Sub-lethal metal toxicity effects on salmonids: a review

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Prepared by Michael H.H. Price

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SkeenaWild Conservation Trust is a regionally based organization. We are dedicated to bringing together governments, First Nations and members of the public in the Skeena Watershed to sustain the long-term health and resilience of the wild salmon ecosystem, while optimizing economic returns to First Nations and local communities.

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Executive summary

Heavy metals are common pollutants of aquatic systems, often associated with human activities. Hard-rock mining, agriculture, urbanization, and industrialization have enabled the release of significant quantities of heavy metals to aquatic ecosystems, sometimes with profound ecological harm.

While metals naturally leach from rock when they are exposed to weathering processes, human activities often speed-up metal leaching through the excavation and exposure of vast quantities of rock from mineral deposits (such as during metal- and coal-mining). Metal leaching can be further accelerated by acidic drainage, which occurs when acid-generating rock is exposed to air and/or water. Acid mine drainage is typically produced in tailings ponds and waste rock dumps at metal- and coal-mining sites, and is characterized by acidic water and high concentration of dissolved metals. These acidic waters can then dissolve and mobilize more heavy metals as they flow across the landscape and contact other minerals and exposed rock. Acid mine drainage is a major source of water contamination in many mining districts on Earth.

Fish are particularly vulnerable to metals because of sensitive organs that are continuously in contact with the environment, and because metals are highly soluble in water. Most metals can disrupt the essential functions of the fish gill (responsible for gas and ion exchange) and the olfactory system (a fish's sense of smell). Even relatively low concentrations of heavy metals can cause harm to fish. The olfactory system specifically plays an essential role in the survival of fish. While there is a wealth of scientific information describing the concentrations of metals that cause death in freshwater fish, much less reported are the potential sub-lethal effects (i.e., negative impacts that do not cause immediate or direct death) on salmon and trout from low metal concentrations.

Herein is a synthesis of the relevant scientific literature describing the 7 most cited heavy metals, and the corresponding lowest concentrations reported to cause sub-lethal effects on salmonids. These metals are: aluminum (Al), cadmium (Cd), copper (Cu), lead (Pb), nickel (Ni), silver (Ag), and zinc (Zn). The lowest concentrations of these metals reported in the literature are compared with the water quality guidelines of British Columbia, so as to determine whether salmonids residing in freshwater systems adjacent to resource extraction industries are protected from metal toxicants.

All of the metals that were examined can negatively impact salmonids to some degree at concentrations below the levels known to cause lethality. Sub-lethal concentrations can alter behaviours related to predator avoidance, foraging, migration, and social interactions, and can cause the physical impairment associated with growth and development, swimming efficiency, and immune system responses. However, most of the metal concentrations reported to invoke sub-lethal effects are above the regulatory limits in British Columbia. Water quality guidelines assigned for Al, Cd, Pb, and Ag would protect against all of the effect concentrations reported in the literature.

There are several instances where sub-lethal effects on salmonids from metals such as Cu, Ni, and Zn have been reported at concentrations below the water quality guidelines of

British Columbia. All of these sub-lethal effects involve either the avoidance of metal-contaminated water by fish or an impaired sense of smell. Several studies report the loss of smell in juvenile rainbow trout, coho salmon, and chum salmon exposed to Cu at concentrations below provincial guidelines. These copper-exposed fish failed to detect near-by predators, and did not exhibit the typical anti-predator response; thus, these fish were more vulnerable to predators, and had lower survival compared to unexposed fish. The disruption of such an anti-predator response in salmonids at ecologically-relevant concentrations below government guidelines is a very real scenario that may have conservation implications – especially considering that British Columbia is the largest producer of Cu in Canada.

Water quality criteria for the protection of aquatic life in British Columbia are not legislated, but rather serve as environmental benchmarks. Specific industrial projects apply for permits to pollute, and the resulting metal concentrations in receiving waters of discharge may be higher than the provincial criteria. Two such examples are the open-pit copper mines (Noranda Bell and Granisle) located on Babine Lake in the Skeena River watershed, where the maximum authorized discharge for dissolved Cu from mine waste-water into the lake is five-fold higher than the regulatory guidelines for each mine.

The impact of heavy metals on fish is complex and depends on the chemical characteristics of water. Acidity (pH), hardness ($CaCO_3$), and organic matter are all complicating factors in the determination of metal toxicity. Thus, differences in acidity, hardness, and/or organic matter of test water may at least partly explain why the effect concentrations for a given metal and fish species can be dissimilar between studies.

There are at least four limitations when applying the reported effect concentrations on salmonids to real-life scenarios: i) the effect concentrations reported in this review are more often the lowest *detected effect*, not the actual *lowest effect concentration*, ii) scientific studies rarely reflect natural exposure conditions, iii) laboratory studies tend to examine metals in isolation, which may not be environmentally realistic or relevant for assessing actual impacts on fish, and iv) dietary metal concentrations are not incorporated into Canada's water quality guidelines despite the likely simultaneous occurrence of both waterborne and dietary routes of metal toxicity.

Research is needed not only to determine threshold concentrations for salmonids, but also to compare the effect concentrations derived from laboratory studies with natural environments, and examine the effects of metal mixtures and dietary toxicity on salmonids. Ultimately, a shift in research emphasis from the routine single metal - single organism - perspective, to population, community, and ecosystem scale is required to achieve a full understanding of the sub-lethal metal toxicity effects on salmonids.

Introduction

Heavy metals are widely occurring pollutants commonly associated with human activities. Hard-rock mining, agriculture, urbanization, and industrialization have mobilized significant quantities of heavy metals to aquatic ecosystems (Boyd 2010; Tierney et al. 2010), sometimes with profound ecological harm (Downs and Stocks 1977; Balistrieri et al. 2002). Impacts to freshwater fish from heavy metals have been particularly well documented (Woody et al. 2010; Dennis and Clair 2012).

Heavy metals enter aquatic systems through natural weathering and leaching processes, which can be greatly accelerated by humans. Metals naturally leach from rock when they are exposed to air and/or water, and the resulting chemical reactions mobilize them into biologically available forms (Wilkin 2007). However, human activities often speed-up this process through the excavation of vast quantities of rock from mineral deposits (such as during metal- and coal-mining), and the subsequent exposure of that material to weathering processes (Kelly 1988; Moore and Luoma 1990; Hogsden and Harding 2012). The leaching process can be further accelerated by acidic drainage, which occurs when sulphide minerals previously "locked" in rock are exposed to air and water, and naturally oxidize without the presence of sufficient quantities of neutralizing minerals (Wilkin 2007). Acid mine drainage is typically produced in tailings ponds and waste rock dumps at mining sites, and is characterized by acidic water (low pH) and high concentration of dissolved metals. Bacteria contribute to metal leaching by catalyzing the reactions and speeding-up the rate in which water becomes acidified. These acidic waters can then dissolve and mobilize more heavy metals as they flow across the landscape and contact other minerals and exposed rock. Acid mine drainage is a major source of water contamination in many mining districts on Earth (Hogsden and Harding 2012).

Fish are extremely vulnerable to metal toxicants because fish have sensitive organs that are continuously in contact with the environment, and because metals are highly soluble in water. For example, the fish gill is a sophisticated, yet delicate, organ with multiple physiological functions that range from gas exchange to excretion of nitrogenous waste (Hogstrand and Wood 1998). High concentrations of most metals can disrupt these functions, damage the gill structurally, and cause suffocation and death (Mallat 1985). Even relatively low concentrations of heavy metals can fatally impair physiological functions (such as the regulation of ions; ionoregulation) of the gill (Wood 1992). The olfactory organ, and its associated nerve cells, is also directly exposed to the environment and thus highly susceptible to damage by metal toxicants in water. Heavy metals can interfere with a fish's sense of smell (olfaction) by blocking the effects stimulated by natural odorants or by directly damaging olfactory receptor sites (Hara et al. 1983; Klaprat et al. 1988). Olfaction plays an essential role in the survival of fish, initiating behaviours such as food gathering, predator avoidance, schooling, defense, navigation between ocean and freshwater habitats, and reproduction, and low concentrations of heavy metals can alter such behaviours and reduce survival (Sandahl et al. 2007; Tierney et al. 2010; McIntyre et al. 2012).

Freshwater and anadromous salmonids (fish in the Salmonidae family, which includes salmon and trout) are key elements of ecosystems (Gende et al. 2002; Hocking and

Reynolds 2011); they play an important role in the cultural foundation of human societies (Campbell and Butler 2010), and coastal economies (Schindler et al. 2010). While the ecological threats posed by metal-mining and other resource-extraction industries are not limited to salmonids, lost and degraded salmon and trout populations threaten a range of human values that define our well-being and sustain our quality of life. Concerns regarding the possible effects of heavy metals on salmonid populations have been raised. However, published findings have generally focused on heavily polluted systems or metal concentrations that cause direct lethality. For example, the Coeur d'Alene mining district in northern Idaho, U.S.A. (grossly disturbed by mining for over 100 years), has been routinely studied for its widespread contamination of water and soils, and impairment of salmon and trout populations (Moore and Luoma 1990; Woody et al. 2010; Mebane 2012). Furthermore, we have a fairly robust understanding of the concentrations for most metals that cause death in fish over short time-periods (e.g., Chapman 1978a; Chapman and Stevens 1978; Buhl and Hamilton 1990; Hansen et al. 2002a). Yet much less reported are the potential sub-lethal effects (i.e., negative impacts that do not cause immediate or direct death of fish) on salmon and trout from low metal concentrations.

Canada is a resource-dependent country with a long tradition of mining activity, and poor record for environmental protection (Lemly 1994). In the western-most province of British Columbia (home to the largest wild salmon abundance in the country, and a national leader in mineral production; copper (Cu) specifically), there are 9 metal-mines in operation, at least 18 proposed, and more than 60 additional locations considered significant exploration projects with the potential to become mines (BCMoM 2013a; Figure 1). The British Columbia government has explicitly proposed to create 8 new mines, and expand 9 existing ones, by 2015. Indeed, "the Province will move quickly and decisively to leverage today's high commodity prices and gain a competitive edge over other global mining jurisdictions" (BCMoM 2013b). Given the speed of the proposed metal-mine development in British Columbia, and the abundance and importance of salmonids to humans and ecosystems in the region, there is an urgent need to assess whether such developments pose a risk to salmonids in aquatic environments.

The following is a synthesis of the known literature on metal toxicity effects on salmonids, and an examination as to whether such metals pose a significant risk. The review is not intended to comprehensively cover the literature regarding all heavy metals and their effects. Instead, this review is focused on sub-lethal effects of the most common metal pollutants, and the resulting lowest concentrations reported to cause an effect (i.e., effect concentrations). To assess whether metals in aquatic environments pose a risk to salmonids in British Columbia, effect concentrations were compared to government regulatory guidelines for the protection of aquatic life. The report is structured such that the described metals are in alphabetical order, each with its suite of reported effects on salmonids. All concentrations described are for waterborne toxicants that are assumed dissolved (as opposed to total concentrations), unless otherwise noted. Dissolved concentrations are ecologically important because they represent the mobile and biologically available amount of a given metal in water, whereas total metal concentrations are the total amount of a given metal in water whether in an available form or not; a dissolved metal concentration is a sub-set of the total metal concentration.
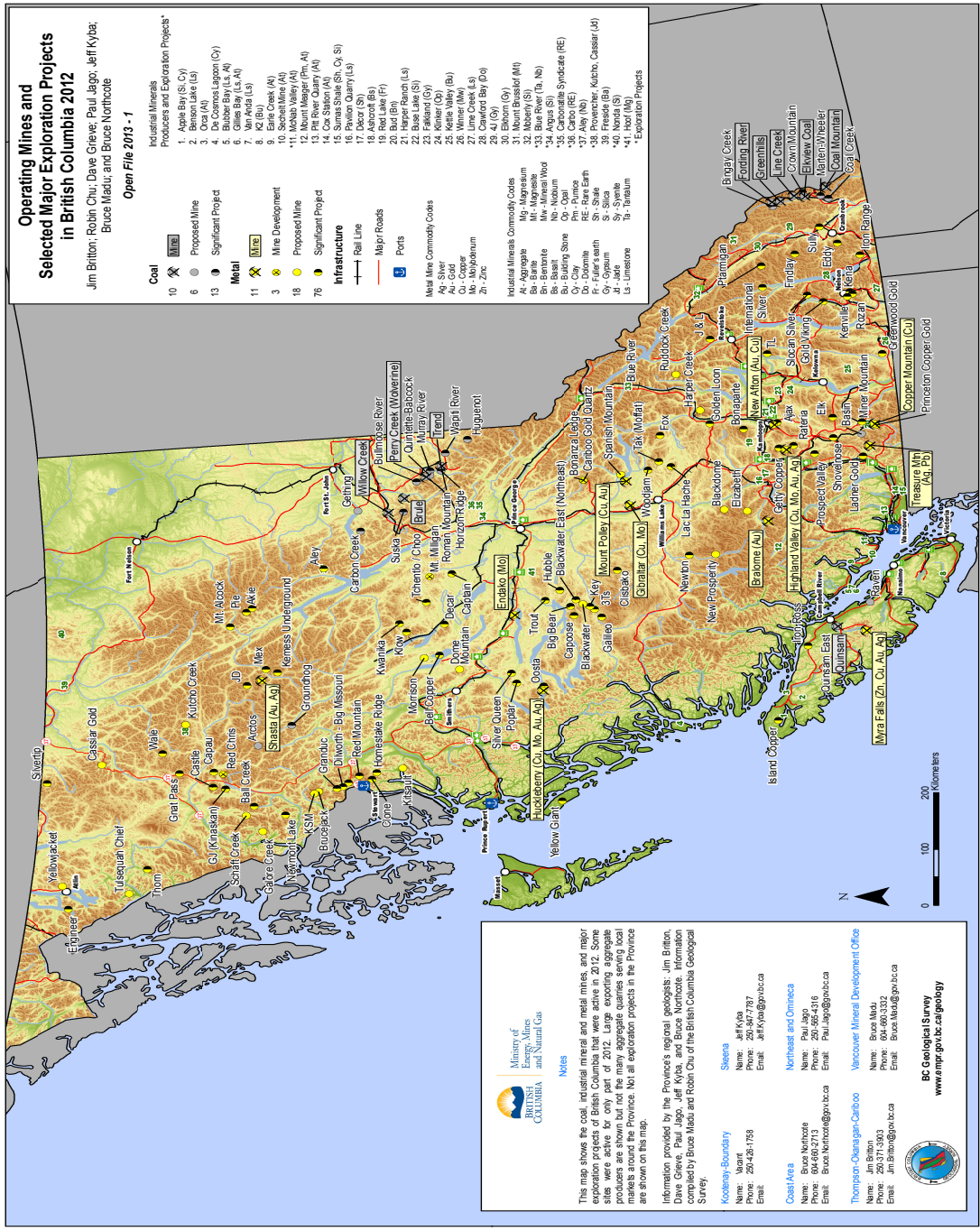
Figure 1. Map of British Columbia and all of the operating, proposed, and potential metal- and coal-mine projects as of 2012 (BCMoM 2013a).

