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Chemosensory mediated behaviors and gene transcription profiles in wild yellow perch (*Perca flavescens*) from metal contaminated lakes



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ABSTRACT

The olfactory system of fish is sensitive to the toxic effects of low concentrations of contaminants. To investigate the effects of long-term metal exposure on olfaction in wild yellow perch (*Perca flavescens*), fish from one clean (Geneva Lake) and two metal-contaminated lakes (Ramsey and Hannah lakes) were collected in and around the metal-mining district of Sudbury, ON. Two different techniques were used to measure the effects of exposure to environmental contamination: (i) behavioral responses were recorded in response to conspecific skin extract and (ii) gene transcription differences in olfactory rosettes were characterized using a novel, 1000-candidate gene yellow perch microarray. Behavioral assays performed on fish from the clean lake demonstrated avoidance of a conspecific skin extract, while fish from metal contaminated lakes showed no avoidance response. A total of 109 out of the 1000 genes were differentially transcribed among the lakes. Most of the differentially transcribed genes were between the two metal contaminated lakes relative to either of the contaminated lakes and the reference lake. No genes were differentially expressed between Geneva Lake (clean) and Hannah Lake (metal contaminated). These results demonstrated that even though the different populations of fish from both Hannah and Ramey lakes were affected at the behavioral level, the impairment of olfaction was not measurable using gene transcriptional changes in olfactory rosettes.

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1. Introduction

Traditional studies designed to characterize metal toxicity in fish have relied on single-metal exposures to model species under the tightly controlled conditions of the laboratory (Wood et al., 2012a, 2012b). These studies have provided a wealth of information about the basic biology of metal exposures, including important routes of uptake, preferential tissue accumulation, and modes of toxicity. Moreover, many of the effects revealed by these studies have been induced only after animals have been exposed to relatively high contaminant concentrations, usually in exposure water having comparatively simple water chemistry relative to natural waters. Much less work has been done to characterize the subtle effects of low, environmentally relevant metal concentrations

in wild fish adapted to either clean or contaminated waters (Couture and Pyle, 2012).

Recently, considerable research attention has been directed towards understanding how dissolved contaminants affect chemosensation and chemical communication in fish (Carreau and Pyle, 2005; Bettini et al., 2006; Sandahl et al., 2006; Blechinger et al., 2007; Kolmakov et al., 2009; Tierney et al., 2010; Dew et al., 2012). Aquatic animals rely on important chemicals in the water that inform about the location of food, the risk of predation, or the reproductive status of potential mates (among others) (Hamdani and Døving, 2007). In other words, information conveyed through chemical communication is essential for maintaining healthy populations. Anything that could disrupt the perception of these important chemical cues, such as environmental contamination, has the potential to cause a significant ecological perturbation by interfering with an animal's ability to find food, avoid predators, or reproduce (Lüriling and Scheffer, 2007). Detecting the presence of a predator is vital for any prey and aquatic organisms are known to use olfaction to evaluate the local risk of predation (Kats and Dill, 1998). Several studies have investigated the fright response in

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aquatic organisms and fishes can recognize the presence of a predator through various cues including conspecific skin extract (Brown, 2003). Impaired anti-predator responses could reduce the ability of fish to detect predators and decrease their chance of survival, which could in turn change predator–prey dynamics to benefit predators, and at a population scale would likely cause changes to the whole ecosystem.

Gene transcription is a sensitive indicator of contaminant exposure, and contaminant-induced gene transcription changes have been measured to determine what effect contaminant exposure will have on an organism (Hogstrand et al., 2002; Snape et al., 2004; Lettieri, 2006; Reynders et al., 2006; Sheader et al., 2006; Moens et al., 2007; Craig et al., 2010). Using microarrays to study sublethal toxicity allows for the analysis of several physiological pathways simultaneously to highlight those pathways that are most sensitive to site-specific contamination. In addition, physiological pathways that may not have been considered to be at risk from environmental contamination could also be found to be affected by the environmental contamination (Denslow et al., 2007). In the only two studies using microarray technology to characterize the effects of a contaminant (in this case copper and/or chlorpyrifos) on gene transcription in olfactory tissues of fish, Tilton et al. (2008, 2011) demonstrated that zebrafish (*Danio rerio*) exposed to copper and/or chlorpyrifos for < 24 h showed significant differences of gene transcription in pooled olfactory tissues. For these studies, olfactory rosettes, telencephalon, and the underlying olfactory bulb were pooled and a commercial zebrafish microarray containing 14,900 transcripts was used to measure gene transcription patterns associated with contaminant exposure. Tilton et al. (2008) showed that copper caused an under-transcription of key genes associated with the olfactory signal transduction pathway such as calcium channels, G-proteins, and olfactory receptors. In 2011 they exposed fish to copper, chlorpyrifos and mixtures of both and found that copper and chlorpyrifos cause their own transcriptional signatures (Tilton et al., 2011). However, the transcriptional signature of the contaminant mixtures was more similar to that in zebrafish exposed to copper (Tilton et al., 2011). One question that remains from that work is whether or not those same gene transcription patterns can be observed in wild fish populations where long-term metal exposure has led to impaired chemosensory function.

