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EFFECTS OF COPPER ON OLFACTION OF COLORADO PIKEMINNOW

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Abstract—Effects of copper on olfaction of Colorado pikeminnow (*Ptychocheilus lucius*) were investigated by exposing fish for 24 or 96 h, then evaluating olfactory ability using a behavioral assay and observing olfactory structures using scanning electron microscopy (SEM). The behavioral assay measured a response known as fright reaction. Failure of exposed fish to demonstrate a fright reaction in the presence of skin homogenate assumed to contain fright pheromone was considered evidence of copper-induced loss of olfactory ability. Regression analysis was used to describe the response of fish as a function of copper concentration at each exposure duration. Olfactory ability declined with increasing copper concentration. For copper concentrations less than 66 μg/L, olfaction was more sensitive to exposure at 24 h than at 96 h. This result suggests that physiological adaptation and recovery of sensory ability occurred despite continuous exposure in the 96-h treatment. Protective mechanisms induced by exposure may have reduced sensitivity to copper by 96 h. Systematic surveys using SEM to detect presence or absence of olfactory receptors confirmed results of behavioral assays. Copper concentrations in one river inhabited by Colorado pikeminnow were compared with effective concentrations estimated by regression. Comparisons suggest that ambient copper concentrations may occasionally inhibit olfaction of wild fish.

Keywords—Copper Colorado pikeminnow Olfaction Behavioral assay Scanning electron microscopy

INTRODUCTION

The Colorado pikeminnow (*Ptychocheilus lucius*) is a large cyprinid endemic to the Colorado River Basin, USA [1]. Historically, the Colorado pikeminnow was widespread in warmwater streams and rivers, but the species was listed as federally endangered in 1967 in response to declining populations [2]. The decline of Colorado pikeminnow is commonly attributed to interactions with introduced fishes, construction of dams, and habitat modification [3,4], but effects of chemical contaminants are increasingly becoming a concern. An important element of Colorado pikeminnow life history is that natural reproduction is preceded by spawning migrations that may exceed 100 km in length. The means of navigation used by Colorado pikeminnow during migration are unknown, but it has been proposed that olfaction is a critical component of the homing process [1]. Many contaminants are known to disrupt olfaction in fish. Short-term sublethal exposure to copper, lead, mercury, nickel, silver, zinc, extremes of pH, and naphthalene inhibit olfactory ability [5–9]. Olfactory receptors are not protected from contaminant exposure by external membranes but come into direct contact with water-borne solutes. Toxic substances may alter detection of olfactory cues through several modes of action, including damaging organelles and enzyme systems, direct interaction with membrane receptor sites, or masking biologically important chemical signals.

The purpose of this investigation was to describe the effect of contaminant exposure on olfactory ability of Colorado pikeminnow. This objective was achieved by exposing Colorado pikeminnow to sublethal concentrations of copper for 24 or 96 h and evaluating olfactory ability using a behavioral assay coupled with observations of olfactory structures using scanning electron microscopy (SEM). Advantages of combining the two techniques were that the behavioral assay required study fish to detect, process, and respond to stimuli in an ecologically relevant fashion, and SEM observations provided evidence of related structural changes of olfactory receptors. The behavioral assay used in this investigation measured a response known as fright reaction in Colorado pikeminnow. The fright reaction is a well-known response of Ostariophysan fishes to fright pheromone, which is released from specialized cells in the epidermis when mechanical damage occurs [10,11]. Fright pheromone is detected by olfaction [10,11]. Consequently, failure of metal-exposed fish to demonstrate a fright reaction in the presence of the pheromone was considered evidence of a copper-induced loss of olfactory ability. Resulting exposure–response relationships were compared with copper concentrations near a Colorado pikeminnow spawning site to evaluate potential for effects on wild fish.

MATERIALS AND METHODS

Experimental animals

Colorado pikeminnow were obtained from Dexter National Fish Hatchery and Technology Center (Dexter, NM, USA). Fish were fed a mixture of live <24-h-old brine shrimp nauplii (Aquarium Products, Glen Burnie, MD, USA) and a commercially prepared flake diet (TetraMin®; TetraWerke, Melle, Germany) twice daily. Colorado pikeminnow were 185 d old (after hatching) when studies were started. Total length and wet weight ranged from 25 to 44 mm and 0.14 to 0.68 g, respectively.