Effect concentrations of metals

Aluminum (Al)
Aluminum is the third most abundant element in the Earth's crust, yet this metal is not known to be biologically essential to aquatic life (Gensemer and Playle 1999). Human communities in North America have been reminded about the importance of Al as a toxicant by recent oil shale mining development, where Al is liberated from the mineral Dawsonite (which is present in some oil shale ore) as a bi-product (Freeman and Everhart 1971). The behaviour of Al in water is highly varied, forming a variety of sensitive complexes that exhibit different effects on fish. In freshwater, labile (cationic/inorganic) forms of Al may be most toxic. Although Al is relatively insoluble at pH 6 to 8, the solubility increases under more acidic and alkaline conditions, at lower temperatures, and in the presence of complexing ligands (Driscoll and Postek 1996). It is the interaction between Al, pH, and calcium (Ca), which generally determines the level of toxicity to fish. For example, in hard (high Ca^{2+} concentration) fresh water, Ca protects fish against the toxic effects of Al so that negative effects occur only at high Al concentrations (Gensemer and Playle 1999).

The fish gill is a multifunctional organ involved in ion regulation and respiration; as such, it is the primary site of toxicity for metals such as Al (Exley et al. 1991; Gensemer and Playle 1999; Monette et al. 2008). Aluminum accumulates both on the surface and within the fish gill epithelium during exposure, which can result in increased branchial permeability and active ion uptake inhibition (Youson and Neville 1987; Booth et al. 1988; Wilkinson and Campbell 1993). Increased permeability of the gill may specifically be caused by the displacement of Ca ions by Al at binding sites. Calcium ions help bind intercellular junctions, and the displacement of Ca by Al results in the weakening of these otherwise tight junctions (Booth et al. 1988; Freda et al. 1991; Monette et al. 2008). Salmonids may be particularly vulnerable to ion regulatory disturbance due to their complex life history of separate freshwater and saltwater phases, and the physiological adaptations required for each (Hoar 1988; McCormick et al. 1998). Mortality rates for juvenile Atlantic salmon (*Salmo salar*) are reportedly high when exposed for days-to-weeks to cationic Al concentrations >45 µg/L (Kroglund et al. 2008), though much higher concentrations (i.e., 5,200 µg/L) are noted to cause death within 96 hours to 50% of juvenile rainbow trout (Freeman and Everhart 1971; Table 1).

<u>Physical impairment</u>
Growth and swim speed
Sub-lethal levels of Al can affect the feeding behavior, growth, and swim speed of salmonids. For example, reduced growth rates have been observed in juvenile brown trout (*Salmo trutta*) exposed to total Al concentrations greater than 27 µg/L (unknown dissolved concentration) in waters with pH below 5.5 (Sandler and Lynam 1987; Table 1). Juvenile rainbow trout exposed to 30.0 µg/L total Al (unknown dissolved concentration) in waters of 5.2 pH showed a 30% reduction in the maximum sustainable swimming speed within 7 days, and these effects were roughly two times greater than for fish exposed only to low pH (i.e., 5.2; Wilson and Wood 1992). In a separate study, juvenile rainbow trout (*Oncorhynchus mykiss*) pre-exposed to 38 µg/L total Al at 5.2-5.4

pH for 36 days suffered impaired swim speed, and the maximum swim speed remained depressed even when fish were subsequently placed in waters with pH of 6.5 and 0 μg/L total Al (Wilson et al. 1994).

Mucous production
Aluminum can accumulate rapidly on the gill lamellae surface of juvenile rainbow trout, and may gradually penetrate within the gill cells themselves over time (Wilson and Wood 1992). Juvenile rainbow trout exposed to 38 μg/L Al at pH 5.2 for 5 days showed a five-fold increase in the number of mucous cells present in the filamental epithelium compared to fish exposed to 0 μg/L Al in waters with pH 5.2 and 6.5 (Wilson et al. 1994). After 34 days exposure to 38 μg/L Al at pH 5.2, juvenile rainbow trout showed a four-fold increase in mucous cells compared to unexposed fish (Wilson et al. 1994), suggesting that fish do not acclimate to Al toxicity. Gill hyperplasia, which is an abnormal increase in cell numbers that can lead to respiratory impairment, may result from Al toxicity. Specifically, positively charged Al binds to the negatively charged fish gill epithelium, causing irritation that results in excessive mucous production, which can then clog gill membranes and lead to severe respiratory impairment (Rosseland and Staurnes 1994; Sparling and Lowe 1996; Klöppel et al. 1997). At minimum, low Al concentrations, especially in waters with pH between 5.0 and 5.6, will cause fitness degradation, and reduce the ability of salmonids to adequately deal with other stressors, such as smoltification (Dennis and Clair 2012).

Migration
Exposure to low levels to Al during long-term and episodic (single or re-occurring episodes lasting several days) events may disrupt the downstream migration of juvenile salmonids and reduce survival in seawater. Several studies have reported that non-lethal Al concentrations can compromise the ability of juvenile Atlantic salmon to balance body fluids (osmoregulation) during smoltification (Staurnes et al. 1995; Magee et al. 2001, 2003; Kroglund et al. 2007). Juvenile Atlantic salmon exposed for three months to 6 (+/-2) μg/L Al showed a 20-30% reduction in survival compared to control fish (Kroglund and Finstad 2003). Juvenile Atlantic salmon exposed to 28-64 μg/L inorganic Al for 2 to 5 days in acidic water (pH 5.4-6.3) also showed reduced seawater tolerance compared to control fish (Monette et al. 2008). Concentrations of inorganic Al of 5-10 μg/L is predicted to cause a 25%–50% reduction in the survival of Atlantic salmon when smolts are exposed for as few as 3 days during seaward migration (Kroglund et al. 2008).

Olfaction
Aluminum may cause physical alteration in the olfactory epithelium of salmonids and influence the electrical properties of olfactory sensory neurons. For example, juvenile rainbow trout exposed to 9.5 μg/L Al in acidic water (pH 4.7) for 2 weeks resulted in loss of receptor cell cilia, anatomically altered olfactory knobs, and clumped microvilli compared to control fish, and showed reduced olfactory nerve responses compared to fish only exposed to acidic water (Klaprat et al. 1988).

<u>Regulatory limits</u>
The government of British Columbia's water quality criteria pertaining to Al for the protection of aquatic life is dependent on pH and exposure duration (BCMoE 2013). Based on the above examples of low effect concentrations, the guidelines appear low enough to protect salmonids from chronic lethal and sub-lethal effects (Figure 2).
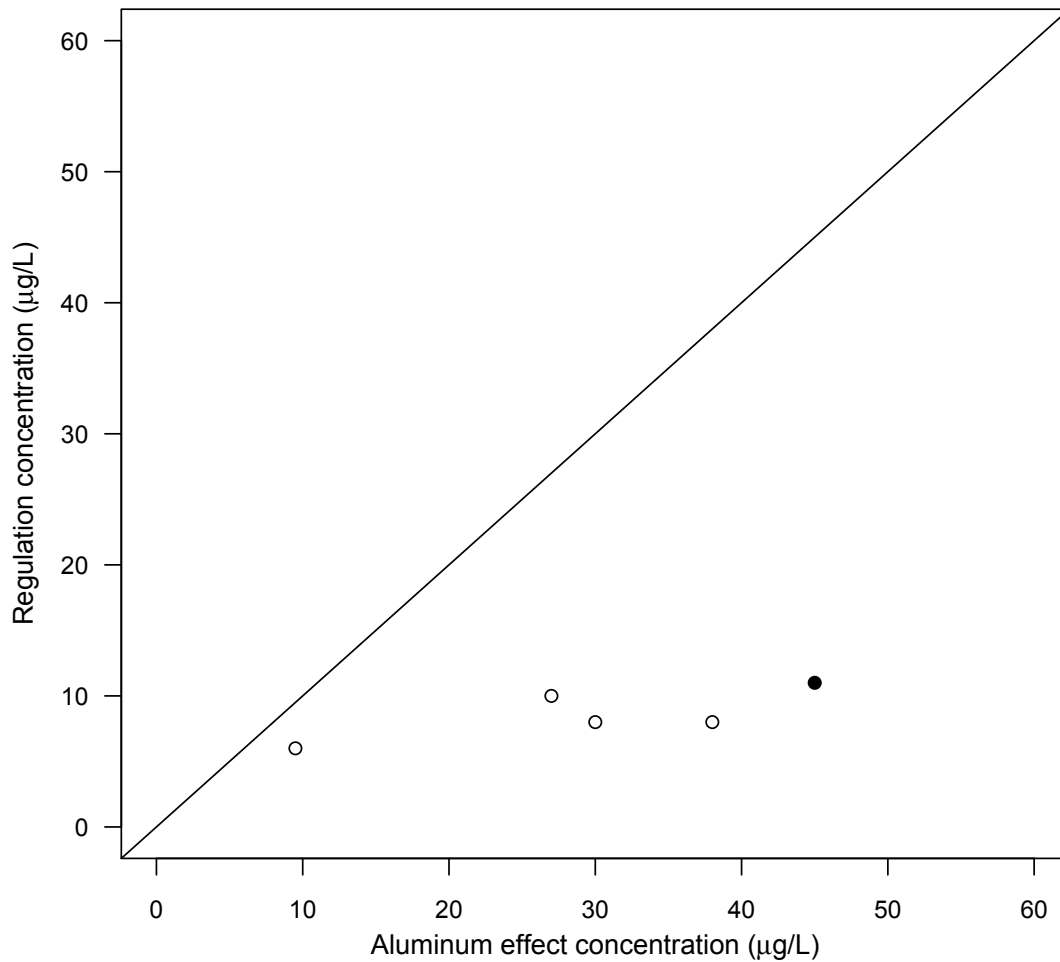
Figure 2. Lowest total and dissolved concentrations of waterborne aluminum (Al) observed to cause chronic sub-lethal (open circle) and lethal (closed circle) effects on salmonids plotted against the British Columbia government's water quality criteria for the protection of aquatic life (Regulation concentration; BCMoE 2013). Circles located above the 1:1 line show Al concentrations that cause an effect on fish at levels below regulation.

Cadmium (Cd)
Cadmium is a biologically non-essential heavy metal that occurs naturally in ores together with Cu, lead (Pb), and zinc (Zn), which is extremely toxic to salmonids at low concentrations (U.S.EPA 2001; Jarup 2003). Major human uses of Cd are in the manufacture of batteries and plastic stabilizers, and the primary sources of Cd pollution include smelter fumes and dusts, fertilizers, and municipal wastewater and sludge discharges (Eisler 1985). Cadmium concentrations are often highest in the localized regions of smelters, mines, or in urban industrialized areas, and wild salmonids are most likely to be affected by Cd in adjacent freshwater systems through waterborne and/or dietary exposure (Franklin et al. 2005). Background levels of Cd in uncontaminated aquatic systems range several orders of magnitude, from 0.05 µg/L to 0.2 µg/L (Korte 1983; Eisler 1985), whereas Cd concentrations in polluted waters are known to reach 200 µg/L (Jezierska et al. 2009).

The toxic nature of Cd is due to its actions as a Ca-antagonist; waterborne Cd mimics Ca, which can cause an imbalance and deficiency of Ca, and eventual death (Wood 2001). The two most important sites of Cd absorption in fish are the gills and the gastrointestinal tract (Szebedinszky et al. 2001). Waterborne Cd enters the gill epithelium through the same pathway as Ca, and effectively blocks active Ca uptake (Verbost et al. 1987, 1989; Playle et al. 1993). Importantly, the pathological effects of Cd are less severe in waters with high Ca. For example, waterborne Ca (in $CaCO_3$; measured as water hardness) can have a strong protective effect against waterborne-Cd toxicity by protecting Ca uptake, and by competitively inhibiting Cd binding to the gills (Playle et al. 1993; Hollis et al. 2001). An additional uptake route of waterborne Cd of fish is through the olfactory epithelium, which contains ciliated olfactory sensory neurons, and is in direct contact with surface waters (McIntyre et al. 2008). Olfactory sensory neurons are responsible for sensory inputs that convey important information about a fish's surrounding environment (McIntyre et al. 2008, 2012). Sub-lethal Cd accumulation in the olfactory system can cause significant behavioural effects relevant to a fish's ability to smell (Scott et al. 2003). Food may also be a significant route for Cd toxicity (Farag et al. 1999; Szebedinszky et al. 2001; Meyer et al. 2005), yet there remains insufficient knowledge on the risk of diet-borne Cd to salmonids. Cadmium concentrations lethal to salmonids range from 0.4 µg/L for juvenile rainbow trout (Hansen et al. 2002a) to 30 µg/L for juvenile sockeye salmon (*Oncorhynchus nerka*; Servizi and Martens 1978).

