INTERNATIONAL PACIFIC SALMON
FISHERIES COMMISSION

PROGRESS REPORT

No. 39

EFFECTS OF SELECTED HEAVY METALS ON EARLY LIFE OF SOCKEYE AND PINK SALMON

BY

J. A. SERVIZI and D. W. MARTENS

NEW WESTMINSTER, B.C.
CANADA
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in the Fraser River System

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ABSTRACT

Sockeye and pink salmon were exposed continuously to cadmium, copper, and mercury under laboratory conditions during the egg to fry stage. Observations of mortality, hatching, development and growth were used to evaluate toxic effects. Acute toxicity was quantified by measurements of median tolerance limit using alevins, fry and smolts.

Mortality, hatching and growth of sockeye salmon during the egg to fry stage were not affected by continuous exposure to 5.7 µg/l cadmium. However, the 168 hr LC50 for first feeding fry was 8 µg/l cadmium.

When exposed to copper, the incipient lethal level was between 37 and 78 for sockeye but between 25 and 55 µg/l for pink salmon during the egg to fry stage. Growth and hatching were no better than mortality as indicators of toxic effects of copper. Copper inhibited egg capsule softening, but associated mortalities during hatching occurred only at concentrations also lethal to eggs and alevins. Copper was concentrated by eggs, alevins and fry in proportion to exposure concentrations. Copper concentrations of 105 and 6.8 ppm in pink salmon eyed eggs and fry, respectively, coincided with mortalities.

Continuous exposure of eggs to 2.5 µg/l mercury caused malformed embryos at hatching. Mortality, growth and hatching were less sensitive to mercury than was malformation. Mercury was concentrated by sockeye and pink salmon in proportion to exposure concentration during the egg to fry stage. Mercury concentrations of 1.87 ppm in eyed eggs coincided with malformed embryos at hatching.

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INTRODUCTION

Heavy metals may enter the aquatic environment for natural and man-caused reasons. Mining, ore-processing, metal finishing and a variety of industrial processes are potential sources of heavy metals in the aquatic environment. These activities raise the possibility that sockeye (Oncorhynchus nerka) and pink salmon (O. gorbuscha) will be exposed to various concentrations of heavy metals. This study measured the effects of copper, cadmium and mercury on selected phases of the freshwater life of sockeye and pink salmon.

METHODS

Water Supply

The control and diluent water supplies were pumped to the laboratory from Cultus Lake via wood stave, plastic, glass and cast iron plumbing.

Filterable residue, alkalinity, hardness, conductivity, carbon dioxide, sulfates, nitrates and nitrites were measured according to Standard Methods (1965). Soluble organic carbon was measured courtesy of the Canada Fisheries Research Board using the Method of Menzel and Vaccaro (1964).

Fish Stocks

Sockeye used in bioassays included hatchery produced alevins and fry from Cultus Lake race, wild smolts captured at a counting fence at the outlet of Cultus Lake, and hatchery produced smolts of the Chilko Lake race. Pink salmon alevins and fry were from the Sweltzer Creek and Seton Creek races. All hatchery fish were produced at Sweltzer Creek Laboratory.

Acute Toxicity

Acute bioassays of copper were of four days (96 hr) duration, but seven day (168 hr) exposures were used during bioassays of cadmium and mercury, since significant mortalities continued beyond 96 hours when fish were exposed to the latter two metals. Acute bioassays were of the continuous flow design, using proportional diluters (Mount and Brungs, 1967) to supply five concentrations of either copper, mercury or cadmium and control water to bioassay chambers. Alevins and fry or smolts were bioassayed in 1.2 or 10 ℓ polyethylene aquaria, respectively. Flows to each aquarium were 0.33 l/min

providing a 95 percent replacement time of 1.5 hours or less (Sprague, 1973). Temperatures were adjusted to suit the natural temperature of the test fish at each stage of development and ranged from 5.5 to 8.8°C. Dissolved oxygen was maintained at saturation without supplemental aeration owing to the constant flow system used.

Copper, cadmium and mercury were supplied from reagent grade $CuSO_4 \cdot 5H_2O$, $CdCl_2$ and $HgSO_4$, respectively. Stock solutions were made in distilled water and lasted two to three days. Stock solutions of mercuric sulfate were acidified with 5 to 10 ml hydrochloric acid per 18 liters of stock.

Each diluter was calibrated using copper to compare actual and nominal concentrations. Copper concentrations were measured by the dibenzyldithiocarbamate colorimetric method (Abbott and Harris, 1962).

Acute toxicity was quantified by determining the 96 or 168 hr LC50's from observations of mortality (Davis and Mason, 1973).

Exposure from Egg to Fry

Sensitivity during the egg to fry life stage was determined by continuous exposure to heavy metals. Following fertilization, water hardening and transfer to Sweltzer Creek Laboratory, eggs were placed in continuous up-flow incubators supplied with water from proportional diluters calibrated as described above.

Three hundred sockeye salmon eggs were placed in each of four 1.35 ℓ incubators at each of five concentrations of metal and in control water. Owing to unavoidable delays, copper, cadmium and mercury were not added to the water until four, eleven and two days, respectively, after fertilization. Water flow to each incubator was initially set at 32 ml/min but was increased to 65 ml/min to maintain dissolved oxygen near saturation throughout the study. The initial flow was sufficient to maintain a 95% replacement time equal to about 1.5 hours (Sprague, 1973).

Five hundred and eighty pink salmon eggs were placed in 5.5 ℓ incubators at each of five concentrations of copper and mercury and in control water. Exposure to metals commenced within 24 hours of fertilization and was terminated when the swim-up fry stage was reached. Water flow to each incubator was 500 ml/min, equivalent to a 95% replacement time of less than one hour.

Water temperatures used during incubation were similar to those normally experienced in the natural environment and ranged from a maximum of about 8.8°C to a minimum of about 5.5°C.

Incubators were examined five days per week and dead eggs or alevins removed and recorded. Samples of 20 or more alevins and swim-up fry were preserved in 10% formalin for measurement of fork length. Samples of eggs and swim-up fry were frozen in 1% HCl for later analysis of copper and mercury content. In addition, eggs, alevins and fry obtained from various natural spawning areas were analyzed for copper and mercury content. Two and five specimens from each sample were measured for copper and mercury content, respectively. Copper was measured by Fisheries and Environment Canada, West Vancouver Laboratory, while mercury was measured at the Fish Inspection Laboratory, Environment Canada.

