Aquatic Toxicology 101 (2011) 295–297

Contents lists available at ScienceDirect

Aquatic Toxicology

journal homepage: www.elsevier.com/locate/aquatox

Short communication

Copper-induced olfactory toxicity in salmon and steelhead: Extrapolation across species and rearing environments

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ARTICLE INFO

Article history:
Received 21 June 2010
Received in revised form 18 August 2010
Accepted 24 August 2010

Keywords:
Copper
Salmonid
Olfaction
Hatchery

ABSTRACT

Recent research has shown that hatchery coho salmon (*Oncorhynchus kisutch*) are vulnerable to the olfactory neurotoxicity caused by copper from urban runoff, pesticide use, and mining activities. To explore the broader application of this data to salmonids living in the wild, we exposed naturally-reared steelhead (*O. mykiss*) to copper (5 and 20 μg/L; 3 h) and measured losses in olfactory function via electro-olfactogram (EOG) recordings. Copper exposure disrupted the olfactory responsiveness of steelhead to an amino acid (L-serine) in a dose-dependent manner that was equivalent to previously published data for hatchery coho. Our findings support extrapolation of copper toxicity data across species and from fish raised in hatcheries to fish in the wild.

Published by Elsevier B.V.

In the western United States, anadromous salmonid species (genus *Oncorhynchus*) are now listed under the U.S. Endangered Species Act (ESA) including distinct populations of coho (*O. kisutch*), Chinook (*O. tshawytshca*), sockeye (*O. nerka*), chum salmon (*O. keta*), and steelhead (*O. mykiss*) (Good et al., 2005). Degraded freshwater quality is one factor contributing to salmon declines, particularly in watersheds with intensive human activity. Copper, for example, is a metal widely used in building materials (e.g., copper roofs and treated lumber), automobile parts (e.g., brake pads), and pesticides (Davis et al., 2001). Consequently, copper is often a pervasive contaminant in urban and agricultural watersheds where juvenile salmon and steelhead rear prior to oceanic migration.

Short-term copper exposures (a few minutes to a few hours) at low, environmentally relevant concentrations (<10 μg/L) have been shown to interfere with salmonid olfaction – specifically, the sensory detection of (and behavioral response to) ecologically important chemical signals in the aquatic environment (Baldwin et al., 2003; Sandahl et al., 2007). Recent research on the neurobehavioral toxicity of copper, however, has largely focused on hatchery-reared coho salmon (e.g., Baldwin et al., 2003; Sandahl et al., 2007) and uncertainties remain about whether wild salmonids and their hatchery counterparts are similarly susceptible to the olfactory toxicity of copper. Behavioral and physiological differences between hatchery and wild salmonids, attributable to different rearing environments, have been documented (see reviews by Einum and Fleming, 2001; Weber and Fausch, 2003). Moreover, the assessment of differences between hatchery and naturally-reared fish can depend on experimental setting (i.e., effects seen in the laboratory might not manifest in natural settings, Riley et al., 2005; Tatara et al., 2008). Lastly, different species of salmon and steelhead may vary in their response to the toxic effects of copper (see, for example, Hansen et al., 1999).

The objective of the current study was to assess whether the olfactory toxicity of copper in a naturally-reared salmonid (in this case, steelhead) is equivalent to that previously reported in hatchery-reared coho in order to determine whether juvenile hatchery-reared coho are reliable surrogates for other at-risk wild salmonids. We focused on juvenile steelhead because several wild steelhead populations along the west coast of the United States have declined to the extent that they now require federal protection under the ESA.

We raised juvenile steelhead under natural stream conditions and used conventional neurophysiological recordings to measure the impacts of dissolved copper on the responsiveness of the olfactory epithelium to a conventional odorant (the amino acid L-serine). The results were compared to previously published toxicity data for hatchery coho using the same recording methods, copper exposure concentrations (5 and 20 μg/L), and fish of a similar size and age (Sandahl et al., 2007).

