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Highlighted Article

Do you smell what I smell? Olfactory impairment in wild yellow perch from metal-contaminated waters

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ABSTRACT

In this study, we sampled yellow perch from three lakes along a metal-contamination gradient and examined their olfactory ability in response to conspecific chemical alarm cues and metal-binding characteristics of their olfactory epithelium (OE). We measured the electrophysiological response at the OE, tested their antipredator behaviour and measured neuronal density at the olfactory rosette and bulb. Yellow perch from contaminated lakes exhibited significantly larger electrophysiological responses to alarm cues than clean lake fish, but showed no antipredator behaviour contrary to clean lake fish. Neuron density did not differ at either the olfactory rosette or bulb between clean and contaminated fish. Unlike fishes raised under laboratory or aquaculture settings, fish from contaminated lakes possessed a functional OE after metal exposure, but similar to laboratory/aquaculture fishes, yellow perch did not exhibit olfactory-mediated behaviours. Thus, wild fish from contaminated lakes can detect chemical stimuli but olfactory signal processing is disrupted which could alter ecological functioning.

1. Introduction

Metal contamination, at elevated concentrations, can pose serious problems for many aquatic organisms. The influx of metals into an aquatic system can cause short- and long-term changes in the physiology and behaviour of fishes (see reviews: Klaprat et al., 1992; Scott and Sloman, 2004). Historically, metal toxicity was often evaluated on the basis of acute lethality toxicity testing which were used to set regulatory guidelines (reviewed in Scott and Sloman, 2004). However, most natural metal-contaminated environments have metal concentrations that are substantially below those required to induce acute toxicity. Acute toxicity tests do not account for effects on ecological activities, such as foraging, reproduction, or assessing predation risk. Although exposure to subacute metal concentrations are not directly lethal to fishes, it may impede a fish's ability to function in an ecological context if their normal physiology and (or) behaviour is altered (Scott and Sloman, 2004). Thus, aquatic organisms from natural ecosystems exposed to metals may not respond in the same manner physiologically and behaviourally to environmentally relevant stimuli as metal-exposed fishes from laboratory or aquaculture settings.

Fishes use chemosensory information to mediate several important ecological activities such as foraging, assessing predation risk, finding mates, reproduction, kin recognition, locating and recognizing conspecifics, assessing habitat quality, and homing/migration (Smith, 1992; Wisenden, 2000; Sandahl et al., 2006). Impairment of chemosensory function can lead to disruption of activities crucial for survival, potentially resulting in large-scale effects that have gone relatively unexplored by the ecological risk assessment community. Waterborne metals such as aluminium (Al), silver (Ag), cadmium (Cd), copper (Cu), mercury (Hg), manganese (Mn), nickel (Ni), and zinc (Zn) have been shown to disrupt olfactory receptor function and (or) olfactory-mediated behaviours in laboratory- or aquaculture-reared fishes (Hara et al., 1976; Tallkvist et al., 1998, 2002; Hansen et al., 1999a, b; Beyers and Farmer, 2001; Baldwin et al., 2003; Persson et al., 2003; Scott et al., 2003; Sandahl et al., 2004, 2006, 2007; Carreau and Pyle, 2005; Bettini et al., 2006). Similarly, exposure to organic contaminants also disrupts olfactory function (Scholz et al., 2000; Sandahl et al., 2004; Scott and Sloman, 2004).

The olfactory epithelium is constantly in direct contact with the aquatic environment and therefore to any contaminants that are present (Klaprat et al., 1992; Baldwin et al., 2003; Sandahl et al., 2004; Bettini et al., 2006). Direct exposure to contaminants

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may result in damage or death of olfactory sensory neurons (OSNs). Previous studies have shown that exposure to 10–25 μg Cu/L over 4 h led to OSN cell death (Hansen et al., 1999b; Baldwin et al., 2003; Bettini et al., 2006) and although OSNs are capable of regenerating it may be weeks before recovery (Zielinski and Hara, 1992). This could account for the disruption of olfactory receptor function and olfactory-mediated behaviours documented in previous studies. All previous studies have been conducted on laboratory- or aquaculture-reared fishes and it is not known whether wild fishes from contaminated lakes exhibit the same olfactory inhibition (but see McPherson et al., 2004).

Industrial activities have been taking place in the Sudbury region (northern Ontario, Canada) for more than a century, resulting in some of the most metal-contaminated environments in the world (Keller and Gunn, 1995). The metals of interest in this region are primarily Cu, Ni, and Zn (Brodeur et al., 1997; Rajotte and Couture, 2002; Couture and Rajotte, 2003; Pyle et al., 2005; see Klinck et al. (2007) for a complete list of metals found in Sudbury area lakes). The 'zone of impact' from industrial activities extends approximately 17,000 km^2 around the center of mining and processing activities and includes approximately 7000 lakes (Keller et al., 1992). Although remediation efforts have been ongoing for decades, several metal-intolerant species (e.g., salmonids and cyprinids) have been extirpated. One of the few metal-tolerant fishes found in lakes inside the zone of impact are yellow perch, *Perca flavescens*. Yellow perch are widely distributed throughout North America (Scott and Crossman, 1979), and occupy important trophic positions in aquatic ecosystems depending on their age and resource availability (i.e., planktivore, benthivore, and piscivore) (Sherwood et al., 2002). Moreover, yellow perch use chemical alarm cues (released from injured conspecifics) to assess predation risk (Mirza et al., 2003). Therefore it is important to study fishes specifically from metal-contaminated systems to determine whether laboratory-generated results are applicable to wild fishes which would provide the highest degree of ecological relevance for environmental risk assessment.

