Impact of dissolved copper on the olfactory system of seawater-phase juvenile salmon

Estimated cost: **$36,767**

Oversight Group: Exposure and Effects Workgroup

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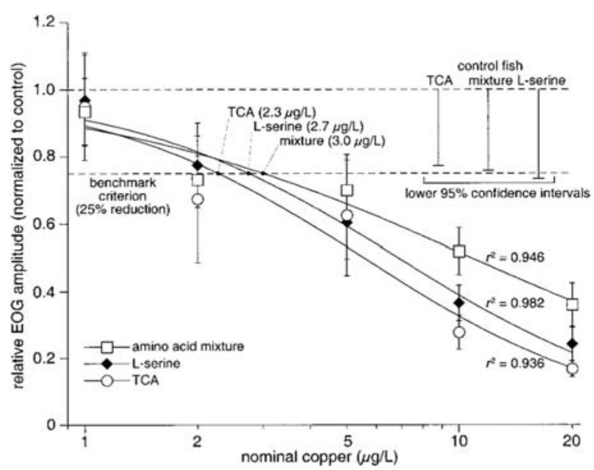
PROPOSED DELIVERABLES AND TIMELINE

Setup, exposures, electrophysiological recordings, and data analysis	June – Sept, 2011
Preparation of report	Sept – Nov, 2011

BACKGROUND

Copper is a ubiquitous contaminant of aquatic systems in urbanized and agricultural areas. In the San Francisco Bay estuary, elevated ambient copper concentrations result from a multitude of anthropogenic sources including, in decreasing order, erosion of buried sediments, inflow from the Sacramento and San Joaquin Rivers, urban and non-urban runoff, anti-fouling marine coatings, urban wastewater, atmospheric deposition, and industrial discharge (CRWQB 2007). Although the source of copper entering the Bay from the Sacramento and San Joaquin Rivers has not been assessed, one possible source is the application of copper-containing pesticides (data available online from the California Department of Pesticide Regulation Pesticide Use Reporting Program www.cdpr.ca.gov/docs/pur/purmain.htm).

In fish, exposures to dissolved copper concentrations that are sublethal are, nonetheless, known to be able to impair peripheral sensory systems (e.g. gustation, mechanosensation, and olfaction). For example, freshwater studies have shown that copper is toxic to fish mechanosensory (Hernandez et al. 2006; Linbo et al. 2006) and olfactory (Hansen et al. 1996; Baldwin et al. 2003) receptor neurons. Furthermore, inhibition of olfactory function is highly correlated with reduced ability to respond to an olfactory alarm cue that triggers anti-predation behavior in juvenile coho salmon (*Oncorhynchus kisutch*, Sandahl et al. 2007). In experiments with predatory cutthroat trout (*Oncorhynchus clarki*), survival of juvenile coho salmon was reduced 3- to 5-fold (McIntyre et al. in prep.). Significantly, these effects on the olfactory system can occur following exposure periods of as little as 30 minutes and increases in dissolved copper concentrations of as little as 3 µg/L (e.g. Baldwin et al. 2003; see Figure).



Dose-response curves and threshold determination for sub-lethal copper olfactory toxicity. Olfactory responses to three different odors were reduced following 30-minute increases in copper. Figure 5 from Baldwin et al. (2003).

Current site-specific copper objectives (SSOs) for San Francisco Bay were derived, in large part, from 96-hour tests on the development of larval *Mytilus edulis*. As per EPA guidance, toxicity tests in site water and standard laboratory waters were compared to account for the local influence of water chemistry on the bioavailability of copper to relevant ligands in this organism. When considering copper neurotoxicity to peripheral sensory systems of sensitive fish taxa, it is important to realize that water chemistry may not have the same influence as on the development of larval *Mytilus edulis*. In tests on peripheral sensory systems in freshwater fishes, hardness and alkalinity provided little to no protection from copper neurotoxicity (McIntyre et al. 2008; Linbo et al. 2009). This differs from the conventional understanding of the influence of water quality on copper toxicity derived from the Biotic Ligand Model (BLM). Dissolved organic matter (DOC) provided some protection against copper neurotoxicity, but not to the extent expected from BLM-based predictions. These results are likely because the copper dynamics modeled by the BLM are based on interactions with ligands at the fish gill, not at sensory epithelia.

Currently, the copper SSOs are higher than concentrations at which effects on olfactory neurophysiology (Baldwin et al. 2003) and behaviour (Sandahl et al. 2007; McIntyre et al. in prep.) were seen in juvenile coho salmon. However, studies on copper neurotoxicity to peripheral sensory systems have thus far been conducted exclusively in freshwater. Given the uncertainty about the protectiveness of seawater from copper olfactory neurotoxicity, it is difficult to extrapolate effects thresholds to the different water chemistry of marine and estuarine waters. Additionally, differences between the biology of freshwater-phase versus seawater-phase juvenile salmon produce another source of uncertainty. The goal of this study is to determine the impacts of dissolved copper of the olfactory system of seawater-phase juvenile salmon in order to compare these effects to those known for freshwater-phase juvenile salmon and the copper SSOs for the San Francisco Bay estuary.