The industrial region of Sudbury, ON, Canada provides an excellent opportunity to study metal-impaired chemical communication in wild yellow perch (*Perca flavescens*) populations (Pyle et al., 2005). Sudbury is a top nickel-producing region in the world (Chau and Kulikovskiy-Cordeiro, 1995). Mining has taken place in the region since the late 1800s which has resulted in acidification and metal contamination in over 7000 lakes in a 17,000 ha industrial “zone of impact” (Keller et al., 1992). The dominant fish species is yellow perch, mainly because of its acid tolerance (Freda and McDonald, 1988) and well-documented ability to tolerate dissolved metals at concentrations elevated significantly above background concentrations (Taylor et al., 2003). Recent studies have demonstrated that yellow perch from metal-contaminated lakes in the Sudbury area have impaired chemosensory function when presented with pure olfactory chemicals (such as amino acids) or natural chemosensory cues (such as conspecific skin extracts containing chemical alarm cues) (Mirza et al., 2009; Azizishirazi et al., 2013). Recently, a novel 1000 candidate-gene yellow perch microarray was developed as a tool for the detection of metal-induced stress and to identify the different mechanisms of sublethal metal toxicity in yellow perch (Bougas et al., 2013). The microarray contains genes associated with a wide variety of cellular process including genes associated with olfaction. The 1000 candidate-gene microarray revealed different mechanisms of the sublethal effects of nickel and/or cadmium in livers of yellow

perch after 45 days of exposure to environmentally relevant concentrations (Bougas et al., 2013).

The objective of this study was to determine if behavioral deficits induced by metal-impaired chemosensory function in wild yellow perch from metal contaminated lakes are linked to gene transcription patterns in olfactory tissues. Such a link could provide insights into the mechanism(s) of toxic action related to metal-impaired olfaction in wild fish populations. To investigate this question, we collected wild yellow perch from a clean lake and two metal-contaminated lakes in the Sudbury region. The natural avoidance response of yellow perch to conspecific skin extract was used to test the olfactory acuity of fish from all lakes. The endpoints used in this experiment were fleeing and avoiding an olfactory-labeled “high risk zone” of a choice maze. Gene transcription patterns of the most exposed olfactory tissue, the olfactory rosette, were examined using the yellow perch microarray, and these expression patterns were analyzed relative to behavioral responses.

2. Materials and methods

2.1. Water sampling

Temperature and pH were measured on site using a YSI 6600 V2 multi-parameter sonde (YSI Inc, Yellow Springs, Ohio). Water samples were collected from Hannah Lake, Ramsey Lake, and Geneva Lake (Azizishirazi et al., 2013) in close proximity to where fish were collected. Samples were stored in 50 mL tubes and capped under water to decrease the headspace, and were split into three groups for subsequent analysis. Total dissolved metal concentrations were determined in 50 mL water samples acidified with 200 μ L of trace metals grade high purity nitric acid (Fisher Scientific, Nepean, ON) passed through a 0.45 μ m syringe filter. After acidification and filtration, samples were stored at 4 °C until analyzed via inductively coupled plasma atomic emission spectroscopy (ICP-AES) by the Lakehead University Instrumentation Laboratory, Thunder Bay, ON, Canada for metal concentrations (Table 1). Dissolved organic carbon (DOC) concentration was measured by the Lakehead University Centre for Analytical Services using a San⁺⁺ Automated Wet Chemistry Analyzer (SKALAR, Breda, the Netherlands). Alkalinity and hardness were measured as previously described (Pyle et al., 2005).

