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# Metallothionein Induction, Growth, and Survival of Chinook Salmon Exposed to Zinc, Copper, and Cadmium

M. Roch and J. A. McCarter

Department of Biochemistry and Microbiology, University of Victoria, Victoria, B.C. V8W 2Y2, Canada

Resident salmon in the Campbell River are exposed to heavy metal contamination which originates from the Westmin Resources Ltd. mine along Myra Creek near the south end of Buttle Lake. In addition, salmon raised by the Quinsam River hatchery must pass through the Campbell River during their migration to salt water.

Clark and Morrison (1982) identified zinc, copper, cadmium and lead as the metals in Buttle Lake which might have reached concentrations that are toxic to aquatic organisms. Zinc, copper and cadmium have been shown to induce hepatic metallothionein in mammals (Kägi and Nordberg 1978) and in fish (Overnell and Coombs 1979, McCarter et al. 1982). Metal contamination in Buttle Lake and waters downstream has resulted in elevated concentrations of hepatic metallothionein in wild (Roch et al. 1982) and caged rainbow trout (Roch and McCarter, in press).

Alderdice and McLean (1982) identified chinook salmon as being at greater acute risk than coho salmon when they are exposed to a combination of zinc, copper and cadmium. Zinc and copper have also been shown to elicit an avoidance response at very low concentrations (Sprague 1965), to interfere with salt water adaptation and to inhibit downstream migration of coho salmon (Lorz and McPherson 1976) and upstream migration of adult Atlantic salmon (Saunders and Sprague 1967).

In order to assess the hazards of metal contamination in the Campbell River to resident and transitory salmonids, chinook salmon eggs obtained from the Quinsam hatchery were exposed to varying degrees of metal contamination to determine survival, effects on growth and hepatic metallothionein concentrations after long term exposure.

## MATERIALS AND METHODS

Chinook salmon (Oncorhynchus tshawytscha) eggs (952) were obtained from the Quinsam hatchery on January 7, 1983 at 460 degree-days after fertilization. The eggs were transported to the University of Victoria toxicology laboratory in a styrofoam container holding ice covered with moss. The eggs were placed on the moss and covered with wet cloths.

The eggs were divided among four Heath trays supplied with dechlorinated, deionized city water mixed with well water to provide a soft water representative of the Campbell River (20-25 mg/L as $CaCO_3$). This water which contained less than 5 µg Zn, 1 µg Cu and 0.5 µg Cd/L, was used as the control and a mixture of zinc, copper and cadmium in a ratio of 400:20:1 was added by peristaltic pump to simulate metal contamination. One of the trays received water from the entrance to John Hart Dam which empties into the Campbell River. This water was transported and stored in polyethylene containers. All water sources were pumped through glass condenser coils in a water bath refrigerated by a chiller to maintain a constant temperature of 10 ± 1°C during the exposure. John Hart Lake water was used throughout the 8 week alevin exposure. When the alevins had completely buttoned-up, the four groups (35 each) were transferred to 20 litre tanks receiving a water flow of 100 ml/min and the group that had been exposed to John Hart Lake water was exposed to a combination of metals simulating that of John Hart Lake for the remaining 13 weeks of the experiment. They were fed at a rate of 2% per day following transfer with mash and 1/32 inch pellet obtained from the Quinsam hatchery.

Heavy metal concentrations in stock solutions were measured by flame atomic absorption spectrophotometry (AAS) and adjusted to within 5% of a Zn:Cu:Cd ratio of 400:20:1. The metal exposure was monitored by measuring zinc concentrations daily and adjusting dilution rates to achieve nominal concentrations. Hardness was measured daily and the well-deionized water mix was maintained between 20 and 25 mg/L as $CaCO_3$.

Static 96 h bioassays were carried out in 15 litre polyethylene containers with appropriate additions of stock solution to a mixture of well and deionized water (23 ± 2 mg/L as $CaCO_3$). Zinc was measured by flame AAS, copper and cadmium by graphite furnace using a Varian AA475. Copper concentrations were 98 ± 31% (mean ± standard deviation) of nominal values and cadmium was 96 ± 23%. All concentrations reported refer to total metal. The 96 h LC50 was determined using 10 alevins per concentration and 95% confidence limits were determined according to Litchfield and Wilcoxon (1949).

Metallothionein concentrations were determined by (a) homogenizing whole alevins (320 degree-days post hatch), and (b) homogenizing 5 or 6 pooled livers (21 weeks post hatch) in ice-cold 0.9% NaCl. The volume was adjusted to 5 ml, the sample was heated at 85°C for 5 min, then 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.).

