Effects of Copper on Fish and Aquatic Resources

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Introduction

The Nushagak and Kvichak river watersheds in Bristol Bay Alaska (Figure 1) together produced over 650 million sockeye salmon during 1956-2011, about 40% of Bristol Bay production (ADFG 2012). Proposed mining of copper–sulfide ore in these watersheds will expose rocks with elevated metal concentrations including copper (Cu) (Figure 1; Cox 1996, NDM 2005a, Ghaffari et al. 2011). Because mining can increase metal concentrations in water by several orders of magnitude compared to uncontaminated sites (ATSDR 1990, USEPA 2000, Younger 2002), and because Cu can be highly toxic to aquatic life (Eisler 2000), this review focuses on risks to aquatic life from potential increased Cu inputs from proposed development.

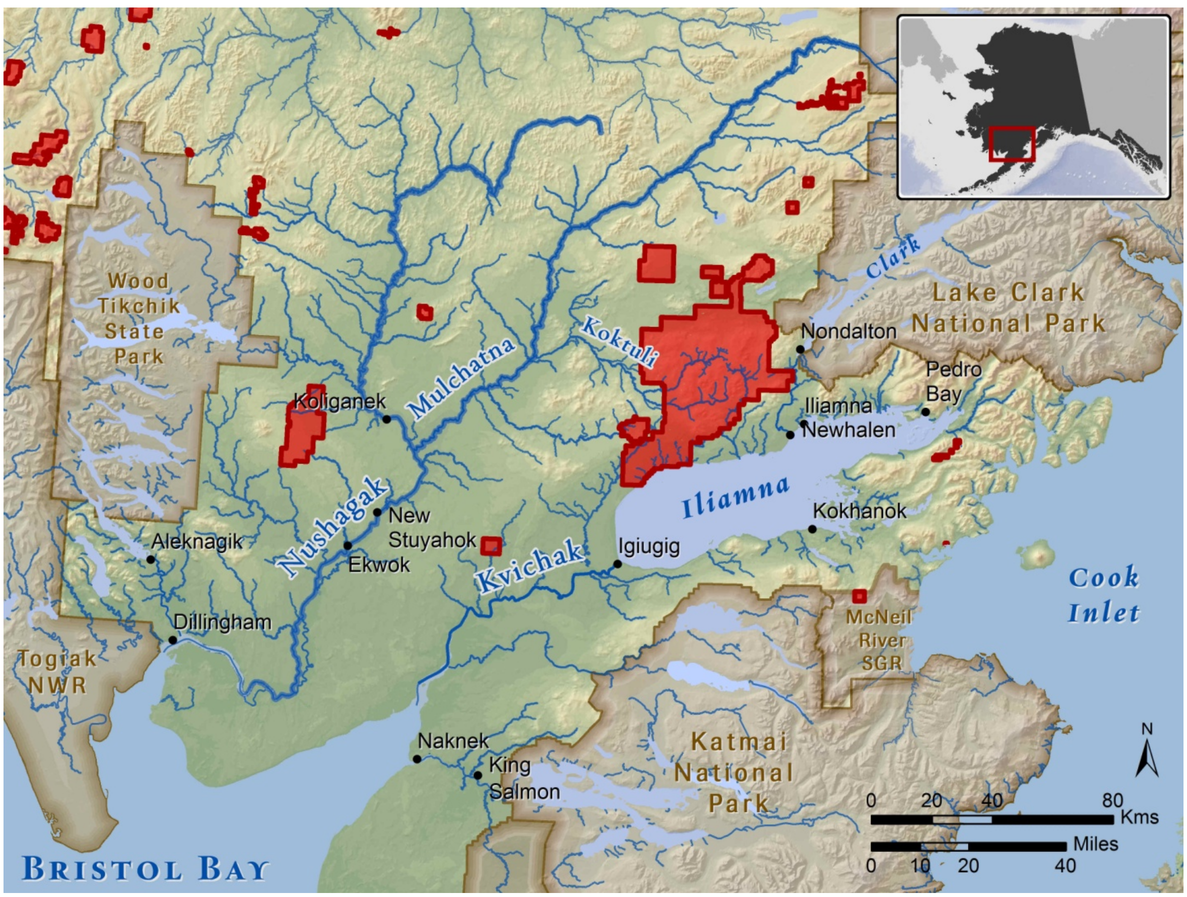

Figure 1. Map showing current mining claims (red) in Nushagak and Kvichak river watersheds as of 2011. Proven low-grade copper sulfide deposits are located in large lease block along Iliamna Lake. Documented salmon streams are outlined in dark blue. Note many regional streams have never been surveyed for salmon presence or absence. Sources: fish data from: www.adfg.alaska.gov/sf/SARR/AWC/index.cfm?ADFG =main.home mine data from Alaska Department of Natural Resources - http://www.asgdc.state.ak.us/

