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MERCURY AND OTHER INORGANIC CONSTITUENTS OF COHO AND PINK SALMON EGGS FROM THE YAKOUN RIVER

Regional Program Report No. 86-12

By

George Derksen

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ABSTRACT

The metal levels of coho and pink salmon eggs incubated in their natural environment were monitored as part of a data collection program prior to the development of a proposed gold mine. Preliminary 1981 coho data indicated the possible accumulation of mercury during incubation. However, additional samples of coho salmon eggs and pink salmon eggs did not indicate any accumulation. Copper and sodium showed the greatest differences between unfertilized and late-eyed eggs for both species.

RÉSUMÉ

Dans le cadre d'un programme de collecte de données entrepris avant la mise en exploitation d'une mine d'or non encore confirmée, on a surveillé le niveau de concentration des métaux dans les oeufs de saumon Coho et de saumon rose incubés dans leur milieu naturel. Les données préliminaires de 1981 concernant le saumon Coho ont laissé prévoir une accumulation de mercure durant l'incubation. Cependant, de nouveaux échantillons d'oeufs de saumon Coho et de saumon rose n'ont révélé aucune accumulation. Pour les deux espèces, c'est le cuivre et le sodium qui ont montré les plus grands écarts entre les oeufs non fécondés et les oeufs arrivés au dernier stage de développement des yeux.

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SUMMARY

Metal levels of pink and coho salmon eggs incubated in their natural environment were monitored as part of a pre-development baseline data program for the proposed Cinola gold mine on Graham Island, Queen Charlotte Islands. Coho eggs collected in 1981/82 indicated there was a potential accumulation of mercury in eggs collected from Barbie Creek. However, additional samples of pink salmon eggs from the Yakoun River and coho salmon eggs from Barbie Creek did not indicate any accumulation. For both coho and pink salmon, there was a significant decrease in the copper and sodium content of late-eyed eggs relative to unfertilized eggs.

Metal levels in salmon eggs may serve as a tool to assess metal contamination of spawning grounds. However, further work is required to assess threshold levels of uptake and field procedures. A program incorporating water quality, sediment quality and salmon egg development could provide useful background data to assess mine projects.

1 INTRODUCTION

In July 1980 Consolidated Cinola Mines Ltd. submitted a Stage I report to the Provincial Steering Committee for Development of New Mines (Fanning and Griffing 1980). The report summarized that the ore, waste rock, soils and tailings may contain anomalously high levels of mercury and other heavy metals. The Cinola project (gold mine) is located on Graham Island (Queen Charlotte Islands) and was identified as having a potential for impacting upon the anadromous fishery resources of the Yakoun River drainage.

It has been well established that many trace metals which enter natural water accumulate to high levels in sediments. Clastic stream sediments serve as spawning substrates for Pacific salmon and resident trout. It is important to maintain the quality of these areas. Several researchers have reported that salmon eggs do accumulate mercury and other metals (Wedemeyer 1968, Zeitoun et al. 1976, Servizi and Martens 1978, Rombough and Garside 1982) and that embryos and alevin concentrate sediment-released metals including mercury (Birge et al. 1977). Salmonid eggs incubated in their natural environment could potentially serve as a useful tool to monitor metal contamination (especially mercury) of spawning grounds.

As part of a pre-development data collection program the Environmental Protection Service (Department of Environment) undertook a monitoring program to assess background metal levels in coho and pink salmon eggs. This report presents data on the metal content of salmon eggs collected between 1981 and 1984 from the Yakoun River drainage. The trace metal levels of Yakoun River and tributary stream sediments has been reported by Derksen 1985.

2 STUDY AREA

The area of the proposed Consolidated Cinola gold mine is shown on Figure 1. Coho salmon have been observed to spawn at several locations in Barbie Creek which transects the mine development area. Large numbers of pink salmon (even year cycle) spawn downstream and upstream of Barbie Creek. Gold Creek which enters the Yakoun River upstream of Barbie Creek is a major coho spawning area. Areas where salmon eggs were collected are shown on Figure 1.

