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Survival and Hepatic Metallothionein in Developing Rainbow Trout Exposed to a Mixture of Zinc, Copper, and Cadmium

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Rainbow trout (Salmo gairdneri) in Buttle Lake on Vancouver Island, B.C. are exposed to metal contamination originating from a copper and zinc mining operation at Myra Falls near the head of the lake. The operation began in 1967 and since then concentrations of metals in the lake have gradually risen due to waste rock acidification (Clark and Morrison, 1982).

Various investigations have been carried out to assess toxicity of the lake water to fish, including static, flow through and in situ exposures (Roch and McCarter, Part 1 and II, 1984a). These exposures were carried out with juvenile rainbow trout (1-5 g). Chapman (1978) reported that the swim-up stage of the steelhead trout (Salmo gairdneri) was more sensitive to copper, zinc or cadmium than the egg or alevin. In order to properly assess the risk to a population of rainbow trout in Buttle Lake, we initiated a long-term exposure of rainbow trout from hatch including the swim-up stage.

Copper, zinc or cadmium are known to induce metallothionein in mammals (Kägi and Nordberg, 1978) and as a mixture of metals, induce hepatic metallothionein in rainbow trout (Roch and McCarter, 1984a). Investigation of hepatic metallothionein concentrations in wild rainbow trout from Buttle Lake and in lakes of the Campbell River downstream showed a correlation with metal concentrations in the water (Roch et al., 1982). Rainbow trout held in situ for 4 weeks showed the same correlation (Roch and McCarter, 1984b). In this report we determined whether or not the degree of contamination was correlated with concentrations of metallothionein in the livers of rainbow trout exposed to the mixture of metals during the early life stages.

Chinook salmon exposed to a mixture of zinc, copper and cadmium during early life stages showed a concentration-dependent retardation of growth (Roch and McCarter, 1984c). The effect of metals on growth of the rainbow trout is reported here.

MATERIALS AND METHODS

The water supply was dechlorinated Victoria city water which was

deionized using two pairs of 9 inch cation and anion resin tanks in series (Culligan, Richmond, B.C.) in order to reduce metal concentrations to below detection limits (Zn <5 µg/L, Cu <1 µg/L, Cd <0.2 µg/L). Tanks were replaced when the copper concentration rose above 2 µg/L. The water was reconstituted to a hardness of 25 mg/L as $CaCO_3$ by mixing well water (280 mg/L as $CaCO_3$) with the deionized water. This hardness is representative of Buttle Lake and most of the freshwater on Vancouver Island. A partitioned head tank received well and deionized water and the mix was controlled by valves.

Rainbow trout (Salmo gairdneri) eggs obtained from Spring Valley Trout Farm in Langley, B.C., were held in uncontaminated water (Zn <5 µg/L, Cu <2 µg/L and Cd <0.2 µg/L) until hatch. Within 24 hours of hatch on January 31, 1984, 150 alevins were placed in each of four containers receiving various concentrations of a mixture of zinc, copper and cadmium. Nominal concentrations included a control (<5 µg Zn/L, <1 µg Cu/L, <0.2 µg Cd/L), 50 µg Zn/L, 100 µg Zn/L and 200 µg Zn/L with copper and cadmium present in a ratio of 400:20:1 (Zn:Cu:Cd). The highest concentration was approximately equivalent to the most contaminated sites of Buttle Lake in 1981. Twenty litre hemispherical tanks received soft (25 mg/L as $CaCO_3$) water at a flow rate of 500 mL/min. A stock solution containing the mixture of metals was added by peristaltic pump at a rate of 0.25 mL/min. Metal concentrations in stock solutions were measured by flame atomic absorption spectrophotometry and adjusted to within 5% of desired concentrations. The exposure was monitored by measuring zinc concentrations daily. Copper and cadmium concentrations in all containers were determined by graphite furnace atomic absorption spectrophotometry. Zinc was measured by flame AAS using a Varian AA 475.

