Olfactory recovery of wild yellow perch from metal contaminated lakes

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A B S T R A C T

Fish depend on their sense of smell for a wide range of vital life processes including finding food, avoiding predators and reproduction. Various contaminants, including metals, can disrupt recognition of chemical information in fish at very low concentrations. Numerous studies have investigated metal effects on fish olfaction under controlled laboratory conditions. However, few have measured olfactory acuity using wild fish in source water. In this study, we used electro-olfactography (EOG) to measure the olfactory acuity of wild yellow perch (Perca flavescens) from a clean lake (Geneva Lake) and two metal contaminated lakes (Ramsey and Hannah lakes) from Sudbury, ON, in their own lake water or in water from the other lakes. The results showed that fish from the clean lake had a greater olfactory acuity than those from metal contaminated lakes when fish were tested in their own lake water. However, when fish from the clean lake were held for 24 h in water from each of the two contaminated lakes their olfactory acuity was diminished. On the other hand, fish from the contaminated lakes held for 24 h in clean lake water showed a significant olfactory recovery relative to that measured in their native lake water. These results show that although fish from a clean lake demonstrated impaired olfaction after only 24 h in metal-contaminated water, fish from metal contaminated lakes showed a rapid olfactory recovery when exposed to clean water for only hours.

1. Introduction

Aquatic animals rely on chemical information in order to mediate vital life processes, such as finding food, avoiding predation, and reproduction (Pyle and Mirza, 2007). Recent work has demonstrated that contamination of aquatic environments can lead to impaired chemosensory function, a process recently coined as ‘info-disruption’ (Lirling and Scheffer, 2007). However, most studies that have demonstrated contaminant effects on chemosensation in fishes have typically relied on tightly controlled laboratory exposures on model fish species (Tierney et al., 2010). Under natural conditions, fish are typically exposed to more than one contaminant simultaneously, exposures occur over the fish’s lifetime as opposed to the short-term exposures used in most laboratory studies, and responses to contaminants are often affected by site-specific exposure conditions (i.e., water quality). Moreover, model species, such as rainbow trout (Oncorhynchus mykiss) or fathead minnows (Pimephales promelas) may not inhabit the contaminated system of interest. Consequently, there is a need to investigate info-disruption in a wild fish to determine if metal induced chemosensory deficit observed under controlled laboratory conditions extrapolate to natural populations.

In order to develop a better understanding of the importance of info-disruption in natural fish populations, fish chemosensory function can be tested in situ or wild fish can be sampled from their natural habitats and tested under laboratory conditions. McPherson et al. (2004) demonstrated that Iowa darters (Etheostoma exile) in contaminated lakes did not avoid traps treated with conspecific alarm cues whereas those from a clean lake could. This result suggested that wild fish in a contaminated lake are unable to detect an important antipredator cue and may be more susceptible to predation than fish from clean lakes where chemosensory function is intact. Mirza et al. (2009) demonstrated that wild yellow perch (Perca flavescens) collected from metal contaminated lakes were unable to respond to standard chemosensory cues in behavioural assays relative to those from clean lakes. However, those same chemosensory-impaired fish showed a significantly greater neurophysiological response to the same standard cues to which they yielded no behavioural response. Although this result might suggest a decoupling between epithelial neurophysiology at the site where the chemosensory cues are detected and higher-order information processing centres related to mounting a behavioural response, it may actually reflect differences in the quality and ionic composition of the water to which the fish was adapted; e.g., elevated Mg concentrations, as proposed by Mirza et al. (2009).

The objective of this study was to compare olfactory acuity in wild yellow perch from clean and metal contaminated lakes and
to determine whether or not observed differences in olfactory acuity are due to permanent olfactory dysfunction or to exposure water quality. A second objective was to determine if olfactory function could recover after only a short exposure to clean lake water if olfactory dysfunction resulted from fish being exposed to contaminated lake water. To address these questions, we conducted a reciprocal cross-exposure study using natural lake water and wild yellow perch from one clean lake, one moderately metal-contaminated, and one metal-contaminated lake. Olfactory acuity was measured using a common neurophysiological technique, electro-olfactography (EOG).

2. Materials and methods

2.1. Study lakes

Three lakes in the industrial region of Sudbury, Ontario, Canada were selected based on previous studies (Fig. 1, Table 1; Keller et al., 2004; Pyle et al., 2005; Couture et al., 2008). Geneva Lake is 70 km northwest of Sudbury and is considered a clean lake given that it is situated outside of the Sudbury industrial zone of influence and has relatively low dissolved metal concentrations (Table 2). Hannah and Ramsey lakes are located within the metal-contaminated Sudbury zone of influence, and are considered metal contaminated and moderately metal contaminated, respectively (Table 2).

