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A Sensory System at the Interface between Urban Stormwater Runoff and Salmon Survival

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Motor vehicles are a major source of toxic contaminants such as copper, a metal that originates from vehicle exhaust and brake pad wear. Copper and other pollutants are deposited on roads and other impervious surfaces and then transported to aquatic habitats via stormwater runoff. In the western United States, exposure to non-point source pollutants such as copper is an emerging concern for many populations of threatened and endangered Pacific salmon (*Oncorhynchus* spp.) that spawn and rear in coastal watersheds and estuaries. To address this concern, we used conventional neurophysiological recordings to investigate the impact of ecologically relevant copper exposures (0−20 μg/L for 3 h) on the olfactory system of juvenile coho salmon (*O. kisutch*). These recordings were combined with computer-assisted video analyses of behavior to evaluate the sensitivity and responsiveness of copper-exposed coho to a chemical predation cue (conspecific alarm pheromone). The sensory physiology and predator avoidance behaviors of juvenile coho were both significantly impaired by copper at concentrations as low as 2 μg/L. Therefore, copper-containing stormwater runoff from urban landscapes has the potential to cause chemosensory deprivation and increased predation mortality in exposed salmon.

Introduction

Human population growth is increasingly concentrated along the coastal margins of countries such as the United States (*1*, *2*). Urbanization and other forms of coastal development increase the runoff of pollutants from terrestrial landscapes to the aquatic environment. Upon completing the most comprehensive review of the nation's management of oceans, coasts, and the Great Lakes in more than three decades, the U.S. Commission on Ocean Policy recently highlighted non-point source pollution as one of the most significant emerging threats to aquatic species (*3*). A similar review by the Pew Ocean Commission found that non-point sources represent the greatest pollution threat to oceans and coasts (*4*). For at-risk aquatic species, the current conservation challenges associated with toxic runoff are global in scope, complex, expanding and poorly understood.

Pavement is a universal feature of urbanized landscapes, and impervious surfaces accumulate chemical pollutants from automobile traffic as well as from other sources (*5*). During rainfall events, these contaminants are mobilized by stormwater (*6*) and transported to rivers, lakes, and estuaries (*7*). Dissolved copper is a particularly pervasive contaminant in urban runoff. This reflects the many industrial, commercial, and residential uses of copper, including the incorporation of the metal into roofing and flashing materials, treated wood, and various pesticide formulations. In addition, vehicle emissions via exhaust and brake pad wear represent major sources of copper in runoff from roads (*8*). Within a particular watershed, the loading of copper to surface waters will depend, in part, on site-specific hydrological characteristics, as well as land cover (e.g., percent impervious surface), vehicle traffic, and rainfall patterns. As an example of measured concentrations in aquatic habitats, recent monitoring of streams in northern California following storm events found dissolved copper at levels that varied from 3.4 to 64.5 μg/L, with a mean of 15.8 μg/L (*9*).

In the present study, we investigate the impact of dissolved copper on juvenile coho salmon (*Oncorhynchus kisutch).* Wild stocks of coho and other species of anadromous Pacific salmon and steelhead are declining throughout much of their natural range in the western U.S. (*10*). Currently, 26 distinct population segments (evolutionary significant units; ref *11*) of coho, chinook (*O. tshawytscha*), sockeye (*O. nerka*), and chum (*O. keta*) salmon as well as steelhead (*O. mykiss*) are listed as either threatened or endangered under the U.S. Endangered Species Act (ESA). In the case of coho, several historical runs have been extirpated throughout California, Oregon, Washington, and Idaho (*12*). To reverse salmon declines, federal, state, and local governments have invested hundreds of millions of U.S. dollars in recent years to conserve and restore the quality of freshwater and estuarine habitats (e.g., ref *13*). Freshwater habitat quality is particularly important for coho salmon that rear for more than a year in lowland streams and ponds before beginning their seaward migration (*14*).

Copper is a neurobehavioral toxicant in fish, and it has been known for more than three decades that the metal disrupts the normal function of the fish olfactory system (*15*). Ultrastructural analyses have shown that dissolved copper damages the olfactory sensory epithelium (*16*−*19*), and previous studies using direct neurophysiological recordings from the fish nose (*15*, *17*, *20*, *21*) or observations of chemosensory behavior (*16*, *22*−*24*) have shown that copper interferes with the ability of fish to detect and respond to chemical signals in aquatic environments. Chemosensory deprivation has important implications for salmon, as these migratory animals rely on their sense of smell to find food, avoid predators, form social dominance hierarchies, navigate from the ocean to freshwater spawning habitats, and assess the reproductive status of prospective mates.

