Aquatic Toxicology 162 (2015) 39–53

Contents lists available at ScienceDirect

Aquatic Toxicology

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

Transcriptional and biochemical markers in transplanted *Perca flavescens* to characterize cadmium- and copper-induced oxidative stress in the field

Michel A. Defo[a], Louis Bernatchez[b], Peter G.C. Campbell[a], Patrice Couture[a,*]

[a] Institut National De La Recherche Scientifique (INRS), Centre Eau Terre Environnement, 490 De La Couronne, Québec, QC G1K 9A9, Canada
[b] Institut De Biologie Intégrative Et Des Systèmes (IBIS), Université Laval, Québec, QC G1V 0A6, Canada

ARTICLE INFO

Article history:
Received 17 January 2015
Received in revised form 19 February 2015
Accepted 20 February 2015
Available online 21 February 2015

Keywords:
Wild fish
Metal pollution
Reciprocal transplantation
Biomarkers
Plasticity traits
Genomic reaction norm

ABSTRACT

Despite recent progress achieved in elucidating the mechanisms underlying local adaptation to pollution, little is known about the evolutionary change that may be occurring at the molecular level. The goal of this study was to examine patterns of gene transcription and biochemical responses induced by metal accumulation in clean yellow perch (*Perca flavescens*) and metal depuration in contaminated fish in a mining and smelting region of Canada. Fish were collected from a reference lake (lake Opasatica) and a Cd, Cu and Zn contaminated lake (lake Dufault) located in the Rouyn-Noranda region (Qc, Canada) and caged for one or four weeks in their own lake or transplanted in the other lake. Free-ranging fish from the same lakes were also collected. Kidney Cd and Cu concentrations in clean fish caged in the contaminated lake increased with the time of exposure, but metal depuration did not occur in contaminated fish caged in the clean lake. After 4 weeks, the major retinoid metabolites analysed, the percentage of free dehydroretinol (dROH) and the retinol dehydrogenase-2 (*rdh-2*) transcription level in liver decreased in clean fish transplanted into the metal-contaminated lake, suggesting that metal exposure negatively impacted retinoid metabolism. However, we observed an increase in almost all of the retinoid parameters analysed in fish from the metal-impacted lake caged in the same lake, which we interpret as an adaptation response to higher ambient metal concentration. In support of this hypothesis, liver transcription levels of microsomal glutathione-*S*-transferase-3 (*mgst-3*) and glucose-6-phosphate dehydrogenase (*g6pdh*) were enhanced in clean fish transplanted into the metal-contaminated lake and this up-regulation was accompanied by an increase in the activities of corresponding enzymes, involved in antioxidant response. However, although in the same fish the transcription level of Cu/Zn superoxide dismutase (*Cu/Zn sod*) was also increased, this did not lead to a change in the activity of the SOD enzyme, suggesting that this upregulation was aimed at maintaining SOD-related antioxidant capacities. In contrast, the transcription level of the *cat* gene, which did not change in contaminated fish, did not compensate for the decrease of CAT activity. After 4 weeks of exposure, some plastic responses of the clean fish were observed when they were transplanted in the metal-contaminated lake. However, probably as a consequence of the prior 80 years of exposure to metals, the contaminated population showed a limited plastic response in the expression of the majority of the candidate genes tested, when they were transplanted in the reference lake.

The overall findings of this field investigation highlight how yellow perch molecularly and biochemically responded to a sudden or relatively long-term exposure (4 weeks) to a cocktail of metals.

1. Introduction

It is broadly accepted that anthropogenic activities are affecting all ecosystems. Among these activities, pollution sources including metal mining and smelting have contributed to drastic changes in environmental conditions that could potentially lead to changes in

* Corresponding author. Tel.: +1 418 559 3825; fax: +1 418 654 2600.

http://dx.doi.org/10.1016/j.aquatox.2015.02.014
0166-445X/

biodiversity (Eisler, 2004). Animals may use three routes (dispersion, phenotypic plasticity or evolutionary adaptation) to better respond to these stressors or shifts in environmental conditions (Hansen et al., 2012). In favourable ecological and physical conditions such as an absence of physical barriers, they will disperse to new locations. However, in most cases the dispersal of fish to suitable nearby habitats is hard to achieve, and therefore they react *via* phenotypic plasticity to express particular traits (Merilä and Hendry, 2014) or *via* evolutionary adaptation if changes in environmental conditions occur over the long term (Hansen et al., 2012). In some cases, populations fail to adapt to anthropogenic stress. For instance, sulphur dioxide emissions from base metal smelters have led to the rapid acidification of lakes and consequent species extinctions (Gonzalez et al., 2013).

Generally, studies dealing with the development of plastic traits have been carried out for only one point in time, thereby ignoring the time dependency of changes in gene expression, the intensity of expression and the nature of genes (same or not) that were involved in the plastic development between two time points (Aubin-Horth and Renn, 2009). When assessing the mechanisms of action of pollutants, it is critical to evaluate the time point when the differences in gene expression occurred, because it can offer a valuable tool to identify genes that trigger plastic development (Aubin-Horth and Renn, 2009). Whereas, adaptive stress response genes may be involved in short exposure periods, additional sets of genes may become involved as the exposure time increases, in order to maintain animal homeostasis (Denslow et al., 2007). In support of this hypothesis, using a cDNA microarray on flounders (*Platichthys flesus*) exposed to different salinities in both short-term (1 day) and long-term (50 days) reciprocal-transplantation experiments between the North sea and the Baltic sea, Larsen et al. (2007) demonstrated that the number of genes significantly and differentially expressed increased with exposure time, indicating a plastic response to a local change in environmental conditions (Larsen et al., 2007).

Compared to most other fish species, the yellow perch (*Perca flavescens*) is considered as a biomonitor because it fulfils several criteria (Giguère et al., 2004). It is abundant and widely distributed across North American freshwater ecosystems and is particularly relevant in metal toxicology studies because it tolerates metals such as cadmium (Cd) and copper (Cu) at high concentrations (Hontela et al., 1995; Hontela et al., 1992; Rajotte and Couture, 2003). This species possesses efficient biochemical defence mechanisms (*e.g.* increasing metallothionein concentrations with increasing metal levels in its tissues; (Giguère et al., 2005), which allow it to live in metal impacted areas. Nevertheless, metal concentrations in yellow perch tissues have been correlated with biological impacts such as the 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, 2002b) and elevated metallothionein concentration (Giguère et al., 2005). In a recent investigation on the mechanisms of Cd and Ni toxicity in yellow perch, we identified transcriptional and functional signatures specific to Cd and Ni exposure. The results indicate that metals, particularly Cd, affect the transcription level of genes and the activity of enzymes involved in oxidative stress response and vitamin A metabolism (Defo et al., 2014a). In an earlier study in evolutionary ecotoxicology, negative correlations between hepatic concentrations of cadmium, but not copper, and genetic diversity have been reported in wild yellow perch living in metal-impacted areas (Bourret et al., 2008). Results from another study carried out in the copper and gold mining region of Rouyn-Noranda have shown that metals, especially Cd, induce changes in the yellow perch genome by selecting alleles that increase the fitness of perch inhabiting metal-impacted lakes (Bélanger-Deschênes et al., 2013). Both studies support the hypothesis that pollution can trigger natural selection.

Gene expression can also be directly informative about population adaptive genetic divergence (Larsen et al., 2007). When attempting to test hypotheses aimed at identifying possible ecological drivers, traits and genes involved in the adaptation to changing environmental conditions, one approach involves the comparison of the transcription levels of genes among different exposure conditions (Côté et al., 2014; Hoffmann and Daborn, 2007; Meier et al., 2014). Among natural populations, variation in gene expression has been shown to play a prominent role in ecological adaptation (Derome and Bernatchez, 2006; Pavey et al., 2011; St-Cyr et al., 2008). The expression of mRNA was reported to explain *ca.* 40% of the variance in protein expression (Abreu et al., 2009) meaning that changes in gene transcription levels do not necessarily involve modifications in the concentrations of the corresponding enzymes (Giuliani et al., 2013; Regoli et al., 2011). It is therefore important to take into account functional and genomic information when designing genomic studies (Furlong, 2011) instead of simply discussing functional outcomes (*e.g.* change in enzymes activities) on the basis of transcriptomic data (Nikinmaa et al., 2013). Change in response to the interactions between an organism and it environment can be observed at different levels including morphological, behavioral or physiological. The generic term of "genomic reaction norm" is generally used to describe the relationship between trait and environment for a given genotype, or to define the extent of plasticity of that trait (Aubin-Horth and Renn, 2009).

In aerobic organisms, oxygen, in its normal configuration (O_2) is somewhat unreactive; however, it can be transformed into reactive oxygen species (ROS) in response to changing environmental or metabolic conditions (Gliszczyńska-Świglo, 2006; Scandalios, 2005). ROS production can lead to cellular oxidative stress, itself inducing lipid peroxidation, DNA and protein damage and other adverse effects (Chen et al., 2013; Leonard et al., 2004). Metals have different modes of generating or increasing cellular ROS (Defo et al., 2014b; Lushchak, 2011). In order to counterbalance oxidative damage, gene transcription levels of cellular antioxidant response and the activities of enzymes involved in these metabolic pathways increase in response to higher cellular ROS (Uren Webster et al., 2013). Studies have reported that yellow perch living in metal impacted areas possess efficient biochemical defence mechanisms (Giguère et al., 2005). Both non-enzymatic antioxidant biomarkers such as vitamin A metabolites and enzymatic antioxidant biomarkers including superoxide dismutase (SOD), catalase (CAT), glutathione-*S*-transferase (GST) and glucose-6-phosphate dehydrogenase (G6PDH) interact together to minimize the damaging effects of ROS in fish (Scandalios, 2005). In a previous laboratory study where yellow perch were separately exposed to aqueous Cd (7.0 nmol/L and 71 nmol/L) and Ni (1020 nmol/L and 10,200 nmol/L) for 6 weeks, we observed that the enzyme activities of liver retinyl ester hydrolase and lecithin dehydroretinyl acyl transferase increased in fish exposed to higher Cd. However, no measurable effect was observed in fish exposed to Ni either at lower or higher Ni concentration (Defo et al., 2014a). Among antioxidant defences, increases in liver CAT and G6PDH activities were observed in fish exposed to higher Cd, however no effects on liver microsomal GST and SOD activity were reported (Defo et al., 2014a). Exposure to Ni did not affect the activities of these enzymes except for CAT where an increase in liver CAT activity was observed at the higher Ni concentration. In contrast to the responses in liver, muscle CAT and GST activities decreased or remained unchanged with metal exposure, except at low Cd concentrations, where the activities increased (Defo et al., 2014a). Levesque et al. (2002) reported that the liver G6PDH activity was low in perch inhabiting the most metal-contaminated lakes, in summer, but this activity remained