Core samples collected from Cu prospects near Iliamna Lake (Figure 1) show high potential for acid generation due to iron sulfides in the rock (NDM 2005a). When sulfides are exposed to oxygen and water sulfuric acid forms, which can dissolve metals in rock. Acid and metals can then be washed into ground and surface waters during rainfall or snowmelt (Cox et al. 1996, Ripley et al. 1996, Gusek and Figueroa 2009). Mine development proposed for the region include open pit and underground block caving with processed mine waste stored in large tailings reservoirs (AKDNR 2012, Ghafarri et al. 2011). Acid can be generated from such development where sulfide rock is exposed in pit walls, waste rock piles, voids created by underground block caving and in tailings reservoirs (Younger et al. 2002, Gusek and Figueroa 2009). Cox et al. (1996) indicated, "Tailings from milling of ore can cause release of acid mine drainage from mine and waste dumps" and that intensity of environmental impact associated with sulfide deposits is "greater in wet climates than in dry climates". Southwest Alaska is a relatively wet region with about 660 mm of precipitation annually (~26 inches/year; Iliamna Airport 2011) and the region is characterized by abundant ground and surface waters (Figure 1; NDM 2005b, Cathcart 2008, PLP 2012).

Copper (Cu) Sulfide Ore

- Mining of Cu sulfide ores exposes sulfides to water and air creating acid, which dissolves Cu; storm water can then carry Cu into ground and surface waters (Cox et al. 1996, Ripley et al. 1996, USEPA 2000, Younger 2002, Gusek and Figueroa 2009).
- Samples from Cu sulfide deposits in Bristol Bay show high potential for acid generation (NDM 2005a).
- Environmental impact from mining sulfide deposits is "greater in wet than in dry climates" (Cox et al. 1996).
- Bristol Bay has a relatively wet climate and abundant ground and surface waters.

Copper in the Environment

Cu naturally occurs in the aquatic environment in low concentrations. Major aquifers of the U.S. have Cu concentrations less than 10 parts per billion[1] (ppb) total Cu (Lee and Helsel 2005), while Canadian freshwaters have 1-8 ppb Cu (ATSDR 1990), and streams in Bristol Bay have 0.04-5.60 ppb Cu (Zamzow 2011). Seawater Cu concentrations are generally less than 1 ppb (Nordberg et al. 2007).

Elevated aquatic Cu concentrations primarily occur near copper mining and smelting facilities and in urbanized areas (Davis et al. 2000, Eisler 2000). Aquatic habitats are susceptible to Cu pollution because they are the ultimate receptor of industrial and urban wastewater, storm water run off, and atmospheric deposition (Nriagu 1979, Davis et al. 2000). Cu is acutely toxic (lethal) to freshwater fish in soft water at low concentrations ranging from 10 – 20 part per billion (NAS 1977). Elevated Cu concentrations observed in mine-impacted Mineral Creek Colorado were as high as 410 ppb (Runkel et al. 2009) and in mine impacted Copperas Brook in Vermont were 4600 ppb (Balistreri et al. 2007). Sansalone et al. (1997) documented urban storm water run off Cu concentrations of 325 ppb. Such Cu concentrations are lethal to fish and aquatic life (Eisler 2000).

Copper in the Aquatic Environment

- Copper naturally occurs at low levels:
    - Major US aquifers < 10 ppb total Cu (Lee & Helsel 2005).
    - Canadian freshwaters = 1-8 ppb total Cu (ATSDR 1990).
    - Bristol Bay headwaters = 0.04-5.6 ppb total Cu (Zamzow 2011).

- Cu is acutely toxic (lethal) to freshwater fish in soft water at low concentrations ranging from 10 – 20 part per billion (NAS 1977).

- Elevated Cu occurs in freshwaters near Cu mining sites & urban areas:
    - Mine-impacted Mineral Creek CO = 410 ppb total Cu (Runkel et al. 2009)
    - Mine-impacted Copperas Brook in VT = 4600 ppb total Cu (Balistreri et al. 2007)
    - Urban storm water run off = 2.1 to 325 ppb total Cu (Sansalone et al. 1997)

[1] One part per billion Cu is equivalent to one drop of Cu diluted in 250 drums (55gal) of water.

About 15 million tonnes of Cu is used worldwide yearly for construction, electrical conduit, agriculture, manufacturing and other uses; one third of this Cu is from recycling the rest is from mining (ATSDR 1990). Because Cu is a non-degradable potentially toxic pollutant that builds up in the environment continued releases are of global concern (Nigaru 1996).

Copper is Both Essential and Toxic to Life

Copper is an essential trace metal necessary for growth and metabolism of all living organisms; humans need approximately 1-2.5 mg daily (1 mg = 0.00001 oz) (Nordberg et al. 2007). In vertebrates, including fish, Cu forms part of many enzymes and glycoprotein, it is important for nervous system function and is necessary for hemoglobin synthesis (Sorensen 1991, Nordberg et al. 2007). Deficiencies are rare as Cu is plentiful in the environment however deficiencies in mammals are linked to anemia, gastrointestinal disturbances, aortic aneurisms, abnormal bone development and death (Aaseth and Norseth 1986). Precise Cu dietary needs and deficiency effects in wild fish and aquatic species are unclear and the subject of ongoing research.