The fish used in the experiment were produced from artificially spawned hatchery-origin steelhead from the Skookumchuck River (Washington State). Fertilized eggs were incubated at the Washington Department of Fish and Wildlife's Bingham Creek Hatchery to the eyed egg stage and then transported to the University of Washington's Big Beef Creek (BBC) Research Station. Upon reaching the fry stage, ~4900 fish were released into a 35 by 5-m screened

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0166-445X/$ – see front matter. Published by Elsevier B.V.
doi:10.1016/j.aquatox.2010.08.011

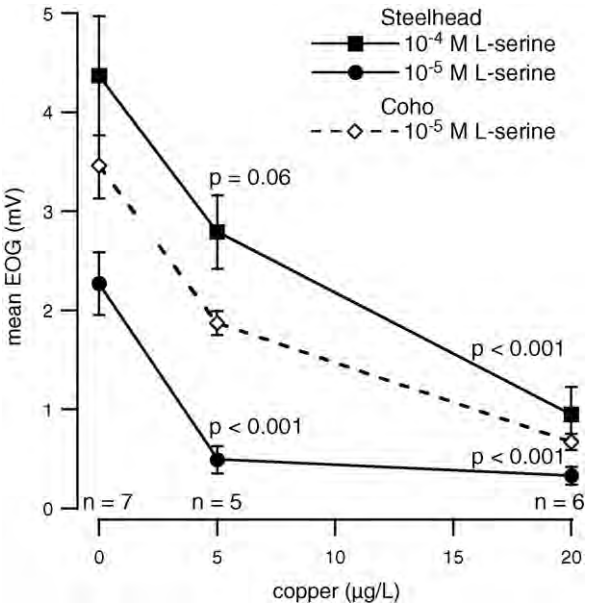

Fig. 1. Naturally-reared steelhead exposed to copper show a significant and dose-dependent decrease in the amplitude of electro-olfactograms (EOGs) evoked by two concentrations of the odorant L-serine (one-way ANOVA, both p values <0.0004). For each concentration, the p values on the graph denote the results of comparisons to the respective controls (Dunnett's post hoc). For comparison, open symbols and the dashed line show data from coho (Sandahl et al., 2007; prior to blank subtraction). Error bars represent one standard error.

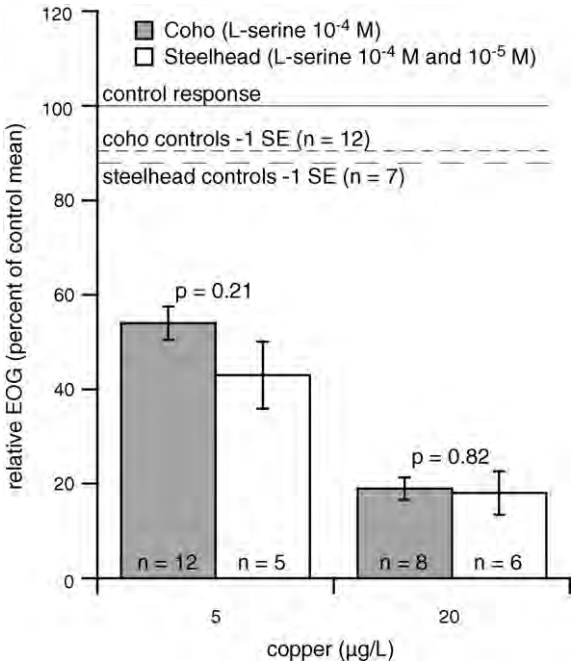

Fig. 2. Copper-induced reductions in electro-olfactograms (EOGs) evoked by L-serine in naturally-reared steelhead are similar to those seen in hatchery-reared coho. Data for coho are from Sandahl et al. (2007). For each species prior to averaging, each EOG was normalized to the mean EOG response magnitude for unexposed controls for the specific concentration. For each copper exposure, p values are the results from t-tests comparing the two species. Error bars represent one standard error (SD).

side channel of BBC for natural rearing (described in Riley et al., 2005). The channel had a gravel-cobble substrate, natural early successional riparian vegetation, and flow of ~0.05 $m^3\ s^{-1}$ originating from artesian springs and Big Beef Creek. Fry used in the assays were collected by seining and held for up to 3 days in an enclosure within the stream channel prior to use. The average daily temperature of the stream channel (±1 standard deviation [SD]) was 11.3 ± 0.9 °C. Copper-exposed animals were 6 months old, with an average fork length and weight (±1 SD) of 4.9 ± 0.8 cm and 1.2 ± 0.6 g. The juvenile coho salmon in the comparison group (Sandahl et al., 2007) were 4–5 months old, with an average fork length and weight (±1 SD) of 4.6 ± 0.4 cm and 0.9 ± 0.2 g.