In this study, we tested the olfactory ability of yellow perch from contaminated and non-contaminated lakes along a metal-contamination gradient around Sudbury, Ontario, Canada. We collected juvenile yellow perch from three different lakes: Hannah Lake (contaminaed), Ramsey Lake (contaminated), and James Lake (reference). First, we measured the integrated extracellular field potential response of yellow perch to stimulation of olfactory epithelium (OE) using electro-olfactogram (EOG) to yellow perch alarm cues. Second, using yellow perch from the same three lakes, we tested their antipredator behaviour in response to yellow perch alarm cues. Third, we examined neuronal cell density at the OE and olfactory bulb to evaluate whether changes in the number of neurons at either site could have contributed to the observed differences among fish from the three lakes. Based on studies with laboratory- and aquaculture-reared fishes, we predicted that compared to yellow perch from reference lakes yellow perch from contaminated lakes should exhibit: (1) lower electrophysiological activity at the OE, (2) reduced olfactory-mediated behaviours, and (3) lower neuron density at the OE.

2. Methods

2.1. Collection and maintenance of animals

In all studies, fish were handled in accordance with the guidelines of the Canadian Council of Animal Care. Juvenile yellow perch were collected by angling from Hannah and Ramsey Lakes (contaminated), and James Lake (reference) in early August and early October 2005 and transported in aerated native lake water to Nipissing University (Fig. 1). Yellow perch were held in 60 l plastic tanks in

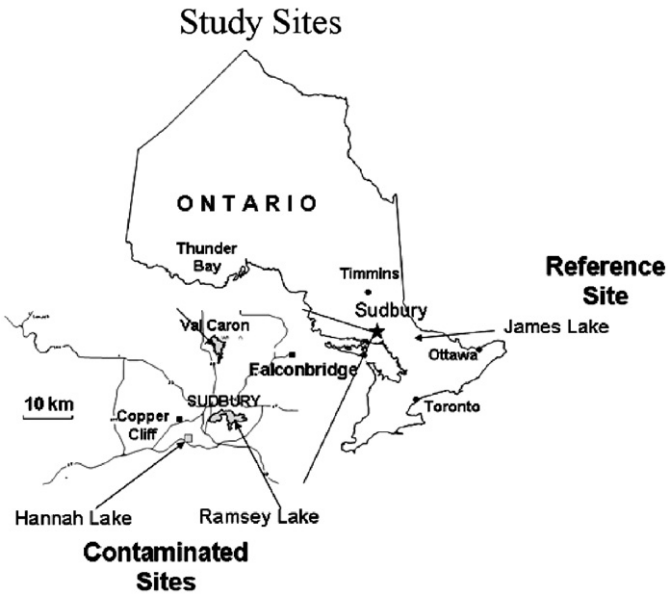

Fig. 1. Map of study sites, where water samples and yellow perch were collected.

North Bay dechlorinated tap water at 20 °C in static water under a 16:8 L:D photoperiod for 48 h before being used in experiments. Fish were not fed during this period because past experience has shown that yellow perch from these northern lakes will not readily feed in the laboratory (personal observation). However, yellow perch from each lake were treated in the same manner prior to and during experimentation. Waterborne concentrations of metals of interest and basic water quality parameters of the study lakes are listed in Table 1. All work reported herein was conducted in accordance with Nipissing University Animal Care Protocols 2005-03-03 and 2005-10-03.

