

The potential effects of cadmium and other mixed metal mining effluents on fish species in Morrison Lake, with particular emphasis on sockeye salmon.

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1. Introduction

1.1 Morrison Lake Overview

Morrison Lake is located in North Central British Columbia. The lake is moderately oligotrophic and dimictic—characterized by strong temperature stratification in the summer, ice cover and weak reverse stratification in the winter, and spring and fall turnover periods (Gottesfeld et al. 2011). Morphometric, limnological, and water quality parameters of the lake (summarized in Table 1) make it a good physical habitat for various fish species, particularly salmonids. Fish species observed in the lake include: chinook salmon, coho salmon, sockeye salmon, cutthroat trout, lake trout, rainbow trout, lake whitefish, rocky mountain whitefish, longnosed sucker, white sucker, northern pikeminnow, peamouth chub, and prickly sculpin (BC Ministry of Environment FISS database, reviewed by Gottesfeld et al. 2011)

Table 1. Morrison Lake morphometry, limnology, and water quality parameters

Parameter	Value	Parameter	Value
Conductivity ^a	57.5-65 µs/cm	Ammonia ^a	1.9-7.3 µg/L
Depth		Fluoride ^a	22-98 mg/L
Maximum ^a	60-63 m	Total Organic Carbon ^a	11 mg/L
Average ^a	21.6 m	Total Phosphate ^a	5.7-12.4 µg/L
Flow ^a	4.5 m ³ /s	Total Nitrate ^a	0.09-0.25 mg/L
Length		Total Sulphate ^a	2.34-2.61 mg/L
Maximum ^b	15,100 m	Total Dissolved Solids ^a	50-59 mg/L
Average ^a	13,000 m	Aluminum ^a	0.03 mg/L
Width		Arsenic ^a	0.26-2.9 µg/L
Maximum ^a	1,500 m	Copper ^a	0.83-1.81 µg/L
Average ^a	879 m	Cyanide ^a	6.3-8.6 µg/L
Surface area ^a	1,330 ha	Iron ^a	0.125 mg/L
Volume ^a	286 Mm ³	Molybdenum ^a	0.15 µg/L
pH ^a	7.2-7.8	Mercury ^a	0.013 µg/L
		Lead ^a	0.05 µg/L
		Selenium ^a	0.6 µg/L
		Zinc ^a	2 µg/L

^a David Bustard and Associates Ltd. 2005

^b Gottesfeld et al. 2011

1.2 Proposed Morrison Copper/Gold Project

The Morrison Copper/Gold Project (“Project”) is proposed in the Morrison-Hearne Hill property (20,000 hectares) located on the east side of Morrison Lake (Babine Watershed), 65 km north-east of Smithers. The proposed Project would be an open pit operation with ore processed in a conventional milling plant and the copper/gold concentrate transported off site. The Project would consist of an open pit mine, tailings storage facility, waste rock dump, processing plant, warehouse, assay lab, and other facilities that transport power to the site (Klohn Crippen Berger 2010). The Project would be under construction 2.5 years, in operation 21.5 years as a zero-surface-discharge facility, and under closure and reclamation for 5 years (Morrison Copper/Gold Project 2011a,b). Data presented by Pacific Booker Minerals, Inc. (PBM) indicates that a number of elements could be released into Morrison Lake at levels that may be of concern to fish populations and human health. These elements include: aluminum, arsenic, cadmium, copper, fluoride, magnesium, mercury, nitrite, nitrate, selenium, and sulphate (Morrison Copper/Gold Project 2010, Klohn Crippen Berger 2010)

1.3 Objective of This Review

This review is a qualitative assessment of existing data and a review of relevant primary literature. The objective of this review is to assess the potential effects of elements released from the Morrison Copper/Gold Project on fish assemblages in Morrison Lake, with particular emphasis on sockeye salmon. The effect of toxicants on salmonids is of particular importance because: (i) they are a predatory species that accumulates high pollutant levels in their edible muscle tissue through the process of biomagnification, and (ii) the consumption of salmonid tissues containing cadmium poses potential long-term risks to human populations and non-human consumers. The effect of released elements on sockeye salmon is of considerable importance because Morrison Lake lies within an important sockeye producing watershed in Canada (Gottesfeld et al. 2011). In previous years, sockeye salmon comprised nearly 72% of the fish found in Morrison Lake open waters, with approximately 500,000 sockeye fry observed at a time (Gottesfeld et al. 2011). Further, sockeye salmon are crucial to the First Nations of the Skeena Watershed for sustenance, as well as social and ceremonial purposes (Gottesfeld et al. 2011).

This review will assess (i) the known effects of cadmium on sockeye salmon and other fish species, (ii) the potential impacts of mixed metal mine effluents on sockeye salmon and other fish species, (iii) the effect of cadmium and other metals on sensitive stages in fish life history, (iv) variability in sensitivity to metals among different fish species, and (v) the adequacy of the BC Water Quality Guidelines standards.

2. The effect of cadmium on sockeye salmon and other fish species

2.1. Cadmium overview

Cadmium is a biologically nonessential heavy metal that occurs naturally in ores together with zinc, lead, and copper (reviewed by Jarup 2003). Concentrations of cadmium in polluted natural waters can range up to 0.2 mg/L (reviewed by Jezierska et al. 2009) and can affect aquatic populations through waterborne or dietary exposure. Exposure to cadmium can cause toxicity and even mortality in a variety of aquatic organisms depending on the range of cadmium concentrations, the species of organism, the life stage of the organism, and the presence of other metal ions (WHO 1992; Table 2 and Table 3). Cadmium accumulates in the organism and its half-life (time required to eliminate half of the accumulated dose) ranges from 24-63 days, depending on species (Harrison and Klaverkamp 1989). There is also some evidence to suggest that cadmium can biomagnify across the freshwater food web (Campbell et al. 2003, Croteau et al. 2005). In humans, cadmium accumulates in the kidneys and has a biological half-life of 10-35 years (WHO 2008). The Minimum Risk Level (or MRL, an estimate of the threshold level that humans can be exposed to on a daily basis without appreciable risk of adverse, non-cancer health effects) for cadmium orally ingested by humans is 0.0001 mg of cadmium/kg/day on a chronic basis (ATSDR 2012).