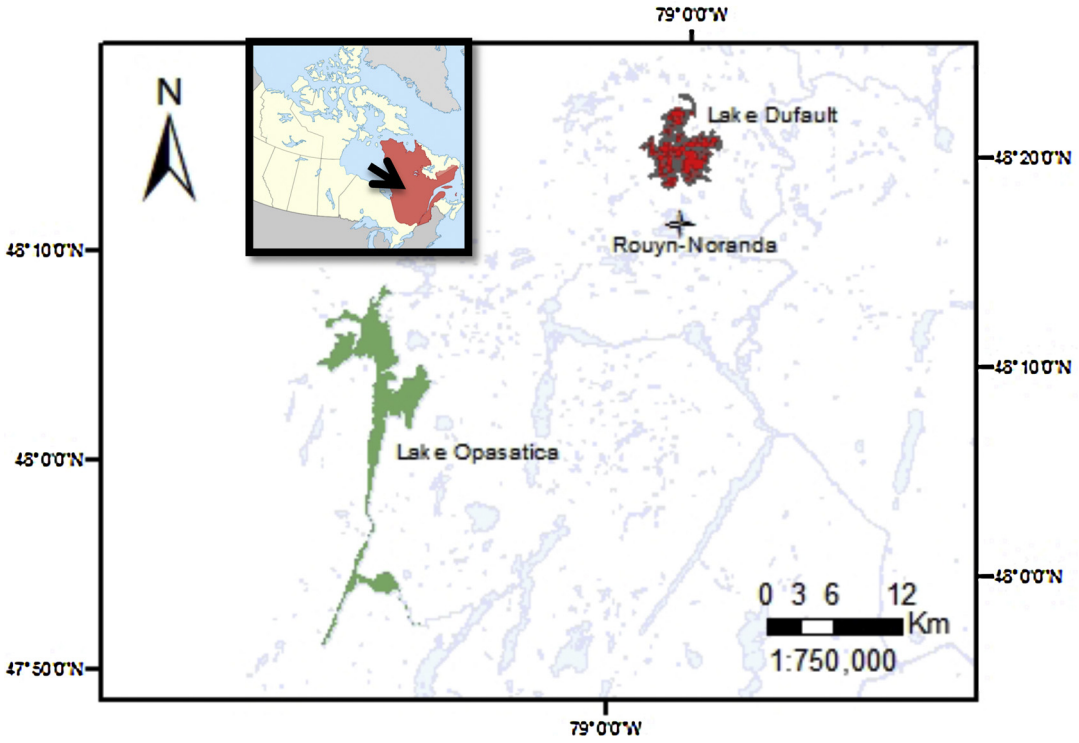

Fig. 1. Study area and lakes sampled. The reference lake is indicated in green and the metal-contaminated lake in red. The cross refers to the point source of metal contamination in the Rouyn-Noranda region of Québec.

high compared to that of their homologs caught in fall of the same year (Levesque et al., 2002).

In the present reciprocal transplantation field experiment using caged fish, transcriptional analysis coupled with biochemical analyses were employed to investigate the effects of metal exposure on retinoid metabolism and oxidative stress response biomarkers in yellow perch. Our specific objectives were: (i) to examine temporal changes in liver transcription levels of a set of toxicologically-relevant genes involved in the oxidative stress response in yellow perch exposed up to four weeks to metals; (ii) to compare gene expression levels with 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; (iii) to compare responses of a population from a clean site to those at a site where the population has been exposed to contaminants for 80 years and could have therefore developed adaptation to cope with the contaminated environment; and (iv) to determine at what time scale changes in the metabolic response appear as a result of metal accumulation or depuration.

2. Materials and methods

2.1. Study area, fish sampling and reciprocal transplantations

Two lakes were selected along the polymetallic concentration gradient in the Rouyn-Noranda region (Québec-Canada) (Fig. 1). Since 1927, a copper smelter has been in operation in this region and metal concentrations (Cd, Cu and Zn) in lakes situated downwind from the smelter are markedly higher than in lakes located upwind (Bonham-Carter et al., 2006). Lake Dufault is closely situated, *ca.* 2 km, downwind from the smelter and was chosen as the metal-contaminated lake (Con) due to historical atmospheric

Table 1
Total aqueous and sediment concentrations of trace metals Cd, Cu and Zn in the study.

Compartment	Water			Sediments			
Metal	[Cd] (nM)	[Cu] (nM)	[Zn] (nM)	[Cd] (μmol/g dw)	[Cu] (μmol/g dw)	[Zn] (μmol/g dw)	References
Opasatica	0.16	44.7	8				(Ponton, 2010)[a]
	0.11	44	31				(Giguère et al., 2004)
	0.30	33	19				(Giguère et al., 2006)
	<0.90	72	27	12	0.88	2.33	(Borgmann et al., 2004)
	<0.03	45.7	55				(Croisetière et al., 2006)
	0.07	64	7				(Fortin et al., 2010)
	0.11	47	5				(Kraemer et al., 2008)
	5.1	287	1194				(Ponton, 2010)[a]
	3.3	158	714				(Ponton, 2010)[a]
Dufault	7.7	254	2425				(Giguère et al., 2004)
	6.7	180	1100				(Giguère et al., 2006)
	3.0	232	577	341	45.9	68.7	(Borgmann et al., 2004)
	3.2	194	510				(Croisetière et al., 2006)
	2.7	127	430				(Fortin et al., 2010)
	5.5	225	1015				(Kraemer et al., 2008)

[a] Lake sampled in 2010 by Dominic Ponton, INRS-ETE, unpublished data.

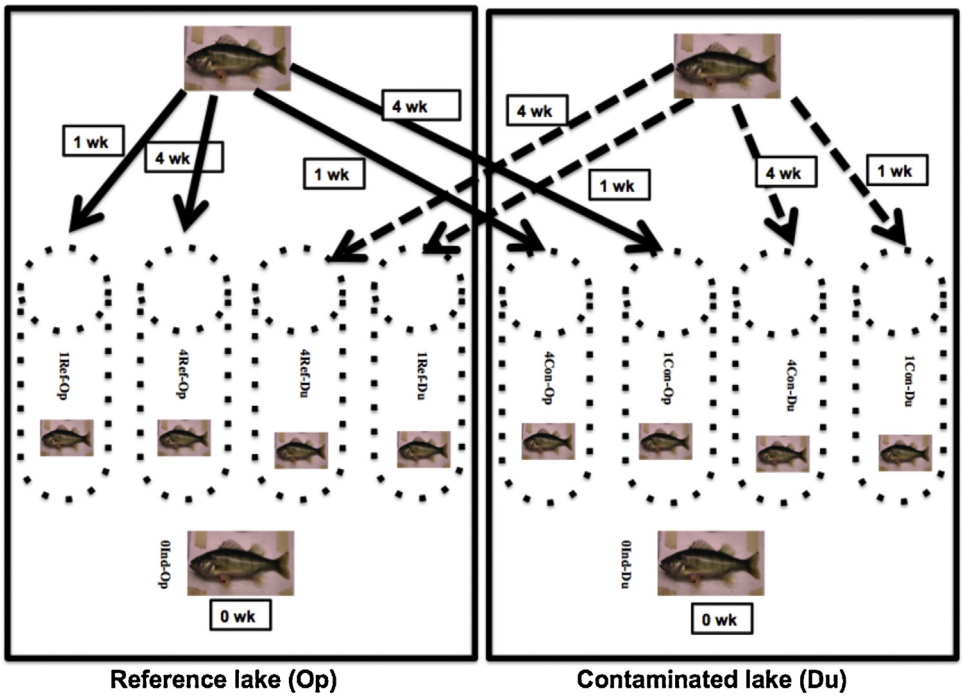

Fig. 2. Experimental design of all exposure conditions: 1Ref-Op, 1Ref-Du, 1Con-Op, 1Con-Du, 4Ref-Op, 4Ref-Du, 4Con-Op, 4Con-Du, 0Ind-Op and 0Ind-Du (see Table 2 for details of the experimental design).

deposition of relatively high levels of metals such as Cd, Cu and Zn (Table 1). This has resulted in increased levels of metals in the water column, sediments and in prey. Lake Opasatica, located *ca.* 30 km upwind of the metal emission site, was selected as the reference lake (Ref.) because of the relatively low metal concentrations in the water column, sediments and prey. Furthermore earlier work has shown that Zn concentrations are tightly controlled in yellow perch (*e.g.* Giguère et al., 2004, 2005).

At the time of first sampling (May 22, 2012), lake Opasatica and lake Dufault water physico-chemical properties were, respectively: pH (7.81 ± 0.03 and 7.53 ± 0.07); oxygen (10.11 ± 0.72 mg/L and 9.01 ± 0.15 mg/L) and temperature (17.6 ± 1.7 °C and 17.2 ± 2.5 °C). Values are expressed as means ± standard error. Other water quality variables were not measured in this study; however, some data were obtained from previous studies carried out in 2010 in both lakes (Dominic Ponton INRS-ETE, unpublished data). The major cation and anion concentrations found in lake Opasatica and lake Dufault were: *ca.* (0.21 and 0.39 mmol/L); Mg (0.11 and 0.11 mmol/L); Na (0.13 and 0.17 mmol/L); K (0.025 and 0.016 mmol/L); Cl (0.09 and 0.20 mmol/L); SO_4 (0.06 and 0.29 mmol/L).

The cage experiments were carried out in spring 2012 according to the approach developed in Kraemer et al. (2005a,b); Kraemer et al. (2005a,b), with slight modification. In brief, planktivorous juvenile yellow perch (2.47 ± 0.07 g; 6.57 ± 0.07 cm; mean ± SE) were collected between May 22–24, 2012 either in lake Opasatica, hereafter Op or in lake Dufault, hereafter Du, and reciprocally transplanted into cages located in both lakes. The cage walls were made of 5 mm mesh netting, allowing free movement of water and food into the cages. A total of 8 cages were used (Fig. 2), creating 4 condition regimes for each exposure period (1 and 4 weeks). The reciprocal-transplantation experiment conditions are explained in Table 2. Also, at the beginning of the transplantation phase and at 1 and 4 weeks, free-ranging fish were collected from each lake. However, only metal analyses were performed on free-ranging fish sampled at 1 and 4 weeks. The effect of caging itself, which compares fish caged in their own lake with free-ranging fish, was examined in another study (Julie Grasset, INRS-ETE, unpublished

Table 2
Exposure condition, percentage of males and yellow perch mortality in the reciprocal transplantation experiment. Opasatica is the reference lake and Dufault is the contaminated lake.

Condition[a]	Percentage of males	Percentage of mortality	Explanation
0Ind-Op	10	–	Free-ranging indigenous lake Opasatica fish sampled at the beginning of experiment
0Ind-Du	10	–	Free-ranging indigenous lake Dufault fish sampled at the beginning of experiment
1Ind-Op	10	–	Free-ranging indigenous lake Opasatica fish sampled at 1week
1Ind-Du	5	–	Free-ranging indigenous lake Dufault fish sampled at 1 week
1Ref-Op	10	27.5	Fish from the reference lake caged in the reference lake for one week
1Ref-Du	15	12.2	Fish from the metal-contaminated lake caged in the reference lake for one week
1Con-Op	5	24.4	Fish from the reference lake caged in the metal-contaminated lake for one week
1Con-Du	0	80.0	Fish from metal-contaminated lake caged in metal-contaminated lake for one week
4Ind-Op	31	–	Free-ranging indigenous lake Opasatica fish sampled at 4 week
4Ind-Du		–	Free-ranging indigenous lake Dufault fish sampled at 4 week
4Ref-Op	47	57.5	Fish from the reference lake caged in the reference lake for four weeks
4Ref-Du	28	68.9	Fish from the metal-contaminated lake caged in the reference lake for four weeks
4Con-Op	27	76.1	Fish from the reference lake caged in metal-contaminated lake for four weeks
4Con-Du	15	50.0	Fish from metal-contaminated lake caged in the metal-contaminated lake for four weeks

[a] The number prefix in the condition column indicates the time of exposure (weeks).

Table 3
Nucleotide sequences of specific partial coding sequence (primer) pairs and PCR products used in RT-qPCR analysis.