2.2. Stimulus preparation

For testing electrophysiological responses, a 10^{-5} M L-alanine standard test stimulus was prepared fresh daily by diluting a fresh 10^{-2} M L-alanine stock solution with dechlorinated municipal water. We also prepared skin extract stimuli (chemical alarm cue) from juvenile rainbow trout (mean ± SD standard length, 8.05 ± 1.11 cm, $n = 11$), James Lake yellow perch (9.17 ± 0.86 cm, $n = 7$), Ramsey Lake yellow perch (9.15 ± 1.78 cm, $n = 10$), and Hannah Lake yellow perch (11.90 ± 1.85 cm, $n = 6$). Although the skin extract is a mixture of skin components of which a proportion are the actual alarm cue, this mixture represents what fishes would be exposed to during a predation event; hence, we refer to the skin extract as an alarm cue. Fish were sacrificed by severing the spinal cord in accordance with guidelines of the Canadian Council on Animal Care. Skin was removed from both sides of each fish and placed into 100 ml of ice-chilled dechlorinated tap water For yellow perch, 81.03 cm^2 (Hannah Lake), 82.64 cm^2 (James Lake), and 80.91 cm^2 (Ramsey Lake) of skin were used to produce yellow perch skin extract stimuli. For rainbow trout 134.43 cm^2 of skin was used; this served as a control for stimuli from an injured fish. Skin was homogenized, then filtered through polywool filter floss to remove any large particles and then diluted in dechlorinated tap water to a final volume of 1600 ml for yellow perch from each lake and 2685 ml for rainbow trout giving us final concentrations of 0.051 cm^2 skin/ml (Hannah Lake), 0.052 cm^2 skin/ml (James Lake), 0.051 cm^2 skin/ml (Ramsey Lake), and 0.050 cm^2 skin/ml rainbow trout. Skin extracts (yellow perch and rainbow trout) were then frozen at −20 °C in 50 ml aliquots until used.

For behavioural trials, 14.12 cm^2 (Hannah Lake), 14.23 cm^2 (James Lake), and 14.09 cm^2 (Ramsey Lake) of skin was removed from three yellow perch from each lake (mean ± SD James Lake: 11.0 ± 1.47 cm; Ramsey Lake: 7.97 ± 0.40 cm; Hannah Lake: 8.63 ± 0.47 cm). Skin was homogenized and filtered (as described above), then diluted in distilled water to make a final volume of 275 ml for each population. This gave us concentrations of 0.051 cm^2 skin/ml (Hannah Lake), 0.052 cm^2 skin/ml (James Lake), and 0.051 cm^2 skin/ml (Ramsey Lake). Stimuli were frozen in 25 ml aliquots at −20 °C until used in the experiment. Rainbow trout stimulus was from the same batch used in the EOG experiments.

We produced a brine shrimp stimulus by placing 1 g of frozen brine shrimp (*Artemia salina*) into 250 ml of dechlorinated water and then filtered through polywool filter floss to remove large particles (final concentration 0.004 g brine shrimp/ml water). The supernatant was used to stimulate swimming activity in yellow perch during the pre-stimulus period. Yellow perch typically remain stationary when isolated; thus, an additional stimulus is required to promote

Table 1
Dissolved metal (μM), cation (mM) concentrations, and water quality parameters in water samples ($n = 5$) collected from Hannah, Ramsey (contaminated) and James (reference) lakes

Lake		Cu	Ni	Zn	Ca	Mg	Na	K	pH	Hardness (mg/l as $CaCO_3$)	Alkalinity (mg/l as $CaCO_3$)
James	Mean	0.02	0.01	0.06	0.23	0.36	0.03	0.01	6.6–7.0	56–61	40–43
	SEM	0.005	0.005	0.009	0.027	0.07	0.009	0.001	–	–	–
Ramsey	Mean	0.14	0.74	0.05	0.34	0.17	2.13	0.04	7.7–8.0	47–54	30–35
	SEM	0.006	0.169	0.010	0.107	0.033	0.05	0.001	–	–	–
Hannah	Mean	0.39	2.26	0.13	0.25	0.16	2.48	0.05	6.8–7.1	40–42	16–18
	SEM	0.005	0.100	0.009	0.044	0.01	0.03	0.007	–	–	–

Note: SEM = standard error of the mean.

swimming/searching activity (Mirza et al., 2003). Brine shrimp stimulus was made fresh before every set of trials.

2.3. Electrophysiological responses

2.3.1. Electro-olfactogram recording

Yellow perch were prepared for EOG experimentation using methodology adapted from Sveinsson and Hara (2000). Yellow perch were anaesthetized in a solution of MS222 (150 mg/l; ethyl 3-aminobenzoate methanesulfonate salt, Sigma, Oakville, ON, Canada) and subsequently immobilized by an epaxial intramuscular injection of Flaxedil (gallamine triethiodide, 3 mg/kg body mass, Sigma, Oakville, ON, Canada) (Sveinsson and Hara, 2000). Anaesthetized and immobilized fish were wrapped in wet tissue paper (to prevent desiccation), leaving the head and tail regions exposed. Fish were secured and electrically grounded in a Plexiglas perfusion chamber where the gills were perfused with a constant supply of oxygenated, dechlorinated water containing 50 mg/l MS222 via a tube inserted into the mouth (Sveinsson and Hara, 2000). The right olfactory rosette was exposed by removing the nasal septum separating the anterior and posterior nares and the exposed olfactory chamber was perfused with dechlorinated water.