Exposure conditions

The 24- and 96-h exposures were conducted following prescribed methods for renewal-acute toxicity tests [12]. Six concentrations were studied for the 24-h exposure (<10, 16.6, 33.3, 66.5, 133, and 266 μg/L copper) and five concentrations

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were studied for the 96-h exposure (<10, 15.0, 30.0, 60.0, and 120 μg/L copper). The 266-μg/L concentration was not used in the 96-h exposure because toxic effects were severe at 24 h, and it was anticipated that too few fish would survive the longer exposure. Hamilton and Buhl [13] reported a 96-h LC50 of 269 μg/L for Colorado pikeminnow exposed to copper under similar water-quality conditions. Concentrations were assigned to replicate (n = 10) 1-L exposure beakers using a balanced, randomized design. Beakers were filled with test solutions to a depth of about 8.5 cm. Exposure solutions were renewed every 24 h. Five fish were randomly assigned to each exposure beaker. Fish were not fed during chemical exposure. Cool-white fluorescent lamps were the only source of illumination, and a 16:8-h light:dark photoperiod was maintained.

Physical and chemical conditions

Dilution water for copper exposures was a mixture of well water and deionized water prepared to match the average hardness in July of the Yampa River near Maybell, Colorado, USA. Water quality for this time and location were selected because of correspondence with spawning activity and presence of a spawning site approximately 95 river-km downstream. Average water quality was estimated by calculating mean hardness of two low (1981 and 1989), medium (1980 and 1982), and high (1984 and 1985) water years (data from U.S. Geological Survey gage 09251000; [14–19]). Target water quality characteristics were hardness, 124 mg/L as $CaCO_3$; alkalinity, 100 mg/L as $CaCO_3$; and pH, 8.3. Dilution water was vigorously aerated for approximately 48 h while being maintained at the test temperature of 20 ± 2°C. Dissolved oxygen, hardness, pH, alkalinity, and specific conductance were measured daily during toxicant exposures. Water temperature was measured continuously with a temperature recorder. Means and ranges for the measured dilution water characteristics were dissolved oxygen, 6.9 (6.5–7.5) mg/L; hardness, 117 (113–120) mg/L as $CaCO_3$; pH, 8.3 (8.1–8.5); alkalinity, 80 (71–88) mg/L as $CaCO_3$; specific conductance, 240 μS/cm, and temperature, 18.5 to 21.0°C.

Copper solutions and analytical procedures

Stock solutions of analytical-grade copper sulfate (Mallinckrodt, Paris, KY, USA) were prepared in deionized water. Exposure concentrations were prepared by pipetting the desired amount of toxicant stock into 1-L glass beakers containing 0.75 L dilution water. Exposure solutions were stirred and transferred to exposure beakers within 30 min of preparation.

Toxicant concentrations were measured at the beginning of exposure periods. On each occasion, two 50-ml samples were collected from separate beakers for each exposure concentration. Unfiltered samples were placed in acid-washed polyethylene bottles, acidified to pH < 2 with analytical-grade nitric acid, and held at 4°C until analyzed by inductively coupled plasma emission spectroscopy (Soil, Water, and Plant Testing Laboratory, Colorado State University, Fort Collins, CO, USA). Chemical analysis confirmed accuracy of exposure concentrations. Measured copper concentrations averaged 92% (standard error = 4.8) of nominal. Measured and nominal concentrations were in close agreement; consequently, statistical analyses were based on nominal concentrations.

Behavioral assay

Skin homogenate that was assumed to contain fright-pheromone was prepared before each assay. Donor Colorado pikeminnow were juveniles obtained from the same mass culture used for study fish. Homogenate preparation involved removing skin posterior to the head and anterior to insertion of the caudal fin. Skin was cut into pieces and homogenized (Omni-mixer, Sorvall, Norwalk, CT, USA) in dilution water to a final concentration of 12.5 g/L.

