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Cadmium-induced olfactory dysfunction in rainbow trout: Effects of binary and quaternary metal mixtures

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ABSTRACT

A functioning olfactory response is essential for fish to be able to undertake essential behaviors. The majority of work investigating the effects of metals on the olfactory response of fish has focused on single-metal exposures. In this study we exposed rainbow trout to cadmium, copper, nickel, zinc, or a mixture of these four metals at or below the current Canadian Council of Ministers of the Environment guidelines for the protection of aquatic life. Measurement of olfactory acuity using an electro-olfactogram demonstrated that cadmium causes significant impairment of the entire olfactory system, while the other three metals or the mixture of all four metals did not. Binary mixtures with cadmium and each of the other metals demonstrated that nickel and zinc, but not copper, protect against cadmium-induced olfactory dysfunction. Testing was done to determine if the protection from cadmium-induced olfactory dysfunction could be explained by binding competition between cadmium and the other metals at the cell surface, or if the protection could be explained by an up-regulation of an intracellular detoxification pathway, namely metallothionein. This study is the first to measure the effects of binary and quaternary metal mixtures on the olfactory response of fish, something that will aid in future assessments of the effects of metals on the environment.

1. Introduction

The olfactory system of fish can be impaired by a wide variety of contaminants, including low, ecologically-relevant concentrations of metals (Tierney et al., 2010). Without a functioning olfactory system fish cannot effectively undertake olfactory-mediated behaviours, including finding food, selecting an appropriate mate, following migratory routes, or evaluating the risk of predation (Lürling and Scheffer, 2007). To date, studies involving the effects of metals on the olfactory response of fish have predominantly focused on single-metal exposures.

Of the studies evaluating the effects of metal mixtures on fish olfaction, two (Azizishirazi et al., 2013; Thompson and Hara, 1977) measured the neurophysiological effects of contaminated lake water, while others (Hansen et al., 1999b; Hartwell et al., 1987a; Hartwell et al., 1987b; Svecevičius, 1999, 2003) measured the behavioural effect of exposure to a metal mixture on the response to a cue composed of the same metal mixture. Short-term exposure (10 min) of arctic char (*Salvelinus alpinus*) to water from a multi-metal-contaminated lake (Ross Lake) or a quaternary mixture of cadmium, copper, nickel, and zinc based on the concentrations found in Ross Lake induced comparable reductions in olfactory function as measured at the olfactory bulb (Thompson and Hara, 1977). Exposure of wild yellow perch (*Perca flavescens*) to water sourced from multi-metal-contaminated lakes in the Sudbury region of Ontario, Canada induced olfactory dysfunction as measured at the olfactory epithelium by electro-olfactogram (EOG) (Azizishirazi et al., 2013). Both studies illustrate that complex mixtures of metals can impair the olfactory response of fish; however, neither study identified which metals were responsible for the impairment, or if there were interactive effects of mixing.

For our present study we selected four metals associated with hard-rock mining operations; cadmium, copper, nickel and zinc. All four metals have previously been demonstrated to impair olfactory-related neurophysiological or behavioural responses of fish following single metal exposures. Of the four metals, copper has received the most attention and has been shown to impair the olfactory response of a wide variety of fish species, including fathead minnows (*Pimephales promelas*) (Carreau and Pyle, 2005; Dew et al.,

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2012; Green et al., 2010), Colorado pikeminnow (*Ptychocheilus lucius*) (Beyers and Farmer, 2001), goldfish (*Carassius auratus*) (Kolmakov et al., 2009), and a number of salmonids including Atlantic salmon (*Salmo salar*) (Winberg et al., 1992), coho salmon (*Oncorhynchus kisutch*) (Baldwin et al., 2011; McIntyre et al., 2008; Sandahl et al., 2007), chinook salmon (*Oncorhynchus tshawytscha*) (Hansen et al., 1999a), chum salmon (*Oncorhynchus keta*) (Sandahl et al., 2006), and rainbow trout (*Oncorhynchus mykiss*) (Baldwin et al., 2011; Hansen et al., 1999a; Hara et al., 1976). The olfactory-related effects of cadmium exposure has also received some attention, as it impairs olfactory responses of common carp (*Cyprinus carpio*) (Takagi et al., 1978), banded kokopu (*Galaxias fasciatus*) (Baker and Montgomery, 2001), zebrafish (*Danio rerio*) (Blechinger et al., 2007), rainbow trout (Scott et al., 2003; Sloman et al., 2003), and coho salmon (Williams and Gallagher, 2013). Very few studies have investigated the effects of nickel and zinc on fish olfaction. Nickel is known to impair the olfactory response of rainbow trout (Brown et al., 1982) and fathead minnows (Dew et al., 2014), while zinc impairs the olfactory response of rainbow trout (Brown et al., 1982), Atlantic salmon (Saunders and Sprague, 1967), and zebrafish (Bloom et al., 1978). While it is important to determine the effects of individual metals on olfaction, fish in effluent receiving waters are often exposed to a complex mixture of contaminants, not single metals (Azizishirazi et al., 2013). In order to understand how fish olfaction is affected by living in receiving waters containing complex mixtures of contaminants, fish must be exposed to both individual metals and a mixture of metals to determine if there are any interactive effects of mixing.

