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Effects of salinity on olfactory toxicity and behavioral responses of juvenile salmonids from copper

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ABSTRACT

Dissolved copper is one of the more pervasive and toxic constituents of stormwater runoff and is commonly found in stream, estuary, and coastal marine habitats of juvenile salmon. While stormwater runoff does not usually carry copper concentrations high enough to result in acute lethality, they are of concern because sublethal concentrations of copper exposure have been shown to both impair olfactory function and alter behavior in various species in freshwater. To compare these results to other environments that salmon are likely to encounter, experiments were conducted to evaluate the effects of salinity on the impairment of olfactory function and avoidance of copper. Copper concentrations well within the range of those found in urban watersheds, have been shown to diminish or eliminate the olfactory response to the amino acid, L-serine in freshwater using electro-olfactogram (EOG) techniques. The olfactory responses of both freshwater-phase and seawater-phase coho and seawater-phase Chinook salmon, were tested in freshwater or seawater, depending on phase, and freshwater-phase coho at an intermediate salinity of 10‰. Both 10‰ salinity and full strength seawater protected against the effects of 50 μg copper/L. In addition to impairing olfactory response, copper has also been shown to alter salmon behavior by causing an avoidance response. To determine whether copper will cause avoidance behavior at different salinities, experiments were conducted using a multi-chambered experimental tank. The circular tank was divided into six segments by water currents so that copper could be contained within one segment yet fish could move freely between them. The presence of individual fish in each of the segments was counted before and after introduction of dissolved copper (<20 μg/L) to one of the segments in both freshwater and seawater. To address whether use of preferred habitat is altered by the presence of copper, experiments were also conducted with a submerged structural element. The presence of sub-lethal levels of dissolved copper altered the behavior of juvenile Chinook salmon by inducing an avoidance response in both freshwater and seawater. While increased salinity is protective against loss of olfactory function from dissolved copper, avoidance could potentially affect behaviors beneficial to growth, survival and reproductive success.

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1. Introduction

Dissolved copper is one of the more pervasive and toxic constituents of stormwater runoff and is commonly found in stream, estuary, and coastal marine habitats of juvenile salmon. Copper enters the aquatic environment from a variety of sources (Davis et al., 2001): these include treated wood products, pesticides, and anti-fouling paint from recreational vessels. Metal-containing dust shed from trucks and automobiles is thought to be the greatest contributor (Davis et al., 2001). Concentrations in stormwater runoff vary greatly depending on time of year and surrounding land use. A study on the Governor Albert D. Rosellini Bridge-Evergreen point on SR-520 in Seattle, WA, which isolated stormwater runoff primarily to road deposition on a busy highway found copper levels between 23 and 300 μg/L (McIntyre et al., 2014). Other measurements of stormwater runoff from outputs in and around Sinclair/Dyes Inlets watersheds' ranged from a median of 1–75 μg/L (Hart-Crowser, 2007). With increasing urbanization, it is likely that animals that inhabit the estuarine and nearshore waters, including listed species of Pacific salmon, will be exposed to copper.

Sublethal levels of dissolved copper commonly found in urban aquatic systems have been shown to disrupt olfactory function

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(Tierney et al., 2010), behavior (Goldstein et al., 1999; Kleerekoper, 1976; McPherson et al., 2004; Scott and Sloman, 2004), and survival (McIntyre et al., 2012), primarily in freshwater. Contaminants entering freshwater usually find their way into the estuarine and nearshore environments where salinities range between freshwater and full seawater. Unfortunately, little work has been done to determine whether dissolved copper affects fish at different salinities. While copper is far less acutely toxic in seawater than it is in freshwater (Blanchard and Grosell, 2006), the presence of sublethal copper concentrations in estuarine and seawater environments has the potential to disrupt normal fish behavior and development for many species, particularly salmonids at a critical life stage. Juvenile Chinook salmon, for example, depend on estuarine habitats to provide food resources for rapid growth and submerged structure provides shelter from predators while transitioning from freshwater to seawater (Healy, 1982; Thorpe, 1994). Loss and degradation of these habitats are considered to be factors contributing to the decline of wild populations of Pacific salmonids throughout their range. Functioning peripheral sensory systems are essential to successfully utilize these habitats, while copper avoidance could mean fish are not able to effectively use these critical habitats.

The toxicity of copper varies depending on fish species, life stage and water chemistry, and the mechanism of toxicity appears to be different in freshwater than in seawater (Chapman, 1978). Studies of the sub-lethal effects of contaminants on fish physiology in freshwater have demonstrated copper-linked neurophysiological effects on the olfactory system in salmonids resulting in loss of smell and olfactory-triggered predator avoidance behaviors (McIntyre et al., 2008) and neurotoxicity in zebrafish (*Danio rerio*) that targets lateral line neurons (Linbo et al., 2009). These studies link copper exposure to physiological damage and related behavioral effects. Olfaction is also important for feeding (Hara, 2006), sibling recognition (Quinn and Busack, 1985), and predator avoidance (Brown, 2003) as well as being critical for stream imprinting as juveniles (Wisby and Hasler, 1954). The sense of smell may also play an important role in avoiding contaminants. Salmon exposed to low levels of dissolved copper have been shown to lose the avoidance response to dissolved copper (Hansen et al., 1999). While no direct connection between olfaction and avoidance of copper has been made, it is likely that olfaction plays a key role.

