Aquatic Toxicology 177 (2016) 324–332

Contents lists available at ScienceDirect

Aquatic Toxicology

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

Combined effects of temperature changes and metal contamination at different levels of biological organization in yellow perch

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ARTICLE INFO

Article history:
Received 15 February 2016
Received in revised form 10 June 2016
Accepted 11 June 2016
Available online 14 June 2016

Keywords:
Fish
Yellow perch
Perca flavescens
Temperature
Metals
Cadmium
Nickel
Metabolic capacities
Gene transcription level
Biometric condition indicators
Oxidative stress

ABSTRACT

In this study, we measured the effects of temperature (9 °C, 20 °C, and 28 °C), metal contamination (cadmium and nickel) and their interaction on yellow perch (*Perca flavescens*) using liver enzymatic and transcriptomic endpoints and biometric indices. Kidney metal concentrations increased with a rise of temperature. The biometric indices analysed (Fulton condition factor, pyloric cæca, hepatosomatic and gonadosomatic indices) generally decreased with an increase of temperature but not with metal contamination. At the enzymatic level, the activity of superoxide dismutase (SOD), involved in antioxidant response, was affected by both temperature and metal contamination, whereas the activity of glucose-6-phosphate dehydrogenase (G6PDH), involved in energy accumulation but also in antioxidant response, was only affected by metal exposure. The response of perch to the stressors at the transcriptional level differed from the metabolic response. In particular, the transcription level of the *cco* and *g6pdh* genes sharply decreased with increasing temperature, while the activities of the corresponding enzymes remained stable. The normal response of the transcription level of the apoptotic gene (*diablo*) to heat stress was also altered in metal-contaminated fish. The combination of metal and temperature stresses also modified the response of antioxidant metabolism induced by these stressors individually. This study contributes to a better understanding of the influences of natural stressors like temperature on biomarkers commonly used in ecotoxicological studies and will facilitate their interpretation in the context of multiple stressors characteristic of field situations.

1. Introduction

Among the major contaminants of concern in the environment, metals can cause devastating effects to aquatic life because of their propensity for bioaccumulation and toxicity. Metal mining and smelting are historically among the most important economic activities in Canada, but the industry has caused large-scale water contamination and impacts on aquatic habitats, in particular by nickel (Ni) and cadmium (Cd) (Csavina et al., 2012; Iles and Rasmussen, 2005; Klinck et al., 2007; Lemly, 1994).

In addition to metal effects, aquatic organisms from mining areas are exposed to numerous natural stressors in their environment. Among these natural factors, changes in water temperature can have important impacts on aquatic fauna and their environment (Cochrane et al., 2009; Rosenzweig et al., 2007). In ectotherms including fish, temperature is a major driver of metabolic and locomotor capacities, although other exogenous and endogenous factors such as food availability and reproductive status can also influence these capacities (Guderley, 2004). In this context, yellow perch (*Perca flavescens*) are particularly relevant to examine combined effects of multiple stressors, as they are considered eurythermal and tolerant to a wide range of physico-chemical conditions (Scott and Crossman, 1974) including metal contamination (Couture and Pyle, 2015; Eastwood and Couture, 2002; Pierron et al., 2009).

Yellow perch exposed to changes in water temperature or to metal contamination can use various defence mechanisms to fight against these stressors. In a previous study (Grasset et al., 2014), we reported that an increase in temperature affected biometric indices (Fulton condition factor (FCF) and pyloric cæca index (PCI)), the activity of glucose-6-phosphate dehydrogenase (G6PDH) and the transcription level of genes involved in apoptosis (the diablo-

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http://dx.doi.org/10.1016/j.aquatox.2016.06.008
0166-445X/

like protein gene, *diablo*) and in energy metabolism and oxidative stress response (*g6pdh*). Metal contamination is also well known to impact biometric indices, enzymatic activities and gene transcription levels in fish. In yellow perch, Cd induces oxidative stress in gills and liver (Giguère et al., 2004). Exposure to Ni resulted in changes in biomarkers of oxidative stress such as an increase in the transcription of *g6pdh* and *cat* (Defo et al., 2014) and of CCO activity (Pierron et al., 2009). Negative effects of Ni on iron metabolism have been demonstrated in yellow perch (Bougas et al., 2013; Wood et al., 2011).

Clearly, there is compelling evidence that both temperature and metal contamination induce various stress responses in yellow perch. In this context, the objective of this study was to investigate the combined effects of temperature changes and metal contamination (Ni or Cd) on yellow perch using biometric indicators (FCF, PCI, hepatosomatic index (HSI) and gonadosomatic index (GSI)), hepatic metabolic capacities (enzyme activities of cytochrome C oxidase (CCO), an indicator of aerobic capacities, nucleoside diphosphate kinase (NDPK), an indicator of biosynthetic capacities, G6PDH and superoxide dismutase (SOD), indicators of antioxidant capacities) and hepatic gene transcription levels (*cco*, *diablo*, *g6pdh* and *sod*).

2. Materials and methods

2.1. Fish

Young of the year yellow perch (*Perca flavescens*) were bought from a fish farm (Trevor Thomas, Abbey Road Fish Farm, Wainfleet, ON) and brought back to the Laboratoire de Recherches en Sciences Aquatiques (LARSA) at Université Laval (Québec, QC). During the acclimation period (2 weeks), perch were kept in a 1 m^3 circular tank at 9 °C, where they were fed daily at 3% of their biomass with frozen brine shrimp. After this period, fish were acclimated in eighteen 40-L glass aquaria for 2 weeks with a gradual increase of temperature up to their experimental temperature. After acclimation, the experimental conditions were initiated.