The acclimation period for each behavioral assay was initiated immediately after conclusion of the 24- or 96-h exposures. Fish and solutions from each replicate were transferred to flow-through observation aquaria receiving dilution water at a rate of 50 ml/min. Observation aquaria were 10 × 20 × 15 cm high, and depth of water was 12 cm. Each aquarium was enclosed within a blind to visually isolate fish. An observation port allowed access for a video camera lens. Fish were allowed to acclimate to observation aquaria for approximately 90 min before being tested for presence of a fright reaction. After the acclimation period, movements of fish in each aquarium were video recorded for 1 min, followed by introduction of skin homogenate and an additional 1 min of video recording. Aliquots (0.1 ml) of skin homogenate were introduced into observation aquaria through injection ports in the water-delivery system, and final concentration of the homogenate in aquaria was 0.625 mg/L. Preliminary studies in which dilution water was injected into the delivery system showed that the procedure did not disturb the fish or elicit a fright reaction. Behavioral assays were conducted so that persons responsible for video camera operation and fright-pheromone injections were unaware (blind) of the experimental treatment assigned to fish within each aquarium. Upon conclusion of behavioral assays, three fish from each experimental treatment were preserved for SEM observations and the remainder were returned to culture facilities. Remaining fish from the 96-h exposure were cultured for 14 d, then were reassayed using the procedure described above to evaluate the potential for recovery of olfactory ability.

Video interpretation

The behavioral reaction to fright pheromone is facilitated by social interactions between fish. Consequently, the combined response of all fish within an observation aquarium (experimental unit) was the unit of measurement. Generally, undisturbed fish were dispersed and oriented in different directions within an aquarium and only occasionally changed their position during the 1-min preexposure observation period. Criteria used to identify a positive fright reaction response were demonstration of one or more of the following behaviors: (1) cover seeking, characterized by fish assembling into a polarized school, then moving toward the bottom of the aquarium and reducing movement; (2) agitation, characterized by rapid movement and frequent turning; and (3) dashing, characterized by one or more fish displaying frenzied behavior, including jumping out of the water, repeated and rapid changes in direction, and swimming against aquarium walls. The criteria were used only to qualify presence or absence of a fright reaction and not to quantify intensity of the response.

Video interpretation was conducted by an observer who was unaware (blind) of the experimental treatment assigned to fish within each aquarium. The criteria were used to interpret behavior during the first minute of each 2-min recording interval in order to identify fish that were likely to give a false-positive response. Fish that demonstrated cover seeking, agitation, or dashing behaviors before introduction of the skin homogenate were excluded from subsequent analysis. Presence

of a fright reaction from remaining fish was evaluated by applying the criteria during the 1-min interval after introduction of the skin homogenate.

Copper concentrations in Colorado pikeminnow habitat

Copper concentrations in the Yampa River near Maybell were described using data from a U.S. Geological Survey gage (09251000). Data were obtained from the U.S. Geological Survey, Water Resources Division, Colorado District (Denver, CO, USA). All data were used for the period of record from September 1974 to September 1991. The number of observations ranged from two to four per year with a total of 29 for total copper and 66 for dissolved copper. Detection limits for total and dissolved copper ranged from 2 to 20 and 1 to 10 μg/L, respectively. Frequency distributions for each constituent were constructed.

Scanning electron microscopy

Scanning electron microscopy was used to examine the olfactory epithelium of study fish for evidence of damage from chemical exposure. Sample preparation and analysis followed Lee [20]. Three fish from the control and each exposure concentration were fixed and preserved for SEM by immersion in 3% glutaraldehyde buffered to pH 7.2 with sodium phosphate. Subsequently, all fish were processed as a batch to eliminate potential bias from variation in preparation techniques. Specimens were rinsed with phosphate buffer and postfixed with 1% osmium tetroxide (Sigma Chemical, St. Louis, MO, USA), then dehydrated in a graded acetone series and critical-point dried in a Polaron apparatus (Bio-Rad, Cambridge, MO, USA). Individual specimens were mounted on aluminum stubs with colloidal silver, sputter coated with gold (20-nm thickness; Hummer VII sputtercoater, Anatech, Alexandria, VA, USA), and examined using a Philips 505 SEM (Einhovn, Holland).

