Temporal variations in kidney metal concentrations and their implications for retinoid metabolism and oxidative stress response in wild yellow perch (*Perca flavescens*)

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ARTICLE INFO

ABSTRACT

The objective of this study was to determine if temporal variations in tissue metal concentrations are related to biomarkers of retinoid metabolism and oxidative stress responses in juvenile yellow perch (*Perca flavescens*). To this end, kidney metal (Cd, Cu and Zn) concentrations were measured in fish sampled in spring and fall 2012 in four lakes representing a wide range of water and sediment metal contamination in the Rouyn-Noranda (Quebec) region. Lakes Opasatica and Hélène were considered as reference lakes while lakes Dufault and Marlon were metal-contaminated. Kidney concentrations of Cd, Cu and Zn varied widely between spring and fall in fish from both clean and metal-contaminated lakes. An inter-lake difference in renal metal concentrations was only observed for Cd, with fish from Lake Marlon consistently displaying higher concentrations. In the spring, the concentrations of liver dehydroretinol, dehydroretinyl palmitate and total vitamin A esters were higher in fish sampled in the most contaminated lake. Strong temporal variations in the concentrations of these metabolites, as well as in the percentage of liver free dehydroretinol and the epidermal retinol dehydrogenase 2 transcription levels, were observed in fish living in the most metal-impacted lake, with generally higher values in the spring. In contrast to liver, in muscle, no clear seasonal variations in the concentrations of dehydroretinol, dehydroretinyl stearate or in the percentage of free dehydroretinol were observed in fish captured in the most contaminated lake. Temporal variations of traditional biomarkers of oxidative stress response were also observed in the most metal-impacted lake. For example, the transcription level of the gene encoding Cu/Zn superoxide dismutase-1 in liver and muscle catalase activity of perch sampled in the most contaminated lake were higher in spring than in fall. Positive relationships were found between kidney Cd concentrations and the transcription level of the gene encoding glucose 6-phosphate dehydrogenase, and all forms of retinoid concentrations in liver in spring, except with the percentage of free dehydroretinol where the correlation was negative. Our results translate to a state of stress caused by Cd and illustrate that temporal variations in tissue metal concentrations affect retinoid metabolism and antioxidant capacities in juvenile wild yellow perch. Overall this study contributes to highlight the importance of considering temporal variations when investigating the consequences of metal contamination on the physiology of wild fish.

1. Introduction

Studies of metal effects on fish are typically conducted under controlled laboratory conditions using waterborne exposure to a single metal, ignoring the possible influences of seasonal variations of physico-chemical parameters (temperature, dissolved oxygen), food availability, fish biological cycle (sexual maturation) and tissue metal concentrations. In yellow perch, seasonal variations of tissue metal concentrations are well known (Couture et al., 2008a; Levesque et al., 2002) and have been correlated to changes in condition (Audet and Couture, 2003; Eastwood and Couture, 2002; Pyle et al., 2008), energy stores (Levesque et al., 2002) and metabolic biosynthetic capacities (Audet and Couture, 2003; Couture et al., 2008b).

Although there is a permanently low concentration of reactive oxygen species (ROS) in aerobic organisms (Lushchak, 2011), cellular concentrations of ROS can quickly increase as a result of adverse changes in the environmental or metabolic conditions of organisms (Scandalios, 2005). An overproduction of ROS can lead to lipid

peroxidation, protein or nucleic acid denaturation and other negative cellular effects (Chen et al., 2013; Leonard et al., 2004; Regoli, 2000). Metals can generate or increase the production of free radicals in the cell through various mechanisms (Defo et al., 2014b; Lushchak, 2011). An excessive level of cellular free radicals can then stimulate the expression of genes involved in the defence against oxidative stress and modulate the activity of enzymes involved in this metabolic pathway (Webster et al., 2013). Enzymatic antioxidants such as superoxide dismutase (SOD), catalase (CAT), glutathione-S-transferase (GST) and glucose-6-phosphate dehydrogenase (G6PDH) can interact to diminish the damaging effects of ROS in fish (Scandalios, 2005). SOD and CAT belong to the vital first line of the cellular defence system against oxidative stress (Yonar, 2013), since the first enzyme is involved in the dismutation of superoxide (O_2^-) into O_2 and H_2O_2, which in turn is converted to H_2O and O_2 by the second enzyme (Yu, 1994).