<u>Sensory impairment</u>
Predation
Low concentrations of Cd for relatively short exposure periods can affect the chemosensory function in prey fish. For example, juvenile rainbow trout exposed to 2 µg/L Cd for 7 days showed a significant reduction in normal predator avoidance behaviour when presented with an alarm substance (i.e., predator skin extract; Scott et al. 2003). The same exposure concentration and duration also inhibited the normal physiological response to stress (the release of plasma cortisol) of juveniles compared to unexposed fish. Importantly, these effects were present after two days in Cd-free water, which suggests that disruptive effects may persist well after exposure has ceased. Fish

exposed to 0.5 µg/L Cd for 7 days also showed a small, but statistically insignificant, disruption of the behavioural response (Scott et al. 2003).

Foraging
Foraging behaviour can be a sensitive indicator of metal toxicant stress on fish (Atchison et al. 1987; Little et al. 1990; Scherer et al. 1992). Several authors have reported a significant relationship between chronic sub-lethal Cd toxicity and reduced predation success in salmonids. For example, adult lake trout (*Salvelinus namaycush*) exposed to 0.5 µg/L Cd for 106-112 days showed a significant reduction in the number of prey captured and consumed compared to unexposed fish (Kislalioglu et al. 1996). A similar study reported that adult lake trout exposed to 0.5 µg/L Cd for 9 months showed reduced predation success compared to control fish when presented with unexposed rainbow trout prey, and foraging success decreased with increasing Cd concentration (Scherer et al. 1997). This same study revealed that unexposed lake trout showed the highest predation success when presented with juvenile rainbow trout previously exposed to 0.5 µg/L Cd for 9 months, though the results were not significantly different from controls. Finally, research by Riddell et al. (2005a, b) showed that exposure to 0.5 µg/L Cd for 30 days can alter the net energy available to juvenile brook trout (*Salvenius fontinalis*) by increasing the activity of individuals, and reducing their prey capture efficiency. Specifically, the capture efficiency of Cd-induced fish declined by 20%, and the activity of individuals increased by 25%, compared to unexposed fish (Riddell et al. 2005b).

Although the particular mechanisms that may have caused the reduction in predation success were not investigated, disruption of the olfactory system during waterborne exposure to Cd may play a role. Cadmium can accumulate in the olfactory system and inhibit olfactory functions in fishes, such as foraging (Hara 1986), and prey detection of predators such brook trout or lake charr may in some way be dependent on olfaction.

Social interactions
The social behaviour of individual fish, and dominance hierarchies within populations, can be altered by sub-lethal levels of Cd. Dominance hierarchies form between a pair and among groups of salmonids living in the confined or natural environment, owing to competition over limited resources such as food or mates (McGeer et al. 2011). Juvenile rainbow trout exposed to 2 µg/L Cd for 24 hours displayed significantly less aggressive attacks during agonistic encounters with non-exposed fish, and had a reduced ability to socially compete and become dominant even after 3 days depuration in clean water (Sloman et al. 2003a). Fish exposed to 0.8 µg/L Cd for 24 hours also showed a decreased tendency to become dominant compared to non-exposed fish, but the results were not statistically significant. When groups of rainbow trout were exposed to Cd during hierarchy formation, hierarchies developed faster than among non-exposed controls (Sloman et al. 2003a). In a separate study, exposure of juvenile rainbow trout to 3.3 µg/L Cd for 24 hours resulted in less aggressive competition than between control fish, and dominance amongst individuals was less easily determined (Sloman et al. 2003b). Furthermore, all Cd pre-exposed fish became subordinate when paired with non-exposed fish.

Both the decreased aggression of individual exposed fish, and faster formation of hierarchies among groups, may in part be attributed to a disruption in the olfactory system. Olfaction is thought to play an important role in the social interactions of salmonids (Brown and Brown 1993; Griffiths and Armstrong 2000), and an inability to detect odours in the water may reduce aggression amongst exposed fish, which in turn may increase the rate of hierarchy formation. However, interference by Cd toxicity with other physiological mechanisms linked to social behaviour, such as neurotransmitters (Winberg and Nilsson 1993) and hormone concentrations (Sloman et al. 2001), is thought to also play a role (Sloman et al. 2003a).

Avoidance

Salmonids can detect and respond to sub-lethal Cd levels. Lake whitefish (*Coregonus clupeaformis*) exposed to 0.2 μg/L Cd demonstrated a dichotomous response pattern where most fish showed avoidance to the source metal while a significant number appeared to be attracted (McNicol and Scherer 1991). The authors postulate that these opposing reactions may be an indication that such concentrations can disorient fish.

Physical impairment

Development

Sub-lethal Cd concentrations can reduce the growth and development of salmonids. Atlantic salmon alevins exposed to 0.47 μg/L Cd showed a significant reduction in growth compared to unexposed fish, and the results further indicated that these fish had a lower growth response threshold around 0.13 μg/L Cd (Rombough and Garside 1982). Rainbow trout alevins exposed to 0.25 μg/L Cd for 56 days weighed significantly less than fish exposed to the same Cd concentration for 35 days (Lizardo-Daudt and Kennedy 2008), and juveniles exposed to 1.0 μg/L Cd for 30 days showed a reduction in growth compared to control fish (Ricard et al. 1998). Finally, juvenile bull trout (*Salvelinus confluentus*) exposed to 0.79 μg/L Cd for 56 days showed a 28% reduction in weight change compared to unexposed fish (Hansen et al. 2002b).

In terms of biological performance (measured as reduced biomass in the population), exposure of juvenile brown trout to 0.87 μg/L Cd, and Atlantic salmon alevins to 1.0 μg/L Cd, caused a 20% and 38% reduction in biomass, respectively, compared with control groups (Rombough and Garside 1982; Brinkman and Hansen 2007). Additionally, juvenile brook trout exposed to 0.5 μg/L Cd for 30 days showed significantly poorer biological health, as measured by condition factor, compared to unexposed fish (Riddell et al. 2005b). The condition of exposed fish declined by 12-18% over a 30-day period, whereas the condition of control fish increased by 34%. The authors hypothesized that Cd-exposed fish shift their preference from nutritionally-rich pelagic prey to nutritionally-poor benthic prey (Riddell et al. 2005b). Importantly, an exposed fish's proximity to contaminated sediment may further exacerbate the sub-lethal effects of Cd on these individuals by intensifying or prolonging exposure through a combination of trophic transfer and altered foraging behavior (Cummins and Wuycheck 1971; Riddell et al. 2005b).

Reproduction

Sub-lethal Cd levels can negatively affect the reproductive functioning in salmonids. Rainbow trout eggs exposed to 0.05 µg/L Cd have been shown to hatch prematurely compared to unexposed eggs; yet exposure of eggs to 2.5 µg/L Cd resulted in delayed hatching, with >90% of eggs having hatched on the last day of the hatching period (Lizardo-Daudt and Kennedy 2008). Exposure of female juvenile rainbow trout to 5 µg/L Cd for 72 hours decreased egg yolk formation (vitellogenesis), and caused endocrine disruption in estrogenic pathways, which are signals that contribute to the function of the reproductive system (Vetillard and Bailhache 2005). Finally, a significant number of adult male brook trout exposed to 3.4 µg/L Cd for 24 weeks showed distressed activity and eventual death in the presence of female spawning behaviour compared to control fish (Benoit et al. 1976).

Immune response
Cadmium can affect stress in salmonids. Juvenile rainbow trout exposed to 1 µg/L Cd for 2 days showed elevated plasma cortisol levels compared to control fish, and a similar response was observed after 30 days (Brodeur et al. 1998; Ricard et al. 1998). Cortisol production is a general adaptation response of fish to stress (Brodeur et al. 1998).

Regulatory limits
The government of British Columbia does not have water quality criteria pertaining to Cd for the protection of aquatic life. Instead, the government relies on the guidelines approved by the government of Canada and the Canadian Council of Ministers of the Environment (CCME 2013). Similar to all metals except Al, guidelines are predicated on the concentration of $CaCO_3$ (hardness) in water. Based on the above examples of acute and chronic low effect concentrations, the federal guidelines appear low enough to protect salmonids from lethal and sub-lethal toxicity (Figure 3).
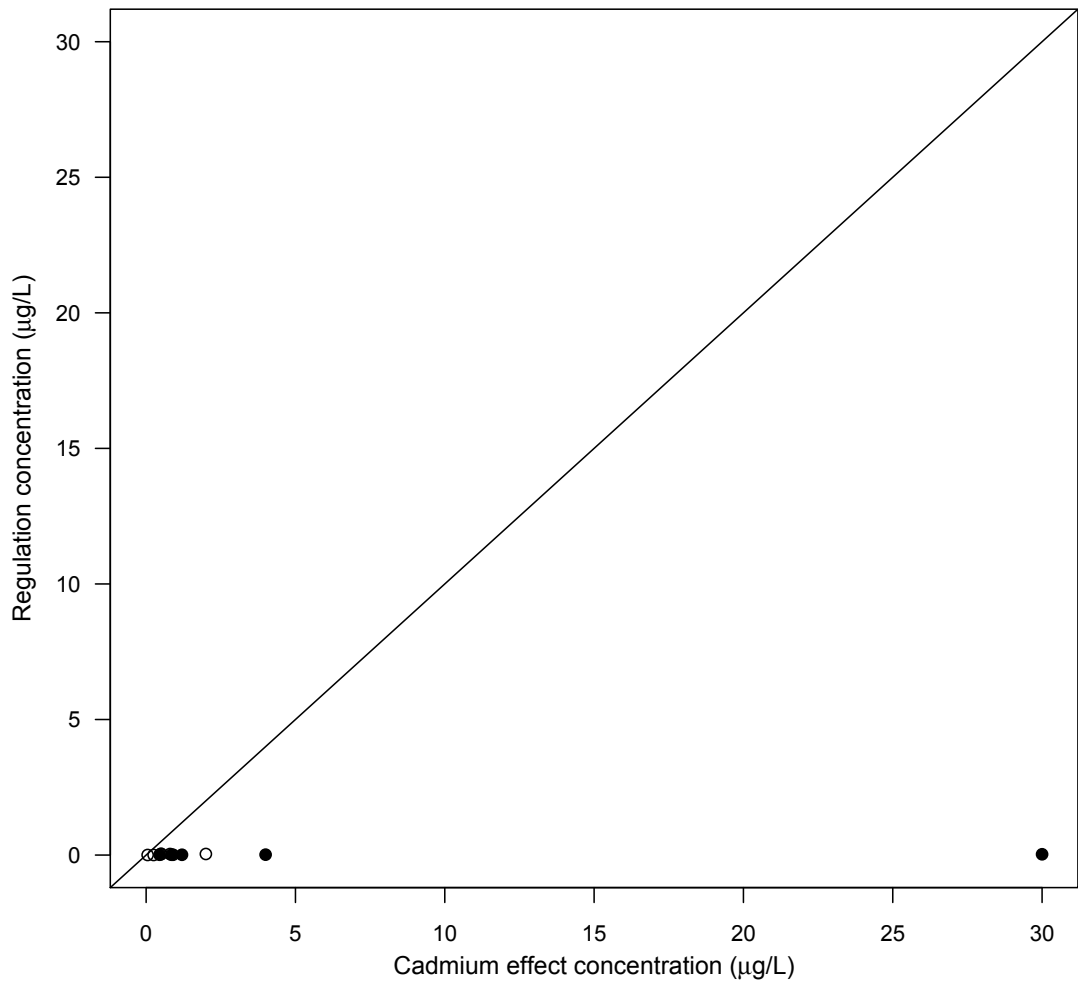
Figure 3. Lowest dissolved concentrations of waterborne cadmium (Cd) observed to cause acute and chronic sub-lethal (open circle) and lethal (closed circle) effects on salmonids plotted against the Canadian Council of Ministers of the Environment criteria for the protection of aquatic life (Regulation concentration; CCME 2013). Circles located above the 1:1 line show Cd concentrations that cause an effect on fish at levels below regulation.

Copper (Cu)
Copper is a biologically essential heavy metal that occurs naturally. Because of its
abundance and availability, Cu was one of the first metals to be worked by humans 7,000
to 8,000 years ago (Schroeder et al. 1966), and continues to be widely used in building
materials, automobile parts, and pesticides (Davis et al. 2001). Input of Cu into aquatic
systems is primarily the result of industrial discharges from metal mines, smelters,
municipal sewage, and agricultural pesticides and fertilizers (Eisler 1998a).
Consequently, Cu is one of the most pervasive contaminants in urban and agricultural
watersheds where salmonids reside (Baldwin et al. 2011).

Copper is one of the most toxic heavy metals in aquatic systems (Eisler 1998a). It is a
neurobehavioral toxicant that interferes with the ability of fish to detect and respond to
chemical signals in aquatic environments (Sandahl et al. 2007), and elevated
concentrations can decrease growth, reproduction, and survival of salmonids (U.S.EPA
2007). These effects can manifest over a period of minutes to hours, and can be
temporary or permanent. Importantly, a large body of scientific literature has shown that
behaviors of salmonids can be compromised at concentrations of Cu that are at or slightly
above ambient (i.e., background) levels (Hecht et al. 2007). Acute lethality in salmonids
can occur at Cu concentrations that range 9-17 µg/L for juvenile rainbow trout (Chapman
1978a; Marr et al. 1999) to 103-240 µg/L for juvenile sockeye salmon (Davis and Shand
1978).