RESULTS

Characteristics of Dilution Water

Since the constituents in water may affect the toxicity of metals to fish, various characteristics of the water supply from Cultus Lake were measured (Table 1). The water can be characterized as medium hard with moderate buffering capacity.

Table 1. Characteristics of Sweltzer Creek Laboratory water supply from Cultus Lake.

Characteristic	Range	Mean
Filterable residue, mg/l	89.5 -101.0	95.4
pH	7.55- 7.80	7.63
Alkalinity, mg/l $CaCO_3$	60.6 - 68.2	62.5
Hardness, mg/l $CaCO_3$	82.9 - 84.4	83.1
Conductivity, µmho/cm	160 -171	167
CO_2, mg/l	2.7 - 5.0	4.2
Soluble Organic Carbon, mg/l	1.74- 3.72	2.58
Sulfate (SO_4) mg/l	23.2 - 27.0	25.1
Nitrate (NO_3-N) mg/l	0.1	0.1
Nitrite (NO_2-N) mg/l	0.1	0.1

Acute Toxicity

Cadmium

The 168 hr LC50 for sockeye salmon exposed to cadmium decreased from 4,500 to 8 µg/l as development proceeded from the alevin to fry stage (Table 2). The lowest concentration of cadmium at which mortalities of sockeye occurred during acute bioassays was 5.6 µg/l when fry were tested at 2,114 degree-days. Sockeye smolts captured during emigration from Cultus Lake were more tolerant than fry.

The 168 hr LC50 for pink salmon alevins exposed to cadmium was 3,650 µg/l but unlike sockeye, there was little decrease in the LC50 as alevins advanced to the fry stage.

Signs of distress noted among alevins, fry and smolts during acute bioassays of cadmium included frequent twitching of the body musculature. When frightened, fish displaying this symptom would swim about the test chamber in a frenzy, lose equilibrium and die within minutes. Death was accompanied by tetanization of the lateral body musculature.

Table 2. Acute toxicity of cadmium to sockeye and pink salmon during 168 hr bioassays.

Life Stage	Av. Wgt. gm	Temp. Units degree-days °F	Cadmium 168 hr LC50, µg/l	
			Sockeye	Pink
Alevin, newly hatched		938		3,650
Alevin, newly hatched		1,080	4,500	
Alevin		1,290		3,160
Alevin		1,423	1,000	
Alevin		1,517	500	
Fry		1,663		2,700
Fry	0.14	1,771	30	
Fry	0.20	2,114	8	
Smolt	5.5		360	

Copper

Sockeye alevin 96 hr LC50's varied with development, commencing at 190 µg/l for newly hatched alevins, decreasing to 100 µg/l mid-way through the alevin stage and increasing to 150 µg/l when the fry stage was reached (Table 3). Sockeye smolt 96 hr LC50's ranged between 170 and 240 µg/l.

Table 3. Acute toxicity of copper to sockeye and pink salmon during 96 hr bioassays.

Life Stage	Av. Wgt. gm	Temp. Units degree-days °F	Copper 96 hr LC50, µg/l	
			Sockeye	Pink
Alevin, newly hatched		1,016		143
Alevin, newly hatched		1,095	190	
Alevin		1,145	200	
Alevin		1,178		87
Alevin		1,350	100	
Alevin		1,391	110	
Alevin		1,545	130	
Fry		1,610		199
Fry		1,771	150	
Smolt	5.5		210	
Smolt	5.5		170	
Smolt	5.5		190	
Smolt	4.8		240	

The tolerance of pink salmon alevins to acute toxicity of copper followed a pattern similar to that for sockeye, with the minimum 96 hr LC50 equal to 87 µg/l occurring part way through the alevin stage.

Greatest sensitivity of sockeye and pink salmon occurred during the alevin stage when the lowest lethal concentrations were 95 and 80 µg/l, respectively.

Mercury

The 168 hr LC50 for sockeye alevins exposed to mercury remained relatively constant with a mean value of 290 µg/l (Table 4). Sockeye fry and smolts were about equally tolerant with an average 168 hr LC50 of 190 µg/l mercury.

The 168 hr LC50 for pink salmon alevins exposed to mercury ranged between 140 and 220 µg/l, which was consistently lower than the value for sockeye alevins.

Table 4. Acute toxicity of mercury to sockeye and pink salmon during 168 hr bioassays.

Life Stage	Av. Wgt. gm	Temp. Units degree-days °F	Mercury 168 hr LC50, ug/l	
			Sockeye	Pink
Alevins, newly hatched		985		220
Alevins, newly hatched		1,071	280	
Alevin		1,162	310	
Alevin		1,229		140
Alevin		1,333	280	
Alevin		1,379		140
Alevin		1,491	300	
Alevin		1,566		210
Fry	0.14	1,771	180	
Smolt	5.5		220	
Smolt	15.4		180	

Egg to Fry Exposure

Cadmium

Cadmium concentrations selected for study apparently had no toxic effect on eggs since mortalities and degree-days to hatching were similar to those in controls (Table 5). Newly hatched alevins appeared to have normal pigmentation, fin development and yolk-sac shape. Although mortalities of alevins increased coincident with cadmium concentration, the mortalities were considered in the normal range and were similar to those experienced by the copper and mercury control groups. Further study would be required to determine the levels of cadmium lethal to eggs and alevins during continuous exposure.

The growth of alevins from hatching to yolk absorption was not affected since control and experimental groups were of similar length at hatching and yolk absorption (Table 6).

Table 5. Mortality and degree-days to 50% hatch for sockeye eggs and alevins exposed continuously to cadmium.

Cadmium µg/l	Mortality, Percent			Degree-Days to 50% Hatch °F
	Egg	Alevin	Egg-to-Fry	
5.7	3.2	1.8	4.6	1,106
3.3	3.3	1.3	4.4	1,099
1.5	3.4	0.9	4.2	1,103
0.9	3.5	0.7	4.0	1,105
0.4	2.3	0.4	2.6	1,106
Control	3.7	0.2	3.8	1,089

Table 6. Length of sockeye alevins incubated at various concentrations of cadmium.

