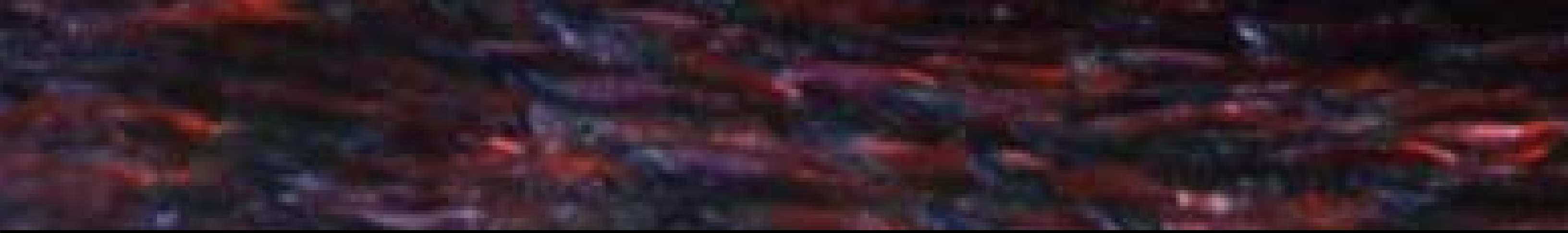

# Copper

## Effects on Freshwater Food Chains and Salmon:

***A review***

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# INTRODUCTION

Copper (Cu) is essential to all living organisms. However, no fatal Cu deficiencies have ever been documented for any aquatic species (Sorensen 1991, Carbonell and Tarazona 1994, Eisler 2000). Furthermore, concentrations just over that required for growth and reproduction can be highly toxic to aquatic species and cause irreversible harm (Hodson et al. 1979, Hall et al. 1988, Sorensen 1991, Eisler 2000, Baldwin et al. 2003). Toxicity of Cu to fish and their food chains is well documented (see reviews by Hodson et al. 1979, Sorensen 1991, Eisler 2000) but can be difficult to predict because many factors (U.S. Environmental Protection Agency 1980, Eisler 2000) influence toxicity including:

- Cu species and concentration;
- water quality including: pH, temperature, hardness, salinity, suspended solids, and organics;
- Cu interactions with other local elements;
- Species of fish or organism, age, size, reproductive condition, and prior Cu exposure.

Cu is slated for extraction by industrialized mining proposals for Bristol Bay (see Pebble Mine Project at: http://www.ndmpebblemine.com/), a region that produces about one third of all Alaska salmon. Salmon and organisms comprising freshwater food chains are very sensitive to heavy metals, trace elements and other contaminants found in mine wastes (Sorensen 1991, Lemly 1994, Eisler 2000). Because Cu is highly toxic to freshwater aquatic organisms, this review focuses on copper's potential effects on salmon and their freshwater food chains. Both lethal and sublethal effects of increasing Cu concentrations in aquatic ecosystems are reviewed.

Knowledge of the established effects of Cu on freshwater species is crucial for informed policy and management decisions. For example, both lethal and sublethal effects of Cu on salmon and their food chains have been demonstrated (see review by Eisler 2000) at concentrations below the Alaska state water quality standards for protection of freshwater species (9 micrograms Cu per liter calculated on 100 mg/L hardness ($CaCO_3$)) and well below the human drinking water standard of 1,300 µg Cu/L (Alaska Department of Environmental Conservation 18 AAC 2006). The poor record of water quality protection by the mining industry (e.g., Kuipers et al. 2005) and related potential for harm to Alaskan sustainable salmon resources raise considerable compatibility issues (Murdoch and Clair 1986, Lemley 1994, Woodward et al 1994, et al. Woodward 1995, Peplow and Edmonds 2005).

Bristol Bay salmon resources are briefly reviewed, followed by a summary of the documented effects of Cu on salmon and various components of freshwater food chains.

## *Bristol Bay Salmon Resources*

Bristol Bay, Alaska supports the world's largest wild sockeye salmon runs, primarily due to many large lakes in the region (Figure 1). Sockeye salmon spawn in tributaries, ponds, and beaches associated with lakes where their young rear one to two years prior to migrating to sea. Major runs of Chinook, coho, chum, and pink salmon also spawn and rear in the region's high quality freshwater habitats.