To determine whether short term (3 h) exposures to dissolved copper at concentrations typical of urban stormwater runoff (0−20 μg/L) interfere with olfaction and olfactory-mediated behaviors in juvenile coho salmon, we used a combination of in vivo neurophysiological recordings from the olfactory epithelium and three-dimensional digital imaging to quantify predator avoidance behaviors that are normally triggered in juvenile salmon by a conspecific chemical alarm pheromone (*25*). For each copper exposure

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concentration, recordings from olfactory sensory neurons were matched to behavioral observations from the same animal. This allowed us to evaluate the sublethal neurobehavioral effects of copper at two different biological scales and to assess the extent to which classical measures of sensory toxicity (i.e., electrophysiology) are predictive of behavioral impairment.

Experimental Procedures

Animals. Coho salmon eggs were obtained from the University of Washington hatchery (Seattle, WA) at the eyed egg stage and raised at the Northwest Fisheries Science Center's hatchery facility under natural photoperiod conditions. Coho parr were maintained in tanks supplied with filtered, dechlorinated municipal water (hereafter referred to as hatchery water; 120 mg/L total hardness as $CaCO_3$, pH 6.6, dissolved oxygen 8.1 mg/L, temperature 11−13 °C) on a single-pass flow system. Fish were raised on standard commercial salmon pellets (Bio-Oregon, Warrenton, OR). Fish were 4−5 months of age with an average (±SD) length of 4.6 ± 0.4 cm and a weight of 0.9 ± 0.2 g.

Preparation of Chemical Alarm Stimulus. A stock alarm substance was prepared by homogenizing approximately 600 cm^2 of skin from 16 juvenile coho in 50 mL of distilled water. The homogenate was then filtered through polyester floss, diluted to a final concentration of 100 cm^2 skin/L in distilled water, mixed, aliquoted into 10 mL glass vials, and stored at −20 °C. Immediately before use, aliquots were thawed, filtered, and diluted 1:100 in hatchery water to a final concentration of 1 cm^2 skin/L. Control blank solutions consisted of hatchery water only. Although the as-yet unidentified alarm substance is unlikely to be a protein (*26*), the pheromone is contained within specialized club cells that are generally distributed throughout skin tissue (reviewed in ref *27*). Thus, the concentration of pheromone is likely to vary in proportion to the protein content of the skin extract. Moreover, protein assays are more precise and more reproducible than estimates of epidermal surface area. Accordingly, we measured the total protein content of the conspecific skin extract using a modified Bradford (*28*) assay (Coomassie Plus-2000 Protein Assay Reagent, Pierce, Rockford, IL). Odor stimulus concentrations are reported as mg (or μg) of protein/L. As a point of reference, 1 cm^2 skin was empirically determined to be equivalent to 5 mg of protein. Moreover, a mechanical disruption of the skin as small as 1 mm^2 (50 μg of protein) would be sufficient to fill 100 L to a concentration of 0.5 μg/L protein, a concentration within the experimental range examined here.

Copper Exposures and Chemical Analysis. Copper-containing exposure solutions were constituted by dissolving copper chloride (Sigma Chemical Co., St. Louis, MO; 99% purity cupric chloride, dihydrate) in distilled water. A total of five stock solutions was prepared, such that adding 100 mL of each stock to 25 L of hatchery water produced nominal dissolved copper concentrations of 0, 2, 5, 10, and 20 μg/L in aerated, 30 L glass exposure aquaria. The exposure aquaria were visually isolated from each other. Prior to the introduction of fish, 100 mL water samples for dissolved copper analysis were collected in acid-washed, Teflon bottles and refrigerated at 4 °C. Fish were then exposed to copper for 3 h. Each fish was treated individually (n = eight to 12 animals per exposure concentration) in separate tanks using freshly prepared copper exposure solutions diluted from a common stock. Individual exposures were staggered to maintain a constant duration between the onset of the copper exposure and the onset of either behavioral or electrophysiological trials. Different combinations of copper-exposed fish were tested on any given day, but at least one fish from the control group was tested on each day. Water temperature, pH, and dissolved oxygen (dO) remained relatively constant over the course of the exposure period, with a mean and range (in parentheses) of 10.8 °C (10−12 °C), pH 6.7 (6.5−7.1), and 8.2 mg/L dO (6.5−9.6 mg/L).