Cadmium µg/l	Length, mm	
	Hatching	Yolk Absorption
5.7	18.7	26.0
3.3	18.5	25.8
1.5	18.5	25.8
0.9	18.6	25.7
0.4	18.6	25.9
Control	18.5	26.0

Copper

Copper was lethal to sockeye eggs at concentrations of 78 µg/l and greater, but was not lethal at concentrations of 37 µg/l and less (Table 7). The original plant of newly fertilized eggs at 174 µg/l experienced 100% mortality but the second plant, using eyed eggs, experienced 85.6% mortality through hatching, indicating that newly fertilized eggs were slightly less resistant to copper than eyed eggs. Alevins were more resistant than eggs to copper, since at 78 µg/l, 48.4% of the eggs died whereas only 3.3% of the alevins succumbed.

Copper was lethal to pink salmon eggs at 55 µg/l but not at 25 µg/l (Table 7). During the alevin stage, pink salmon experienced 42.4% mortality at 55 µg/l but sockeye mortality was only 3.3% at 78 µg/l. Thus, pink salmon alevins were more susceptible to copper toxicity than were sockeye.

The number of degree-days required for 50% hatch of sockeye eggs was unaffected by copper concentrations of 78 µg/l and less (Table 7). However, hatching of pink salmon eggs was delayed about 5 days at 55 µg/l copper.

Table 7. Mortality and degree-days to 50% hatch for sockeye and pink salmon eggs and alevins during continuous exposure to copper. [a]

	Copper µg/l	Mortality, Percent			Degree-Days to 50% Hatch °F
		Egg	Alevin	Egg-to-Fry	
Sockeye Salmon	174	100		100	
	174[b]	85.6	78.4	98.3	
	78	48.4	3.3	50.0	1,128
	37	2.4	1.4	3.8	1,125
	18	1.2	1.0	2.1	1,127
	9	1.3	0.8	2.0	1,132
	Control	1.9	1.5	3.4	1,130
Pink Salmon	103	100		100	
	103[b]	100		100	
	55	38.2	42.4	64.4	1,064
	25	3.0	3.4	6.3	1,003
	13	4.4	1.8	6.1	1,006
	6	3.2	3.4	6.5	1,009
	Control	1.9	3.6	5.4	1,009

[a] Exposure commenced with fertilized, water hardened eggs except where noted.

[b] Exposure commenced with eyed eggs.

Approximately 74% of sockeye alevin mortality at 174 µg/l copper was caused because alevins were trapped in a partially hatched condition with the head and yolk protruding from the egg capsule. Similarly, about 16% of pink salmon alevin mortality at 55 µg/l copper occurred when alevins were unable to shed the egg capsule. Normally the egg capsule softens and breaks away readily upon hatching. However, visual inspection revealed that the capsule retained sufficient rigidity at 174 and 55 µg/l to maintain a spherical shape in spite of the alevin emerging part way. At 78 and 25 µg/l the capsules split enough to permit escape of sockeye and pink salmon alevins, respectively, but the two hemispheres remained joined as though hinged. Copper appeared to affect rigidity of sockeye and pink salmon egg capsules at 18 and 13 µg/l, respectively, but did not influence hatching success.

Examination of alevins upon hatching indicated pigmentation, fin development and yolk-sac shape were identical among experimental and control groups. However, sockeye and pink salmon alevin bodies were significantly shorter than controls at 18 and 6 µg/l copper (Dunnett's procedure, Steel and Torrie, 1960), respectively (Table 8). The difference in body length became insignificant at yolk absorption except for pink salmon exposed to 55 µg/l, where fish were about 13% shorter than the control group. Such a result was not unexpected at 55 µg/l since mortality during the alevin stage was 42.4%.

Table 8. Length of sockeye and pink salmon alevins exposed to copper.

	Copper µg/l	Length, mm	
		Hatching	Yolk Absorption
Sockeye	78	18.0	25.6
	37	18.9	25.4
	18	19.2	25.9
	9	19.4	25.5
	Control	19.6	25.7
Pink	55	18.9	27.8
	25	18.9	31.0
	13	19.4	31.5
	6	20.2	32.0
	Control	20.7	31.8

The copper contents of eyed pink salmon eggs exposed to copper in the laboratory were proportional to exposure concentration (Table 9, Figure 1). Although analyses of copper content were limited by available time to two specimens from each sample, the copper content was virtually identical in each pair except for alevins from the Thompson River. A copper content of 105 ppm in eyed eggs coincided with high mortalities caused by exposure to 55 µg/l copper, whereas mortalities were similar to controls among eggs containing 63 ppm copper. A copper content of 6.8 ppm in pink salmon fry at yolk absorption was associated with mortalities. The copper contents of sockeye and pink salmon eggs and alevins collected from natural environments were 11 ppm or less. Fry from natural environments contained 1 ppm copper at yolk absorption.