2.2. Fish collection

All experiments were conducted in accordance with the guidelines of the Canadian Council of Animal Care. Fish were collected from Geneva Lake, Ramsey Lake, and Hannah Lake using seine nets and angling in June 2011 for use in gene transcription experiments, and in June 2012 for behavioral experiments. At each collection site, 20 fish for behavioral experiments and twelve fish for the gene expression experiment were randomly selected. Randomly-selected fish were transported to Laurentian University, Sudbury, ON, in aerated native lake water for gene expression and behavioral experiments.

2.3. Behavioral assessment

2.3.1. Maintenance of the fish

Fish collected from each of the three lakes were kept in their native lake water in 30 L plastic tanks for 24 h to acclimate to laboratory conditions. Each tank was aerated and water was changed every twelve hours using fresh water from each lake. The temperature of the holding water was 25 ± 1 °C and the photoperiod was 16:8 light:dark.

2.3.2. Experimental design

Conspecific skin extract was made fresh prior to each behavior trial. Donor fish were sacrificed with a sharp blow to the head. Skin was removed from both sides of two yellow perch from Geneva, Hannah, and Ramsey lakes. In three separate watch glasses (one per source of fish), 10 ± 0.5 cm² of skin was chopped using fine dissecting scissors and a scalpel. Native lake water (1 L) was used to dilute the skin extract to a concentration of 10 cm²/L. The solution was mixed for 5 min and allowed to settle for 5 min, after which the top 800 mL was poured in 50 mL plastic tubes. Troughs measuring 70 cm \times 20 cm \times 15 cm ($L \times W \times H$) were used as behavior mazes for this experiment. Lines were drawn on the edge of the trough (visible from the top) to divide the maze into three zones. Each distal segment of the maze was 27 cm long with a middle zone of 15 cm. Mazes were filled using 8 L of native lake water for the fish to be tested. Fish were randomly assigned to the mazes and allowed to acclimate to the trough for 20 min prior to delivering the stimulus and

Table 1

Concentrations of dissolved metals and cations as well as water quality parameters in water samples ($n=3$ per lake) from Hannah, Ramsey, and Geneva lakes in June 2011 and June 2012.

| Lake | | Cu | Fe | Mn | Ni | Zn | Ca | K | Mg | Na |
|---------------|-----------------------------|------------------------|-------|------|--|-----|--|-----|----------------------------|------|
| | | $(\mu\text{g L}^{-1})$ | | | | | (mg L^{-1}) | | | |
| Geneva (2011) | Mean | 0.9 | 24.8 | 10.0 | 4.0 | 2.5 | 2.7 | 0.4 | 0.7 | 0.9 |
| | SEM | 0.2 | 2.0 | 0.5 | 0.9 | 0.5 | 0.2 | 0.1 | 0.1 | 0.1 |
| Ramsey (2011) | Mean | 12.6 | 124.4 | 33.0 | 44.5 | 5.1 | 16.5 | 1.5 | 4.9 | 52.8 |
| | SEM | 0.5 | 10.0 | 2.5 | 3.0 | 1.9 | 0.7 | 0.1 | 0.2 | 3.0 |
| Hannah (2011) | Mean | 17.7 | 81.3 | 19.5 | 70.1 | 4.2 | 11.5 | 1.6 | 3.7 | 54.9 |
| | SEM | 1.5 | 4.0 | 2.3 | 3.5 | 0.8 | 0.7 | 0.2 | 0.4 | 3.5 |
| Geneva (2012) | Mean | 0.7 | 21.6 | 16.9 | 4.1 | 2.5 | 2.5 | 0.4 | 0.6 | 0.8 |
| | SEM | 0.1 | 4.0 | 0.9 | 0.5 | 0.5 | 0.3 | 0.1 | 0.1 | 0.1 |
| Ramsey (2012) | Mean | 7.9 | 11.9 | 12.0 | 30.1 | 7.8 | 14.8 | 1.4 | 4.4 | 47.5 |
| | SEM | 0.8 | 3.0 | 0.6 | 1.9 | 0.9 | 0.8 | 0.1 | 0.3 | 2.0 |
| Hannah (2012) | Mean | 11.5 | 16.3 | 7.4 | 44.2 | 4.2 | 8.8 | 1.4 | 4.4 | 47.5 |
| | SEM | 0.6 | 2.0 | 0.3 | 5.0 | 0.9 | 0.7 | 0.1 | 0.3 | 2.0 |
| Lake | Temp ($^{\circ}\text{C}$) | pH | | | Alkalinity (mg L^{-1} as CaCO_3) | | Hardness (mg L^{-1} as CaCO_3) | | DOC (mg L^{-1}) | |
| Geneva (2011) | 22–23 | 6.8–6.9 | | | 10–12 | | 8.7–10.5 | | 3.8–3.9 | |
| Ramsey (2011) | 22–24 | 7.8–7.9 | | | 25–28 | | 58.8–63.9 | | 3.9–4.2 | |
| Hannah (2011) | 23–25 | 7.2–7.4 | | | 16–18 | | 40.5–47.3 | | 3.5–3.7 | |
| Geneva (2012) | 22–24 | 6.7–6.8 | | | 8–10 | | 7.5–9.9 | | 3.7–3.9 | |
| Ramsey (2012) | 24–25 | 7.9–8.1 | | | 17–19 | | 51.8–58.3 | | 4.0–4.1 | |
| Hannah (2012) | 25–26 | 6.7–6.8 | | | 14–15 | | 32.1–37.7 | | 3.6–3.8 | |