Annual salmon runs to Bristol Bay vary; from 1956 to 2006 the runs ranged from 3.5 to 65 million fish, averaging approximately 31.3 million fish. The Alaska Department of Fish and Game reported the following 20-year average salmon harvests (1986-2005) in their most recent management report (Salomone et al. 2007):

Average Commercial Salmon Harvests

- 24 million sockeye salmon
- 70,000 Chinook
- 103,000 coho
- 922,000 chum
- 261,000 even-year pinks

Subsistence users annually harvested an average of about 150,000 salmon, while sport fishers harvested an average of 40,000 salmon, primarily Chinook and coho during this same time frame (Salomone et al. 2007).

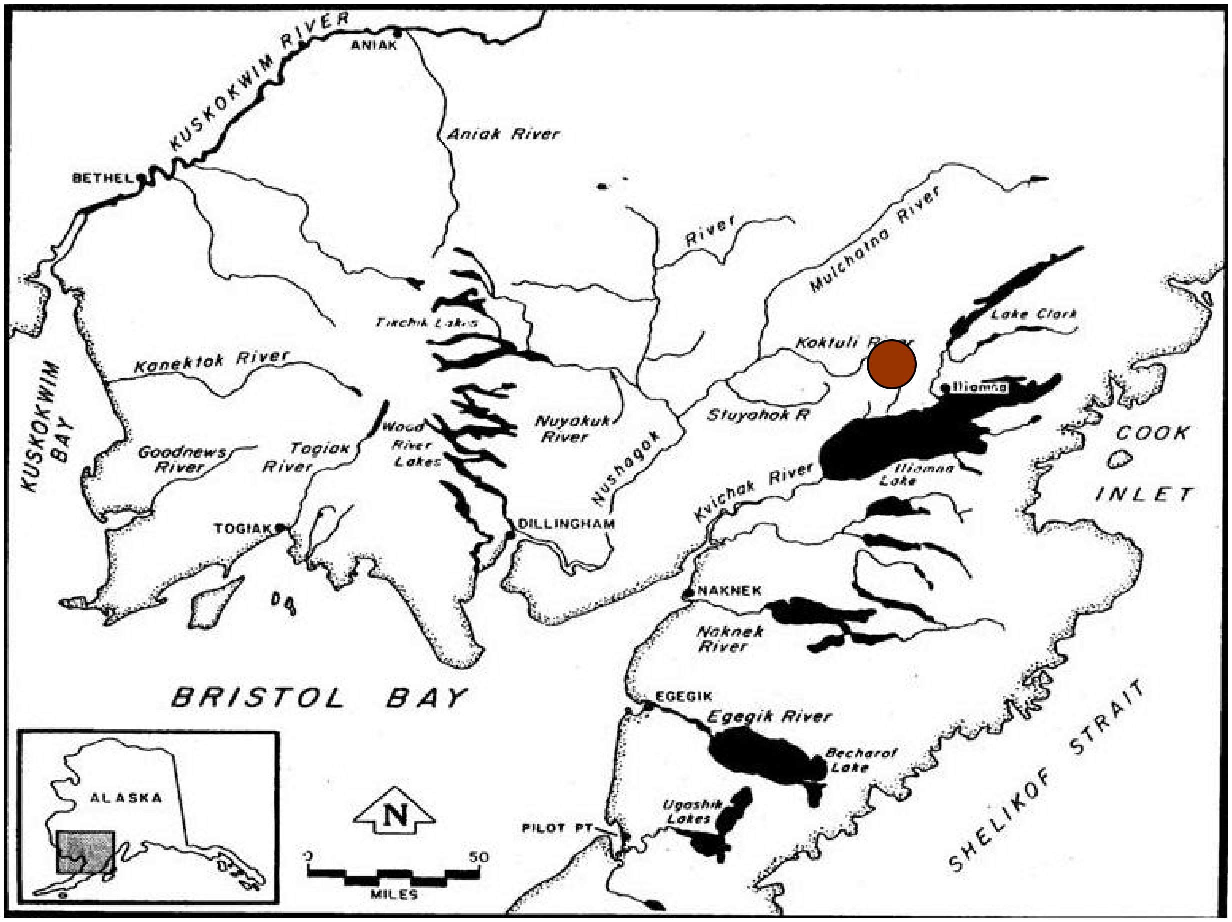


Figure 1. Map of Bristol Bay and major lake drainages. Map scale prohibits inclusion of hundreds of tributaries that are essential salmon habitat. Dot indicates proposed mining development (see Pebble Mine: http://www.ndmpebblemine.com/)