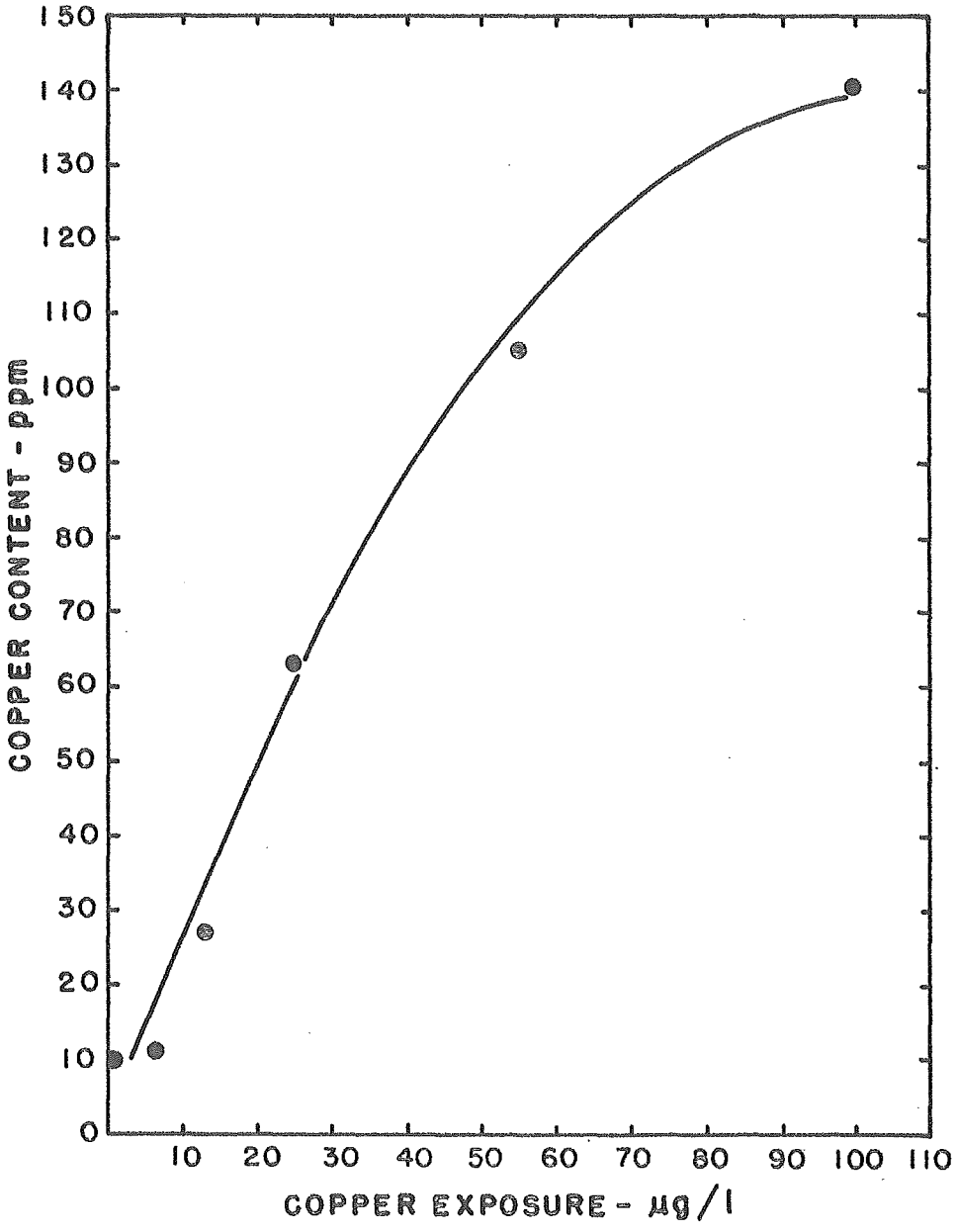

Figure 1. Copper content of eyed pink salmon eggs exposed to copper in the laboratory.

Table 9. Copper content of sockeye and pink salmon collected from natural environments and pink salmon exposed to copper in the laboratory.[a]

	Sample Source or Copper Exposure µg/l	Copper Content, ppm wet wgt.			
		Eggs, Early Development	Eyed Eggs	Alevin	Yolk Absorption
Sockeye	Cultus Lake (Lindell Beach)		9		1
	Weaver Creek		6.3		
Pink	103		140		6.8
	55		105		2
	25		63		1
	13		27		1
	6		11		1
	Control		10		
	Seton Creek	2.9	3		
	Thompson R.			5.2-11	
	Sweltzer Creek	6.4	4.3		1

[a] Samples obtained from laboratory experiments are designated by exposure concentrations.

Mercury

Mercury was highly toxic to sockeye and pink salmon eggs with 100% mortality occurring during the pre-eyed stage at 9.3 and 8.5 µg/l, respectively (Table 10). Mortality was high at 4.3 and 5.2 µg/l for sockeye and pink salmon, respectively, when exposure commenced with freshly fertilized eggs. However, when exposure of sockeye and pink salmon commenced with eyed eggs at 9.3 and 5.2 µg/l mercury, respectively, mortalities were similar to controls in both egg and alevin stages.

There was no significant effect by mercury on degree-days to 50% hatch but malformed embryos were evident among both sockeye and pink salmon. The malformations ranged from slight cranial flexures to coiled bodies. Sockeye embryos incubated at 4.3 µg/l mercury experienced 45.6% malformations (Table 11). At 5.2 µg/l, 81.3% of the pink salmon embryos from the original groups of eggs incubated since being fertilized and water hardened were malformed. Graphical presentation of the data shows a sharp increase in malformations for mercury concentrations exceeding 2.5 µg/l (Figure 2).

Table 10. Mortality and degree-days to 50% hatch for sockeye and pink salmon eggs and alevins during continuous exposure to mercury.[a]

	Mercury µg/l	Mortality, Percent			Degree-Days to 50% Hatch °F
		Egg	Alevin	Egg-to-Fry	
Sockeye Salmon	17.8	100		100	
	17.8[b]	0.6	8.5	9.0	
	9.3	100		100	
	9.3[b]	0.2	4.3	4.3	
	4.3	24.9	8.4	33.7	1,097
	2.5	3.8	0.7	4.7	1,098
	1.0	6.5	0.8	7.3	1,104
	Control	3.6	1.5	5.0	1,098
Pink Salmon	8.5	100		100	
	8.5[b]	0	3.2	3.2	
	5.2	88	100	100	
	5.2[b]	0	1.5	1.5	
	2.5	5.4	5.3	10.4	1,008
	1.0	3.4	3.7	7.0	1,006
	0.5	3.4	3.1	6.3	1,007
	Control	3.4	2.3	5.6	1,010

[a] Exposure commenced with fertilized, water hardened eggs, except where noted.

[b] Exposure commenced with eyed eggs.

Table 11. Malformed sockeye and pink salmon embryos at hatching following continuous exposure to mercury since fertilization.[a]

Sockeye Salmon		Pink Salmon	
Mercury µg/l	Malformations Percent	Mercury µg/l	Malformations Percent
17.8[b]	35.3	8.5[b]	92.3
9.3[b]	1.7	5.2	81.3
4.3	45.6	5.2[b]	32
2.5	1.2	2.5	4.5
1.0	0.4	1.0	1.4
		0.5	0.7
Control	0.21	Control	0.3

[a] Exposure commenced with fertilized, water hardened eggs, except where noted.

[b] Exposure commenced with eyed eggs.