Gene abbreviations	Name and functions	Specific primers	PCR products
β-actin	Reference gene	F: 5′- GCCTCTCTGTCCACCTTCCA-3′ R: 5′- GGGCCGGACTCATCGTACT-3′	From Pierron et al. (2014)
rdh-2	Photoreceptor associated retinol dehydrogenase 2 (retinoid and oxidative stress metabolism)	F: 5′-AGTCAAGCAGTGCATCAACAAT-3′ R: 5′- CATGCGAACAACACCAAAGAAG-3′	151
cat	Catalase (oxidative stress metabolism)	F: 5′- GTCTTTCTTGTTCAGCGATCGA-3′ R: 5′- GTAGAAACGTTCACCATCAGCA-3′	106
mgst-3	Microsomal glutathione *S* transferase-3 (oxidative stress metabolism)	F: 5′- CCTTCCTCTACAGCTGGATCAT-3′ R: 5′- TGAATACCTGCTCCTTGTCACT-3′	114
g6pdh	Glucose 6 phosphate dehydrogenase (pentose phosphate and oxidative stress metabolism)	F: 5′- ACGAGAGGCTGATATTGGATGT-3′ R: 5′- TCCATATGTGTAAGGGATGGGG-3′	146
sod	Cu/Zn superoxide dismutase (oxidative stress metabolism)	F: 5′-TGAGCAGGAGGAGGGTTCATCCCC-3′ R: 5′-CCTGCACTGATGCACCCGTTTGT-3′	123

F: forward primer. R: reverse primer.

data). Each cage initially contained 30–40 fish, and at the end of each exposure period the remaining fish (up to a maximum of 20) were sampled from each cage. The percentage of fish mortality in each cage is reported in Table 2. Lengths and weights were recorded and sex determined. Livers were dissected for molecular and biochemical analyses, kidneys for metal analyses and pyloric cæca for biometric analyses. Tissues were immediately frozen and stored in liquid nitrogen in the field. Upon arrival in the laboratory, samples were placed in a freezer at −80 °C where they were kept until used for analyses. The fish manipulation protocol was approved by the Ministère des Ressources Naturelles et de la Faune du Québec and by the Comité Institutionnel de Protection des Animaux (CIPA) of INRS.

2.2. Calculation of fish condition index (Kn) and corrected pyloric cæca weights

The relative condition factor (Kn) was calculated using the Eq. $Kn = (Wf/L^{3.16}) \times 100$ (where "Wf" is fish weight, "L" is the total fish length) (Gauthier et al., 2009). The corrected pyloric cæca weights were calculated according to the method described elsewhere (Gauthier et al., 2011) with 2.6 g as the standard fish weight and 0.92 as the allometric exponent.

2.3. Analyses of kidney metal concentrations, liver quantitative RT-PCR, determination of retinoid and protein concentrations and enzyme activities

Cadmium, Cu and Zn were analyzed in kidney tissues after digestion in trace metal grade nitric acid according to Pierron et al. (2009), using an inductively coupled-plasma atomic emission spectrophotometer (ICP-AES). Internal standards were within 10% of nominal values in all cases and mean metal recoveries from the reference material analyzed (TORT-2, lobster hepatopancreas, National Research Council of Canada, Ottawa, ON) were 94.8 ± 0.4% for Cd, 85.3 ± 1.3% for Cu and 89.0 ± 0.9% for Zn (mean ± S.E.).

RNA isolation, cDNA synthesis and analysis of liver gene transcription levels were performed in accordance with methods and technical standardization procedures cited in Defo et al. (2014a). The partial coding sequences (primers) used in this study are listed in Table 3.

All the biochemical parameters, including liver retinoid concentrations and enzyme activities (CAT, SOD, G6PDH and GST) analyzed in this study were conducted using the protocol described by Defo et al. (2014a), without modification.

2.4. Data analysis

To test for differences in biometric parameters, metal concentrations, enzyme activities and retinoid concentrations among experimental conditions, we used one-way analysis of variance (ANOVA), after checking the assumption of normality (Levene test, $p > 0.05$). We also used ANOVA to evaluate the differences in gene transcription levels among conditions and to determine the degree of plasticity (genomic reaction norm) among experimental conditions. When the probability was significant ($p < 0.05$), multiple comparison tests (Tukey HSD test) were used to identify differences between two conditions. When the assumption of normality was not met, and when $\log_{10}$ or box-cox (Peltier et al., 1998) transformation of the data was unable to normalize the distribution, non-parametric paired Wilcoxon tests were applied. For vitamin A determination, statistical analyses were performed without considering sex as an explanatory variable. A probability of $p < 0.05$ indicates a statistically significant difference from the control experiment condition. Results were expressed as means ± standard error (SE). The relationships between individual kidney metal concentrations and liver gene transcription level, biochemical analysis or biometric parameters were investigated using the non-parametric Spearman rank correlation test. Analyses were performed using JMP 9.0 software.

3. Results

3.1. Kidney metal accumulation dynamics

Kidney metal concentrations reflected the metal contamination status of the two studied lakes. At the beginning of the experimentation ($t = 0$), metals accumulated in kidneys of free ranging-fish from the reference lake Opasatica (0Ind-Op) (Fig. 3A) were 4.6 and 1.3 times lower for Cd and Cu, respectively, than those for fish in the metal-impacted lake Dufault (0Ind-Du) (Fig. 3B). This trend was confirmed in indigenous free-ranging fish sampled at $t = 1$ week. Kidney metal concentrations in free-ranging fish collected at $t = 4$ weeks in the metal-impacted lake (Fig. 3B) were also significantly higher ($p < 0.05$) for Cd, Cu and Zn (4.5, 1.9 and 1.5 times, respectively) than those in fish from the reference lake (Fig. 3A).

Metal accumulation patterns in yellow perch kidneys varied over the transplantation period. Kidney Cd concentration of fish from reference lake Opasatica caged in metal-impacted lake Dufault (4Con-OP) increased over time in comparison to reference fish caged in lake Opasatica (4Ref-Op) (Fig. 3A). The increase was not significant after 1 week of exposure (1Con-Op), but a significant ($p < 0.05$) increase was observed at the end of experiment (4Con-Op) (Fig. 3A), although the Cd concentration was lower than that of fish living naturally in lake Dufault (4 Ind-Du) (Fig. 3B). A similar but more pronounced trend was also seen for Cu (Fig. 3A). No trend in kidney Zn accumulation was observed in caged fish (Fig. 3A). Unlike the results obtained in the contamination experiment, kidney Cd, Cu and Zn concentrations of fish from the metal-impacted lake transplanted into the reference lake were not different from those in fish that remained caged in lake Dufault (Fig. 3B).

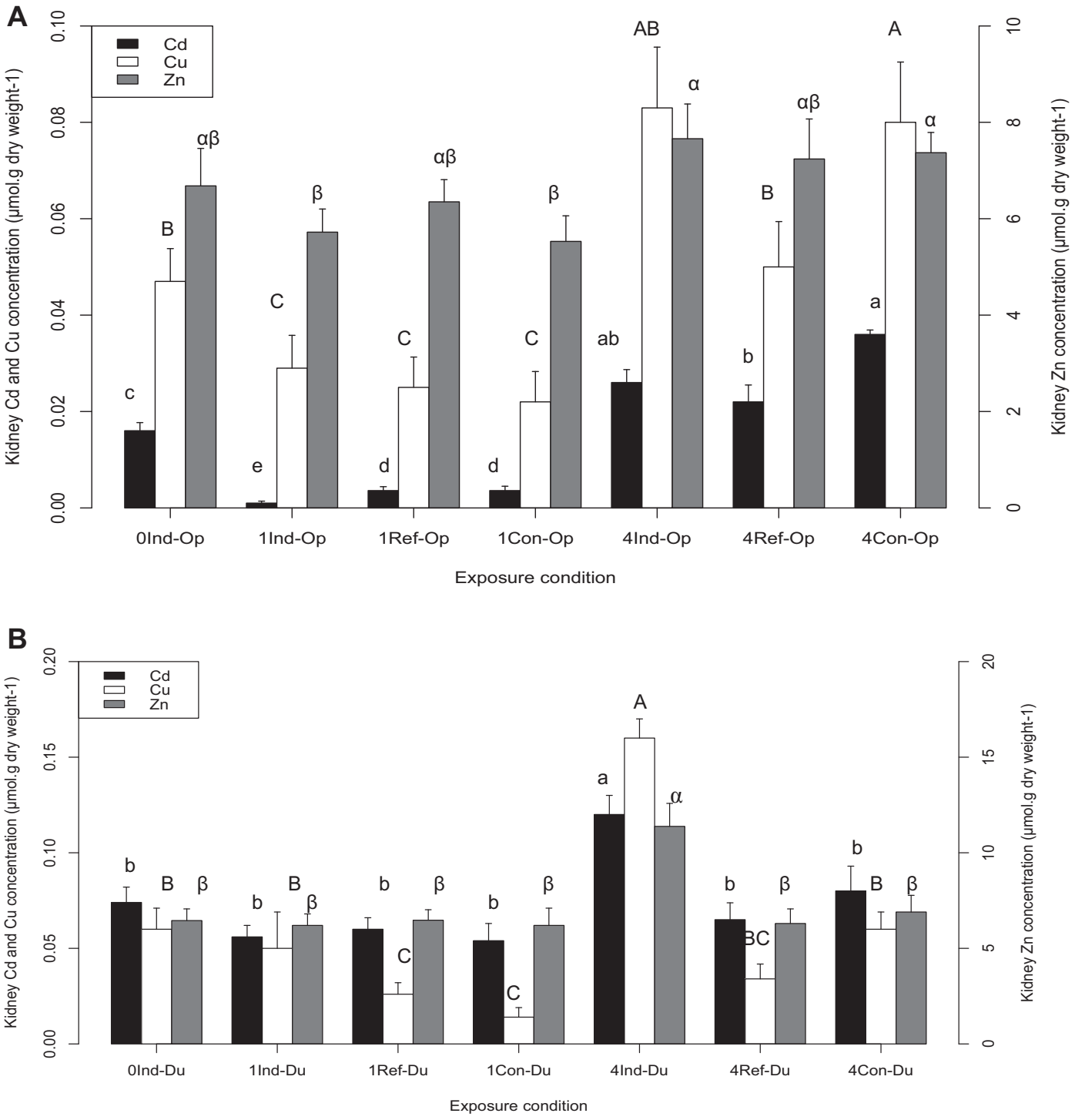

Fig. 3. Total cadmium, copper and zinc concentrations in the kidney of indigenous yellow perch sampled after 1 and 4 weeks for the contamination experiment (Fig. 3A; lake Opasatica specimens) or for the depuration experiment (Fig. 3B; lake Dufault specimens). Free-ranging perch from lake Opasatica (Ind-Op) and lake Dufault (Ind-Du), respectively, represent control fish for contamination and depuration experiments. Con-Op and Con-Du denote fish caged in lake Dufault, while Ref-Op and Ref-Du represent fish caged in lake Opasatica. The number prefix in the exposure conditions indicates the time of exposure (weeks). Bars are means ± standard error (SE) ($11 \leq N \leq 20$/condition). Means designated with different letters for a given metal are significantly different (p-value < 0.05). Different letters (capital letters for Cd, lower case letters for Cu and greek letters for Zn) represent comparisons of kidney metal concentrations among exposure conditions.