The Bristol Bay commercial salmon fishery is considered one of the few sustainable fisheries in the world (Hilborn et al. 2003) which has endured since 1893. This sustainability is attributed, in part, to the hundreds of diverse smaller spawning salmon populations that comprise the whole. These small spawning populations often differ from each other in important characteristics and specific adaptations to natal habitats (Hilborn et al. 2003). Such populations are not replaceable as illustrated by extinctions and hundreds of failed attempts to mitigate for losses (e.g.; Nehlsen et al. 1991, Wood 1995, Allendorf et al. 1997, Nimmo et al. 1998, Harper and Quigley 2000, Van Cleve et al. 2004). Gradual loss of small adapted populations, due to habitat degradation or loss, affects both productivity and long term sustainability of regional salmon runs. For example, Atlantic salmon once supported viable commercial and subsistence fisheries, but are now listed as endangered by the U.S. Fish and Wildlife Service. The American Fisheries Society considers at least 214 Pacific Coast anadromous salmonid populations to be "at risk" of extinction, while at least 106 once abundant populations are already extinct (Nehlsen et al. 1991, Slaney et al. 1996); the primary cause of declines was habitat loss or degradation.

## *Copper Effects on Freshwater Food Chains and Salmon*

Lethal and sublethal effects to fish and the aquatic food chain can occur below 9 μg Cu/L (Eisler 2000), the current Alaska standard for protection of aquatic species (calculated on 100 and well below the 1300 μg Cu/L standard for human drinking water (Alaska Department of Environmental Conservation 18 AAC 2006). Unfortunately, data to accurately assess the even broader ecosystem impacts from increased Cu loads are lacking. The following facts are important to consider relative to developments that will increase bioavailability of Cu in freshwater habitats of Bristol Bay:

1. Toxicity tests to determine lethal levels and sublethal effects of Cu and other heavy metals are lacking for most Alaskan fish species (Hodson et al. 1979, Eisler 2000), all of which are used for subsistence (Krieg et al. 2005).

2. Many species of freshwater plants and animals die within 96 hours at waterborne concentrations of 5.0 to 9.8 μg/L; sensitive species of mollusks, crustaceans and fish die at 0.23 to 0.91 μg/L within 96 hr (Eisler 2000)

3. There is a lack of information on how multispecies aquatic food chains are affected by Cu and how aquatic organisms cycle Cu through aquatic ecosystems (Hodson et al. 1979, Sorensen 1991, Eisler 2000).

4. Numerous elements in addition to Cu, such as zinc, cadmium, mercury, iron, lead, aluminum, ammonia, and selenium, are often released at hard rock sulfide ore mining sites in a unique "cocktail"; such effects of multiple element releases are not well studied nor understood and effects may be additive, synergistic, or antagonistic (Hodson et al. 1979, Eisler 2000). Federal and State water quality limits for metals *do not* take these interaction effects into account.

5. The numerous parameters affecting Cu toxicity dictate site and species specific studies to determine acceptable exposure levels in the specific ecosystems of interest.

## *Copper Sources*

Copper occurs naturally at low levels in air, soil and water (Table 1). Activities such as mining and smelting of copper, industrial emissions, sewage, municipal wastes, fertilizers, and pesticides increase copper levels in the biosphere (Nriagu 1979a, Eisler 2000). Atmospheric Cu originates primarily from human activities (73%) such as Cu production (e.g. mining and smelting) and combustion of fossil fuel (coal, gas), the rest is from natural sources (Nriagu 1979a). Precipitation of atmospheric fallout is a significant source of Cu to the aquatic environment in mining and industrial areas and deposition patterns vary relative to prevailing winds, precipitation and intensity of industrial activity (Nriagu 1979a, USEPA 1980, Eisler 2000, Maret and MacCoy 2003). For example, in lakes near Sudbury, Ontario, an active copper and nickel mining region, total Cu concentrations decreased with distance from the mining site (Stokes et al. 1973).