APPLICABLE RMP OBJECTIVES AND MANAGEMENT QUESTIONS

4.3 What ecological risks are caused by pollutants of concern? This study will determine what levels of dissolved copper pose a risk to the olfactory system of seawater-phase juvenile salmon.

4.6 Which forms of pollutants cause impairment? In addition to being present as free copper ions (Cu^{2+}), dissolved copper will form complexes (i.e. species) with other constituents of water (e.g. hydroxyl ions, carbonates, and dissolved organic molecules). These other species are considered to be much less toxic with respect to acute mortality and development. However, some of them may still be toxic to the olfactory system. The ability of seawater to form copper complexes, therefore, may provide less protection to the olfactory system of salmon than expected based on acute mortality.

5.1 What percentage of the Estuary is supporting beneficial uses? This study will address whether copper SSOs are likely to protect the olfactory system of juvenile salmon in the SF Bay estuary from sublethal impairment.

APPROACH AND OBJECTIVE

This study will be based on previous NOAA studies that measured copper olfactory toxicity in freshwater-phase juvenile salmon (Baldwin et al. 2003; Sandahl et al. 2007; McIntyre et al. 2008). As was done in these previous studies, the impact of copper exposure on the sensitivity of the salmon olfactory system to odors will be measured using direct electrophysiological recordings (electroolfactograms; EOGs) from the olfactory epithelium (Baldwin et al. 2003). While the effect of copper on the EOGs of seawater-phase salmon has not been measured, the methods have been adapted to record EOGs in seawater-phase cutthroat trout (Labenia et al. 2007). The experiments will be performed at the Northwest Fisheries Science Center's (NWFSC) Mukilteo Field Station (Mukilteo, WA) using Chinook salmon (*Oncorhynchus tshawytscha*). Fertilized eggs will be obtained from a local hatchery and reared in freshwater at Mukilteo until fish are the appropriate age for smolting. The majority of the fish will then be transitioned to seawater and maintained in seawater for the duration of the experiment, while a subset will remain housed in freshwater. Water samples will be collected for analyses of water chemistry parameters (e.g. salinity and DOC) and copper concentrations.

Determine the threshold for effects of dissolved copper on the olfactory system of seawater-phase juvenile salmon. The proposed studies are expected to be initiated in June 2011 and should be complete by the end of the fiscal year (October 2011). To determine the effect of copper on the olfactory system, odor-evoked EOGs will be obtained from fish using a standard odorant, the amino acid L-serine. Fish will be either unexposed to copper (controls) or exposed for a short period (30 minutes) to one of at least 3 copper concentrations (ranging from 2-20 µg/L, but higher if needed). Copper-induced changes in the sensitivity of the olfactory system will be measured as a dose-dependent reduction in the amplitude of the odor-evoked EOGs from which an effect threshold for copper can be determined (e.g. see figure above). While the focus of the EOG recordings will be on seawater-phase juvenile Chinook salmon, fish that weren't smolted will be available, if necessary, to confirm the impact of copper on the olfactory system of freshwater-phase Chinook.

Since DOC is known to affect the toxicity of copper to the olfactory system (McIntyre et al. 2008), exposures will be performed at three different DOC concentrations representative of the SF Bay (e.g. 2, 4, and 6 mg/L). To the extent possible, the source of DOC used to amend the local seawater (which is ~2 mg/L DOC) will have copper binding properties similar to that of DOC from the SF estuary. The aim of the study will be to determine the effects threshold for the olfactory toxicity of copper in seawater-phase juvenile salmon at various DOC concentrations in order to compare this with thresholds measured from freshwater-phase salmon and with the copper SSOs in the SF Bay estuary.

REQUESTED BUDGET:

Biological Technician contractor salary (4 months)	$19,767
Equipment	$6,000
Supplies	$3,000
Travel	$2,000
Copper and water analyses	$6,000
Total	**$36,767**

NOAA contributions will include fish, fish husbandry, additional equipment and salary support (e.g. technicians and PI).

Cited literature:

Baldwin, D. H., J. F. Sandahl, J. S. Labenia, and N. L. Scholz. 2003. Sublethal effects of copper on coho salmon: Impacts on nonoverlapping receptor pathways in the peripheral olfactory nervous system. Environmental Toxicology and Chemistry **22**:2266-2274.

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Linbo, A. O., C. M. Stehr, J. P. Incardona, and N. L. Scholz. 2006. Dissolved copper triggers cell death in the peripheral mechanosensory system of larval fish. Environmental Toxicology and Chemistry **25**:597-603.

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