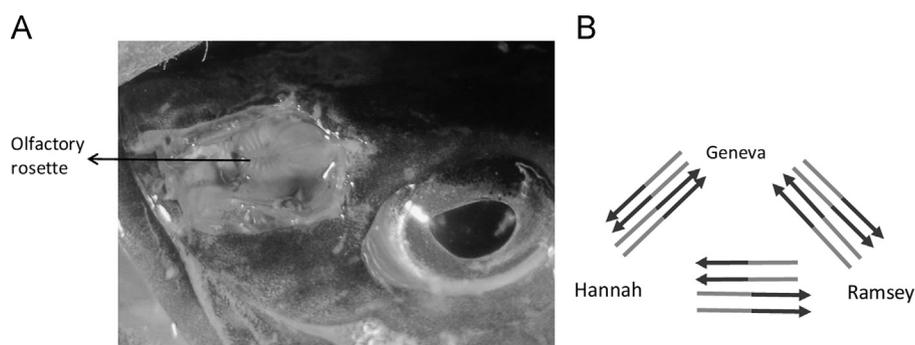


Fig. 1. A. Dissection of an olfactory rosette from yellow perch. B. Experimental design of the microarray experiment. Each arrow represents a microarray, the black end of which represents red fluorescent dye, with the gray end representing green fluorescent dye.

blank. After the acclimation period, 50 mL of stimulus (conspecific skin extract) or blank (native lake water) was delivered to either end of the maze using syringes and aeration tubing from behind a curtain. The end of the trough maze receiving either the stimulus or blank was randomized for each behavioral trial. After allowing 2 min for the cue to diffuse throughout the maze, fish movement was recorded for 8 min using a Logitech C615 high definition web camera (Logitech, Mississauga, ON). All videos were scored by an observer for fish position in the maze in 10 s intervals and the number of entries into each end of the maze. To reduce bias the observer was blind to the position of the stimulus or blank.

2.3.3. Statistical analysis

Behavioral data were analyzed using R, version 2.15.2 (R Development Core Team, 2012). Two separate analyses were performed on the behavioral data, the time spent in the stimulus zone versus the time spent in the blank zone, and the number of entries into the stimulus zone. A paired t -test was used to determine if there was a difference between time spent in the stimulus versus time spent in the blank zone for fish from each lake. The number of entries into the stimulus zone was compared between fish from Geneva Lake and either of the other two lakes using two Student's t -tests. Prior to Student's t -tests an outlier was identified in the data for Geneva Lake using a Grubb's test and removed. The significance level, α , was set a priori to 0.05. However, p -values were adjusted using a Holm's correction to compensate for an increased probability of committing a Type 1 statistical error owing to serial hypothesis testing.