TABLE 1. Effects of Dissolved Copper on the Swimming Behavior of Coho Salmon[a]

copper nominal (μg/L)	copper measured (μg/L)	pre-stimulus swimming speed (cm/s)	post-stimulus swimming speed (cm/s)	freeze responses (fraction)
0	0.3 ± 0.2	5.6 ± 0.4	1.4 ± 0.3	11/12
2	1.9 ± 0.4	6.0 ± 0.3	3.7 ± 0.7	6/12*
5	4.7 ± 0.6	5.6 ± 0.3	4.8 ± 0.7	3/12*
10	10.2 ± 1.6	5.2 ± 0.5	4.1 ± 0.5	2/12*
20	16.8 ± 1.7	2.3 ± 0.4*	2.4 ± 0.5	1/8*

[a] Measured copper values are from three composite samples for each treatment group taken at the start of the exposure period. A freeze response was a 50% or greater reduction in locomotory activity (see Experimental Procedures). Data are presented as mean ± SE or as fractions representing the number of responders over the total number of fish tested. For pre-stimulus swimming speed and freeze responses, asterisks represent a statistical difference from controls ($p < 0.05$, one-way ANOVA with a Dunnett's post hoc and Fisher's exact test, respectively).

Nominal exposure solutions were analyzed for total dissolved copper by an outside laboratory using inductively coupled plasma mass spectrometry (Frontier Geosciences, Seattle, WA). The background concentration of total dissolved copper in the hatchery water was 0.3 μg/L. Copper recovered from exposure tanks ranged from 84 to 102% of nominal values (Table 1). Accordingly, copper exposures are hereafter expressed in terms of nominal concentrations.

Quantitative Analysis of Predator Avoidance Behaviors. Following a 3 h exposure, the behavioral response of each juvenile coho to a chemical predation cue was monitored using a computer-assisted, three-dimensional data acquisition system (*29*). The experimental design was modified slightly from Scholz et al. (*25*). For the behavioral trials, individual fish were transferred to a clean 30 L glass aquarium filled with 25 L of hatchery water. Continuous, closed circulation mixing in the aquarium was provided by a small aquarium pump. Conspecific skin extract was injected into the behavioral observation tank via a 50 cm length of Tygon tubing. Initial tests with dye indicated an even distribution of odor stimulus throughout the tank within approximately 1 min.

The three-dimensional position of fish was monitored using two orthogonally placed Firewire digital cameras (Fire-i, Unibrain Inc., San Ramon, CA) connected to a laptop computer (iBook, Apple Computer, Cupertino, CA), as previously described (*29*). In brief, the two cameras acquired simultaneous images of the fish from the front and side of the tank every 2 s. Each pair of images was then analyzed to determine the position of the fish via triangulation, with a correction for refraction. The three-dimensional distance between subsequent pairs of images (divided by 2 s) was used to calculate the swimming speed at each time point.

Trials began by transferring individual control or copper-exposed fish to the observation tank and then allowing them to acclimate for 30 min. A baseline, pre-stimulus swimming speed for each animal was subsequently recorded for a 3 min interval (t = −180−0 s). Following this, a small volume of the chemical alarm substance (0.5 mL; 5 mg of protein/L) was injected into the circulation system (t = 0 s) to achieve a final diluted concentration of 0.1 μg of protein/L in the observation chamber. The post-stimulus swimming speed of the fish was then monitored for an additional 4 min. To allow for differences in odorant dispersal as well as differences in the initiation of the avoidance response among animals, we selected a fixed 30 s interval (t = 45−75 s) to measure the post-stimulus swimming speed. On the basis of initial trials,