Cu is toxic at higher concentrations and mammals (including humans) evolved efficient Cu regulatory systems for uptake, distribution, storage and excretion (Nordberg et al. 2007). In mammals, excess Cu is generally absorbed into gastrointestinal cells and excreted when cells slough (Eisler 2000). Overdoses of Cu are documented and symptoms in humans for 44 mg Cu/L and less include gastrointestinal distress, nausea, vomiting, headache, dizziness, and metallic taste in mouth; higher doses can cause coma and death (NAS 2000). Humans afflicted with Wilson's disease, children under one year, people with liver damage, chronic disease, and diabetes are more susceptible to Cu poisoning (Nordberg et al. 2007).

Cu is one of the most toxic elements to aquatic species, at levels just above that needed for growth and reproduction it can accumulate and cause irreversible harm to some species (Hall et al. 1988, Sorensen 1991, Carbonell and Tarazona 1994, Eisler 2000, Kapustka et al. 2004, Tierney et al. 2010). Cu is acutely toxic (lethal) to freshwater fish via their gills in soft water at concentrations ranging from 10 – 20 ppb (NAS 1977).

Alaska Copper Water Quality Standards

Copper and other metals released from mining and urban sites can contaminate water sources and affect fish through water or food borne exposure (Sorensen 1991, Peplow and Edmonds 2000, Younger et al. 2002, Clearwater et al. 2002, Lapointe et al. 2011). Fish and aquatic organisms are very sensitive to increased Cu concentrations in water however Cu toxicity depends, in part, on water quality.

Waterborne Cu exists in a variety of forms with the dissolved form dCu (cupric ion Cu^{2+}) considered most toxic to aquatic life (Eisler 2000, USEPA 2007). Some water parameters affecting Cu toxicity include: whether water is soft or hard, pH, anions and dissolved organic carbon (DOC). Toxicity of Cu to aquatic life varies with:

1) **Water hardness**: dCu is more lethal in soft compared to hard waters rich in cations (e.g., Ca^{2+} and Mg^{2+}) as cations reduce bioavailability of dCu and thus toxic effects (Pagenkopf 1983, Paquin et al. 2002).
2) **pH**: Cu is more toxic under acidic conditions ($pH < 6$);
3) **Anions and dissolved organic carbon (DOC)** bind to dCu creating compounds, which reduce dCu concentrations and toxic effects (Niyogi and Wood 2004, USEPA 2007).

The Alaska Department of Environmental Conservation uses an Aquatic Water Quality (AWQ) criteria to protect freshwater species from increased Cu inputs (ADEC 2010). Acute AWQ Cu criteria address lethal effects of Cu using a regression model (Figure 2) of lethal dCu concentrations as a function of water hardness; the model also takes into account pH and alkalinity (Stephan et al. 1985, USEPA 2007).

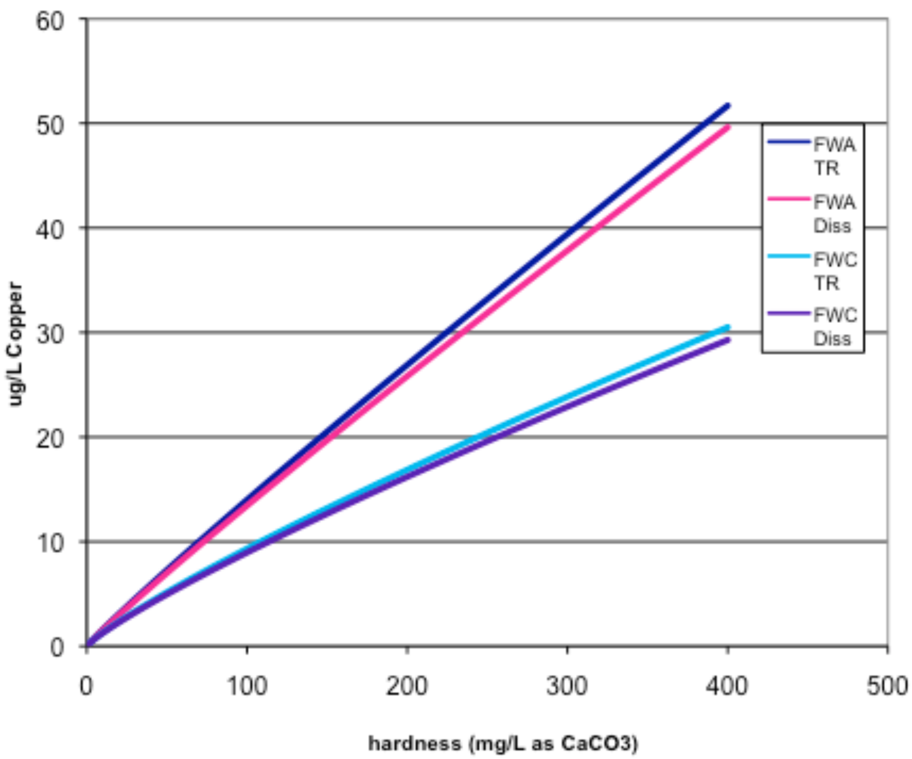

Figure 2. Graph depicting Alaska acute and chronic Aquatic Water Quality criteria for copper as a function of hardness. FWA = fresh water acute or lethal concentrations; TR = total recoverable Cu; Diss = dissolved Cu, FWC= freshwater chronic concentrations. (ADEC 2010).