Many studies have shown avoidance of copper in freshwater (Giattina et al., 1982), but only two have been conducted in seawater (Koltes, 1985; Labenia et al., 2006). While copper avoidance was not the primary objective of either of these studies, results are consistent with responses in freshwater. Copper concentrations however, were at the high end of what occurs in the environment (100 μg/L and 40 μg/L respectively). The paucity of studies examining the effects of copper on fish in seawater has led to an information gap concerning the effects of copper on fish in the estuarine and nearshore environment. One important component of salmon habitat that has been well studied in freshwater is submerged physical structure, usually in the form of woody debris (Roni et al., 2002). The importance of submerged structure as fish make the transition from fresh to seawater and move out into the estuary and nearshore is less well understood but the potential for the disruption of normal behavior and use of the estuarine environment may have implications for success at this life stage. Avoidance of copper may limit the use of otherwise suitable habitat. Alternately, preference for habitat may increase the risk of exposure to contaminants.

To evaluate how changes in salinity affect the response of juvenile salmon to dissolved copper concentrations typical of urban estuaries, we conducted two separate laboratory experiments.

To assess whether salinity alters the impact of copper on olfactory function in juvenile salmonids, neurophysiological responses to an introduced odorant were recorded from the olfactory epithelium before and after exposure to dissolved copper. To examine conditions typical of estuaries, fish were tested in different salinities and at pre and post-smolt life stages. This was done with both coho (*Oncorhynchus kisutch*) and Chinook (*Oncorhynchus tshawytscha*) juveniles. Also, whether the presence of copper alters the behavior of salmon by inducing an avoidance response was tested using a multi-chambered experimental tank. This behavioral tank allowed us to determine attraction to submerged structure, avoidance of copper, and the interaction between the two.

We were able to demonstrate that copper reduces the olfactory response in freshwater but not in an intermediate salinity of 10‰ or in full seawater. Copper induced an avoidance response in both freshwater and seawater. Additionally, fish were attracted to submerged structure in freshwater though not in seawater but that attraction disappeared when copper was present.

2. Methods

2.1. Animals

2.1.1. Olfactory function

Fall Chinook salmon were acquired as fry from the Wallace River hatchery (Sultan, WA) and transported to the NOAA Fisheries Mukilteo Field Facility in Mukilteo, WA for rearing and experiments. Fish were fed standard commercial salmon pellets (Bio-Oregon, Warrenton, OR) and held in a 1.8 m diameter circular fiberglass tank on a recirculating freshwater system supplied by carbon filtered city water (100–300 mg/L total hardness as $CaCO^3$, pH 7.1–7.3, temperature 8–12 °C, oxygen 9–12 mg/L) until smolted over one week. Following transition to seawater, fish were held in flow-through, sand-filtered seawater, (salinity 30‰, pH 7.8, oxygen 9.5 mg/L, temperature 11 °C). This water also served as the background solution for the EOG recordings. Fish used during the experiment were a mean (±SD) fork length (FL) of 12.7 (±0.7) cm and a weight of 23.5 (±4.5) g.

Coho salmon (*O. kisutch*) were reared at the Northwest Fisheries Science Center's (NWFSC) hatchery facility in Seattle, WA. Freshwater coho were maintained on recirculating dechlorinated municipal water (10 °C, pH 7.2, hardness 85–100 mg/L). Freshwater coho used in the study were (mean ± SD) 13.7 ± 1.2 cm FL, and 30.8 ± 8.0 g. Seawater coho used in the study were transitioned over the course of a week to a recirculating seawater system (11 °C, pH 8–8.2), and were 16.9 ± 1.4 cm FL and 56.7 ± 17.4 g.

2.1.2. Copper avoidance

Fall Chinook salmon were acquired as eyed eggs from the University of Washington's School of Fisheries hatchery (Seattle, WA) and transported to the NOAA Fisheries Mukilteo Field Facility in Mukilteo, WA for rearing and experiments. Fish were fed standard commercial salmon pellets (Bio-Oregon) and held in a 1.8 m diameter circular fiberglass tank on a recirculating freshwater system supplied by carbon filtered city water (100–300 mg/L total hardness as $CaCO^3$, pH 7.1–7.3, temperature 8–12 °C, oxygen 9–12 mg/L) until smolted (Meador et al., 2006). Prior to smolting, forty fish to be used in the freshwater portion of the experiment were transferred to an indoor, circular, 1.2 m diameter tank on a separate recirculating freshwater system supplied by the same water source. The remaining fish were smolted and held on flow-through, sand-filtered seawater, (salinity 30‰, pH 7.8, oxygen 9.5 mg/L, temperature 10–12 °C). The recirculating system for the freshwater trials of the experiment was replenished from city water passed through a 5 μm filter and an activated carbon filter and chilled to between 12 and 14 °C. Seawater for the experiment was sand filtered and passed through a 20 μm cartridge filter and 10 μm bag filter. Temperature was maintained at 10–12 °C.