2.2. Water sampling and analysis

On-site measurements of pH and temperature were taken using a YSI 6600 V2 multiparameter sonde (YSI Inc, Yellow Springs, Ohio). Water samples were collected from each lake where fish were collected. For each sample one 15 ml container was rinsed three times, containers were submerged and capped under water. Half of the water samples were acidified using 50 μl of trace metals grade high purity nitric acid (Fisher Scientific, Nepean, ON). The acidified samples were passed through a 0.45 μm syringe filter and stored at 4 °C until analysed for metals. The other half of the water sample was passed through a 0.45 μm syringe filter and stored at 4 °C until analysed for dissolved organic carbon (DOC) concentration. Metal concentrations were measured using inductively coupled plasma atomic emission spectroscopy (ICP-AES) (Varian, Mississauga, ON). The DOC concentration was measured with a San Plus Automated Wet Chemistry Analyzer (SKALAR, Breda, The Netherlands). All analytical work was performed by the Lakehead University Centre for Analytical Services (LUCAS), which is accredited through the Canadian Association for Laboratory Accreditation (CALA). All QA/QC procedures followed internal standard operating procedures of LUCAS, including analysis of National Institute of Standards and Technology (NIST) traceable reference material standards. Alkalinity and hardness were determined as previously described (Pyle et al., 2005). All measurements of metal concentrations are summarised in Table 2 with measurements of water quality in Table 3.

2.3. Collecting and maintenance of fish

Fish were collected in early August 2011 using seine nets and angling. Animals were transported back to the lab in aerated lake water and maintained in the lab in static aerated native lake water under a 16:8 light:dark photoperiod and ambient temperature (22 ± 1 °C). All animals were held for 24 h prior to exposures. Animals were not fed during the acclimation or exposure periods.

2.4. Experimental design

Yellow perch from Geneva Lake (clean lake), Ramsey Lake, and Hannah Lake (contaminated lakes) were held in their native lake water for 24 h to acclimate to

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### Table 1

<table>
<thead>
<tr>
<th>Lake</th>
<th>Decimal latitude</th>
<th>Decimal longitude</th>
<th>Lake area (ha)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Geneva</td>
<td>46.7552</td>
<td>−81.5561</td>
<td>356.4</td>
</tr>
<tr>
<td>Ramsey</td>
<td>46.4712</td>
<td>−80.9784</td>
<td>792.2</td>
</tr>
<tr>
<td>Hannah</td>
<td>46.4458</td>
<td>−81.0367</td>
<td>27.7</td>
</tr>
</tbody>
</table>

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Fig. 1. Map of study lakes in the Sudbury region (redrawn and modified from Couture et al., 2008).
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Table 2
Dissolved metal and cation concentrations in water samples (n = 3 per lake) from Hannah Lake, Ramsey Lake, and Geneva Lake.

<table>
<thead>
<tr>
<th>Lake</th>
<th>Cu (µg l⁻¹)</th>
<th>Fe (µg l⁻¹)</th>
<th>Mn (µg l⁻¹)</th>
<th>Ni (µg l⁻¹)</th>
<th>Zn (µg l⁻¹)</th>
<th>Ca (mg l⁻¹)</th>
<th>K (mg l⁻¹)</th>
<th>Mg (mg l⁻¹)</th>
<th>Na (mg l⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Geneva</td>
<td>Mean 1.6</td>
<td>21.6</td>
<td>16.9</td>
<td>4.3</td>
<td>2.5</td>
<td>2.7</td>
<td>0.8</td>
<td>0.7</td>
<td>1.8</td>
</tr>
<tr>
<td></td>
<td>SEM 0.3</td>
<td>4.0</td>
<td>0.9</td>
<td>0.5</td>
<td>0.0</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Ramsey</td>
<td>Mean 8.6</td>
<td>38.4</td>
<td>14.0</td>
<td>32.5</td>
<td>1.7</td>
<td>16.8</td>
<td>1.7</td>
<td>5.3</td>
<td>55.9</td>
</tr>
<tr>
<td></td>
<td>SEM 1.5</td>
<td>3.0</td>
<td>0.5</td>
<td>2.0</td>
<td>0.1</td>
<td>0.3</td>
<td>0.2</td>
<td>0.3</td>
<td>2.0</td>
</tr>
<tr>
<td>Hannah</td>
<td>Mean 13.7</td>
<td>84.3</td>
<td>12.4</td>
<td>48.2</td>
<td>4.2</td>
<td>10.0</td>
<td>1.5</td>
<td>3.6</td>
<td>50.8</td>
</tr>
<tr>
<td></td>
<td>SEM 2.3</td>
<td>3.0</td>
<td>0.6</td>
<td>2.0</td>
<td>1.2</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
</tbody>
</table>