In a previous laboratory study where yellow perch were separately exposed to aqueous Cd or Ni for 6 weeks, we observed an increase in liver CAT activity for both metal exposures, but no measurable effect on liver SOD activity was reported (Defo et al., 2014a). In contrast to the responses in liver, muscle CAT activity generally decreased or remained unchanged with metal exposure. In that study, no effects of metal exposure on liver microsomal glutathione-S-transferase (mGST) were reported. The first enzyme of the pentose phosphate pathway, G6PDH, catalyses the conversion of glucose-6-phosphate to 6-phosphogluconate, resulting in NADPH production (Ho et al., 2007), required for reduced glutathione metabolism, which in turn plays a role in the protection against oxidative damage (Efferth et al., 2006). In our previous study we reported that liver transcription levels of *g6pdh* were generally enhanced in Cd-exposed fish and this up-regulation was accompanied by an increase in the activity of the corresponding enzyme (Defo et al., 2014a). In another laboratory study, where yellow perch were exposed to a combination of heat and metal stress for seven weeks, the normal responses of antioxidant capacities (SOD and G6PDH activities) were affected in Cd- and Ni-exposed fish compared to control fish (Grasset et al., 2016).

In a field study carried out in the spring, we observed that the liver transcription level of microsomal *gst-3* decreased in metal-contaminated yellow perch compared to control fish, although no change in the activity of the corresponding enzyme was found. The activity of liver CAT increased in those fish, but this variation was not reflected by the *cat* gene transcription level (Defo et al., 2015). Bougas et al. (2016) reported that genes encoding for antioxidant-related proteins, namely *Cu/Zn sod, gst-mu3* and *mgst*, were overexpressed in liver of uncontaminated yellow perch chronically transplanted into a metal-contaminated lake for 4 weeks in the spring (Bougas et al., 2016). A decrease in *sod-1* gene transcription level was associated with an increase in hepatic Cd in a study investigating the mechanisms involved in metal stress in yellow perch collected along a metal contamination gradient in the Rouyn-Noranda (QC) and Sudbury (ON) mining regions (Pierron et al., 2009). In another field study investigating metal effects on wild yellow perch living in two Canadian mining regions, Giguère et al. (2005) showed that metal-contaminated fish expressed a reduced capacity for antioxidant defence, including lower values of liver glutathione reductase activity and glutathione concentrations, compared to their homologs living in clean lakes. The decrease in these indicators of antioxidant capacities was linked to an increase in metallothionein concentrations, which would have played a role in the protection against metal-induced oxidative stress (Giguère et al., 2005).

The only study examining links between seasonal variations of yellow perch tissue metal concentrations and oxidative stress response parameters is that of Levesque et al. (2002). In their study, liver G6PDH activity was high in yellow perch living in the most metal-contaminated lake in the fall, but this activity was low when fish were sampled in summer of the following year (Levesque et al., 2002). However, because of some limitations in their study design, such as a difference in sampling years, it is not possible to use their results to evaluate the

possible seasonal variation in G6PDH activity in fish living in metal-impacted lakes. Note that in a later study, carried out in the spring of 2012, liver transcription levels of *g6pdh* were enhanced in wild yellow perch collected from a clean lake and then caged in a metal-contaminated lake, and this up-regulation was accompanied by an increase in the activity of the corresponding enzyme (Defo et al., 2015).

The structure of retinoids, also called vitamin A, is close to that of retinol. Retinoids are essential for diverse physiological functions such as reproduction, vision and growth. Furthermore, several retinoids possess antioxidant properties (Alpsoy et al., 2009). Although we studied the effects of chronic metal exposure on retinoid metabolism in wild perch (Defo et al., 2012), the seasonal variations of fish retinoid metabolism and their relationships with tissue metal concentrations are to our knowledge unknown.