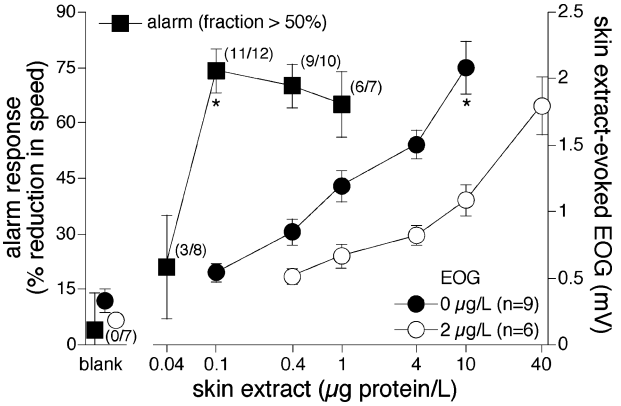

FIGURE 1. Odorant stimulus−response curves were determined for both alarm (closed squares) and EOG responses (closed circles) to skin in control (unexposed fish) and for EOG responses to skin in fish exposed to 2 μg/L of copper (open circles). Fractions within parentheses correspond to the proportion of fish tested in each group that showed a >50% reduction in activity (number of fish responding/total number of fish tested). A slight EOG response was observed when the perfusion of the olfactory chamber was switched to hatchery water alone (blank stimulus). Unlike the behavioral responses, the EOG response did not plateau at higher concentrations of skin extract. In both graphs, error bars represent one standard error. Asterisks denote the skin extract concentration used in subsequent copper exposure experiments.

this interval included the behavioral responses of almost all the fish. The magnitude of the response was quantified by comparing the change (reduction) in swimming speed over the pre- and post-stimulus intervals (Supporting Information, Figure S1A). Additionally, the reaction to alarm pheromone was scored as a predator avoidance response if the animal exhibited motionlessness, as indicated by a reduction in swimming speed of 50% or more. To reduce inter-animal variability arising from risk-taking behavior (i.e., motivation to forage in the face of a predation threat), we did not feed juvenile coho during behavioral trials and thus did not monitor food strikes (*25*).

Odor-Evoked Neurophysiological Recordings from the Coho Olfactory Epithelium. Once the behavioral observations were complete, odor-evoked EOGs were recorded from the peripheral olfactory epithelium of each juvenile coho using established procedures (*30*). Fish were anaesthetized in tricaine methanesulfonate (MS-222; 50 mg/L) and transferred to a vibration isolation table for electrophysiological recordings. For each animal, the EOG evoked by an odorant was measured in triplicate and then averaged to produce a single response value. The size or amplitude of the EOG was expressed as the negative phasic displacement (in millivolts) of the evoked peak relative to the pre-stimulus electrical baseline (refs *20* and *21* and Supporting Information Figure 1B).

Odorant solutions were prepared daily from concentrated stocks of conspecific skin extract (alarm substance), the amino acid L-serine, and the bile salt taurocholic acid (TCA) dissolved in hatchery water. The olfactory chamber of each animal (with the nare intact) was perfused with a sequence of the three different odorants: skin extract (10 μg of protein/L), l-serine (10^{-5} M), and TCA (10^{-6} M). L-Serine and TCA are well-studied odorants in salmon and were included for the purposes of comparing the results of this study to previous investigations (*21*). At these concentrations, all three odorants elicit similar, robust EOGs from the olfactory epithelium of unexposed animals. Fish were euthanized by decapitation after recording EOGs.

Initial Alarm Substance Range-Finding Experiments. Several range-finding experiments were performed to measure the behavioral and physiological responses of fish to a range of skin extract dilutions. For the alarm behavior, unexposed juvenile coho salmon were presented with the skin extract at nominal concentrations of 0 (hatchery water blank), 0.04, 0.1, 0.4, and 1.0 μg of protein/L (n = seven to 12 animals per concentration) with each fish tested with only one skin extract dilution. For the physiological response, EOGs from a separate group of unexposed fish were recorded in response to five dilutions of skin extract (0.1−10 μg of protein/L; n = eight to nine fish per stimulus dilution). Finally, to evaluate the effects of copper on the stimulus-response relationship for the alarm substance, a third group of fish was exposed to 2 μg/L of copper for 3 h, and EOG responses to skin extract were then recorded at dilutions ranging from 0.4−40 μg of protein/L (n = three to six fish per dilution).

Statistical Analysis. The electrophysiological and behavioral measures were analyzed using either one-way analysis-of-variance (ANOVA) to test for statistical differences between groups (followed by a Dunnett's test for comparisons with controls), Fisher's exact test (for freeze responses), or regression analysis to test for concentration-dependent relationships. Paired t-tests were used to determine differences in pre-stimulus baseline activity and post-stimulus activity for antipredator responses. Correlations were determined by using the Pearson correlation procedure. Statistical analyses and graphing were performed with GraphPad Prism 4.0 (San Diego, CA) and SAS Institute JMP 5.1 (Cary, NC).