Copper exposures followed the methods of Sandahl et al. (2007). Each week a 25 mg/L copper stock solution was made from copper chloride (Sigma Chemical Co., St. Louis, MO, USA; 99% purity $CuCl_2$, dihydrate) dissolved in BBC well water. Nominal exposure concentrations of 0, 5, or 20 µg/L were prepared by adding the appropriate amount of stock solution to 25 L of BBC water in aerated 30 L glass exposure aquaria. Individual fish were exposed in separate tanks for 3 h. Although different exposure concentrations were tested on any given day, at least one fish from the control group (0 µg/L) was tested on each day. Measured dissolved copper concentrations (ICP-MS; Frontier Geosciences, Seattle, WA, USA) of samples of the BBC water, the 20 µg/L exposure tank, and the stock solution were <0.04 µg/L (below detection limit), 16.6 µg/L, and 21.6 mg/L, respectively. Copper exposures are hereafter expressed as nominal concentrations. BBC water had a hardness of 58 mg/L (as $CaCO_3$), alkalinity of 52 mg/L ($CaCO_3$), and pH of 7.5 (Am-Test, Seattle, WA).

Odor-evoked electro-olfactograms (EOGs) were recorded from the peripheral olfactory epithelium of each steelhead using established procedures (Baldwin et al., 2003; Sandahl et al., 2007). Odorant solutions containing the amino acid L-serine dissolved in BBC water (10^{-5} and 10^{-4} M) were prepared daily from a concentrated stock. The two stimulus concentrations were chosen because they evoked baseline olfactory responses bracketing the response to 10^{-4} M L-serine previously reported for hatchery coho (see Fig. 1; Sandahl et al., 2007). While intriguing, the slightly larger response to 10^{-4} M L-serine in wild-reared steelhead may reflect differences in experimental conditions (e.g., exposure water, odorants present in the background water, minor differences in electrode placement, etc.) rather than a species difference in the sensitivity to L-serine. Rather than comparing the absolute amplitudes of the two studies, the olfactory toxicity in steelhead was compared to data for coho (Sandahl et al., 2007) by normalizing each olfactory response to the mean for the study's unexposed controls. For the steelhead data, for each fish the responses to the two L-serine stimulus concentrations (10^{-5} and 10^{-4} M) were combined to obtain a single average L-serine response relative to controls.

The amplitudes of L-serine-evoked olfactory responses in unexposed steelhead and fish exposed to 5 and 20 µg/L copper are shown in Fig. 1. For both L-serine stimulus concentrations, a 3 h copper exposure produced a dose-dependent loss of olfactory function (one-way ANOVA; both p values <0.0004). The dose–response curves were also similar to that of coho (Sandahl et al., 2007). More specifically, as shown in Fig. 2, the reductions in olfactory sensitivity following exposures to both 5 and 20 µg/L were not statistically different between naturally-reared juvenile steelhead and hatchery juvenile coho (t-test; $p > 0.05$).

These findings are consistent with previous studies that have evaluated the olfactory toxicity of copper in salmonids over the past three decades. By various different analytical measures, copper has been shown to disrupt olfaction and olfactory-mediated behaviors in hatchery-reared fish (reviewed in Tierney et al., 2010) including Chinook (Hansen et al., 1999), coho (Baldwin et al., 2003), chum (Sandahl et al., 2006), and steelhead as well as Atlantic salmon (*Salmo salar*) and non-anadromous rainbow trout (*O. mykiss*) (Hara et al., 1976; Hansen et al., 1999). Where electrophysiological recording methods have been used, the threshold for copper neurotoxicity has been remarkably similar across species. For example, our observation of a significant loss of olfactory responsiveness at copper concentrations of 5 µg/L (for both coho and steelhead) is

very close to the threshold for olfactory toxicity (~7 μg/L) reported for rainbow trout by Hara and colleagues as early as 1976 (Hara et al., 1976).