To address this question, yellow perch were sampled in four lakes varying in metal contamination, in the spring and the fall, two contrasting seasons for this early spring spawner. Spring sampling was carried out after reproduction, whereas fall sampling was performed after the summer growth season. Tissue metal and retinoid concentrations as well as the transcription levels and enzymatic activities of biomarkers of antioxidant capacities were measured. The main objective of this work was to determine if the transcription levels of a set of genes involved in retinoid metabolism and oxidative stress responses, as well as the corresponding biochemical parameters, followed seasonal changes in tissue metal concentrations.

2. Materials and methods

2.1. Study area and fish sampling

The investigation area for this study is situated in the Rouyn-Noranda region, in north-western Quebec, Canada. Mining and smelting activities carried out in this region since 1927 have led to extensive environmental contamination by metals, particularly Cd, Cu and Zn (Campbell et al., 2008; Perceval et al., 2002) and adverse effects on aquatic living organisms (Couture et al., 2008b). Fish sampling was conducted in spring (late May) and in fall (early September) 2012. Four lakes were selected based on known water and sediment metal concentrations. Lakes Opasatica and Hélène were considered as reference lakes while lakes Dufault and Marlon, situated close to the Horne smelter, were considered metal-contaminated (Fig. 1).

For each lake and season, a seine net was used to collect 20 juvenile yellow perch (3.5 ± 0.2 g; 7.0 ± 0.1 cm; mean of the sample pool $\pm$ SE). Fish of similar size were selected in order to diminish the variability of the studied parameters as related to age. Fish were dissected according to the method described elsewhere (Defo et al., 2012, 2014a,b). Briefly, after recording lengths and weights, the liver was dissected, weighed and kept for molecular, biochemical and biometric analyses, whereas the kidney was sampled for metal analyses and the pyloric cæca weighed for biometric analyses. Since fish were juvenile and immature, sex could not be determined. 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, corrected pyloric cæca weights and hepatosomatic index

Fulton condition factor (K) is the index commonly used when assessing the general body condition of fish. This metric is associated with recent feeding activity and with somatic energy reserve accumulation (Gauthier et al., 2009). This index is based on the assumption of isometric growth, i.e., fish size increases according to a cubic relationship.
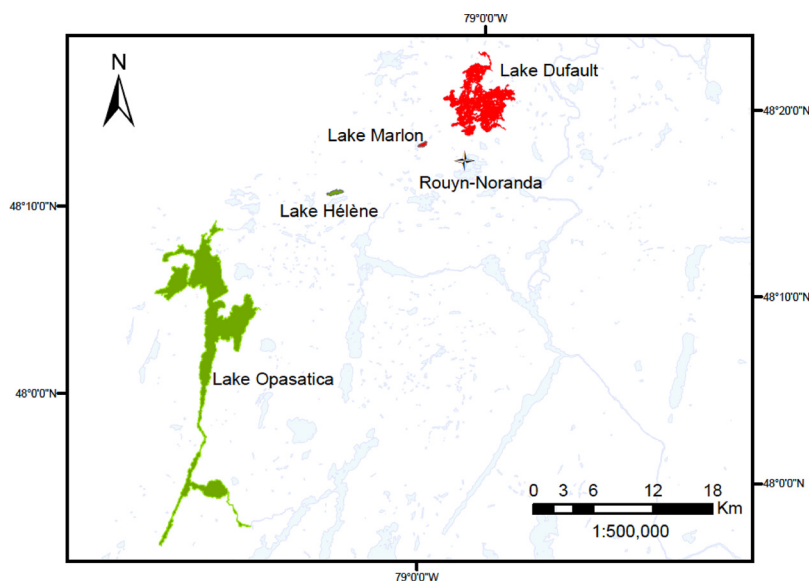
However, in most species, growth is allometric. The relative index (Kn) removes biases related to size and allows the comparison of fish from the same population or from different populations, irrespective of size (Baigún et al., 2009). The relative condition factor Kn was calculated as follows: $Kn = (W_f/L_f^b) \times 100$, where "$W_f$" is fish weight, "$L_f$" is fish length and "b" is the slope of the logarithmic relation between mass and length of all perch sampled (Gauthier et al., 2009), which corresponded to 3.16 in the present study. The corrected pyloric cæca weights were calculated according to the method described by Gauthier et al. (2011), using 2.6 g, the median value of the dataset, as the standard fish weight, and 0.92 as the allometric exponent.