Results

Neurobehavioral Responses to a Chemical Predation Cue over a Range of Stimulus Concentrations. The onset of predator avoidance behavior usually occurred within 30−60 s after the introduction of skin extract to the observation tank. This brief delay presumably reflected variability in the time required for the skin extract to circulate throughout the tank, the position of the fish in the tank at the time of stimulus introduction, and variation in inter-animal behavior. In a typical response, juvenile coho oriented to the direction of water flow and began a rapid fanning motion of the pectoral fins. This sculling or freezing behavior served to hold the fish in a relatively fixed position. Responsive fish also tended to slowly settle toward the bottom of the tank (Supporting Information, Movie S1). Although the stereotypical antipredator response was a rapid onset of motionlessness, the duration of the response varied, with some animals freezing for tens of seconds and others freezing for several minutes (not shown).

The degree of acclimation to the observation tank was consistent across the groups of fish, as indicated by a comparable amount of baseline (pre-stimulus) swimming activity among groups (mean ± SE; 5.2 ± 0.2 cm/s; one-way ANOVA, $p > 0.5$). Fish presented with hatchery water only (blank) showed no change in swimming speed over the pre- and post-stimulus observation period (paired t-test, $p > 0.5$), and no animals exhibited the stereotypical freezing behavior in response to a blank stimulus. The swimming speed of fish presented with skin extract at 0.04 μg of protein/L was slightly diminished, with a 21 ± 14% (mean ± SE) reduction in speed relative to the pre-stimulus interval (paired t-test, $p = 0.12$). However, three of eight animals exhibited motionlessness or freezing. A more pronounced antipredator response occurred when the alarm stimulus concentration was increased to 0.1 μg of protein/L. This included a 74 ± 6% reduction in speed relative to controls (paired t-test, $p < 0.001$) and a freezing response in 11 of 12 animals. Behavioral changes were similarly pronounced at higher stimulus concentrations (0.4 and 1.0 μg of protein/L; paired t-test, $p < 0.001$).

Odor-evoked field potential recordings from the olfactory epithelium of coho indicated a concentration-dependent increase in EOG amplitude in response to skin extract (0.1−10 μg of protein/L; n = eight to nine fish per stimulus concentration, Figure 1), with evoked extracellular potentials ranging from 0.2−1.7 mV, after subtraction of the response to a blank solution (hatchery water only). At a concentration of skin extract of 0.1 μg of protein/L, the EOG responses were indistinguishable from responses to the blank solution ($p >$ 0.05, one-way ANOVA followed by Dunnett's post hoc). Thus, for juvenile coho, the measured neurophysiological detection threshold for conspecific skin extract under these experimental conditions was between 0.1 and 0.4 μg of protein/L. Exposure to 2 μg/L of copper for 3 h reduced the EOG responses to all skin extract concentrations, effectively shifting the concentration relationship to the right (Figure 1). For copper-exposed fish, responses to concentrations of skin extract of 1 μg of protein/L or less were indistinguishable from blank responses ($p >$ 0.05, one-way ANOVA followed by Dunnett's post hoc), indicating an increase in response threshold by about 1 log unit.

In summary, the conspecific skin extract elicited measurable electrophysiological and behavioral responses from juvenile coho salmon at concentrations either above or at 0.1 μg of protein/L. The behavioral stimulus-response curve was steep, with the skin extract evoking maximal predator avoidance behaviors at a concentration that was below the lowest concentration detectable via olfactory neurophysiology. Also, since motionlessness was observed in response to alarm substance at a concentration subthreshold for evoked EOGs (0.04 μg of protein/L), the behavioral measurement appears to be the more sensitive of the two experimental assays. On the basis of these initial observations, a stimulus concentration of 0.1 μg of protein/L was used for subsequent behavioral trials involving copper-exposed fish. To elicit a robust EOG response, the olfactory chamber was perfused with 10 μg of protein/L during neurophysiological experiments.

Relative Thresholds for Neurophysiological and Behavioral Impairment in Juvenile Coho Exposed to Dissolved Copper. To determine the relative impacts of short-term copper exposures (3 h; 2−20 μg/L) on olfactory sensitivity and predator avoidance behavior, we exposed individual fish to copper, monitored a behavioral response to 0.1 μg of protein/L of skin extract, and then recorded odor-evoked EOGs from each animal's olfactory epithelium using conspecific skin extract and two other natural odorants (the amino acid L-serine and the bile salt TCA) as stimuli. The Supporting Information includes examples of paired ethograms and olfactograms for four control fish and four animals exposed to 10 μg/L of copper (Figure S2) and a movie showing the behavioral responses of a control fish and a fish exposed to 10 μg/L of copper (Movie S1).