Chinook smolts (65) were transported to the University of Victoria from the Quinsam hatchery on June 2, 1983 in a 50 litre polyethylene carboy supplied with oxygen and immersed in ice water. The

smolts were allowed to acclimate to a metal free soft water (25 mg/L as $CaCO_3$) for 3 days prior to addition of zinc, copper and cadmium in a ratio of 400:20:1. Twelve smolts were exposed to (a) metal-free water, (b) metal concentrations representative of John Hart Lake, and (c) metal concentrations 3-fold those of John Hart Lake at 14 ± 1°C for seven days.

Following metal exposure each group of smolts was transferred to a 50 litre polyethylene container of sea water obtained from Cattle Point, Oak Bay. The sea water was diluted to 28 $^{0}/_{00}$ salinity with deionized water using a hydrometer to determine salinity. The smolts were exposed for 24 h as described by Clarke and Blackburn (1977) at 11 ± 1°C. Blood was then collected in ammonium-heparinized capillary tubes after severing the caudal peduncle. The blood was centrifuged at 11,000 rpm for 2 min, 5 µl of plasma was drawn off and diluted to 5.0 ml with distilled water. Plasma sodium concentrations were measured by flame emission at 590 nanometers and concentrations were verified using a 140 mmole Na/L flame photometer standard.

Chinook salmon smolts, tagged at the Quinsam hatchery with metal wire inserts in the nose and removal of the adipose fin were released from the hatchery on 5 May 1982. Fish were caught in seine nets in the estuary of Campbell River on 7, 10 and 17 of May. The fish were immediately frozen on dry ice and sent to the University of Victoria. The livers were excised without allowing the fish to thaw and metallothionein values were determined as described above. One control group of fish was taken on 3 May before release. Another was exposed to running sea water for 7 days at 11°C in Pacific Biological Station, Nanaimo, B.C.

## RESULTS AND DISCUSSION

Bioassays conducted after hatch, four weeks post hatch and 8 weeks post hatch (Figure 1) show greatest metal tolerance after hatch, least tolerance 4 weeks after hatch and a slight increase in tolerance at the button-up stage. The alevins that were exposed to metals showed some enhanced tolerance after 4 weeks and again at 8 weeks. Survival at concentrations up to 232 µg Zn, 12 µg Cu and 0.6 µg Cd/L did not differ from the controls (Table 1) from hatch to button-up nor for the remainder of the 21 week exposure.

Table 1. Mortality during 8 weeks (620 degree days) after hatch of juvenile chinook salmon exposed to the concentrations of metals shown.

| Metal concentration (µg/L) (mean ± standard deviation, n = 38) | | | |
|---|---|---|---|
| Zn | Cu | Cd | % Mortality |
| 232 ± 56 | 11.6 ± 2.8 | 0.6 | 7 |
| 135 ± 41 | 6.8 ± 2.1 | <0.5 | 8 |
| 43 ± 7 | 1.9 ± 1.0 | <0.5 | 6 |
| <5 | <1 | <0.5 | 6 |

During the last two days of the experiment a number of fish died in the tank designed to simulate metal concentrations in John Hart Lake. The reason for the mortality could not be determined and may have been due to a disease which infected this group. As much higher concentrations of metals did not result in mortality it is unlikely to be due to metal exposure.

The combination of metals did not have a significant effect on dry or wet weight during the first four weeks after hatch (Table 2) but had a pronounced effect on the growth of chinook fry (Figure 2) over the 21 week exposure and a significant reduction ($P < 0.05$) was apparent at a mean concentration as low as 50 µg Zn, 2.5 µg Cu and <0.5 µg Cd/L, which was approximately one fourth of the highest concentration which did not result in greater mortality than the control. Long term growth therefore appears to be a very sensitive measure of the deleterious effect of this mixture of heavy metals.