Sensory impairment
Olfaction
Olfactory impairment can manifest within minutes, with recovery rates being time and
dose dependent. The inhibitory effects of 5 µg/L Cu on juvenile coho salmon
(*Oncorhynchus kisutch*) have been observed within 10 minutes, and a 30-minute
exposure was sufficient to produce the maximal reduction in odor detection (Baldwin et
al. 2003). A 7-day continuous exposure of 4.4 µg/L Cu to juvenile coho produced similar
results (Sandahl et al. 2004), but also suggests that the olfactory system of salmonids may
not be able to acclimate to continuous Cu exposure (Hecht et al. 2007). While olfactory
system recovery may be relatively quick (i.e., ≤1 day) when exposure time and
concentrations are low (Baldwin et al. 2003; Sandahl et al. 2006), recovery can take
weeks or months where sensory cell death occurs (Evans and Hara 1985; Moran et al.
1992; Sandahl et al. 2007). Hansen et al. (1999a) showed that a 4-hour exposure to 25
µg/L Cu caused a significant loss of olfactory receptor neurons in juvenile Chinook
salmon (*Oncorhynchus tshawytscha*) and rainbow trout. Impairment recovery was
quickest for fish exposed to lower Cu concentrations, yet no recovery was evident in
Chinook and rainbow trout exposed to >50 µg/L and >100 µg/L, respectively (Hansen et
al. 1999a). Similarly, Hara (1981) reported that no recovery was evident in fish exposed
to 320 µg/L, and Hara et al. (1976) reported that recovery rates for juvenile rainbow trout
exposed to 50 µg/L Cu were slower with increasing exposure times.

Several studies have estimated effect thresholds for reduced sensory responses in juvenile
coho salmon exposed to Cu. Sandahl et al. (2004) produced threshold estimates for
juvenile coho salmon of 4.4 µg/L and 11.1 µg/L Cu exposure, which corresponded to

reductions in odor recognition of 20% and 50%, respectively. Baldwin et al. (2003) estimated a 25% reduction in olfactory response of juvenile coho exposed to a concentration of Cu that ranged from 2.3 µg/L to 3.0 µg/L. Finally, Hecht et al. (2007) estimated a 29.2% reduction in olfactory response, and 31.8% reduction in alarm response, for juvenile coho exposed to between 0.44 µg/L and 1.42 µg/L Cu. Although these benchmark concentrations are derived using data from coho salmon, thresholds are considered applicable to other salmonids given the similar range of olfactory toxicity responses to comparable Cu exposures (Hecht et al. 2007; Baldwin et al. 2011).

Social interactions
The social behaviour of individual fish may influence the accumulation of Cu in the olfactory system. Within a dominance hierarchy, the social status of juvenile rainbow trout was found to affect the uptake of Cu. For example, sub-ordinate fish had a greater tendency to accumulate Cu from the water, and these fish consequently displayed higher tissue burdens when exposed to 30 µg/L Cu for 48 hours (Sloman et al. 2002).

Avoidance
Where distinct Cu gradients are present (e.g., near a point-source discharge), salmonids may use their sense of smell to detect and avoid contaminated waters. Several studies have reported that juvenile salmonids rearing in freshwater avoid Cu concentrations ranging from 0.7 µg/L to 7.3 µg/L (Sprague et al. 1965; Giattina et al. 1982; Hansen et al. 1999b; Svecevicius 2007), with Chinook salmon, rainbow trout, and Atlantic salmon, all displaying avoidance behavior in waters with Cu concentrations ≤ 2.4 µg/L. A recent study estimated that Cu concentrations as low as 0.84 µg/L for rainbow trout and 0.91 µg/L for Chinook salmon produced an avoidance response in 20% of the test population (Meyer and Adams 2010). An avoidance response to Cu-contaminated water may ensure that fish select favorable habitat conditions for survival, but also indicates that fish habitat is lost when contaminated (Saucier et al. 1991; Baldwin et al. 2003).

Long-term sub-lethal Cu exposure may impair a fish's avoidance response to higher Cu concentrations. For example, juvenile Chinook salmon exposed to 2 µg/L Cu for 25 to 30 days showed no preference for clean water versus contaminated water, and failed to avoid waters with Cu concentrations higher than 2 µg/L, including a failure to avoid Cu-contaminated water of 21 µg/L (Hansen et al. 1999b). Prior to acclimation to 2 µg/L Cu, Chinook salmon consistently avoided waters up to 21 µg/L Cu (Hansen et al. 1999b). The failure to avoid higher Cu concentrations suggests that the sensory mechanism responsible for avoidance responses was impaired by the long-term sub-lethal concentration of 2 µg/L Cu, which could result in further impairment of sensory-dependent behaviors essential for survival, or result in mortality if fish are later exposed to higher concentrations.

Migration
Sub-lethal Cu exposure may delay the upstream migration of salmonids to spawning habitat, and induce downstream movement by adults away from spawning grounds. The upstream spawning migration of Atlantic salmon has been reported to be interrupted by Cu concentrations of 20 µg/L (Sprague et al. 1965; Sutterlin and Gray 1973), with reverse

downstream migrations occurring whenever Cu concentrations exceeded 16.8 μg/L to 20.6 μg/L (Sprague et al. 1965; Saunders and Sprague 1967; Hecht et al. 2007). Copper levels higher than 38.4 μg/L are thought to completely prevent upstream migration by spawning Atlantic salmon (Saunders and Sprague 1967). There is also observational evidence that the spawning migration of Chinook salmon may be interrupted at Cu concentrations between 10 μg/L and 25 μg/L (Hecht et al. 2007). Furthermore, the effectiveness of home-stream water as an attractant to Atlantic salmon can be altered by Cu concentrations as low as 44 μg/L (Sutterlin and Gray 1973).

Low levels of Cu exposure can disrupt the downstream migration of juvenile salmonids and reduce survival in seawater. For example, yearling coho salmon exposed to ≥5 μg/L Cu exhibited delayed downstream migration to the ocean and reduced seawater survival, compared to unexposed control fish (Lorz and McPherson 1976). Migration success for juveniles decreased more with higher Cu concentrations and increasing exposure time. A 40% reduction in downstream migration success over a distance of 6.4 km was observed for juvenile coho exposed to 30 μg/L Cu for 72 hours, and a 76% decline in survival occurred for juveniles exposed to 20 μg/L Cu for 144 hours followed by transfer to seawater, compared to control fish (Lorz and McPherson 1976). Juvenile coho exposed to 15 μg/L Cu for 7 days in freshwater followed by transfer to seawater resulted in 40% mortality compared to 100% survival of unexposed fish (Schreck and Lorz 1978). Finally, juvenile sockeye salmon exposed to 30 μg/L Cu in freshwater for 144 hours and transferred to seawater for 24 to 48 hours also demonstrated incomplete smoltification and increased mortality compared to control fish (Davis and Shand 1978).

Predation
Low levels of Cu can cause a loss in sensory capacity for salmonids, and interfere with a fish's ability to detect and respond to chemical signals. Juvenile salmon in natural environments typically alter their behaviour when alerted by the smell of predators to avoid being captured; studies show that low levels of Cu can disrupt this anti-predator response. For example, exposure of 5 μg/L Cu impaired the neurophysiological response of juvenile coho to odorants within minutes (Baldwin et al. 2003). Similar impairment of olfactory function has been reported for juvenile steelhead trout exposed to 5 μg/L Cu for 3 hours (Baldwin et al. 2011), juvenile chum salmon (*Oncorhynchus keta*) exposed to 3 μg/L Cu for 4 hours (Sandahl et al. 2006), and juvenile coho exposed to 3.6 μg/L for 7 days (Sandahl et al. 2004). When the chemical odor is conspecific skin extract (i.e., a chemical cue of predator threat), unexposed fish reduce their swimming speed on average by 75% as an anti-predator response. However, juvenile coho exposed to 2.0 μg/L Cu for 3 hours and then presented with conspecific skin extract showed significant impairment of predator avoidance behaviours; fewer fish became motionless compared to pre-exposure (Sandahl et al. 2007). In a separate study, upstream predator cues presented to juvenile coho previously exposed to 5.0 μg/L Cu for 3 hours did not elicit an alarm response in contrast to control fish (McIntyre et al. 2012). Importantly, Cu-exposed juvenile coho were more vulnerable to predation by cutthroat trout, as measured by attack latency, survival time, and capture success rate; and, pre-exposing predators to similar Cu concentrations did not improve the evasion success of coho prey (McIntyre et al. 2012).

<u>Physical impairment</u>
Growth and swim speed
Sub-lethal Cu exposure can alter swimming and feeding behaviour. Rainbow trout fry exposed to 9.0 μg/L Cu showed a 10% reduction in critical swim speed, and the same effect was observed with juvenile rainbow trout exposed to 5.0 μg/L Cu in low pH water (Waiwood and Beamish 1978a, b). Juvenile brook trout exposed to 6 μg/L Cu showed a three-fold increase in swimming activity within minutes compared to pre-exposure activity levels (Drummond et al. 1973). However, the increase in activity did not equate to an increase in feeding behaviour, as these same fish showed a 40% reduction in foraging after 2 hours of exposure to 6 μg/L Cu (Drummond et al. 1973). The concomitant decrease in feeding behaviour with increased activity may best be explained by the need of fish to increase water flow across the gills for oxygen diffusion due to suffocation from gill damage and/or clogging of the lamellae with mucus, which is a direct effect of Cu toxicity on fish (Scarfe et al. 1982).

Several authors have reported reduced growth rates in Cu-exposed fish. For example, juvenile brook trout exposed to 3.4 μg/L Cu for 1-23 weeks showed a reduction in growth by 15-25% compared to control fish (McKim and Benoit 1971). Rainbow trout fry exposed to 4.6 μg/L Cu for 20 days experienced significantly reduced growth during the same period, and a 40-day exposure to 9.0 μg/L Cu resulted in a 45% reduction in mean body mass relative to control fish (Marr et al. 1996). A reduction in growth by 20% relative to control fish occurred in juvenile rainbow trout exposed to 4.0 μg/L Cu, and the same effect was observed in fish exposed to 2.0 μg/L Cu in low pH water (Waiwood and Beamish 1978a). Cu-induced growth suppression may be associated with depressed appetite and decreased food consumption (Lett et al. 1976; Waiwood and Beamish 1978b), but may more likely involve a metabolic cost related to metal detoxification (Dixon and Sprague 1981; Marr et al. 1995, 1996).

Immune response
Low level Cu exposure can stress fish, suppress resistance to pathogens, and increase susceptibility to secondary stressors. Brook trout fry exposed to 6 μg/L Cu for 5-20 hours showed increased cough frequencies, which is indicative of stress (Drummond et al. 1973). Juvenile coho salmon exposed to 18.2 μg/L for 30 days showed significantly reduced immune response to *Vibrio anguillarum*, the etiological agent of the fish disease known as vibriosis (Stevens 1977). Finally, coho fry exposed to 13.8 μg/L Cu for 7 days showed reduced survival after handling and confinement (Schreck and Lorz 1978), an indication that Cu exposure may increase the vulnerability of salmonids to secondary stressors such as disease and predator pursuits.

Reproduction
Low-level Cu concentrations are able to disrupt the reproductive performance and spawning behaviour of exposed fish. For example, adult brown trout exposed to 10 μg/L Cu for 4 days and then presented with female pheromones produced significantly less milt than control fish, and control fish demonstrated more pre-spawning behaviours than exposed fish (Jaensson and Olsen 2010).

Although British Columbia's water quality criteria for Cu do adequately protect salmonids from lethal effects, they often do not protect salmonids from acute and chronic sub-lethal effects based on the above examples of low effect concentrations (Figure 4). Several effects have been documented in fish exposed to Cu concentrations that are lower than the set criteria. However, it is important to note that the government criteria are based on *total* Cu, whereas the above examples of low effect concentrations exclusively pertain to *dissolved* Cu; the actual concentration of Cu that is dissolved, and thus available as a toxicant to fish in freshwater, tends to be lower than the total Cu concentration.
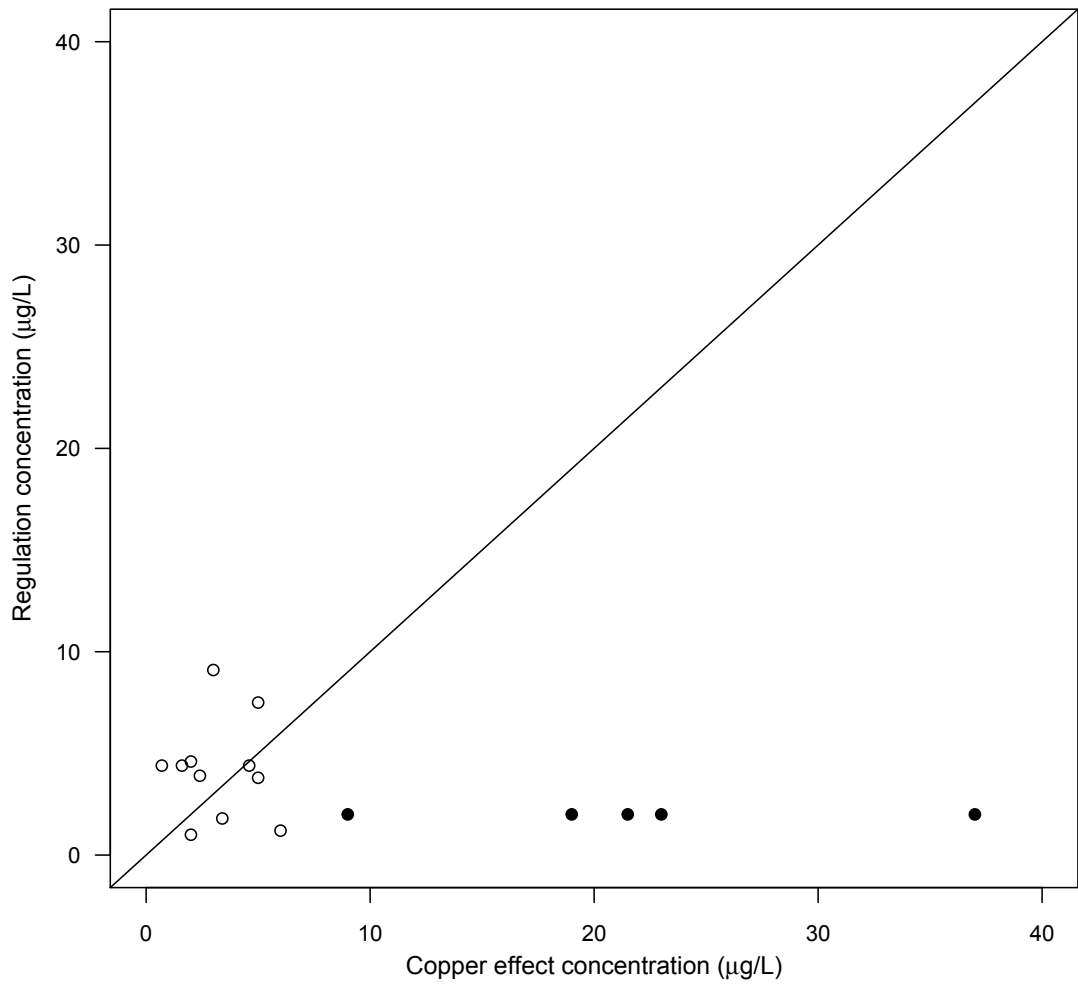
Figure 4. Lowest dissolved concentrations of waterborne copper (Cu) observed to cause acute and chronic sub-lethal (open circle) and lethal (closed circle) effects on salmonids plotted against the British Columbia government's water quality criteria for the protection of aquatic life (Regulation concentration; BCMoE 2013). Circles located above the 1:1 line show Cu concentrations that cause an effect on fish at levels below regulation.