EOG responses were differentially recorded (difference between the recording and reference electrodes) using saline (0.9%)-gelatine (4%) filled capillary tubes (tip diameter, 60–80 μm) bridged to Ag/AgCl electrodes (Type MEH8, WP Instruments, Sarasota, FL, USA) filled with 3 M KCl. The recording electrode was placed in the olfactory chamber, close to but not touching the olfactory epithelium, near the posterior-most lamellae to obtain the largest response to a standard (10^{-5} M L-alanine; Sigma, Oakville, ON, Canada) and a minimal response to a dechlorinated water blank. A reference electrode was placed on the surface of the skin posterior to the perfused naris (Sveinsson and Hara, 2000). EOG responses were amplified using a DC headstage and DC preamplifier (AD Instruments, Colorado Springs, CO, USA) and recorded using a computer-assisted data acquisition system (model ML 750, AD Instruments, Colorado Springs, CO, USA) and Chart® version 5 software.

2.3.2. Experimental protocol

Experimental stimuli were delivered one at a time to the nares of the fish using a gravity-flow stimulus delivery system for five seconds, after which the flow of dechlorinated water was immediately restored. Two minutes were left between each stimulus delivery to minimize olfactory receptor adaptation to odourants. Before testing began fish were allowed to acclimate in the Plexiglas perfusion chamber for 15–20 min. Using a randomized block design, four stimuli were delivered in random order: (1) dechlorinated water (blank), (2) 10^{-5} M L-alanine (standard cue), (3) yellow perch skin extract (conspecific alarm cue), and rainbow trout skin extract (control for odour of an injured fish). Each stimulus was delivered three times consecutively. At the time of testing, gill perfusion water and all experimental stimuli were at room temperature (18–22 °C). Ten fish per lake were used in these experiments.

A subset of five yellow perch from each lake also received two additional types of yellow perch skin extract in order to determine if yellow perch from each lake respond differently to alarm cues produced by non-native yellow perch; i.e., yellow perch skin extract donors came from the other two lakes.

2.4. Behavioural assays

Juvenile yellow perch from each lake were exposed to either: yellow perch skin extract, rainbow trout skin extract or dechlorinated tap water ($n = 15$ per treatment for James and Hannah Lakes; Ramsey lake $n = 12$ for yellow perch skin extract, $n = 13$ for rainbow trout skin extract, $n = 12$ for dechlorinated tap water). We tested 45 yellow perch from each of James and Hannah Lakes and 37 yellow perch from Ramsey Lake (James Lake: 11.0 ± 1.01 cm SL, 17.3 ± 3.25 g; Ramsey Lake: 8.37 ± 1.67 cm SL, 9.03 ± 4.98 g; Hannah Lake: 9.03 ± 1.62 cm SL, 10.5 ± 5.3 g). Trials were conducted in 37-l aquaria ($50 \times 25 \times 30$ cm^3). Each test aquarium had a single air stone located in the middle of the back wall of the short side of the tank. A plastic tube was situated next to the air stone for introduction of a chemical stimulus into the tank. Tanks were wrapped with black plastic on three sides to visually occlude test fish from those in adjacent tanks to avoid any visual influences from other fish.

All yellow perch were tested individually and exposed to conspecific alarm cues from their respective populations to mimic natural exposure conditions. Previous studies have shown that juvenile yellow perch respond to alarm cues from conspecifics both in groups and individually (Mirza et al., 2003), resulting in adaptive antipredator behaviour, such as reduced movement, reduced foraging, tighter group cohesion or area avoidance (Lima and Dill, 1990). All yellow perch were tested in clean North Bay dechlorinated tap water (median pH: 7.0, range: 6.7–7.6; alkalinity: 23.4 ± 2.5 mg/l as $CaCO_3$; hardness: 35.6 ± 3.9 mg/l as $CaCO_3$, $n = 13$).

Trials were conducted after a 24 h period of acclimation to test tank conditions. Each trial was 17 min in length and consisted of an 8-min pre- and 8-min post-stimulus period, with a 1-min stimulus introduction period between the pre- and post-stimulus periods. At the beginning of each trial, 60 ml of water was removed from the tank through the stimulus injection tube with a syringe and discarded. This removed any stagnant water in the injection tube. A second and third 60 ml syringe of water was removed and retained. We then injected 5 ml of brine shrimp stimulus and slowly flushed it through the injection tube with one of the two retained 60 ml syringes at a rate of 1 ml/s. After the pre-stimulus period, 5 ml of the test stimulus was injected into the tank and again flushed slowly through the stimulus delivery line with the 60 ml of water at the same rate. Dye trials indicated that it took approximately 30 s for the test stimulus to distribute to all parts of the tank. All trials were conducted using direct observation. After trials fish were euthanized as described above and used for histological analysis (see below).

2.5. Neuron density counts

To ensure that the neuron density analysis was conducted blind to group designation, all animals were coded prior to tissue harvesting. Six adult fish from each lake (used in behavioural tests) were included in the histological analysis.

2.5.1. Tissue preparation and sectioning

Animals were sacrificed by severing the spinal column. Subsequently, both olfactory rosettes and the olfactory bulb were extracted from each fish and drop fixed in 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.3). Tissue blocks remained in fixative for an additional 72 h, before being placed in three ten minute washes of phosphate buffer followed by 1% osmium tetroxide for 1 h. The tissue blocks were then dehydrated in a graded series of ethanol solutions and embedded in Spurr's embedding medium (Ladd Research Industries, Burlington, Vermont).