Scanning electron microscopy was used only to confirm results of behavioral assays because preparation and viewing of specimens is labor and time intensive. Observations were made on fish from controls and from the lowest copper concentrations that inhibited the fright-reaction response in all replicates. It was anticipated that olfactory receptors in exposed fish would be less abundant compared with control fish. To reduce potential for investigator bias, examinations of olfactory epithelium were restricted to the same general regions of the sensory surface. Target regions were systematically surveyed by conducting adjacent parallel scans at ×10,000 magnification with a viewing field of 11.5 × 9.0 μm for 15 min. Presence or absence of ciliated receptor cells was recorded.

Statistical analysis

Logistic regression was used to analyze the binomial response (present or absent) for fright reaction as a function of concentration and exposure duration. Proc Genmod (with options link = logit, dist = binomial, dscale; [21]) was used to describe the response as a function of the independent variables. The full regression model had the form

$$\text{logit}(p) = \beta_0 + \beta_1 C + \beta_2 T + \beta_3 CT$$

where p = probability of response to skin homogenate, logit(p) = natural log[$p/(1 - p)$], β_0 = intercept, β_1, β_2 = coefficients for the linear terms of main effects, C = exposure concentration (μg/L), T = exposure time (h), and β_3 = coefficient of cross products. The coefficient of cross products tests for equal slopes of the 24- and 96-h concentration–response relationships. A significant coefficient suggests that the regression lines are not parallel and further analysis is not required to demonstrate that the concentration–response relationships are different from each other. It also suggests that the effect of copper was not consistent at both durations of exposure, i.e., there was an interaction of main effects. When an interaction is detected, statistical tests of main effects cannot be simply interpreted [22]. Coefficient β_1 represents the test for effects due to concentration gradient; β_2 represents the test for effects due to exposure duration.

Table 1. Maximum-likelihood parameter estimates and significance probabilities for a regression model describing probability of fright-reaction response to skin homogenate by Colorado pikeminnow after exposure to copper for 24 or 96 h

Parameter	Estimate	Standard error	p
β_0, intercept	2.70	2.87	0.347
β_1, $\log_{10}$(concentration)	−1.86	1.69	0.308
β_2, exposure time	0.144	0.0901	0.0269
β_3, $\log_{10}$(concentration) × exposure time	−0.0792	0.0520	0.0427

[a] Estimates for the regression equation logit(p) = $\beta_0 + \beta_1 C + \beta_2 T + \beta_3 C T$, where p = probability of response to skin homogenate, logit(p) = natural log [$p/(1 - p)$], C = exposure concentration (μg/L), and T = exposure time (h).

Concentrations of copper estimated to inhibit olfaction in 1 and 50% of the test organisms (EC1 and EC50) were also calculated for each exposure duration using Proc Probit (with options d = logistic inversecl lackfit; [23]). In this case, the EC1 was arbitrarily selected as a conservative threshold for adverse effects on an endangered species. Transformations ($\log_{10}$) were used if they improved the fit of regression models. Graphical analyses of data and residual plots were conducted to confirm that regression models were appropriate and to evaluate compliance with statistical assumptions.

RESULTS

Behavioral assay

Olfactory ability declined as a function of copper concentration after 24- or 96-h exposure, but slopes of the two concentration–response relationships were not parallel (Table 1). There was an interaction ($p = 0.0427$) between the effects of copper exposure and exposure time. As a consequence of this interaction, the influence of copper on olfaction at each exposure time must be evaluated separately. To facilitate evaluation, a graph of the probability of response to skin homogenate as a function of exposure concentration was constructed using the regression model (Fig. 1). At copper concentrations below 66 μg/L (i.e., the point where the lines cross), olfaction was more sensitive to exposure at 24 h than at 96 h; at higher concentrations, the opposite relationship was observed.

Behavioral assays conducted after a 14-d recovery period showed that Colorado pikeminnow exposed to 60.0 μg/L copper for 96 h regained olfactory ability (Table 2). Immediately after exposure, 5 of 10 positive responses to skin homogenate were elicited from fish in this experimental treatment, whereas after the recovery period, positive responses were detected in eight of nine remaining replicates.