**Table 1.** Mean concentration of copper in air, water, and soil from a range of areas. MAX = maximum concentration recorded; μg/m$^3$=microgram per cubic meter; μg/L = micrograms per liter which is comparable to ppb = parts per billion.

| **Material and Concentration** | Observed Concentration | Reference |
|---|---|---|
| **AIR μg/m$^3$** | | |
| Remote areas | Usually < 0.001; MAX= 0.012 | Nriagu 1979a |
| Urban areas | 0.15–0.18; MAX = 1.6 | Nriagu 1979a |
| Near copper smelters | 1-2; MAX = 5.0 | ATSDR 1990<br>USEPA 1980 |
| **FRESHWATERs μg/L** | | |
| Canada | 1-8 | ATSDR 1990 |
| Uncontaminated waters | 1-7 | Schroeder et al. 1966 |
| Contaminated waters | 50-100 | Schroeder et al. 1966 |
| **Lake Sediments** | | |
| Lake sediments 3-5 km from smelter; Sweden | 707-2531 | Johnson et al. 1992 |
| Lake sediments 50-80 km from smelter; Sweden | 37-54 | Johnson et al. 1992 |
| **Glaciers, μg/kg fresh weight** | 0.2 | Veleminsky et al. 1990 |
| **Coal, μg/kg dry weight** | 17,000 | Nriagu 1979a |
| **SOILs mg/kg dry weight** | | |
| Global | 2-250 | ATSDR 1990, Aaseth and Norseth 1986 |
| Near copper production facility | 7000 | ATSDR 1990 |
| Rocks, crustal and sedimentary | 24-45 | Schroeder et al. 1966<br>Nriagu 1979a |

Heavy metals, such as copper, zinc, lead, and mercury can increase in aquatic systems due to both natural weathering and mining-caused acid rock drainage (ARD). When sulfide rock is exposed to air and water, sulfuric acid can form and dissolve heavy metals (e.g., Cu) making them more available to aquatic species. The proposed Pebble Mine Project in Bristol Bay contains sulfide ores (1% - 5%; Northern Dynasty Mines 2005) and ARD is a probable outcome of mining there (Kuipers et al. 2006). The extent to which ARD will develop and the potential for increased heavy metal loads to aquatic ecosystems in Bristol Bay is currently unknown.

## *Lethal and Sublethal Effects of Copper on the Freshwater Food Chain*

Cu affects salmonid ecosystems from the bottom of the food chain to top predators, as demonstrated in hundreds of studies documenting both lethal and sublethal effects in aquatic systems (see reviews by Hodson et al. 1979, Sorenson 1991, and Eisler 2000). Increases in dissolved Cu above normal background levels can reduce productivity of key links in aquatic food chains, including algae, zooplankton, insects and fish (Table 2).

Table 2. Copper effects on representative species comprising the freshwater salmonid food chain. µg/L = micrograms per liter; hr = hours; d = days; wk = week; MATC = Maximum acceptable toxicant concentration: low value is highest concentration tested with no measurable effect with chronic exposure, higher value is lowest concentration tested producing a measurable effect.

| **Freshwater Organism, Cu µg/L Concentration and notes** | **Effects** | **References** |
|---|---|---|
| Algae | | |
| *Chlamydomonas* spp. | | |
| 18 µg Cu/L 24 hr | Reduction in flagella | Winner and Owen 1991 |
| 21 µg Cu/L 7 d | Growth normal | Schafer et al. 1994 |
| 32 µg Cu/L 7 d | 50% decline in growth | Schafer et al. 1994 |
| *Chlorella* spp. | | |
| 1.0 µg Cu/L | Reduced growth | USEPA 1980 |
| 6.3 µg Cu/L | Photosynthesis inhibited | |

Table 2. Continued.