2.1.1. Waterborne exposure

Waterborne cadmium can mimic calcium to some extent and is taken up by fish primarily through the branchial calcium transport pathway (e.g. Niyogi and Wood 2004, Niyogi et al. 2008). Acute toxicity occurs when active calcium transport is prevented by blocked calcium channels and/or inhibited calcium ATPase transporters (Verbost et al. 1987, Reddy et al. 1988). Biomolecular functions can also be impaired if cadmium binds to proteins cysteine or glutathione (Baldisserotto et al. 2004, Campbell et al. 2008). One result of cadmium induced toxicity is reduced calcium levels in the organism (Sauer and Watabe 1988, Verbost et al. 1989).

2.1.2. Dietary exposure

Mechanisms of dietary uptake and toxicity of cadmium are not well understood, though dietary exposure has been shown to be considerably less toxic to fish than waterborne (Handy 1993). Cadmium enters the gut where absorption can occur through calcium (e.g. Franklin et al. 2005) or iron transport pathways (Cooper et al. 2006, Kwong and Niyogi 2009, Kwong et al. 2011). Cadmium then enters the gills where it can stimulate the induction of metallothionein, a metal binding protein (Dang et al. 2001). Cadmium has also been shown to accumulate in freshwater invertebrates which are common prey items for salmonids (Spehar et al. 1978).

2.2. The effects of cadmium on fish

Cadmium readily accumulates in various tissues of fishes (e.g. gills, liver, kidneys, and gonads) and causes physiological disturbances including decreased growth, impaired development, endocrine disruption, and disrupted gene transcription (Table 3). It should be noted that studies investigating the effects of cadmium on fish are largely focused on short term exposures ranging from hours to weeks. This time scale is not indicative of the risks associated with chronic or lifetime exposure to cadmium. The US EPA water quality criterion for cadmium has been set at a criterion maximum concentration of 0.002 mg/L, and a criterion continuous concentration of 0.00025 mg/L, assuming a water hardness of 100 mg CaCO₃/L (US EPA 2001). The BC water quality guidelines are set at 0.000024 mg/L assuming a water hardness of 90 mg CaCO₃/L. Projected cadmium levels to be released into Morrison Lake from the Morrison Copper/Gold Project are expected to reach concentrations of up to 0.0005 mg/L in the final effluent and up to 0.000025 mg/L in the 10 m mixing zone from the treated water discharge diffuser (Klohn Crippen Berger 2010). These levels of cadmium could affect various fish species present in Morrison Lake including: chinook salmon, chub, coho salmon, lake trout, lake whitefish, pike minnows, rainbow trout, sculpins, sockeye salmon, and suckers. The documented effects of cadmium on different fish species are discussed below, with particular emphasis on the effects of cadmium on sockeye salmon, when information is available.

2.2.1. Acute Toxicity/Mortality

Cadmium has been shown to significantly bioaccumulate in various tissues (e.g. gills, intestine, liver, kidney, muscle, gonads) of multiple species of teleost fish (Table 3). The estimated half-life of cadmium in fish is 24-63 days (Harrison and Klaverkamp 1989). In salmonids, 4 month exposure to 5 mg/kg dietary cadmium caused cadmium to accumulate in the gut, kidney, and muscle of Atlantic salmon (Berntssen et al. 2001), and 1 month exposure to 0.003 mg/L waterborne cadmium resulted in cadmium accumulation in the gills, liver, kidney, and carcass of rainbow trout (Hollis et al. 2001). Cadmium has been shown to cause genotoxic effects that damage DNA and can potentially result in mutations (Bagdonas and Vosyliene 2006, Ahmed et al. 2010, Cambier et al. 2010). Exposure to cadmium also leads to mortality in various fish species, including salmonids (e.g. Atlantic salmon: Peterson et al. 1985, Coho salmon: Chapman and Stevens 1978, Steelhead salmon: Chapman and Stevens 1978, Sockeye salmon: Servizi and Martens 1978). The toxicity of cadmium to salmonids varies based on life stage and species of fish (Table 2), and is more toxic in waters of low calcium content. The 96-h LC50 (concentration that kills 50% of the test group in 4 days) for cadmium in salmonids can range from 0.001 to 0.027 mg/L in steelhead and chinook salmon in the alevin, swim up, parr, and smolt life stages (Chapman 1978a). In contrast, exposure to cadmium for over a week resulted in 50% mortality

when adult steelhead salmon and male coho were exposed to 0.0052 mg/L and 0.0037 mg/L of cadmium, respectively (Chapman and Stevens 1978).

2.2.2. Metabolism, Activity, & Growth

Cadmium has been shown to alter the behavior and activity levels of teleost fishes (Kislalioglu et al. 1996; Farag et al. 2003; Szczerbik et al. 2006, Eissa et al. 2010). Further, exposure to cadmium can interfere with metabolic activities. A glossary of some of the pertinent metabolic parameters in fish is provided in Glossary 1. Exposure to cadmium in teleost fishes has been shown to reduce food intake and assimilation rates (Ferrari et al. 2011), alter metabolic rate (Ferrari et al. 2011), alter glycogen and phosphorus reserves (Hallare et al. 2005, Annabi et al. 2011, Malekpouri et al. 2011), decrease growth rate (Campbell et al. 2003, Szczerbik et al. 2006, Rasmussen et al. 2008, Cao et al. 2010), and alter expression of genes and enzymes involved in metabolism (Hontela and Lacroix 2006, Szczerbik et al. 2006, Cao et al. 2010, Lin et al. 2011).