3.2. Fish condition index (Kn) and corrected pyloric cæca weights

In the metal contamination experiment (lake Opasatica to lake Dufault), analysis of biometric parameters revealed little difference among the experimental conditions. Indeed transplanted fish (1Con-Op and 4Con-Op) displayed higher condition factors than their corresponding control fish (1Ref-Op) and (4Ref-Op), respectively (Table 4). However, no change in pyloric cæca weights was observed either at 1 week or 4 weeks in transplanted fish compared to control fish (Table 4). Transferring fish from the contaminated lake to the reference lake(lake Dufault–lake Opasatica) did not significantly affect the biometric parameters analysed, except for the fish transplanted for 4 weeks (4Ref-Du), which displayed higher condition factors than control fish (4Con-Du) (Table 4). A significant

Table 4
Number of fish in each sampling group (N), relative condition index (Kn), corrected pyloric cæca weight and major vitamin. A concentrations in liver of indigenous free-ranging yellow perch from lake Opasatica (0Ind-Op) and lake Dufault (0Ind-Du) and in yellow perch caged in lake Dufault (Con-Op and Con-Du) or in lake Opasatica (Ref-Op and Ref-Du) sampled after 1 and 4 weeks (see Section 2.1). Values are mean ± SE and means within columns designated with different letters are significantly different (p-value < 0.05).

		Biometric parameters		Retinoid metabolism[ϒ]		
Condition[*]	N	Relative index condition (Kn)	Corrected pyloric cæca wet weight (mg)	[dROH][√]	[dROH-palmitate][√]	[Total vitamin A esters][√]
0Ind-Op	20	0.62 ± 0.01^{b}	10.0 ± 0.7^{ab}	0.024 ± 0.008^{b}	0.078 ± 0.017^{c}	0.124 ± 0.028^{c}
0Ind-Du	20	0.59 ± 0.01^{b}	10.3 ± 0.9^{ab}	0.050 ± 0.013^{a}	0.103 ± 0.031^{c}	0.174 ± 0.055^{bc}
1Ref-Op	20	0.61 ± 0.01^{b}	9.2 ± 0.9^{abc}	0.016 ± 0.007^{b}	0.072 ± 0.031^{c}	0.120 ± 0.052^{c}
1Ref-Du	20	0.63 ± 0.01^{b}	10.8 ± 0.9^{ab}	0.026 ± 0.010^{b}	0.112 ± 0.020^{c}	0.197 ± 0.034^{bc}
1Con-Op	20	0.70 ± 0.03^{a}	11.2 ± 1.0^{ab}	0.015 ± 0.006^{b}	0.084 ± 0.03^{c}	0.145 ± 0.053^{c}
1Con-Du	8	0.62 ± 0.03^{b}	12.7 ± 1.9^{a}	0.025 ± 0.007^{b}	0.125 ± 0.020^{c}	0.237 ± 0.040^{bc}
4Ref-Op	17	0.65 ± 0.02^{b}	5.8 ± 0.4^{cd}	0.012 ± 0.002^{bc}	0.250 ± 0.035^{b}	0.319 ± 0.070^{b}
4Ref-Du	14	0.72 ± 0.03^{a}	7.3 ± 0.8^{bcd}	0.020 ± 0.004^{b}	0.432 ± 0.063^{a}	0.632 ± 0.092^{a}
4Con-Op	11	0.77 ± 0.02^{a}	4.8 ± 0.3^{d}	0.008 ± 0.003^{c}	0.144 ± 0.027^{c}	0.224 ± 0.037^{bc}
4Con-Du	20	0.65 ± 0.02^{b}	5.0 ± 0.4^{d}	0.032 ± 0.008^{a}	0.385 ± 0.036^{a}	0.645 ± 0.071^{a}

ϒ $N = 6$.
[*] The number prefix in the condition column indicates the time of exposure (weeks).
√ Expressed in μmol/g liver wet weight.

relationship was observed between Kn and kidney Cu concentrations ($\rho = 0.23$; $p = 0.0002$) and between pyloric cæca weights and kidney Cu concentrations ($\rho = -0.19$; $p = 0.014$), for all fish pooled.

3.3. Changes in liver retinoid levels during metal accumulation and depuration

As expected, all-trans-3,4-didehydroretinoids were the predominant form of vitamin A found in yellow perch liver. After 4 weeks of metal exposure, the concentration of dehydroretinyl palmitate (dROH-palmitate) and the main form of vitamin A storage significantly decreased in fish from lake Opasatica caged in lake Dufault (4Con-Op) in comparison to control fish (4Ref-Op) (Table 4).

The percentage of free dehydroretinol (dROH) also significantly decreased in fish from lake Opasatica transplanted in lake Dufault (4Con-Op) in comparison to control fish (4Ref-Op) (Fig. 4A; left panel).

There were no significant differences among any of the forms of vitamin A analysed in fish from the metal-impacted lake that were caged in their own lake or in the reference lake (Table 4 and Fig. 4A; right panel), except for the concentration of free liver dROH, which decreased in fish caged in lake Opasatica after 4 weeks (4Ref-Du) (Table 4).

3.4. Response of molecular biomarkers of oxidative stress during metal accumulation and depuration

In the metal accumulation experiment, liver *rdh-2* transcription levels were lower in fish exposed to metals compared to controls (1Ref-Op and 4Ref-Op), and the decrease was significantly more pronounced after 4 weeks of exposure compared to 4Ref-Op) (Fig. 4A; left panel). Liver *cat* transcription levels were not significantly different in fish exposed to metals compared to controls (Fig. 4B; left panel). However, liver *mgst-3* (Fig. 4C; left panel), *g6pdh* (Fig. 4D; left panel) and *Cu/Zn sod* (Fig. 4E; left panel) transcription levels were significantly higher in fish exposed to metals compared to controls, and the increases were more pronounced after 4 weeks of exposure.

In the metal depuration experiment, liver *cat* (Fig. 4B; right panel), *mgst-3* (Fig. 4C; right panel) and *Cu/Zn sod* (Fig. 4E; right panel) transcription levels had decreased after 1 week. However no change in liver *rdh-2* (Fig. 4A; right panel) and *g6pdh* (Fig. 4D; right panel) transcription levels was observed compared to controls. After 4 weeks, the transcription levels of the measured genes were no longer different, except for liver *rdh-2,* which had a transcription level 1.9 times higher in fish exposed to low metals (4Ref-Du) than in control fish (4Con-Du) (Fig. 4A; right panel).

Positive correlations were observed between kidney Cu concentrations and *Cu/Zn sod*, *mgst-3* and *g6pdh* transcription levels; correlations were not significant with kidney Cd concentrations (Fig. 5). We chose not to present correlations with Zn because no significant results were obtained between the studied parameters and kidney Zn concentrations.

3.5. Changes in oxidative stress related enzyme activities during metal accumulation and depuration

After 4 weeks, the activity of liver CAT had decreased in fish transplanted from lake Opasatica to lake Dufault (4Con-Op) (Fig. 4B; left panel). In contrast, the activities of liver GST (Fig. 4C; left panel) and G6PDH (Fig. 4D; left panel) were significantly higher in fish that had been exposed to high metal concentrations (4Con-Op), relative to the control (4Ref-Op). Liver SOD activity had not increased significantly as a consequence of metal exposure after 4 weeks (Fig. 4E; left panel). No effect of metal depuration was detected on the activity of the analysed enzymes when fish were transplanted from lake Dufault to lake Opasatica (4Ref-Du), relative to the corresponding control (4Con-Du) ($t = 4$ weeks; Fig. 4B–E; right panel).

Although gene transcription levels were not correlated with kidney Cd concentrations, the corresponding biochemical parameters analysed were positively correlated except for liver SOD activity, for which a negative but significant correlation was observed (Fig. 5).

3.6. Metal genomic reaction norm: response to a changing environment

Differentials in gene transcription levels showing the representation of trait values in relation to changes in environmental conditions (clean fish caged in the contaminated lake, contaminated fish caged in the clean lake, or free ranging fish caged in their own lake) were estimated after 1 week (Fig. 6 left panel) and 4 weeks (Fig. 6 right panel). Among the five genes analysed, the results clearly indicate that patterns of genomic reaction norms vary depending on fish origins and sampling time. After 1 week none of the genes analysed were transcriptionally induced or inhibited in either the metal contamination or the metal depuration experiments (Fig. 6 left panel). With regard to three of the

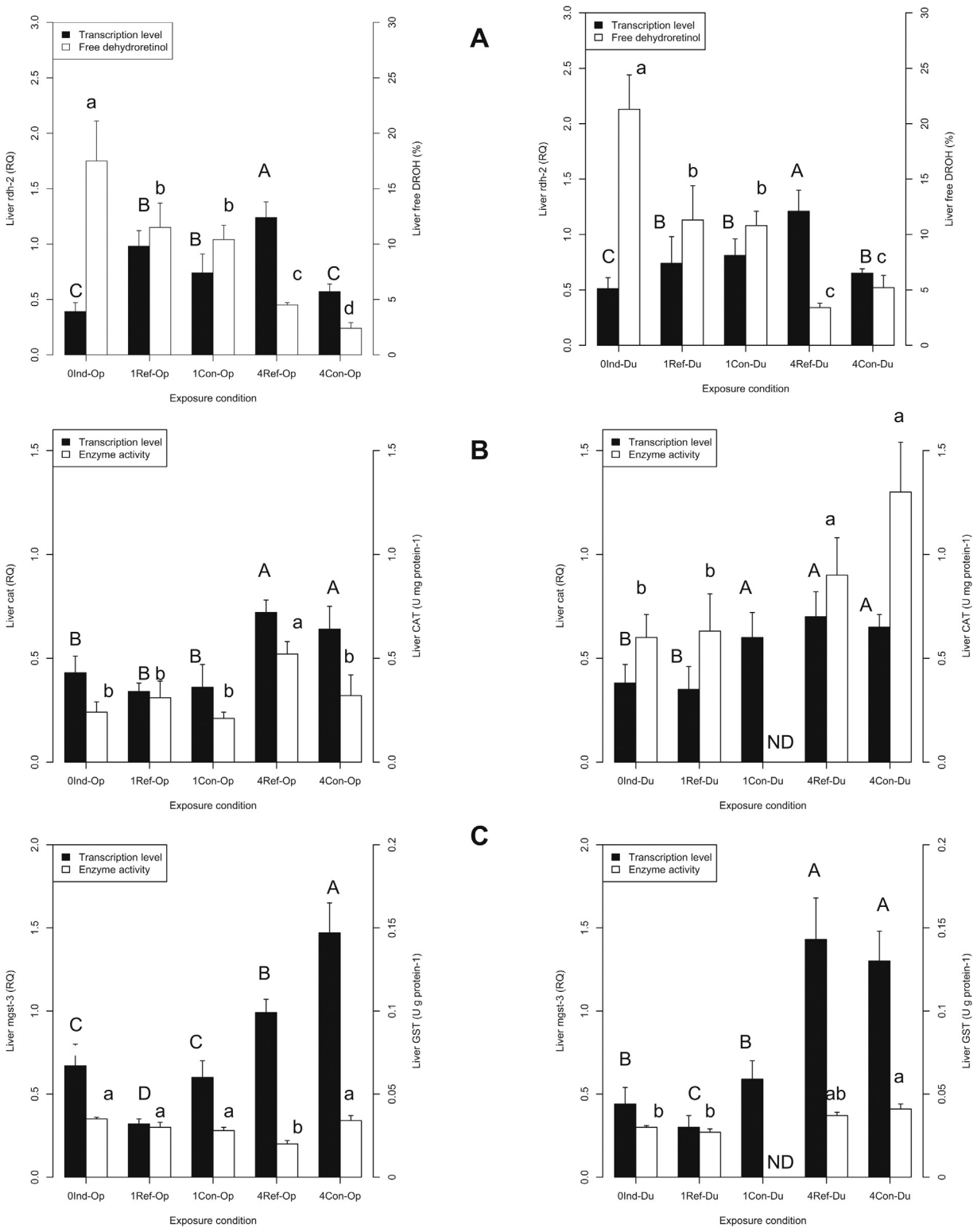

Fig. 4. Liver gene transcription level (mean ± SE) of *rdh-2* (A), *cat* (B), *mgst-3* (C), *g6pdh* (D) and *Cu/Zn sod* (E) and corresponding biochemical analysis of indigenous yellow perch sampled after 1 and 4 weeks for the contamination experiment (lake Opasatica specimens; left panel) or for the depuration experiment (lake Dufault specimens; right panel). Free-ranging perch from lake Opasatica (Ind-Op) and lake Dufault (Ind-Du), respectively, represent control fish for contamination and depuration experiments. Con-Op and Con-Du denote fish caged in lake Dufault, while Ref-Op and Ref-Du represent fish caged in lake Opasatica (see Section 2.1). The number prefix in the exposure condition indicates the time of exposure (weeks). Means designated with different letters for a given parameter are significantly different (p-value < 0.05; $6 \leq N \leq 8$/condition). Different letters (capital letters for gene transcription level and lower case letters for biochemical analysis) represent comparisons of each parameter among exposure conditions. ND: not determined.