2.4. Gene transcription

2.4.1. Total RNA extraction

Randomly selected fish were euthanized in a 2 L bath of 200 mg/L MS-222, (Syndel Laboratories Ltd, Nanaimo, BC) buffered with sodium bicarbonate (Fisher Scientific, Nepean, ON) within 10 min of arriving at the laboratory. Sacrificed fish

from each lake were divided into 4 groups, and olfactory rosettes of all three fish in each group were collected and pooled together. Skin covering both nares of each perch (located dorsally between the eyes and snout of the fish) were removed using a scalpel and forceps (Fig. 1A). The olfactory rosette was located in each olfactory chamber, grasped with forceps, and the underlying connective tissue and olfactory nerve were cut with a scalpel. The olfactory rosettes were removed and stored in *RNAlater*[®] Solution (QIAGEN Inc, Toronto, ON) in 1.5 mL tubes, refrigerated overnight, and then stored at -80°C until RNA was extracted. Prior to RNA extraction, all six rosettes were thawed, disrupted and homogenized together using a motorized homogenizer (Silentcrusher M, Heidolph, Elk Grove Village, IL). Total RNA was extracted using the PureLink[®] RNA Mini Kit (Life Technologies Inc, Burlington, ON) according to manufacturer's instructions. Extracted RNA was stored at -80°C until used.

2.4.2. Labeling and cDNA hybridization

The quality and integrity of the total RNA was checked using an Experion Automated Electrophoresis Station and RNA HighSens Chips (Bio-Rad, Hercules, CA). For each sample, 1.5 μg total RNA was retro-transcribed and the cDNA samples were labeled using the Genisphere 3DNA Array 350 Kit, Invitrogen's Superscript II retro-transcriptase, and *Cyanine3* and *Cyanine5* fluorescent dyes (Genisphere), following the procedures described at <http://genisphere.com/products/3dna-array-detection/support> (Genisphere Array 350 Protocol). Four samples from each lake were compared and analyzed through a loop design (Fig. 1B) for a total of twelve microarrays. The loop design included pair-wise direct comparisons among samples of the different lakes. Each pooled sample was technically replicated on two bi-colored microarrays and dye-swapped.

2.4.3. Data acquisition, preparation, and statistical analysis

Scan, localization and quantification of the spots, and data analysis were conducted as described by Bougas et al. (2013). Using a mixed model ANOVA, we tested for the presence of significant lake effects (Geneva, Hannah, and Ramsey)

with the “Array” term included as a random effect, and “Dye” and “Lake” included as fixed effects (Fs, with 1000 sample ID permutations). A False Discovery Rate correction (FDR=0.1) was applied within the R/MAANOVA package to reduce the Type I error rate associated with multiple comparisons and the corrected p -values were used to determine the significance of differential gene transcription levels.

A posteriori tests were conducted with 1000 permutations for the three possible comparisons to generate a list of genes with significant differential expression among the different lakes (FDR=0.1).

2.4.4. Functional classification

Gene ontology (GO) and assessment of significant differential representation of functional classes was performed in the Blast2Go software as described by Bougas et al. (2013).

3. Results

3.1. Behavioral experiment

When given the choice between a zone of a maze containing a blank cue or an alarm cue, yellow perch collected from Geneva Lake actively avoided the alarm cue by spending 6.8 fold more time in the zone containing the blank relative to the zone containing the alarm cue ($t_7=4.56$, $p < 0.01$; Fig. 2), while yellow perch from Ramsey Lake and Hannah Lake showed no preference for either zone ($t_{10}=1.24$, $p=0.36$ and $t_7=-0.31$, $p=0.76$, respectively; Fig. 2).

Fish from Ramsey Lake entered the zone containing alarm cue three-fold more frequently than fish from Geneva Lake ($t_{16}=-3.14$, $p < 0.01$; Fig. 3), while fish from Hannah Lake entered the alarm cue containing zone 4 times more frequently than fish from Geneva Lake ($t_{9,7}=-3.02$, $p < 0.02$; Fig. 3).

3.2. Gene transcription

3.2.1. Gene transcription differences

A total of 109 out of the 1000 analyzed genes showed a significant differential transcription between at least one pair of lakes (FDR < 0.1, $p < 0.015$). The comparison between Ramsey and Hannah lakes showed more differentially transcribed genes ($n=91$) compared to 64 and 0 differentially transcribed genes in Ramsey Lake versus Geneva Lake and Geneva Lake versus Hannah Lake, respectively (Fig. 4) and most of the differentially transcribed genes between Hannah–Ramsey and Geneva–Ramsey were the same (Fig. 4). In the Ramsey Lake and Hannah Lake comparison, 54 genes in Ramsey Lake fish were under-transcribed (average \log_2 of

