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Waterborne cadmium and nickel impact oxidative stress responses and retinoid metabolism in yellow perch

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ABSTRACT

In this experiment, we studied the transcriptional and functional (enzymatic) responses of yellow perch (*Perca flavescens*) to metal stress, with a focus on oxidative stress and vitamin A metabolism. Juvenile yellow perch were exposed to two environmentally relevant concentrations of waterborne cadmium (Cd) and nickel (Ni) for a period of 6 weeks. Kidney Cd and Ni bioaccumulation significantly increased with increasing metal exposure. The major retinoid metabolites analyzed in liver and muscle decreased with metal exposure except at high Cd exposure where no variation was reported in liver. A decrease in free plasma dehydroretinol was also observed with metal exposure. In the liver of Cd-exposed fish, both epidermal retinol dehydrogenase 2 transcription level and corresponding enzyme activities retinyl ester hydrolase and lecithin dehydroretinyl acyl transferase increased. In contrast, muscle epidermal retinol dehydrogenase 2 transcription level decreased with Cd exposure. Among antioxidant defences, liver transcription levels of catalase, microsomal glutathione-S-transferase-3 and glucose-6-phosphate dehydrogenase were generally enhanced in Cd-exposed fish and this up-regulation was accompanied by an increase in the activities of corresponding enzymes, except for microsomal glutathione-S-transferase. No consistent pattern in antioxidant defence responses was observed between molecular and biochemical response when fish were exposed to Ni, suggesting a non-synchronous response of antioxidant defence in fish exposed to waterborne Ni. There was a general lack of consistency between muscle transcription level and enzyme activities analyzed. The overall findings from this investigation highlight the usefulness of transcriptional and biochemical endpoints in the identification of oxidative stress and vitamin A metabolism impairment biomarkers and the potential use of multi-level biological approaches when assessing environmental risk in fish.

Abbreviations: ANOVA, Analysis of variance; BHT, Butylated hydroxytoluene; BSA, Bovine serum albumin; CAT, Catalase; CCO, Cytochrome c oxidase; cDNA, Complementary DNA; CDNB, Chloro-2,4-dinitrobenzene; DMSO, Dimethyl sulfoxide; DPPC, Dipalmitoylphosphatidylcholine; dREH, Dehydroretinyl ester hydrolase; DROH, Dehydroretinol; G6PDH, Glucose-6-phosphate dehydrogenase; GPx, Glutathione peroxidase; GST, Glutathione-S-transferase; HCd, High Cd concentration corresponding to waterborne exposure of fish to 8 μg/L (71 nmol/L) of Cd; HNi, High Ni concentration corresponding to waterborne exposure of fish to 600 μg/L (10,200 nmol/L) of Ni; HPLC, High-performance liquid chromatography; Kn, Relative condition index; LARSA, Laboratoire Régional des Sciences Aquatiques; LCd, Low Cd concentration corresponding to waterborne exposure of fish to 0.8 μg/L (7.0 nmol/L) of Cd; LdRAT, Lecithin dehydroretinyl acyl transferase; LNi, Low Ni concentration corresponding to waterborne exposure of fish to 60 μg/L (1020 nmol/L) of Ni; LRAT, Lecithin retinyl acyl transferase; LSI, Liver somatic index; MGST, Microsomal glutathione-S-transferase; mgst-3, Microsomal glutathione-S-transferase-3; MTs, Metallothioneins; NADH, Nicotinamide adenine dinucleotide; PBS, Phosphate saline buffer; RBP, Retinol binding protein; REH, Retinyl ester hydrolase; Rdh-2, Epidermal retinol dehydrogenase 2; ROH, Retinol; ROS, Reactive oxygen species; RT-PCR, Reverse transcription polymerase chain reaction; RQ, Relative quantification; SE, Standard error; SOD, Superoxide dismutase; TCEP, Tris 2-carboxyethyl phosphine; TTR, Transthyretin.

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1. Introduction

Metal pollution is a major environmental hazard affecting the health of freshwater biota. The yellow perch (*Perca flavescens*) is a good candidate as a biomonitor of aquatic metal pollution for several reasons (Giguère et al., 2004). It is widely distributed across North American freshwater ecosystems and is particularly relevant in metal toxicology studies because it tolerates the presence of metals such as cadmium (Cd) and nickel (Ni) at high concentrations tolerated by few other fish (Hontela et al., 1995, 1992; Rajotte and Couture, 2003). The bioaccumulation of metals in its tissues reflects local contamination and corroborates its sedentary behavior (Campbell et al., 2003). Additionally, metal concentrations in yellow perch tissues have been correlated with biological impacts such as metabolic imbalance of vitamin A_2 (Defo et al., 2012), impairment of metabolic capacities (Couture and Kumar, 2003), poor condition and overall health (Couture et al., 2008b; Rajotte and Couture, 2002), elevated metallothionein concentrations (Giguère et al., 2005), and reduction in genetic diversity at both neutral and coding regions of the genome (Bourret et al., 2008; Bélanger-Deschênes et al., 2013).

An emerging priority in biomonitoring is to identify relevant biological impacts in sentinel species providing informative elements about the adverse outcomes induced by environmental stressors (Chandurvelan et al., 2013). Effects of contaminants can be assessed at different levels of biological organization, from molecular to community levels (Ankley et al., 2010). To this end, biomarkers describe biochemical, cellular, physiological or behavioral changes that can be measured in cells, tissues or whole organisms as a result of bioaccumulation or effects of toxicant exposure (Depledge et al., 1995; Tsangaris et al., 2010). Biomarkers can be used as premonitory warning signals of environmental disturbance, by providing the causal link between the presence of toxicants and an ecological effect (Walker et al., 2006).