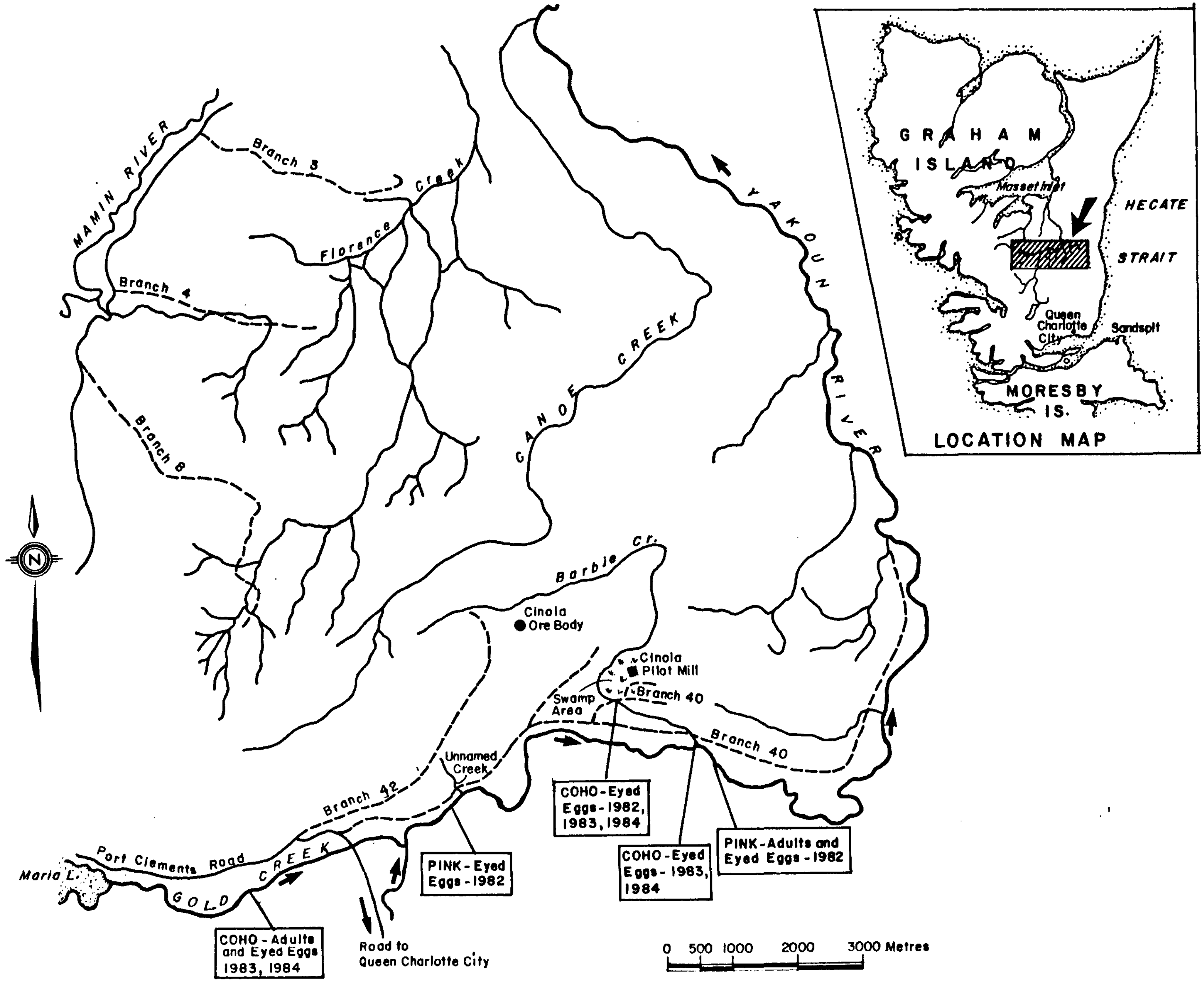

FIGURE I GENERAL LOCATION OF SALMON EGG COLLECTION SITES

3 METHODS

3.1 Laboratory

Metal analyses were performed at the EPS/DFO laboratory in West Vancouver (Anon 1979, Appendix I). The 1981/82 mercury samples were analyzed by Flameless Atomic Absorption spectrophotometry. Later mercury samples were analyzed on a Pharmica Model 100 Mercury Monitor. All the other metals were analyzed by Inductively Coupled Argon Plasma (ICAP) emission spectrophotometry. ICAP analytical procedures are routinely checked against NBS reference (bovine liver) samples (Table 1). There is a good agreement between mean results of ICAP analyses and reference tissue levels.