Lead (Pb)

Lead is a biologically non-essential element that is present in nearly all surface waters (Eisler 1988). Most lead measurements from pristine aquatic systems in British Columbia are less than 1 μg/L (Nagpal 1987). Naturally occurring Pb has three oxidative states: metal, Pb (ii), and Pb (iv). While Pb (ii) is the primary state found in water, Pb (iv) is found in extreme conditions. Lead in the form of Pb (iv) compounds can also be produced artificially and released into the environment. For example, tetraethyl Pb (which was a widely used agent in gasoline) has been one of the principal sources of anthropogenic Pb due the subsequent release of emissions from gasoline and waste oil combustion (Nagpal 1987). The manufacture of Pb chemicals and batteries, incineration of refuse, and the effluent generated from mining, smelting, milling, sewage treatment facilities, leachate from landfills, and agricultural run-off are also primary sources of Pb in aquatic environments (Eisler 1988).

The major route of uptake for Pb in fish occurs across the gill (Hodson et al. 1978). Lead is similar to Cd in that the metal is a Ca-antagonist and neurotoxin (Sorensen 1991) that may affect the behaviour of salmonids (Sloman et al. 2003b). Lead accumulates in the bones and tissues of fish, and in high enough concentrations can impair the function of the liver, kidney, and spleen (Haider 1964), and can cause spinal deformities and death (Davies and Everhart 1973). Concentrations between 1,000 μg/L for juvenile rainbow trout (Rogers et al. 2003) and 3,362 μg/L for juvenile brook trout can cause death to 50% of fish in 96 hours (Holocombe et al. 1976).

Physical impairment

Lead exposure can inhibit essential physiological functions in salmonids. The exposure of juvenile rainbow trout to 13 μg/L Pb for 2 weeks caused a reduction in red blood cell enzyme (delta-aminolevulinic acid dehydratase; ALA-D) activity, and the activity was significantly reduced after 4 weeks compared to control fish; at 4 months, enzyme activity of exposed fish was reduced to 60% of control fish levels (Hodson 1976). The effect of Pb on ALA-D increases both with concentration and exposure time (Hodson 1976). Delta-aminolevulinic acid dehydratase is responsible for the production of hemoglobin, an essential oxygen-transport protein in red blood cells. In a follow-up study, Hodson et al. (1977) reported a 20% reduction in ALA-D activity in juvenile rainbow trout exposed to 10 μg/L Pb after only 2 weeks compared to control fish. Red blood cell enzyme activity of juvenile brook trout was inhibited by 20-45% only during exposure to 90-100 μg/L Pb for 2 weeks; 50-60 μg/L Pb over the same time period had little effect (Hodson et al. 1977). The maximum acceptable toxicant concentration for juvenile rainbow trout exposed to Pb has been estimated at between 3.0 μg/L and 13 μg/L in waters of alkalinity between 26 mg/L and 90 mg/L (Davies and Everhart 1973; Hodson 1976).

Development

Salmonids exposed to Pb can develop physical abnormalities. Juvenile rainbow trout exposed for 6 weeks during the eyed-egg stage to 7.6 μg/L Pb in soft water developed blacktail abnormalities (Davies et al. 1976). Roughly 40% of fish exposed as eggs to 13.2 μg/L Pb over the same time period developed blacktails, and 5.5% and 3.6% also

developed eroded caudal fins and curved spines, respectively (Davies et al. 1976). Physical deformities in juvenile brook trout increased with each generation exposed to Pb. For example, the percentage of second-generation alevins at hatch with curved spines was 5% when exposed to 119 μg/L total Pb (unknown dissolved concentration) compared to control fish, whereas the percentage of third-generation alevins at hatch with curved spines increased to 21% (Holocombe et al. 1976).

Reproduction
Sub-lethal Pb exposure can cause endocrine dysfunction in fish. For example, Ruby et al. (1993) reported decreased transformation of spermatogonia to spermatocytes in sexually maturing male rainbow trout exposed to 10 μg/L Pb for 12 days. Additionally, two-year old female rainbow trout exposed to 10 μg/L Pb for 12 days showed significantly reduced oocyte (cells from which eggs develop) growth compared to control fish (Ruby et al. 2000).

Regulatory limits
The government of British Columbia's criteria pertaining to Pb for the protection of aquatic life are low enough to protect salmonids from sub-lethal effects, based on the above examples of chronic low effect concentrations (Figure 5), and are very conservative to protect fish from lethal effects (Table 2; not shown on Figure 5).

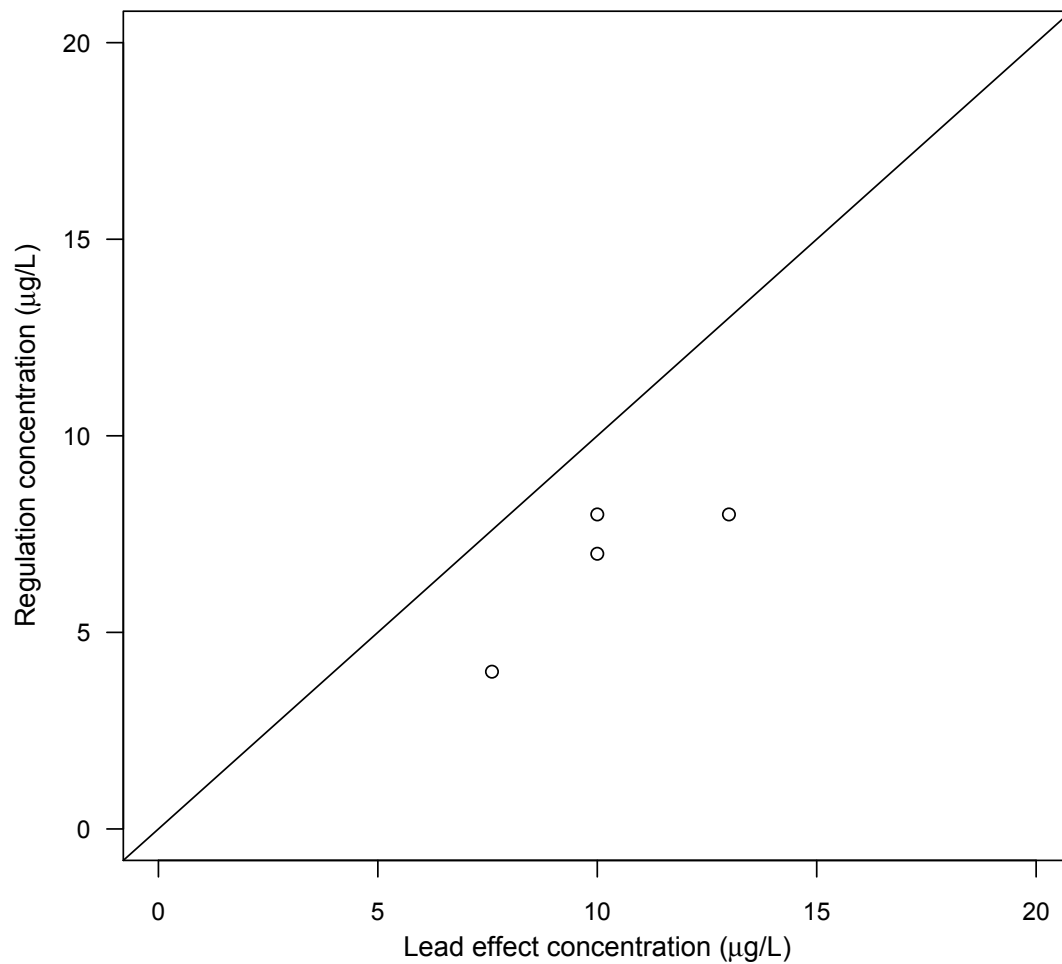**Figure 5.** Lowest dissolved concentrations of waterborne lead (Pb) observed to cause chronic sub-lethal effects on salmonids (lethal concentrations exceed the figure scale) plotted against the British Columbia government's water quality criteria for the protection of aquatic life (Regulation concentration; BCMoE 2013). Circles located above the 1:1 line show Pb concentrations that cause an effect on fish at levels below regulation.

Nickel (Ni)
Nickel is a biologically essential element for the normal growth of fish (Eisler 1998b).
The metal is a common component of natural freshwaters due to erosion and weathering,
and levels of Ni generally range 1-10 μg/L in unpolluted areas (U.S.EPA 1980a), though
human activities have contributed greatly to the more recent loadings in terrestrial and
aquatic ecosystems. Mining, smelting, refining, fossil fuel combustion, and waste
incineration are some of the most common contributors of Ni to the environment (Eisler
1998b). Nickel concentrations are comparatively elevated in fishes near Ni smelters, Ni-
Cd battery plants, sewage outfalls, metal mines, and generally heavily polluted areas.

Nickel is a respiratory toxicant, and the gill is a key site of toxicity in fish; this is in
contrast to most other metals that are ionoregulatory toxicants (Pane et al. 2003).
Significant structural alterations to the brachial epithelium (i.e., swelling of the gill
surface) have been observed in salmonids exposed to Ni (Hughes et al. 1979), which can
lead to diffusive limitations of high performance gas exchange during intense swimming
episodes (Pane et al. 2005). The large swelling of the respiratory surface is thought to be
the result of profound Ni-induced disturbances in blood gases and acid–base balance,
such as observed in juvenile rainbow trout by Pane et al. (2003). Concentrations of Ni as
high as 8,100 μg/L can cause death to 50% of juvenile rainbow trout within 96 hours
(Nebeker et al. 1985).

Sensory impairment
Avoidance
Salmonids respond to Ni in different ways at sub-lethal concentrations. At 6 μg/L total Ni
(unknown dissolved concentration), juvenile rainbow trout showed a 40% increase in
time spent in the area of the experimental tank with toxicant water compared to control
fish; yet these same fish detected and avoided the toxicant water when total Ni
concentrations reached 10-19 μg/L (Giattina et al. 1982). The concentration that caused a
50% reduction in the amount of time fish spent in an area relative to control times was
estimated at 23.9 μg/L total Ni (unknown dissolved concentration; Giattina et al. 1982).

Physical impairment
Locomotion
Chronic exposure of fish to sub-lethal Ni concentrations can result in respiratory toxicity
in the form of altered gill morphology, and impaired swim performance and oxygen
consumption patterns. For example, juvenile rainbow trout exposed to 384 μg/L Ni for 12
and 24 days resulted in small (~7%; not statistically significant) decreases in critical
swim speed compared to un-exposed fish (Pane et al. 2005). After 34 days of exposure to
394 μg/L Ni, juvenile rainbow trout showed 33% reduction in maximal oxygen
consumption rate, and 38% decrease in aerobic activity, compared to control fish (Pane et
al. 2005). Importantly, Pane et al. (2005) report that the aerobic capacity of exposed fish
remained depressed despite a subsequent clean-water exposure period of 38 days, and
suggest that such an impairment may reduce the overall fitness of juvenile rainbow trout
by impairing predator avoidance, prey capture, and migration success.