Fishes are considered to be sensitive to metal-induced oxidative stress (Kubrak et al., 2012; Souid et al., 2013), resulting from an imbalance between the amount of cellular antioxidants and that of ROS (reactive oxygen species) (Defo et al., 2014; Vertuani et al., 2004). Yet, studies have reported that yellow perch living in metal impacted areas possess efficient biochemical defence mechanisms (Giguère et al., 2005). Depletion of cellular antioxidants, or an overproduction of ROS, can disturb this equilibrium leading to oxidative stress (Scandalios, 2005), which can lead to an impairment of cellular repair mechanisms (Dorval and Hontela, 2003). Both non-enzymatic antioxidant biomarkers such as vitamin A metabolites and enzymatic antioxidant biomarkers including SOD (superoxide dismutase), CAT (catalase), GST (glutathione-S-transferase) and G6PDH (glucose-6-phosphate dehydrogenase) interact together to minimize the damaging effects of ROS in fish (Scandalios, 2005).

As argued by Ankley et al. (2010), linking the responses to chemical interactions at different biological organization levels provides a critical basis for predictive approaches in environmental risk assessment. Cadmium and Ni are both non-essential metals for fish. These metals are known to be toxic at low levels and can cause oxidative stress in aquatic organisms (Kubrak et al., 2013; Vertuani et al., 2004). They can be found in sediments, the water column, and biota at elevated levels as a consequence of anthropogenic activities (Luoma and Rainbow, 2008), such as mining and smelting. In a previous study on yellow perch chronically exposed to metals in the field, we reported a negative correlation between hepatic Cd concentrations and liver transcription levels of genes encoding for enzymes involved in the metabolism of retinoids (Pierron et al., 2011). Subsequent research showed that the percentage of hepatic free dehydroretinol decreased, suggesting that Cd inhibits the enzymes and the binding proteins involved in retinoid homeostasis (Defo et al., 2012). Yellow perch from Cd-contaminated lakes had significantly higher concentrations of liver dehydroretinol and dehydroretinyl esters than did fish from reference lakes. To our knowledge, the only other study reporting simultaneous molecular and biochemical responses to metal exposure on antioxidant enzymes in fish is that of Henrik Hansen et al. (2007). Trout (*Salmo trutta*) from an environment with low metal levels were transferred to a polluted river with higher Cd and Zn concentrations for 15 days. Although a significant correlation between Cd accumulation and the transcription level of some antioxidant genes was reported, the changes of enzymes activities and gene transcription levels were inconsistent (Henrik Hansen et al., 2007), suggesting that changes in gene transcription levels do not necessarily lead to modifications in the activity of corresponding enzymes (Giuliani et al., 2013; Regoli et al., 2011).

In our investigation of the mechanisms of toxicity of Cd and Ni in yellow perch, we have identified transcriptional signatures specific to Cd and Ni exposure. The results indicate that Cd and Ni affect the transcription level of genes involved in several metabolic pathways including oxidative stress and vitamin metabolism (Bougas et al., 2013). The general objective of the present study was to investigate at two biological organization levels (molecular and biochemical) the effects of waterborne Cd and Ni exposure on oxidative stress response biomarkers in yellow perch. Renal metal concentrations were used as indicators of metal accumulation. Our specific objectives were: (i) to examine changes in liver and muscle transcription levels of a set of toxicologically-relevant genes involved in oxidative stress response in yellow perch exposed to Cd and Ni; and (ii) to compare gene expression levels to corresponding biochemical endpoints (enzyme activities and retinoid storage) in order to determine if metal-induced changes in gene transcription levels lead to significant physiological effects. This study will contribute to a better understanding of the role of perturbations in retinoid metabolism and oxidative stress in chronic metal toxicity.

2. Materials and methods

2.1. Fish and maintenance conditions

Juvenile yellow perch were obtained from a private fish supplier (Trois-Rivières, Québec, Canada) between September and October 2011, and transferred to the LARSA (Laboratoire Régional des Sciences Aquatiques) at Université Laval (Québec). Fish were acclimated in a flow-through holding tank (1 m^3) for 4 weeks with aerated, dechlorinated water kept at 18 °C and photoperiod set at 12-h light:12-h dark. One week prior to the beginning of exposures, fish were randomly selected and transferred to aquaria (40 L), where acclimation continued. Water of the same chemical composition was used for both the acclimation phase and the experimental treatments: pH 7.2–7.3; Ca 0.35 mmol/L; Mg 0.073 mmol/L; Na 0.70 mmol/L; K 0.036 mmol/L; Cl 0.48 mmol/L; SO_4 0.22 mmol/L; alkalinity 0.22 mmol/L. Temperature was adjusted to 20 °C and oxygen maintained at 100% saturation.

2.2. Experimental treatments

Each exposure group (aquarium) consisted of 15 fish with an average total biomass of 250 g per aquarium. Perch were exposed to low and high Cd and Ni nominal water concentrations as follows: control (0 μg/L; 0 nmol/L), low Cd concentration (LCd, 0.8 μg/L; 7 nmol/L), high Cd concentration (HCd 8 μg/L; 71 nmol/L), low Ni concentration (LNi, 60 μg/L; 1020 nmol/L), and high Ni concentration (HNi, 600 μg/L; 10,200 nmol/L). Each condition was