Excess embedding medium was removed and thick sections (1 μm) were taken from the olfactory rosettes and olfactory bulbs. Thick sections were stained with toluidine blue (2%) in order to quantify the number of neurons in the tissue volume. Thick sections were photographed using a digital camera affixed to a Leica light microscope (400 × original magnification).

2.5.2. Neuron counting

Estimates of the number of neurons were determined using an unbiased dissector technique (Black et al., 1990). This stereological technique produces an estimate of the neuronal density in a given volume of tissue. This method is necessary to account for changes in neuronal density due to hypertrophy or tissue

processing effects, and to ensure that neurons are only counted once regardless of their size and configuration.

Neurons were distinguished from glial or support cells by the presence of a central nucleolus within a pale nucleus. Neurons were counted by comparing pairs of adjacent thick sections in the series. Neurons were counted if they were observed in one of the sections within the area limited by an unbiased sampling frame (400 μm^2), but not observed in the other section in the dissector pair. This ensured that neurons were only counted once regardless of the number of images in which they appeared. The symbol Q was used to denote the total number of neurons counted in each series. The total volume for neuronal counting (V_{neur}) was derived as: $V_{neur} = A \times H$, where A is the area of the counting frame and H the total thickness of the series. Neuron density (N_{neur}) was calculated as: $N_{neur} = Q/V_{neur}$. These data were subsequently converted to neurons per mm^3 which served as the final units of analysis.

For the rosette tissue, randomly selected sampling regions were chosen within bands of sensory neurons, as these bands contain the primary sensory neurons involved in olfaction. In the olfactory bulbs, the randomly chosen sampling frames were selected from the peripheral region of the bulb.

2.6. Water sample analysis

Water samples from each of the three lakes were filtered through a 45 μm nylon filter and the dissolved Cu, Ni, and Zn concentrations were determined using GFAAS (Varian GTA 120, Varian Scientific, Mulgrave, Australia). A certified reference material for trace metals (TE90-4) from the National Research Council of Canada was used to ensure accuracy. The concentrations of Ca^{2+}, K^+, Na^+, and Mg^{2+} in water samples were measured using FAAS (Varian SpectrAA-240 FS, Varian Scientific, Australia) against standard curves produced using reference standards from Fisher Scientific, USA.

2.7. Statistical analysis

In the EOG trials, we conducted 2 MANOVAs to determine if there was a significant interaction of the response of yellow perch from each lake and stimulus (10^{-5} M L-alanine, native yellow perch skin extract, rainbow trout skin extract) on the integrated extracellular field potential at the OE of yellow perch. Subsequent one way ANOVAs were conducted with Dunnett's post hoc analysis to determine if significant differences exist when comparing the extracellular field potentials at the OE of yellow perch from the two contaminated lakes to that of James Lake yellow perch. The second MANOVA was conducted to determine whether the EOG response to yellow perch alarm cues from different donor populations i.e., each lake were different.

In the behavioural trials, during both the pre- and post-stimulus periods we recorded time spent moving, dashing (a burst of rapid, disorientated swimming), and freezing (remaining motionless in the water column or on the substrate for a minimum of 30 s). Changes between the pre- and post-stimulus periods were calculated (post-stimulus minus pre-stimulus), and the difference in changes among treatments was analysed using a 2-way ANOVA testing for main effects of lake, stimulus, and lake × stimulus interactions followed by Tukey HSD post-hoc analysis. Dashing and freezing were analysed using Chi-square tests for independence followed by post hoc pairwise comparisons. We compared neuron density at the olfactory epithelium and olfactory bulb from yellow perch among the three lakes with a one-way ANOVA. Differences among comparative groups were considered statistically significant when $p \leqslant 0.05$.

3. Results

Yellow perch from clean and contaminated lakes exhibited significantly different EOG responses at the olfactory epithelium when exposed to L-alanine, native yellow perch alarm cue and rainbow trout skin extract (Table 2). When exposed to native yellow perch skin extract, yellow perch from the contaminated lakes (Ramsey and Hannah Lakes) exhibited higher extracellular field potentials (2.7 and 2.3 times greater, respectively) than yellow perch from the reference lake (James Lake) (Fig. 2). Similarly, yellow perch from Ramsey and Hannah lakes had increased EOG responses to rainbow trout skin extract (2.3 and 1.8 times greater, respectively) and L-alanine (3.3 and 1.9 times greater, respectively) compared to responses of James Lake yellow perch (Fig. 2). Yellow perch from all three lakes responded equally to yellow perch skin extract from all three donor populations (Table 2).