Static 96 hour bioassays (Table 2) were carried out in 15-L polyethylene containers with appropriate additions of stock solutions to a mixture of deionized water (25 mg/L as $CaCO_3$) according to the recommendations of Sprague (1973). All concentrations are reported as total metal. Dissolved concentrations were 0.93 ± 0.5, 0.59 ± 0.11 and 0.84 ± 0.28 (means ± standard deviation) of total metals for zinc, copper and cadmium respectively in twelve samples. LC50 concentrations and 95% confidence limits were calculated according to Litchfield and Wilcoxon (1949). Flow-through bioassays with rainbow trout fry (0.7-1.3 g) were done to compare lethal response to individual metals with the mixture. These tests were done at 10 ± 2°C and in water of 23 ± 2 mg/L hardness as $CaCO_3$.

Metallothionein concentrations were determined by homogenizing 2-4 pooled livers in ice cold 0.9% NaCl. The volume was adjusted to 2 mL, the sample was heated at 85°C for 5 min, cooled in ice and filtered (Whatman GF/A) to remove coagulated protein. Metallothionein was measured in an aliquot of filtrate by differential pulse polarography (Olafson and Sim, 1979) using the model 174A polarographic analyzer (Princeton Applied Research, Princeton, N.J., U.S.A.).

On February 29, 1984 at 340 degree days after hatch, the number of fish in each tank was equalized at 60 and the trout were fed Silver Cup (Murray, Utah) starter feed at a rate of 5.6% body weight per day.

On April 4, 1984, fish were obtained from the Western Fish Toxicology Station in Corvallis, Oregon in order to compare the response of these rainbow trout to our own. The rainbow trout (150) were transported in two 50 litre polyethylene containers by truck. Total transportation time was about 24 hours and only 1 fish died in transit. Continuous flow bioassays (Table 5) were initiated between April 19 and 23, 1984 using the rainbow trout obtained from Corvallis, rainbow trout from Spring Valley Trout Farm, Langley, B.C., and steelhead from Fraser Valley Trout Hatchery in Abbotsford, B.C. All stocks were acclimated to the same conditions for at least two weeks prior to testing. The tests were carried out in perforated polyethylene enclosures (10 fish per 500 ml enclosure) under identical conditions of temperature (14° ± .5°C) and hardness (24 ± 4 mg/L as $CaCO_3$). Static bioassays (96 H) were done after mixing Corvallis or University of Victoria water with deionized water to achieve the same hardness of 26 mg/L as $CaCO_3$ (Table 6).

RESULTS AND DISCUSSION

Mortality at all concentrations (Table 1) was minimal throughout the experiment at concentrations up to 120 µg Zn, 6 µg Cu, and 0.3 µg Cd/L, but pronounced mortality occurred at 150 degree days post hatch at a concentration of 215 µg Zn, 11 µg Cu and 0.5 µg Cd/L. The hardness during the experiment was 23.7 ± 3.4 mg/L as $CaCO_3$ (mean ± standard deviation, n = 112) and the temperature during the first phase of the experiment (hatch to swimup) ranged between 10.0 and 11.5°C and during the second phase between 11.0 and 14.5°C. Copper concentrations were 114 ± 32% of nominal concentrations and cadmium concentrations were 111 ± 28% based on a ratio of 400:20:1 (Zn:Cu:Cd).

Table 1. Mortality of Spring Valley rainbow trout during exposure to a mixture of metals.

Nominal concentration µg Zn/L	Actual concentration µg Zn/L	Mortality Jan 31 - Feb 29	Mortality Feb 29 - May 21
Control	<5	0/150	1/60
50	65 ± 16[a]	0/150	1/60
100	120 ± 17	0/150	1/60
200	215 ± 27	42/150	3/60

[a]Mean ± standard deviation, n = 107.

Few studies have been carried out which assess the toxicity of a mixture of metals to salmonids and predictions of toxicity are often based on individual metal toxicities. Lloyd (1961) and Sprague and Ramsay (1965) showed that the toxicity of a mixture of zinc and copper to rainbow trout (Salmo gairdneri) or Atlantic salmon (Salmo salar) was additive.