2.3. Analysis of kidney metal concentrations

In this study, metal concentrations were measured in kidney since livers were too small and had to be prioritized for other endpoints (biochemical and molecular analysis). Kidneys were freeze-dried before digestion in trace metal grade nitric (67–70%). Kidney Cd, Cu and Zn were analyzed 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: $94.8 \pm 0.4\%$ for Cd, $85.3 \pm 1.3\%$ for Cu and $89.0 \pm 0.9\%$ for Zn (mean $\pm$ S.E.).

2.4. Analyses of gene transcription levels, retinoid and protein concentrations and enzyme activities

The partial coding sequences (primers) used in this study are listed in Table 1. RNA isolation, cDNA synthesis and analysis of liver and muscle gene transcription levels were performed in accordance with methods and technical standardization procedures described in Defo et al. (2014a). All biochemical and molecular parameters, including tissue retinoid concentrations (dehydroretinol, dehydroretinyl stearate, dehydroretinyl palmitate and other esters),enzyme activities (CAT, SOD, G6PDH and GST) and gene transcription levels (gene encoding photoreceptor associated retinol dehydrogenase 2, (*rdh-2*), *cat, Cu/Zn sod-1, g6pdh* and *mgst-3*) analysed in this study were conducted using the protocol described in Defo et al. (2014a), without modification. Due to the low quality of liver RNA, Hélène Lake samples from the spring sampling period were not considered for enzymatic and gene expression analyses.

2.5. Data analysis

Statistical analyses were performed using JMP 9.0 software (SAS Institute) and results were expressed as means $\pm$ standard error (SE). To test for differences in biometric parameters, kidney total metal concentrations, tissue enzyme activities and retinoid concentrations, tissue gene transcription level, among lakes and seasons, we used two-way analysis of variance (ANOVA), after checking the assumption of normality (Levene test, $p > 0.05$). When the probability of between-lake differences was significant ($p < 0.05$), multiple comparison tests (Tukey HSD tests) were used to identify groups that differed significantly from one another. When the assumption of normality was not met, and when $\log_{10}$ or Box-Cox (Peltier et al., 1998) transformations of the data were unable to normalize the distribution, non-parametric Wilcoxon tests were applied. The relationships between individual kidney metal concentrations and tissue gene transcription levels, biochemical and biometric parameters were investigated using the non-parametric Spearman rank correlation test.

3. Results

3.1. Metal concentrations in yellow perch kidney

Whether in the spring or fall, kidney Cd concentrations clearly reflected the inter-lake contamination gradient (Fig. 2). A factor of 60 was observed between the kidney Cd concentrations of fish from the cleanest lake and fish from the most contaminated lake in spring, while in fall this factor was 32. In both seasons, tissue Cd concentrations were lower in fish from uncontaminated lakes than in those from metal-contaminated lakes (Fig. 2). Except in Hélène Lake, a seasonal effect was observed in yellow perch kidney Cd concentrations, but seasonal variations varied among lakes, with higher values in the spring in Lake Dufault fish, but higher values in the fall in Opasatica and Marlon fish (Fig. 2).

Kidney Cu concentrations were lower in fish from Opasatica compared to other lakes in the spring, but in the fall, values decreased in fish from all lakes except for Opasatica and became similar among study sites (Fig. 2). Unlike for Cd and Cu, no differences in kidney Zn concentrations were observed among lakes in fish caught in the spring, whereas in the fall, fish living in metal-contaminated lakes showed lower kidney Zn concentrations compared to those inhabiting cleaner lakes (Fig. 2). Except in Marlon fish, seasonal variations in kidney Zn concentrations were observed, with higher values in the fall for cleaner

Table 1
Specific partial coding sequence (primer) pairs and PCR products used in RT-qPCR analysis.