TABLE 1 NBS REFERENCE SAMPLES

METAL (ug/g) (wet weight)	EPS Bovine Liver NBS 1577		NBS REFERENCE Bovine Liver NBS 1577	
	$\overline{x}$	S.D.	$\overline{x}$	S.D.
Cu	199	10	193	10
Zn	146	8	130	10
Fe	263	9	270	20
Ca	125	12	123	-
Mg	580	23	605	-
Na	2330	97	2430	130

3.2 Field

Between 1981 and 1983 the field approach to obtain salmon eggs to assess metal content changed.

3.2.1 1981 Preliminary Evaluation of Coho Salmon Eggs.

In mid-October 1981, egg samples were collected from three ripe coho salmon from the Yakoun River. The eggs were carefully stripped and placed (using disposable gloves) into whirlpac sample bags and then frozen. On March 11, 1982 pre-hatch eggs (late-eyed stage) from three gravel areas of Barbie Creek were collected. Spawning was observed at these sites the previous fall. A hydraulic sampler was used to stir up the redd area which was encircled with a modified Hess circular sampler. The eggs drifted downstream into the sampler net. The contents of the net were placed into a clean polyethylene bowl and live eggs were removed with clean plastic tweezers. Eggs were rinsed with distilled water from a squirt bottle, placed in whirl pac bags and then frozen over dry ice. Three samples of eyed eggs were collected but whether they were from distinct redds of three different adults or several pockets from the same female is not known. The section of creek was only 60 cm across and consisted of small areas of road bed gravel overlying a peat base.

3.2.2 1982 Evaluation of Pink and Coho Salmon Eggs. On September 14, 1982 egg samples were stripped from ten ripe pink salmon netted from a spawning area downstream of Barbie Creek (Figure 1). Eggs were stripped into a clean polyethylene bowl, poured into whirl pac bags and then frozen over dry ice. Pre-hatch eggs (late-eyed stage) samples were collected from spawning areas downstream and upstream of Barbie Creek on November 24, 1982. The pink salmon eggs were collected from previously marked redds with a hydraulic sampler and net. The contents of the net were placed in a clean polyethylene sample bowl, live eggs were picked with clean plastic tweezers, rinsed with distilled water from a squirt bottle, placed in a whirl pac and then frozen over dry ice. The eggs had just begun to hatch as a few alevin were noted in a few of the net samples.

Coho salmon eggs were obtained from five ripe females collected from the Yakoun River in mid-October 1982. One sample was collected from Barbie Creek on October 26, 1982. The eggs were stripped and carefully placed (using disposable gloves) into whirl pac bags and then frozen. On February 24, 1983 coho eggs (late-eyed stage) were collected from two areas in lower Barbie Creek (just above Yakoun River confluence) and from two areas sampled in spring 1982. Samples were treated in the same fashion as the pink eggs.

3.2.3 <u>1983 Evaluation of Coho Salmon Eggs</u>. In 1983 a different approach was taken to assess metal levels over the incubation period. The intent was to obtain eggs from six parental females, fertilize them individually onsite and then plant duplicate boxes of 200 eggs/per box in Barbie Creek and Gold Creek. However, only a limited sample size was obtained due to difficulty in obtaining brood stock. While flows in Gold Creek were condusive to planting the boxes, flows in Barbie Creek were very high and it was difficult to place the boxes. Eggs from three females were obtained from Gold Creek on November 1, 1985, fertilized and water hardened for three hours. The water hardened eggs were placed in 12 cm x 7.0 cm x 15 cm acid washed polyethylene sample bottles that had been previously drilled with 2.5 mm holes. The sample bottles were filled with acid washed 3.5 cm diameter flexirings. Duplicate bottles for each female were placed in Gold Creek and from two females in lower Barbie Creek. The bottles were recovered March 28, 1984.

From each parental female duplicate samples for metal analysis were taken from unfertilized and three hour Gold Creek water hardened eggs. One sample from each egg box was collected. A sample was analyzed with the chorion and without the chorion. The chorion was removed using clean stainless steel tweezers. By March 28, 1983 some of the eggs had begun to hatch. Percent mortality was determined from the dead and live egg count.