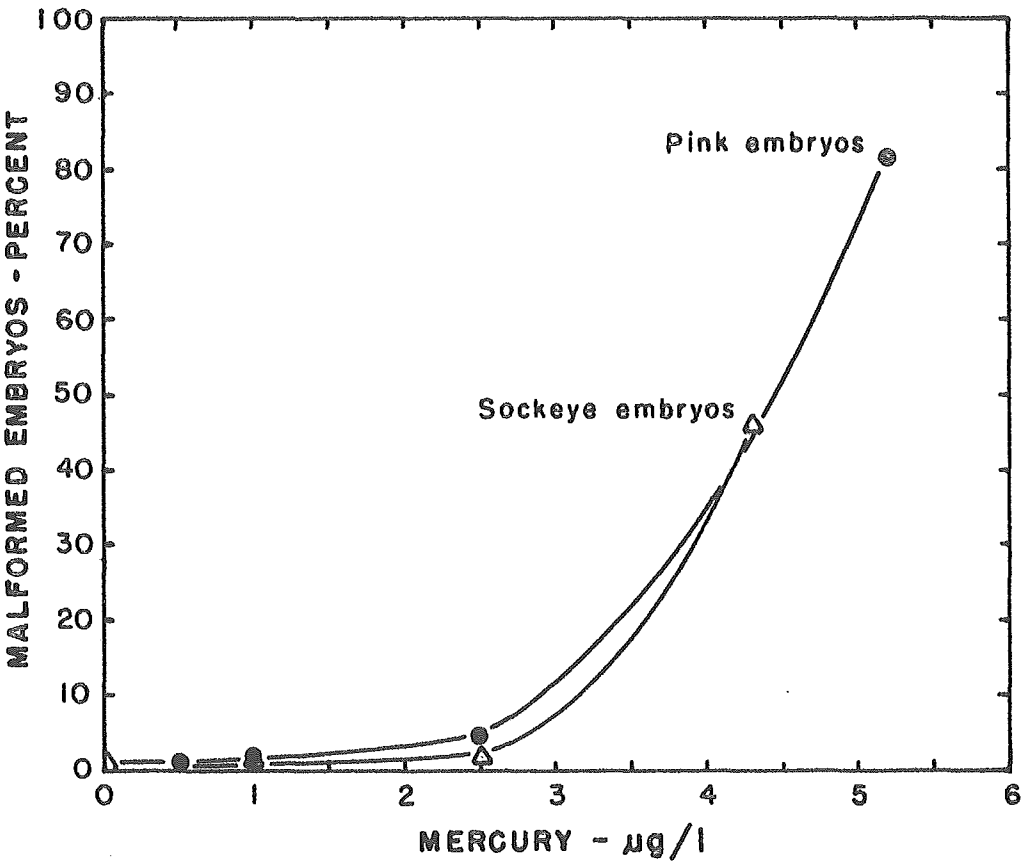

Figure 2. Occurrence of malformed sockeye and pink salmon embryos at hatching following exposure to mercury since fertilization.

Malformed sockeye alevins were not evident upon hatching from eggs exposed to 17.8 and 9.3 µg/l mercury since being eyed, but tiny hemorrhagic spots (petechiae) were observed in body musculature. As growth proceeded, petechiae disappeared and malformed alevins occurred, reaching 35.3 and 1.7% in 17.8 and 9.3 µg/l mercury, respectively. In the case of pink salmon eggs incubated at 5.2 µg/l mercury since being eyed, 32% of the alevins were malformed compared to 81.3% when exposure commenced after water hardening.

Mortality and malformation data indicate these toxic effects were greatest when exposure to mercury commenced soon after fertilization instead of after eyeing.

Growth data were compared for concentrations of 4.3 µg/l mercury and less since alevins at higher concentrations were first exposed to mercury as eyed eggs, whereas at lower concentrations exposure commenced with fertilized, water hardened eggs. Malformed alevins were excluded when measurements of length were made. At hatching, sockeye and pink salmon alevins exposed to 2.5 and 1.0 µg/l mercury or more, respectively, were shorter than control groups (Dunnett's procedure, Steel and Torrie, 1960) (Table 12). However, size differences became insignificant among sockeye and pink salmon at yolk absorption, except those exposed to 4.3 and 2.5 µg/l mercury, respectively.

Table 12. Length of sockeye and pink salmon alevins exposed to mercury.

	Mercury µg/l	Length, mm	
		Hatching	Yolk Absorption
Sockeye	4.3	17.4	24.7
	2.5	18.2	25.5
	1.0	18.8	25.6
	Control	18.7	25.7
Pink	2.5	19.3	30.4
	1.0	20.0	31.2
	0.5	20.1	31.5
	Control	20.4	31.5

Sockeye and pink salmon concentrated mercury during incubation at all levels of exposure (Table 13). Mercury contents of sockeye and pink salmon eggs and fry collected from the natural environment were in the range of 0.026 to 0.042 ppm. These are similar to values reported for the Fraser River (Northcote, Johnston and Tsumura, 1975). However, the mercury contents of

sockeye and pink salmon control fry were 0.19 and 0.36 ppm, respectively. These levels may have occurred due to contamination of control water by mercury in the laboratory. A concentration of 5.10 ppm in eyed sockeye eggs coincided with high incidence of mortality and malformations. Mortalities of pink salmon eggs were 88%, coincident with a mercury content of 3.79 ppm in eyed eggs. Malformed alevins were evident when the mercury content of eyed eggs was 1.87 ppm.

Table 13. Mercury content of sockeye and pink salmon eggs and fry exposed to mercury in the laboratory and collected from natural environments. [a]

	Sample Source or Mercury Exposure µg/l	Mercury Content, ppm wet wgt.		
		Newly Fertilized Eggs	Eyed Eggs	Yolk Absorption
Sockeye	4.3	0.032	5.10	6.85
	2.5	0.032	2.60	4.27
	Control	0.032	0.12	0.19
	Cultus (Lindell Beach)	0.032	0.04	0.03
Pink	8.5	0.024	6.00	-
	5.2	0.024	3.79	-
	2.5	0.024	1.87	7.55
	1.0	0.024	0.97	2.58
	0.5	0.024	0.48	1.29
	Control	0.024	0.074	0.36
	Sweltzer Creek	-	0.026	0.042

[a] Samples obtained from laboratory experiments are designated by exposure concentrations.