In our own experiments with rainbow trout fry in discrete exposures, 7 day LC50's were 380 µg Zn/L, 50 µg Cu/L and 1.35 µg Cd/L. The LC50 of the mixture of zinc, copper and cadmium in a rato of 400:20:1 was 425 µg Zn, 21 µg Cu and 1.1 µg Cd/L or 2.35 toxic units, showing moderate antagonism. Because of the variability in toxicity due to changes in metal ratios it is essential that water quality criteria be developed on a site and metal ratio-specific basis.

Bioassays conducted with alevins within 48 hours of hatch (Table 2) showed that they were more resistant to metals than at the swim-up stage (300 degree days post hatch). Fry that had been exposed to metals until swim-up stage had acclimated to the metal mixture. Resistance was correlated to the concentration of metals to which they had previously been exposed.

Table 2. Resistance to metals during development.

Stage	Degree days	LC50 (95% CI)	Temperature	Hardness
Alevin	20	490 (420-570)[a]	12 ± 1°C	25 ± 2
Swim-up fry				
(control)	300	225 (205-248)[a]	12 ± 1°C	25 ± 2
(50 µg Zn/L)	300	370 (336-407)[a]	12 ± 1°C	25 ± 2
(100 µg Zn/L)	300	480 (429-538)[a]	12 ± 1°C	25 ± 2
Fry	940	390 (358-425)[a]	12 ± 1°C	26 ± 1
Fry	940	310 (270-360)[b]	13 ± 1°C	22 ± 1

[a]Static. (96 H)
[b]Flow through. (96 H)

Metallothionein concentrations in the livers of the rainbow trout after 16 weeks of exposure from hatch (Table 3) showed a significant correlation with the metal concentrations in the water ($r = 0.947$, $P < 0.01$, $n = 41$).

Table 3. Hepatic metallothionein in rainbow trout exposed to a mixture of metals (Zu:Cu:Cd: = 400:20:1) for 16 weeks.

Metal concentration µg Zn/L	Hepatic metallothionein mean ± standard deviation nmol/g	
<5	48.4 ± 11.5	n = 10
65	90.6 ± 19.6**	n = 10
120	123.6 ± 24.7**	n = 10
215	201.7 ± 21.4**	n = 11

**Significantly different from control ($P<0.01$).

The acclimation to metals that is evident at the swim-up stage is likely due to enhanced rates of metallothionein synthesis by those fish that have been exposed to metals. Experiments using ^{35}S-cysteine to measure the rate of metallothionein synthesis in coho salmon (Oncorhynchus kisutch) have shown that the rate is dependent on the concentration of metal in the water (McCarter and Roch, 1984). Although the quantity of tissue available in this experiment was not sufficient for protein fractionation, experiments with larger rainbow trout exposed to the same mixture of metals

have shown that the metallothionein is primarily copper-thionein (Roch and McCarter, 1984a) even though zinc concentrations in the water are 20 times the concentrations of copper. Ley et al. 1983 have reported high levels of copper-thionein in rainbow trout. Elevations in the copper content of the low molecular weight protein are accompanied by elevation in copper content of the high molecular weight proteins in the livers of both wild and laboratory exposed trout (Roch et al., 1982; Roch and McCarter, 1984a).

Growth of the rainbow trout was significantly ($P < 0.05$) retarded at the highest concentration (215 µg Zn, 11 µg Cu and 0.5 µg Cd/L) after 12 weeks of feeding and a total exposure of 16 weeks (Table 4). The variation in weight also increased with concentration as evident in the standard deviation and range.

Table 4. Weight and length of rainbow trout exposed to a mixture of metals for 16 weeks.

Concentration	Wet weight (g)	Range	Length (cm)
<5 µg Zn/L	1.31 ± 0.35, n = 27	0.84 - 2.10	5.20 ± 0.37
65 µg Zn/L	1.44 ± 0.30, n = 28	0.97 - 1.88	4.98 ± 0.40
120 µg Zn/L	1.11 ± 0.48, n = 30	0.47 - 2.40	4.63 ± 0.61
215 µg Zn/L	1.07 ± 0.56, n = 41*	0.34 - 2.49	4.52 ± 0.85

*Significantly different from control ($P<0.05$).