Table 2
MANOVA and ANOVA results for the interaction and main effects of chemical stimuli and yellow perch skin donors on the extracellular field potential recorded at the olfactory epithelium (electrophysiological experiments) and time spent moving (behavioural experiments) of wild juvenile yellow perch from clean and contaminated lakes

	F	df	p
Electrophysiological experiments			
MANOVA			
Chemical stimuli × lake	11.98	6, 48	<0.0001
ANOVA			
Yellow perch alarm cue	18.12	2, 26	<0.0001
Rainbow trout skin extract	23.41	2, 26	<0.0001
L-alanine	43.57	2, 26	<0.0001
MANOVA (donor population)			
Lake × Yellow perch alarm cue	1.35	6, 20	0.2833
Behavioural experiments			
Two-way ANOVA			
Chemical stimuli × lake	11.91	4, 118	<0.0001

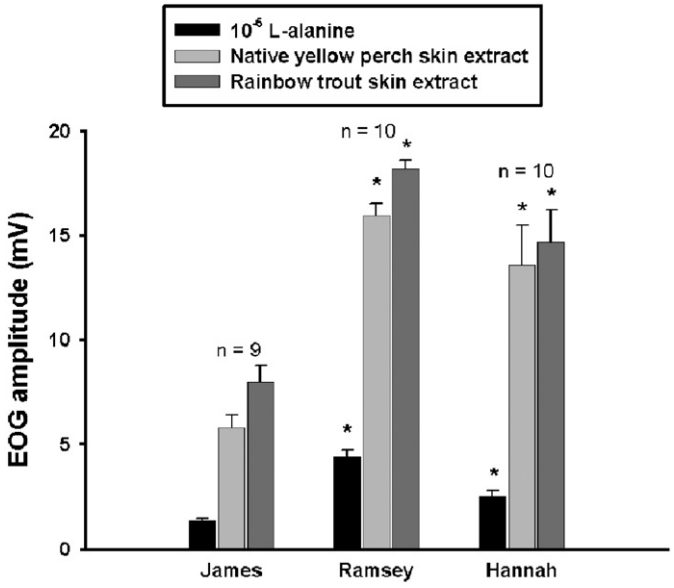

Fig. 2. Mean extracellular field potential response at the olfactory epithelium ± S.E.M. of juvenile yellow perch from three lakes collected along a metal-contamination gradient (James: low contamination, Ramsey: intermediate contamination and Hannah: high contamination) exposed to native yellow perch skin extract, and two control stimuli, 10^{-5} M L-alanine and rainbow trout skin extract (control for odour of injured fish). Asterisks indicate a significant difference from James Lake yellow perch for each corresponding odourant at $p \leqslant 0.05$ (see text for statistical details).

Testing the antipredator behaviour of yellow perch, we found that perch from different lakes responded differentially to chemical stimuli after we stimulated initial activity during the pre-stimulus period with the brine shrimp cue (Table 3; Fig. 3). Yellow perch from James Lake (reference) exposed to conspecific alarm cue decreased activity compared to both rainbow trout skin extract (14.9 times) and control water (18.2 times) (Fig. 3). Similarly, yellow perch from James Lake exposed to yellow perch alarm cues decreased activity more than yellow perch from either of the contaminated lakes which slightly increased activity when exposed to yellow perch alarm cues (Fig. 3). Yellow perch from both contaminated lakes did not exhibit any differential response to rainbow trout extract or dechlorinated water, in any of the behavioural tests (Fig. 3).

Table 3
Percentage of individuals exhibiting freezing behaviour in wild juvenile yellow perch exposed to either dechlorinated tap water, rainbow trout skin extract or yellow perch skin extract

Lake	Stimulus	n	Percentage
James (reference)	Control water	15	0
	Rainbow trout extract	15	7
	Yellow perch extract	15	60*
Ramsey (contaminated)	Control water	13	0
	Rainbow trout extract	12	0
	Yellow perch extract	13	0
Hannah (contaminated)	Control water	15	0
	Rainbow trout extract	15	0
	Yellow perch extract	15	0

Yellow perch were collected from three different lakes in northern Ontario representing a metal-contamination gradient.
*Denotes a significant difference at $p<0.001$.

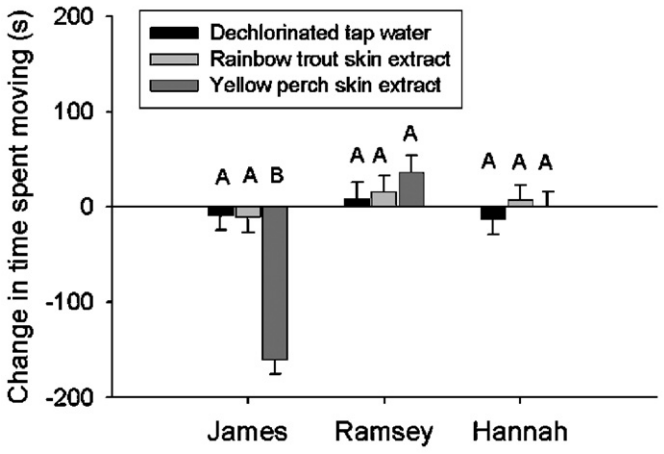

Fig. 3. Mean ± S.E.M. of change (post-stimulus minus pre-stimulus) in time spent swimming (s) of juvenile yellow perch from three lakes along a metal-contamination gradient. Perch from each lake were exposed to: (1) native yellow perch skin extract, (2) rainbow trout skin extract or (3) dechlorinated tap water. Different letters indicate a significant difference ($p \leqslant 0.05$, $n = 12$–15 per treatment, see text for statistical details).