Glossary 1. A glossary of pertinent metabolic parameters in fish

Parameter	Description
glucocorticoid receptor	binds cortisol and glucocorticoids; regulates genes controlling development, metabolism, and immunity;
glucose	primary source of energy;
hepatosomatic index	liver:body weight, indicator of energy reserve status;
lactate	produced during metabolism and exercise;
metabolic enzymes	key catalysts involved in metabolism (e.g. glycogen phosphorylase);

Cadmium has been shown to affect metabolic parameters in salmonids at dietary and waterborne exposure concentrations as low as 125 mg/kg and 0.005 mg/L, respectively (Table 3). In Atlantic salmon, exposure to 125 mg/kg of dietary cadmium for 4 months reduced lipids, proteins, glycogen, and energy reserves (Berntssen and Lundebye 2001), whereas exposure to 0.01 mg/L waterborne cadmium for 8 hours increased plasma glucose and lactate, and reduced liver glycogen (Soengas et al. 1996). In rainbow trout, 30 day exposure to 60 000 mg/kg of dietary cadmium or 0.005 mg/L waterborne cadmium decreased the hepatosomatic index, and

decreased condition factor and body weight (Adiele et al. 2011). Exposure to 0.4 mg/L waterborne cadmium increased plasma glucose and decreased liver glycogen (Hontela et al. 1996), and exposure to 0.1 mg/L waterborne cadmium delayed growth hormone expression (Jones et al. 2001). No studies to date have investigated the effect of cadmium on metabolic parameters in sockeye salmon.

Table 2. Toxicity of cadmium to different life stages of salmonids

Salmonid Species	Life Stage	Exposure Period	LC50 (mg/L)	Water Hardnes (mg CaCO ₃ /L)
Atlantic salmon	alevin ^d	24 hr	1.5	19-28
Chinook salmon	alevin ^b	96 hr	0.026	24
	swim up ^b	96 hr	0.0018	24
	parr ^b	96 hr	0.0035	24
	smolt ^b	96 hr	0.0029	24
	adult ^a	215 hr	0.0037	20-23
Sockeye salmon	fry ^c	160 hr	0.03	82.9-84.4
Steelhead salmon	alevin ^b	96 hr	0.027	24
	swim up ^b	96 hr	0.0013	24
	parr ^b	96 hr	0.001	24
	smolt ^b	96 hr	0.0029	24
	adult ^a	408 hr	0.0052	28-90

LC50 – concentrations that kills 50% of test individuals; ^aChapman and Stevens 1978,

^bChapman 1978a, ^cServizi and Martens 1978, ^dRombough and Garside 1980

Table 3. Overview of general effects of cadmium in teleost fishes

Species	Life Stage	Effect	Duration of Exposure	Type of Exposure			Reference
				Dietary mg/kg	Waterborne (mg/L)	<i>in vitro</i> mg/L	
Salmonids							
Atlantic salmon	Parr	tissue accumulation, gut cell death	4 months	5			Berntssen et al. 2001
	Parr	tissue accumulation, induced metallothionein expression	8 weeks	125			Dang et al. 2001
	Parr Parr	reduced energy reserves decreased liver glycogen, increased plasma glucose and lactate, increased plasma cortisol	4 months 8 h	125		0.01	Berntssen and Lundebye 2001 Soengas et al. 1996
Chinook salmon	Parr	disrupted gill morphology	2 days		0.01		Devos et al. 1998
Coho salmon	juvenile	50% mortality	96 h		0.002-0.026		Chapman 1978a
	juvenile	50% mortality	1 week		0.0037		Chapman and Stevens 1978
Rainbow trout	juvenile	tissue accumulation	1 month		0.003		Hollis et al. 2001
	juvenile	tissue accumulation	4 week	34-39			Kwong et al. 2011
	juvenile	tissue accumulation, induced metallothionein expression	30 days			0.003	Hollis et al. 2001
	Adult	reduced condition factor, reduced body weight	30 days	60000		0.005	Adiele et al. 2011
	juvenile	decreased liver glycogen, increased plasma glucose	2h-1 week			0.4	Hontela et al. 1996
	embryo	delayed growth hormone expression	2 weeks			0.1	Jones et al. 2001
	juvenile	altered plasma cortisol	48 h			0.001	Brodeur et al. 1998
	juvenile	Impaired kidney function	30 days				0.001 Brodeur et al. 1998
juvenile	Increased plasma cortisol	2-4 h			0.4	Hontela et al. 1996	
juvenile	suppressed cortisol production, disrupted corticosteroid synthesis	4 h				0.002 Sandhu and Vijayan 2011	
juvenile	decreased vitellogenesis, endocrine disruption	72 h			0.005	Vetillard and Bailhache 2005	
Sockeye salmon	juvenile	50% mortality	160 h		0.03		Servizi and Martens 1978
Steelhead salmon	juvenile	50% mortality	96 h		0.001-0.027		Chapman 1978a
	juvenile	50% mortality	1 week		0.0052		Chapman and Stevens 1978

Table 3 (continued). Overview of general effects of cadmium in teleost fishes

Species	Life Stage	Effect	Duration of Exposure	Type of Exposure			Reference
				Dietary (mg/kg)	Waterborne (mg/L)	<i>in vitro</i> (mg/L)	
Cyprinids							
Common carp	juvenile	tissue accumulation	30 days		0.03		Ghiasi et al. 2011
	juvenile	altered behavior and activity levels	4 days		0.6		Eissa et al. 2010
	juvenile	reduced food intake and assimilation rates, altered metabolic rate	2 wk		0.15		Ferrari et al. 2011
Goldfish	juvenile	altered energy reserves, disrupted levels and ratios of serum ions	14 days		1-2		Malekpouri et al. 2011
	juvenile	tissue accumulation, altered behavior and activity levels, decreased growth rate, altered gene and enzyme expression, reduced gonad:body weight, inhibited ovulation	3 years	100-10000			Szczerbik et al. 2006
Sheepshead minnow Zebrafish	Larvae	tissue accumulation	7 days	0.3			Dangre et al. 2010
	Adult	tissue accumulation, induced metallothionein expression	21 days		0.4		Chouchene et al. 2011
	Adult	tissue accumulation, damaged DNA altered energy reserves, impaired development, caused malformations	21 days 48 h		0.01 0.25-10		Cambier et al. 2010 Hallare et al. 2005
Other fish species							
Gilthead sea bream	juvenile	induced heat shock proteins, increased plasma cortisol, decreased ATPase activity	11 days	1.25			Garcia-Santos et al. 2011
Japanese flounder	juvenile	tissue accumulation, decreased growth rate, altered gene and enzyme expression	80 days		0.012-0.048		Cao et al. 2010
Marine grunts	juvenile	tissue accumulation, induced metallothionein expression	4 weeks	30-60	0.06		Dang and Wang 2009
Mosquitofish	juvenile	altered energy reserves, impaired kidney function, disrupted gill components	30 days		0.4		Annabi et al. 2011
Silver catfish	juvenile	tissue accumulation	14 days		0.414		Pretto et al. 2011