Gene abbreviations	Name	Functions	Specific primers	PCR products
β-actin	Reference gene		F: 5'- GCCTCTCTGTCCACCTTCCA-3' R: 5'- GGGCCGGACTCATCGTACT-3'	From Pierron et al. (2009)
rdh-2	Photoreceptor associated retinol dehydrogenase 2	Retinoid and oxidative stress response	F: 5'-AGTCAAGCAGTGCATCAACAAT-3' R: 5'- CATGCGAACAACACCAAAGAAG-3'	151
cat	Catalase	Oxidative stress response	F: 5'- GTCTTTCTTGTTCAGCGATCGA-3' R: 5'- GTAGAAACGTTCACCATCAGCA-3'	106
Cu/Zn-sod	Cu/Zn superoxide dismutase	Oxidative stress response	F: 5'-TGAGCAGGAGGAGGGTTCATCCCC-3' R: 5'-CCTGCACTGATGCACCCGTTTGT-3'	123
mgst-3	Microsomal glutathione S transferase-3	Oxidative stress response	F: 5'- CCTTCCTCTACAGCTGGATCAT-3' R: 5'- TGAATACCTGCTCCTTGTCACT-3'	114
g6pdh	Glucose 6 phosphate dehydrogenase	Pentose phosphate pathway and oxidative stress response	F: 5'- ACGAGAGGCTGATATTGGATGT-3' R: 5'- TCCATATGTGTAAGGGATGGGG-3'	146

F: Forward primer; R: Reverse primer.

lakes and in the spring for the contaminated Lake Dufault (Fig. 2).

3.2. Metal effects on biometric parameters

Within a season, sampled fish were similar in terms of weight and length except for those from the metal-contaminated lake Dufault, where fall captures were significantly smaller than those from the other lakes (Table 2).

In the spring, fish from Marlon Lake showed a higher Kn value than fish from the other lakes, whereas in the fall, this metric did not vary among lakes (Table 2). A seasonal effect was observed in fish condition factors in lakes Opasatica and Dufault, with fish caught in the fall displaying higher condition factors than in the spring (Table 2). While the Kn was correlated positively with kidney Cu concentrations in the spring ($\rho = 0.2384$; $p = 0.0293$), in the fall, positive relationships were found between this metric and kidney Cd and Zn concentrations ($\rho = 0.2495$; $p = 0.0192$ and $\rho = 0.4109$; $p = 0.0002$, respectively).

Corrected pyloric cæca weights did not vary among lakes in either spring or fall, but values for the spring captures were consistently higher than in the fall (Table 2). A negative correlation was observed between corrected pyloric cæca weights and kidney Zn concentrations

Table 2
Weight, length, relative condition index (Kn) and corrected pyloric cæca weight of indigenous yellow perch sampled in four lakes in the spring and fall 2012 in the Rouyn Noranda region (N = 20/lake). Values are mean ± SE and means within columns designated with different letters are significantly different (p-value < 0.05).