Table 2. Wet and dry weights of chinook alevins exposed to metals for 4 weeks (310 degree days) after hatch.

| Zn:Cu:Cd = 400:20:1 | | |
|---|---|---|
| [Zn] | Wet weight (g) (n = 10) | Dry weight (g) |
| 249 ± 61 | .3344 ± .0314 | .0825 ± .0053 |
| 137 ± 38 | .3477 ± .0328 | .0807 ± .0059 |
| 42 ± 7 | .3292 ± .0235 | .0777 ± .0058 |
| <5 | .3594 ± .0170 | .0814 ± .0045 |

Metallothionein concentrations in whole alevins after four weeks of metal exposure (Table 3a) do not show an increase because the synthesis of metallothionein occurs primarily in the liver which is only 1% of the body weight of the fish. Hepatic metallothionein concentrations in chinook fry showed marked elevations in proportion to the metal concentrations to which the fish were exposed for 21 weeks (Table 3b). These extremely elevated concentrations reflect a marked biological response to the high concentrations of metals which are entering the fish (Table 4) a response which is amplified at higher concentrations because of the salmon's inability to grow.

Table 3. Metallothionein (MT) concentrations in (a) whole alevins after 4 weeks of exposure to metals after hatch, (b) livers of chinook salmon exposed for 21 weeks to a combination of metals (Zn:Cu:Cd = 400:20:1). Results are expressed in microamperes of polarographic activity per gram of liver.

| (a) [Zn] µg/L $\bar{x}$ ± S.D. n = 20 | MT in whole alevins $\bar{x}$ ± S.D. n = 5 | (b) [Zn] µg/L $\bar{x}$ ± S.D. n = 112 | MT in liver $\bar{x}$ ± S.D. | |
|---|---|---|---|---|
| 249 ± 61 | 57 ± 18 | 213 ± 53 | 1176 ± 102 | n = 5 |
| 137 ± 38 | 80 ± 14 | 128 ± 35 | 841 ± 102 | n = 5 |
| 42 ± 7 | 79 ± 20 | 51 ± 22 | 200 ± 105 | n = 2 |
| <5 | 72 ± 26 | <5 | 181 ± 37 | n = 5 |

Table 4. Metal concentrations in chinook salmon liver after 21 weeks of exposure to metals (Zn:Cu:Cd = 400:20:1).

| [Zn] in water | Concentrations of metals in liver (µg/g wet weight) Zn | Cu | Cd | |
|---|---|---|---|---|
| <5 | 52 ± 7 | 18 ± 2 | 0.4 ± 0.3 | n = 5 |
| 51 ± 22 | 59 ± 22 | 33 ± 20 | 0.8 ± 0.6 | n = 2 |
| 128 ± 35 | 110 ± 15 | 117 ± 12 | 3.9 ± 1.5 | n = 5 |
| 213 ± 53 | 144 ± 14 | 148 ± 17 | 2.8 ± 1.1 | n = 5 |

Chinook smolts exposed to metals for one week and faced with a salt water challenge did not have higher plasma sodium levels than the controls (Table 5) indicating that the metal exposure does not markedly reduce their ability to adapt to salt water. The mean plasma sodium concentrations are approximately 10% higher than the values obtained by John Blackburn at the Nanaimo Biological Station for the same group of fish and some mortality occurred during the metal exposure and salt water challenge in both exposed and control fish. The mortality may be due to the stress of the lengthy transport of fish from the Quinsam Hatchery to Victoria and may have contributed to the higher plasma sodium concentrations which we observed.

In Table 6, it can be seen that concentrations of metallothionein in livers of chinook salmon released from Quinsam hatchery were not significantly elevated during passage of the fish from the hatchery through the polluted waters of Campbell River and residence in the estuary. The concentrations of zinc and copper in the Campbell River were 36 ± 5 and 1.4 ± 0.4 µg/L respectively (mean ± standard deviation, n = 6) during January to April 1982 (Alderdice and McLean 1982). The metallothionein values of fish