The EC1s and 95% confidence limits for 24- and 96-h copper exposures were 2.61 (0.331, 6.24) and 18.3 (1.58, 30.0)

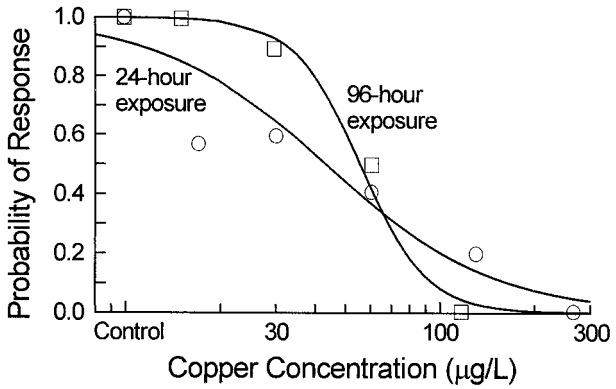

Fig. 1. Probability of response to skin homogenate for Colorado pikeminnow exposed to copper for 24 (circles) or 96 h (squares). The mean response for all replicates at each exposure concentration is shown. Probability 1 = response; 0 = no response.

μg/L total copper; EC50s were 43.3 (28.5, 69.0) and 56.0 (39.3, 86.6) μg/L total copper, respectively. Comparing these estimates with total copper concentrations in the Yampa River (Fig. 2) revealed that the 24-h EC1 was equaled or exceeded by about 52% of the field samples. The 96-h EC1 was equaled or exceeded by about 14% of the samples. For dissolved copper, the 24- and 96-h EC1s were equaled or exceeded by about 57 and 2% of field samples, respectively. The EC50 values were approached or exceeded on one or two occasions during the 17-year period of record.

Scanning electron microscopy

The SEM surveys always detected one or more ciliated receptor cells in control fish but did not detect receptor cells immediately after exposure in fish in the other copper treatments examined (Table 3). Observations confirmed that Colorado pikeminnow regenerated olfactory receptor cells after 96-h exposure to 60.0 μg/L copper. Ciliated receptor cells were detected in the olfactory epithelium of all three fish from this experimental treatment after the 14-d recovery period.

DISCUSSION

Mechanism for variable sensitivity

We exposed Colorado pikeminnow to a gradient of copper concentrations and evaluated the influence on olfaction by challenging fish to detect and respond to a skin homogenate assumed to contain fright pheromone and by determining presence or absence of ciliated receptor cells in exposed fish. In general, olfactory ability declined with increasing copper concentration. However, an unexpected result was that the response to copper varied with duration of exposure. For copper concentrations less than 66 μg/L, olfaction was more sensitive to exposure at 24 h than at 96 h. This response is inconsistent with the general principle of toxicology that, for a given con-

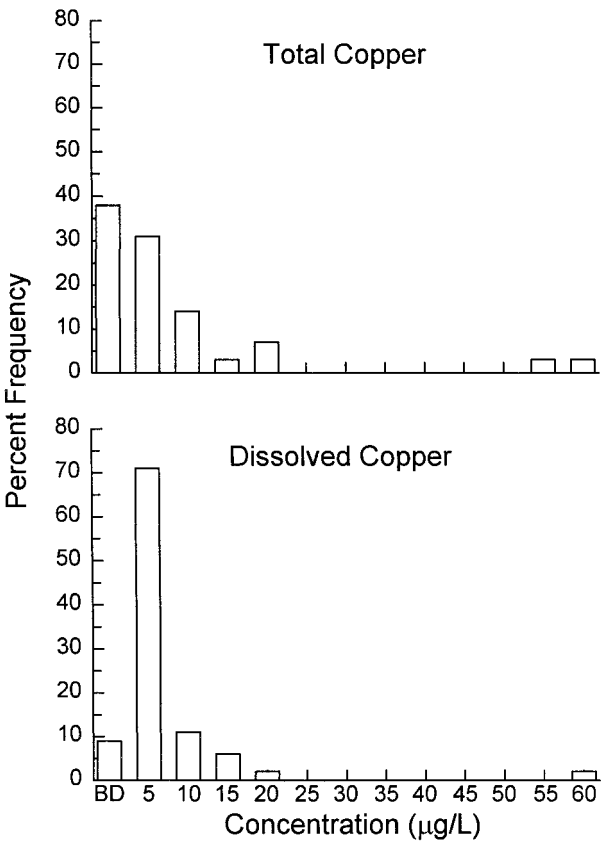

Fig. 2. Frequency of observation of copper concentrations in the Yampa River near Maybell, CO, USA, for the period of record from September 1974 to September 1991 (n = 29 for total copper, 66 for dissolved copper). Histograms represent number of observations less than or equal to the label value in the interval between each category. BD = below detection.