Regulatory limits

The government of British Columbia does not have water quality criteria pertaining to Ni; instead, the government relies on the guidelines approved by the Canadian Council of Ministers of the Environment (CCME 2013). Based on the above examples of low effect concentrations, the federal guidelines at times are not low enough to protect salmonids from sub-lethal effects (Figure 6). There are two examples where juvenile rainbow trout exhibited non-normal behaviour when exposed to acute concentrations of Ni that were lower than the regulatory concentration. The first involved an attraction response to 6 μg/L Ni, and the second involved an avoidance response by 50% of fish to 23.9 μg/L Ni; the regulatory concentration based on the same water hardness as the study is set at 25 μg/L. Importantly, the concentrations that were shown to evoke a response in fish were measured as *total* Ni, and the actual *dissolved* concentrations were likely lower.
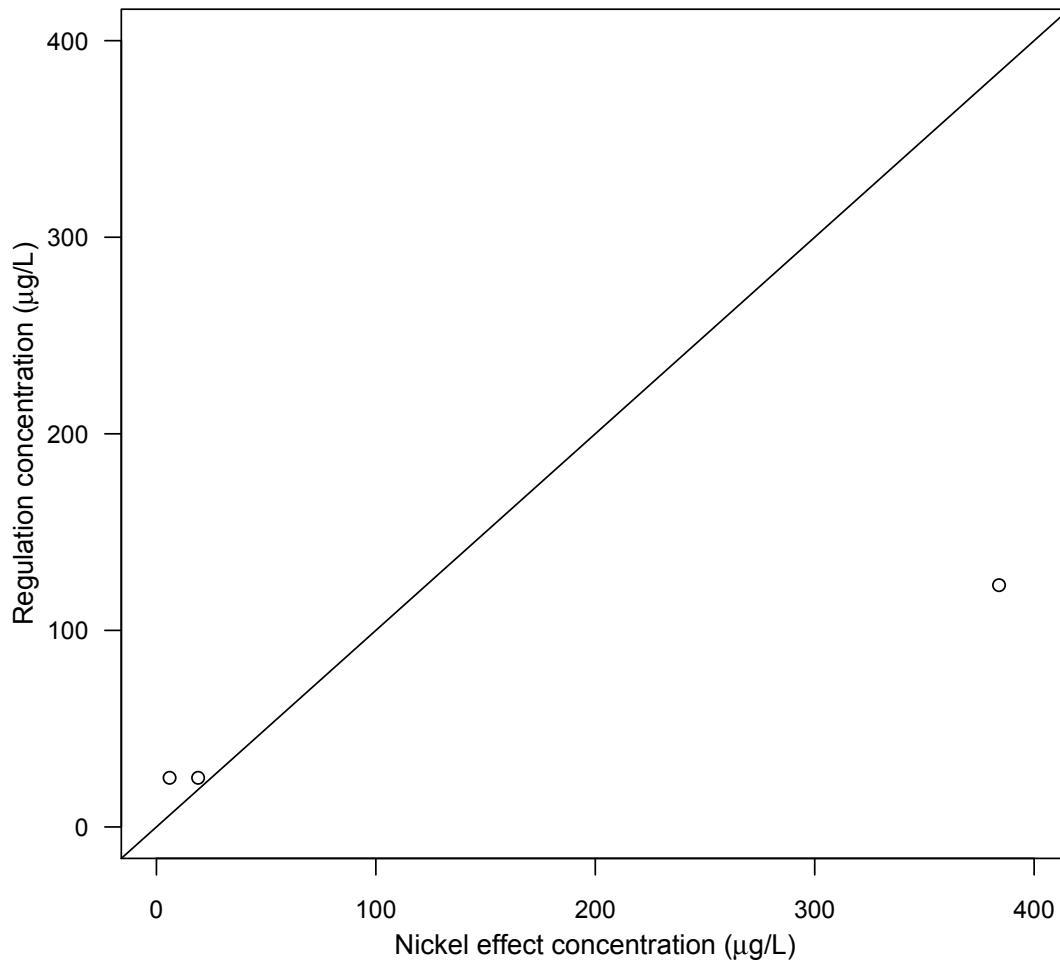
Figure 6. Lowest total concentrations of waterborne nickel (Ni) observed to cause acute and chronic sub-lethal effects on salmonids (lethal concentrations exceed the figure scale) plotted against the Canadian Council of Ministers of the Environment criteria for the protection of aquatic life (Regulation concentration; CCME 2013). Circles located above the 1:1 line show Ni concentrations that cause an effect on fish at levels below regulation.

Silver (Ag)

Silver is a rare and biologically non-essential element that is one of the most toxic metals known to aquatic organisms when in its ionic form (Ag^+; Davies et al. 1978; Hogstrand et al. 1996; Galvez and Wood 2002). Silver is commonly recovered as a byproduct from the smelting of Ni, and in the ores of Cu, Pb, gold (Au), platinum (Pt), and Zn, but is also naturally elevated in crude oil (Eisler 1996). In recent times, the principal industrial use of Ag was in the manufacture of photographic imaging materials, electrical and electronic products, coins, jewelry, and medicinal products such as antiseptics and germicides (Eisler 1996). Silver is commonly found in low concentrations (range = 0.09-0.55 μg/L) in natural waters, yet concentrations in biota tend to be highest near sites of sewage effluent and metal mines (U.S.EPA 1980b).

The mechanism of Ag toxicity in salmonids involves the blockage of sodium (Na^+) and chloride (Cl^-) transport at the gills (Wood et al. 1999). This inhibition can result in reductions in plasma Na^+ and Cl^- levels, and the decrease in plasma ions will eventually lead to circulatory failure and death of the fish (Hogstrand and Wood 1998; Morgan et al. 2005). Concentrations of Ag as low as 6.5 μg/L can cause death to 50% of juvenile rainbow trout within 96 hours (Davies et al. 1978).

<u>Physical impairment</u>
Development and mobility
Sub-lethal Ag exposure can alter feeding behaviour, growth, and swim speed. Food consumption by juvenile rainbow trout exposed to 5 μg/L Ag decreased by 23% compared to unexposed fish (Galvez and Wood 2002). These same fish were significantly smaller than unexposed fish after 10 days of exposure, and weighed 22% less than control fish after 23 days. Additionally, specific growth rates of exposed fish were reduced by 70% compared to unexposed fish, which further resulted in food-conversion efficiencies of exposed fish that were 58% lower than those measured in unexposed fish (Galvez and Wood 2002). In two separate studies, juvenile rainbow trout exposed to 0.1 μg/L and 0.17 μg/L Ag were significantly smaller (in mean length and weight) than unexposed fish after 60 days (Davies et al. 1978; Nebeker et al. 1983). The maximum acceptable toxicant concentration based on the lowest significant effect level for these fish was estimated to be < 0.1 μg/L Ag (Nebeker et al. 1983). Premature hatching of eggs and retarded sac-fry development as a result of exposure to 0.17 μg/L Ag was reported in rainbow trout by Davies et al. (1978). Finally, with regards to mobility, 5 days exposure to 5 μg/L Ag reduced the critical swim speed of juvenile rainbow trout by 14% compared to control fish (Galvez and Wood 2002).

Physiological response
Juvenile rainbow trout exposed to 5 μg/L Ag for 5 and 10 days showed reduced plasma Na^+ concentrations of 23% and 18%, respectively, compared to unexposed fish; however, plasma Na^+ concentrations returned to control concentrations after 15 days exposure (Galvez and Wood 2002). Similarly, mean plasma Cl^- concentrations were significantly reduced in fish exposed to 0.1 μg/L Ag on day 15, and 5 μg/L Ag on days 5 and 10; juveniles exposed to 5 μg/L Ag showed reductions in plasma Cl^- concentrations of 21% and 17% by days 5 and 10, respectively (Galvez and Wood 2002).

<u>Regulatory limits</u>
The government of British Columbia's criteria pertaining to Ag for the protection of aquatic life is low enough to protect salmonids from chronic lethal and sub-lethal effects, based on the above examples of low effect concentrations (Figure 7).

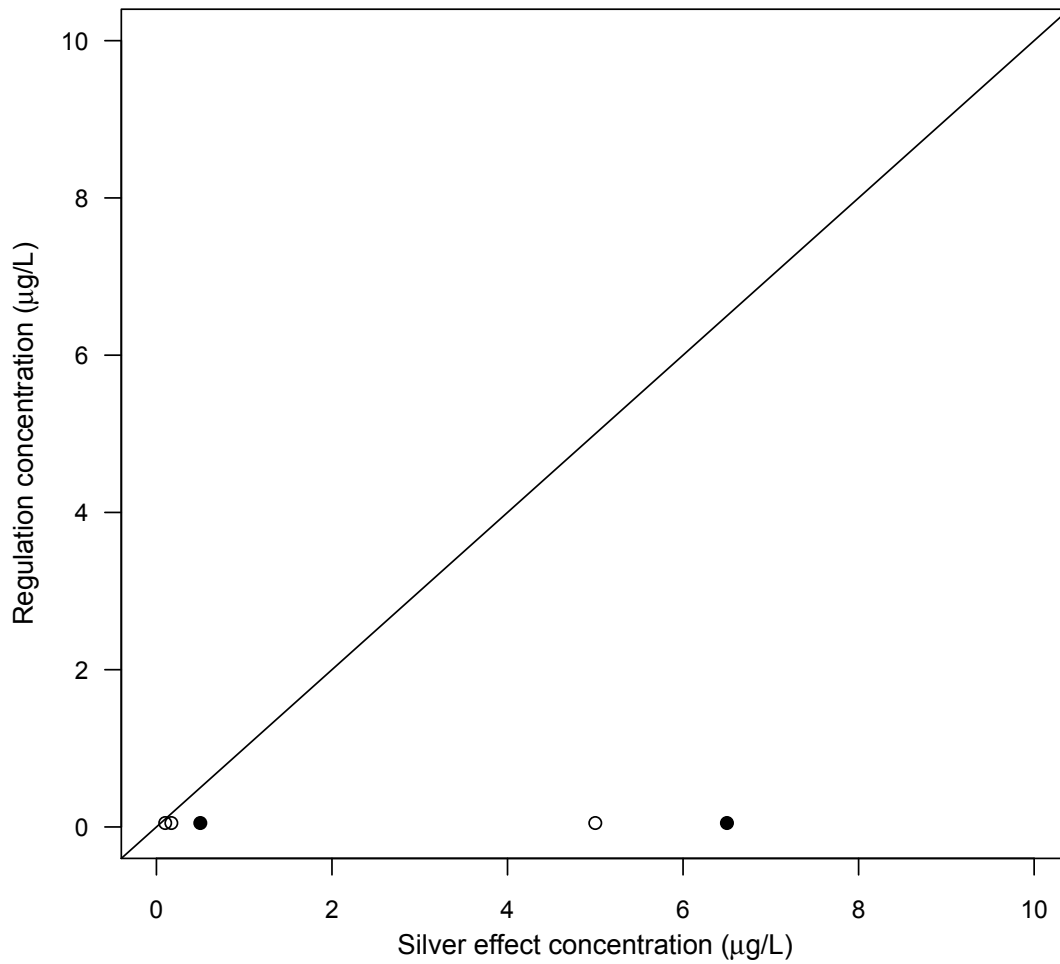**Figure 7.** Lowest dissolved concentrations of waterborne silver (Ag) observed to cause chronic sub-lethal (open circle) and lethal (closed circle) effects on salmonids plotted against the British Columbia government's water quality criteria for the protection of aquatic life (Regulation concentration; BCMoE 2013). Circles located above the 1:1 line show Ag concentrations that cause an effect on fish at levels below regulation.

Zinc (Zn)

Zinc is a ubiquitous element that is biologically essential for the normal growth, physiology, and development of fish in minute quantities, but becomes toxic when in excess of cellular requirements (Wood 2001). Zinc is also one of the most common contaminants in aquatic systems, and tends to occur in elevated concentrations adjacent to areas of urban run-off, industrial discharges, and soil erosion (Bowen et al. 2006). The primary anthropogenic sources of Zn in the environment are from mining activities and metal smelters, though the production and use of Zn in die castings metal, alloys, rubber, and paints may also lead to its release to receiving systems through various waste streams.

Zinc is a Ca-antagonist, and sub-lethal concentrations of waterborne Zn can competitively inhibit the uptake of Ca^{2+} by fish at the gill (Hogstrand et al. 1995). Zinc and Ca^{2+} compete for the same sites on the gills of fish, hence the protective effect of increased water hardness ($CaCO_2$) on fish exposed to Ca-antagonist metals such as Zn. However, as the concentration of Zn relative to Ca increases in freshwater systems, the more Zn will effectively bind to sites on the fish's gill and outcompete Ca. The result is an accumulation of Zn on the gills, a decrease in branchial ionoregulation (i.e., the maintenance of the concentrations of the various ions in the body fluids relative to one another), and eventual death (Skidmore 1970). Concentrations between 93 µg/L for juvenile rainbow trout (Chapman 1978a) and 749 µg/L for juvenile sockeye salmon (Chapman 1978b) can cause death to 50% of fish in 96 hours.

Sensory impairment

Avoidance

Sub-lethal Zn exposure may induce avoidance of rearing habitat for salmonids. Estimates of threshold concentrations for avoidance (i.e., the lowest concentration that causes at least 50% of fish to show significant avoidance) of juvenile rainbow trout to Zn are reported to be 8.6 µg/L (95% confidence limits range 7.3-10.3 µg/L; Sprague 1968). Importantly, a decrease in water temperature raised the avoidance threshold for fish. For example, the threshold avoidance for juvenile rainbow trout exposed to 17°C water was 7.3 µg/L, whereas exposure to 9.5°C resulted in an estimated threshold avoidance of 8.4 µg/L; though the differences were not statistically significant (Sprague 1968). Juvenile Atlantic salmon exposed under similar laboratory conditions also showed avoidance to Zn, with an estimated threshold concentration of 53 µg/L (range = 27-104 µg/L; Sprague 1964). The difference in avoidance thresholds between the two species is thought to be the result of differences in behaviour characteristics (i.e., while Atlantic salmon tend to be less mobile, rainbow trout are active swimmers that may become more aware of toxicant gradients), rather than a difference in sensory perception (Sprague 1968).

Physical impairment

Immune response

Sub-lethal exposure of salmonids to waterborne Zn can induce physiological stress and reduce immune responses. For example, juvenile rainbow trout exposed to 81 µg/L Zn for 1 day showed significantly higher plasma glucose levels compared to control fish, and the rise in glucose was attributed to, and a sign of, stress (Wagner and McKeown 1982).

In a separate study of juvenile rainbow trout, fish exposed to 10 µg/L Zn for 30 days showed significantly inhibited immune response compared to control fish (Sanchez-Dardon et al. 1999).

Regulatory limits
There is one example of where juvenile rainbow trout exhibited avoidance behaviour when exposed to a chronic concentration of Zn that was lower than British Columbia's regulatory concentration (Figure 8). At 8.6 µg/L, 50% of fish avoided the toxicant compared to control fish; the regulatory concentration based on the same water hardness as the study is set at 33 µg/L. Regulatory concentrations do adequately protect salmonids from acute mortality.