A greater proportion of yellow perch from the reference lake exhibited freezing behaviour when exposed to conspecific alarm cues than yellow perch from the contaminated lakes ($\chi^2 = 17.78$, df = 2, $p<0.0001$; Table 3). James lake yellow perch also exhibited higher freezing behaviour when exposed to conspecific alarm cue than when exposed to rainbow trout skin extract or control water ($\chi^2 = 13.37$, df = 2, $p = 0.001$; Table 3). Finally, neuron counts did not differ statistically in either the olfactory rosettes ($F_{2,15} = 0.144$, $p = 0.867$, Table 4) or olfactory bulbs ($F_{2,15} = 0.880$, $p = 0.435$, Table 4) in yellow perch among the three lakes.

4. Discussion

The results from this study are the first to demonstrate that wild juvenile yellow perch from metal-contaminated lakes exhibit olfactory activity at the olfactory epithelium, but do not respond to ecologically relevant chemical cues with olfactory-mediated behaviours. Moreover, there was no difference in OSN density at either the olfactory rosette or olfactory bulb among yellow perch from the three lakes. This is contrary to what has been reported

Table 4
Neurons per unit volume in the olfactory rosettes and olfactory bulbs of yellow perch collected from three different lakes in northern Ontario representing a metal-contamination gradient

Lake	Olfactory rosettes (per mm^3)	Olfactory bulb (per mm^3)
James (reference)	$1.63 \times 10^6 \pm 8.42 \times 10^4$	$15{,}589 \pm 965.88$
Ramsey (contaminated)	$1.73 \times 10^6 \pm 1.56 \times 10^4$	$17{,}881 \pm 1632.45$
Hannah (contaminated)	$1.71 \times 10^6 \pm 1.41 \times 10^4$	$17{,}184.22 \pm 1054.01$

for laboratory- or aquaculture-reared fishes acutely exposed to metals. Laboratory- or aquaculture-reared fishes have been shown to have decreased activity at the olfactory epithelium and (or) olfactory-mediated behaviours (Hansen et al., 1999a, b; Baldwin et al., 2003; Scott et al., 2003; Sandahl et al., 2004, 2006, 2007). Additionally, acute metal exposures also result in OSN death (Hansen et al., 1999b; Baldwin et al., 2003; Bettini et al., 2006), but we found no difference in OSN density in yellow perch from clean and contaminated environments. Therefore, fish that have spent multi-generations in contaminated environments do not show the same responses as laboratory- and aquaculture-reared fishes suggesting that caution should be taken when extrapolating results from the laboratory to the field under certain ecological contexts i.e., chronic multi-generational exposure.

Although research on the effects of metals and organic pollutants has been ongoing for years, relatively little research has been conducted on wild-caught fishes from metal-contaminated environments. In the Sudbury area lakes, yellow perch are the typically the dominant species and fish species diversity is limited (Keller et al., 2007). We collected yellow perch from the wild that had been chronically exposed to elevated metal concentrations throughout their lives, and for successive generations over the last 25–28 years (Mr. G. Morgan, Laurentian University Cooperative Freshwater Ecology Unit, pers. comm., 2007). Yellow perch were able to adapt to acidic and metal-contaminated conditions. Olfactory sensory neuron density at both the OE and olfactory bulb did not differ among the three lakes. In laboratory studies with laboratory- and aquaculture-reared fishes (salmonids and Tilapia), OSN death is seen after 4 h of exposure to waterborne Cu (Hansen et al., 1999b; Baldwin et al., 2003; Bettini et al., 2006). Moreover, 30 min to 4 h exposure to waterborne Cu (reflective of episodic point source exposures such as runoff) is sufficient to suppress the electrophysiological response at the OE (Hansen et al., 1999a; Baldwin et al., 2003; Sandahl et al., 2004, 2006, 2007). However, we were able to record electrophysiological activity in yellow perch from both contaminated lakes indicating that the OE was functional.

Interestingly, electrophysiological responses of the OE were of similar strength between both contaminated lakes, but was significantly higher than that recorded for the clean lake. This may suggest a difference in olfactory sensitivity between yellow perch from clean and contaminated environments. Alternatively, the difference in olfactory responses may be related to the high concentration of Mg^{2+} in James Lake. Magnesium ions have been known to inhibit cAMP-activated currents in OSNs (Kleene, 1995). When extracellular Mg^{2+} is high, Mg^{2+} can physically block cyclic nucleotide-gated (CNG) channels of the OSN preventing Ca^{2+} from entering the cell. The concentration of Mg^{2+} in the clean lake was twice that of the contaminated lakes. Excess Mg^{2+} could be present in the mucus covering the OE and block CNG channels in the OE leading to lower EOG recordings in James Lake yellow perch. Further work is needed to determine the role of Mg^{2+} on the OE responses of James Lake yellow perch.