Dissolved copper inhibited olfactory responses to all three odorants (skin extract, L-serine, and TCA) in a concentration-dependent manner (ANOVA, $p < 0.001$, Figure 2A). In unexposed animals, the mean EOG responses to 10 μg of protein/L of skin extract, 10^{-5} M L-serine, and 10^{-6} M TCA were 1.2, 2.8, and 4.0 mV, respectively. At the lowest copper exposure concentration (2 μg/L), the mean skin extract-evoked EOG amplitude was 0.6 mV, a significant reduction relative to controls (ANOVA, Dunnett's test, $p < 0.01$). At 20 μg/L of copper, EOG responses to all three odorants were nearly abolished. The data for each odorant were also closely fit ($r^2 \geq 0.97$) by a nonlinear regression to a sigmoidal function, $\text{EOG} = \text{max}/(1 + (\text{copper}/\text{EC}_{50})^{\text{slope}})$, which was applied previously for juvenile coho (*21*). For each of the regressions shown in Figure 2A, the mean olfactory response of the control group was used to define the value of max in the previous equation.

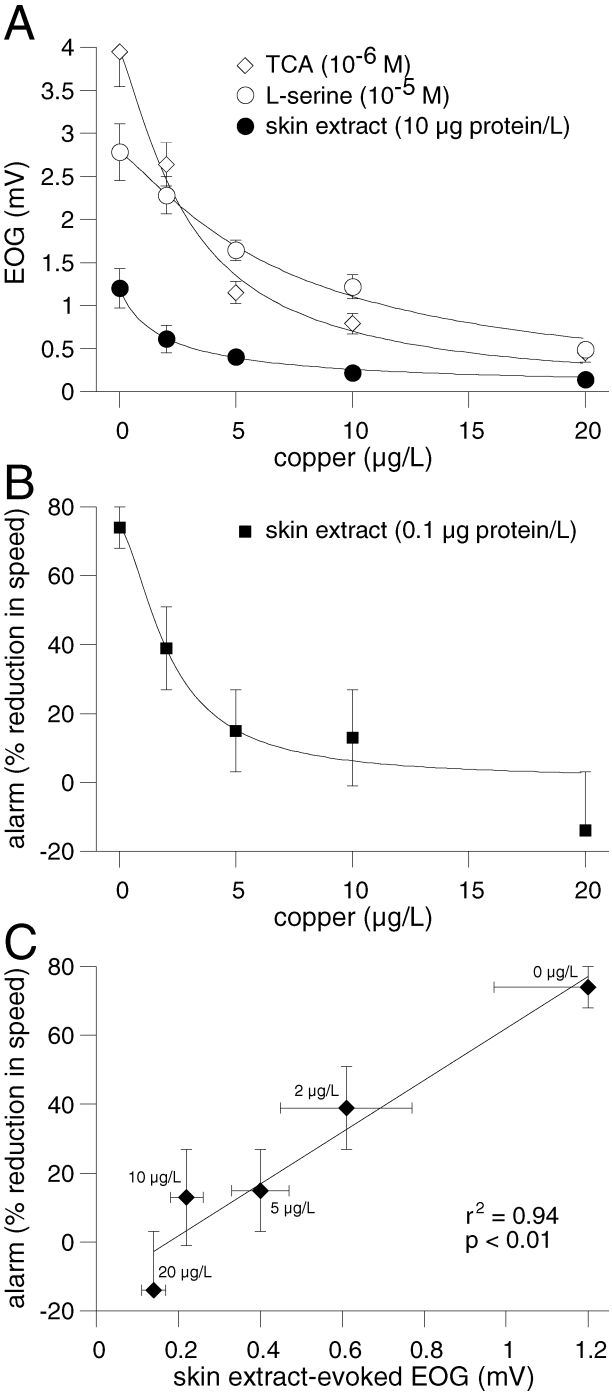

FIGURE 2. Exposure to copper diminished olfactory sensitivity and alarm behavior in juvenile coho. (A) Electro-olfactogram (EOG) responses to skin extract (10 μg of protein/L), L-serine (10^{-5} M), and taurocholic acid (TCA, 10^{-6} M) were inhibited at increasing copper exposure concentrations. Note that, in contrast to Figure 1, the EOG responses shown here were blank-subtracted. The results of nonlinear regressions are shown with solid lines (see Results for details). (B) Copper exposure also reduced the alarm response elicited by 0.1 μg of protein/L of skin extract in a dose-dependent manner. The result of a nonlinear regression is shown with a solid line (see Results for details). (C) Paired physiological and behavioral response means were highly correlated (i.e., fish with reduced olfactory sensitivity showed reduced alarm behavior). Error bars in all graphs represent one standard error.