Freshwaters in mine leases near Iliamna Lake (Figure 1) are "soft" (low hardness of 1 to 31 mg/L; Zamzow 2011); corresponding lethal dCu concentrations at this hardness range from 0.18 to 4.46 ppb dCu respectively (Figure 2); chronic dCu toxicity ranges from 0.18 to 3.29 ppb. This is because soft waters are limited in their ability to ameliorate toxic effects of increased Cu inputs (Figure 2). Cusimano et al. (1986) found that 50% of exposed rainbow trout died in 96 hours at a concentration of 2.8 ppb Cu in water of 9.2mg/L hardness. Taylor et al. (2000) found that Cu was approximately 20 times more toxic to 1- to 2-g rainbow trout in soft water (20 mg/L) than in hard water (120 mg/L as $CaCO_3$). Increases in dCu concentrations can be lethal at very low concentrations in soft waters (NAS 1986, Eisler 2000, Scott and Sloman 2004).

Criticisms of the hardness based AWQ include the fact that Cu can reduce a salmon's sense of smell by 50% at increases of just 2 ppb dCu over baseline; *hardness does not significantly reduce this effect but dissolved organic carbon can* (McIntyre et al. 2008, Sandahl et al. 2007). Another fish sensory system "the lateral line" is comprised of neurons (hair cells) that provide fish information on their environment including vibrations, water flow and other parameters; the lateral line enables schooling, predator avoidance, feeding, and orientation to water flows. In a recent study, fish exposure to dCu concentrations of ≥20 ppb for 3 hours destroyed 20% of hair cells (Linbo et al. 2006). *Hardness only slightly reduced toxicity of Cu to the lateral line* but DOC caused a greater reduction in Cu toxicity. Linbo et al. (2009) determined increasing organic carbon (0.1– 4.3 mg/L) increased concentrations at which dCu destroyed 50% of lateral line hair cells from approximately 12 ppb to 50 ppb. Waters within and near Cu mining leases in Bristol Bay are soft with low DOC concentrations (Table 1; Zamzow 2011). Site-specific dCu toxicity studies using natural hardness and DOC levels documented in proposed mining leases are currently lacking.

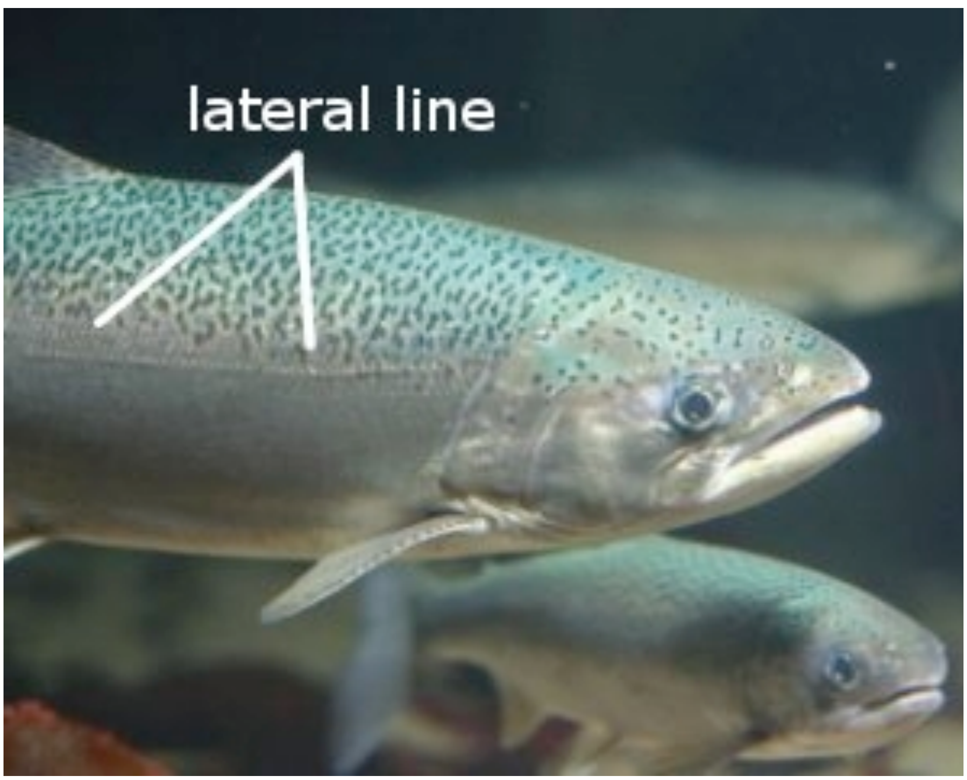

Table 1. Water quality parameters for study sites within and near mining leases located near Iliamna Lake, Alaska, from Zamzow (2011).