Our own experiments with chinook salmon, Oncorhynchus tshawytscha (Roch and McCarter, 1984c) and those of Finlayson and Verrue (1980) demonstrated that reduced growth is a very sensitive measure of the toxicity of a mixture of metals to this species. Significant reductions in growth were apparent at a concentration 0.15 of the LC50 for swim-up chinook salmon. Rainbow trout do not seem to be as sensitive in this respect; growth retardation was not detectable at a concentration 0.5 of the LC50 for swim-up rainbow trout.

Continuous flow bioassays conducted with various stocks of rainbow trout showed variable sensitivity to a mixture of metals (Zn:Cu:Cd =400:20:1) among the different groups (Table 5). Although it was not possible to obtain different stocks at the same stage of development, Spring Valley rainbow trout may be more resistant than the Corvallis rainbow trout at approximately the same stage of development. The steelhead stock was close to the swim-up stage and was probably more sensitive for this reason.

Table 5. 96 H LC50 of Salmo gairdneri from different origins.

Stock origin	Degree days after hatch	LC50 µg Zn/L	95% CI
Spring Valley - rainbow trout	940	310	267 - 360
Corvallis - rainbow trout	790	140	120 - 164
Fraser Valley - steelhead	405	180	160 - 210

[a]Zinc, copper cadmium, 400:20:1.

A comparison of metal toxicity in University of Victoria water and Corvallis water using static bioassays showed no difference in toxicity when the same stock was used, Zn:Cu:Cd = 400:20:1, (Table 6) and the water was made up to the same hardness.

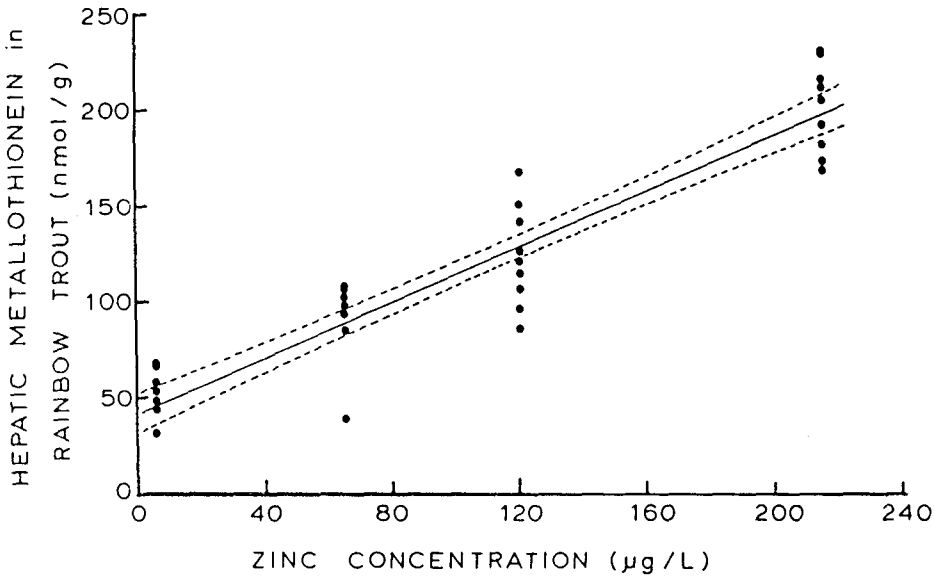

Figure 1. Hepatic metallothionein concentrations of rainbow trout exposed to a mixture of metals (Zn:Cu:Cd = 400:20:1) for 16 weeks after hatch. The dotted lines are 95% confidence limits of the regression line.

Table 6. Static bioassays carried out at University of Victoria.

Stock	Water	96 H LC50 (95% CI)	Hardness (T = 12 ± 1°C)
Fraser Valley Steelhead Wt. 0.18 ± 0.02	Corvallis	160 (127 - 202)	26.4 ± 0.7 n=5
	U. Victoria	160 (133 - 182)	26.5 ± 0.5 n=5
Corvallis Rainbow trout Wt. 0.46 ± 0.15	Corvallis	200 (168 - 238)	26.4 ± 0.2 n=5
	U. Victoria	210 (174 - 254)	26.0 ± 1.0 n=5
Spring Valley Rainbow trout Wt. 0.31 ± 0.10 g	Corvallis	400 (350 -456)	26.3 ± 0.5 n=5
	U. Victoria	390 (358 - 425)	26.6 ± 0.2 n=5

The results show that there is considerable variability in response depending on the source of the rainbow trout and the stage of development but that the differences are not due to water quality.