Table 2. Results of behavioral assays after exposure to copper for 24 or 96 h or after a 14-d recovery period; values are number of positive behavioral responses/replicates assayed

24-h exposure		96-h exposure		14-d recovery[a]	
Concentration (μg/L)	Positive responses	Concentration (μg/L)	Positive responses	Concentration (μg/L)	Positive responses
<10[b]	10/10	<10[b]	10/10	<10[b]	9/9
16.6	5/9	15.0	10/10	15.0	8/9
33.3	6/10	30.0	8/9	30.0	9/9
66.5	4/10	60.0	5/10	60.0	8/9
133	2/10	120	0/6	ND[c]	ND
266	0/10				

[a] Fish were held in control water for 14 d following 96-h exposure to copper concentrations.
[b] Control.
[c] ND = not determined because few fish recovered from copper exposure.

Table 3. Results of scanning electron microscopy examinations after exposure to copper for 24 or 96 h or after a 14-d recovery period; values are number of fish with one or more ciliated receptor cells/fish examined

24-h exposure		96-h exposure		14-d recovery[a]	
Concentration (μg/L)	Receptors detected	Concentration (μg/L)	Receptors detected	Concentration (μg/L)	Receptors detected
<10[b]	3/3	<10[b]	3/3	<10[b]	3/3
266	0/3	60.0	0/3	60.0	3/3
		120	0/3	ND[c]	ND

[a] Fish were held in control water for 14 d following 96-h exposure to copper concentrations.
[b] Control.
[c] ND = not determined because few fish recovered from copper exposure.

centration, the magnitude of toxic effect increases with exposure time.

One explanation for the observed response is that sensitivity of the sensory system or receptors changed with time. If this explanation is correct, then compensatory mechanisms induced by initial exposure must have facilitated physiological adaptation and the development of tolerance within 96 h. A time-dependent response of the olfactory system has been observed by other investigators [24] and is consistent with the general adaptation syndrome [25,26]. The general adaptation syndrome describes the response of physiological systems to stressors as a three-phase time-dependent process that includes an initial loss of ability caused by exposure followed by a period of physiological adaptation that ends when compensating mechanisms are unable to sustain the level of activity required to offset effects of the stressor. Our data suggest that the initial loss of ability occurred during the first 24 h of exposure and was reflected by the lower response rate of fish to skin homogenate. By 96 h, induction of protective mechanisms allowed recovery of olfactory ability, which increased the frequency of detection and response to skin homogenate in copper concentrations <66 μg/L. Fish exposed to higher copper concentrations either were not able to compensate or needed more time for protective mechanisms to be induced sufficiently to offset effects of exposure.

Other data support our hypothesis that loss of olfactory ability followed by recovery can occur within 96 h. Several investigators have reported that olfactory receptors of fish are damaged or destroyed in <24 h by exposure to copper concentrations ranging from 0.02 to 7,600 mg/L [27–31]. In addition, investigators have reported that regeneration of olfactory cells after exposure to copper occurs within 8 d to 12 weeks [7,24,30,32] and that effects and recovery of ability are exposure dependent [24,31]. Copper concentrations in most studies of structural damage to olfactory cells are much higher than those in our investigation. Consequently, other copper-specific data documenting repair of structural damage in the olfactory organ are not available for the concentration range and 96-h exposure duration that we used. However, Cancalon [33] observed regeneration of olfactory receptors in fish within 96 h of exposure to low concentrations (0.03–0.1%) of a detergent. Thus, it is possible that protective mechanisms in Colorado pikeminnow were induced and regeneration of olfactory receptors occurred within the 96-h exposure period.