Region	Sample Date	Hardness (mg/L)	Alkalinity (mg/L)	Lab pH	DOC (mg/L)
North of Pebble Prospect	May 2009	17-20	21-22	7.2	3-4
	June 2009	10-23	4-25	7.1-7.6	2-4
	June 2010	28-30	25-34	7.2-7.3	2-4
South Fork Koktuli	May 2009	7-14	9-16	6.3-7.0	1-3
	June 2009	13-18	11-20	7.1-7.6	1-3
	June 2010	13-32	14-42	6.9-7.2	1-3
North Fork Koktuli	May 2009	8-14	9-12	6.2-6.9	4-6
	June 2009	7-23	11-28	7.3-7.7	2-4
	June 2010	14-29	18-40	7.1	1-2
Upper and Lower Talarik	May 2009	11-17	14-20	6.6-7.2	2-4
	June 2009	14-31	20-31	7.3-7.6	1-2
	June 2010	23-43	26-47	7.2-7.6	1-2
Southwest of Pebble Prospect	May 2009	1-6	0.5-5	5.4-6.5	5
	June 2009	1-15	4-22	5.5-7.2	1-3
	June 2010	13-17	15-25	6.7-6.9	3

The Alaska hardness based acute (lethal) AWQ is under-protective for numerous species comprising aquatic food chains. A recent review of 75 published reports by Scannell (2009) for the Alaska Department of Fish and Game indicated the hardness based AWQ was under-protective in acute (lethal) Cu toxicity studies for:

- 5% of reviewed fish test values,
- 14% of reviewed aquatic invertebrate test values,
- 40% of freshwater mussel test values,
- 38% of zooplankton test values.

Zooplankton, invertebrates and mussels are important components of the aquatic food chain that sustain sockeye, coho and Chinook salmon while they rear one to three years in freshwater as well as other important subsistence species.

The USEPA (2007) recently recommended use of a more complex AWQ model for Cu because the hardness model may sometimes be under or overly protective and site specific studies required to fine-tune models can be expensive. The so-called Biotic Ligand Model (BLM) uses 10 parameters including temperature, pH, dissolved organic carbon (DOC), calcium, magnesium, sodium, potassium, sulfate, chloride and alkalinity to calculate AWQ for aquatic species (USEPA 2007). The BLM can be useful in predicting acute or lethal effects to aquatic life that occur at fish gills in waters of different chemistries (Niyogi and Wood 2004). But BLM assumptions have been questioned and failures of the model to protect aquatic life are documented (Slaveykova and Wilkinson 2005, Welsh et al. 2008). For example, *the BLM did not adequately predict Cu toxicity to trout in soft water and underestimated acute toxicity at higher pH and chronic toxicity at lower pH* (Ng et al. 2010). Another issue with the BLM is that DOC varies in form and consequently can limit accurate Cu toxicity predictions of the BLM (Wood et al. 2011). *Further research is needed to resolve inadequacies of the BLM to ensure salmon and aquatic species are protected from increased Cu inputs*.

Alaska Water Quality Standards for Copper

- Alaska uses a hardness based Aquatic Water Quality (AWQ) criteria to protect aquatic species from lethal effects of increased Cu inputs. It is under protective of many fish forage species and new research indicates the AWQ may not protect salmon sensory inputs that enable mating, predator avoidance, feeding, and homing (McIntyre et al. 2008, see review by Tierney et al. 2010).

- Cu is lethal at very low concentrations in soft waters compared to hard waters (Taylor et al. 2000). Waters in proposed mine claims of Bristol Bay are very soft (Zamzow 2011).

- Cu can impair behaviors important to survival and reproduction by reducing a fish's sense of smell and/or orientation ability; water hardness does not reduce Cu impacts to sensory systems as much as dissolved organic carbon does (Sandahl et al. 2007, McIntyre et al. 2008).

- The USEPA (2007) recommends use of a new AWQ called the Biotic Ligand Model (BLM) to determine Cu criteria. It has promise, but does not adequately predict Cu toxicity in soft waters nor does it account for variation in ability of dissolved organic matter (DOC) to bind Cu reducing its toxicity (Ng et al. 2010, Wood et al.2011)

Copper Toxicity to Aquatic Organisms

Toxicity of Cu to aquatic organisms depends on its "bioavailability" or its potential to transfer from water or food to a receptor (e.g., gills, olfactory neurons, etc.) on an organism where toxic effects can occur. Toxic effects of Cu are classified as "acute" or lethal and "chronic" where sublethal exposures result in reduced growth, immune response, reproduction and/or survival. Adverse effects have been demonstrated on various fish "receptors" including gills, olfactory receptors, and lateral line cilia and scientists are now learning more about how Cu affects fish DNA and molecules. Copper is known to reduce fish resistance to diseases; it disrupts migration (i.e., fishes avoid copper-contaminated spawning grounds); alters swimming; causes oxidative damage; impairs respiration; disrupts osmoregulation structure and pathology of kidneys, liver, gills, and other stem cells; impacts mechanoreceptors of lateral line canals; impairs functions of olfactory organs and brain; is associated with changes in behavior, blood chemistry, enzyme activities, corticosteroid metabolism and gene transcription and expression (Table 2; Hodson et al. 1979, Knittel, 1981, Rougier et al. 1994, Eisler 2000, Craig et al. 2010, Tierney et al. 2010).

Cu is acutely toxic to freshwater fish via the gills in soft water at concentrations ranging from 10 – 20 ppb (NAS 1977). Marr et al. (1998) demonstrated that 50% of rainbow trout died when exposed to 14 ppb Cu and 20% died when exposed to 8 ppb Cu in waters of about 25 mg/L hardness. Playle et al. (1993a) demonstrated a dose-response relationship between 120 hour mortality of rainbow trout juveniles and Cu gill accumulation after 24 hours. Playle et al. (1993b) showed that Cu binding to fish gills and how much metal bound to gill was related to dCu concentrations. Waters in mine claims near Iliamna Lake are both very soft with low DOC concentrations (Zamzow 2011).