2.2.3. Stress, Immunity, & Cellular Responses

A description of some of the pertinent stress, immunity, and cellular response parameters in fish is provided in Glossary 2. In general, exposure to cadmium in teleost fishes has been shown to increase the expression of heat shock proteins (Garcia-Santos et al. 2011), increase plasma cortisol levels (Garcia-Santos et al. 2011), and induce the expression of metallothionein (Dang and Wang 2009, Chouchene et al. 2011); these physiological and cellular stress responses are all potentially energetically costly. Exposure to cadmium can also impair kidney function (Annabi et al. 2011), alter genes involved in immunity and the stress response (Lin et al. 2011), and cause damage to DNA through single nucleotide modifications (Cambier et al. 2010); these effects can impact the health status of the fish.

Glossary 2. A glossary of pertinent stress, immunity, and cellular response parameters in fish

Parameter	Description
glucocorticoid receptor	binds cortisol and glucocorticoids, regulates genes controlling development, metabolism, and immune response;
heat shock proteins	indicate cells have been exposed to stress, associated with increased cortisol levels (e.g. Iwama et al. 1999, Roberts et al. 2010);
heme oxygenase	catalyzes the degradation of heme;
kidney/renal/glomeruli	kidneys filter waste and toxins from the blood to prevent osmoionic failure;
metallothionein	protein that binds heavy metals, helps protect against metal toxicity;
oxidative stress endpoints	indicate toxic effects are damaging cells (e.g. changes in activity of enzymes such as catalase CAT, superoxide dismutase SOD; concentrations of antioxidants such as GSH; increase in lipid peroxidation, LPO);

Cadmium has been shown to affect stress, immunity, and cellular response parameters in salmonids at dietary, waterborne, and *in vitro* concentrations as low as 125 mg/kg, 0.001 mg/L, and 0.001833 mg/L, respectively. In Atlantic salmon, 4 month exposure to 125 mg/kg of dietary cadmium led to gut cell death (Berntssen et al. 2001), 8 week exposure to 125 mg/kg of dietary cadmium stimulated the expression of metallothionein (Dang et al. 2001), and 8 hour exposure to 0.010 mg/L waterborne cadmium increased plasma cortisol (Soengas et al. 1996). In rainbow trout, 2 day exposure to 0.001 mg/L waterborne cadmium altered plasma cortisol levels (Brodeur et al. 1998), 30 day exposure to 0.001 mg/L cadmium *in vitro* impaired kidney function (Brodeur et al. 1998), 30 day exposure to 0.003 mg/L waterborne cadmium stimulated expression of metallothionein (Hollis et al. 2001), 2 to 4 hour exposure to 0.4 mg/L waterborne cadmium increased plasma cortisol (Hontela et al. 1996), and 4 hour exposure to 0.001833

mg/L cadmium *in vitro* suppressed cortisol production and disrupted genes for corticosteroid synthesis (Sandhu and Vijayan 2011).

2.2.4. Osmoregulation & Respiration

A glossary of some of the pertinent osmoregulatory and respiratory parameters in fish is provided in Glossary 3. Exposure to cadmium in teleost fishes has been shown to disrupt serum levels and ratios of ions (Lin et al. 2011, Malekpouri et al. 2011), decrease sodium/potassium ATPase activity (Garcia-Santos et al. 2011), and disrupt gill components (Annabi et al. 2011).

Glossary 3. A glossary of pertinent osmoregulatory and respiratory parameters in fish

Parameter	Description
ions (Na, Cl, Ca, K)	role in osmoionic homeostasis of body fluids;
gill epithelium	facilitates absorption, filtration, and gas exchange;
gill lamellae	facilitate exchange of oxygen and CO ₂ between water and blood;

Cadmium can affect osmoregulatory and respiratory parameters in salmonids at waterborne exposure concentrations as low as 0.01 mg/L. In Atlantic salmon, exposure to 0.01 mg/L of waterborne cadmium for 2 days disrupted parr and smolt gill morphology (Devos et al. 1998).

2.2.5. Reproduction

Exposure to cadmium in teleost fishes has been shown to inhibit estrogen receptors (Le Guevel et al. 2000), reduce the gonadosomatic index (ratio of fish gonad weight to body weight) (Szczerbik et al. 2006), prevent ovulation (Szczerbik et al. 2006), impair growing embryos, and cause malformations in embryos and larvae (Hallare et al. 2005).

In salmonids, cadmium has been shown to alter reproductive parameters at waterborne concentrations as low as 0.005 mg/L. In rainbow trout, exposure to 0.005 mg/L of waterborne cadmium for 72 hours decreased vitellogenesis and caused endocrine disruption in estrogenic pathways (Vetillard and Bailhache 2005).

3. The effect of mixed metals on sockeye salmon and other fish species

3.1. Overview

A number of elements may be released from the Morrison Copper/Gold Project into the Morrison Lake at levels of concern and exert their adverse effects as mixtures. These elements include: aluminum, arsenic, cadmium, copper, fluoride, magnesium, mercury, nitrite, nitrate, selenium, sulphate, and acid pH (Klohn Crippen Berger 2010, Morrison Copper/Gold Project 2010). While the accumulation of mixed metals is variable based on season, type of metal, type of fish tissue and fish species, previous studies have shown that interactions between metal ions can initiate oxidative stress and genotoxicity (damage to DNA) in fish (Moriwaki et al. 2008). Bioaccumulation of elements (e.g. cadmium, copper, lead, zinc) occurs when an element accumulates in an organism's tissues due to absorption whereas biomagnification occurs when the concentration of an element (e.g. mercury) increases across the food chain as a result of persistence and food web dynamics. Alsop and Wood (2011) showed that there is a common pathway of metal uptake and a common mechanism of acute toxicity across groups of metals in zebrafish. In rainbow trout, Kamunde and MacPhail (2011a) reported that cadmium accumulation was enhanced when fish were exposed to metal mixtures rather than single metals. Literature dealing with the effect of mixed metals combined with cadmium and other stressors on fish, with particular emphasis on sockeye salmon, is discussed below. While numerous studies address the effects of single metals (e.g. arsenic, copper, zinc, selenium or mercury) on fish (e.g. Sorensen 1991), this report will focus on the combined effects of cadmium and other pertinent stressors on fish species present in Morrison Lake.