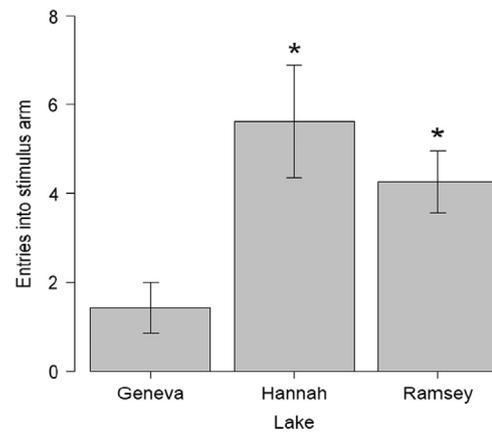


Fig. 3. Number of times fish from Geneva, Ramsey and Hannah lakes entered into the zone of the behavioral maze receiving a conspecific alarm cue. An asterisk denotes a significant difference from Geneva Lake fish, $p \leq 0.05$.

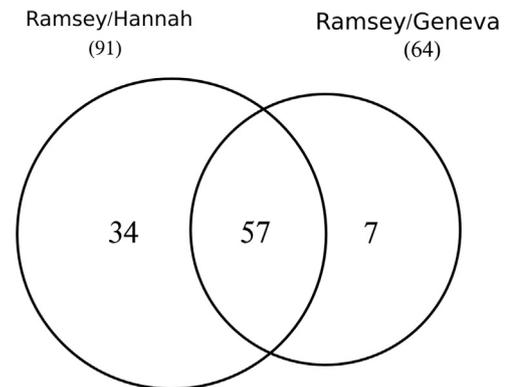


Fig. 4. Venn diagram representing the number of differentially transcribed genes between Ramsey/Hannah and Ramsey/Geneva comparisons (FDR=0.1).

fold change: -0.19) and 37 genes were over-transcribed (average \log_2 of fold change: 0.41) relative to Hannah. In the Ramsey and Geneva comparison, 35 genes from Ramsey Lake fish were under-transcribed (average \log_2 of fold change: -0.2) and 29 genes were over-transcribed (average \log_2 of fold change: 0.39) relative to Geneva (Table S1).

3.2.2. Functional categories of differentially transcribed genes

Gene ontology annotation was used to identify over-represented GO terms (Fisher tests, $p < 0.05$) in the lists of genes that were significantly differentially transcribed among the Geneva and Ramsey lakes and among Hannah and Ramsey lakes. The results of this analysis are shown in Table 2. Only the GO biological processes with a minimum of five genes are represented. The GO analysis identified eight biological processes from the list of differentially transcribed genes among the Ramsey and Hannah lakes and seven biological processes from the list of differentially transcribed genes among Ramsey and Geneva lakes. Some of these over/under-represented processes are identified in both lists of significant genes from the two comparisons (e.g., ribosome biogenesis, translation, cellular component biogenesis, localization, response to chemical stimulus and cellular homeostasis). In some of these processes (e.g., tetrapyrrole metabolic process, ribosome biogenesis, translation and transmembrane transport), 100 percent of the genes were either over-transcribed or under-transcribed in Ramsey Lake fish compared to fish from Hannah or Geneva lakes. Under-transcription of all genes was observed for ribosome biogenesis and translation in Ramsey versus Hannah comparison

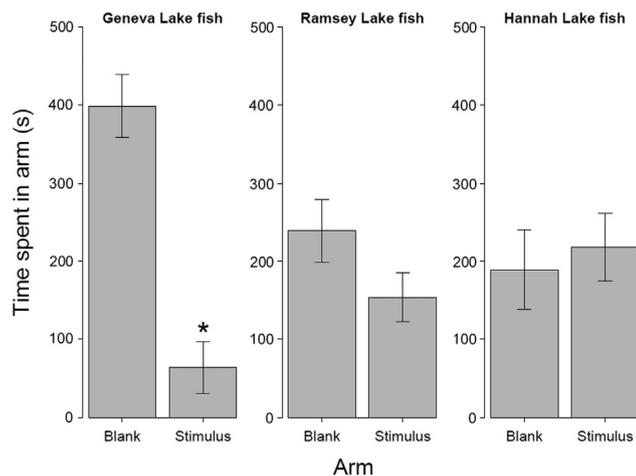


Fig. 2. Mean time spent by fish from Geneva ($n=9$), Ramsey ($n=11$) and Hannah ($n=9$) lakes in either stimulus or blank zone (\pm SEM). All trials were conducted in native lake water. An asterisk denotes a significant difference between the time spent in zone receiving the stimulus (conspecific alarm cue) relative to the zone receiving the blank (native lake water), $p \leq 0.05$.