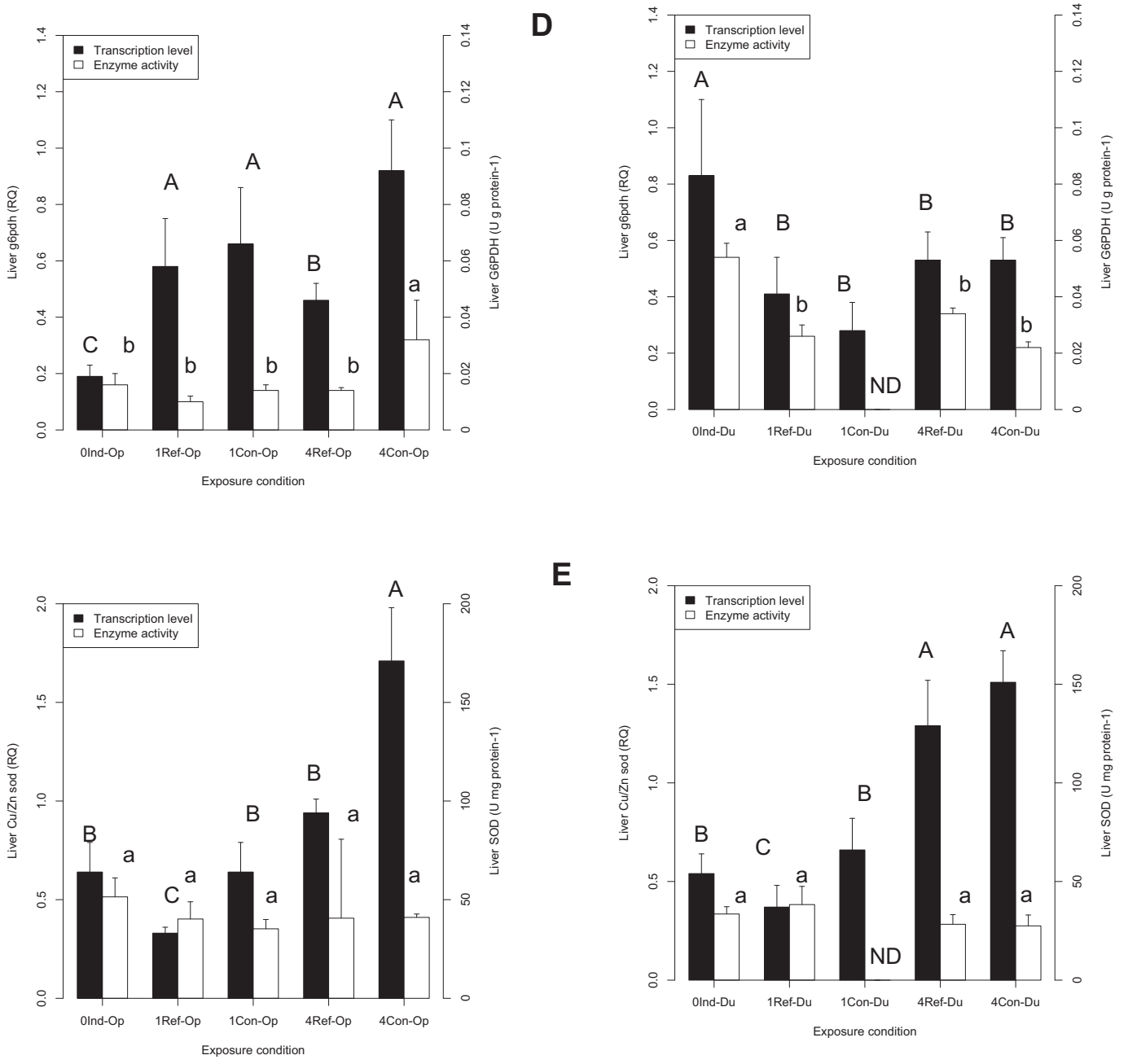

Fig. 4. (*Continued.*)

analysed genes (*Cu/Zn sod*, *mgst-3* and *g6pdh*), it appears that fish from lake Dufault showed a reduced response relative to fish from lake Opasatica where no difference in expression was observed between the rearing environments; whereas, this was the case for lake Dufault perch.

After 4 weeks the analysis of the depuration effect revealed a clear response only with *rdh-2* gene, where the gene increased in expression (Fig. 6A; right panel). However, in the metal contamination experiment this gene was under-expressed in comparison with their homologs that remained caged in clean lake (Fig. 6A; right panel). Catalase gene expression was not transcriptionally induced or inhibited in fish from lake Opasatica caged in lake Dufault (Fig. 6B; right panel). In contrast, a more pronounced and progressive response was noticed at the end of the experiment for *Cu/Zn sod, mgst-3* and *g6pdh* in fish from lake Opasatica caged in lake Dufault (Fig. 6C–E, respectively, right panel).

4. Discussion

We have inevitably collected and analysed only specimens found alive at the end of each experiment. Caging induced a high rate of mortality, especially after four weeks (Table 2). Nevertheless, survivors appeared healthy and their condition index and feeding activity illustrated by the corrected pyloric cæca weights were comparable to values in free-ranging fish. Mortality in cages was likely due to factors other than contamination, since there was no evidence that it was higher in fish caged in the contaminated lake. In the absence of predators, dominance and competition for food resources in restricted environments like cages or aquaria lead to differential growth rates among individuals increasing over time, which can induce a selection for healthier, faster-growing individuals. Regardless of the causes of mortality, our data suggest that although survivors may differ from

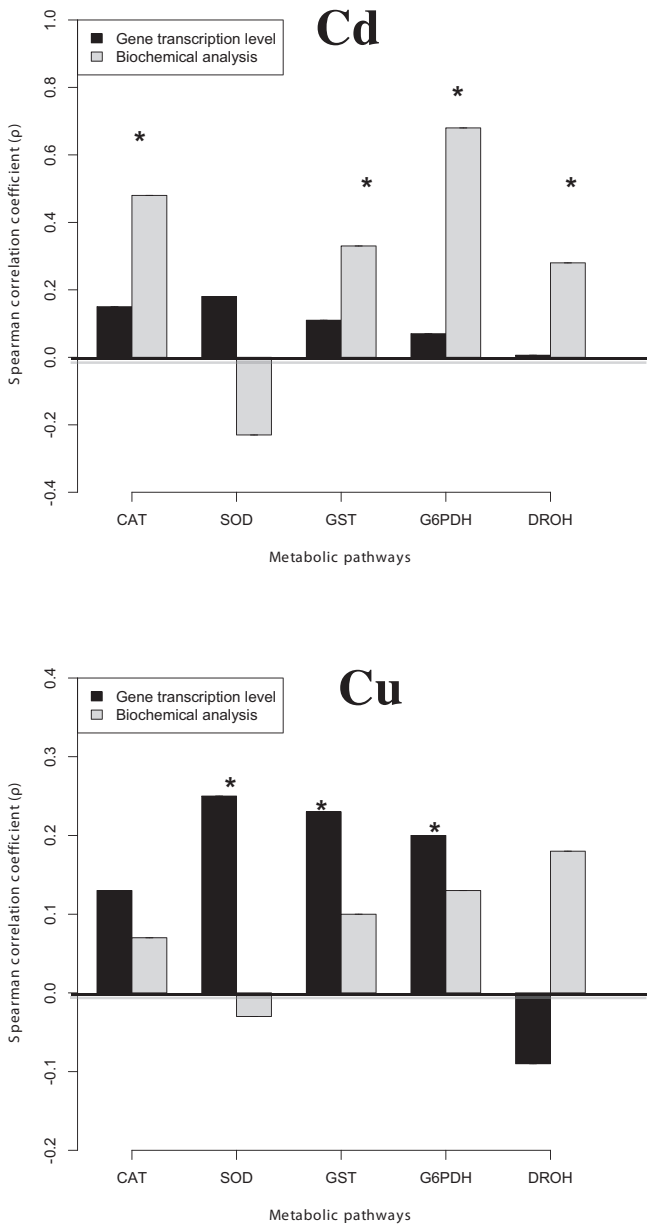

Fig. 5. Spearman correlation coefficients (ρ) between kidney Cd and Cu concentrations and (i) gene transcription level of liver *cat*, *Cu/Zn sod*, *mgst-3*, *g6pdh* and *rdh-2*, (ii) corresponding biochemical analysis of indigenous free-ranging yellow perch from lake Opasatica (0Ind-Op) and lake Dufault (0Ind-Du) (both control lakes for contamination and depuration experiments at the start of the experiment or 0 week, respectively) and in yellow perch caged in lake Dufault (Con-Op and Con-Du) or in lake Opasatica (Ref-Op and Ref-Du) sampled after 1 and 4 weeks. ($60 \leq N \leq 80$), $^{*}p < 0.05$.

their population of origin in terms of variability of the biomarkers examined, fish sampled in cages remain comparable among themselves.

4.1. Effects of metal contamination and depuration on kidney metal concentration

Although we did not measure the concentrations of dissolved metals in water or sediment in this study, Campbell et al. (2008) reported a 22-fold difference in aqueous Cd concentration (the main metal responsible for deleterious effects on aquatic organisms) between the two study lakes. Others authors (Table 1) have reported major differences in aqueous metal concentrations between the reference lake Opasatica and the contaminated lake Dufault, ranging roughly from 22 to 70-fold for Cd, 2 to 6-fold for Cu and 58 to 78-fold for Zn. Important differences were also observed in the sediment metal concentrations of the two lakes, with factors of 28, 52, 29.4 between lake Dufault and lake Opasatica for Cd, Cu and Zn, respectively.

In this study, liver tissue was prioritized for biochemical and molecular analysis and kidney for metal analysis. Previous studies have reported a good correlation between liver and kidney metal concentrations in indigenous yellow perch and demonstrate that kidney metal contamination is a suitable surrogate for estimating metal accumulation (Couture et al., 2008a,b; Kraemer et al., 2005a,b).

In response to the differences in ambient metal concentrations, kidney metal (especially Cd) accumulation was much lower in fish living in the reference lake (lake Opasatica) than in fish from the metal-impacted lake (lake Dufault) (Fig. 3A and B), thus confirming the contamination status of both study lakes. Unsurprisingly, metal-naïve fish caged in the contaminated lake had increased their renal Cd and Cu concentrations after 4 weeks of exposure. Unlike the case for Cu, kidney Cd concentrations in transplanted fish had not reached levels similar to those of indigenous caged fish from lake Dufault, indicating that a steady-state was not reached during the experimental period. Cadmium, a non-essential metal, may have accumulated more slowly in comparison to Cu or Zn, which are both essential metals. Our study agrees with that of Kraemer et al. (2005a), who observed an increase in kidney Cd concentrations after yellow perch where transplanted from lake Opasatica to lake Dufault for 30 days. However, in their study, no clear temporal trend in kidney Cu concentrations was observed.