Dissolved copper also disrupted odor-evoked predator avoidance behaviors (Figure 2B and Table 1). For juvenile coho exposed to copper at concentrations up to 10 μg/L, pre-stimulus baseline swimming activity was indistinguish-

able from controls (5.6 ± 0.4 cm/s, Table 1). By contrast, fish exposed to 20 μg/L of copper for 3 h were noticeably lethargic, as indicated by a reduction in the mean baseline activity in these fish relative to controls (2.3 ± 0.4 cm/s; ANOVA, $p < 0.05$). The alarm pheromone triggered an average reduction in swimming speed of 74 ± 6% (mean ± SE) among unexposed animals. The behavioral change was highly significant (paired t-test, $p < 0.001$), with all but one of the unexposed animals ($n = 11$ of 12 fish) becoming motionless during the post-stimulus interval (Table 1). While the reduction in swimming speed among fish exposed to 2 μg/L of copper (39 ± 12%) was significant (paired t-test, $p < 0.01$), fewer animals (n = six of 12) became motionless. At higher copper concentrations (5, 10, and 20 μg/L), there were no significant reductions in swimming speed (paired t-tests, $p > 0.1$), and the majority of fish did not become motionless. The effect of copper on the alarm reaction also showed a reasonable fit to the same sigmoidal function as the EOG responses ($r^2 = 0.80$, Figure 2B). The duration of the alarm reaction for the few fish that did respond to the pheromone at these higher copper exposures was generally shorter than for controls (e.g., 24 s for the one 20 μg/L exposed fish versus 114 ± 27 s for the 11 unexposed fish). Overall, however, too few of the copper-exposed fish responded to allow for a comparison of duration (not shown).

A direct comparison of the inhibitory effects of dissolved copper on the sensory biology and behavior of juvenile coho is shown in Figure 2C. The relationship between olfactory inhibition and diminished alarm response was significantly correlated (Pearson $r = -0.97$, $r^2 = 0.94$, $p < 0.01$). From the slope of the correlated measures (linear regression, slope = 75 ± 11), a ~25% decrease in olfactory function corresponds to a ~29% decrease in the magnitude of the pheromone-mediated predator avoidance behavior. Consequently, the relative impacts of dissolved copper exposure are similar at these two different scales of biological organization.

Discussion

Our current findings provide an important link between habitat degradation (i.e., dissolved copper exposure) and changes in the sensory-mediated behavior of threatened and endangered Pacific salmon. More specifically, we have shown that short-term exposures to dissolved copper diminish the olfactory sensitivity of juvenile coho salmon and that this loss of sensory function leads, in turn, to a failure to initiate predator avoidance behaviors in response to a conspecific olfactory stimulus. For salmonids, the detection of chemical alarm cues is important for predator recognition and learning (reviewed by ref *31*) as well as for surviving encounters with predators (*32*). Notably, these neuroethological effects of copper occur at concentrations that are well within the lower range of measured copper levels in surface waters of urban and urbanizing watersheds (e.g., 3−64 μg/L; ref *9*).

The effective range of chemical alarm pheromone-mediated signaling in aquatic systems is likely to vary with the strength of the signal at the source (i.e., the degree of damage to the skin of another fish), the turbulent dispersal of the chemical cue, and the sensory capabilities of the receiver. By interfering with chemosensation in the receiver, dissolved copper will effectively reduce the active space over which a conspecific alarm signal is effective. Moreover, copper-exposed fish may simply fail to respond to a predation cue at concentrations that would normally trigger anti-predator behaviors in uncontaminated systems. The neurobehavioral basis for this shift can be seen in Figure 1 and is illustrated in Figure 3. In the present study, the EOG response of juvenile salmon following a 3 h exposure to copper at 2 μg/L was reduced by ~40% over the entire range of odor concentrations (Figure 1), thereby shifting the stimulus−response curve to the right nearly a log unit. This