Table 2

Over-represented processes categories in the Ramsey versus Hannah and Ramsey versus Geneva comparisons (Fisher's exact test, significance threshold: p -value=0.05, <http://www.blast2go.com/b2ghome>). Abbreviations: OG: over transcribed gene, UG: under transcribed gene, fold change are \log_2 transformed.

| Comparison | Processes category | Number of genes | Percent OG | OG fold change | UG fold change | |
|-------------------------------|--------------------------------|-------------------------|------------|----------------|----------------|-------|
| Ramsey versus Hannah | Tetrapyrrole metabolic process | 5 | 100 | 0.34 | – | |
| | Metal ion homeostasis | 5 | 60 | 0.27 | –0.2 | |
| | Cellular homeostasis | 6 | 67 | 0.26 | –0.2 | |
| | Ribosome biogenesis | 10 | 0 | – | –0.2 | |
| | Translation | 15 | 0 | – | –0.19 | |
| | Cellular component biogenesis | 16 | 17 | 0.43 | –0.24 | |
| | Response to chemical stimulus | 17 | 59 | 0.27 | –0.24 | |
| | Localization | 23 | 39 | 0.48 | –0.23 | |
| | Ramsey versus Geneva | Cellular homeostasis | 5 | 60 | 0.2 | –0.19 |
| | | Transmembrane transport | 6 | 0 | – | –0.19 |
| Ribosome biogenesis | | 7 | 0 | – | –0.15 | |
| Translation | | 11 | 0 | – | –0.16 | |
| Cellular component biogenesis | | 11 | 27 | 0.38 | –0.25 | |
| Response to chemical stimulus | | 12 | 58 | 0.27 | –0.34 | |
| Localization | | 17 | 47 | 0.44 | –0.29 | |

and in the Ramsey versus Geneva comparison. In addition, under-transcription of all genes was also observed for transmembrane transport in Ramsey Lake fish compared to Geneva Lake fish and over-transcription of all genes was found for tetrapyrrole metabolic process in Ramsey Lake fish compared to Hannah Lake fish.

4. Discussion

This study demonstrates that chronically exposed wild yellow perch from a contaminated environment cannot respond behaviorally to a conspecific alarm cue. This impaired chemosensory function in fish from metal-contaminated lakes mirrors a study by Mirza et al. (2009) in which yellow perch from Hannah Lake and Ramsey Lake had a reduced anti-predator response when compared to fish from a control lake, James Lake. In their study, experiments were performed in laboratory water, while our experiments were performed in native lake water. Previously, it was demonstrated that the source water for exposures was an important consideration for using a neurophysiological technique (electro-olfactography; EOG) to measure olfaction; however, it appears that wild yellow perch from contaminated lakes fail to respond to conspecific alarm cue, despite being tested in native lake water or clean laboratory water, which is not true for neurophysiological (i.e., EOG) endpoints (Azizishirazi et al., 2013).

Previous laboratory studies indicate that fish continuously exposed to metals can recover olfactory function after an initial impairment (Beyers and Farmer, 2001; Dew et al., 2012). The results of the current study indicate that chronically exposed yellow perch from contaminated lakes show an impaired behavioral response to a conspecific alarm cue relative to fish from a clean lake. One explanation for this discrepancy is that fish from our study were naturally exposed to contaminants for their entire lives, while laboratory fish used in the previous studies were exposed to contaminants for only a short period. Dew et al. (2012) showed that adult fathead minnows (*Pimephales promelas*) recovered olfactory function during continuous exposure to sublethal concentrations of copper. In contrast, when the same species was exposed to almost the same concentration of copper during embryonic development, fish were not able to detect predators, and did not recover from chemosensory dysfunction after 84–96 days in clean water (Carreau and Pyle, 2005). These studies suggest that the ability of fish to recover from contaminant-impaired olfaction is related to the life-stage exposed to chemosensory-impairing contaminants. In the current study, wild yellow perch would have been exposed to

contaminants during embryonic development; therefore, recovery of olfactory function in contaminated water is unlikely, which is consistent with our data. In addition, Beyers and Farmer (2001) and Dew et al. (2012) used a single-contaminant (i.e., copper), which was added to laboratory water under controlled exposure conditions, whereas wild fish inhabit waters with variable exposure conditions and are exposed to a mixture of contaminants and modifying factors (Table 1). It is plausible that differences in water quality, as well as the fact that the wild fish used in the present study were likely exposed to contaminants during embryonic development, in part explain the lack of chemosensory recovery in continuous exposure found in this study.