No significant loss of metals from the kidney for either sampling period was observed in fish caught in the metal-impacted lake and held within the reference lake (Fig. 3B). Although not surprising, this result indicates that the biological depuration times of kidney Cd, Cu and Zn are longer than 30 days. To our knowledge, the only other study reporting metal depuration in indigenous fish is that of Kraemer et al. (2005b). Juvenile yellow perch living in a metal-impacted lake (high Cd, Cu and Zn) were held for up to 75 days in cages within a reference lake (low Cd, Cu and Zn). Gill and gut Cd concentrations decreased rapidly, but the biological half lives of Cd in the kidney and liver were 52 and 75 days, respectively. The depuration of excess Cu also occurred more rapidly in the liver and gut compared to the kidney and gills (Kraemer et al., 2005b). Our study combined with theirs suggests that metal depuration rates are metal-and tissue-specific.

Although our experiment took place within a single season (spring), it is important to note the increase in kidney metal concentrations in free-ranging fish that occurred over the experimental period, particularly in fish collected at $t = 4$ weeks. However, a decrease in kidney Cd and Cu concentrations was observed in free-ranging fish sampled at $t = 1$ week in comparison to those collected at the beginning of the experiment. Food availability likely played a role in metal uptake during our sampling period. Indeed, fish from the reference lake caged in the metal-impacted lake displayed higher values of Kn, a metric associated with recent feeding activity, and higher pyloric cæca weights, reflecting higher feeding rate and food conversion efficiency (Bergot et al., 1981; Gauthier et al., 2008), compared to their homologs caged in the reference lake. The positive relationship observed between Kn and kidney Cu concentrations in our study further supports the idea that tissue metal accumulation during the caging experiment was likely enhanced due to increased feeding activity ($\rho = 0.23$; $p = 0.0002$). However, we cannot rule out that the increase in metal accumulation is due to higher aqueous metal exposure or to an increase in the proportion of trophically available metal in prey (Luoma and Rainbow, 2005).

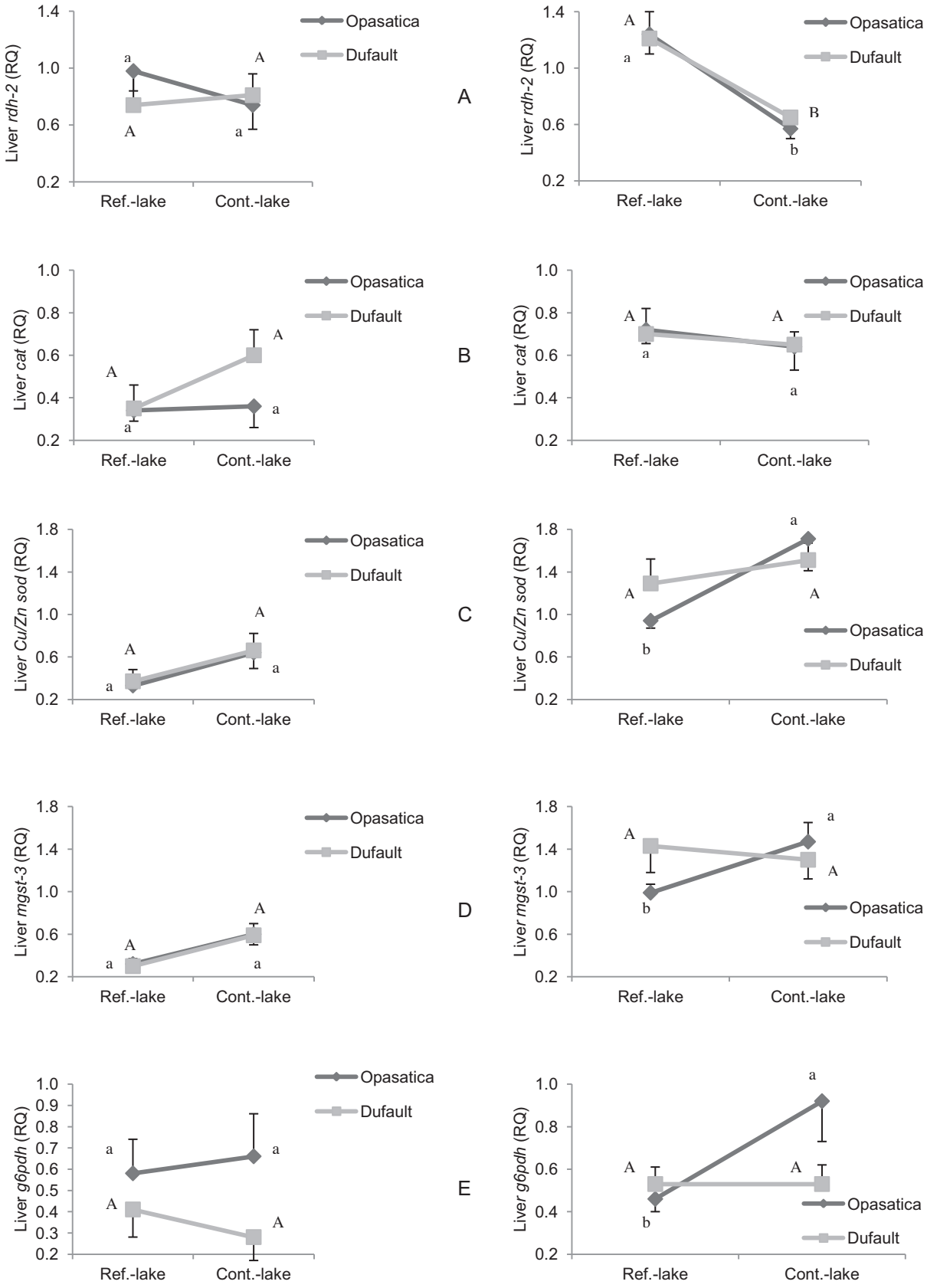

Fig. 6. Genomic reaction norm of *rdh-2* (A), *cat* (B), *Cu/Zn sod* (C), *mgst-3* (D) and *g6pdh* (E) of indigenous yellow perch from lake Opasatica and lake Dufault caged in lake Dufault (Cont.-lake) or in lake Opasatica (Ref.-lake) and sampled after 1 week (left panel) and 4 weeks (right panel) of exposure. Means designated with different letters are significantly different (p-value < 0.05). Capital letters represent comparison between fish from lake Dufault caged in contaminated and references lakes, and lowercase letters indicate comparison between fish from lake Opasatica caged in reference and contaminated lakes. Values (expressed in relative quantification) are mean and standard errors (SE).

4.2. Effect of metal contamination on liver retinoid metabolism

In the present field study, the hepatic retinoid concentrations of fish originating from lake Opasatica decreased after 4 weeks exposure to metals, suggesting that exposure to high Cd and Cu levels negatively impacted yellow perch liver retinoid metabolism. Although this result contrasts with a previous study where we reported positive correlations between kidney Cd concentrations and liver dehydroretinoids (free and esterified form) in wild fish collected in metal impacted lakes (Defo et al., 2012), it is supported by our recent laboratory study where hepatic retinoid concentrations had decreased after a 6-week exposure to waterborne Cd and Ni (Defo et al., 2014a). After 4 weeks, exposure to elevated aqueous Cd and Cu concentrations caused a down-regulation of the transcription level of liver *rhd-2,* a gene encoding for the synthesis of enzymes involved in the oxidation of retinol to retinaldehyde for retinoic acid biosynthesis (Lee et al., 2009). This result contrasts with our recent study where we reported that liver *rhd-2* was markedly up-regulated in fish chronically exposed in a cocktail of metals in the field (M.A. Defo, INRS-ETE, unpublished data). The discrepancy between these studies could be due to multiple factors such as the exposure time (4 weeks here versus chronic exposure); the nature of fish sampled (captivity against free-ranging fish) and the interactions of multiple stressors found in the natural environment (for example diet quality and availability) or individual biometric parameters such as fish sex, size and age, that probably affected retinoid metabolism (Defo et al., 2014b). We also observed that a down-regulation of this gene was coupled with a decrease in the percentage of liver free dROH. This synchronous reaction at the molecular and biochemical levels indicates that metals negatively impact retinoid metabolism at both levels of biological organization. This result is in agreement with our previous studies where we reported negative correlations between kidney Cd concentrations and the percentage of liver free dROH in fish inhabiting metal-impacted lakes (Defo et al., 2012).

However, the current investigation also demonstrated that when perch living naturally in a metal-impacted lake were caged in the same lake, their liver retinoid concentrations (free dROH, dROH-palmitate and total vitamin A esters) and the percentage of free liver dROH were all significantly higher than those of metal-naïve perch caged in the same metal-impacted lake for 4 weeks. This observation of higher retinoid parameters in the metal-contaminated fish caged in their own lake suggests a compensatory mechanism in order to counteract the direct effects of metal induced oxidative stress (Defo et al., 2012). It is likely that more than 80 years of metal pollution have led to the selection of traits that enhanced the retinoid metabolic capacity of wild perch. Our present finding is in agreement with our previous report where we demonstrated that adult perch from Cd-impacted lakes displayed significantly higher concentrations of liver dROH and dROH-esters than fish from reference lakes (Defo et al., 2012). Lake Dufault perch may have acquired some tolerance to metals through physiological acclimatization or adaptation. For example, a recent study showed that metal contamination has driven rapid adaptive evolution by favoring allelic selection of genes that could increase yellow perch fitness in metal-impacted areas (Bélanger-Deschênes et al., 2013). Also, higher nucleoside diphosphate kinase (NDPK) activity, an indicator of biosynthetic capacities, was reported in yellow perch inhabiting a metal-impacted lake in the region of Sudbury (Rajotte and Couture, 2002a). In support of these higher physiological capacities, fish from lake Dufault caged in the same lake exhibited higher liver aerobic capacities (higher cytochrome-c-oxidase activity) than did fish from lake Opasatica held captive in lake Dufault for 4 weeks (M.A. Defo, INRS-ETE, unpublished data).

4.3. Effect of metal depuration on liver transcriptional and biochemical markers analysed

In contrast to the yellow perch from lake Opasatica caged in the contaminated lake Dufault, fish from lake Dufault transplanted in Opasatica did not show changes in transcriptional and biochemical response after 1 and 4 weeks, except for liver *rdh-2* where the exposure to low aqueous Cd and Cu concentrations caused an up-regulation of the transcription level of this gene.

Uptake rates, tissue bioaccumulation levels and depuration rates of metals are important parameters that define metal toxicity. The results indicate that depuration time in this study (4 weeks) was not enough to significantly limit the production of ROS, resulting in no changes in the transcription levels and in the activities of the antioxidant enzymes, compared to the control group (4Con-Du). In support of this, no significant loss of metals from the kidney for either sampling period was observed in fish caught in the metal-impacted lake and held within the reference lake. Our results suggest that *rhd-2* transcription level was up-regulated probably in order to activate the synthesis of retinoid metabolites involved in fish growth. In support of this hypothesis, we found that fish transplanted from the metal-contaminated lake to the clean lake displayed higher condition factors and heavier pyloric cæca weights than their corresponding control fish (4Con-Du), although not significantly for the latter parameter.