lab conditions. Baseline olfactory acuity was measured in fish held for 24 h in their native lake water after the acclimation period. To determine the effect of contaminated lake water on olfactory acuity of fish from a clean lake, fish from Geneva Lake were held in water from Ramsey and Hannah lakes for 24 h prior to measuring olfactory acuity using exposure water. To determine if recovery of olfactory acuity could be seen by holding fish from contaminated lakes in clean water, fish from Ramsey and Hannah lakes were held in Geneva Lake water for 24 h and olfactory acuity was measured using Geneva Lake water. In all cases, olfactory acuity was measured using EOG (see below).

2.5. Electrophysiological responses

The methods used for EOG analysis were modified from those described by Mirza et al. (2009). Water used to irrigate the olfactory epithelium of each fish was the same as its exposure water. For this study, two different cues, l-alanine and taurocholic acid (TCA), were used. Evidence has demonstrated that amino acids (like l-alanine) and bile salts (like TCA) differently engage the subtypes of olfactory neurons, inducing specific responses in distinct areas of the bulbs (Harmdani and Daving, 2007). By using both stimuli we can determine how each type of olfactory neuron responds. Solutions of l-alanine (10⁻³ M) and TCA (10⁻⁴ M) were made fresh each day in water from the same sources as the exposure water used for each fish. Each stimulus was delivered at least three times to each fish, with a minimum of 2 min between deliveries of any given cue in order to mitigate potential olfactory attenuation. The response to the appropriate blank (i.e., the lake water used to dissolve each stimulus) was also measured. The order of the stimulus delivery (l-alanine, TCA, or a blank) was randomized to ensure there was no bias due to order of delivery.

The raw EOG amplitude for each stimulus delivery was determined by measuring the difference between the baseline EOG response and the maximum response to the stimulus. The raw EOG amplitudes measured to each of the stimuli for an individual fish were then averaged and corrected by subtracting the response elicited by the blank. These corrected values were then averaged across all fish of a specific exposure, such that all data represent mean blank-corrected EOG amplitudes.

2.6. Statistical analysis

All statistical analyses were performed using R, version 2.13.0 (R Development Core Team, 2011), with graphics made using the sciplot package (Morales et al., 2010). A fixed-effects ANOVA was used to determine if there were differences among the responses to each cue in fish from each lake exposed to their own lake water. A Dunnett’s test was then performed to compare the responses of fish from the two metal contaminated lakes to the responses of fish from Geneva Lake. The same analysis (a fixed-effects ANOVA followed by a Dunnett’s test) was performed to determine if there was a significant difference between the responses of Geneva Lake fish exposed to water from all three lakes, with exposure to native water as the control. In addition, this analysis was used for a comparison between the response of fish from Hannah and Ramsey Lake held in Geneva Lake water with the response of Geneva Lake fish in their native water as the control. For experiments where fish from contaminated lakes (e.g., Hannah and Ramsey) were exposed to either their own water or Geneva Lake water, independent-samples t-tests were used to determine if there was a significant difference. For all analyses, significance was set at α = 0.05. Parametric testing was performed prior to each analysis, with a log₁₀ transformation being used to regain parametric assumptions, if needed.

3. Results

3.1. Baseline response of fish from clean and metal contaminated lakes

Yellow perch from Ramsey Lake and Hannah Lake showed a lower EOG response to both cues when held in their own lake water relative to those from Geneva Lake held in their own lake water (l-alanine F(2, 7) = 8.967, p < 0.02, Fig. 2A; TCA F(2, 7) = 8.037, p < 0.02, Fig. 2B). The response to 10⁻³ M l-alanine by fish from Ramsey Lake was 17 percent of the response from Geneva Lake fish, and the response of Hannah Lake fish was 28 percent of Geneva Lake fish. When 10⁻⁴ M TCA was used as the stimulus, the response of fish from Ramsey Lake was 10 percent of the response of Geneva Lake fish, and the response of Hannah Lake fish was 24 percent of the response of fish from Geneva Lake.