4 RESULTS AND DISCUSSION

4.1 1981 Preliminary Evaluation of Coho Salmon Eggs

The results for the coho salmon eggs collected in 1981/82 are reported in Table 2. The results indicate that there is a significantly higher (T-test, $p < .05$) mercury content in the eyed egg ($\bar{x}$ = .23 ug/g dry weight) than the unfertilized eggs ($\bar{x}$ = 0.12 ug/g). Other inorganic constituents that were significantly different included copper and sodium which were lower in eyed eggs. Compared to the other two samples, one eyed egg sample appeared to have anomalously low levels of calcium, magnesium and sodium. The mercury results indicated that further sampling should be conducted to confirm the higher mercury level in developing eggs.

4.2 1982 Evaluation of Pink and Coho Salmon Eggs

The results for the pink salmon eggs collected in 1982 are reported in Table 3 (Appendix II). The results do not indicate any significant difference between the mercury content of unfertilized and eyed eggs from the Yakoun River. Inorganic constituents that did indicate significant differences (T-test, $p < .05$) included calcium which was greater in the eyed egg and copper, iron and sodium which were lower.

The results for the coho salmon eggs collected in 1982/83 are reported in Table 4. There is no significant difference between the mercury content of unfertilized eggs and eyed eggs from Barbie Creek. Values were less than the upper detection limit of 0.05 ug/g in all cases. Inorganic constituents that did indicate significant differences ($p < .05$) included iron which was greater in eyed eggs and copper and sodium which were lower in eyed eggs. The coho eyed egg samples #3 and #4 from one Barbie Creek site had much higher iron levels (135-188 ug/g) than those from the sample #1 and #2 site (53.5-78.6 ug/g).

4.3 1983 Evaluation of Coho Salmon Eggs

The results for the coho eggs collected in 1983/84 are reported in Table 5. Results do not indicate any significant differences between the mercury content of unfertilized eggs and eyed eggs from Gold Creek or Barbie

TABLE 2 COHO SALMON EGGS 1981/82 - METAL CONTENT

METAL (ug/g dry weight)	YAKOUN RIVER (Unfertilized Eggs)*					BARBIE CREEK (Eyed Egg)**				
	1	2	3	$\bar{x}$	S.D.	1	2	3	$\bar{x}$	S.D.
Hg	.12	.12	.12	<u>.12</u>	0.0	.25	.26	.19	<u>.23</u>	.04
						(.18)	(.15)	(.15)	(.16)	(.02)
Cu	15.8	15.0	12.0	<u>14.4</u>	1.8	6.7	3.9	5.6	<u>5.4</u>	1.4
Zn	94.7	70.2	78.8	81.2	12.4	71.5	68.5	51.2	63.7	10.9
Fe	94.7	51.4	45.3	63.8	26.9	175	165	875	405	407
Ca	1540	1520	1480	1510	30	1370	1820	282	1160	790
Mg	1650	1450	1760	1620	157	1400	1240	129	939	704
Na	1290	1780	1140	<u>1400</u>	335	780	620	80	<u>493</u>	367

* eggs from three adults, collected mid-October 1981

** eggs from three gravel areas of Barbie Creek, collected March 11, 1982

—— = underlined means are significantly different $p < .05$.

( ) = results are for dead eggs from same sample

TABLE 3 PINK SALMON EGGS 1982 - METAL CONTENT

METAL (ug/g dry weight)	UNFERTILIZED EGGS*			EYED EGGS**		
	$\bar{x}$	S.D.	n	$\bar{x}$	S.D.	n
Hg	.04	.03	10	.06	.04	9
Cu	15.0	2.3	10	3.2	.3	9
Zn	50.8	9.0	10	52.6	4.4	9
Fe	57.7	13.3	10	47.2	3.9	9
Ca	1340	59	10	1530	68	9
Mg	1400	98	10	1390	96	9
Na	2080	370	10	680	140	9