| Freshwater Organism, Cu Concentration and notes | Effects | References |
|---|---|---|
| Rotifers | | |
| *Brachionus* spp. | | |
| 2.0 – 5.0 µg Cu/L | MATC | Janssen et al. 1994 |
| 14 µg Cu/L 5 hr | 50% impairment of swimming | Janssen et al. 1994 |
| 25 µg Cu/L 5 hr | 100% immobilized | Janssen et al. 1994 |
| 26 µg Cu/L 24 hr | 50% mortality | Janssen et al. 1994 and Ferrando et al. 1993 |
| Molluscs | | |
| Freshwater mussel; *Anodonta* spp. | | |
| 2.1 µg Cu/L 72 hr | Glochidial valve closure inhibited by 50%; reduced host infection | Huebner and Pynnonen 1992 |
| 5.3 µg Cu/L 48 hr | 50% decline in valve closure rate | |
| *Villosa iris* | | |
| 27-29 µg Cu/L 24 hr | Valve closure reduced 50% | Jacobson et al. 1993 |
| Freshwater snail; *Biomphalaria* spp. | | |
| 60 µg Cu/L 60 hr | Lethal | Cheng 1979 |
| *Gammarus pseudolimnaeus* | | |
| <4.6 µg Cu/L 15 wk (2 generations) | No adverse effect | Arthur and Leonard 1970 |
| 4.6 – 8 µg Cu/L | MATC @ 45 mg CaCO3/L | USEPA 1980 |
| 6.2 – 12.9 µg Cu/L 5 wk | Decreased survival | Arthur and Leonard 1970 |
| 20 µg Cu/L 4 d | LC50 | Arthur and Leonard 1970 |
| Daphnids | | |
| *Daphnia pulex* | | |
| 0.003-0,3 µg Cu/L 21d | Increased reproduction | Roux et al. 1993 |
| 3 µg Cu/L 3 wk | Impaired reproduction | Roux et al. 1993 |
| 5 µg Cu/L 70 d | No change in reproduction; decreased survival on day 58 | Ingersoll and Winner 1982 |
| 20-37 µg Cu/L 2d | LC50 | Ingersoll and Winner 1982 |
| *Daphnia pulicaria* | | |
| 7.2-11.4 µg Cu/L 4 d | LC50@44-48 mg CaCO3/L | Roux et al. 1993 and |
| 17.8-27.3 µg Cu/L 4d | LC50 @ 95-245 mg CaCO3/L | Dobbs et al. 1994 |
| *Daphnia magna* | | |
| 5.9 µg Cu/L 3 wks | Reduced growth (10%) | USEPA 1980 |
| 10 µg Cu/L 4 d | LC50@ 45 mg CaCO3/L | USEPA 1980 |
| 10 µg Cu/L life cycle | Inhibited reproduction | |
| Macroinvertebrate Communities<br>11.3 µg Cu/L 10 d | 75% decline in abundance of Lab specimens; field streams 44% decline; 56% decline in number of taxa in lab vs. 10% in field sites. | Enserink et al. 1991<br>USEPA 1980<br>USEPA 1980<br>Clements et al. 1990 |
| Aquatic Insects | | |
| Midge, *Tanytarsus dissimillis*; 16.3 µg Cu/L 10 d | LC50 | USEPA 1980 |
| *Chironomus* spp; 10, 20, 100, 150, or 200 µg Cu/L for 3 wk @50 mg CaCO3/L | Significant concentration dependent decline in salivary gland gene activity≥ 20 µg Cu/L | Aziz et al. 1991 |
| Species mix: 25 µg Cu/L for 10 d in outside experimental channels | Caddisflies declined by 16-30%<br>Chironomids: 80% decline<br>Mayflies:67-100% decline in abundance | Clements et al. 1992 |

Table 2. Continued.

| Freshwater Organism, Cu Concentration and notes | Effects | References |
|---|---|---|
| *Arctic Grayling (Thymallus arcticus)* | | |
| 2.65 µg Cu/L 96 hours; swimup | LC50 | Buhl and Hamilton 1990 |
| 9.6 µg Cu/L; fry | LC50 | Buhl and Hamilton 1990 |
| White sucker *Catostomus commersoni* | | |
| 12.9 -33.8 µg Cu/L | MATC @ 45 mg CaCO3/L | USEPA 1980 |
| Northern Pike; *Esox lucias* | | |
| 34.9 – 104.4 µg Cu/L | MATC @ 45 mg CaCO3/L | USEPA 1980 |