3.2. Decreased response of fish from clean lake in metal contaminated lakes water

The EOG response of Geneva Lake fish to either cue was reduced when they were held in water from Ramsey Lake or
Hannah Lake as compared to the response when the same fish were held in their native water (L-alanine $F_{(2, 9)}=8.857, p < 0.01$, Fig. 3A; TCA $F_{(2, 9)}=6.235, p < 0.02$, Fig. 3B). There was a reduction in EOG response to $10^{-3}$ M L-alanine of 75% percent when Geneva Lake fish were held in Ramsey Lake water, and a 59 percent decrease when they were held in Hannah Lake water. In response to $10^{-4}$ M TCA, fish from Geneva Lake held in Ramsey Lake water exhibited a 75 percent reduction in EOG response relative to those from Geneva Lake held in their own lake water. There was no significant difference detected between the response of Geneva Lake fish held in their source water and Hannah Lake water to $10^{-4}$ M TCA, even though there was an apparent 58 percent decrease in response due to the exposure with Hannah Lake water.

3.3. Increased response of fish from metal contaminated lakes in clean lake water

Yellow perch from Ramsey Lake held in Geneva Lake water for 24 h had significantly increased EOG response to both cues, a 3.4 fold increase in response to $10^{-3}$ M L-alanine ($t = -5.25, df = 3.68, p < 0.008$, Fig. 4A) and a 14.2 fold increase in response to $10^{-4}$ M TCA ($t = -4.25, df = 2.74, p < 0.01$, Fig. 4B) compared to the response of the same fish held in their own lake water. Fish from Hannah Lake, however, only showed a significantly increased response by 5.8 fold to $10^{-3}$ M L-alanine ($t = -9.45, df = 3.80, p < 0.001$, Fig. 4C) when held in Geneva lake water as compared to water from their source lake. Even though the response to $10^{-4}$ M TCA was not statistically different ($t = -3.6, df = 2.19, p = 0.082$, Fig. 4D), there was an apparent 5.2 fold increase in EOG response when Hannah Lake fish are held in Geneva Lake water as compared to water from their source lake. A comparison of the response of yellow perch from all three lakes held in Geneva Lake water for 24 h demonstrated that fish from Hannah Lake had a 2.4 fold higher response to L-alanine as did fish from Geneva Lake ($F_{(2, 7)}=5.275, p < 0.01$ Fig. 5A). There was no difference between the response to L-alanine with fish from Geneva Lake and Ramsey Lake. There was no significant difference detected in the response of fish from all three lakes held in Geneva Lake water to TCA ($F_{(2, 7)}=2.247, p = 0.763$ Fig. 5B).

4. Discussion

In contrast to previously reported results (Mirza et al., 2009), this study demonstrates that yellow perch from metal contaminated lakes show impaired EOG response relative to yellow perch.
from a clean lake. Water quality parameters of all three lakes are comparable (Table 3). Few studies have compared olfactory acuity of wild fish from clean and metal contaminated lakes. In an in situ trap experiment, Iowa darters avoided antipredator cue in a clean lake but not in a metal-contaminated lake (McPherson et al., 2004). Behavioural responses of perch from clean and contaminated lakes showed a similar trend to that shown by Iowa darters, in that perch from a clean lake avoided an antipredator cue, while perch from contaminated lakes did not (Mirza et al., 2009).

In this study, exposing fish from a clean lake to water from contaminated lakes resulted in a significant reduction in olfactory acuity as measured by EOG. These exposures represent complex mixtures of contaminants, as the concentrations of numerous metals (Table 2) are elevated in the contaminated lakes relative to the clean lake. Several studies investigating single metal (such as copper) exposures show impairment of olfaction in a wide variety of fish species such as the pike minnow (Ptychocheilus lucius) (Beyers and Farmer, 2001), coho salmon (Oncorhynchus kisutch) (Baldwin et al., 2003), chum salmon (Oncorhynchus keta) (Sandahl et al., 2006), goldfish (Carassius auratus) (Kolmakov et al., 2009) and fathead minnow (Pimephales promelas) (Green et al., 2010; Dew et al., 2012). To our knowledge, no studies have been performed to investigate the effect of a metal contaminated lake’s water on the olfactory function of wild fish. Further work is required to elucidate how complex mixtures in metal contaminated lakes affect olfactory acuity and behavioural responses of fish.