Sockeye and pink salmon eggs and alevins concentrated mercury in proportion to the level of exposure (Figure 3). Thus contamination of natural water by mercury would be reflected in mercury contents of eggs and fry.

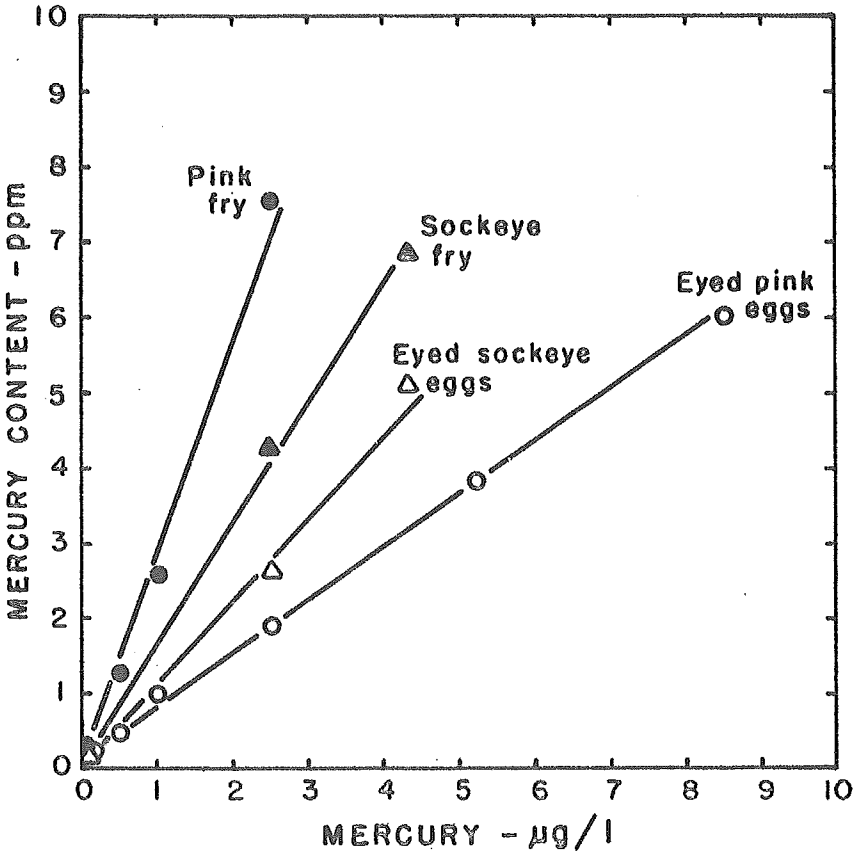

Figure 3. Mercury content of sockeye and pink salmon eggs and fry exposed to mercury in the laboratory.

DISCUSSION

Cadmium

Hyperactivity and tetany reported herein for sockeye and pink salmon exposed to cadmium have been reported for other species. Eaton (1974) reported hyperactivity followed by tetany among bluegill (Lemomis macrochiris Rafinesque) exposed to 80 µg/l cadmium. After eight weeks exposure, hyperactivity leading to death was reported among brook trout (Salvelinus fontinalis) exposed to 6.4 µg/l cadmium (Benoit, et al, 1976). Significant numbers of first and second generation brook trout males became hyperactive and died during spawning at 3.4 µg/l cadmium. Hyperactivity was not observed at 1.7 µg/l. The lowest concentration of cadmium which caused mortalities among sockeye fry was 5.6 µg/l. Thus there was little difference between the concentrations which were lethal to brook trout and sockeye.

Sockeye and pink salmon eggs and alevins were not affected by continuous exposure to 5.7 µg/l cadmium but this concentration was lethal to sockeye fry in seven days. A somewhat similar response was noted among brook trout eggs and alevins which were not affected by 3.4 µg/l cadmium, whereas juveniles exposed to this concentration for 16 weeks experienced retardation of growth. It is evident that the fry and juvenile developmental stages were more sensitive to cadmium than were eggs or alevins. The reason for the difference in sensitivity was not evident from the studies reported herein or cited above. Furthermore, there was only a small decrease in tolerance of pink salmon to cadmium during 168 hr acute bioassays as they progressed from alevins to fry. Since it is unlikely that pink salmon are far more tolerant of cadmium than sockeye, it is possible that sensitivity of this species increased at a point later in fry development than that bioassayed.

Water chemistry is believed to influence toxicity of metals to aquatic life. The incipient lethal level for rainbow trout (Salmo gairdnerii) exposed to cadmium was reported to occur between 0.7 and 1.5 µg/l in soft water (hardness 31 mg/l) and between 13.5 and 21.0 µg/l in hard water (hardness 326.6 mg/l) (Davis, 1976). The difference in toxicity of cadmium in hard and soft water was suspected to be caused by some antagonistic reaction inhibiting the toxicity of cadmium in hard water. A toxic response at 5.6 µg/l cadmium as reported herein for sockeye fry was obtained at a mean hardness of 83.1 mg/l and is within the foregoing range reported for rainbow trout.

The National Academy of Sciences (1973) proposed maximum permissible concentrations of 0.4 to 3 µg/l cadmium in soft and hard water, respectively, to protect crustaceans or the eggs and larvae of salmon. To protect aquatic life in general, it was recommended that cadmium not exceed 4 µg/l in water with hardness less than 100 mg/l. However, using results of chronic and acute toxicity studies on a variety of fish and applying a safety factor, Sauter et al (1976) recommended a maximum permissible concentration of 0.01 µg/l cadmium. The latter value is about 0.13% of the 168 hr LC50 for sockeye fry.