Copper and Fish Behavior

Effects of contaminants on fish behavior are now a topic of intensive research (see review by Tierney et al. 2010, McIntyre et al. 2012). Fish behavior is linked to individual survival and reproduction- if a salmon returns to spawn but instead gets eaten by a bear then that fish's genes are out of the gene pool. Because copper impairs key senses such as smell it has potential to impair complex fish behaviors important to survival and reproduction.

Copper can impair olfaction in fish and hardness based dCu WQS are less protective at the fish nose than at the fish gill (McIntyre 2008). Fish are highly sensitive to odors in their environment and can detect natural chemical cues over long distances, such as mating pheromones, at concentrations in the parts per billion or parts per trillion (ppt) (Laberge and Hara 2003, Belanger et al. 2006). Approximately one hundred different olfactory receptors receive and trigger critical physiological and/or behavioral responses in fish (Tierney et al. 2010), such as sperm production (Waring et al. 1996), predator recognition and avoidance (Brown and Smith 1997, Hirovan et al. 2000, McIntyre et al 2012), food location (Hara 2006), kin recognition (Quinn and Busack 1986), recognition of conspecifics (Brown and Smith 1997, Hirovan et al. 2000), migration (Groot et al. 1986), homing (Hasler and Schlotz 1983) and reproduction (Moore and Waring 1996, Waring et al. 1996). Impairment of such behaviors has potential to affect population biodiversity if behaviors such as feeding, predator avoidance, homing, migration and spawning are affected (Table 2).

Table 2. Selected examples of adverse effects of copper on salmonids. Table after NOAA (2007).

Species	Effect	Concentration ppb	Effect Statistic*	Hardness	Exposure duration	Reference
Coho juvenile	Reduced olfaction and alarm response	0.18-2.1	EC_{10} – EC_{50}	120	3 hours	Sandhal et al. 2007
Coho juvenile	Increased predation	5	LOEC	56	3 hours	McIntyre et al. 2012
Chinook juvenile	Avoidance	0.75	LOEC	25	20 min	Hansen et al. 1999a
Rainbow trout	Avoidance	1.6	LOEC	25	20 min	Hansen et al. 1999a
Chinook juvenile	Loss of avoidance ability	2	LOEC	25	21 days	Hansen et al. 1999b
Coho juvenile	Reduced downstream migration	5	LOEC	95	6 days	Lorz and McPherson 1976, 1977
Rainbow trout	Loss of homing ability	22	LOEC	63	40 weeks	Marr et al. 1996
Rainbow trout	Reduced growth	2.8	EC10	25	120	Marr et al. 1996
Rainbow trout	Increased disease susceptibility	3.9	LOEC	36.6	96 hr	Baker et al. 1983
Hatchery coho & wild steelhead	Impaired olfaction	5	LOEC	58	3 hours	Baldwin et al. 2011

*ECp = effective concentration adversely affecting (p) percent of the test population or percent of measured response, e.g., 10% for an EC10, etc.; LOEC= Lowest Observed Adverse Effect Concentration, but note not all concentrations were tested.

Hatchery and wild salmon and steelhead exposed to 5 ppb and 20 ppb for 3 hours at 58 mg/L hardness showed impaired olfaction (Baldwin et al 2011). Sandahl et al. (2007) demonstrated that coho salmon exposed to just 2 ppb increases in Cu for 3 hours at 120 mg/L hardness showed significantly impaired olfactory detection of predator alarm cues and a 50% decline in normal predator avoidance response; impairment in ability to detect and avoid predators can be lethal. McInryre et al. (2008) determined that neither pH nor hardness significantly reduced Cu toxicity on olfactory neurons but dissolved organic carbon did.

Salmonids avoid waters with low dCu contamination, which disrupts their normal migration patterns. For example, coho salmon yearlings held in 5 – 30 ppb Cu for as little as 6 days showed altered downstream migration patterns (Lorz and McPherson 1977). Chinook avoided at least 0.7 ppb Cu whereas rainbow trout avoided at least 1.6 ppb dissolved Cu (Hansen et al. 1998). Laboratory avoidance of Cu by rainbow trout was observed at 0.1, 1.0 and 10 ppb Cu (Folmar 1976). Birge et al. (1993) and others demonstrated that salmon and other fish can be attracted to very high concentrations of dCu (4,560 ppb), which is lethal. In a review by Scannell (2009), a personal communication citation for B. Shephard (2008) suggested avoidance was the only behavioral endpoint with effects lower than the hardness chronic criterion. However, recent studies on salmon olfaction and lateral line systems indicate hardness plays a lesser role in reducing toxic effects of Cu than DOC.