When fish from metal contaminated lakes were exposed to water from a clean lake for 24 h, there was a dramatic increase in response to chemosensory cues as compared to fish from the same lake tested in native lake water. This increased response means that olfactory-impaired fish from metal contaminated lakes can quickly recover their ability to respond to cues once the contamination is removed. This recovery was seen for both the moderately contaminated (Ramsey) and contaminated (Hannah) lakes. Recovery of olfactory acuity after exposure to low, ecologically relevant concentrations of copper has been observed after the exposure has been removed in fathead minnows (Green et al., 2010), coho salmon (Baldwin et al., 2003), and chum salmon (Sandahl et al., 2006). Recovery from short term exposure to higher concentrations of copper has been shown to require longer recovery periods before olfactory responses are fully restored in goldfish (Kolmakov et al., 2009). The need for a longer recovery period is most likely due to copper inducing damage in olfactory sensory neurons (OSNs) in the olfactory epithelium. Recovery after being exposed to high copper concentrations has been shown to occur in Tilapia mariae (Bettini et al., 2006), which recovered 10 days after copper was removed. Copper-induced cell death is most likely through an apoptotic mechanism owing to the demonstration of copper-induced apoptosis in rainbow trout OSNs (Julliard et al., 1996). Since recovery of the EOG response of yellow perch was within 24 h in our study, it is likely that OSNs were not damaged by the mixture of metals in their environment, but instead impaired through a similar mechanism to the studies using ecologically-relevant copper concentrations detailed above (Baldwin et al., 2003; Sandahl et al., 2006; Green et al., 2010). An alternate explanation could be that only mature OSNs were affected by the metal exposure such that an intact stem cell layer could regenerate and replace the damaged OSNs.

Contaminated fish tested in clean water responded to both stimuli similarly to clean fish tested in clean water after a 24 h acclimation period, with one exception: Hannah Lake fish yielded a stronger response to l-alanine when tested in clean water than clean fish. This elevated response beyond that of the control fish reflects similar results reported by Mirza et al. (2009), where contaminated fish were tested in clean lab water and showed a stronger response to standard chemosensory stimuli than controls. Although the mechanism for this response is unknown, it may be anadaptive compensatory response by OSNs to maintain chemosensory function in the presence of neurotoxic environmental contaminants.

In all conditions, when either l-alanine or TCA were used, similar trends were observed. As l-alanine and TCA were used to measure different OSN subtypes, the similar trends demonstrate that the toxicity and recovery measured during the experiments was due to a general effect on the olfactory system, and not due to specific OSN subtypes being impaired. In addition, regardless of the source of the fish used, whenever fish were exposed to Geneva Lake water and the EOG response to TCA was measured, there was a high variability to the data. More work is needed to understand why this phenomenon occurred.

Olfactory recovery after chronic exposure to low, environmentally-relevant metal concentrations has not been investigated before this study. This chemosensory recovery after chronic metal exposure may account for the observed differences in the baseline responses in fish from clean and metal-contaminated lakes observed in this study, and those reported by Mirza et al. (2009). Their study used wild yellow perch, as did ours; however, their fish were acclimated to laboratory water and all experiments were performed using laboratory water (i.e., clean water). It is likely that their study showed a higher EOG response to different cues from fish from contaminated lakes because yellow perch from metal contaminated lakes were acclimated to clean water. This acclimation to clean water could have led to a recovery of olfactory response to cues in fish from metal contaminated lakes and may explain their results. Any future experiments involving olfaction of wild fish should be performed using water from their source habitats. Conducting experiments in native lake water also improves the ecological relevance of the results since lake water is their natural environment and reflects the water condition that fish deal within their habitat. Furthermore, this observation demonstrates that olfactory impairment of fish from metal contaminated lakes can be reversed if the contamination is removed. This observation is important for lake remediation because it demonstrates that olfactory impairment of fish from metal contaminated lakes have the ability to recover once the lake recovers.
5. Conclusions

This study demonstrates that yellow perch inhabiting metal contaminated lakes have an impaired olfactory response to standard chemosensory cues compared to fish from a clean lake. In addition, exposure of fish from clean lake to a mixture of different contaminants in metal contaminated lake water for 24 h water inhibits their olfactory function. Furthermore, fish that have spent their lives in metal contaminated lakes recover quickly (i.e., 24 h) after being transferred to clean water. Taken together, there are two major implications from this study. First, when performing research on the olfactory response of wild fish it is essential that the experiments be conducted in source water. Second, fish in metal contaminated lakes have impaired olfactory acuity, however, this impairment can be reversed when the contaminants are removed. These outcomes could improve our ability to evaluate the ecological risk of low-level metal release to freshwater, which can eventually lead to improved environmental policies and guidelines that can effectively protect sensitive freshwater ecosystems.

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References


Morales M. with code developed by the R Development Core Team, with general advice from the R-listserv community and especially Duncan Murdoch, 2010. sciplot: Scientific Graphing Functions for Factorial Designs., R package version 1.0-7.