Copper

The toxicity of copper to aquatic life is influenced by water chemistry. In general, copper is more toxic in soft water than hard water but recent studies stress that hardness per se is not the determining factor. Detailed equilibrium calculations using data from bioassays where alkalinity, pH, hardness and total copper concentrations were different indicated copper (II) is the chemical species toxic to fishes and that alkalinity is the factor controlling copper (II) concentration (Pagenkopf, Russo and Thurston, 1974). Sylva (1976) concluded that inorganic and organic complexation and adsorption and precipitation processes are capable of reducing copper (II) levels to very low values even in the presence of high levels of total copper. Zitko and Carson (1976) concluded that neither calcium nor magnesium affects the lethality of copper. It was concluded that, due to the strong influence of copper complexation on toxicity, results of tests in various natural waters cannot be directly compared on the basis of total copper or dissolved copper measurements alone (Andrew, Biesinger and Glass, 1977). The difference in toxicity of copper associated with water chemistry is illustrated by the following results for rainbow trout. The 96 hr LC50 of rainbow trout was estimated at 100 µg/l copper in water of 14 mg/l hardness using data reported by Grande (1967). Alkalinity was not reported but normally would be low in such soft water. On the other hand, the 96 hr LC50 ranged between 250 and 680 µg/l copper for 365 mg/l hardness (Lett, Farmer and Beamish, 1976).

The 96 hr LC50 for coho salmon (O. kisutch) decreased from 74 to 60 µg/l copper from November to May when smoltification occurred (Lorz and McPherson, 1976). As shown herein, the 96 hr LC50 for sockeye salmon smolts ranged between 170 and 240 µg/l, while the LC50 for pink salmon fry was 199 µg/l.

Alkalinity and hardness were 73 and 91 mg/l $CaCO_3$, respectively, in the tests with coho and 63 and 83 mg/l, respectively, in tests with sockeye and pink salmon. Since alkalinity and hardness were similar in the foregoing tests of Pacific salmon, other chemical factors may have influenced toxicity, or coho were less tolerant of copper than sockeye and pink salmon.

Hazel and Meith (1970) reported mortalities of 12% for chinook salmon (O. tshawytscha) alevins exposed to 21 µg/l copper. These studies were conducted in water of alkalinity and hardness equal to 21 and 44 mg/l $CaCO_3$, respectively. For the data reported herein the incipient lethal level was between 37 and 78 µg/l for sockeye alevins, but between 25 and 55 µg/l for pink salmon in water with alkalinity and hardness equal to 63 and 83 mg/l, respectively. The difference in tolerance to copper among these three Pacific salmon may be related in part to species differences, but the lower alkalinity of water used in tests of chinook salmon would also be a factor in toxicity according to Pagenkopf, Russo and Thurston (1974).

Using data reported by Grande (1967) it was estimated that 50% mortality of Atlantic salmon (Salmo salar)and rainbow trout eggs occurred at 12 and 46 µg/l copper, respectively, in water with hardness equal to 14 mg/l $CaCO_3$. Alkalinity was not reported but would be low in a natural soft water. Sockeye and pink salmon egg mortalities were 48.4 and 38.2% at 78 and 55 µg/l copper, respectively, the lower tolerance to copper observed for Atlantic salmon and rainbow trout eggs may be related to water chemistry and/or species differences.

The rigidity of egg capsules at hatching was affected by 13 µg/l copper and rigidity increased in proportion to copper concentration. A portion of the mortality among sockeye and pink salmon at hatching was apparently caused because alevins were trapped in a partially hatched condition at 174 and 55 µg/l copper, respectively. McKim and Benoit (1971) reported 25% of brook trout egg mortality occurred when the embryos tried to emerge from the eggs. Normally the egg capsule is softened enzymatically prior to hatching to enable the alevin to exit. Since the amount of softening appeared inversely proportional to copper concentration, it is assumed that copper inhibited enzymatic processes responsible for softening the egg capsule. Grande (1967) suggested that zinc acted in a similar manner on Atlantic salmon eggs.

Degree-days to hatching were unaffected by copper except at 55 µg/l where a delay was noted in hatching of pink salmon eggs. Grande (1967) reported hatching of Atlantic salmon eggs was delayed when exposed to zinc. Delay in hatching would be expected since egg capsules were not softened as normally occurs. However, in contrast to these results, hatching of brook trout eggs exposed to copper preceded controls (McKim and Benoit, 1971). A reason for the difference in observations was not evident.

Sockeye and pink salmon alevin exposed to 18 and 6 µg/l copper, respectively, were shorter than controls but at yolk absorption, only pink salmon exposed to 55 µg/l were shorter. Thus, size at yolk absorption was affected only at those concentrations of copper which were lethal to alevins. Similar results were noted by Hazel and Meith (1970) and Sauter et al (1976), who reported alevins of chinook salmon and brook trout, respectively, exposed to copper were shorter than controls at those concentrations where alevin mortality exceeded that of controls. McKim and Benoit (1971) reported brook trout grew more slowly at all concentrations of copper tested, including those which were not lethal in the alevin stage. However, after 23 weeks growth rates equalled those of controls among all groups of surviving alevins. The foregoing observations concerning growth may indicate acclimation was occurring, since rainbow trout were reported to adapt to concentrations of copper up to one-half the LC50 value during growth studies (Lett, Farmer and Beamish, 1976).

The copper contents of pink and sockeye salmon fry from control and natural environments were 1 ppm on a wet weight basis (Table 9). The mean copper content of muscle tissue of adult chinook and sockeye salmon collected from the Fraser River was of similar magnitude, being 0.55 and 0.73 ppm respectively, on a wet weight basis (Northcote, Johnston and Tsumura, 1975).

Copper contents of 6.8 ppm and 105 ppm in fry and eyed eggs, respectively, coincided with high mortality among pink salmon eggs and alevins. Further work would be required to determine whether a consistent relationship exists between copper contents of eggs or fry and toxic effects. However, since the copper content of eyed eggs was proportional to the exposure concentrations, copper content may be a useful investigative tool for documenting chronic copper contamination.

The most sensitive effects noted among sockeye and pink salmon exposed to copper were egg capsule rigidity and copper content of eyed eggs. Alevin length at hatching was influenced by exposure to copper at sub-lethal levels, but at yolk absorption, length was no better indicator of toxic effect than mortality. Degree-days to hatching were unaffected by copper except pink salmon were delayed at lethal levels.

Two factors which may affect survival of sockeye and pink salmon but which were not examined herein, are adaptation to seawater and effect of copper on food organisms. Exposure of coho salmon smolts to 20 µg/l copper for 144 hr noticeably decreased subsequent survival in seawater, and the percentage of smolts migrating downstream was reduced following exposure to as little as 5 µg/l for 3960 hr (Lorz and McPherson, 1976). Daphnia magna, a zooplankton representative of fish food organisms, was reported to have a 48 hr LC50 equal to 60 µg/l copper and a 16% reduction in reproduction occurred at 22 µg/l (Biesinger and Christensen, 1972).