3.2.1. Acidity

One potential impact of the Morrison Copper/Gold Project of concern to the aquatic biota is the acidification of Morrison Lake. A third party review by Robertson Geoconsultants Inc. (RGC) suggests that many of the predictions made by Pacific Booker Minerals Inc. are dependent on pH of approximately 8, so mitigation efforts to maintain a stable pH will be a very important component when evaluating the potential toxicity of released elements from the mine (Robertson Geoconsultants Inc. 2011). Previous studies have shown that acidification of surface waters can increase the accumulation of methyl mercury, lead, and cadmium by fish (Spry and Wiener 1991), and increase the toxicity of copper to fish. Further, Parker and McKeown (1987) reported that sockeye salmon exposed to acidified waters at pH 4.0 to 5.0 were characterized by reduced egg and alevin survival, delayed embryo hatching, reduced efficiency of yolk to tissue conversion in alevins, and decreased percent hatch. Peterson et al. (1985) reported that in Atlantic salmon, accumulation of cadmium and mortality occurred more frequently under low pH conditions.

3.2.2. Aluminum

Aluminum can adversely affect aquatic life through several mechanisms (Exley et al. 1991). No study to date has investigated the combined effects of cadmium, aluminum, and other metals on sockeye salmon, but Salbu et al. (2008) reported that metal mixtures of aluminum and cadmium enhanced the toxicity of cadmium to the gills of juvenile Atlantic salmon and activated the physiological stress response. A similar effect can be expected to occur in other members of the family Salmonidae, including the sockeye salmon, since the physiological, morphological and biochemical characteristics are often similar in species belonging to the same family.

3.2.3. Arsenic

Arsenic is a widely distributed metalloid, occurring in rock, soil, water and air (reviewed by Jarup 2003). Projected arsenic levels to be released into Morrison Lake from the Morrison Copper/Gold Project are expected to reach concentrations of up to 0.01 mg/L in the final effluent and up to 0.00005 mg/L in the 10 m mixing zone from the treated water discharge diffuser (Klohn Crippen Berger 2010). The BC Water Quality Guidelines and the CCME guidelines require that arsenic levels in BC waters are less than 0.005 mg/L. No study to date has characterized the mixed effects of arsenic and cadmium on sockeye salmon, but numerous studies have investigated the combined effects of cadmium, arsenic and other heavy metals in other fish species and observed metal accumulation and an induction of oxidative stress endpoints (Foran et al. 2004, Falco et al. 2006, Kelly et al. 2008, Ebrahimi and Taherianfard 2011, Raissy et al. 2011, Vieira et al. 2011, Oliva et al. 2012). The induction of oxidative stress endpoints indicates a potential disruption of cellular processes and damage to cell membranes, DNA, and proteins through lipid peroxidation caused by reactive oxygen species

3.2.4. Copper

Projected copper levels to be released into Morrison Lake from the Morrison Copper/Gold Project are expected to reach concentrations of up to 0.015 mg/L in the final effluent and up to 0.00075 mg/L in the 10 m mixing zone from the treated water discharge diffuser (Klohn Crippen Berger 2010). The BC Water Quality Guidelines and the CCME guidelines require that copper levels in BC waters are less than 0.002 mg/L. No study to date has investigated the mixed effects of copper and cadmium on sockeye salmon, but numerous studies have characterized the combined effects of cadmium, copper and other metals in other fish species and observed metal accumulation, induction of stress responses and oxidative stress endpoints, and induction of metallothionein during day to month exposure concentrations ranging from 0.0158-0.03 mg/L of copper and <0.001-0.015 mg/L of cadmium (Chapman and Stevens 1978, Chapman 1978a, Marr et al. 1995, Lundebye et al. 1999,

Berntssen et al. 2000, Foran et al. 2004, Hansen et al. 2006, Hansen et al. 2007, Kelly et al. 2008, Moriwaki et al. 2008, Jezierska et al. 2009, Atli and Canli 2011, Kamunde and MacPhail 2011a, Kamunde and MacPhail 2011b, Kamunde and MacPhail 2011c, Jaffal et al. 2011, Pierron et al. 2011, Maceda-Veiga et al. 2012, Oliva et al. 2012).

3.2.5. Fluoride

No study to date has investigated combined effects of cadmium, fluoride, and other metals on sockeye salmon. In rainbow trout, 96 hr LC50 values (the concentrations that kills 50% of fish) increased from 51 to 193 mg of fluoride per liter as water hardness rose from 17 to 385 mg/L CaCO₃ (Pimentel and Bulkley 1983). Fluoride toxicity to aquatic organisms has been reviewed by Camargo (2003) and concentrations below 0.5 mg F⁻/L have been recommended to protect invertebrates and fishes in soft waters with low ionic content.

3.2.6. Magnesium

Projected magnesium levels to be released into Morrison Lake from the Morrison Copper/Gold Project are expected to reach concentrations of up to 120 mg/L in the final effluent from the treated water discharge diffuser (Klohn Crippen Berger 2010). No study to date has investigated the combined effects of cadmium, magnesium, and other heavy metals on sockeye salmon. In gibel carp fed 2900 mg/kg of magnesium for 3 months, negative impacts on survival, growth, and ATPase activity were observed (Han et al. 2011).