Potential protective mechanisms

There are several potential protective mechanisms that may decrease effects of long-term exposure to copper on olfactory receptors by sequestering, eliminating, or reducing absorption of toxicants. First, exposure to copper has been shown to increase mucus production in fish [24,34]. Mucus provides a protective coat over olfactory sensory cells. Odorants or contaminants must diffuse through the mucus layer before they can stimulate or inhibit receptor cells [35]. Increased mucus production induced by contaminant exposure increases thickness of the mucus coat [34]; consequently, time required for toxic solutes to diffuse to olfactory receptors may increase. Miller and Mackay [36] showed that mucus is a strong copper chelater. Thus, in addition to the physical advantages of a thicker mucus coat, the affinity of mucus for contaminants may prevent diffusion to olfactory receptors, and sloughing of excess mucus may physically remove chelated toxicants from the olfactory chamber. Whitear [37] hypothesized that chemoreceptors in fish may have a neuroendocrine link to surrounding cells in the epidermis. If this function exists for olfactory receptors of fish, it may influence mucus secretion of the epidermis by controlling goblet and superficial epithelial cells. Such an association would provide a basis for a feedback mechanism in which olfactory receptor cells could optimize local mucus production by increasing it when harmful solutes are present and decreasing it under normal conditions to improve sensitivity to information-containing odorants.

A second potential protective system is induction of detoxifying mechanisms. The olfactory epithelium of a number of fishes contains high levels of cytochrome P-450 monooxygenase, which can be activated by exposure to heavy metals [9]. Presence of this enzyme system in olfactory tissues suggests the ability to eliminate or sequester toxic solutes.

A third potential protective system involves a sequestering function of melanophores in the olfactory organ. After 60-d exposure to copper at 20 μg/L, Julliard et al. [38] observed a correspondence between regeneration of olfactory structure in rainbow trout (*Oncorhynchus mykiss*) and increasing metal concentrations in melanosomes in melanophores. They suggest that the correspondence is evidence of a mechanism that contributes to recovery of olfactory ability despite continued copper exposure.

Implications of this investigation

The regenerative capacity and variable sensitivity of the olfactory system of fish is probably an evolutionary adaptation to injury during normal life [9]. These abilities may give a false impression that there is a margin for error when assessing potential effects of environmental contaminants on fish. However, evidence suggests that fish that are acclimated to chemical exposure suffer reduced olfactory ability compared with unexposed fish [24]. In addition, intermittent loss and recovery of olfactory ability can potentially have a high biological cost. Olfaction in fishes facilitates a variety of ecological interactions including predator avoidance, feeding in patchy environments, mating, and migration [39]. Although the temporal scale of these behaviors may be short (e.g., predator avoidance may occur in seconds), they facilitate intense interactions that have important outcomes like survival or death.

Results of this study and available water-quality data suggest that copper concentrations in the Yampa River may occasionally inhibit olfactory ability of resident Colorado pikeminnow. The Yampa River was selected for comparison in this study because data are available that describe its water quality characteristics, adult Colorado pikeminnow inhabit the river all year, and spawning occurs in the river from late June through early August. However, the Yampa River is considered to be one of the more pristine large rivers in the Colorado River Basin. Conditions in other rivers may warrant greater concern.

There are a variety of contaminant sources in the Colorado River Basin including natural hot springs, municipal releases, irrigation returns, and historic mining. The potential for existing sources of contamination to result in adverse ecological effects is compounded by demand for water in the West. As resources are diverted for municipal, industrial, and agricultural uses, less water will be available for dilution. If increases in water use outpace the process of pollution abatement within the Colorado River Basin, frequency and magnitude of contaminant-induced toxic effects will increase even if contaminant inputs remain constant. Geographical distribution of Colorado pikeminnow has been greatly reduced and reproducing

populations currently inhabit less than 25% of their former range [1]. There will be no refugia for Colorado pikeminnow if remaining habitat becomes unsuitable for any life-stage requirement. Given that many human-induced changes responsible for decline of Colorado pikeminnow are still present in the Colorado River Basin, it is important to prevent further anthropogenic degradation and be proactive about predicting how existing conditions may change in the future.

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