Artificial rearing environments and genetically based differences between hatchery and wild salmonids (within and between species) are known sources of variation in physiology, morphology, and behavior (Einum and Fleming, 2001; Weber and Fausch, 2003). Surprisingly, however, there has been little research to assess how this variation might influence physiological susceptibility to toxic chemical contaminants. Our comparison of the olfactory neurotoxicity data for dissolved copper across salmonid species (coho and steelhead) raised in different environments (natural and hatchery) suggests negligible variation across both factors. While this study does not address the potential for genetically based differences in olfactory toxicity among hatchery and wild populations, given the lack of differences across species, it seems unlikely that the olfactory toxicity of copper would differ within a species across different genetic stocks.

Although copper olfactory toxicity research has largely focused on hatchery-reared juvenile coho salmon there are myriad inputs of copper into freshwater habitats of anadromous salmonids throughout the western United States. Therefore, copper poses a threat to wild-reared juveniles of numerous salmonid species, many of which have current population abundances at or near their historical lows, such as steelhead. Our findings suggest that substantial differences in the vulnerability of the olfactory system of hatchery and wild fish to at least copper are unlikely. Resource managers, therefore, might use the olfactory toxicity information for coho when considering the potential consequences of copper exposure to steelhead and possibly other at-risk but less-studied species.

Acknowledgements

We thank Cathy Laetz, Michelle Havey, Jeff Atkins, Abby Tillotson, Rob Endicott, and Henry Desmarais for technical support and the UW School of Fisheries and Gordy George for facility support at BBC. This work was funded in part by the NOAA Coastal Storms Program.

References

Baldwin, D.H., Sandahl, J.F., Labenia, J.S., Scholz, N.L., 2003. Sublethal effects of copper on coho salmon: impacts on non-overlapping receptor pathways in the peripheral olfactory nervous system. Environ. Toxicol. Chem. 22, 2266–2274.

Davis, A.P., Shokouhian, M., Ni, S., 2001. Loading estimates of lead, copper, cadmium, and zinc in urban runoff from specific sources. Chemosphere 44, 997–1009.

Einum, S., Fleming, I.A., 2001. Implications of stocking: ecological interactions between wild and released salmonids, Nordic. J. Freshwater Res. 75, 56–70.

Good, T.P., Waples, R.S., Adams, P. (Eds.), 2005. Updated status of federally listed ESUs of West Coast salmon and steelhead. U.S. Dept. Commerce, NOAA Tech. Memo. NMFS-NWFSC-66, 598 p.

Hansen, J.A., Rose, J.D., Jenkins, R.A., Gerow, K.G., Bergman, H.L., 1999. Chinook salmon (*Oncorhynchus tshawytscha*) and rainbow trout (*Oncorhynchus mykiss*) exposed to copper: neurophysiological and histological effects on the olfactory system. Environ. Toxicol. Chem. 18, 1979–1991.

Hara, T.J., Law, Y.M.C., Macdonald, S., 1976. Effects of mercury and copper on the olfactory response in rainbow trout *Salmo gairdneri*. J. Fish. Res. Board Can. 33, 1568–1573.

Riley, S.C., Tatara, C.P., Scheurer, J.A., 2005. Aggression and feeding of hatchery- and naturally-reared steelhead fry in a laboratory flume and a natural stream. Can. J. Fish. Aquat. Sci. 62, 1400–1409.

Sandahl, J.F., Miyasaka, G., Koide, K., Ueda, H., 2006. Olfactory inhibition and recovery in chum salmon (*Oncorhynchus keta*) following copper exposure. Can. J. Fish. Aquat. Sci. 63, 1840–1847.

Sandahl, J.F., Baldwin, D.H., Jenkins, J.J., Scholz, N.L., 2007. A sensory system at the interface between urban stormwater runoff and salmon survival. Environ. Sci. Technol. 41, 2998–3004.

Tatara, C.P., Riley, S.C., Scheurer, J.A., 2008. Environmental enrichment in steelhead (*Oncorhynchus mykiss*) hatcheries: field evaluation of aggression, foraging, and territoriality in natural and hatchery fry. Can. J. Fish. Aquat. Sci. 65, 744–753.

Tierney, K.B., Baldwin, D.H., Hara, T.J., Ross, P.S., Scholz, N.L., Kennedy, C.J., 2010. Olfactory toxicity in fishes. Aquat. Toxicol. 96, 2–26.

Weber, E.D., Fausch, K.D., 2003. Interactions between hatchery and wild salmonids in streams: differences in biology and evidence for competition. Can. J. Fish. Aquat. Sci. 60, 1018–1036.