Starting at the bottom the bottom of the food chain, at just 1.0 µg Cu/L green algae (*Chlorella* spp.) growth declined, at 5.0 µg Cu/L photosynthesis declined, and at 6.3 µg Cu/L photosynthesis was inhibited in a mixed algae culture (USEPA 1980). Zooplankton feed on algae and their growth and reproduction are affected by food availability. Declining algae production causes declining zooplankton production (Urabe 1991, Müller-Navarra and Lampert 1996), reducing food availability for species, such as sockeye salmon (*Oncorhynchus nerka*), that feed on zooplankton.

Zooplankton, a preferred food of sockeye salmon, are directly affected by Cu. *Daphnia pulex,* the common water flea, increased reproductive rates when cultured for 21 days at 0.003 – 0.3 µg Cu/L, but had impaired reproduction when held at 3.0 µg Cu/L for 15 days (Roux et al. 1993). The concentrations at which 50% mortality of a *Daphnia* culture (LC50) occurred was at 20-37 µg Cu/L for 48 hours (Roux et al. 1993, Dobbs et al. 1994, Ingersoll and Winner 1982). *Bosmina longirostris,* another food of sockeye salmon, were 50% immobilized when held for 48 hours at 1.4 µg Cu/L without food, and at 3.7 µg Cu/L with food (Koivisto et al. 1992). Their growth declined when held for 15 days at 16 µg Cu/L and survival declined at 18 µg Cu/L (Koivisto and Ketola 1995).

Aquatic insects, an important fish food, are sensitive to dissolved Cu. In an experimental stream treated with 25 µg Cu/L for 10 days, mayflies suffered 67-100% mortality, chironomids 80%, and caddisflies 16-30% (Clements et al. 1992).

Note that adverse impacts to the salmonid food chain can occur below the Cu criterion for aquatic life in Alaska (9 µg Cu/L), and lethal levels are well below the human drinking water standard, which in Alaska is 1,300 µg Cu/L (Table 2).

## Sublethal Effects of Copper on Salmon

Copper can harm fish at levels below that which causes mortality (Table 3). Concentrations below the accepted criterion for aquatic life in Alaska (< 9 µg Cu/L) have produced the following documented effects on fish:

a. Impair their sense of smell (olfaction)
b. Interfere with normal migration.
c. Impair their ability to fight disease (immune response).
d. Make breathing difficult
e. Disrupt osmoregulation
f. Impair their ability to sense vibrations via their lateral line canals (a sensory system that helps fish avoid predators)
g. Impair brain function
h. Change their enzyme activity, blood chemistry and metabolism
i. Can delay or accelerate natural hatch rates (Sorenson 1991).

### *Copper Impairs Olfaction*

Copper can impair or destroy a fish's ability to smell (olfaction), which can be fatal. Salmon use their keen sense of smell to identify predators, prey, kin, and mates - mixing up any of these relationships can be detrimental or fatal (Hasler and Schlotz 1983, Groot et al. 1986, Stabell 1987, Olsen 1998, Brown and Smith 1997, Hirovan et al. 2000, Quinn and Busack 1985, Moore and Waring 1996). An increase of just 2.3 to 3.0 µg Cu/L of dissolved Cu above background levels was enough to interfere with behaviors tied to olfaction in juvenile coho salmon; from 1.0 to 20.0 µg Cu/L affected their sense of smell within 10 minutes regardless of water hardness (Baldwin et al. 2003). Rainbow trout olfaction was impaired when exposed to 8.0 µg Cu/L for 2 hours (Hara et al. 1977).

## *Copper Interferes with Migration*

Anadromous salmon memorize or "imprint" a complex map of chemical smells as they migrate from natal freshwaters to saltwater. When they to return to natal habitats to spawn, they follow their nose using this memorized map (Hasler and Schlotz 1983). This behavior is called "homing" and because it isolates small breeding populations in space and time, genetic divergence and population specific adaptations evolve among local populations (Foerester 1968, Taylor 1991, Woody et al. 2000, Hilborn et al. 2003).