* collected September 14, 1982 downstream of Barbie Creek

** collected November 24, 1982 upstream and downstream of Barbie Creek

___ = underlined means are significantly different

TABLE 4 COHO SALMON EGGS 1983 - METAL CONTENT

METAL (ug/g dry weight)	YAKOUN RIVER* (Unfertilized Eggs)								BARBIE CREEK** (Eyed Eggs)					
	1	2	3	4	5	6	$\overline{x}$	S.D.	1	2	3	4	$\overline{x}$	S.D.
Hg	< .03	< .05	< .04	< .05	< .05	< .05	< .05	0.0	< .05	< .05	< .04	< .05	< .05	0.0
Cu	13.1	9.9	11.5	13.2	9.4	9.4	<u>11.1</u>	1.8	1.7	2.1	2.3	2.7	<u>2.2</u>	.4
Zn	69.3	53.7	61.3	65.8	52.5	56.3	59.8	6.8	61.4	57.4	61.1	57.2	59.3	2.3
Fe	35.2	53.8	34.4	47.8	27.3	24.7	<u>37.2</u>	11.4	53.5	78.6	188	139	<u>115</u>	60.6
Ca	1610	1320	1540	1740	1350	1430	<u>1500</u>	162	1420	1260	1270	1270	<u>1310</u>	77
Mg	1570	1400	1460	1340	1620	1630	1500	121	1370	1360	1480	1450	1410	59
Na	1120	1230	1340	1300	1310	1030	<u>1220</u>	122	820	860	620	740	<u>760</u>	106

* (1-5) eggs from adult coho collected mid-October 1982 from Yakoun River
(6) eggs from adult coho collected October 26, 1982 from Barbie Creek

** eyed eggs collected from four gravel areas in Barbie Creek February 24, 1983, (1-2) Barbie at Yakoun and (3-4) Barbie at pilot mill road

___ = underlined means are significantly different

TABLE 5 COHO SALMON EGGS 1984 - METAL CONTENT

METAL (ug/g dry weight)	GOLD CREEK: Unfertilized (n=6)			GOLD CREEK: Water Hardened (n=6)			GOLD CREEK: Late-Eyed Egg With Chorion			GOLD CREEK: Late-Eyed Egg Without Chorion			BARBIE CREEK: Late-Eyed Egg With Chorion		
Parental Female	i	ii	iii	i	ii	iii	i	ii	iii	i	ii	iii	i	ii	iii
Hg i	< .02	< .03	< .03	.04	.08	< .02	.02	< .02	< .03	< .02	< .02	< .02	< .03	< .02	-
Hg ii	.07	< .02	< .03	< .02	.03	.02	< .02	< .02	.02	-	< .02	< .02	< .03	< .02	-
overall $\bar{x}$ (S.D.)	.03 (.02)			.04 (.02)			.02			.02			.02		
Cu i	10.6	11.2	11.0	13.2	9.9	10.3	3.8	10.0**	2.4	3.5	3.1	2.4	3.4	3.0	-
Cu ii	10.4	13.0	10.2	13.0	14.0	8.5	3.3	2.9	2.2	-	3.6	2.3	3.0	4.3	-
overall $\bar{x}$ (S.D.)	11.1 (1.0)			11.5 (2.2)			<u>2.9</u> (0.3)			<u>3.0</u> (0.3)			<u>3.4</u> (0.3)		
Zn i	71.9	71.4	76.1	70.7	75.1	75.2	74.1	84.6	76.8	73.3	77.7	82.0	77.9	79.6	-
Zn ii	73.0	78.7	71.4	71.9	76.7	69.4	69.2	80.2	73.0	-	83.9	79.8	69.2	78.2	-
overall $\bar{x}$ (S.D.)	73.8 (3.0)			73.2 (2.9)			76.3 (5.5)			<u>79.3</u> (4.1)			76.2 (4.7)		
Fe i	35.1	32.4	29.9	40.5	27.8	29.4	49.5	101	36	42.2	42.4	37.1	104	97.3	-
Fe ii	28.3	36.2	28.8	34.3	30.1	24.0	43.7	68.8	34.7	-	45.5	36.7	99.2	117	-
overall $\bar{x}$ (S.D.)	31.8 (3.3)			31.0 (5.7)			<u>55.6</u> (25.4)			<u>40.8</u> (3.8)			<u>104</u> (8.9)		
Ca i	1530	1150	1190	1470	1220	1200	1570	1360	1410	1570	1390	1500	1610	1240	-
Ca ii	1530	1240	1150	1470	1240	1120	1470	1460	1360	-	1550	1540	1450	1150	-
overall $\bar{x}$ (S.D.)	1300 (182)			1290 (148)			1440 (80)			<u>1510</u> (72)			1360 (207)		
Mg i	1700	1550	1800	1630	1600	1740	1700	1690	1570	1540	1510	1660	1750	1710	-
Mg ii	1730	1800	1650	1660	1640	1610	1570	1600	1500	-	1670	1590	1660	1670	-
overall $\bar{x}$ (S.D.)	1710 (83)			1650 (50)			<u>1610</u> (77)			<u>1590</u> (71)			1700 (41)		
Na i	1340	2040	2260	632	801	832	1280	1510	1250	999	1100	1220	408	553	-
Na ii	1120	2380	1890	648	791	755	1220	1480	1220	-	1230	1160	509	578	-
overall $\bar{x}$ (S.D.)	1840 (506)			<u>743</u> (84)			<u>1330</u> (133)			<u>1140</u> (95)			<u>512</u> (75)		

