol, a hormone involved in the ''general stress response'' (Norris et al. 1996).

We also observed degeneration of the mucosal epithelium of the pyloric caeca of fish fed the South Fork and Cataldo diets. This finding is of special concern because the pyloric caecae are important in the digestive function of trout, and Woodward et al. (1995) noted this same result in trout fed diets containing macroinvertebrates with elevated concentrations of metals (Clark Fork River, Montana). Additionally, we noted vacuolated neuroglia cells in fish fed the Cataldo diet. Balaza et al. (1986) noted the toxic effects of lead on endothelial cells, neurons, and the glia of the brain. Thus, the vacuolated neuroglial cells may compromise the integrity of axons in the glia.

The induction of metallothionein requires energy that may otherwise be used for growth. Reduction in growth following metal exposures has been associated with metallothionein induction (Marr et al. 1995). Metallothionein induction in early life stage fish has previously been difficult to observe because the measurements were performed on homogenized, whole fish (Woodward et al. 1995) instead of dissected livers. Metals from the South Fork diet or water alone did not induce metallothionein; instead, a combination of the two induced metallothionein. However, we did not observe reduced growth in the South Fork–4× exposure even though metallothionein was induced in fish from the exposure. If metallothionein induction is a precursor of reduced growth, a longer experiment may have resulted in reduced growth in fish exposed to South Fork–4×.

There is some indication that metallothionein was induced by the Cataldo diet alone, without the water exposure. Because of high mortality, only one sample was taken from the Cataldo–0× and Cataldo–4× exposures, but both these measurements were twice the South Fork–0× and North Fork–4× measurements. Although no statistical significance can be placed on these measurements, the Cataldo diet may have induced metallothionein in survivors. Further research would be necessary to determine if the Cataldo diet alone causes an increase in metallothionein, but these data are more evidence that metals in the Cataldo diet were present in a form that affects fish health.

Measurements of fish health indicated reduced fitness of fish fed the South Fork diet in the absence of effects on growth. The effects measured on feeding activity, histology, and metallothionein would likely result in reduced growth and survival of these fish under natural conditions. Reduced feeding activity could be detrimental to young-of-the-year fish when energy is required to escape predators, find prey, and compete for cover. The combination of reduced feeding activity with degeneration of gut tissue could result in hampered digestion of the already smaller amounts of food consumed. These changes may be exacerbated further by the effects observed in the kidney and the induction of metallothionein. In the laboratory, the excessive availability of food and the lack of predators reduces the adverse effects of diets contaminated with metals.

It should also be noted that the 4× water concentration of Cd, Pb, and Zn exacerbated many effects caused by the South Fork and Cataldo diets. Although the aqueous concentrations of each of these metals were four times the chronic water quality criteria established by the U.S. Environmental Protection Agency for the protection of aquatic life, they were at or below the concentrations often measured in the CDA River near Cataldo (Harenberg et al. 1994; Brennan et al. 1995). Thus, these concentrations are environmentally relevant in the CDA River and may serve to further compromise wild trout.

The nutritional content of the diets collected from the CDA River may also have contributed to the toxicological effects observed, because the South Fork and Cataldo diets provided approximately 15% less energy content than did the North Fork diet. However, fish were overfed to compensate for these differences. Also, the histological abnormalities indicate that factors other than nutritional content of the diet affected fish health. We suggest that further research be directed at determining if the metals in contaminated sites affect the nutritional content of invertebrate diets.

Fish fed Biodiet during this study had the greatest caloric and protein contents with 332 kcal/100 g and 54.4% protein. Commercial diets are formulated to obtain maximum growth, a situation not observed with natural diets. Thus, the use of invertebrate diets from reference streams leads to conservative statistical comparisons during these feeding studies.

Our study supports previous findings and also supports the hypothesis that organometallic complexes in diets are keys to the efficient transfer of metals across the gut (Vighi 1981; Harrison and Curtis 1992). The Cataldo diet, which had lower concentrations of metals than the South Fork diet, actually caused greater accumulations of metals in fish tissues. Fish fed the Cataldo diet exhibited the most deleterious effects on growth and survival. Therefore, organometallic complexes in that diet may have enhanced the uptake of those metals across the gut wall.

In summary, fish feeding on invertebrates in the CDA River below the Bunker Hill smelting complex are at risk of reduced fitness. The risk is greatest for early life stage fish, whose diet is restricted to benthic macroinvertebrates. Additionally, concentrations of metals less than or equal to those frequently measured in the water at Cataldo exacerbate dietary effects. This study demonstrated that the diet collected from Cataldo caused the most deleterious effects. This finding is of special concern because cutthroat trout have been observed in the river near Cataldo. If trout remain and feed in that area, their health will likely be compromised.

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