Fish exposed to sublethal or chronic Cu concentrations can potentially suffer the following direct and indirect effects and further study is needed:

a. Impaired neurological and brain function (Baldwin et al. 2003; Tierney et al 2010),
b. Impaired reproduction (Pickering et al. 1977),
c. Impaired predator detection and avoidance (Baldwin et al. 2003, Sandahl et al. 2007, McIntyre et al. 2012),
d. Impaired ability to find food (Drummond et al. 1973),
e. Impaired ability to recognize members of their own species (Quinn and Busack 1985),
f. Impaired ability to recognize siblings (Hara 1986, Brown and Brown 1992),
g. Impaired "homing" ability (Scholz et al. 1976, Baldwin et al. 2003),
h. Impaired migration behaviors (Lorz and McPherson 1976),
i. Impaired growth (Scudder et al. 1988).
j. Depressed immune response (Knittel 1981, Rougier et al. 1994)

Exposure to contaminants that can change a fish's ability to interpret chemical cues that influence homing precision, reproductive behavior, identification of predators, prey, kin or mates represents a threat to genetic integrity of salmon stocks. Sustainability of Bristol Bay fisheries is reliant, in part, on genetic integrity and biodiversity of stocks (Hilborn et al. 2003, Schindler et al. 2010). Population structure is positively associated with genetic diversity and resilience to disturbance such that large, highly structured populations have high genetic diversity and probability of persistence over time as environmental conditions change (Giesel 1974, Altukhov 1981). In contrast, small, panmictic (geographically limited) populations with limited population structure are vulnerable to inbreeding, demographic stochasticity, genetic drift and thus, reduced evolutionary potential, and increased probability of extinction (Cornuet and Luikart 1996; Luikart *et al.* 1998; Soulé and Mills 1998).

Dietborne Copper

Studies investigating toxic effects of Cu on fish have primarily focused on waterborne Cu toxicity but food borne Cu paths in and through aquatic food chains is of increasing interest. Recent studies indicate Cu uptake efficiency is similar for diet and water (Clearwater et al. 2002). Diet borne Cu caused a quantitatively more important effect on gene transcription levels for proteins involved in energy metabolism, metal detoxification and protein protection compared to fish exposed to waterborne Cu (Lapointe et al. 2011). Similar to other heavy metals, copper can accumulate in fish tissues from ingestion (Dallinger and Kautzky 1985, Clearwater et al. 2002, Vineeta et al 2007, Gundogdu et al. 2009). Vineeta et al. (2007) fed fish high concentration Cu diets and observed Cu accumulated in descending order from gill>kidney>liver>muscle. Toxic concentrations of dietborne Cu have been described for rainbow trout, carp and channel catfish, but data appear contradictory (see review by Clearwater et al. 2002). In one study, rainbow trout dietary copper toxicity occurred at 730 mg Cu/kg and maximum tolerable level was 665 mg Cu/kg, adverse effects included reduced growth, increased feed:gain ratios, food refusal and elevated liver copper levels (Lanno et al. 2003). Clearwater et al. (2002) determined Cu toxicity occurred at daily intake levels of 1–15 mg/kg body weight per day (depending on life stage) for Atlantic salmon and at 35–45 mg/ kg body weight per day for rainbow trout (*Oncorhynchus mykiss*). Further studies are needed to improve understanding of potential effects of ingested Cu because once released into the environment Cu can accumulate in aquatic sediments and continue to recycle into aquatic food webs.

Copper and the Freshwater Food Chain

Aquatic food chains and energetic pathways are organized in a hierarchical way (Figure 3) and Cu can be transferred through aquatic food chains McGreer et al. (2003). Copper can affect salmonid ecosystems from the bottom of the food chain to top predators (see reviews by Hodson et al. 1979, Sorenson 1991, Eisler 2000). Studies on cumulative adverse effects of Cu on productivity of aquatic food chains are lacking however, *numerous studies document adverse effects on freshwater algae, zooplankton, and mussels at levels below Aquatic Water Standards* (see review by Scannell 2009) which could result in reduced prey abundance and quality to support fish growth and reproduction (Wootton 1994).

Copper is one of the most toxic metals to unicellular algae, which form the base of the salmonid food chain. Photosynthetic algae production (*Chlorella* spp.) can decline at just 1.0 – 2 ppb Cu and photosynthesis can be inhibited at 5.0 to 6.3 ppb (USEPA 1980, Franklin et al. 2002). Zooplankton feed on algae and their growth and reproduction are affected by food availability; declines in algae production can cause declines in zooplankton production (Urabe 1991, Müller-Navarra and Lampert 1996), which implies reduced food for fish that feed on zooplankton such as sockeye salmon.

Zooplankton are the preferred food of juvenile sockeye salmon, which rear in lakes one to two years prior to seaward migration. Zooplankton are highly sensitive to acute Cu effects and studies in waters of high hardness show Cladocera may not be adequately protected by current Alaska AWQ criteria (Bossuyt et al. 2005) particularly because freshwaters in and near Bristol Bay mine claims are very soft and have low levels of dissolved organic carbon (Zamzow 2011, also see review by Scannell 2009).