* all samples consisted of 16 eggs
** not used in mean calculation
—— = underlined means are significantly different than unfertilized eggs $p < .05$

Creek. Inorganic constituents that did indicate significant differences included iron which was greater in eyed eggs and copper and sodium which were less in eyed eggs. Differences between the eyed eggs from Gold Creek and Barbie Creek included higher levels of iron and lower levels of sodium in Barbie Creek eggs. After water hardening the sodium content was 743 ug/g compared to 1840 ug/g in unfertilized eggs. While Barbie Creek late-eyed eggs maintained a low sodium level (512 ug/g) the late-eyed eggs in Gold Creek had a mean concentration of 1330 ug/g.

The mean survival of eggs in baskets from Gold Creek was 85% while in Barbie Creek it was 12%. Additional handling and transport of water hardened eggs plus adverse flow conditions during the planting would have reduced survival of Barbie Creek eggs. In addition, Barbie Creek baskets were heavily sedimented compared to Gold Creek baskets.

4.4 Metal Content of Salmon Eggs and Alevin

Fletcher and King 1978 reported that for migrating sockeye salmon, concentrations of copper and zinc were significantly lower in mature ovaries than ovaries obtained at the initiation of the spawning migration. Calcium and magnesium concentratons did not differ between the two groups. However, the total amount (per 100 g body weight) of zinc, calcium and magnesium in the ovaries was significantly greater at the end of the spawning migration while copper did not differ. They hypothesized that almost all of the ovarian zinc, calcium and magnesium was obtained from body stores other than the liver. The metal levels in this study reflect the content of mature ovaries.

Wedemeyer 1968 reported that for coho salmon eggs, the eggs accumulated zinc in the proportion of approximately 70% bound to the chorion, 26% in the perivitelline fluid, about 2% in the yolk and about 1% in the embryo. Zinc uptake involved physiochemical sorption to the chorion together with passive diffusion into the yolk and embryo. Servizi and Martens, 1978 exposed sockeye and pink salmon eggs to cadmium, copper and mercury. They reported that the copper content of eyed pink salmon eggs exposed to copper was proportional to the exposure concentration. Sockeye and pink salmon eggs and alevin concentrated mercury in proportion to the level of exposure.

Klaverkamp et al 1983 reported that preliminary studies in their laboratory suggested that selenium, but not mercury, accumulation in lake trout eggs depends upon the ionic strength of the solution surrounding the eggs. Rombough and Garside 1982 reported that cadmium uptake by Atlantic salmon eggs was rapid, with dose-dependant saturation levels reached within 24 hours and maintained until hatch. The cadmium content of newly hatched alevins was much lower but directly porportional to that of eggs. The relatively low concentration in newly hatched alevins indicated that most of the metal in the egg was associated with the zona radiata (chorion) but appreciable amounts penetrated the zona radiata and were absorbed by the embryo. Peterson et al 1985 reported that for Atlantic salmon eggs, that usually most of the cadmium is taken up by the chorion and the yolk accumulates the least. Chorionic cadmium concentrations were greatly dependent on incubation pH and with levels much lower at lower pH levels. Dabrowski 1976 reported that the accumulation of arsenic in rainbow trout eggs was related to the arsenic concentration and to the arsenic compound used.