At the end of the experiment, perch were sacrificed by a blow to the head, immediately measured, weighed and dissected. Pyloric cæca, gonads, liver, kidney and muscle were collected and frozen in liquid nitrogen. All procedures were approved by our institutional animal care committee and followed the guidelines of the Canadian Council on Animal Care.

2.2. Experimental conditions

Each condition, described below, was replicated in two separate aquaria each containing 22 fish, yielding a total of 44 fish per condition. Fish were divided among various projects, and a number were set aside for method optimizations. For this project, in order to examine physiological and transcriptomic endpoints, 16 fish were randomly sampled from each experimental condition: eight yellow perch were used to measure gene expression (for qRT-PCR) and eight were used to measure enzyme activities. After acclimation, yellow perch were exposed to combined stressors for seven weeks. Three metallic conditions were used: control (no metal), cadmium exposure and nickel exposure. Fish from each metal exposure condition were exposed to one of three different temperatures (9 °C, 20 °C and 28 °C), yielding nine conditions. The oxygen level and daily ration were the same for the exposure as they were during the acclimation period. Metal concentrations were chosen based on previous studies in our lab and were similar to concentrations measured in contaminated Canadian lakes (eg. Couture et al., 2008; Defo et al., 2012). They were measured twice a week in each aquarium and adjusted as required using stock solutions of Cd and Ni with certified concentrations of 1000 μg/L in 4% HNO_3 (SCP Science, ICP Standard item # 140-051-485 for Cd 140-051-285 for Ni). Measured aqueous metal concentrations were statistically identical in aquaria adjusted at different temperatures and did not vary over the seven weeks of exposure. For Cd, measured water concentrations were 3.99 ± 0.21 μg/L (35.5 ± 1.9 nM) (mean ± SEM, n = 84; 3 temperatures, 2 aquaria per temperature, two samplings per week). Values in Ni exposure aquaria were 606.1 ± 13.4 μg Ni/L (10.3 ± 0.2 μM) (n = 84). Concentrations of both metals in control aquaria were consistently below 0.01 μg/L. Aqueous concentrations of Cd and Ni were measured using an inductively coupled plasma mass spectrometer (ICP-MS, Thermo Elemental, Model X-7). The instrument response was calibrated with certified standard solutions (SCP Science, ICP Standard item # 140-051-485 for Cd 140-051-285 for Ni) and possible instrument drift was controlled for by inserting blanks and standards every 10 samples.

2.3. Sample analysis

2.3.1. Biometric measures

Fish condition was estimated using four biometric measurements. The Fulton condition factor (FCF) was calculated with the following equation: FCF = (weight (g)/length $(cm)^3$) × 1000 (Nash et al., 2006). The pyloric cæca index (PCI) was calculated as described by Gauthier et al. (2008), using the following equation: $Wcorr = (Wf/Wm)^{b*}Wc$, where Wcorr is the corrected weight of the pyloric cæca, Wm is the mean of the fish weight for the dataset, Wf is the fish weight, b is the slope of the logarithmic relationship between fish weight and cæcum weight (0.869 for this dataset) and Wc is the uncorrected weight of pyloric cæca. The pyloric cæca index is a good indicator of recent feeding history of fish and of food availability in their environment (Gauthier et al., 2008). The hepatosomatic index (HSI) and the gonadosomatic index (GSI) were calculated using the equations described by Lloret and Rätz (2000): HSI = (liver weight (g)/fish weight (g)) × 100 and GSI = (gonad weight (g)/fish weight (g)) × 100.

2.3.2. Metal concentrations

Kidney samples used for metal measurement were lyophilized in acid-washed (15% HNO_3) Eppendorf® tubes. Certified reference material (TORT-2 from the Canadian National Research Council) and blanks were submitted to the same treatment as samples in order to determine analytical accuracy. After lyophilisation, freeze-dried samples were digested in trace metal grade nitric acid at a ratio of 100 μL of HNO_3 for 1 mg of dried sample for 2 days at room temperature and for 24 h in trace metal grade hydrogen peroxide at a ratio of 40 μL for 1 mg of dried sample. The samples were then diluted in ultrapure water (in order to obtain 1 mL/mg of dry weight) and concentrations of Cd and Ni were measured using an ICP-MS (Thermo Elemental, Model X-7) (Bougas et al., 2013).

2.3.3. Enzyme and protein assays

Protein assays followed the Bradford protocol elaborated for microplates by Bio-Rad (Bio-Rad Protein Assay). Enzyme activities were measured in triplicate using a UV/Vis spectrophotometer (Varian Cary 100) on a microplate at room temperature (20 °C). Reactions were recorded over a period of five minutes and a linear portion of at least three minutes was used in order to calculate reaction rates. Activities were expressed as international units (IU)/g wet weight.

All chemicals were bought from Sigma-Aldrich (Canada). Livers were homogenized in ice-cold buffer (pH 7.5, 20 mM HEPES, 1 mM EDTA, 0.1% Triton X-100) for three bursts of 20 s using an Ultra Turrax T25 tissue homogenizer.

Reaction conditions for the enzymes involved in energy production, biosynthetic and anaerobic capacities were as follows: