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Technical White Paper

An overview of sensory effects on juvenile salmonids exposed to dissolved copper: Applying a benchmark concentration approach to evaluate sublethal neurobehavioral toxicity

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Executive Summary

Dissolved copper is a ubiquitous surface water pollutant that causes a range of adverse acute, chronic, and sublethal effects in fish as well as in aquatic invertebrates and algae. This technical white paper is a summary and targeted synthesis regarding sensory effects to juvenile salmonids from low-level exposures to dissolved copper (dCu). As such, the material presented in this paper does not reflect official policy of the National Marine Fisheries Service, but serves to summarize research on dCu and its impacts on salmonid sensory systems. This document is a snap shot of the existing information; undoubtedly, new information will become available that enhances our understanding of copper's effect on listed salmonids and their supporting habitat.

A large body of scientific literature has shown that fish behaviors can be disrupted at concentrations of dCu that are at or slightly above ambient concentrations (i.e. background). In this document, background is defined as surface waters with less than 3 ug/L dCu as experimental water had background dCu concentrations as high as 3 ug/L dCu. Sensory system effects are generally among the more sensitive fish responses and underlie important behaviors involved in growth, reproduction, and ultimately survival (i.e. predator avoidance). Recent experiments on the sensory systems and corresponding behavior of juvenile salmonids contribute to more than four decades of research and show that dCu is a neurotoxicant that directly damages the sensory capabilities of salmonids at low concentrations. These effects can manifest over a period of minutes to hours and can persist for weeks.

In this paper, benchmark concentrations (BMC) were calculated for dCu using an U.S. Environmental Protection Agency (EPA) methodology to provide examples of effect thresholds to assist in .evaluating effects of activities that deliver dissolved copper to surface waters. Benchmark concentrations ranged from 0.18 – 2.1 µg/L corresponding to reductions in predator avoidance behavior from approximately 8 – 57%. The BMC examples represent the dCu concentration (above background [where background is less than or equal to 3 ug/L]) expected to affect juvenile salmonids' ability to avoid predators in fresh water. These concentration thresholds for juvenile salmonid sensory and behavioral responses fall within the range of other sublethal endpoints affected by dCu such as behavior, growth, and primary production, 0.75-2.5 µg/L.

Point and non-point source discharges from anthropogenic activities frequently exceed these thresholds by one, two, and sometimes three orders of magnitude and can occur for hours to days. The United States Geological Survey (USGS) ambient monitoring results for dCu representing 811 sites across the U.S. detected concentrations ranging from 1-51 µg/L with a median of 1.2 µg/L. Additionally, typical dCu concentrations originating from road runoff from a California study were 3.4 - 64.5 µg/L, with a mean of 15.8 µg/L. Taken together, the information reviewed and presented herein indicates that impairment of sensory functions important to survival of juvenile salmonids is likely to be widespread in many freshwater aquatic habitats. Impairment of these essential behaviors may occur following ten minutes of exposure and continue for hours to days depending on concentration and duration. Due to these acute, sublethal responses i.e. within minutes, it is unlikely that avoidance or acclimation play significant roles in reducing the

effects of short term anthropogenic increases of dCu to juvenile salmonid sensory systems.

We also discuss the bioavailability of copper in aquatic habitats including the effects of water chemistry on olfactory toxicity. Avoidance behavior studies on salmonids exposed to dCu are summarized as well as representative studies of acute, chronic, and sublethal effects to salmonids. Given the large body of literature on copper and responses of aquatic ecosystems, we focused on a subset of fish sensory system studies relevant to anadromous salmonids.

Abstract:

Dissolved copper (dCu) is a ubiquitous, toxic pollutant in U.S. surface waters. Four decades of research with dCu, indicate toxicity to multiple fish endpoints including fish sensory systems and behaviors. This document summarizes literature on the effects of dCu to salmonid sensory systems and conducts a targeted analysis on a recent sensory system behavioral dataset. The review portion of the document discusses peer reviewed and gray literature (see Appendix) on the effects of dCu on salmonid sensory systems, associated sensory-mediated behaviors, and physiology and is intended to facilitate understanding of the effects of dCu on sensory system mediated behaviors that are important to survival, reproduction, and distribution of salmonids. The review does not address the effects of dCu on salmonid habitats although copper is also highly toxic, at low ug/L concentrations, to aquatic plants and invertebrates.

The targeted analysis was conducted with data from a recent experiment on fish olfaction and predator avoidance behavior. Results from this experiment showed that increases in dCu impaired the ability of juvenile salmonids to smell and by extension reduced their capacity to detect and respond to alarm signals (conspecific skin extracts). Impaired olfaction manifested over a period of minutes in juvenile coho. Olfaction and behavioral impairment endpoints were significantly correlated ($r^2 = 0.94$) and indicated statistically significant effects (alpha =0.05) at all concentrations tested for olfaction (2, 5, 10, 20 ug/L) and at 5, 10, and 20 µg/L for alarm response (inhibition of swimming speed reductions). However, no experimental treatments were tested below 2 µg/L which corresponded to an approximately 50% reduction in olfactory function and a 47% reduction in alarm response.

To address this critical uncertainty, we conducted a benchmark concentration (BMC) analysis with the olfactory dataset. The analysis produced BMC estimates ranging from 0.18 (BMC_{10}) - 2.1 (BMC_{50}) µg/L which corresponded to approximately 8 – 57% estimated reductions in predator avoidance response. These results indicate juvenile salmonid sensory systems and their mediated ecologically relevant behaviors are particularly sensitive to low ug/L increases. Impairment of olfaction in juvenile salmonids can manifest in minutes, last for minutes to weeks (depending on dose), and potentially result in population level consequences. These sensory effects are discussed in the context of site specific issues including the bioavailability of dCu.

Acronyms and Glossary

Acute exposure – short term continuous exposure generally 96 hrs or less

BLM- Biotic Ligand Model

Chronic exposure – longer term continuous or pulsed exposures generally greater than 96 hrs

Confidence interval (CI) - A confidence interval is a random interval constructed from data in such a way that the probability that the interval contains the true value can be specified before the data are collected.

dCu – dissolved copper

DOC- dissolved organic carbon

EC_p – effective concentration adversely affecting (p) percent of the test population or percent of measured response, e.g., 10% for an EC_{10}, etc.

EOG- Electro-olfactogram

LC_{50} - the aqueous concentration that kills 50% of the test population

Lower-bound 90% confidence interval - is the lower half of the 90% confidence interval of the mean

Lower-bound 95% confidence interval - is the lower half of the 95% confidence interval of the mean

LOEC - Lowest observable effect concentration

Mean - is the average of the response values in a treatment population. Numerically it represents the sum of the individual response values divided by the number of individuals in a treatment.

mV- millivolts

NOAEL - No observable adverse effect level

NOEC- No observable adverse effect concentration

ORN- olfactory receptor neuron

ppb – part(s) per billion, equivalent to ug/L

Relative departure - is a prescribed change in response e.g. the concentration at which a 10% effect is predicted.

Statistical departure – uses statistical methods to select a prescribed change e.g. applying the 90% or 95% lower-bound confidence interval of the mean of the control response to select the value at which an individual salmonid's olfaction is impaired.

Introduction

Copper, a naturally occurring element, is an essential micronutrient for plants and animals, but is also recognized as a priority pollutant under the U.S. Clean Water Act. Historical and current anthropogenic activities have mobilized significant quantities of copper. Vehicle emissions and brake pad dust [1], pesticides [2], industrial processes, mining, and rooftops [3, 4] are a few of the sources that contribute copper to the environment. These uses may lead to the unintended and, in some circumstances, intended introduction of copper into aquatic ecosytems [5, 6]. Once introduced into the aquatic environment, copper is detected in multiple forms. It can be dissolved or bound to organic and inorganic materials either in suspension or in sediment. This so called speciation of copper is dependent on site specific abiotic and biotic factors. Copper is an element, so once introduced, it will persist indefinitely, cycling through ecosystems. Copper in its dissolved state is worthy of particular scrutiny as it is highly toxic to a broad range of aquatic species including algae, aquatic invertebrates, and fishes (including anadromous salmon and steelhead within the *Oncorhynchus* and *Salmo* genera).

Currently, anadromous salmonid populations inhabit waters of Alaska, Oregon, Washington, California, Idaho, and Maine (Atlantic salmon [*Salmo salar*]). Dissolved copper (referred to as dCu herein) is consistently detected in salmonid habitats including areas important for rearing, migrating, and spawning [7, 8]. Dissolved copper is known to affect a variety of biological endpoints in fish (e.g. survival, growth, behavior, osmoregulation, sensory system, and others; reviewed in [9]). More than forty years of experimental results show that sensory systems of salmonids are particularly sensitive to dissolved copper. Recent experimental evidence showed that juvenile sensory system mediated behaviors are also affected by short term exposures to dCu.

Given the ecological significance of these behaviors to salmonids, it is important to characterize the potential effects from dCu. The growing body of scientific literature indicates that dCu is a potent neurotoxicant that directly damages the sensory capabilities of salmonids at low concentrations (see discussion below). These concentrations may stem from anthropogenic inputs of dCu to salmonid habitats. Salmonid sensory systems mediate ecologically important behaviors involved in predator avoidance, migration, and reproduction. Impairment of these behaviors can limit an individual salmonid’s potential to complete its lifecycle and thus may have adverse population level consequences.

The purpose of this paper is to: (1) summarize information on the effects of dCu to the sensory systems of juvenile salmonids in freshwater (also see Appendix); (2) conduct a benchmark concentration analysis to generate examples of dCu effect thresholds; and (3) to discuss site-specific considerations for sensory system effects. As such, this white paper focuses on a single contaminant (dCu), two relevant sensory system endpoints (olfaction and alarm response behavior), and a single salmonid life stage (juvenile, < 10 months old).

Previous studies on the effects of copper

Examples of copper's effect on a suite of selected biological endpoints from laboratory and field exposures are presented in Table 1. Additionally, the Appendix contains a targeted review and summary of some of the previous studies showing copper's effect on salmonid behavior, including avoidance and migratory disruptions. A supplemental bibliography is also attached for further information on salmonid sensory systems. The following analysis of sensory effects on juvenile salmonids primarily emphasizes recent and ongoing research conducted at the National Oceanic Atmospheric Administration's Northwest Fisheries Science Center. However, the phenomenon that copper and some other trace metals can interfere with chemoreception, alter behaviors, and influence the movements of fish was first described at least 40 years ago, and a large body of knowledge on the adverse effects of dCu has subsequently developed (Table 1).

Table 1. Selected examples of adverse effects with copper to salmonids or their prey.

Species (lifestage)	Effect	Effect concentration (µg/L) (Note A)	Effect statistic	Hardness (mg/L) (Note B)	Exposure duration	Source/ Notes
	Sensory and behavioral effects					
Coho salmon (juvenile)	Reduced olfaction and compromised alarm response	0.18 - 2.1	EC_{10} - EC_{50}	120	3 hours	[10]
Chinook salmon (juvenile)	Avoidance in laboratory exposures	0.75	LOEC	25	20 minutes	[11]
Rainbow trout (juvenile)	Avoidance in laboratory exposures	1.6	LOEC	25	20 minutes	[11]
Chinook salmon (juvenile)	Loss of avoidance ability	2	LOEC	25	21 days	[11]
Atlantic salmon (juvenile)	Avoidance in laboratory exposures	2.4	LOEC	20	20 minutes	[12]
Atlantic salmon (adult)	Spawning migrations in the wild interrupted	20	LOEC	20	indefinite	[12]

Species (lifestage)	Effect	Effect concentration (µg/L) (Note A)	Effect statistic	Hardness (mg/L) (Note B)	Exposure duration	Source/ Notes
Chinook salmon (adult)	Spawning migrations in the wild apparently interrupted	10 – 25	LOEC	40	indefinite	[13]
Coho salmon	Delays and reduced downstream migration of dCu-exposed juveniles	5	LOEC	95	6 day	[14, 15]
Rainbow trout	Loss of homing ability	22	LOEC	63	40 weeks	[16]
	Ecosystem effects					
	Ecosystem function: Reduced photosynthesis	2.5	LOEC	49	~ 1 year	[17]
	Ecosystem structure: loss of invertebrate taxa richness in a mountain stream	5	LOEC	49	~ 1 year	[18]
	Other sublethal effects					
Chinook salmon	Reduced growth (as weight)	1.9	EC_{10}	25	120 days	[19]
Rainbow trout	Reduced growth (as weight)	2.8	EC_{10}	25	120 days	[20]
Coho salmon	Reduced growth (as weight)	21 – 22	NOEC	24 - 32	60 days	[21]
Steelhead	Reduced growth (as weight)	45 – > 51	NOEC	24 - 32	60 days	[21]
	Direct Lethality (Note C)					
Chinook salmon (fry)	Death	19	LC_{50}	24	96 h	[22]
Coho salmon (fry)	Death	28 – 38	LC_{50}	20 – 25	96 h	[15]

Species (lifestage)	Effect	Effect concentra-tion (µg/L) (Note A)	Effect statistic	Hardness (mg/L) (Note B)	Exposure duration	Source/ Notes
Steelhead/ Rainbow trout (fry)	Death	9 – 17	LC_{50}	24 – 25	96 h	[22, 23]
Coho salmon (adult)	Death	46	LC_{50}	20	96 h	[24]
Steelhead (adult)	Death	57	LC_{50}	42	96 h	[24]
Coho salmon (juvenile)	Death	21 – 22	NOEC	24 – 32	60 days	[21]
Steelhead (juvenile)	Death	24 – 28	NOEC	24 - 32	60 days	[21]
Steelhead (egg-to-fry)	Death	11.9	EC_{10}	25	120 days	[19]

Abbreviations: LOEC – Lowest observed adverse effect concentration. Most LOEC values given are not thresholds, but were simply the lowest concentration tested; NOEC – No observed adverse effect concentration; LC_{50} – the concentration that kills 50% of the test population; ECp – effective concentration adversely affecting (p) percent of the test population or percent of measured response, e.g., 10% for an EC10, etc.; Indefinite – field exposures without defined starting and ending times

Note: A. Effects and exposure durations stem from laboratory and field experiments, therefore in some experiments multiple routes of exposure may be present i.e. aqueous and dietary, and water chemistry conditions will likely differ (see reference for details).

Note: B. Hardness is reported as it can influence the toxicity of copper.

Note: C. Acute sensitivity of salmonids to copper probably varies by life-stage, and the swim-up fry stage is probably more sensitive than older juvenile life stages such as parr and smolts, or adults.

Effects to anadromous salmonids' sensory systems exposed to dissolved copper

The salmonid olfactory sensory system relies on neurons (ciliated receptors) to detect and respond to cues in the aquatic environment. The receptors are in direct contact with the aqueous environment. Olfactory receptors detect chemical cues that are important in finding food, avoiding predators, navigating migratory routes, recognizing kin, participating in reproduction, and avoiding pollution. A pair of olfactory rosettes composes the peripheral portion of the olfactory system in a fish's nose (Figure 1A). Each rosette contains olfactory sensory neurons that respond to dissolved odors in water as they pass through the olfactory chamber (Figure 1B) where the olfactory receptors lie (Figure 1C). These chemical cues convey important information about the surrounding environment and underlie salmonid behaviors critical to completion of anadromous lifecycles.

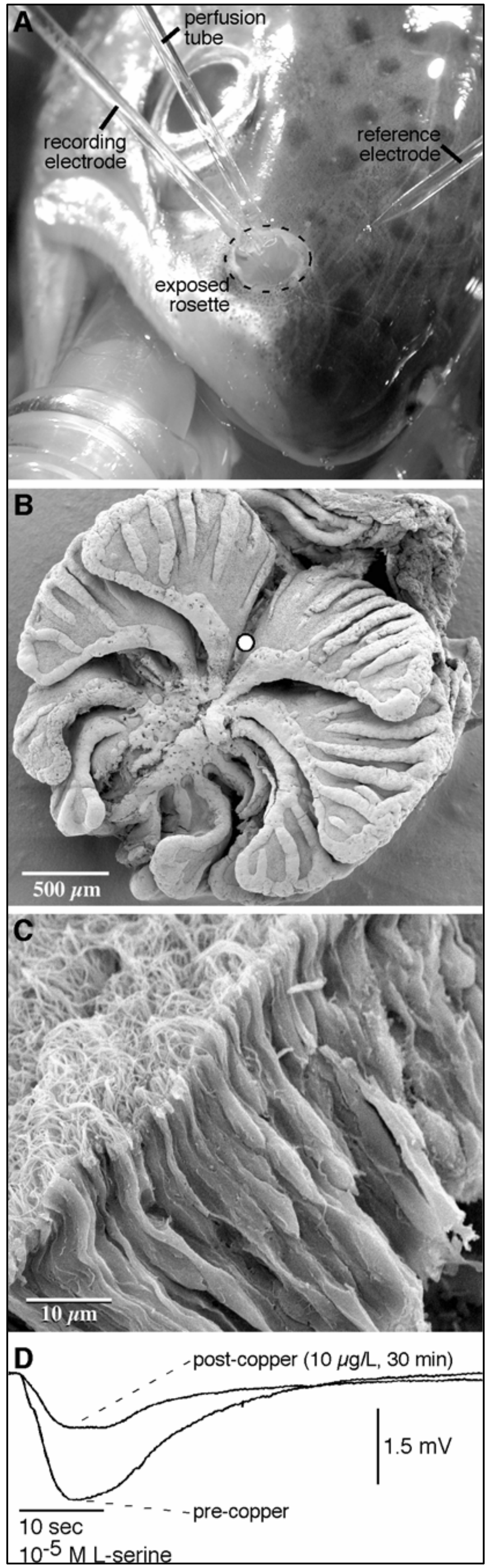


Figure 1. Recording methods and features of the salmon peripheral olfactory system. A) Photograph showing the rostrum of a coho salmon during the recording of electro-olfactograms (EOGs). The mouthpiece provides chilled, anaesthetized water to the gills, while the perfusion tube delivers odor-containing solutions to the olfactory cavity. The recording electrode in the olfactory cavity and reference electrode in the skin monitor the response of the olfactory system to an odor. B) Scanning electron micrograph showing a rosette, located within an olfactory chamber of a juvenile coho salmon. Each rosette consists of lamellae (lobes) covered by an epithelium containing regions of sensory neurons. The open circle denotes the location and approximate size of the tip of the recording microelectrode. C) Scanning electron micrograph showing a cross-section from a region of sensory epithelium of a lamella. In the upper left is the apical surface containing the cilia and microvilli of the olfactory receptor neurons (ORNs). The dendrites and somata of the ORNs appear in the center within the epithelium, while the axons of the ORNs emerge from the basal surface at the lower right to produce the olfactory nerve. D) Typical odor-evoked EOGs obtained from a salmon before and after exposure to copper. A 10-second switch to a solution containing 10^{-5} M L-serine is shown with a horizontal bar. The EOG evoked by the odor pulse consists of a negative deflection in the voltage. A 30-minute exposure to copper reduced the amplitude of the EOG evoked in the same fish by 57%. Figure adapted from Baldwin and Scholz [25].

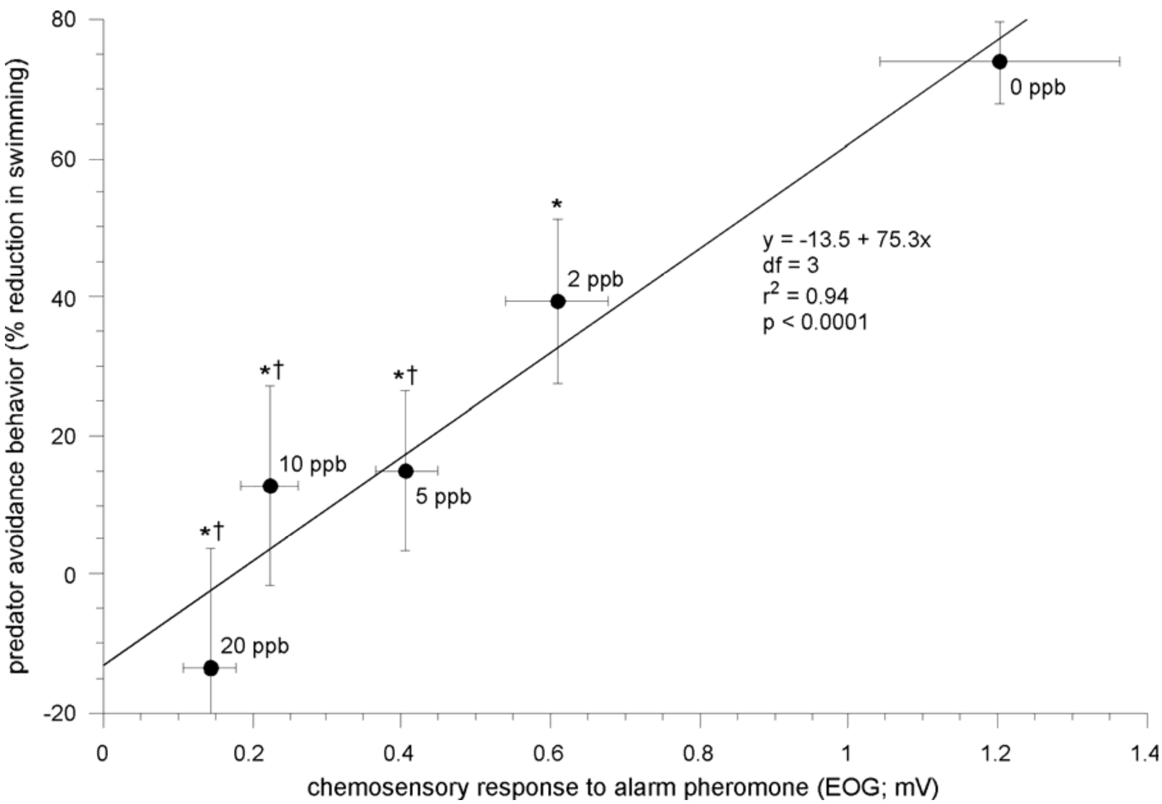
The precise mechanism by which dCu damages the olfactory system remains unknown, although direct exposure to dCu can impair and destroy olfactory sensory neurons [10, 26-28]. Impairment of olfaction (i.e. smell) can be measured by an electrophysiology technique called the electro-olfactogram (EOG) (Figure 1) [25, 28, 29]. The EOG measures olfactory receptor function in fish. Reductions in the amplitude of the EOG of copper-exposed fish compared to unexposed fish reflect functional losses in sensory capabilities. Dissolved copper's toxic effect to olfactory sensory neurons is observable as a reduction in or elimination of the EOG amplitude to a recognizable odor (Figure 1D).

Several recent studies highlight some important aspects of copper olfactory toxicity [10, 27, 30]. Baldwin et al. [27] found that the neurotoxic effects of copper in coho manifest over a timescale of minutes. At 10 minutes, EOG amplitude reductions were observed in juvenile coho exposed to 2, 5, 10, and 20 µg/L dCu above experimental background (3 µg/L). After 30 minutes at 2 µg/L dCu above experimental background, the EOG amplitude from juvenile coho salmon to odors was reduced by approximately 25% compared to controls; in 20 µg/L dCu after 30 minutes by approximately 80%. Sandahl et al. [30] found similar effects following 7 days of exposure (both in EOG reductions and copper concentrations). This result indicated that the juvenile olfactory system cannot adapt to, and correct for, continuous copper exposure for durations up to 7 days.

Recently, using EOG measurements in combination with a predator avoidance assay, Sandahl et al. [10] presented the first evidence that impaired olfaction (smell) resulted in a direct suppression of predator avoidance behavior (alarm response) by juvenile coho at environmentally relevant dCu exposures (≥2.0 µg/L; 3 hr exposure). Unexposed juveniles (control treatment) reduced their swimming speed on average by 74% (alarm response) in response to an alarm odor (conspecific skin extract). A reduction in swimming speed is a typical predator avoidance response for salmonids and many other fish. In unexposed fish, the alarm odor elicited a mean EOG response of 1.2 mV. Juvenile coho exposed to 2-20 µg/L copper exhibited measurable reductions in both EOG (50-92%) and alarm response (47 - >100%) [derived from Figure 2 in Sandahl et al., [10]]. Juvenile coho exhibited statistically significant decline in antipredator behavior at 5, 10, and 20 µg/L dCu (Figure 2).

Importantly, no concentrations of dCu below 2 µg/l were tested. This is particularly troubling because all concentrations tested (between 2-20 µg/L) significantly affected olfaction e.g. reductions in EOG amplitudes of ~ 50 - 92%. Because individual juvenile coho were significantly affected at the lowest concentration tested (2 µg/L), uncertainty remains at what concentrations salmonid olfaction is first impaired. The results of this last study provide evidence that juvenile salmon exposed to sublethal dCu concentrations at 2 µg/L (resulting in approximately 50% reductions in EOG), and likely even lower, might not recognize and respond to a predation event, and therefore have an increased risk of being eaten by other fishes or birds, an event referred to as ecological death [31].

Figure 2. Copper-induced reductions in juvenile salmonid olfactory physiology and behavior are significantly correlated. Fish exposed to dCu (3h) showed reduced olfactory sensitivity and corresponding reduction in predator avoidance behavior. Values represent treatment means (with copper exposure concentration labeled to the right); error bars represent one standard error; n=8-12 individual coho salmon; * represents a statistically significant difference in olfactory response (EOG data) compared to controls (one-way ANOVA with Dunnett post hoc test, $p<0.05$); †represents statistically significant difference in behavioral response to skin extract (% reduction in swimming) compared to controls (one-way ANOVA with Dunnett post hoc test, $p <0.05$). The line represents a statistically significant linear regression based on treatment means ($n = 5$; $p < 0.0001$; $r^2 = 0.94$). 1 ppb = 1 µg/l. Adapted from Figure 2C Sandahl et al [10].



Typically dCu concentrations in road runoff are well within the range affecting anti predator behavior (3.4 - 64.5 µg/L, with a mean of 15.8 µg/L [8]). The length of exposure is also likely to be sufficient, as stormwater runoff durations may range from a few minutes to several hours [5]. Fish may regain their capacity to detect odors fairly quickly in some cases; physiological recovery of olfactory neuron function is dose-dependent and occurs within a few hours at low copper concentrations (i.e., <25 µg/L dCu; [27]). However long-term damage is also documented. In the case of olfactory neuron cell death (i.e. ≥25 µg/l copper [11, 26]) recovery is on the order of weeks [32] and in some cases months [33].

Interestingly, another fish sensory sytem, the lateral line, is also sensitive to dCu. It is composed of mechanosensory neurons (hair cells) that collect data from the aquatic environment. Specifically, the neurons detect vibrations and other forms of water movement in the aquatic environment; thereby mediating shoaling, pursuit of prey, predator avoidance, and rheotaxis (flow orientation). In a recent study, dissolved copper (i.e., ≥20 μg/L; 3 hr exposure) killed 20% of zebra fish hair cells [34]. As mentioned earlier, juvenile Chinook olfactory epithelial cells may also be killed by increases in dCu, highlighting the similar sensitivity of olfactory and lateral line receptors to dCu. Consequently, dCu may damage or destroy either or both of these important sensory systems. Currently, we are not aware of any research on the effects of dCu to salmonid lateral lines, although the comparable sensitivities of olfactory and lateral line neurons suggest dCu affects these neurons as well.

In this paper, a benchmark dose (concentration) analysis [35] is applied to recent data from dose-response experiments on juvenile salmonids exposed to dCu [10] to determine the exposure concentrations that may adversely affect salmonid sensory systems . In previous studies, BMCs were determined for olfactory responses, however concomitant behavioral responses were not measured [27, 30]. The BMC analysis conducted herein determined concentrations of dCu that could be expected to affect juvenile salmonid olfaction and, by extension, alarm response behavior involved in predator avoidance.

Application of the benchmark concentration analysis

The benchmark concentration (BMC), also referred to as a Benchmark dose method, has been used since 1995 by agencies such as the Environmental Protection Agency (EPA) to determine No Observable Adverse Effect Level (NOAEL) values. The method statistically fits dose-response-data to determine NOAEL values [35]. This is in contrast to other methods (e.g. using an analysis of variance) that rely on finding a No Observable Effect Concentration (NOEC) and Lowest Observable Effect Concentration (LOEC) to establish the NOAEL. Multiple difficulties arising from the traditional approach of selecting a NOAEL from dose-response data were previously identified by EPA. Specific short comings associated with traditional methods included: 1) arbitrary selection of a NOAEL based on scientific judgments; 2) experiments involving fewer animals produced higher NOAELs; 3) dose-response slopes were largely ignored; and 4) the NOAEL was limited to the doses tested experimentally [35]. These as well as other concerns with selection of an NOAEL led to the development of an alternative approach, the BMC analysis. The BMC approach uses the complete dose-response dataset to identify a NOAEL, thereby selecting an exposure concentration that may not have been tested experimentally.

The BMC is statistically defined as the lower confidence limit for a dose that produces a predetermined adverse effect relative to controls. This effect is referred to as the benchmark response [BMR]) [35]. Unlike the traditional method of selecting the NOAEL (e.g. establishing a NOEC) the BMC takes into account the full range of dose-response data by fitting it with an appropriate mathematical model. These can be linear, logarithmic, sigmoidal, etc. The BMR is generally set near the lower limit of responses (e.g. an effect concentration of 10%) that can be measured directly in exposed, or affected, animals.

In the present context, a BMC approach was used to estimate thresholds for dCu's sublethal effects on the chemosensory physiology and predator avoidance behaviors of juvenile coho salmon [10]. An example of this approach is shown in Figure 3. This methodology has been used previously to determine toxicity thresholds in Pacific salmon [27, 30, 36]. The dose-response relationship for copper's effect on the EOG was described by fitting the data with a sigmoid logistic model:

$$y = m/[(1+(x/k)^n]$$

where m = maximum EOG amplitude (fixed at the control mean of 1.2 mV)

y = EOG amplitude

x = copper concentration

k = copper concentration at half-maximum EOG amplitude (EC_{50})

n = slope

For this non-linear regression, the average olfactory response of the control fish to a natural odor was used to constrain the maximum odor evoked EOG (m in the above equation). Consequently, the control fish were not used in the regression other than to set *m*. The regression incorporated the individual response of each exposed fish (n = 44 total) rather than the average values for each exposure group. As shown in Figure 3, the sigmoid logistic model was a very good fit for both the sensory and behavioral data (r^2= 0.94; $p<0.0001$). Benchmark concentrations were then determined based on the concentration at which the estimated curve intersected benchmark responses.

Results of the benchmark concentration analysis

Examples of benchmark concentrations and responses are presented in Figure 3 and Table 2. The EPA methodology recommends using the concentration that represents a 10% reduction in response compared to controls when limited biological effects data are available [37]. This is the BMC_{10} and is synonymous with the concentration producing an effect of 10% (EC_{10}), in this case a 10% reduction in the recorded amplitude of the salmon's chemosensory response (EOG). Since the predicted fish EOG response at the BMC_{10} falls well within the olfactory response of unexposed juveniles i.e. 95% CI (control fish; Figure 3), it is more than likely that this individual response (1.08 mV) at the BMC_{10} (0.18 ug/L) would not be detectable or biologically significant as an adverse response. This highlights that a BMC based purely on a relative departure (e.g. BMC_{10}) may not account for the variability of olfactory responses in unexposed fish.

Other BMCs were derived using statistical criteria to determine benchmark responses. For example, Table 2 shows two BMCs that were determined using the statistical departure of the lower-bound confidence interval (CI) of the control mean (unexposed fish), 1.2 mV (either the 90 or 95% CI). The selection of different CIs results in different BMCs. The CI derived BMCs represent a reasonable estimate of when an individual salmonid is likely to have a significant reduction in olfaction and a concomitant reduction in predator avoidance behavior. The relative departures from controls in Table 2 are equivalent to effective concentrations for olfactory inhibition, i.e. at the lower-bound 90% CI a BMC of 0.59 µg/L equates to a $BMC_{24.2}$. Said another way, the BMC analysis predicts a substantial 24.2 % reduction in olfaction (i.e. EOG amplitude) at 0.59 µg/L dCu. At the lower-bound 95% CI a 29.2% reduction in olfaction is predicted to occur at 0.79 ug/L.

The BMC_{50} is equivalent to the EC_{50} for olfactory responses (2.1 µg/L) and is very similar to the lowest observable effect concentration (LOEC) of 2 µg/L. Since the EC50 approximately equals the LOEC, it is almost certain that effects to juvenile salmonid's olfaction will occur at lower concentrations than those measured. Therefore it is appropriate and useful to apply a BMC analysis to these data to predict effects occurring between 0 and 2 µg/L dCu. The predicted effect thresholds for sensory responses in juvenile coho ranged from 0.18 - 2.1 µg/L which corresponded to reductions in predator avoidance behavior (i.e. reduced alarm response) of 8 - 57%. Comparatively, the other two studies that conducted a BMC approach with salmon olfaction datasets (e.g. EOG measures) estimated dCu BMCs from 3.6 – 10.7 ug/L (BMC_{20}-BMC_{50}) [17] and 2.3 - 3.0 ug/L (BMC_{25}) [15].

Together these three studies highlight that different experimental conditions including age of fish, exposure duration, and experimental background of dCu may influence BMCs. Importantly, of the three experiments that derived BMCs for olfactory impairment, the Sandahl et al [10] (i.e. the data set used in this white paper) empirically linked impaired olfaction to an ecologically relevant behavior i.e. reduced alarm behavior (Figure 2). Therefore, we believe that the dCu BMC analysis in this white paper is the most ecologically relevant of the three studies.

Figure 3. Using a benchmark concentration approach to estimate a threshold for dCu toxicity in the salmonid olfactory system. Filled circles represent treatment means; error bars represent the 95% confidence interval for each mean (n = 8-12 individual coho salmon). An asterisk indicates a statistically significant difference in the size of the olfactory response (EOG data) compared to controls (one-way ANOVA with Dunnett post hoc test, $p<0.05$). The line represents a statistically significant non-linear regression based on individual fish ($n = 44$; $p < 0.0001$; $r^2 = 0.55$). The gray band shows the 95% confidence band for the non-linear regression. The regression used a standard sigmoid function with the maximum constrained to the control mean (1.2 mV; indicated by the upper horizontal dashed line). Therefore, the control fish were not included in the non-linear regression. The lower bound of the 95% confidence interval of the control mean (0.85 mV) is indicated by the lower horizontal dashed line and is an example of a benchmark response (BMR). The large open circle shows where the regression line crosses the BMR and denotes the corresponding benchmark concentration (BMC) which, in this case, is a dCu concentration of 0.79 ug/L. Horizontal and vertical lines through the open circle highlight the 95% confidence intervals for the BMC based on the results of the non-linear regression. The small open circle shows where the regression line crosses the BMR (1.08 mV) and denotes the corresponding BMC_{10} (0.18 ug/L) at which a 10% reduction in olfactory capacity is expected. Data from Sandahl et al. [10].

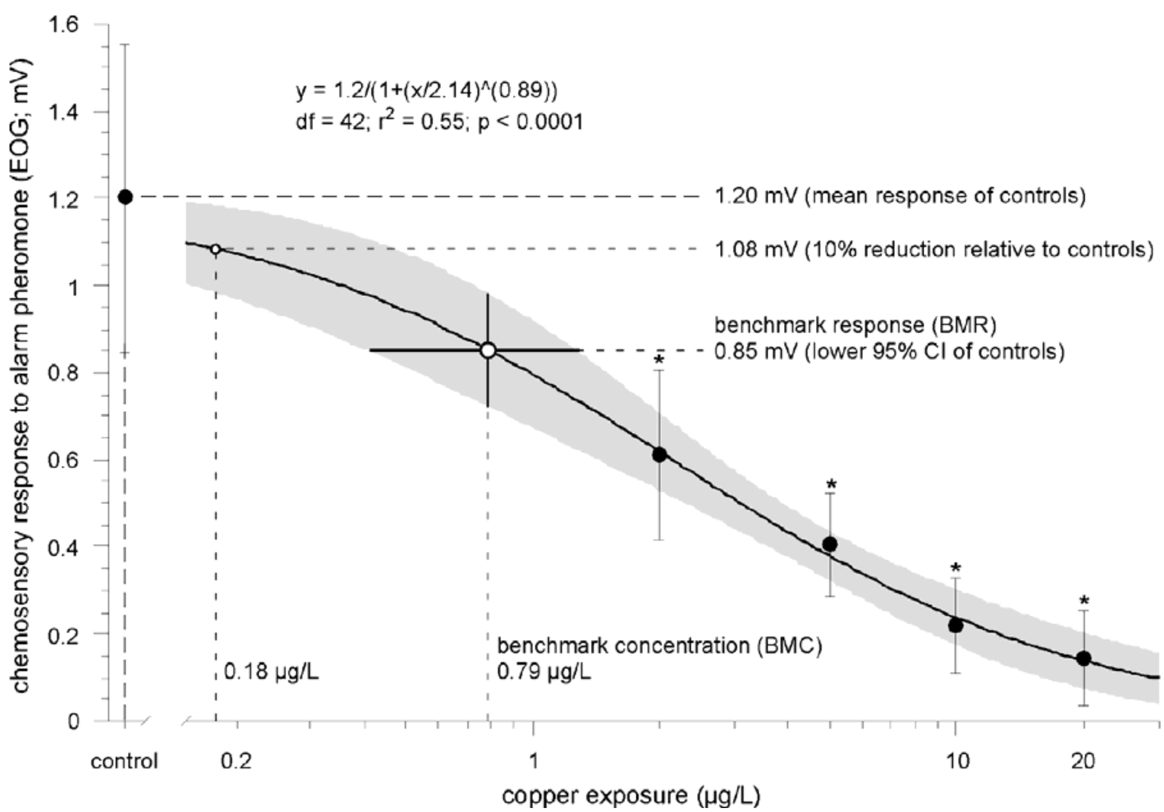


Table 2. Benchmark concentrations and benchmark responses for juvenile salmon exposed to dCu for 3 hr. Response values are a loss of olfactory function, or reduction in a chemosensory response to an alarm pheromone as measured via EOG recordings. Behavioral impairment indicates a predicted decrease in predator recognition and avoidance as indicated by a reduced alarm response. CI = confidence interval; NA = not applicable.

Benchmark Responses[1] Departure from mean of controls		**Benchmark Concentrations**[2]		**Behavioral Impairment (predicted)**[3] Departure from mean of controls
Statistical[4] (CI of control mean)	Relative[5] (% reduction in olfactory response)	Value[6] (µg/l)	95% CI[7] (µg/l)	Relative[8] (% reduction in alarm response)
NA	10.0	0.18	0.06 - 0.52	8.3
Lower 90%	24.2	0.59	0.30 - 1.16	25.6
Lower 95%	29.2	0.79	0.44 - 1.42	31.8
NA	50.0	2.10	1.60 - 2.90	57.2

[1] the predetermined level of altered response or risk at which the benchmark dose (concentration) is calculated (EPA/630/R-94/007; 02/1995)
[2] the dose (concentration) producing a predetermined, altered response for an effect (EPA/630/R-94/007; 02/1995)
[3] based on the linear regression shown in Figure 2; note behavioral responses were determined by inputting the Benchmark response value (EOG, mV) into the regression equation
[4] location of the value with respect to a confidence interval of the mean of the controls
[5] amount of reduction in the olfactory response represented by the value relative to the mean of the controls
[6] corresponding concentration, see Figure 3 and text for calculation method
[7] confidence interval for the value based on the non-linear regression
[8] amount of reduction in alarm response represented by the value relative to the mean of the controls

Discussion of site specific considerations for sensory system effects

Below, we identify several issues to consider when applying the benchmark concentrations to real world aquatic ecosystems.

These BMCs reflect expected impairment of chemosensory systems from short term increases of dCu above ambient concentrations.
Specifically, the BMCs are predicated on anthropogenic increases of dCu to salmon habitats. Effects to juvenile salmonid olfaction are expected following a few minutes of exposure. Salmonids are capable of regulating the amount of internal copper via uptake

and elimination processes. These so called homeostatic mechanisms (such as metallotheionein induction) can reduce copper's toxic effects and may result in acclimation. Consequently, fish may tolerate certain copper exposures without showing overt toxicological responses, however at higher levels these mechanisms ultimately fail. The BMC examples presented in Table 2 are not expected to be alleviated by juvenile salmonid homeostatic mechanisms. This is supported by the effect concentrations presented in Table 1 and the Appendix.

Although acclimation could theoretically reduce the toxicity of dCu to the salmon olfactory system, initial evidence indicates that this is not likely for pulsed or short term exposures lasting less than a week [11]. For other measures of copper toxicity from long term exposures, evidence suggests that acclimation may not occur (Table 1, Appendix).

These BMC examples represent short term increases of dCu above ambient concentrations in surface waters (defined here as < 3 µg/L) [10, 27, 30]. It is uncertain whether fish sensory systems acclimated to higher ambient concentrations (i.e. >3 µg/L) will respond differently to additional anthropogenic loading, which might then lead to different threshold concentrations for olfaction and behavior.

These BMCs reflect the impact of dissolved copper on olfaction and predator avoidance behavior.

In salmonid habitats fish are rarely exposed to dCu only. In fact, exposure to complex mixtures of other toxic compounds (e.g. metals, pesticides, PAHs, etc.,) in conjunction with other multiple stressors (e.g. elevated temperatures, low dissolved oxygen, etc.,) is the norm. Equally important are exposure routes other than the water column, such as consumption of contaminated prey items (dietary) or contact with contaminated sediments. Threshold examples (BMCs) presented here are based solely on juvenile salmonids exposed to dCu. Presently, these thresholds are uncertain for multiple routes of exposure and complex mixtures of contaminants for olfaction. That being said, several studies demonstrate greater than expected toxicity to other fish endpoints from mixtures of metals [12, 38]. For example, mixtures containing zinc and copper were found to have greater than additive toxicity to a wide variety of aquatic organisms including freshwater fish [9], and other metal mixtures also yielded greater than additive toxic effects at low dissolved metal concentrations [39]. The toxic effects of metals to salmonids are also likely to be exacerbated by poor water quality conditions, including elevated temperatures, low dissolved oxygen, pesticides, and polycyclic aromatic hydrocarbons. While the interactions of multiple stressors and mixtures are beyond the scope of this document, they warrant careful consideration in site specific assessments.

These BMCs were derived from experiments using a single freshwater source (de-chlorinated, soft municipal water). Hardness, alkalinity, and dissolved organic carbon (DOC) are known to alter the bioavailability of dissolved copper in surface waters to the gills of fish. These water chemistry parameters can therefore influence the potential for dCu exposure in the field to cause an acute fish kill. Acute copper lethality via the gill route of exposure is typically estimated using the Biotic Ligand Model (BLM; reviewed by [40]). However, recent unpublished research by McIntyre et al. [41] suggest that these parameters may have less influence on olfactory responses especially when compared to ambient levels of hardness, alkalinity, and DOC.

The USGS has monitored hardness, alkalinity, and DOC for more than 10 years in many West Coast river basins including the Willamette River Basin, Puget Sound Basin, Yakima River Basin, and the Sacramento-San Joaquin River Basin (National Water Quality Assessment Program [NAWQA]. Several at risk species of anadromous salmonids inhabit these basins. The monitoring data indicate that surface waters within these basins typically have very low hardness and alkalinity and seasonally-affected DOC concentrations. Hardness, alkalinity, and DOC levels found in most freshwater habitats occupied by Pacific salmonids would be unlikely to confer substantial protection against dCu olfactory toxicity [27], [41-43].

Recent experimental results suggest that significant amelioration of olfactory toxicity due to hardness is unlikely in typical Pacific salmonid freshwater habitats. The experiment showed that hardness at 20, 120, and 240 mg/L Ca (experimentally introduced as $CaCl_2$) did not significantly protect juvenile coho from olfactory toxicity following 30 minute laboratory exposures to 10 µg dCu/L above an experimental background of 3 µg/L [27]. In another experiment, a 20 µg dCu/L exposure (30 minutes) in water with low hardness and alkalinity and no DOC produced an 82% inhibition in juvenile coho olfactory function [41]. A hardness of ≥ 82 mg/L Ca was needed to reduce the level of olfactory inhibition to ≤50% at 20 ug/l dCu [41]. However, 82 mg/L was never exceeded in any of the surface water samples from USGS sampled NAWQA basins [41].

Typical alkalinity values from Pacific Northwest and California streams are also unlikely to protect salmonids from olfactory toxicity (NAWQA surface water data). In fact, 0.4% of stream samples contained alkalinity levels sufficient to cut dCu's toxic impact to juvenile salmonids olfactory system in half [41]. Decreases in dCu's olfactory toxicity were obtained with large increases in alkalinity [41]. However, increasing water hardness and alkalinity had some protective effect against the olfactory neurotoxicity of dCu in coho salmon, but the effects were small with olfactory function rising to ~30% of normal (or 15% increase in olfactory function) across the range of average hardness and alkalinity levels in sampled NAWQA basins [41]. Bjerselius et al., [43] and Winberg et al., [42] found that hardness and alkalinity provided limited amelioration of olfactory responses in juvenile Atlantic salmon exposed to dCu.

Increases in DOC showed greater protection to dCu compared to increases in alkalinity and hardness. Twenty-nine percent of USGS surface water samples from West Coast basins had a DOC concentration sufficient to limit olfactory impairment to 50 percent or less at 20 µg dCu /L [41]. However, only 2% of all samples contained a DOC concentration (8 mg/L) sufficient to completely protect the olfactory responses of juvenile coho at 20 µg dCu /L [41]. This information underscores the importance of evaluating site specific DOC data to address its potential influence on olfactory toxicity.

Accordingly, we consider the BMC thresholds presented in this document to be broadly applicable to most Pacific salmonid freshwater environments as typical hardness, alkalinity, and DOC concentrations are unlikely to confer substantial protection against dCu olfactory toxicity.

Dissolved copper's effect on salmonid olfaction in saltwater environments remains a recognized data gap and it is unclear whether the derived BMC thresholds apply to salt water environments. Estuarine and near shore salt water environments, despite their higher salinity (in part due to increased cation concentrations) and hardness may or may not confer protection against dCu-induced olfactory toxicity. One source of this uncertainty is whether or not free copper (Cu^{2+}) is the sole species of copper responsible for olfactory toxicity. In freshwater, evidence suggests that Cu^{2+} is not the only toxic species that adversely affects olfaction [41] and other fish endpoints including mortality [40]. Other copper species e.g. CuOH (Cu^{1+}) will also bind to the gill producing copper toxicity [40]. While the physiology of a salmonid's olfaction in freshwater environments is well characterized, it is unclear whether the physiological changes to olfactory systems in estuarine and marine environments alter the toxicity of dCu.

Using the Biotic Ligand Model we calculated an acute Criterion Maximum Concentration (CMC). The United States Environmental Protection Agency (EPA) sets acute water quality criteria by calculating an acute Criterion Maximum Concentration (CMC) [44]. The CMC is an estimate of the highest concentration of a material in surface water to which an aquatic community can be exposed briefly without resulting in an unacceptable effect [45].We calculated an acute CMC using the Biotic Ligand Model (BLM) [46]. Interestingly, the estimated acute CMC based on the BLM using measured and estimated water quality parameters from Sandahl et. al. [10] was 0.63 µg/L with a range from 0.34 to 3.2 µg/L, while the EPA hardness-based acute CMC [45] was 6.7 µg/L. Because the BLM-based acute criterion is sensitive to pH and DOC, the range of measured test pH values (6.5 to 7.1) and the range of estimated DOC values (0.3 to 1.5 mg/L) produced this range of BLM-based acute criterion values. It is also interesting that the acute CMC range (0.34 - 3.2 ug/L) overlapped with the olfactory based BMC range (0.18 – 2.1 ug/L).

Juvenile salmonids may or may not be able to avoid short term increases in dCu. Salmonids will actively avoid water containing dCu if they can detect it. However, if salmonids avoid optimal rearing and spawning habitats reductions in growth and reproductive success may occur. One study showed that chinook salmon no longer avoided copper following a 20 day exposure at a low, environmentally realistic concentration (2 ug/L) [11]. Since salmonids have the ability to avoid areas with elevated copper, in theory, if these areas were limited and did not interfere with migratory routes, juveniles might simply bypass them. Smith and Bailey [47] and Mebane [13] give examples of deriving regulatory "zones of passage" around wastewater discharges that were based upon salmonid avoidance responses. However, in areas with diffuse, nonpoint source pollution, or multiple point source discharges it may be difficult to determine "zones of passage", or available zones of passage may not even exist. Environmental circumstances may force fish to be exposed to copper they would otherwise detect and avoid, or fish will avoid using important habitats. The "zones of passage" concept would likely not apply to rearing or spawning habitats affected by dCu.

Anthropogenic loading of dCu to surface waters often occurs as stormwater runoff and other types of short term, pulsed inputs lasting a few minutes to hours and in some cases days. In this context, dCu's effect on olfaction manifests in as little as 10 minutes [27]. Recovery of affected olfactory sensory function will require hours to weeks depending on the extent of olfactory damage, which depends on both concentration and duration of exposure [28]. Acute exposure can inhibit olfactory function for months if exposure is sufficient to cause death to sensory neurons (25ug/L [11,26]) [33]. The impacts of copper on fish olfaction will likely be cumulative if full recovery is not achieved between pulses of exposure.

These BMCs were derived using data from juvenile coho salmon.
The examples of BMC thresholds were derived from data based on juvenile Coho salmon (4-5 month old; mean of 0.9 grams [wet weight]). These BMC examples are generally applicable to juvenile salmonids. Three hour exposures of four month old steelhead to a similar range of dCu produced comparable reductions in EOG as seen in four month old coho (Baldwin et al.,personal communication). Studies on 10 month old juvenile coho had similar reductions in olfaction compared to 4 month old fish [27, 30]. Juvenile chum salmon (2-3 month old) also showed a dose dependent reduction in EOG amplitude following exposure to dCu (3-58 ug/L) [28]. Taken together these data support applying the BMC threshold examples broadly to juvenile salmonids. While olfaction is certainly critical to all salmonid lifestages, the application of these thresholds to other life stages (i.e. smolts and adults) remains uncertain.

Conclusions

Dissolved copper (dCu) is a ubiquitous, bioavailable pollutant that can directly interfere with fish sensory systems and by extension important behaviors that underlie predator avoidance, juvenile growth, and migratory success (see appendix). Recent research shows that dCu not only impairs sensory neurons in a salmonid's nose, but also impairs juvenile salmonids' ability to detect and respond to predation cues. A juvenile salmonid with disrupted predator avoidance behaviors stands a much greater risk of being eaten and therefore the likelihood of surviving to reproduce is reduced. Whether this individual behavioral effect impacts a given population will depend, in part, on the number of the individuals affected and the status of the population (numbers, distribution, growth rate, etc.).

In this paper, benchmark concentrations (BMC) were calculated using an EPA methodology to provide effect threshold examples for juvenile salmonids' sensory systems. The BMC examples represent the dCu concentration (above background or ambient levels [where background is less than or equal to 3 ug/L) expected to affect juvenile salmonids' ability to avoid predators in fresh water. Benchmark concentrations ranged from 0.18 – 2.1 µg/L corresponding to reductions in predator avoidance behavior (an alarm response) from approximately 8 – 57%. Taking into account the olfactory responses of unexposed fish (e.g. control treatment), a more biologically relevant range of BMCs is 0.59 – 2.1 ug/L (Table 2). This second range of BMC thresholds for juvenile salmonid sensory and behavioral responses is similar to or slightly less than documented effect concentrations to other copper-affected sublethal endpoints such as behavior, and growth., 0.75 - 2.5 µg/L. These levels may also affect other organisms in the ecosystem upon which salmonids depend, for feeding and sheltering (Table 1 and Appendix).

The primary objective of this paper was to present examples of threshold concentrations for effects of dCu on a critical aspect of salmonid biology, olfaction. A secondary objective of this paper was to summarize a selection of recent and historical information related to the effects of dCu on salmonid sensory systems. Importantly, this overview is not a comprehensive summary of the myriad effects of copper to anadromous salmonids. However, several conclusions were made based on the studies reviewed thus far and in the appendix concerning juvenile salmonids. First, salmonid's and other fish's behavior can be disrupted at dCu concentrations in the low ppb range. Second, reduced growth and impaired swimming performance resulted following dCu exposures as discussed in the appendix. These effects may result in increased susceptibility to predation and may result in population level consequences. Third, in some freshwater systems it is likely that acute toxicity occurs from brief pulses of elevated dCu concentrations.

Taken together, the information reviewed and presented herein indicates that significant impairment of sensory functions important to survival of threatened and endangered juvenile salmonids is likely to be widespread in many freshwater aquatic habitats. Impairment of these essential behaviors may occur following ten minutes of exposure and continue for hours to weeks depending on concentration and duration. Due to these acute, sublethal responses i.e. within minutes, avoidance or acclimation are unlikely to reduce the effects of short term anthropogenic increases of dCu to juvenile salmonids.

It remains uncertain how and to what degree these short term dCu exposures of juvenile salmonids affect salmonid populations. What is certain is that salmonids use their sense of smell to avoid predation events, participate in reproduction, migrate, avoid poor water quality, and feed. Each of these olfactory-mediated behaviors is important for successful lifecycle completion.

This technical white paper is a summary and targeted synthesis regarding sensory effects to juvenile salmonids from low-level exposures to dissolved copper (dCu). As such, the material presented in this paper serves to summarize research on dCu and its impacts on salmonid sensory systems. This document is a snap shot of the existing information; undoubtedly, new information will become available that enhances our understanding of copper's effect on salmonid populations and their supporting habitat.

Appendix:
Other salmonid sensory effects of dissolved copper

In this appendix, results are highlighted from several studies that we thought were particularly relevant, including comparing the concentrations that have caused sensory effects to concentrations causing lethality or growth reductions in field and laboratory experiments. As such, the following review is not an exhaustive summary of copper's adverse effects to anadromous salmonids. We emphasize studies that were conducted in waters with low alkalinity and hardness (< 50 mg/L as calcium carbonate), and if reported, low concentrations of dissolved organic material. These conditions were emphasized since we believe these are the most relevant water quality conditions for an area of particular concern to us – freshwater habitats used by juvenile salmonids in the Pacific Northwest and California, USA.

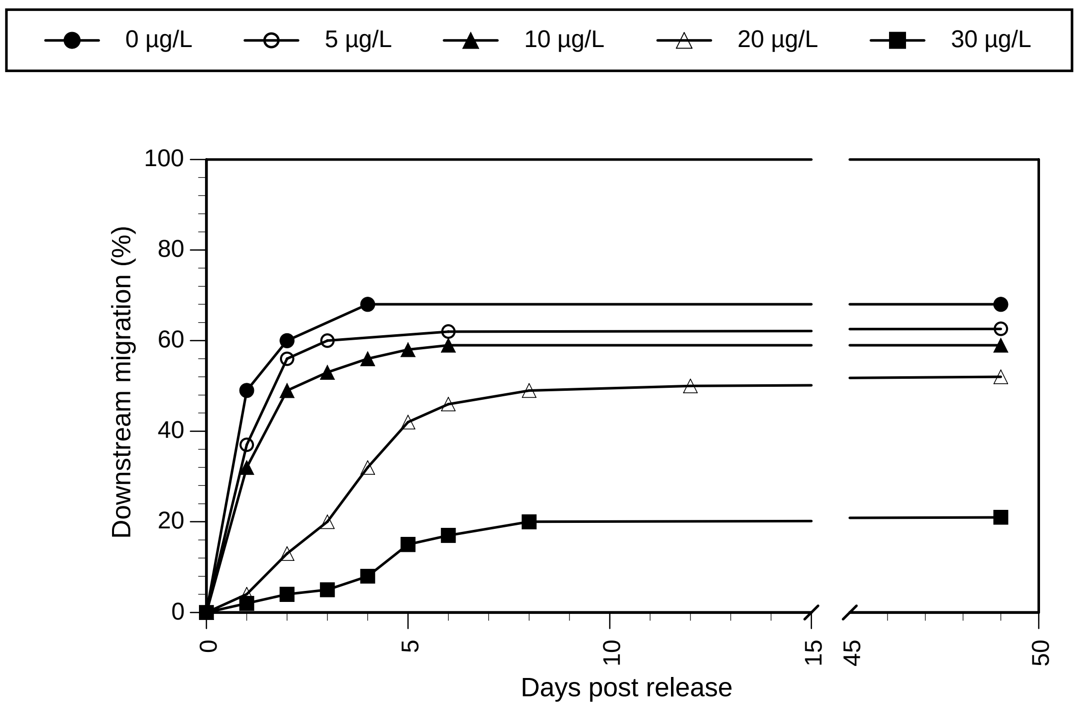
Migratory disruption

Laboratory and field experiments with salmonids have shown avoidance of low concentrations of copper, disruption of downstream migration by juvenile salmonids, loss of homing ability, and loss of avoidance response to even acutely lethal concentrations of copper follow long term habituation to low level copper exposure. Saucier et al. [16] examined the impact of a long-term sublethal copper exposure (22 ug/L; 37-41 weeks in duration) on the olfactory discrimination performance in rainbow trout. When controls were given a choice between their own rearing water or other waters, they significantly preferred their own rearing water, whereas both copper-exposed groups showed no preference. They concluded that their results demonstrate that a long-term sublethal exposure to copper, as it commonly occurs under "natural" conditions, may result in olfactory dysfunction with potential impacts on fish survival and reproduction.

Field studies have reported that copper impairs both upstream spawning migration of salmonids and downstream out migration of juveniles. Avoidance of copper in the wild has been demonstrated to delay upstream passage of Atlantic salmon moving past copper-contaminated reaches of the river to their upstream spawning grounds, unnatural downstream movement by adults away from the spawning grounds, and by increased straying from their contaminated home stream into uncontaminated tributaries. Avoidance thresholds in the wild of 0.35 to 0.43 toxic units were about 7-times higher than laboratory avoidance thresholds (0.05 toxic units) perhaps because the laboratory tests used juvenile fish rather than more motivated spawning adults. For this study 1.0 toxic unit was defined as an incipient lethal level, ILL (essentially a time independent LC_{50}), of 48 µg/l in soft water [12, 48]. Studies of home-water selection with returning adult salmon showed that addition of 44 µg/l copper to their home-water reduced the selection of their home stream by 90% [49]. Releases of about 20 µg/l from a mine drainage into a salmon spawning river resulted in 10 - 22% repulsion of ascending salmon during four consecutive years compared to 1- 2% prior to mining [49]. The upstream spawning migration of Chinook salmon in Panther Creek, Idaho may have been interrupted during the 1980s and early 1990s when the fish encountered dCu concentrations of 10- 25 µg/l. In Panther Creek, the majority of spawning habitat and historical locations of Chinook spawning were high in the watershed, upstream of copper discharges. However, Chinook were only observed spawning below the first major diluting tributary, a point above which copper concentrations averaged about 10- 25 µg/l during the times of the spawning observations [13, 50].

Sublethal copper exposure has been shown to interfere with the downstream migration to the ocean of yearling coho salmon. Lorz and McPherson [14, 15] and Lorz et al. [51] evaluated the effects of copper exposure on salmon smolts' downstream migration success in a series of 14 field experiments. Lorz and McPherson [14, 15] exposed yearling coho salmon for six to 165 days to nominal copper concentrations varying from 0 - 30 µg/l. They then marked and released the fish during the normal coho migration period and monitored downstream migration success. The fish were released simultaneously, allowing for evaluation of both copper exposure concentrations and exposure duration on migration success. All dCu exposures resulted in reduction of migration compared with unexposed control fish. Migration success decreased with both increasing copper concentrations and increased exposure time for each respective concentration. Exposure to 30 µg/l dCu for as little as 72 hours caused a considerable reduction in migration (~60%) compared to control fish. The reductions in migration following short-term exposures to dCu are illustrated in Figure 4, which was re-drawn from Lorz and McPherson [14]. Following exposure to 30 ug/L dCu, 80% of coho did not reach the migratory point in 49 days. These concentrations (5-20 ug/L) were one-tenth to one-third the 96-hour LC_{50} for the same stock of juvenile coho salmon in the same water. Lorz et al. [51] further tested downstream migration with yearling coho salmon previously exposed to copper, cadmium, copper-cadmium mixtures, zinc, and copper-zinc mixtures. Copper concentrations in all tests were held at 10 µg/l. In all cases, the copper exposed fish again had poorer migratory success than did controls. The other metals did not show the dose-dependent result found for copper. These studies suggest that exposure to copper concentrations at levels found in streams subject to nonpoint copper pollution may impair downstream migration, a result of direct and indirect effects to salmon smolts, including reproductive success.

Figure 4. Reduction in downstream migration of yearling coho salmon following 6-days exposure to copper at various concentrations. Redrawn from Lorz and McPherson [14], their figure 19.



Laboratory avoidance studies

Studies have shown that salmonids can detect and avoid copper at low concentrations when tested in troughs or streams that allow them to choose between concentration gradients. To our knowledge, the lowest copper concentration reported to cause avoidance in laboratory conditions was 0.1 µg/L [52]. However, these results may have low applicability to ambient conditions because copper exposure concentrations were not analytically verified. Avoidance thresholds of 2 µg/l copper have been reported for Atlantic salmon (*Salmo salar*), concentrations that are less than one-tenth of acute LC50 values [48]. Giattina et al. [53] reported that rainbow trout appeared to detect copper concentrations down to 1.4 to 2.7 µg/L, because declines in residence time started to occur at these lower concentrations. However, the responses were only statistically significant at 4.4 to 6.4 µg/l depending on whether fish were exposed to a gradually increasing or abruptly increasing concentration gradient respectively. At exposure to extremely high dCu levels e.g. 330-390 µg/L, trout showed diminished avoidance and sometimes attraction to acutely lethal concentrations [11,53,54].

Chapman [54] reported that long-term sublethal copper exposures had impaired the avoidance performance of salmonids. Steelhead trout, acclimated to low copper levels by surviving about three-months early life stage toxicity testing, subsequently failed to avoid much higher, acutely lethal concentrations. Following about three-month continuous exposure to 9 µg/l copper (from fertilization to about 1-month after swim up) the copper-acclimated fish and control fish with no previous copper exposure were exposed to a range of copper concentrations from 10 to 80 µg/l in avoidance-preference testing. The tests used the same counter flow avoidance-preference test chambers described by Giattina et al. [53]. The acclimated trout failed to avoid even the highest copper concentrations while most of the unexposed fish avoided all concentrations.

Hansen et al. [11] and Marr et al. [55] conducted a variety of behavioral and other toxicity studies with Chinook salmon and rainbow trout exposed to copper. In these studies they used well water that was diluted with deionized water and spiked with copper to obtain a hardness, alkalinity, and pH that simulated those in Panther Creek, a mine-affected stream in Idaho. The avoidance response of the Chinook salmon was statistically significant for 0.8 and 2.8 -22.5 µg/L copper but was not significant for a 1.6 µg/L copper treatment. Since the avoidance responses (percent time spent in test water) were similar between the 0.8, 1.6, and 3 µg/L treatments, but the 1.6 µg/L treatment had fewer replicates than the other treatments (10 vs. 20), the lack of statistical significance for the 1.6 µg/L treatment was probably an artifact of the different sample sizes than a true lack of response. Rainbow trout consistently avoided copper at concentrations of 1.6 µg/L and above. To simulate avoidance responses that might result upon exposing fish to background levels of copper, Hansen et al. [11] acclimated both Chinook salmon and rainbow trout to 2 µg/L copper for 25 days, and repeated the avoidance experiments. They observed that the avoidance response of Chinook salmon was greatly dampened such that no copper treatments resulted in statistically significant responses. In contrast, the avoidance response of rainbow trout was unaffected by the acclimation. This dramatic difference between Chinook salmon and rainbow trout avoidance was so unexpected that Hansen et al. [11] ran a second set of experiments that yielded the same results. Background dCu concentrations (<4 µg/L) are commonly observed in natural waterways, yet Chinook salmon failed to avoid any higher dCu concentrations following an acclimation to a nominal 2 µg dCu/L. Importantly, if Chinook salmon will not avoid any dCu concentrations following acclimation to low dCu concentrations, the behavioral defense against chronic and acute exposures to dCu is lost, and high mortality or chronic physiological effects are probable if subsequent higher levels of dCu exposure occur. Unlike Chinook salmon, dCu-acclimated rainbow trout preferred clean water and avoided higher dCu concentrations. Other differences between Chinook salmon and rainbow trout avoidance responses to copper were that addition of 4 and 8 mg/L dissolved organic carbon (DOC) did not appreciably affect the avoidance response of Chinook salmon to copper, nor did altering pH across a range of 6.5 to 8.5. In contrast, the addition of DOC (4 and 8 mg/L) did reduce the avoidance response of rainbow trout to copper. Although variable, avoidance responses of rainbow trout were slightly stronger at pH 7.5 and 8.5 than at 6.5 [55].

A further repeated finding from these laboratory avoidance tests was that although rainbow trout, steelhead, and Chinook salmon avoided low concentrations of dCu, they were apparently intoxicated and sometimes attracted to very high concentrations [11, 53, 54]. The direct relevance of laboratory avoidance studies to the behaviors of fish in the wild is debatable since in natural waters fish likely select and move among habitats based on myriad reasons such as access to prey, shelter from predators, shade, velocity, temperature, and interactions with other fish. In contrast, laboratory preference/avoidance tests are commonly conducted under simple, highly artificial conditions to eliminate or minimize confounding variables other than the water characteristic of interest. Laboratory tests may overestimate the actual protection this behavior provides fish in heterogeneous, natural environments [56-58].

However, at least one study suggested that experimental avoidance responses observed with salmonids are relevant to fish behaviors in the wild. From 1980-1982, sub-lethal levels of a contaminant (fluoride) from an aluminum mill at the John Day Dam on the Columbia River were associated with a significant delay in salmon passage and decreased survival [59]. Salmon took an average of 36 hours to pass up the fish ladder at the Bonneville and McNary dams compared to 157 hours delay at the John Day Dam. Greater than 50% mortality occurred between the Bonneville and McNary dams (above and below the John Day dam), compared to about 2% mortality associated with the other dams. Damkaer and Dey [59] introduced similar levels of the contaminant in streamside test-flumes alongside a salmon spawning stream (Big Beef Creek, Washington). Significant numbers of adult Chinook salmon failed to move out of their holding area and continue upstream; those that did move upstream chose the non-contaminated side of the flume. By adjusting the dose, Damkaer and Dey [59] predicted a threshold detection limit for avoidance by salmon. The mill subsequently reduced its release of the contaminant to below these experimental threshold levels that did not show a response in the streamside tests. Afterwards, fish passage delays and salmon mortality between the dams decreased to 28 hours and <5%, respectively [59]. This study suggested that the delay due to avoidance of a chemical affected the spawning success of migrating adult salmonids. These results are also consistent with the field studies of salmon migration in copper-contaminated streams and from laboratory avoidance/preference testing. Experimental avoidance/preference testing thus appears to be relevant to fish behavior in nature.

Other adverse effects

The focus of this literature synthesis is sensory effects of copper on juvenile salmonids. However, other adverse effects of copper to salmonids reported in the literature include weakened immune function and disease resistance, increased susceptibility to stress, liver damage, reduced growth, impaired swimming performance, weakened eggshells, and direct mortality [19, 20, 60-66] . While a comprehensive review of other adverse effects of copper on fish is beyond the scope of this synthesis, we discuss several studies of interest below.

Stevens [65] reported that pre-exposure to sublethal levels of dCu interfered with the immune response and reduced the disease resistance in yearling coho salmon. Juvenile coho salmon were vaccinated with the bacterial pathogen *Vibrio anguillarum* prior to copper exposure to investigate the effects of copper upon the immune response and survival. Following copper exposure (9.6 - 40 µg/L), surviving juveniles were challenged under natural conditions to *V. anguillarum,* the causative agent of vibriosis in fish. Vibriosis is a disease commonly found in wild and captive fish from marine environments and has caused deaths of coho and Chinook salmon. Coho were exposed to constant concentrations of dCu for about one month at levels that covered the range from no effect to causing 100% mortality, 9.6 - 40 µg/L. The antibody titer level against *V. anguillarum* was significantly reduced in fish exposed to 13.9 µg/L of dCu when compared to that developed in control fish. The survivors of the dCu bioassays were then exposed in saltwater holding ponds for an additional 24 days to the *V. anguillarum* pathogen. The unvaccinated, non-dCu exposed control fish had 100% mortality and the vaccinated, non-dCu exposed fish had the lowest mortality. The vaccinated, dCu-exposed fish had increasing mortality corresponding to the lower antibody titer levels which in turn corresponded to the increasing dCu exposure levels. Therefore, dCu exposure can significantly reduce a fish's immune function and disease resistance at concentrations as low as 13.9 µg/L following 30 days of exposure [65].

Schreck and Lorz [60] studied the effects of copper exposure to stress resistance in yearling coho salmon. Fish that were exposed for 7 days to 15 µg/L dCu and unexposed control fish were subjected to severe handling and confinement stress. Copper-exposed fish survived this additional stress for a median of 12-15 hours while control fish experienced no mortality at 36 hours. Schreck and Lorz concluded that exposure to copper placed a sublethal stress on the fish which made them more vulnerable to handling and saltwater adaptation. Further, they hypothesized that dCu exposure may make salmonids more vulnerable to secondary stresses such as disease and pursuit by predators.

Exposure of brook trout eggs to 17.4 µg dCu/L for 90 days resulted in weakened chorions (eggshells) and embryo deformities. After hatching, poor yolk utilization and reduced growth were demonstrated. These overall weakened conditions may reduce survival chances in the wild [67, 68]. Copper accumulation in the liver of rainbow trout caused degeneration of liver hepatocytes, which resulted in reduced ability to metabolize food, reduced growth, or eventual death [17, 63, 69]. Waiwood and Beamish [61], Chapman [19], Seim et al. [70], McKim and Benoit [62], and Marr [20] have also observed reduced growth of salmonids in response to chronic copper exposures as low as 1.9 ug/L. Waiwood and Beamish [66] reported that rainbow trout exposed to copper levels had reduced swimming performance (10, 15, 20, 30 ug/L dCu) and reduced oxygen consumption (25, 40 ug/L dCu) apparently due to gill damage and decreased efficiency of gas exchange.

In sum, there is a large body of literature showing that behavior of salmonids and other fishes can be disrupted at concentrations of dCu that are only slightly elevated above background concentrations. Further, dCu stress has been shown to increase the cost of maintenance to fish and to limit oxygen consumption and food metabolism. Reduced growth may result in increased susceptibility to predation, and impaired swimming ability may result in reduced escape reaction and prey hunting, with a possible consequence of reduced survival at the population level. We summarize selected examples of effect concentrations reported with copper for several different types of effects in Table 2. In general, typical copper exposures probably do not kill juvenile salmonids directly until concentrations greater than about 10 times that of sensory thresholds, and then only if the concentrations are sustained for at least several hours. In selecting these examples, we sought to list representative effects and concentrations rather than extreme values that could be gleaned from the literature. However, the selected examples do not constitute an exhaustive review of the effects of copper to fish; more general reviews of effects of copper to fish and other aquatic organisms are available elsewhere [9, 17, 46, 71].

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