Seim et al 1984 reported that whole- alevin copper concentrations indicated concentration-dependent bioaccumulation. Intermittent exposure produced greater copper bioaccumulation than continuous exposures producing the same daily mean exposure concentration. Metal content in eggs was not measured. Birge et al 1977 reported that trout embryo and alevin concentrated sediment-released mercury to high levels in the tissue. In addition, trout eggs cultured over natural sediments with varying degrees of metal contamination gave hatching frequencies which correlated closely with levels of sediment metal contamination.

4.5 Future Study Needs to Assess Spawning Stream Quality Relative to Sensitive Mine Site Developments

When a mine development is located near salmonid bearing streams and the potential for impact is perceived to be high, then a high level of baseline monitoring should be conducted. The potential sources of impact on a stream would likely include sedimentation during construction and mine operations plus heavy metal contamination if point source discharges occur. Monitoring might generally encompass four aspects: sediment quality (particle size, permeability); surface and intragravel water quality; metal content of fine sediment fractions and egg to fry survival and metal content.

During the process of collecting baseline data for the proposed Cinola goldmine, several methods were used to evaluate different aspects of streambed quality. Like the results of this study, some of the baseline data is of a developmental nature (Derksen 1985, Derksen 1986). A study approach to assess the spawning ground quality of areas that could be impacted upon is presented in Appendix III. Methods development and continued research using the egg and alevin stages is viewed as an area that could be valuable in the environmental assessment of mine developments. The initial approach might be of the traditional nature and include an assessment of gravel quality and water quality. If any of those categories indicate deteriorating quality, then instream work to assess impacts on the egg/alevin stages could be initiated. Alternatively, the assessment of egg or alevin quality in terms of metal content and incubation success might be incorporated as part of the initial baseline evaluation.

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APPENDICES

APPENDIX I GENERAL BIOTA PREPARATION PROCEDURE

1) Label and weigh a sufficient supply of clean plastic vials. Use computer program #BIOWT to set up a weight file (or to add to an existing file) and use the following conventions:

   a) Vials are identified by a "vial number" - the vial number consists of a letter prefix and a number suffix, i.e. "G23".
   The prefix letter "A" is reserved for reference materials and should not be used for regular samples.

   b) Biota weight file names should consist of a single letter prefix followed by a series of numbers and/or letters, to maximum of six characters, i.e. "P1015A". The first letter in the file name should be the same as the prefix letter used for all vial numbers, i.e. P46, used in that file. The file name "SRM" is reserved for standard reference materials and all vials in this file should have "A" as their prefix letter.

   c) The maximum number of entries in a weight file is 199, try to have 100-150 entries for maximum efficiency and a minimum number of active files.

   d) Assign prefix letters in alphabetical order, start with "B" in the beginning of the year. Some ongoing projects may warrant assigning a single letter for all samples in that project.

2) Homogenize wet tissue samples in a stainless steel blender (if samples are small enough to fit into vial, this step may be omitted). Fill a labelled vial approximately 3/4 full with tissue homogenate. Rinse the blender well with deionized water between samples.

3) Weigh vial + wet sample.

4) Cover sample vials with a "Kimwipe", securing it with a rubber band, place samples in freezer and freeze solid. Exercise care when covering samples - rubber bands contain significant levels of zinc and cadmium.

5) Place frozen samples into freeze drier, allow at least 24 hours to dry - excessively wet samples and a "loaded" freeze drier may extend drying time to two or three days.

6) Weigh vial + dry sample.

7) Pulverize dry sample in a coffee grinder or Wiley mill. Clean coffee grinder well between samples by blowing out with compressed air. Excessively oily samples may coat the grinder, this deposit may be removed by grinding one or two loads of rice, cleaning grinder with a KIMWIPE and then blowing out with compressed air. Very small samples may be pulverised by using a glass rod or stainless steel spatula to grind sample material in the vial.

8) Into a marked petri dish weigh approximately 0.3 g. of freeze dried sample, ensure that the petri dish has been tared properly before adding sample material. All dishes must be covered with watch glasses immediately after sample material has been weighed to reduce contamination. N.B. To weigh replicate sample, prefix the dish number with the letter "R" then the dish number, i.e. R369.