If salmon cannot smell, or if the chemical signature of a salmon's natal stream changes, then fish returning to spawn will not recognize their natal stream. The vast majority of salmon home to and spawn in natal habitats (95%-99%; Quinn 2005), and this behavior is an adaptation to increase individual reproductive success. Alteration of natural adaptive behaviors such as homing, migration and spawning due to water pollution can reduce wild salmon survival and change population structure. 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 (Giesel 1974, Altukhov 1981). In contrast, small, populations are vulnerable to inbreeding, random demographic and environmental changes, genetic drift and thus, reduced evolutionary potential, and increased probability of extinction (Cornuet and Luikart 1996, Luikart et al. 1998, Soulé and Mills 1998). Because long-term sustainability of the Bristol Bay fishery is attributed, in part, to the biodiversity of the many smaller spawning populations (Hilborn 2003), and because biodiversity is maintained by precise homing, acceptable increases in pollutants that can affect salmon homing are critical to consider and address, relative to Bristol Bay.

Salmonids avoid waters with low levels of dissolved Cu contamination, disrupting their normal migration patterns. For example, coho salmon yearlings held in 5 – 30 μg Cu/L for as little as 6 days showed altered downstream migration patterns (Lorz and McPherson 1977). Chinook avoided at least 0.7 μg Cu/L whereas rainbow trout avoided at least 1.6 μg Cu/L (Hansen et al. 1998). Laboratory avoidance of Cu by rainbow trout was observed at 0.1, 1.0 and 10 μg Cu/L (Folmar 1976). Oddly, Birge et al. (1993) and others demonstrated that salmon and other fish are attracted to very high concentrations of dissolved Cu (4,560 µg Cu/L), which are lethal (Table 3).

## *Copper Impairs Fish Immune Response*

Fish, like humans, tend to become ill when stressed and Cu is a stress agent that increases both infection and death rates (Rougier et al. 1994). Steelhead trout exposed to 7 and 10 µg Cu/L for 96 hours had a higher death rate from the bacterial disease “redmouth” (*Yersinia* spp.) than non-exposed control fish (Knittel, 1981). Chinook and rainbow trout showed reduced resistance to a wide array of bacterial infections after exposure to 6.4, 16.0, and 29.0 ppm Cu after 3, 7,14, and 21 days (Baker et al. 1983). Rainbow trout stressed by dissolved Cu required half the number of pathogens to induce a fatal infection than non-exposed fish (Baker et al.1983). Fish mortalities caused by long term, low level exposure to stress agents, such as Cu, are difficult to detect compared to mass mortalities caused by a single acute event, such as a single contaminant spill. Because aquatic species that comprise the aquatic food chain will suffer delayed mortality and adverse effects from sublethal Cu exposure, many populations could decline unnoticed.

Table 3. Effects of copper on salmonids. LC10 indicates that 10% of tested fish died after the indicated time period and LC50 indicates 50% of tested fish died after the indicated time period.

| **Species , Cu concentration** | **Effects** | **References** |
|---|---|---|
| ***Chinook salmon*** | | |
| 10-38 µg Cu/L for 96 hours | LC50 in soft water | EPA 1980 |
| 19 µg Cu/L for 200 hours: swimup stage | LC50 | EPA 1980 |
| 20 µg Cu/L for 200 hours; alevins | LC50 | EPA 1980 |
| 26 µg Cu/L for 200 hours; smolts | LC50 | EPA 1980 |
| 30 µg Cu/L for 200h; parr | LC50 | EPA 1980 |
| 54-60 µg Cu/L for for 96 hours; fry | LC50 | Hamilton and Buhl 1990 |
| 78-145 µg Cu/L for 24 hours; fry | LC50 | Hamilton and Buhl 1990 |
| 85-130 µg Cu/L for 96 hours | LC50 in hardwater | EPA 1980 |

*Table 3. Continued.*

| | | |
|---|---|---|
| ***Coho salmon*** | | |
| 5-30 µg/L for up to 72 days; yearlings | Altered downstream migration patterns, reduced gill function, reduced survival. Appetite depressed at >20 ppb | Lorz & MCPherson 1977 |
| 15.1-31.9 µg/L for 96 hours; juveniles | LC50 | Buhl and Hamilton 1990 |
| 18.2 µg /L for 31 then put in seawater | Reduced survival | Stevens 1977 |
| 24.6 µg /L for 31 days; fingerlings | Reduced survival; survivors did not adapt to seawater | Stevens 1977 |
| 26 µg /L for 96 hours; alevins | LC50 at 25 mg $CaCO_3$/L | EPA 1980 |
| 46 µg /L for 96 hours; adults | LC50 at 20 mg $CaCO_3$/L | EPA 1980 |
| 60 µg /L for 96 hours; smolts | LC50 at 95 mg $CaCO_3$/L | EPA 1980 |
| 60-74 µg /L for 96 hours; yearlings | LC50 at 95 mg $CaCO_3$/L | EPA 1980 |
| ***Rainbow Trout*** | | |
| 0.1 µg /L for 1 hour | Avoidance by fry | EPA 1980 |
| 7.0 µg /L for 200 hours; smolts | Depressed olfactory response | Hara et al. 1977 |
| 9.0 µg /L for 200 hours; swimup | LC10 | EPA 1980 |
| 13.8 µg /L for 96 hours; juveniles | LC50 | Buhl and Hamilton 1990 |

## *Copper Interacts with Other Elements*

Areas near hard rock and coal mines, smelters, coal-fired generators, and urban areas commonly release multiple metals such as zinc (Zn), cadmium (Ca), lead (Pb), aluminum (Al), mercury (Hg), selenium (Se), molybdenum (Mo), magnesium (Mg), nickel (Ni) and iron (Fe). Few studies exist on the effects that multiple metal “cocktails” have on fish and aquatic food chains, and combined effects can be more toxic than any single element. For example, Cu and zinc (Zn) often co-occur; a 6:1 ratio of soluble Zn:Cu caused additive toxicity to fish in hard water, meaning that together the elements were more toxic to fish then either alone (Sorensen 1991). Rainbow trout exposed to sublethal concentrations of Cu, Cu + low Zn, or Cu + high Zn consistently exhibited depressed levels of lymphocytes and elevated levels of neutrophils, two white blood cell types key to immune function (Dethloff et al. 1999).

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 catfish (*Ictalurus* spp.) treated with a 1:1 ratio of Cu:Zn declined predictably under an additive model up to a concentration of ~1 ppm, then mortality rates increased at higher that predicted rates for a synergistic effect (Birge and Black 1979).

## Summary

Copper occurs naturally in the environment at low levels; high levels are recorded for regions where hard rock and coal mining, smelting and refining occur and in areas near industrial and municipal waste sites (Nriagu 1979a, Eisler 2000). Contamination levels in the aquatic environment generally decline with increasing distance from industrial activity, and are also dependent on prevailing winds, and precipitation patterns (Nriagu 1979a, USEPA 1980).

Copper causes lethal and sublethal effects to aquatic organisms and interacts with numerous inorganic and organic compounds that affect its bioavailability and toxicity. Toxicity depends on environmental factors that change through time and space (e.g. temperature and water quality) and on affected organism's species, age, size, and reproductive condition. The proposed Pebble Mine Project in Bristol Bay contains sulfide ore with acid generating potential which can dissolve heavy metals, such as copper and zinc, making them available to fish and aquatic organisms. Similar sulfide ore mines release Cu, other heavy metals, and pollutants. This raises questions regarding the type of metals "cocktail" that can be produced if mining occurs in Bristol Bay, and how salmon will be affected.

Significant adverse effects on salmon olfaction, migration, and immune response, can occur at Cu levels below the Alaska criterion for protection of aquatic species. Sublethal effects of dissolved Cu are documented for all levels of the aquatic food chain, from algae to top predators (Tables 2 and 3). Toxicity tests are lacking for most Alaskan species, all of which are used for subsistence. The Alaska Department of

Environmental Conservation uses a simple hardness based formula to calculate acceptable pollution levels for Cu, which does not take into account the myriad of parameters that influence Cu toxicity nor interactive effects with other pollutants. Therefore, Alaskan salmon and their food resources are not adequately protected from adverse effects that can result from increasing Cu pollution to aquatic habitats.

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