Microarray analysis using the olfactory rosettes of fish from different lakes demonstrated some differential gene transcription patterns. However, data from the 1000-gene yellow perch microarray used to monitor gene transcription patterns in olfactory rosettes was not able to predict olfactory impairment of yellow perch from metal contaminated habitats. A great number of differentially transcribed genes was expected between Hannah and Geneva lakes due to the dramatic difference in metal concentrations between the two lakes, but this was not seen (Table 2). Yellow perch from Hannah Lake showed olfactory impairment using neurophysiological and behavioral endpoints, however, no differences in gene transcription were seen between the fish from these two lakes (Mirza et al., 2009; Azizishirazi et al., 2013; this study). In previous studies, microarray assays showed promising potential for predicting toxicity in fish (Hogstrand et al., 2002; Reynders et al., 2006; Sheader et al., 2006; Moens et al., 2007; Bougas et al., 2013). The 1000 gene yellow perch array used in this study has previously been used and was able to detect the gene transcription changes in liver in a 45-day exposure to sublethal concentrations of nickel and/or cadmium (Bougas et al., 2013). In terms of olfaction, microarray analyses have been used to study the differential gene transcription in the olfactory tissues of zebrafish in response to various contaminants (Tilton et al., 2008; Tilton et al., 2011). In addition to species and microarray dissimilarity, one major difference between our study and research conducted by Tilton et al. (2008, 2011) was that the exposure time in their study was 24 h, whereas in our study fish were exposed to contaminants for their entire lives. In our study, however, because the chronic contaminant exposure in Hannah Lake, it is plausible that the transcription of genes in olfactory sensory neurons (OSNs) compensated for the effect and reached a new steady state at the time of sampling. This speculation may explain why no changes have been observed in the transcription of genes in OSNs, although this hypothesis remains to be rigorously tested.

The greatest number of differentially transcribed genes was observed between Hannah and Ramsey lakes, the two metal-contaminated lakes in the study. In addition, most of the differentially transcribed genes between Hannah–Ramsey and Geneva–Ramsey were the same (Fig. 4), which suggests that a factor other than contaminant exposure most likely had the strongest effect on gene transcription in olfactory tissues. Considering that no genes were differentially transcribed between Geneva Lake and Hannah Lake fish and the similarity of differentially transcribed genes between Hannah–Ramsey and Geneva–Ramsey leads to the conclusion that one or more factors in Ramsey Lake fish caused this differential transcription.

Higher concentrations of iron in Ramsey Lake water at the time of sampling in 2011 compared to the time of sampling in 2012 could be responsible for changes in gene transcription of fish from Ramsey Lake (Table 1). Over transcription of genes like ferritin H subunit, ferritin heavy subunit and ceruloplasmin which all have a role in iron metabolism, support the role of Fe in gene transcription changes of fish from Ramsey Lake compared to fish from Hannah or Geneva lakes. In addition, since Ramsey Lake is situated in the city of Sudbury and receives contamination from storm water, it is possible that some other non-metal contaminant could be responsible for the differential gene transcription in fish from Ramsey Lake (Roberts et al., 2005).

Measuring gene transcription with cDNA microarrays represents a snapshot of gene transcription at a specific time in a specific tissue (Neumann and Galvez, 2002) and transcription of genes can change quickly (Tadiso, et al., 2011; Beggel et al., 2012). On the other hand, the conditions in a lake are variable and many chemical and physical factors, such as temperature and dissolved oxygen, can vary on a time scale ranging from hours to months or even years. Therefore, it is likely that the relatively short-term changes in the conditions of the environment could influence gene transcription and induce “noise” in gene transcription profiles (Van der Meer et al., 2005; Bougas et al., 2013).

5. Conclusions

In conclusion, fish from metal contaminated lakes around Sudbury, ON are olfactory-impaired, as demonstrated by behavioral testing. However, no link could be made between the behavioral and transcriptional levels of biological organization. This incongruity leads to the conclusion that the impairment of olfaction of chronically exposed wild fish would not necessarily be measurable using gene transcriptional changes in olfactory rosettes. Fortunately, classical techniques such as measuring behavioral responses can still be used to investigate olfactory impairment of fish from metal contaminated sites.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at <http://dx.doi.org/10.1016/j.ecoenv.2014.04.045>.

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