Lake	Fish weight (g)	Fish length (cm)	Relative condition index (Kn)	Corrected pyloric cæca wet weight (mg)
Spring				
Hélène	2.37 ± 0.16[b]	6.63 ± 0.10[b]	0.66 ± 0.01[bc]	8.13 ± 0.66[ab]
Opasatica	2.44 ± 0.10[b]	6.58 ± 0.10[b]	0.62 ± 0.01[cd]	8.10 ± 0.64[ab]
Dufault	2.29 ± 0.21[b]	6.49 ± 0.16[b]	0.59 ± 0.01[d]	10.35 ± 0.98[a]
Marlon	2.63 ± 0.13[b]	6.19 ± 0.12[b]	0.73 ± 0.01[a]	9.65 ± 0.96[a]
Fall				
Hélène	6.53 ± 0.41[a]	8.63 ± 0.17[a]	0.69 ± 0.01[ab]	4.88 ± 0.30[c]
Opasatica	6.03 ± 0.58[a]	8.25 ± 0.30[a]	0.70 ± 0.01[ab]	5.01 ± 0.31[c]
Dufault	1.58 ± 0.07[b]	5.66 ± 0.08[b]	0.65 ± 0.01[bc]	6.60 ± 0.76[bc]
Marlon	4.70 ± 0.30[a]	7.76 ± 0.15[a]	0.70 ± 0.01[ab]	4.64 ± 0.26[c]
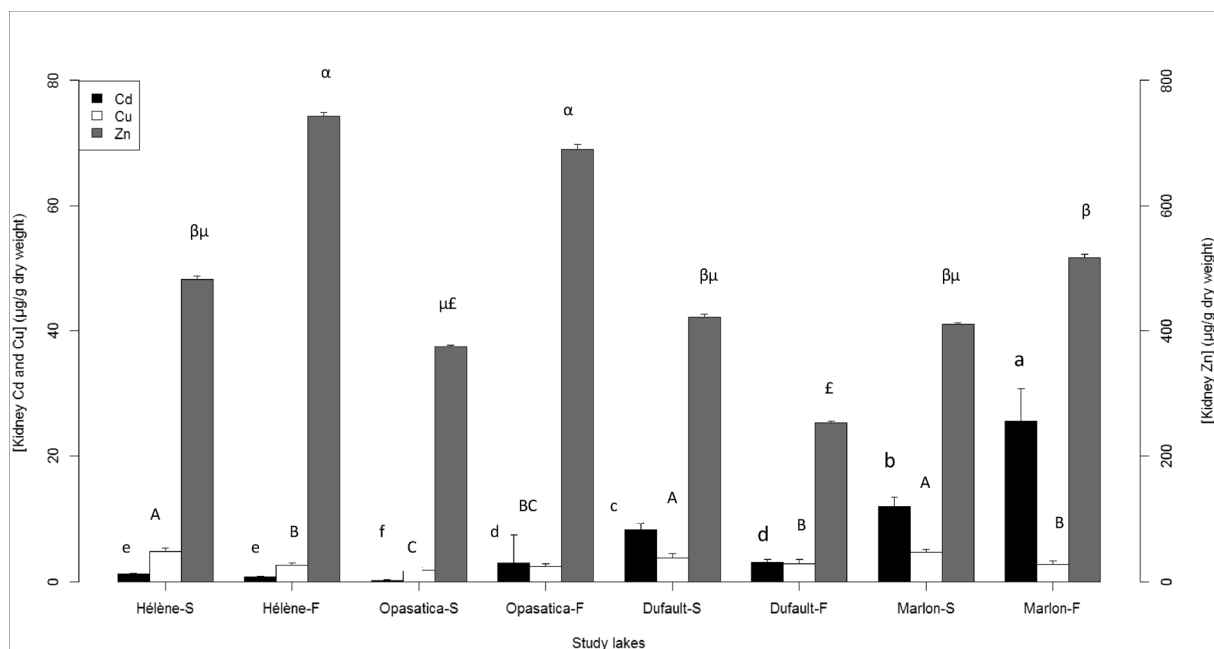
Fig. 2. Total Cd, Cu and Zn concentrations measured in the kidney of juvenile yellow perch collected from four lakes in Rouyn-Noranda region in the spring (S) and in the fall (F) 2012. Values are means ± SE (N = 20/lake). Means designated with different letters (lowercase for Cd, uppercase for Cu and Greek for Zn) are significantly different among lakes and seasons (p-value < 0.05). On the "x" axis, lakes were classified according to the metal concentration gradient.

Table 3

Major vitamin A concentrations in liver and muscle of wild yellow perch sampled in four lakes in spring and fall 2012 in the Rouyn-Noranda region. Values are expressed in nmol g^{-1} of wet tissue (mean ± SE, $6 \leq N \leq 8$/ lake). Means within columns designated with different letters are significantly different (p-value < 0.05).

Lakes	Liver retinoids			Muscle retinoids	
	Dehydroretinol	Dehydroretinyl palmitate	Total esters of vitamin A	Dehydroretinol	Dehydroretinyl stearate
Spring					
Hélène	16.0 ± 5.0[b]	23.0 ± 6.0[d]	40.0 ± 10.0[d]	0.160 ± 0.030[a]	0.200 ± 0.010[a]
Opasatica	24.3 ± 7.8[b]	79.0 ± 17.5[c]	125.0 ± 28.3[c]	0.093 ± 0.005[b]	0.220 ± 0.010[a]
Dufault	45.3 ± 13.2[a]	103.4 ± 31.4[b]	175.8 ± 55.6[b]	0.150 ± 0.010[a]	0.170 ± 0.010[b]
Marlon	50.4 ± 11.2[a]	197.2 ± 28.1[a]	342.3 ± 52.3[a]	0.160 ± 0.020[a]	0.170 ± 0.010[b]
Fall					
Hélène	1.6 ± 0.2[d]	26.2 ± 10.1[d]	41.1 ± 15.3[d]	0.077 ± 0.007[b]	0.130 ± 0.006[c]
Opasatica	1.2 ± 0.5[d]	30.0 ± 11.4[d]	44.0 ± 16.3[d]	0.080 ± 0.007[b]	0.120 ± 0.006[c]
Dufault	6.2 ± 1.6[c]	69.5 ± 24.4[c]	110.0 ± 41.1[c]	0.160 ± 0.020[a]	0.150 ± 0.010[b]
Marlon	2.3 ± 1.2[d]	41.0 ± 13.1[d]	66.2 ± 21.2[d]	0.100 ± 0.010[b]	0.130 ± 0.010[c]

in both spring (ρ = -0.2691; p = 0.0165) and fall (ρ = -0.8353; p < 0.0001).

3.3. Relationships among metal concentrations, tissue retinoid concentrations and rdh-2 transcription levels

3.3.1. Liver

Within a season, yellow perch from metal-contaminated lakes displayed higher liver levels of dehydroretinol, dehydroretinyl palmitate and total esters of vitamin A, although differences were not significant in Marlon Lake fall samples (Table 3). We observed a consistent seasonal variation in retinoid concentrations of the fish populations examined. Liver retinoid concentrations were higher in the spring than in the fall, except in Hélène Lake where no difference was observed in the levels of esterified retinoids (Table 3). A significant and positive correlation between all forms of retinoids analysed and kidney Cd concentration was observed in the spring but not in the fall (Table 4).

In both seasons, although liver retinoid concentrations increased in fish captured in metal-impacted lakes, the percentage of free dehydroretinol generally decreased in those fish (Fig. 3). Furthermore, the percentage of free dehydroretinol strongly varied seasonally and was about 4 times higher in spring than in fall samples. A negative correlation between the percentage of free liver dehydroretinol and kidney Cd concentrations was observed in the spring, in contrast to the fall, when a positive correlation was found between this parameter and renal Cu concentrations (Table 4).

In the spring, the transcription level of the liver *rdh-2* gene was higher in fish from metal-contaminated lakes compared to fish from Opasatica (not measured in Hélène Lake), although not significantly in Dufault Lake samples (Fig. 4). No significant differences existed among the fall samples. Seasonal variations in liver *rdh-2* transcription level were only significant in the most metal-contaminated lake (Marlon), with two-fold higher mean values in the spring compared to the fall (Fig. 4).

3.3.2. Muscle

In the spring, muscle dehydroretinol concentrations were comparable among lakes except for fish from Opasatica Lake, where values were much lower (Table 3). Muscle dehydroretinol concentrations decreased in the fall in fish from Hélène and Marlon Lakes but remained unchanged in fish from Opasatica and Dufault Lakes. In the spring, no significant relationships were found between muscle dehydroretinol concentrations and kidney metal concentrations, but in the fall, this parameter decreased with increasing kidney Zn concentrations (Table 4).