Metallothionein (MT) strongly binds and sequesters cadmium and is present in olfactory tissues of fish (Espinoza et al., 2012; Roy et al., 2012). In some fish species, exposure to cadmium is sufficient by itself to induce MT expression; however, exposure to low concentrations of cadmium for short periods of time does not induce MT in rainbow trout (Hollis et al., 2001; Kay et al., 1987; Thomas et al., 1983; Williams and Gallagher, 2013). Interestingly, co-exposure of cadmium and other metals induces MT production in rainbow trout. Exposure of rainbow trout to cadmium resulted in its binding to non-MT proteins, while pretreatment with zinc resulted in cadmium binding to MT (Thomas et al., 1985). In another fish species (tilapia) co-exposure of cadmium with calcium results in increased production of MTa (one of the two major isoforms of MT in fish) in contrast to no increase in expression following individual exposures with either cadmium or calcium (Wu et al., 2007).

The purpose of the present study was to determine the effects of single metal exposures and metal mixtures of cadmium, copper, nickel, and zinc on rainbow trout olfactory function using concentrations at or below the current Canadian Council of Ministers of the Environment (CCME) guidelines for the protection of aquatic life (CCME, 2001). The guideline values for cadmium (0.05 μg/L), copper (3.6 μg/L), and nickel (136.6 μg/L), were determined by using equations that base the guideline for each metal on the hardness of the receiving waters. The guideline value for zinc is 30 μg/L regardless of hardness. In freshwater ecosystems, the concentration of cadmium is usually less than 0.5 μg/L (McGeer et al., 2012). A survey of streams in Europe demonstrated that there was a range of cadmium from <0.002 μg/L (pristine) to 1.25 μg/L (contaminated) (Pan et al., 2010). The range of nickel found in pristine waterways in Canada can range from 0.1–10 μg/L, while a contaminated waterway can have 50–2000 μg/L (Chau and Kulikovsky-Cordeiro, 1995). The concentration of zinc in waterways ranges from 0.02 μg/L in pristine rivers to >1000 μg/L in contaminated areas, however, zinc rarely exceeds 50 μg/L in a freshwater ecosystem (Hogstrand, 2012). Normal concentrations of copper in Canadian surface and lake waters range from 1 to 80 μg/L, with it rarely exceeding 5 μg/L (Canadian, 1987). Therefore, with the exception of the nickel exposure, which represents a moderately contaminated environment, the concentrations used in the present study are within the normal concentration range for each metal in a freshwater ecosystem.

Table 1
Selected water quality parameters and dissolved metal concentrations of dechlorinated Lethbridge municipal water.

Parameter	Measured value
Cadmium	<17 ng/L[a]
Copper	1.1 ± 0.06 μg/L
Nickel	<2 μg/L[a]
Zinc	<3 μg/L[a]
Calcium	44.2 ± 0.2 mg/L
Magnesium	18.5 ± 0.0 mg/L
Sodium	22.0 ± 0.1 mg/L
Potassium	1.6 ± 0.0 mg/L
Temperature	11.5–12.5 °C
Dissolved organic carbon (DOC)	1.7 ± 0.1 mg/L
pH	7.5–8.2
Calculated hardness	133.1 mg/L as $CaCO_3$

[a] Detection limit.

We determined electro-olfactograms (EOGs), a neurophysiological technique that measures an odour-evoked extracellular field potential at the olfactory epithelium in response to olfactory stimuli. Two olfactory stimuli, L-alanine and taurocholic acid (TCA), were used to determine the response of two olfactory sensory neuron (OSN) classes within the olfactory epithelium; microvillous (responds to L-alanine) and ciliated (responds to TCA) OSNs (Dew et al., 2014; Laframboise and Zielinski, 2011). A similar technique was used with coho salmon, demonstrating different cues (in that case TCA and L-serine) could be used to determine the effect of a contaminant on non-overlapping OSN populations (Baldwin et al., 2003). Quantitative PCR (qPCR) was employed to determine if impairment of EOG function was associated with increases in abundance of mRNA encoding metallothionein α and β (*mta* and *mtb*) isoforms in olfactory tissues. Subsequent binary mixtures with the metals and with electrolytes were done to elucidate the non-additive interaction found with the quaternary mixture, and to determine if such effects were due to the electrolyte-mimicking properties of copper, nickel, and zinc. This research represents the first study to determine the additivity of a metal mixture on the olfactory system of fish.

2. Materials and methods

2.1. Animals

Juvenile rainbow trout were obtained from the Allison Creek Trout Brood Hatchery Station and held in the Aquatic Research Facility at the University of Lethbridge. Rainbow trout were held in an aerated recirculation system at 12 °C under a 16 h:8 h light:dark photoperiod. Fish were acclimated to laboratory conditions for a minimum of two weeks and were fed a maintenance diet (0.5% body weight per day) of commercial trout chow (EWOS, Surrey, BC, Canada) once a day. Samples of the holding water were collected, filtered through a 0.45 μm filter, and acidified to pH 3.0 with trace metals grade nitric acid (Fisher Scientific, Toronto, ON, Canada) to determine metal concentrations. Dissolved metal concentrations were determined by inductively coupled plasma mass spectrometry (ICP–MS) by ALS Environmental (Thunder Bay, ON, Canada). Holding water quality data are reported in Table 1.

2.2. Electro-olfactogram experiments

The EOG responses of rainbow trout were measured as previously described, with modifications required for working with rainbow trout (Dew et al., 2012; Green et al., 2010). Fish were