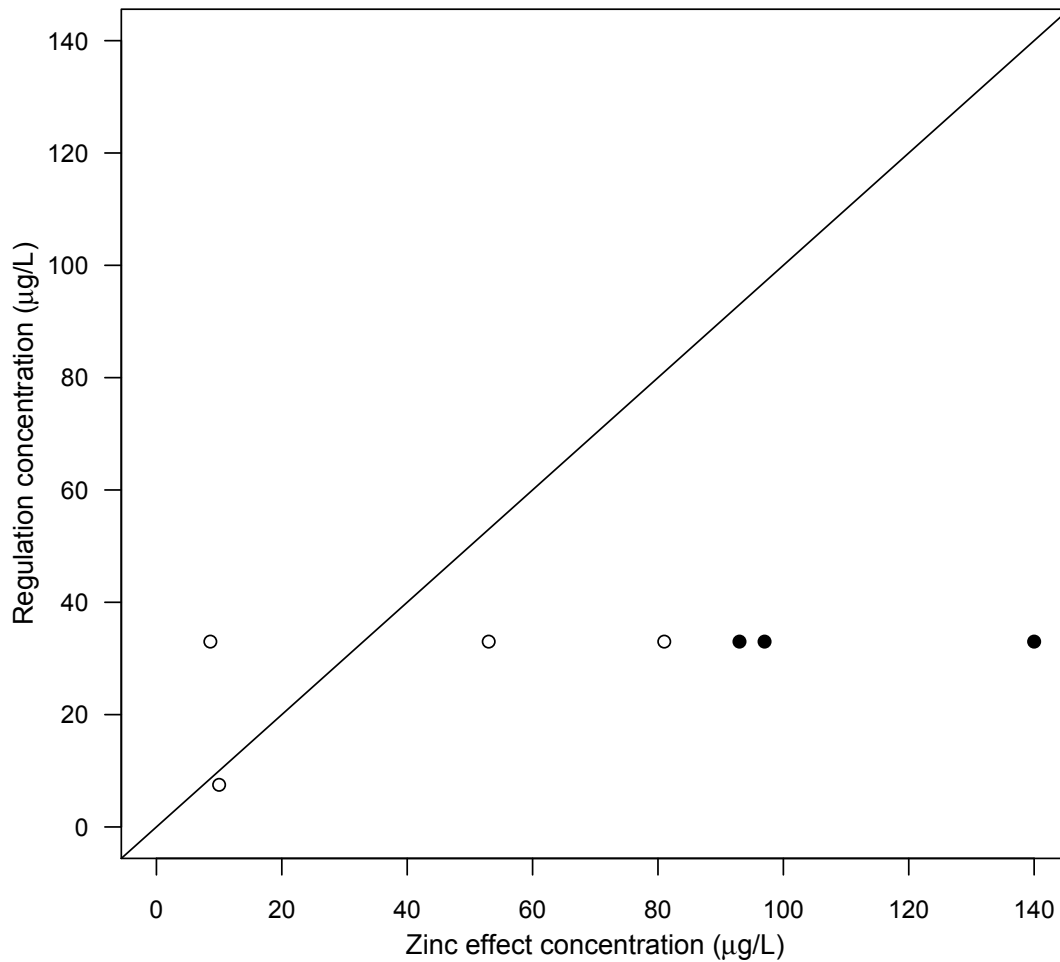**Figure 8.** Lowest dissolved concentrations of waterborne zinc (Zn) observed to cause acute and chronic sub-lethal (open circle) and lethal (closed circle) effects on salmonids plotted against the British Columbia government's water quality criteria for the protection of aquatic life (Regulation concentration; BCMoE 2013). Circles located above the 1:1 line show Zn concentrations that cause an effect on fish at levels below regulation.

Metal Mixtures
One complicating factor in an assessment of the toxicity potential for any particular heavy metal is that, unlike laboratory studies that often examine metals in isolation, multiple metals typically occur and interact in aquatic systems (Boyd 2010). Toxicological studies that focus on the effects of single metals may not be environmentally realistic or relevant for assessing actual impacts on fish. Combinations of heavy metals may behave in three ways: additively (one metal acts independently from another, and the toxic effect of each metal in combination is the same as the effect of the individual metals), synergistically (different metals interact, and the toxic effect of the combined metals is greater than the additive effects of the individual metals), or antagonistically (different metals interact, but the toxic effect of the combined metals is less than the additive effects of the individual metals; Boyd 2010).

Additive effects
Mixtures of metals can illicit responses similar to the individual metals. Adult Chinook salmon preferred to spawn in waters relatively free of metals contamination compared to an adjacent tributary polluted with Cd (7 µg/L), Cu (2 µg/L), Pb (23 µg/L), and Zn (2,200 µg/L) in the Coeur d'Alene River, Idaho (Goldstein et al. 1999). Similarly, a study of adult Atlantic salmon during their spawning migration upstream in the Miramichi River, New Brunswick, reported that 22% of spawning fish avoided upstream waters with sub-lethal Cu (20 µg/L), and Zn (260 µg/L) by returning prematurely downstream (Saunders and Sprague 1967). The concentrations of these metal mixtures are similar to those avoided for individual metals. However, juvenile brown trout exposed to low levels of Pb (<1.7 µg/L), in a mixture of Cd (0.6 µg/L), Cu (6.5 µg/L), and Zn (32 µg/L) for 30 minutes showed a significant avoidance response compared to unexposed fish (Woodward et al. 1995); the Pb concentration used in this study was considerably lower than those cited above and the 26 µg/L Pb reported to induce avoidance behaviour in juvenile rainbow trout by Giattina and Garton (1983). A similar study with juvenile cutthroat trout reported avoidance behaviour when exposed to low levels of Pb (0.6 µg/L) in a mixture of Cd (0.30 µg/L), Cu (6.0 µg/L), and Zn (28 µg/L). Importantly, these same fish did not avoid water containing only 0.6 µg/L Pb (Woodward et al. 1997). Only when Cu or Zn were added did fish show an avoidance response, which suggests that Cu and/or Zn are the metals that fish are negatively responding to.

Synergistic effects
Metal mixtures can illicit responses in salmonids at lower concentrations than the individual metals. For example, the LC50 (lowest concentration that causes death in 50% of fish) for bull trout ranged from 0.83 to 0.88 µg/L Cd when exposed only to Cd, whereas the LC50 in a mixture with Zn was 0.51 µg/L Cd (Hansen et al. 2002a). A study that examined a mixture of Cu and Zn reported that the combination of the two metals reduced the avoidance threshold of fish by an order of magnitude below that for each metal tested individually. In combination, 0.4 µg/L Cu and 6.1 µg/L Zn produced an avoidance reaction in juvenile Atlantic salmon, whereas the individual thresholds were 2.3 µg/L Cu, and 53 µg/L Zn (Sprague 1964). Sprague and Ramsay (1965) also reported that lethal concentrations of mixtures of Cu and Zn on juvenile Atlantic salmon act two or three times faster than the metals singly. The threshold of avoidance for juvenile

rainbow trout exposed to metal mixtures has been estimated at 1.2 μg/L Cu, 0.11 μg/L Cd, 0.32 μg/L Pb, and 5 μg/L Zn (Hansen et al. 1999c), which is less than the single-metal avoidance concentrations for Cu and Zn, and may indicate metal interactions and synergy. With regards to physiological effects, while sub-lethal concentrations of Cd alone (and not Pb alone) induced disturbances to the normal Ca^{2+} influx at the gill of juvenile rainbow trout, the addition of Pb plus Cd exacerbated these effects in a synergistic fashion (Birceanu et al. 2008). Finally, mixtures of Cu and Al, and Cu and iron (Fe) were more than additive in their toxicity to ova of brown trout (Sayer et al. 1991).

Antagonistic effects
Metals such as Zn and Pb can reduce the negative effects of metals in isolation when combined in a mixture. Juvenile rainbow trout exposed to individual doses of sub-lethal concentrations of Cd, Zn, and mercury (Hg) experienced reduced immune system responses compared to control fish (Sanchez-Dardon et al. 1999). However, when Zn was combined with either Cd or Hg, the immune responses of exposed fish were similar to unexposed fish (i.e., no changes occurred). Finally, accumulation of Pb and Cd on the gills of juvenile rainbow trout were less than additive (i.e., antagonistic effect) when combined in a metal mixture likely because of the competition between these metals for binding sites (Birceanu et al. 2008).

Discussion
Sub-lethal toxicity of salmonids is a common consequence of low concentrations of heavy metals in aquatic systems. Indeed, all of the metals that were examined can negatively influence the physiology of salmonids to some degree at concentrations far below lethal levels. Sub-lethal concentrations can alter behaviours related to predator avoidance, foraging, migration, and social interactions, and can cause the physical impairment associated with growth and development, swimming efficiency, and immune system responses. Despite several complicating factors for studies of toxicant impacts on fish, metals such as Cu can cause effects at concentrations below regulatory limits in British Columbia, which is a concern for wild salmonid populations there.

Both the provincial and federal governments of Canada assign water quality guidelines for the protection of aquatic life, which includes fishes. While the two sets of guidelines are generally comparable, federal criteria tend to be more sensitive to metal toxicity for aquatic life (CCME 2013; BCMoE 2013). In British Columbia, federal guidelines are referred to in circumstances when guidelines for a particular metal have not been set; Cd and Ni are two such examples.

Most of the metal concentrations reported to invoke sub-lethal effects on salmonids are above the regulatory limits in British Columbia. For example, water quality guidelines assigned for Al, Cd, Pb, and Ag would protect against all of the effect concentrations that were found reported in the literature. However, there are several instances where sub-lethal effects on salmonids from metals such as Cu, Ni, and Zn have been reported at concentrations below the water quality guidelines of British Columbia and the federal government of Canada (Figure 9). All of these sub-lethal effects involve either avoidance behaviour or impaired olfaction. Avoidance of polluted water is one of the most sensitive responses of fish to toxicants, which enables them to survive in the perturbed environment (Sprague and Drury 1969), yet ultimately results in lost habitat. Alternatively, the inhibition of olfactory responses to predators by juvenile rainbow trout, and juvenile chum and coho salmon (Sandahl et al. 2006, 2007; Baldwin et al. 2011; McIntyre et al. 2012) is arguably the most ecologically severe sub-lethal consequence of metal toxicity. Copper-exposed salmonids are more vulnerable to predators (McIntyre et al. 2012), and have lower survival compared to unexposed fish. The disruption of such an anti-predator response in salmonids at effect concentrations below that assigned by the government of British Columbia is a very real scenario that may have implications for wild salmonid populations – especially considering that British Columbia is the largest producer of Cu in Canada.

Water quality criteria for the protection of aquatic life in British Columbia are not legislated, but rather serve as environmental benchmarks (BCMoE 2013). Specific industrial projects apply for permits to pollute, and the resulting metal concentrations in receiving waters of discharge may be higher than the provincial criteria. Two such examples are the open-pit copper mines (Noranda Bell and Granisle) located on Babine Lake in the Skeena River watershed, where the maximum authorized discharge for dissolved Cu from mine waste-water into the lake is five-fold higher than the regulatory guidelines for each mine (Remington 1996).
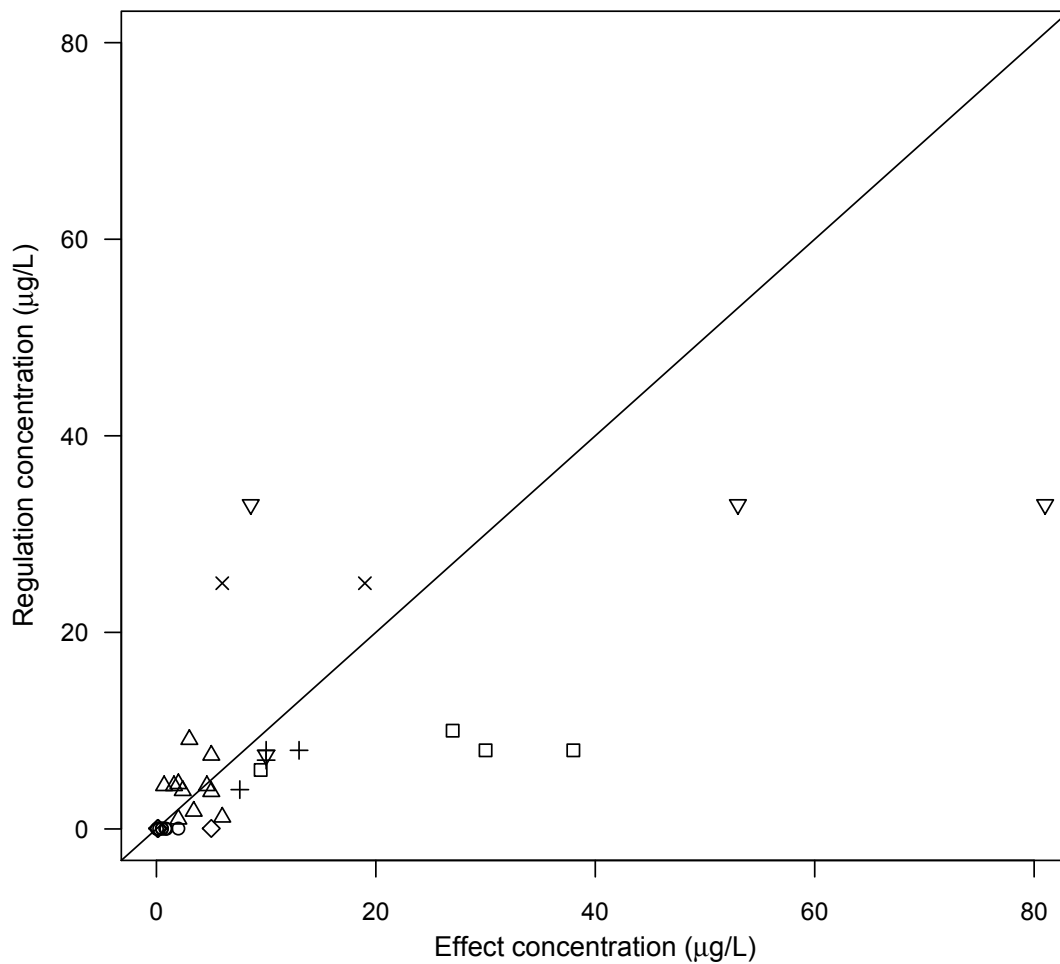
Figure 9. Lowest dissolved concentrations of waterborne metals observed to cause acute and chronic sub-lethal effects on salmonids plotted against the British Columbia government (BCMoE 2013) and Canadian Council of Ministers of the Environment (CCME 2013) water quality criteria for the protection of aquatic life (Regulation concentration). Symbols are represented as following: Al (□), Cd (◊), Cu (△), Ni (×), Pb (+), and Zn (∇); those located above the 1:1 line show concentrations that cause an effect on fish at levels below regulation.