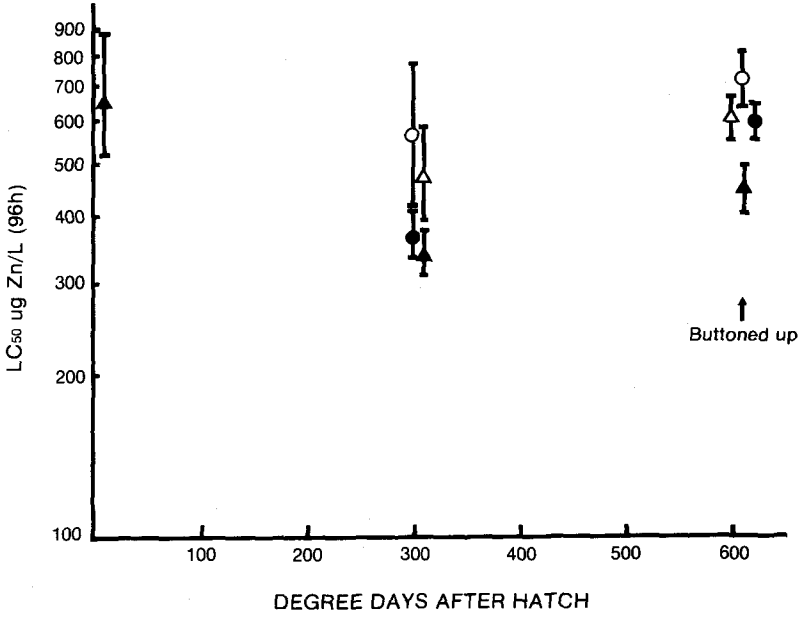


Figure 1. LC50 (96h) and 95% confidence interval of a mixture of zinc, copper and cadmium (Zn:Cu:Cd = 400:20:1) during chinook alevin development in (▲) metal-free water (●) John Hart Lake water, (△) a metal mixture approximately 2× the concentration of John Hart Lake, (○) 4× John Hart Lake.

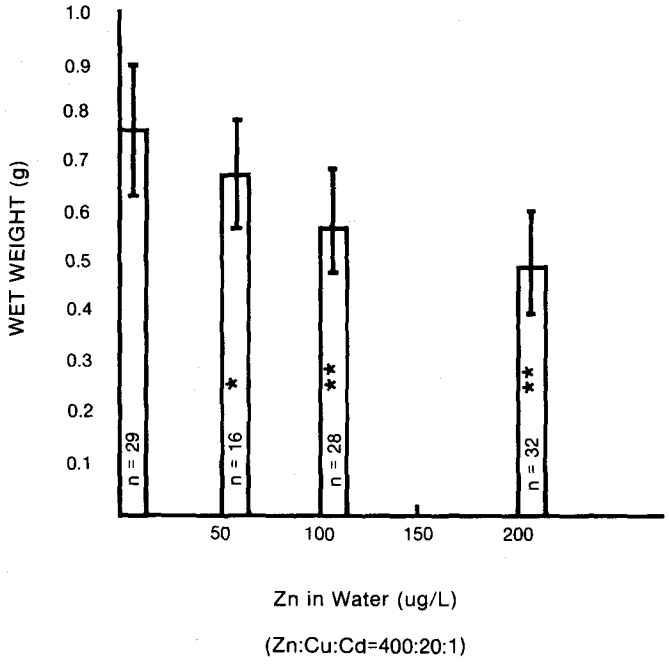


Figure 2. Mean wet weight and 95% confidence interval of chinook salmon exposed to a combination of zinc, copper and cadmium (400:20:1) for 21 weeks after hatch. *significantly less than the control (p 0.05), **(p 0.01).

released from the hatchery may be compared with those in Table 3 which show marked elevations in response to exposure of fish to metals in the laboratory for 21 weeks. These data suggest that fish released from the hatchery are not adversely affected by metals in the water of Campbell River.

Table 6. Hepatic metallothionein in tagged chinook salmon prior to release from Quinsam hatchery compared with fish caught in the estuary of Campbell River at known times after release or after exposure for 7 days to sea water.

| Treatment | | n | Fish weight (g) | Fork length (cm) | Metallothionein (μA/g) |
|---|---|---|---|---|---|
| None | 3 May 82 | 9 | 4.5 ± 0.3 | 7.6 ± 0.2 | 37.7 ± 7.4 |
| Sea water | 7 days | 10 | 4.4 ± 1.8 | 7.6 ± 1.1 | 58.8 ± 17 |
| Released | 5 May 82 | | | | |
| Caught | 7 May 82 | 19 | 3.2 ± 1.0 | 6.7 ± 0.7 | 23.6 ± 6.0 |
| | 10 May 82 | 20 | 3.3 ± 0.8 | 6.5 ± 0.6 | 28.7 ± 17 |
| | 17 May 82 | 13 | 4.6 ± 2.2 | 7.5 ± 0.9 | 40.6 ± 11.4 |
| | 9 Nov 82 | 4 | 63.5 ± 16 | 16.5 ± 1.6 | 47.2 ± 6.6 |

Concentrations of metals in the Campbell River appear to be safe for resident chinook salmon during the sensitive developmental stages but may have a marginal effect on growth. Concentrations should not be allowed to exceed 50 μg Zn, 2.5 μg Cu and <0.5 μg Cd/L as a mixture.

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