3.2.7. Mercury

Mercury can be converted to organic compounds, such as methyl mercury, that are very stable and accumulate in the food chain (reviewed by Jarup 2003). No study to date has investigated the mixed effects of mercury and cadmium on sockeye salmon, but numerous studies have explored the combined effects of cadmium, mercury, and other heavy metals in other fish species and observed positive correlations between mercury and body size and impaired reproduction (Spry and Wiener 1991, Foran et al. 2004, Falco et al. 2006, Kelly et al. 2008, Dietrich et al. 2010, Ebrahimi and Taherianfard 2011, Raissy et al. 2011, Vieira et al. 2011, Maceda-Veiga et al. 2012).

3.2.8. Nitrite/Nitrate/Ammonium

Nitrate waste is one projected byproduct of blasting. No study to date has investigated combined effects of cadmium, nitrite/nitrate, and other metals on sockeye salmon. Elevated levels of ammonia in the body have been shown to have a number of deleterious effects on fish, including mortality, and factors such as stress and activity can increase the toxicity of ammonia to fish (reviewed by Randall and Tsui, 2002).

3.2.9. Selenium

Selenium is an essential element nutritionally required for maintenance of health and normal function of key enzymes such as glutathione peroxidase and heme oxidase. At concentrations only slightly higher than required, selenium becomes toxic and exerts genotoxic and carcinogenic effects (reviewed by Valdiglesias et al. 2010). In fish, the most documented effect of selenium toxicity is abnormal embryonic development and malformations (teratogenicity) in fish larvae (Janz et al. 2010). Waterborne selenite has been shown to be more toxic than selenate to different salmonid species (Hamilton and Buhl 1990), but organic selenium in the diet also exerts toxic effects on salmonids (Hamilton et al. 1990). Projected selenium levels to be released into Morrison Lake from the Morrison Copper/Gold Project are expected to reach concentrations of up to 0.028 mg/L in the final effluent and up to 0.0014 mg/L in the 10 m mixing zone from the treated water discharge diffuser (Klohn Crippen Berger 2010). The CCME guidelines require that selenium levels in BC waters are less than 0.001 mg/L. No study to date has investigated the mixed effects of selenium and cadmium on sockeye salmon, however developmental malformations have been documented in salmonids exposed to excess selenium (Holm et al. 2005, Rudolph et al. 2008).

3.2.10. Sulphate

Projected sulphate levels to be released into Morrison Lake from the Morrison Copper/Gold Project are expected to reach concentrations of up to 1700 mg/L in the final effluent and up to 85 mg/L in the 10 m mixing zone from the treated water discharge diffuser (Klohn Crippen Berger 2010). The BC Water Quality Guidelines require that sulphate levels in BC waters are less than 50 mg/L. No study to date has investigated the mixed effects of sulphate and cadmium on sockeye salmon. Sulphate is one product of the oxidation of sulfide (along with other products including polysulfides, elemental sulfur, thiosulfate, and sulfite), and is associated with the generation of highly acidic conditions. The documented effects of sulfide on freshwater fishes include reduced swimming endurance, tissue irritation and cell death, lower food consumption, inhibited spawning and egg production, reduced egg survival, increased developmental deformities, and reduced survival and growth when sulfide concentrations exceed 0.45 μM H_2S (reviewed by Bagarinao 1992).

4. The effect of cadmium and other metals on sensitive stages of fish development

4.1. Overview

Sockeye salmon spend a period of their lives in freshwater and a considerable period of time out in sea. Fertilized eggs are buried in gravel nests called redds, eggs hatch within a few weeks-months into alevins with attached yolk sacs. Alevins emerge from the gravel and become fry three to six weeks later. Fry develop into parr that feed and grow in their native habitat before becoming smolts in one to three years. Smolts migrate to the ocean and develop into mature salmon within two to three years, and adult salmon return to their native freshwater habitat to spawn after two to three years at sea. The Morrison Lake sockeye salmon spawn in Morrison River or upstream in Morrison Lake or Tahlo Creek, and mature in Morrison Lake (Gottesfeld et al. 2011). The early developmental stages of these fish in their native freshwater habitat can be particularly sensitive to water pollution, and disruption of developmental processes during the embryonic period can reduce offspring quantity and quality. Further, a number of studies have suggested that metals and other toxicants can accumulate in reproductive tissues of adult fish and diminish the ability to produce viable gametes and healthy offspring (e.g. Pelgrom et al. 1995, Larsson et al. 1998). A review of literature investigating sensitive stages of fish development, with particular emphasis on salmonids, is presented below.

4.2. Metal accumulation in reproductive tissues

Waterborne metals including cadmium can accumulate in the gonads of teleost fishes and adversely affect gamete production, viability, or development at exposure concentrations ranging from 0.005 to 5.5 mg/L over days to months (e.g. Allen 1995, Pelgrom et al. 1995, Larsson et al. 1998, Ebrahimi and Taherianfard 2011). The accumulation of metals in gonads can also alter sex hormone levels (Ebrahimi and Taherianfard 2011), and appears to affect female fish more than males (Jaffal et al. 2011).

4.3. Sensitivity of early fish stages to metals

Early stages of fish development are particularly sensitive to cadmium and other mixed metals (e.g. Hallare et al. 2004). In salmonids, early exposure to cadmium can alter the rate and timing of hatching, disrupt sperm motility, or lead to deformations and mortality in newly hatched larvae (e.g. Chapman 1978a, Rombough and Garside 1980, Dietrich et al. 2010). In Atlantic salmon, exposure to 0.00047 mg/L of cadmium during development (nearly twenty times the BCWQG of 0.000024 mg/L) impaired the growth of alevins, and mortalities were observed at concentrations as low as 0.0082 mg/L (Rombough and Garside 1980). The alevin

stage of development was found to be most sensitive to cadmium in Atlantic salmon (Rombough and Garside, 1980) and Chinook salmon (Chapman 1978a). Studies investigating the heightened sensitivity of newly-hatched sockeye alevins to chronic and episodic exposure to low pH suggest that the alvein stage of sockeye salmon may also be most sensitive to cadmium (Parker and McKeown 1987). Servizi and Martens (1978) reported that the 160-h LC50 for sockeye salmon exposed to cadmium declined from 4.5 mg/L at hatch to 0.03 mg/L at the fry stage, and Baker et al. (2009) found that trace metal concentrations were greater in sockeye smolts than adults. The issue of heightened sensitivity to cadmium during early fish stages is of particular importance when considering that shoreline habitat in Morrison Lake is of critical importance to sockeye salmon spawning (Gottesfeld et al. 2011). Spawning areas along the shoreline and at the mouth of creeks could be more heavily impacted by water quality changes induced by seepage and effluent discharges during periods of altered flow in Morrison Lake and its associated tributaries.