In terms of survival and growth of the rainbow trout used in this experiment, 120 µg Zn, 6 µg Cu and 0.3 µg Cd/L must be considered as safe if the fish are exposed continuously from hatch. However, the fry exposed for 16 weeks to 65 µg Zn, 3.3 µg Cu and <0.2 µg Cd/L showed significantly elevated hepatic metallothionein concentrations. A computer plot using the SAS program (SAS Institute, Box 8000 Cary, N.C., U.S.A. 27511) shows the regression line and 95% confidence limits of hepatic metallothionein in rainbow trout as a function of concentration of metals in the water (Figure 1).

It is likely that a measureable induction of hepatic metallothionein occurs at even lower levels than the 65 µg Zn, 3.3 µg Cu and <0.2 µg Cd/L concentration examined here, indicating a biological effect but one of questionable significance at the population level.

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REFERENCES

Chapman GA (1978) Toxicities of cadmium, copper and zinc to four juvenile stages of chinook salmon and steelhead. Trans Am Fish Soc 107:841-847

Clark MJR, Morrison TO (1982) Impact of the Westmin Resources Ltd. mining operation on Buttle Lake and the Campbell River watershed. BC Ministry of Environment

Finlayson BJ, Verrue KM (1980) Estimated safe zinc and copper levels for chinook salmon, Oncorhynchus tshawytscha in the Upper Sacramento River, California. Calif Fish Game 66:68-82

Kägi JHR, Nordberg M (1979) Metallothionein. Birkhauser Verlag, Basel

Ley HL, Failla ML, Cherry DS (1983) Isolation and characterization of hepatic metallothionein from rainbow trout (Salmo gairdneri). Comp Biochem Physiol 74B:507-513

Litchfield JT, Wilcoxon F (1949) A simplified method of evaluating dose-effect experiments. J Pharmac Exp Ther 96:99-113

Lloyd R (1961) The toxicity of mixtures of zinc and copper sulphates to rainbow trout (Salmo gairdneri Richardson) Ann Appl Biol 49:535-538

McCarter JA, Roch M (1984) Chronic exposure of coho salmon to sublethal concentrations of copper III. Kinetics of metabolism of metallothionein. Comp Biochem Physiol 77C:83-87

Olafson RW, Sim R (1979) An electrochemical approach to quantitation and characterization of metallothioneins. Anal Biochem 100:343-351

Roch M, McCarter JA, Matheson AT, Clark MJR, Olafson RW (1982) Hepatic metallothionein in rainbow trout (Salmo gairdneri) as an indicator of metal pollution in the Campbell River system. Can J Fish Aquat Sci 39:1596-1601

Roch M, McCarter JA (1984a) Hepatic metallothionein production and resistance to heavy metals by rainbow trout (Salmo gairdneri) I. Exposed to an artificial mixture of zinc, copper and cadmium. Comp Biochem Physiol 77C:71-75

Roch M, McCarter JA (1984b) Hepatic metallothionein production and resistance to heavy metals by rainbow trout (Salmo gairdneri) II. Held in a series of contaminated lakes. Comp Biochem Physiol 77C:77-82

Roch M, McCarter JA (1984c) Metallothionein induction, growth, and survival of chinook salmon exposed to zinc, copper and cadmium. Bull Environ Contam Toxicol 32:478-485

Sprague JB (1973) The ABC's of pollutant bioassay using fish. In (J Cairns Jr, KL Dickson, eds) Biological methods for the assessment of water quality. ASTM STP 528. American Society for Testing and Materials. Philadelphia, Pennsylvania, pp 3-60

Sprague JB, Ramsay BA (1965) Lethal levels of mixed copper-zinc solutions for juvenile salmon. J Fish Res Board Can 22:425-432
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