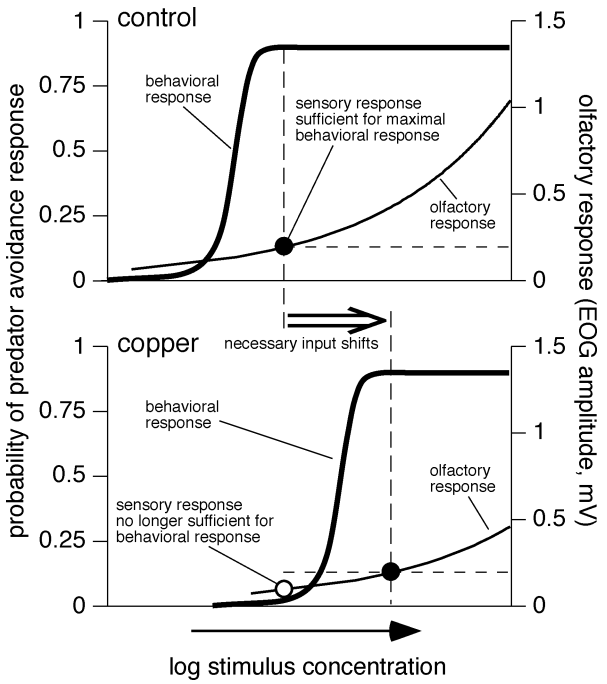

FIGURE 3. Conceptual model to illustrate how shifts in olfactory sensitivity can result in corresponding shifts in predator avoidance behavior. On the basis of the data in Figure 1, sigmoidal and power functions were used to approximate the behavioral and olfactory stimulus−response curves, respectively. In this theoretical model, a threshold concentration of alarm pheromone (left vertical dashed line) is required to generate an olfactory response (horizontal dashed line) that will be sufficient to trigger an alarm response in unexposed fish. Following exposure to copper, a shift in olfactory sensitivity increases the strength of the stimulus needed to reach this physiological and behavioral threshold (right vertical dashed line), and the previous stimulus now effectively fails to elicit the alarm behavior.

shift will increase with higher copper exposures, as evidenced by the continued reduction in EOGs following exposure to 5, 10, and 20 μg/L (Figure 2A). Consequently, as the dissolved copper content in surface waters increases, the responsiveness of the peripheral olfactory system to a predation cue will diminish until it falls below the threshold required to initiate an appropriate behavioral response (Figure 3). Therefore, a likely outcome in salmon habitats is that copper-exposed fish will make behavioral decisions that are inappropriately risky for a particular ecological situation (*33*). The consequences of this for actual rates of predation on juvenile salmon have not been determined, and this remains an important area for future research.

Salmon will avoid copper originating from point sources with defined environmental gradients (e.g., ref *23*). However, such spatial gradients are unlikely to be present in watersheds contaminated with diffuse non-point source runoff. For fish that are unable to avoid stormwater, the toxic effects of copper will be reversible, with physiological recovery taking place over the course of several hours following low-dose exposures (*21*). At higher concentrations, including those sufficient to trigger cell death in the sensory epithelium (i.e., ≥25 μg/L; ref *17*), the regeneration of olfactory neurons may take place over days or weeks. In either case, intermittent rainfall can be expected to drive a dynamic process of neurobehavioral toxicity and recovery among salmon in urban creeks.

Finally, our current results in juvenile coho should be applicable to other fish species in urbanizing watersheds worldwide. In addition to coho (this study and refs *20* and *21*), dissolved copper has been shown to impair olfaction in chinook salmon (*17*, *23*), rainbow trout (*15*, *18*, *24*), brown

trout (*Salmo trutta*; ref *19*), fathead minnow (*Pimephales promelas*; ref *22*), Colorado pikeminnow (*Ptychocheilus lucius*; ref *16*), and tilapia (*34*). It is also likely that the neurotoxic effects of copper extend beyond the olfactory networks that underlie predator avoidance behavior. For example, Baldwin et al. (*21*) found that copper reduces the sensitivity of coho salmon to distinct classes of natural odorants in a similar, dose-dependent manner. This suggests that copper is a general-purpose inhibitor of fish olfaction and may thus interfere with a wide range of chemosensory behaviors. Last, two recent studies indicate that dissolved copper is also toxic to fish lateral line neurons (*35*, *36*) and thus may also disrupt mechanosensory behaviors such as shoaling, prey capture, and predator evasion. For these reasons, non-point source stormwater runoff from roads has the potential to interfere with a wide variety of behaviors in a diversity of fish species.

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Supporting Information Available

Measured behavioral and EOG responses of fish (Figure S1); examples of the behavioral and EOG responses from four control fish and four fish exposed to 10 μg/L of copper (Figure S1); and behavioral responses of a control fish and a fish exposed to 10 μg/L of copper (Movie S1). This material is available free of charge via the Internet at http://pubs.acs.org.

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