Sockeye salmon fry in Morrison Lake utilize all portions of the lake, including deep waters (Gottesfeld et al. 2011). Previous studies have suggested that the utilization of deep waters by sockeye salmon is an important mechanism that facilitates continued residence of sockeye fry in lakes (Hoar 1954). It is of particular importance to note that the treatment effluent diffuser proposed by the Morrison Copper/Gold Project is expected to be placed at the bottom of Morrison Lake in the proximity of sockeye salmon at their most sensitive and early stages of development.

5. Variability in sensitivity to metals among different fish species

No single study has compared cadmium toxicity among a large number of salmonid species. Cappon (1987) found a comparable bioaccumulation of cadmium in the tissues of coho salmon, chinook salmon, lake trout, and brown trout after lifelong exposure to cadmium in a freshwater lake. Chapman (1978a) reported that steelhead salmon are more sensitive to cadmium than Chinook salmon, and Hansen et al. (2002) reported that rainbow trout are two times as sensitive to cadmium as bull trout. Results from multiple studies investigating the effect of cadmium on salmonids (summarized in Table 2 of Section 2), suggest that early life stages of sockeye salmon display a similar tolerance to cadmium (after controlling for the effects of water hardness on cadmium toxicity) when compared with chinook salmon, coho salmon, or steelhead salmon. The toxicity of molybdenum also appears to be relatively comparable across different salmonid species (Reid 2002). The sensitivity to metals and other elements, however, can vary considerably across different species of salmonid based on the type of element studied. Chapman (1978b) noted that sockeye salmon appear to be relatively tolerant of zinc (96-h LC50: 0.749 mg/L) when compared with other salmonid species including

the cutthroat trout (96 h LC50: 0.090 mg/L), chinook salmon (96-h LC50: 0.463 mg/L), and steelhead salmon (0.136 mg/L). In contrast, the toxic effects of acidity appear to be more significant in sockeye salmon than some other salmonids. Parker and McKeown (1987) reported that 55% of sockeye eggs survived to the eyed stage at a pH of 4.6 in contrast to an estimated survival of 82% of brook trout eggs to the eyed stage under the same conditions.

6. BC Water Quality Guidelines

A number of elements may be released from the Morrison Copper/Gold Project into Morrison Lake at levels of concern, but only cadmium is projected to be at concentrations that exceed the BC Water Quality Guidelines several fold. Exceedance of the cadmium threshold could potentially have major adverse effects on all salmonids in Morrison Lake and is likely to lead to loss of the salmon populations. A third party review by Robertson Geoconsultants Inc. (RGC) that evaluated hydrogeology and water quality components of the proposed project suggests that other elements (including sulphate, copper, and zinc) could also potentially exceed the BCWQGs. In the RGC review, selected elements were used to assess the adequacy of the BCWQGs by comparing levels reported by the BCWQGs with levels that have been documented to be toxic to fish (Table 4). Our literature review indicates that the BCWQGs are adequate to protect different species of freshwater fish that could be exposed to trace elements in Lake Morrison, but that there are a number of issues related to the release of effluents from the mine that need to be considered.

First, the TSF pond water quality after closure and at the end of pit filling is predicted to contain cadmium at concentrations much greater than the BCWQGs. RGC highlighted that the present predictions assume no attenuation or absorption of cadmium as the TSF seepage water passes through the groundwater paths (Robertson Geoconsultants Inc. 2011). This could result in mine site stream water concentrations of cadmium greater than 10-fold higher than BCWQGs, which could be released into Morrison Lake at a similar level of water quality. High projected releases of cadmium could result in steady state concentrations throughout Morrison Lake that exceed the BCWQGs. If steady state conditions are not met, sections of the lake will be characterized by even higher concentrations of cadmium. Further, flows of potentially acid generating waste rock contaminated groundwater could deteriorate Morrison Lake water quality if the water level in the open pit is higher than in Morrison Lake. Estimates of the level of released elements into Morrison Lake should be based on worst case scenarios for comparison to BCWQGs, in case mitigation efforts are insufficient to bring waterborne discharges in line with BCWQGs over the long term.

Second, RGC outlined that the estimated “lag time” before the TSF contaminant plume reaches Morrison Lake may be an overestimate (Robertson Geoconsultants Inc. 2011). RGC suggested that PBM conduct a sensitivity analysis to evaluate how flow and transport parameters will affect the timing and peak of concentrations of elements reaching Morrison Lake since steady state ambient levels of several elements could exceed BCWQGs if elements are not dispersed throughout Morrison Lake (Robertson Geoconsultants Inc. 2011).

Finally, water hardness is a factor that could significantly impact the toxicity of released elements. The toxicity of cadmium and copper to steelhead salmon, resulting in 50% mortality, is increased with decreasing water hardness (Table 4), and the toxicity of copper and zinc has been shown to substantially increase with decreasing water hardness (Lloyd 1960, Ebrahimpour et al. 2010). The BCWQG values reported by PBM are based on a water hardness of 90 mg CaCO₃/L, whereas the mean baseline water hardness in Morrison Lake is 29 mg CaCO₃/L. As a result, the BCWQG values reported by PBM may be too high to protect different fish species in Morrison Lake from adverse effects caused by cadmium and other elements released into the lake and associated watersheds.

