Ecotoxicology and Environmental Safety 106 (2014) 239–245

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

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

Ali Azizishirazi [a], William A. Dew [b], Berenice Bougas [c], Mehdi Dashtban [d], Louis Bernatchez [e], Greg G. Pyle [a,b,*]

[a] Department of Biology, Lakehead University, Thunder Bay, Ontario, Canada P7B 5E1
[b] Department of Biological Sciences, University of Lethbridge, 4401 University Drive, Lethbridge, Alberta, Canada T1K 3M4
[c] Institut National de la Recherche Scientifique, Centre INRS Eau Terre et Environnement, 490 rue de la Couronne, Québec, Québec, Canada G1K 9A9
[d] School of Environmental Sciences, University of Guelph, Guelph, Ontario, Canada N1G 2W1
[e] Département de biologie, Institut de Biologie Intégrative et des Systèmes (IBIS), Université Laval, Québec, Québec ,Canada G1V 0A6

ARTICLE INFO

Article history:
Received 28 February 2014
Received in revised form
28 April 2014
Accepted 29 April 2014
Available online 21 May 2014

Keywords:
Olfactory toxicity
Behavioral deficits
Gene transcription
Microarray
Yellow perch

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.

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 (Lürling 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

* Corresponding author at: Department of Biological Sciences, University of Lethbridge, 4401 University Drive, Lethbridge, Alberta, Canada T1K 3M4.
Fax: +1 403 329 2082.
E-mail address: gregory.pyle@uleth.ca (G.G. Pyle).

http://dx.doi.org/10.1016/j.ecoenv.2014.04.045
0147-6513/

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 Kulikovsky-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 μL of trace metals grade high purity nitric acid (Fisher Scientific, Nepean, ON) passed through a 0.45 μm 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 cm^2 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 cm^2/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 × 20 cm × 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