The National Academy of Sciences (1973) recommended 10% of the 96 hr LC50 as a safe level for aquatic life exposed to copper in freshwater. However, Sauter et al (1976) applied a safety factor to chronic exposure and acute toxicity data for a variety of fish, and recommended a maximum permissible concentration of 0.1 µg/l copper. This value is 0.10 and 0.12%, respectively, of the minimum 96 hr LC50's reported herein for sockeye and pink salmon.

Mercury

The acute toxic action of mercuric chloride was reported exerted in gill epithelium where severe necrosis was noted, probably causing death by asphyxia (Wobeser, 1975). The LC50 for rainbow trout exposed to mercuric chloride decreased from 903 to 280 µg/l mercury at 10°C as exposure time was lengthened from 24 to 96 hr LC50 (Wobeser, 1975; McLeod and Pessah, 1973). In comparison, the 168 hr LC50 for sockeye fry and smolts averaged 190 µg/l mercury while for pink salmon the single value obtained was 210 µg/l. Whether sockeye and pink salmon were less tolerant than rainbow trout could not be determined from these data because the longer exposure time of sockeye and pink salmon to mercury would be partly responsible for the lower LC50 values.

Mercuric chloride was lethal to carp eggs at 3,000 µg/l mercury during an exposure of 60-72 hours (Huckabee and Griffith, 1974). It was suggested that mercury may have reacted with sulfhydryl groups in the outer membrane of carp eggs to cause death. Sockeye and pink salmon eggs were far more sensitive to mercury than carp eggs since mortalities occurred at 4.3 µg/l. The difference in sensitivity may be related to the difference in exposure time, since carp eggs hatch in 60-72 hours whereas salmon eggs require several weeks or a few months to hatch. Thus salmon eggs have a longer period than carp eggs to accumulate mercury to harmful levels.

The occurrence of malformed alevins indicated that the toxic effect of mercury was exerted on the developing embryo rather than on the outer membrane as was suggested by Huckabee and Griffith (1974) for carp eggs. Further evidence of the sensitivity of early development to mercury was seen when the percentage of malformed sockeye and pink salmon alevins increased with exposure concentration (Figure 2). In another case, development of brook trout embryos failed to proceed beyond the neural keel stage when exposed to 2.93 µg/l mercury in the form of methylmercuric chloride (McKim et al, 1976).

Deformities were not limited to exposure during early embryonic development since about 1.7% of sockeye alevins exposed to 9.3 µg/l mercury became deformed after hatching. In a somewhat similar case, brook trout exposed to methylmercuric chloride at a concentration of 2.93 µg/l mercury for 20 weeks suffered deformities of the trunk (McKim et al, 1976).

Lengths of sockeye and pink salmon fry at yolk absorption indicated growth of alevins was significantly less than controls at 4.3 and 2.5 µg/l mercury, respectively. Thus growth and teratogenic effects were about equally sensitive to mercury. Similarly, in studies using brook trout, growth during the alevin-juvenile life phase was no more sensitive than hatchability of eggs to toxic effects of mercury (McKim et al, 1976). On the other hand, cough response of brook trout increased significantly when 3 µg/l mercury was added to Lake Superior water in the form of mercuric chloride (Drummond, Olson and Batterman, 1974). This response was believed suitable to predict safe concentrations of mercuric chloride.

Mercury was concentrated in brook trout and rainbow trout when they were exposed to mercuric chloride and methylmercuric chloride under laboratory conditions (McLeod and Pessah, 1973; Wobeser, 1975; McKim et al, 1976).

In each case mercury was concentrated in tissues in proportion to the exposure concentration, including those at which there was no evidence of harmful effect. Uthe, Atton and Royer (1973) concluded from studies using rainbow trout that concentrations of mercury in rainbow trout were good monitors of mercury pollution.

The mercury contents of sockeye and pink salmon eggs and fry also reflected the history of exposure. Lethal effects were observed when the mercury content of eyed eggs equalled 3.79 ppm. Teratogenic effects were noted when the mercury content of eyed eggs equal 1.87 ppm. It appears both eggs and fry could serve as useful tools in studies of mercury contamination in natural environments. However, further work would be required to determine whether the mercury content values cited above represent suitable criteria upon which to judge potential harmful effects of mercury to sockeye and pink salmon.

The following criteria were specified for protection of aquatic life from mercury poisoning and are based primarily on known effects of methylmercury (National Academy of Sciences, 1973).

a) Mercury content of aquatic organisms should not exceed 0.5 ppm on a wet weight basis,

b) The total mercury concentration in unfiltered water should not exceed 0.2 µg/l at any time or place, and

c) The average total mercury concentration in unfiltered water should not exceed 0.05 µg/l.

These criteria are well below concentrations reported herein as being harmful to sockeye and pink salmon.

CONCLUSIONS

1. Sockeye salmon fry had a 168 hr LC50 equal to 8 µg/l cadmium and were less tolerant than eggs, alevins or smolts.
2. The incipient lethal level was between 37 and 78 µg/l copper for sockeye but between 25 and 55 µg/l for pink salmon during the egg to fry stage. Growth and hatching were no better than mortality as indicators of toxic effects of copper.
3. Pink salmon concentrated copper during the egg to fry stage in proportion to exposure concentration. Copper concentrations of 105 and 6.8 ppm in pink salmon eyed eggs and fry, respectively, coincided with mortalities.
4. Copper interfered with the egg capsule softening process. Some mortalities resulted because embryos were trapped in a partially hatched condition, but these occurred at copper concentrations which were otherwise lethal.
5. Malformed embryos occurred when sockeye and pink salmon eggs were exposed continuously to 2.5 µg/l mercury commencing shortly after fertilization. Mortality, growth and hatching were less sensitive to mercury than was malformation.
6. Sockeye and pink salmon concentrated mercury during the egg to fry stage in proportion to exposure concentration. Mercury concentrations of 1.87 ppm in eyed eggs coincided with teratogenic effects in embryos at hatching.

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