The impact of heavy metals on fish is complex and depends on the chemical characteristics of water. Acidity (pH), hardness (CaCO₃), and organic matter are complicating factors in the determination of metal toxicity. For example, acidification of surface waters can increase the toxicity of metals to fish (Cusimano et al. 1986: Spry and Wiener 1991). The solubility of metals such as Al, are particularly susceptible to speciation as a direct function of pH, with the more toxic forms developing in acidic water (Freeman and Everhart 1971). Likewise, water hardness can influence the toxicity of metals to fish. Most heavy metals become more toxic in softer water, and this is likely due (in part) to the decrease in Ca^{2+} ions associated with softer water (Wood et al. 1999; Morgan et al. 2005; Monette et al. 2008). Some heavy metals enter the fish gill epithelium through the same pathway as Ca, and can effectively block active Ca uptake and result in ion imbalance of fish (Verbost et al. 1987, 1989; Playle et al. 1993). An increase in Ca via increasing water hardness can help out-compete metals at cellular binding sites that might otherwise result in the weakening of the tight junctions responsible for ion regulation (Booth et al. 1988; Freda et al. 1991; Monette et al. 2008). Thus, a difference in acidity and/or hardness of test water may at least partly explain why the effect concentrations for a given metal and fish species are often dissimilar between studies.

While acidity and hardness can influence the toxicity of metals to fish, the amount of organic matter in water (particularly in the form of dissolved organic carbon; DOC) may have a greater effect. In a study on the toxic effects of Cu on juvenile coho for example, the olfactory capacity of fish was partially restored by increasing DOC, whereas it was not affected by a change in acidity, and only slightly improved with increasing water hardness (McIntyre et al. 2008). A separate study showed that LC50s (the lowest concentration shown to cause an effect in 50% of the test population) can vary widely depending on the amount of DOC in the water (Ryan et al. 2004; Sciera et al. 2004), which has not been shown for acidity or hardness. Additionally, the effect of Cu on the olfactory system of adult brown trout and juvenile Chinook salmon was considerably reduced as a result of the amount of DOC in the test water (Jaensson and Olsen 2010; Kennedy et al. 2012). As a result, it has been recommended that DOC concentrations be considered when evaluating the potential impact of Cu on fish olfaction. However, one complication with this recommendation is that other heavy metals can potentially reduce the amelioratory effects of DOC through competition for binding sites with DOC (Kennedy et al. 2012).

There are at least four limitations when applying the reported effect concentrations on salmonids to real-life scenarios. First, the effect concentrations reported in this review are more often the lowest *detected effect*, not the actual *lowest effect concentration*. While the lowest *detected effect* describes the lowest concentration of a metal that was tested and found to cause an effect on fish, the *lowest effect concentration* is the actual lowest concentration of a metal that can cause an effect on fish. For example, Sandahl et al. (2007) showed that juvenile coho exposed to ≥2 µg/L Cu for 3 hours exhibited a suppression in predator avoidance behaviour (a lowest *detected effect*); yet, concentrations below 2 µg/L were not tested. Thus, uncertainty remains as to the precise threshold for olfactory impairment. This is also true for the olfactory impairment of

juvenile rainbow trout (Baldwin et al. 2011), and juvenile chum salmon (Sandahl et al. 2006). Further research is needed to determine threshold concentrations for most metals on salmonids.

Second, scientific studies rarely reflect natural exposure conditions. Most of the studies reported in this review were performed in a laboratory, where conditions for fish are near optimal. Parameters such as water flow, temperature, and food all tend to be favorable for fish and constant throughout the experimental period (Pyle and Merza 2007). However, fish in natural settings are typically forced to cope with sub-optimal conditions, and are frequently exposed to multiple stressors (Hecht et al. 2007); these added stressors may or may not alter the toxic effects of heavy metals. Not only can the chemical properties of water influence the availability of toxicants (Newman and Unger 2003), but the nutritional status of fish may influence the uptake of toxicants from the environment (Holmstrup et al. 2010). Thus, the measured toxicity of a particular metal at a given concentration in the laboratory may be less than for fish in contaminated waters.

Third, laboratory studies tend to examine metals in isolation, which may not be environmentally realistic or relevant for assessing actual impacts on fish. This is because fish are more often exposed to an assortment of metals, as well as organic chemical pollutants, in contaminated aquatic systems (Boyd 2010). Of the three ways that metals can behave (antagonistically, additively, or synergistically) when combined in a mixture, the greatest concern for fish is one of synergy. There are examples of mixtures of Cd/Zn, Cd/Pb, Cd/Cu/Pb/Zn, Cu/Al, Cu/Fe, and Cu/Zn with resulting effects on bull trout, rainbow trout, brown trout, and Atlantic salmon that were more than additive (Sprague 1964; Sprague and Ramsay 1965; Sayer et al. 1991; Hansen et al. 1999c; Birceanu et al. 2008). Although a review of the relevant literature on mixed metals suggests that studies more often report synergistic effects than the other two behaviour types (an indication that laboratory studies may underestimate sub-lethal effects on salmonids), future research is needed.

Finally, dietary metal concentrations are not incorporated into Canada's water quality guidelines despite the likely simultaneous occurrence of both waterborne and dietary routes of metal toxicity. The results reported in this literature synthesis only describe waterborne effects of metals on fish; yet, the consumption of metal-contaminated prey is also a common route of toxicity for predatory animals such as salmonids. Dietary Cu may at times be more important than waterborne Cu at reducing survival of salmonids during early life stages (Woodward et al. 1994). Importantly, waterborne and dietary metal exposures occur simultaneously in aquatic environments, and sub-lethal toxic effects of waterborne metals in salmonids may be exacerbated by dietary uptake. For example, the switch in feeding preference from motile to non-motile (benthic) prey by juvenile brook trout as a result of Cd-exposure is hypothesized to exacerbate the effects of Cd by intensifying or prolonging exposure through a combination of trophic transfer and altered foraging behavior (Riddell et al. 2005b). Yet, the water quality guidelines for heavy metals assigned by the governments of British Columbia or Canada do not factor the toxic effects of chronic dietary loading in the regulatory context. This may be because

there is insufficient knowledge on the risk of diet-borne metals to salmonids, and is an important area of future research.

To conclude, heavy metals are common persistent pollutants of aquatic ecosystems that can routinely cause sub-lethal effects in salmonids. Sub-lethal concentrations can alter behaviours related to predator avoidance, foraging, migration, and social interactions, and impair growth and development, swimming efficiency, and immune system responses. Within the regulatory context of the government of British Columbia and Canada's water quality guidelines, Cu is the metal of highest concern for wild populations. Research is needed not only to determine threshold concentrations for salmonids, but also to compare the effect concentrations derived from laboratory studies with natural environments, and examine the effects of metal mixtures and dietary toxicity on salmonids. Ultimately, a shift in research emphasis from the routine single metal - single organism - perspective, to population, community, and ecosystem scale is required to achieve a full understanding of the sub-lethal metal toxicity effects on salmonids.

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Appendix Table. Lowest waterborne metal concentrations observed to cause sub-lethal and lethal effects in various species of salmonids during specified life-cycle periods. Species abbeviations are: rainbow trout (RbT), Atlantic salmon (AtS), brown trout (BnT), lake charr (LkC), brook trout (BkT), bull trout (BlT), cutthroat trout (CtT), arctic grayling (ArG); Chinook salmon (CkS), coho salmon (CoS), sockeye salmon (SkS), and chum salmon (CmS). Phase refers to the life-cycle periods: egg (E), juvenile (J), and adult (A). All concentrations are measured as *dissolved* unless denoted [t], which refers to *total* metal concentration, and * signifies the concentration that causes death in 50% of fish exposed. Source numbers refer to corresponding literature cited.

Metal	Species (phase)	Effect	Effect concentration (µg/L)	Water hardness (mg/L)	Exposure duration	Source
Al		**Sub-lethal effects**				
	RbT					
	(J)	Impaired olfactory nerves	9.5	75	14 d	85
	(J)	Reduced swim speed	30.0[t]	-	7 d	182
	AtS					
	(J)	Reduced survival	6.0	-	-	88
	BnT					
	(J)	Reduced growth	>27.0	-	42 d	138
		Direct lethality				
	RbT					
	(J)	Death	5,200*	41	40 d	47
Cd		**Sub-lethal effects**				
	RbT					
	(E)	Premature hatching	0.05	7	294 d	94
	(J)	Reduced growth	0.25	7	56 d	94
	(J)	Reduced alarm response	2.0	120	7 d	149
	(J)	Competitive impairment	2.0	120	24 hr	154
	LkC					
	(A)	Reduced prey capture	0.5	81	106-112 d	84
	BkT					
	(J)	Reduced prey capture	0.5	156	30 d	126
	(J)	Reduced condition	0.5	156	30 d	127
	BnT					
	(J)	Reduced biomass	0.9	31	30 d	10
		Direct lethality				
	RbT					
	(J)	Death	0.4-0.5*	30	120 hr	59
	(A)	Death	5.2*	54	17 d	23
	BlT					

(J) ArG	Death	0.8-0.9*	30	120 hr	59
(J) BnT	Death	4.0*	41	96 hr	17
(J) CkS	Death	1.2*	29	96 hr	10
(J) CoS	Death	1.8-3.5*	23	96 hr	21
(J)	Death	2.0*	22	9 d	23
(A)	Death	3.7*	22	9 d	32
BkT	Death	2.4*	44	96 hr	20
SkS (J)	Death	30.0*	84	160 hr	150
Cu	**Sub-lethal effects**				
CkS					
(J)	Habitat avoidance	0.7	25	20 min	57
(J)	Reduced avoidance	2.0	25	21 d	57
RbT					
(A)	Habitat avoidance	1.0	284		167
(J)	Habitat avoidance	1.6	25	20 min	57
(J)	Reduced growth	4.6	25	20 d	100
(J)	Impaired olfaction	5.0	58	3 hr	3
(J)	Reduced swim speed	6.0	30	120 hr	179
CoS					
(J)	Impaired olfaction and alarm response	2.0	24-32	3 hr	137
(J)	Reduced alarm response and survival	5.0	56	3 hr	105
(J)	Impaired migration	5.0	89-99	144 hr	95
(J)	Reduced stress resistance	13.8	90	8 d	146
(J)	Reduced disease resistence	13.9	20-83	30 d	165
AtS					
(J)	Habitat avoidance	2.4	20	20 min	161
(A)	Impaired migration	20.0	20	Indefinite	161
CmS					
(J)	Impaired olfaction	3.0	75	4 hr	136
BkT					
(J)	Reduced growth	3.4	45	1-23 wk	106
(J)	Increased cough frequency	6.0	44-46	24 hr	35
(J)	Reduced feeding	6.0	44-46	2 hr	35
BnT					
(A)	Reduced spawning	10.0	10	4 d	79

	SkS					
	(J)	Impaired migration and survival	30.0	36-46	144 hr	29
		Direct lethality				
	ArG					
	(J)	Death	2.6-49.3*	41	96 hr	17
	RbT					
	(J)	Death	9-17*	24-25	96 hr	21, 101
	(J)	Death	14-36*	41	96 hr	17
	CoS					
	(J)	Death	15-32*	41	96 hr	17
	(J)	Death	21-22	24-32	60 d	115
	CkS					
	(J)	Death	19*	24	96 hr	21
	CtT					
	(J)	Death	37*	18	96 hr	170
	SkS					
	(J)	Death	103-240*	36-46	96 hr	29
Pb		**Sub-lethal effects**				
	RbT					
	(J)	Physical deformity	7.6	28	42 d	27
	(J)	Reduced enzyme activity	10	135	14 d	88
	(A)	Reduced oocyte growth	10	121-125	12 d	132
		Direct lethality				
	RbT					
	(J)	Death	1000*	120	96 hr	128
	BkT					
	(J)	Death	3362*	44	96 hr	75
Ni		**Sub-lethal effects**				
	RbT					
	(J)	Attraction behaviour	6^t	28	20 min	51
	(J)	Avoidance behaviour	$>19^t$	28	20 min	51
	(J)	Reduced swim speed	384	140	12 d	121
	(J)	Reduced aerobic capacity	394	140	34 d	121
		Direct lethality				
	RbT					
	(J)	Death	8100*	33	96 hr	118
Ag		**Sub-lethal effects**				
	RbT					
	(J)	Reduced weight and length	0.1	36	60 d	117
	(J)	Reduced growth and swim speed	5	120	5-10 d	48
		Direct lethality				
	RbT					
	(J)	Death	0.5	36	21 d	117

	(J)	Death	6.5*	26	96 hr	28
Zn		**Sub-lethal effects**				
	RbT					
	(J)	Habitat avoidance	8.6	13-15	20 min	159
	(J)	Reduced immune response	10	-	30 d	134
	(J)	Increased stress	81	6-6.5	1 d	177
	AtS					
	(J)	Habitat avoidance	53	18	20 min	157
		Direct lethality				
	RbT					
	(J)	Death	93*	24	96 hr	21
	CkS					
	(J)	Death	97*	24	96 hr	21
	BnT					
	(J)	Death	140*	10	96 hr	42
	SkS					
	(J)	Death	749*	22	96 hr	22