A functional OE did not translate into a behavioural response in yellow perch from the contaminated lakes. Yellow perch from the two contaminated lakes did not exhibit antipredator behaviour in response to conspecific alarm cues whereas yellow perch from the clean lake did. Yellow perch collected from Canadian Shield lakes do not readily feed in the lab (pers. obs.) and this may influence antipredator responses. Fishes that have been food deprived have been shown to not elicit a fright response to conspecific alarm cues (reviewed in Wisenden and Chivers, 2006). However, in our study yellow perch from all three lakes did not eat in the laboratory and were handled in exactly the same manner i.e., periods of food deprivation in the lab were identical for yellow perch from all three lakes. Yellow perch from the clean lake exhibited antipredator behaviour to conspecific alarm cues, while yellow perch from the contaminated lakes did not. Similarly, in a field study we found that Iowa darters (*Etheostoma exile*) from contaminated environments did not avoid areas labelled with conspecific alarm cues compared to darters from clean environments (McPherson et al., 2004). Moreover, we have held fathead minnows from a contaminated lake in the laboratory (for 1 week and for 6 weeks) fed *ad libitum* and no antipredator behaviour is observed when exposed to conspecific alarm cues (Mirza et al., unpubl. data). Collectively, these results suggest that fishes from metal-contaminated lakes are olfactory impaired and a lack of antipredator response is not due to food deprivation.

A lack of behavioural response may be due to effects of metal accumulation within the olfactory system. Different metals may be sequestered in different parts of the olfactory system and brain. Metals such as methyl Hg, Mn, and Pb are transported to the olfactory bulb and can be passed on to the olfactory cortex and other brain regions (Tallkvist et al., 1998; Persson et al., 2003). Other metals such as inorganic Cd, Hg, and Ni enter the neuronal cell body and are transported to the terminal end of the axon, but do not pass into the olfactory bulb (Tallkvist et al., 1998; Persson et al., 2003; Scott et al., 2003). Similarly, Persson et al. (2003) also found Zn within the glomerular and granular layers of the olfactory bulb, but no evidence that Zn passes into higher brain centers. Julliard et al. (1995) found that Cu is not sequestered in the olfactory cell, but stored in melanophores of the lamina propria (the region surrounding the axon outside the myelin sheath) after a 60 d exposure to Cu. In all cases, metals are found downstream of the receptor which could potentially alter signal processing resulting in a lack of behavioural response. Recent studies have shown that trace metals disrupt monoaminergic neuronal systems and the endocrine system in the brain which contribute to altering dominance behaviour thereby disrupting social hierarchies in salmonids (reviewed in Sloman, 2007).

To fully appreciate how fish from metal-contaminated environments cope with toxicity, more studies must be conducted on wild-caught fishes. Under natural conditions, aquatic organisms use chemical information to avoid predators, locate food, find potential mates, assess habitat quality, form social hierarchies and recognize kin. Impairment of the olfactory/chemosensory system that interferes with any of these ecological activities may alter population and community structure. Our study also demonstrates the importance of using behavioural assays in ecotoxicological studies. If we had only conducted the electrophysiological study, then we may have drawn a false conclusion that yellow perch from metal-contaminated lakes were not olfactory impaired. Scott and Sloman (2004) argue that behaviour provides the link between physiological and ecological processes. Another important consideration is the ecological context in which the animals are exposed to contaminants. Episodic exposure to contaminants may provide one set of results while chronic exposure may provide another. During an episodic exposure to metals, the function of the receptor may be impaired thereby inhibiting behavioural responses. Conversely, multi-generational chronic exposure to metals appears to inhibit signal processing (but not receptor functioning) thus a lack of behavioural response. By considering the ecological context of exposure we can gain deeper understanding of ecosystems and apply more appropriate environmental risk assessments to provide the highest degree of ecological relevance.

5. Conclusions

We found that yellow perch from metal-contaminated lakes do not respond with olfactory-mediated behaviours when presented with ecologically relevant chemical stimuli. However, yellow perch from the contaminated lakes exhibited a significantly greater EOG response compared to yellow perch despite not exhibiting a fright response to conspecific alarm cues. There were no differences in OSN density at either the olfactory rosette or olfactory bulb among the three populations of yellow perch, thus changes in neuron density are not the causal factor for a lack of antipredator behaviour in yellow perch from metal-contaminated lakes. Further studies are needed to determine the underlying mechanisms of metal-induced olfactory impairment, further characterize metal-OE binding in wild yellow perch, and how the ecological context of metal exposure influences olfactory ability in wild fishes.

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