4.4. Relationships between gene transcription levels and functional responses

This is the first study examining the time course of the effects of metal exposure on transcriptional and functional regulation in metal-naïve wild perch caged in a contaminated lake. We have identified sets of genes that were differently expressed and consistently associated with biochemical responses to metal (especially Cd) stress, suggesting that the transcriptome and biological function of fish can be significantly altered in response to Cd exposure in the wild. Although all genes analysed are involved in antioxidant defense mechanisms, the responses of individual genes was variable depending on the duration of metal exposure. Consistent with the hypothesis that Cd induced oxidative stress and therefore might increase the level of cellular ROS, an increase in the transcription level of most of the genes studied was observed.

Combining molecular analysis and biochemical responses, our study has demonstrated that genes involved in the response to metal-induced oxidative stress were differentially expressed in fish from lake Opasatica following 4 weeks of acclimatization to a cocktail of metals in lake Dufault, compared to their homologs caged in lake Opasatica. Moreover we observed differences in the activities of the corresponding enzymes. Specifically, after 4 weeks, yellow perch transplanted into the metal-impacted lake displayed high transcription levels of liver *g6pdh*, *mgst-3*, *Cu/Zn sod*, presumably in order to reduce the level of ROS generated in the metal-exposed fish down to levels noted in the reference fish. Furthermore, the up-regulation of some of the studied genes resulted in an increase in enzyme activities (*e.g.* GST and G6PDH), indicating a synchronous reaction at the molecular and biochemical levels to counteract the oxidative stress engendered by metal exposure. Typically, translational responses are expected to follow the transcriptional responses (Nikinmaa and Rytkönen, 2011). Our results suggest that in metal-contaminated environments regulation of the relevant metabolic pathways might be achieved at both levels of biological organization. Our results are in agreement with our previous laboratory study where we reported consistent relationships between liver *mgst-3* and *g6pdh* transcription levels (up-regulation) and corresponding enzyme activities (increasing) in yellow perch exposed to Cd (Defo et al., 2014a).

Although we reported a high transcription level of liver *Cu/Zn sod* in fish originating from lake Opasatica and transplanted into lake Dufault, there was no measurable effect of metals on SOD activity, suggesting that regulation of liver transcription is only sufficient to maintain SOD activity. It is not surprising that gene transcription levels of some antioxidants increase whereas no change is reported at the functional level (Giuliani et al., 2013). As mentioned above, there is a delay between transcription and translation in eukaryotic cells and multiple regulation steps are involved in transcription and translation (Regoli et al., 2011).

Unlike the responses of liver GST and G6PDH activities, liver CAT activity had decreased with metal exposure after 4 weeks; however, no change in *cat* transcription level was observed. These results suggest that, for this parameter, the response at the catalytic level is more sensitive to metal-induced oxidative stress than is the transcriptional response when wild perch are exposed to Cd and Cu in the field for a relatively long time. A decrease in CAT activity is often compensated for by an increase in other antioxidant enzyme activities, as an alternative mechanism for ROS removal (Regoli et al., 2011); antioxidant defence enzymes act co-operatively or synergistically in order to afford an optimal protection against oxidative stress (Bagnyukova et al., 2006). The present results contrast with our previous laboratory observations of a good agreement between liver *cat* transcription level (up-regulation) and CAT activity (increase) when perch were exposed to Cd for 6 weeks (Defo et al., 2014a). The difference between the two studies could be due to the multiple stress factors that operate in the natural environment. Indeed some oxidative stress biomarkers may be more sensitive to metal pollution due to synergistic or combined effects of factors, such as higher temperature, that may exert their influence in natural environment (Lapointe et al., 2011).

4.5. Time scale of changes in metabolic responses and metal genomic reaction norm

This field experiment has added a temporal dimension to our previous study in which we reported the impact of increasing metal concentrations on retinoid metabolism in wild perch (Defo et al., 2012). Although multiple stressors co-exist in natural environments, we hypothesized that the results of our present field manipulation experiment would probably be comparable to those of our recent laboratory study, in which we exposed perch to an environmentally relevant concentration of Cd for six weeks. Indeed, as in our previous laboratory experiment, we found that liver transcription levels of some antioxidant defence genes (*mgst-3*, *g6pdh*) were enhanced and that this up-regulation was accompanied by an increase in the activities of the corresponding enzymes (Defo et al., 2014a).

In addition to the intensity of contamination, it is important to determine the time point at which adverse effects of pollutants may occur in order to better understand the impacts of metal contamination in natural populations. In this reciprocal-transplantation experiment, plastic responses were generally observed after 4 weeks of exposure for all of the genes analysed, except for *cat* for which the response might take more than 4 weeks. However, the responses of the transcription levels of liver *mgst-3* and *Cu/Zn sod* began after 1 week. This result suggests that the number of genes involved in oxidative stress responses may vary over time (Denslow et al., 2007). Indeed, 1 week of exposure to Cd and Cu may be insufficient to induce changes in the activities of antioxidant enzymes analysed.

Studies have reported specific genomic reaction norms in response to changes of environmental conditions such as temperature in brown trout (Meier et al., 2014) or salinity in eels (Côté et al., 2014). To our knowledge, our's is the first study reporting a genomic reaction norm to metals using reciprocal transplant experiments directly in the natural environment. Although a loss of plasticity was observed in the contaminated population, our results support an important role for phenotypic plasticity in explaining yellow perch adaptation to metal-induced oxidative stress when clean fish from lake Opasatica were transferred into lake Dufault. Analysis of a metal contamination effect showed a clear plastic effect for almost all genes analysed at the end of the experiment. Similar patterns of plasticity (progressive) were observed in the expression of *Cu/Zn sod*, *mgst-3* and *g6pdh*. This degree of plasticity could be linked to the extent to which fish are combatting oxidative stress. However, *rdh-2* expression showed different patterns of plasticity, suggesting that yellow perch used different strategies to cope with metal-induced oxidative stress. Finally, *cat* expression did not show any variation between the two groups of caged fish, indicating that *cat* is the gene with the least plastic expression among those studied. The different genomic reactions observed between lake Opasatica yellow perch transplanted into lake Dufault and those caged in their own lake of origin, after 4 weeks, possibly reflect an adaptive plastic response to the change in the environment. This is likely the case since they were causally linked with plastic responses observed at the biochemical level, except for the catalase pathway. Since all of the genes analysed are involved in the cellular response to oxidative stress, our study supports the idea that the genomic response of naïve perch to metal exposure is strongly oriented towards combatting oxidative stress.

In contrast to the yellow perch from lake Opasatica caged in the contaminated lake Dufault, fish from lake Dufault transplanted in Opasatica did not show a pronounced plastic response after 4 weeks. This difference in genomic reaction norms for at least three genes supports the hypothesis of genetic differences between the two populations. Also, the two previous studies indicated that increased metal exposure led to an important reduction in diversity (Bélanger-Deschênes et al., 2013; Bourret et al., 2008). Such a loss of diversity could perhaps impact on the potential for maintaining a plastic response. Overall then, our results support the hypothesis that more than 80 years of metal exposure may have driven rapid evolutionary change in the lake Dufault population genome (Bélanger-Deschênes et al., 2013; Bourret et al., 2008). To generalize this hypothesis would require the analysis of more populations.

Other factors, such as epigenetic effects (induction of phenotypic variation without DNA sequence alteration) (Pierron et al., 2014; Richards, 2006), may also explain the observed differences in gene expression between the two fish groups (Pierron et al., 2014). Indeed, although the yellow perch populations used in this study were similar in body weight and length and originated from the same region with similar natural macro-environments, epigenetic mechanisms could exert a crucial influence on phenotypic plasticity and on the interactions between the fish and their environment, thereby modifying gene expression and contributing to environmentally-induced phenotypic changes (Angers et al., 2010).

5. Conclusion

This study indicates that a 4-week exposure period in a contaminated environment is sufficient for metal-naïve juvenile perch to significantly increase their kidney Cd and Cu concentrations. However, more time is necessary to depurate metals that have accumulated over the course of their lives. Our results clearly show that a four-week exposure of fish from the clean lake to metals negatively affected indicators of retinoid metabolism and increased oxidative stress related parameters, at both the molecular and biochemical levels. Also, this study reveals that perch from clean populations may partly adjust to contamination by plastic responses and that such responses occur within the first 4 weeks of exposure; the contaminated population tended to express a

higher, albeit non-plastic response, possibly as a consequence of more than 80 years of directional selective pressure associated with exposure to contaminants. Globally, our results indicate that in metal-impacted lakes, yellow perch manage to ensure their continued presence by increasing their capacity to synthesize retinoid metabolites. The overall findings from this field investigation highlight how yellow perch molecularly and biochemically deal with sudden or long-term exposures to a cocktail of metals. This study will contribute to a better understanding of how perch manage to live and adapt in natural environments impacted by metals.

Acknowledgments

This study is part of a Collaborative Research and Development project (P. Couture, Principal Investigator) funded by the Natural Sciences and Engineering Research Council of Canada (NSERC, Ottawa, ON, Canada; grant number RDCPJ 379611) and by Vale Limited (Rio de Janeiro, Brazil). The Canada Research Chair program supports P.G.C. Campbell and L. Bernatchez. The assistance of Bérénice Bougas, Julie Grasset, Mariem Fadhlaoui, Antoine Caron and Ophélie Drevet was invaluable throughout the field experimentation. We wish to acknowledge the contribution of Guillaume Côté during the optimization of RT-PCR parameters. The authors are also grateful to the INRS-ETE support staff without whom this study could not have been completed, in particular Stéfane Prémont, Stéphane Moise and Anissa Bensadoune for their technical assistance in retinoid and metal analyses. We are also grateful to the anonymous referees who constructively commented on a previous version of this manuscript.

References

Abreu, R.D., Penalva, L.O., Marcotte, E.M., Vogel, C., 2009. Global signatures of protein and mRNA expression levels. Mol. BioSyst., 5.

Angers, B., Castonguay, E., Massicotte, R., 2010. Environmentally induced phenotypes and DNA methylation: how to deal with unpredictable conditions until the next generation and after. Mol. Ecol. 19, 1283–1295.

Aubin-Horth, N., Renn, S.C.P., 2009. Genomic reaction norms: using integrative biology to understand molecular mechanisms of phenotypic plasticity. Mol. Ecol. 18, 3763–3780.

Bagnyukova, T.V., Chahrak, O.I., Lushchak, V.I., 2006. Coordinated response of goldfish antioxidant defenses to environmental stressors. Aquat. Toxicol. 78, 325–331.

Bélanger-Deschênes, S., Couture, P., Campbell, P.G.C., Bernatchez, L., 2013. Evolutionary change driven by metal exposure as revealed by coding SNP genome scan in wild yellow perch (*Perca flavescens*). Ecotoxicology 22, 938–957.

Bergot, P., Blanc, J.M., Escaffre, A.M., 1981. Relationship between number of pyloric caeca and growth in rainbow trout (*Salmo gairdneri* Richardson). Aquaculture 22, 81–96.

Bonham-Carter, G.F., Henderson, P.J., Kliza, D.A., Kettles, I.M., 2006. Comparison of metal distributions in snow, peat, lakes and humus around a Cu smelter in western Quebec, Canada. Geochem. Explor. Environ. Anal. 6, 215–228.

Bourret, V., Couture, P., Campbell, P.G.C., Bernatchez, L., 2008. Evolutionary ecotoxicology of wild yellow perch (*Perca flavescens*) populations chronically exposed to a polymetallic gradient. Aquat. Toxicol. 86, 76–90.