Table 4. Comparison of toxicity values reported in the literature, estimated release rates in Morrison Lake, and BC Water Quality Guidelines

	Species	Duration	Route	Effect	Reference	Hardness (mg CaCO ₃ /L)	Threshold (mg/L)	PBM max (mg/L) ^a	RGC max (mg/L) ^b	BCWQG (mg/L) ^c
Cd	Atlantic salmon	70 d	water	↓ growth	Peterson et al. 1983	13	0.002	0.000032	0.000082	0.000024
	Chinook salmon	200 h	water	10% mortality	Chapman 1978a	24	0.0012-0.018	0.000032	0.000082	0.000024
	Coho salmon	215 h	water	50% mortality	Chapman and Stevens 1978	22	0.0037	0.000032	0.000082	0.000024
	Pink salmon	168 h	water	50% mortality	Servizi and Martens 1978	83	2.7-3.7	0.000032	0.000082	0.000024
	Rainbow trout	47 d	water	↓ survival	Woodworth and Pascoe 1982	98.6	0.1	0.000032	0.000082	0.000024
	Rainbow trout	62 d	water	↓ survival	Dave et al. 1981	100	0.005	0.000032	0.000082	0.000024
	Rainbow trout	11 d	water	50% mortality	Majewski and Giles 1981	82	0.016	0.000032	0.000082	0.000024
	Sockeye salmon	168 h	water	50% mortality	Servizi and Martens 1978	83	0.008-4.5	0.000032	0.000082	0.000024
	Steelhead salmon	200 h	water	10% mortality	Chapman 1978a	24	0.0007-0.006	0.000032	0.000082	0.000024
	Steelhead salmon	408 h	water	50% mortality	Chapman and Stevens 1978	54	0.0052	0.000032	0.000082	0.000024
Cu	Chinook salmon	200 h	water	10% mortality	Chapman 1978a	24	0.014-0.018	0.0032	0.011	0.0036
	Coho salmon	168 h	water	50% mortality	Buckley et al. 1982	276	0.22	0.0032	0.011	0.0036
	Fathead minnow	3 mo	water	↓ reproduction	Pickering et al. 1977	200	0.037	0.0032	0.011	0.0036
	Fathead minnow	11 mo	water	↓ reproduction	Mount 1968	198	0.032-0.034	0.0032	0.011	0.0036
	Fathead minnow	11 mo	water	↓ survival	Mount and Stephen 1969	31	0.0184	0.0032	0.011	0.0036
	Rainbow trout	30-70 d	water	mortality	McKim et al. 1978	44-50	0.032	0.0032	0.011	0.0036
	Steelhead salmon	200 h	water	10% mortality	Chapman 1978a	24	0.007-0.019	0.0032	0.0011	0.0036
Se	Rainbow trout	90 d	water	mortality	Hunn et al. 1987	272	0.047*	0.00097	0.0005	0.002
	Bluegill sunfish	365 d	water	reduced survival	Hermanutz et al. 1992	179	0.01	0.00097	0.0005	0.002
	Bluegill sunfish	365 d	water	100% mortality	Hermanutz et al. 1992	179	0.03	0.00097	0.0005	0.002

^a Morrison Copper/Gold Project 2010 (based on 30 day averages; assumes effluent flow of 214 m³/hr, TSF seepage flow of 191 m³/hr, and lake hardness of 90 mg/L), ^b Robertson Geoconsultants Inc. 2011, ^c Derived from US EPA water quality criterion for maximum concentration of cadmium $[e^{(1.0166[\ln(\text{hardness})]-3.924)}]$, criterion continuous concentration $[e^{(.7409[\ln(\text{hardness})]-4.719)}]$ (USEPA 2001). *Indicates studies carried out with selenite, † indicates studies carried out with selenate.

7. Conclusions

The objective of this review was to assess the potential effects of elements released from the Morrison Copper/Gold Project on fish assemblages in Morrison Lake, with particular emphasis on sockeye salmon.

(i) Literature investigating the known effects of cadmium on sockeye salmon and other fish species was reviewed and an overview of results is provided in Table 3 and Table 4. The effects of cadmium on different species of fish include: tissue accumulation, altered metabolism and growth, induced cellular stress and immunity responses, altered osmoregulation and respiration, impaired reproduction, and mortality. The concentrations of cadmium projected for release into Morrison Lake could likely adversely impact the salmonid populations in the lake and the associated watershed.

(ii) Literature investigating the potential impacts of mixed metal mine effluents on sockeye salmon and other fish species was reviewed. The combination of cadmium and several other parameters (including acid pH, aluminum, and copper) can increase the accumulation and toxicity of elements released in the effluent. Further, the concentration of copper that is projected to be released into Morrison Lake (0.0032 mg/L) is approximately double the concentration of copper found to induce biomarkers of oxidative stress in brown trout (0.0158 mg/L: Hansen et al. 2006) and other fish species (0.0018-0.002 mg/L: Oliva et al. 2012). The concentration of copper that has been projected by third party review (RGC) to be released into Morrison Lake (0.011 mg/L) is only slightly below the concentration of copper reported by Chapman (1978a) to induce 50% mortality over 96 hours in Chinook salmon (0.019-0.038 mg/L) and exceeds the BCWQGs (0.0036 mg/L). Thus, mixed metal effluents released into Morrison Lake are likely to adversely impact salmonid populations in the lake and associated watersheds.

(iii) Literature investigating the toxicity of cadmium and other metals was reviewed across various life stages, and the toxicity of cadmium on salmonids was found to vary based on the life stage and species investigated (Table 2). Sockeye salmon that utilize deep portions of Morrison Lake, especially at their early and highly sensitive stages of development as fry, could be particularly impacted near the proposed Project effluent diffuser.

(iv) Literature investigating the variability in sensitivity to metals among different fish species was reviewed and is outlined in Table 3. Different salmonid species appear to respond similarly to the toxic effects of metals.

(v) The adequacy of the BC Water Quality Guidelines standards was examined with respect to elements that are projected to be released into Morrison Lake and was found to be adequate based on the published literature. The BCWQG values reported by Pacific Booker Minerals Inc. and used for comparison, however, were based on a water hardness of 90 mg CaCO₃/L when the mean baseline water hardness in Morrison Lake is 29 mg CaCO₃/L. As such, the selected BCWQG values may not be sufficient to protect fish species in Morrison Lake from adverse effects. Further, given the large variability in the toxicity and concentration of released elements based on seepage estimates, diffuser dilution calculations, and other environmental parameters (e.g. calcium levels and water hardness), the toxicity of elements projected to be released into Morrison Lake needs to be evaluated based on biologically relevant estimates that consider worst case scenarios.

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