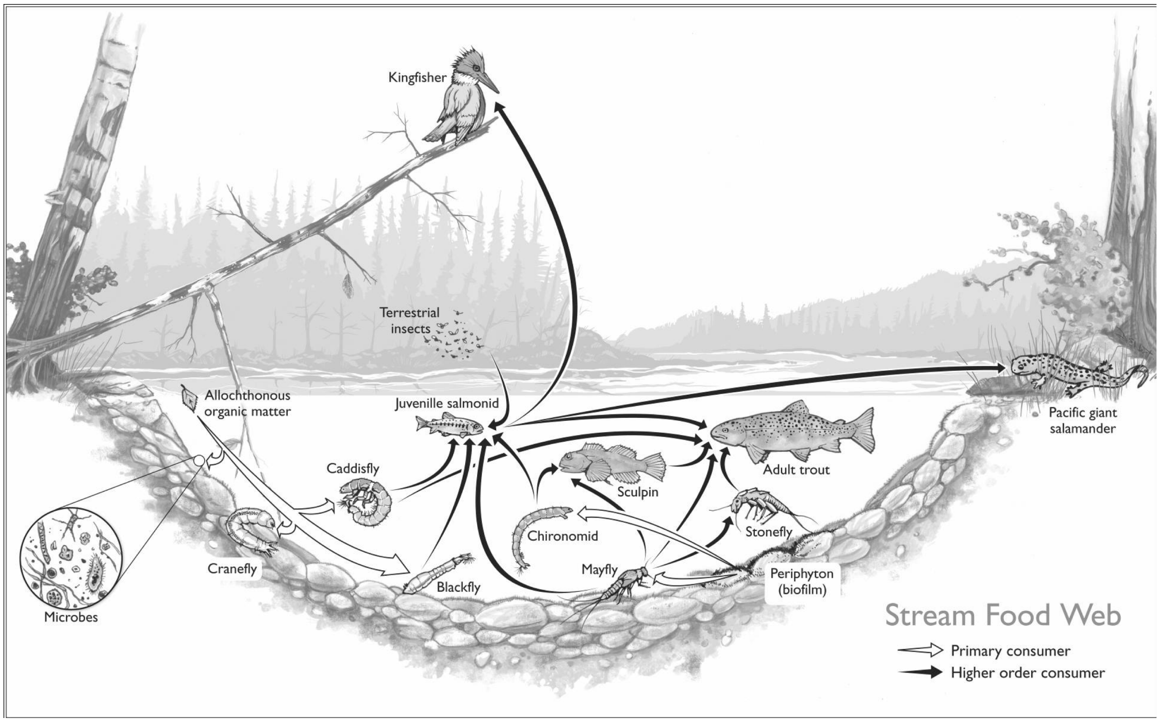

Figure 3. A simplified aquatic food web depicting hierarchical nature of energy flow from organic matter falling into a stream (Allochthonous) to microbes, periphyton, aquatic insects, to top fish and avian predators. Algae form the base of the food chain and derive energy from the sun and nutrients from water.

Freshwater mussels and gastropods (snails) live in sediments and are filter feeders. They recycle dead organic matter in lake and river bottoms and they in turn are prey for fish. For example, freshwater mussels and snails are a primary food of humpback whitefish (Brown 2007), which in turn are prey for larger fish and are a preferred subsistence species for residents of the Kvichak River watershed (Fall et al 1996, 2006). Wang et al. (2007a, 2007b, 2007c) showed growth and survival of freshwater mussels were impaired in relatively hard waters (160-190 mg/L) at *Cu concentrations below Alaska AWQ criteria* (also see review by Scannell 2009).

Interactions Occur Among Metals

Hard rock mines and urban areas release complex mixtures of metals and metalloids such as zinc (Zn), cadmium (Ca), lead (Pb), aluminum (Al), mercury (Hg), selenium (Se), molybdenum (Mo), magnesium (Mg), nickel (Ni) and iron (Fe). Multiple metal discharges effects on fish and aquatic food chains are limited, but show complex chemical interactions and reactions that can be difficult to predict as concentrations of elements comprising mixtures will vary in space and time. Dethloff et al. (1999) investigated changes in the blood, brain biochemistry, and immune system of rainbow trout caused by exposure to sublethal concentrations of Cu and Zn, two metals that generally occur together at Cu sulfide mines (Finlayson and Ashuckian 1979, Roch and McCarter 1984, Woodward *et al*. 1995). They found fish exposed to various mixtures of sublethal Cu and Zinc (Zn) showed no significant effects on survival, weight, and hepatic metal concentrations – typically measured parameters that indicate stress, but did show increased Cu gill concentrations, elevated brain acetylcholinesterase, reduced lymphocytes, and elevated monocytes and neutrophils (both are white blood cell types that play a key role in immune function). This study showed alterations in important physiological parameters that are not generally measured for metal effects.

Interactions between Cu and Zn can be more than additive, with mixtures of the two metals causing higher rates of mortality in fish than expected based on each element alone (Sprague and Ramsey 1965, Sorenson 1991, Eisler 2000). Once inside an organism, elements exist in a specific form and ratio to other elements and will interact directly or indirectly based on a multitude of parameters (Sandstead 1976, Sorenson 1991). For example, survival from egg to hatch of a catfish (*Ictalurus* spp.) treated with a 1:1 ratio of Cu:Zn declined predictably under an additive model up to a concentration of ~1 mg/L, then mortality rates increased at higher than predicted rates, causing a synergistic effect (Birge and Black 1979).

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