Chen, Q., Zhang, R., Li, W.M., Niu, Y.J., Guo, H.C., Liu, X.H., Hou, Y.C., Zhao, L.J., 2013. The protective effect of grape seed procyanidin extract against cadmium-induced renal oxidative damage in mice. Environ. Toxicol. Pharmacol. 36, 759–768.

Côté, C.L., Castonguay, M., Kalujnaia, M.S., Cramb, G., Bernatchez, L., 2014. In absence of local adaptation, plasticity and spatially varying selection rule: a view from genomic reaction norms in a panmictic species (*Anguilla rostrata*). BMC Genom. 15.

Couture, P., Busby, P., Gauthier, C., Rajotte, J.W., Pyle, G.G., 2008a. Seasonal and regional variations of metal contamination and condition indicators in yellow perch (*Perca flavescens*) along two polymetallic gradients I. Factors influencing tissue metal concentrations. Hum. Ecol. Risk Assess. 14, 97–125.

Couture, P., Kumar, P.R., 2003. Impairment of metabolic capacities in copper and cadmium contaminated wild yellow perch (*Perca flavescens*). Aquat. Toxicol. 64, 107–120.

Couture, P., Rajotte, J.W., Pyle, G.G., 2008b. Seasonal and regional variations in metal contamination and condition indicators in yellow perch (*Perca flavescens*) along two polymetallic gradients III. Energetic and physiological indicators. Hum. Ecol. Risk Assess. 14, 146–165.

Defo, M.A., Bernatchez, L., Campbell, P.G.C., Couture, P., 2014a. Waterborne cadmium and nickel impact oxidative stress responses and retinoid metabolism in yellow perch. Aquat. Toxicol. 154, 207–220.

Defo, M.A., Pierron, F., Spear, P.A., Bernatchez, L., Campbell, P.G.C., Couture, P., 2012. Evidence for metabolic imbalance of vitamin A2 in wild fish chronically exposed to metals. Ecotoxicol. Environ. Saf. 85, 88–95.

Defo, M.A., Spear, P.A., Couture, P., 2014b. Consequences of metal exposure on retinoid metabolism in vertebrates: a review. Toxicol. Lett. 225, 1–11.

Denslow, N.D., Garcia-Reyero, N., Barber, D.S., 2007. Fish 'n' chips: the use of microarrays for aquatic toxicology. Mol. BioSyst. 3, 172–177.

Derome, N., Bernatchez, L., 2006. The transcriptomics of ecological convergence between 2 limnetic coregonine fishes (Salmonidae). Mol. Biol. Evol. 23, 2370–2378.

Eisler, R., 2004. Mercury hazards from gold mining to humans, plants and animals. Rev. Environ. Contam. Toxicol. 181, 139–198.

Furlong, E., 2011. Molecular biology: a fly in the face of genomics. Nature 471, 458–459.

Gauthier, C., Campbell, P.G.C., Couture, P., 2008. Physiological correlates of growth and condition in the yellow perch (*Perca flavescens*). Comp. Biochem. Physiol. 151A, 526–532.

Gauthier, C., Campbell, P.G.C., Couture, P., 2009. Condition and pyloric caeca as indicators of food web effects in fish living in metal-contaminated lakes. Ecotoxicol. Environ. Saf. 72, 2066–2074.

Gauthier, C., Campbell, P.G.C., Couture, P., 2011. Enzymatic correlates of energy status in wild yellow perch inhabiting clean and contaminated environments. Environ. Toxicol. Chem. 30, 2148–2156.

Giguère, A., Campbell, P.G.C., Hare, L., Cossu-Leguille, C., 2005. Metal bioaccumulation and oxidative stress in yellow perch (*Perca flavescens*) collected from eight lakes along a metal contamination gradient (Cd, Cu, Zn, Ni). Can. J. Fish Aquat. Sci. 62, 563–577.

Giguère, A., Campbell, P.G.C., Hare, L., McDonald, D.G., Rasmussen, J.B., 2004. Influence of lake chemistry and fish age on cadmium, copper, and zinc concentrations in various organs of indigenous yellow perch (*Perca flavescens*). Can. J. Fish Aquat. Sci. 61, 1702–1716.

Giuliani, M.E., Benedetti, M., Arukwe, A., Regoli, F., 2013. Transcriptional and catalytic responses of antioxidant and biotransformation pathways in mussels *Mytilus galloprovincialis* exposed to chemical mixtures. Aquat. Toxicol. 134–135, 120–127.

Gliszczyńska-Świglo, A., 2006. Antioxidant activity of water soluble vitamins in the TEAC (trolox equivalent antioxidant capacity) and the FRAP (ferric reducing antioxidant power) assays. Food Chem. 96, 131–136.

Gonzalez, A., Ronce, O., Ferriere, R., Hochberg, M.E., 2013. Evolutionary rescue: an emerging focus at the intersection between ecology and evolution. Philos. Trans. R. Soc. London Ser. B Biol. Sci. 368, 1610, 20120404.

Hansen, M.M., Olivieri, I., Waller, D.M., Nielsen, E.E., 2012. The gem working group, monitoring adaptive genetic responses to environmental change. Mol. Ecol. 21, 1311–1329.

Hoffmann, A.A., Daborn, P.J., 2007. Towards genetic markers in animal populations as biomonitors for human-induced environmental change. Ecol. Lett. 10, 63–76.

Hontela, A., Dumont, P., Duclos, D., Fortin, R., 1995. Endocrine and metabolic dysfunction in yellow perch, *Perca flavescens*, exposed to organic contaminants and heavy metals in the St. Lawrence river. Environ. Toxicol. Chem. 14, 725–731.

Hontela, A., Rasmussen, J.B., Audet, C., Chevalier, G., 1992. Impaired cortisol stress response in fish from environments polluted by PAHs, PCBs, and mercury. Arch. Environ. Contam. Toxicol. 22, 278–283.

Kraemer, L.D., Campbell, P.G.C., Hare, L., 2005a. Dynamics of Cd, Cu and Zn accumulation in organs and sub-cellular fractions in field transplanted juvenile yellow perch (*Perca flavescens*). Environ. Pollut. 138, 324–337.

Kraemer, L.D., Campbell, P.G.C., Hare, L., 2005b. A field study examining metal elimination kinetics in juvenile yellow perch (*Perca flavescens*). Aquat. Toxicol. 75, 108–126.

Lapointe, D., Pierron, F., Couture, P., 2011. Individual and combined effects of heat stress and aqueous or dietary copper exposure in fathead minnows (*Pimephales promelas*). Aquat. Toxicol. 104 (1–2), 80–85.

Larsen, P.F., Nielsen, E.E., Williams, T.D., Hemmer-Hansen, J., Chipman, J.K., Kruhøffer, M., Grønkjær, P., George, S.G., Dyrskjøt, L., Loeschcke, V., 2007. Adaptive differences in gene expression in European flounder (*Platichthys flesus*). Mol. Ecol. 16, 4674–4683.

Lee, S.A., Belyaeva, O.V., Kedishvili, N.Y., 2009. Biochemical characterization of human epidermal retinol dehydrogenase 2. Chem. Biol. Interact. 178, 182–187.

Leonard, S.S., Harris, G.K., Shi, X., 2004. Metal-induced oxidative stress and signal transduction. Free Radical Biol. Med. 37, 1921–1942.

Luoma, S.N., Rainbow, P.S., 2005. Why is metal bioaccumulation so variable? Biodynamics as a unifying concept. Environ. Sci. Technol. 39, 1921–1931.

Lushchak, V.I., 2011. Environmentally induced oxidative stress in aquatic animals. Aquat. Toxicol. 101, 13–30.

Levesque, H.M., Moon, T.W., Campbell, P.G.C., Hontela, A., 2002. Seasonal variation in carbohydrate and lipid metabolism of yellow perch (Perca flavescens) chronically exposed to metals in the field. Aquat. Toxicol. 60, 257–267.

Meier, K., Hansen, M.M., Normandeau, E., Mensberg, K.L.D., Frydenberg, J., Larsen, P.F., Bekkevold, D., Bernatchez, L., 2014. Local adaptation at the transcriptome level in brown trout: evidence from early life history temperature genomic reaction norms. PLoS One 9 (1), e85171.

Merilä, J., Hendry, A.P., 2014. Climate change, adaptation, and phenotypic plasticity: the problem and the evidence. Evol. Appl. 7, 1–14.
Nikinmaa, M., McCairns, R.J., Nikinmaa, M.W., Vuori, K.A., Kanerva, M., Leinonen, T., Primmer, C.R., Merilä, J., Leder, E.H., 2013. Transcription and redox enzyme activities: comparison of equilibrium and disequilibrium levels in the three-spined stickleback. Proc. Biol. Sci. R. Soc. 280, 2012–2974.
Nikinmaa, M., Rytkönen, K.T., 2011. Functional genomics in aquatic toxicology-do not forget the function. Aquat. Toxicol. 105, 16–24.
Pavey, S.A., Sutherland, B.J., Leong, J., Robb, A., Schalburg, K., Hamon, T.R., Koop, B.F., Nielsen, J.L., 2011. Ecological transcriptomics of lake-type and riverine sockeye salmon (*Oncorhynchus nerka*). BMC Ecol. 2011, 11–18.
Peltier, M.R., Wilcox, C.J., Sharp, D.C., 1998. Technical note: application of the box-cox data transformation to animal science experiments. J. Anim. Sci. 76, 847–849.
Pierron, F., Bourret, V., St-Cyr, J., Campbell, P.C.G., Bernatchez, L., Couture, P., 2009. Transcriptional responses to environmental metal exposure in wild yellow perch (Perca flavescens) collected in lakes with differing environmental metal concentrations (Cd, Cu, Ni). Ecotoxicology 18, 620–631.
Pierron, F., Baillon, L., Sow, M., Gotreau, S., Gonzalez, P., 2014. Effect of low-dose cadmium exposure on DNA methylation in the endangered European eel. Environ. Sci. Technol. 48, 797–803.
Rajotte, J.W., Couture, P., 2002a. Effects of environmental metal contamination on the condition, swimming performance, and tissue metabolic capacities of wild yellow perch (*Perca flavescens*). Can. J. Fish. Aquat. Sci. 59, 1296–1304.
Rajotte, J.W., Couture, P., 2002b. Effects of environmental metal contamination on the condition, swimming performance, and tissue metabolic capacities of wild yellow perch (*Perca flavescens*). Can. J. Fish. Aquat. Sci. 59, 1296–1304.
Rajotte, J.W., Couture, P., 2003. Morphometric and metabolic indicators of metal stress in wild yellow perch (*Perca flavescens*) from Sudbury, Ontario: a review. J. Environ. Monit. 5, 216–221.
Regoli, F., Giuliani, M.E., Benedetti, M., Arukwe, A., 2011. Molecular and biochemical biomarkers in environmental monitoring: a comparison of biotransformation and antioxidant defense systems in multiple tissues. Aquat. Toxicol. 105, 56–66.
Richards, E.J., 2006. Inherited epigenetic variation – revisiting soft inheritance. Nat. Rev. Genet. 7, 395–401.
Scandalios, J.G., 2005. Oxidative stress: molecular perception and transduction of signals triggering antioxidant gene defenses. Braz. J. Med. Biol. Res. 38, 995–1014.
St-Cyr, J., Derome, N., Bernatchez, L., 2008. The transcriptomics of life-history trade-offs in whitefish species pairs (*Coregonus sp.*). Mol. Ecol. 17, 1850–1870.
Uren Webster, T.M., Bury, N., van Aerle, R., Santos, E.M., 2013. Global transcriptome profiling reveals molecular mechanisms of metal tolerance in a chronically exposed wild population of brown trout. Environ. Sci. Technol. 43, 8869–8877.