For any given lot of samples, the number of replicate samples run for that lot should be at least the square root of the number of samples in that lot, i.e. for 16 sample do 4 replicates and for 19 samples do 5 replicates. Each group of samples should have at least one blank (empty dish) in duplicate and at least one reference material (i.e. Bovine Liver, Oyster) in duplicate. All blanks and refernece materials should be entered in the "SRM" weight file and their vial numbers should have a prefix letter "A". Dummy entries for vial #, vial + wet sample weight, and vial + dry sample weight of "+9", "+10", and "+10" must be entered in the SRM weight file before dish sample weights are entered.

9) Load coverless dishes into plasma asher and ash overight - see asher operating instructions. Each asher load should contain one reagent blank in duplicate, one reference material (i.e. Bovine, Oyster) in duplicate, and one duplicate sample.

10) Remove samples from asher, cover with watch glasses, digest as follows:

I. For 0.3 g. of Dried Sample

a) Add 2.25 ml HNO_3 - warm on hotplate at setting of 2.5,
b) Allow dishes to cool, then add 2.25 ml HNO_3 and 0.75 ml HCl, warm on hotplate to dissolve material.
c) Allow dishes to cool, transfer contents quantitatively to labelled centrifuge tubes, make up to 25.0 ml with DI Water, mix well,
d) Label 30 ml acid washed bottles with the VIAL # and DISH #,
   i.e. VIAL # P36
   DISH # 289
e) Transfer sample solutions to the labelled 30 ml acid washed bottles, and
f) Label the caps of the 30 ml acid washed bottles with the last three digits of the sample number (i.e. 546 for sample number 840546).

II. For 0.1 g of Dried Sample

a) Add 0.9 ml HNO_3 - warm on hotplate at setting of 2.5,
b) Allow dishes to cool, then add 0.9 ml HNO_3 and 0.3 ml HCl, warm on hotplate to dissolve material,
c) Allow dishes to cool, transfer contents quantitatively to labelled centrifuge tubes, make up to 10.0 ml with DI Water, mix well,
d) Label 30 ml acid washed bottles with the VIAL # and DISH #,
   i.e. VIAL # P94
   DIAL # 835
e) Transfer sample solutions to the labelled 30 ml acid washed bottles, and
f) Label the caps of the 30 ml acid washed bottles with the last three digits of the sample number (i.e. 739 for sample number 840739).

APPENDIX II PINK SALMON EGG METAL CONTENT 1982

METAL	REPLICATE											
(ug/g dry weight)	1	2	3	4	5	6	7	8	9	10	$\overline{x}$*	S.D.
Hg (unfertilized)	.03	.03	< .02	.03	.03	< .02	.03	< .02	.10	.10	.04	.03
(eyed)	.10	.09	.09	.10	.03	< .02	.03	< .02	.03	-	.06	.04
Cu (unfertilized)	14.8	15.1	14.4	14.5	16.4	20.5	11.7	13.5	15.8	13.6	15.0	2.3
(eyed)	3.2	2.9	3.2	3.1	3.1	3.7	3.4	3.5	2.9	-	3.2	.3
Zn (unfertilized)	54.1	42.9	45.5	53.7	56.2	57.9	33.2	52.5	65.0	46.6	50.8	9.0
(eyed)	48.9	59.8	45.9	51.1	49.9	54.7	53.0	58.3	52.2	-	52.6	4.4
Fe (unfertilized)	46.3	82.4	51.8	49.7	60.0	76.2	42.4	47.0	64.8	56.2	57.7	13.3
(eyed)	44.9	54.6	42.6	49.7	44.7	43.2	49.7	49.0	46.1	-	47.2	3.9
Ca (unfertilized)	1350	1290	1380	1310	1410	1340	1220	1340	1360	1420	1340	59
(eyed)	1490	1430	1570	1580	1520	1540	1520	1660	1470	-	1530	68
Mg (unfertilized)	1490	1530	1410	1460	1320	1460	1220	1280	1380	1430	1400	98
(eyed)	1300	1440	1440	1450	1430	1540	1260	1270	1350	-	1390	96
Na (unfertilized)	1940	2540	1990	1510	2000	2240	1950	1650	2240	2730	2080	370
(eyed)	511	850	630	560	730	559	575	820	870	-	680	140

* detection limit used in mean calculation

APPENDIX III STUDY NEEDS TO ASSESS STREAM QUALITY