Unlike in the liver, muscle retinoids were mainly stored in the form of dehydroretinyl stearate. In the spring, fish from metal-contaminated lakes displayed lower muscle dehydroretinyl stearate concentrations than those sampled in less metal-contaminated lakes. Conversely, in the fall, fish from Dufault Lake had higher concentrations than fish from other lakes (Table 3). Muscle dehydroretinyl stearate concentrations were higher in the spring, except in fish from Dufault, where the

Table 4

Spearman correlations (ρ) and their significance levels (p) among kidney metal concentrations and biomarkers analysed in yellow perch ($42 \leq N \leq 72$) from the Rouyn-Noranda region, Quebec Canada in spring and fall 2012.

Tissues	Parameters	Spring				Fall					
		Cd		Cu		Cd		Cu		Zn	
		ρ	p	ρ	p	ρ	p	ρ	p	ρ	p
Liver	[Dehydroretinol]	0.4659	0.0210								
	[Dehydroretinyl palmitate]	0.6417	0.0007								
	[Total esters of vitamin A]	0.6374	0.0008								
	Percentage of dehydroretinol	−0.4112	0.0500	0.5253	0.0252			0.5758	0.0040	−0.5548	0.0049
	g6pdh[‡]	0.6502	0.0002					0.4204	0.0166		
	G6PDH[¶]										
	GST[¶]										
	CAT[¶]										
Muscle	[Dehydroretinol]									−0.7461	< 0.0001
	[Dehydroretinyl stearate]	−0.5449	0.0060							−0.5279	0.0080
	Percentage of dehydroretinol					−0.5687	0.0037			−0.5881	0.0025
	SOD[¶]									0.4374	0.0326

[‡] Gene transcription level.

[¶] Enzyme activity.
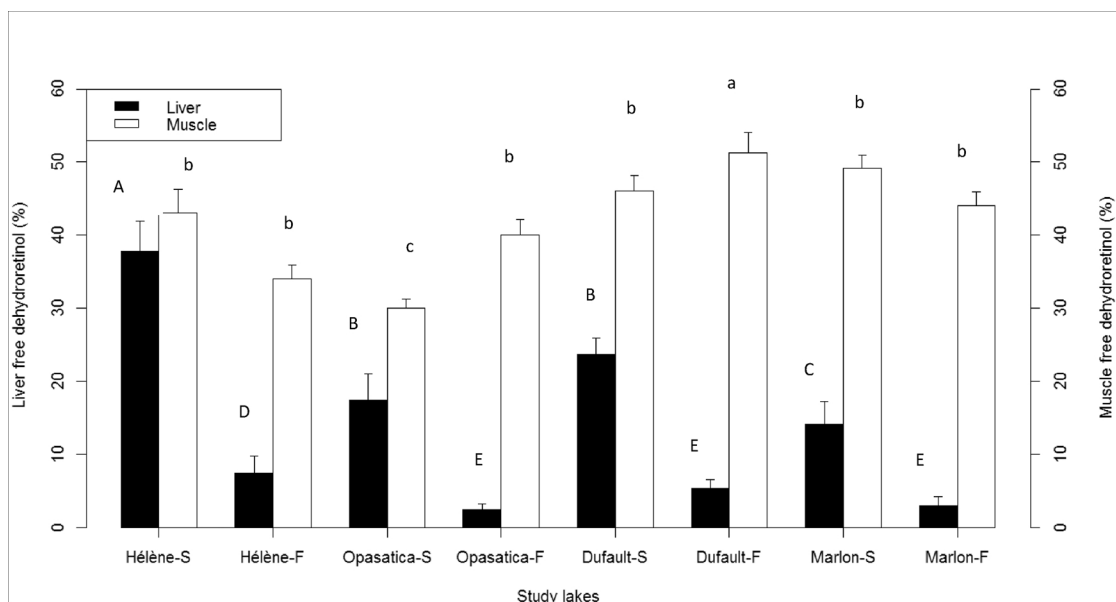
Fig. 3. Percentage of free dehydroretinol in liver and muscle of juvenile yellow perch caught in four lakes in Rouyn-Noranda region in the spring (S) and in the fall (F) 2012. Bars represent means ± SE (6 ≤ N ≤ 8/lake). Values that do not share a common letter (lowercase for liver and uppercase for muscle) are significantly different among lakes and seasons (p-value < 0.05).
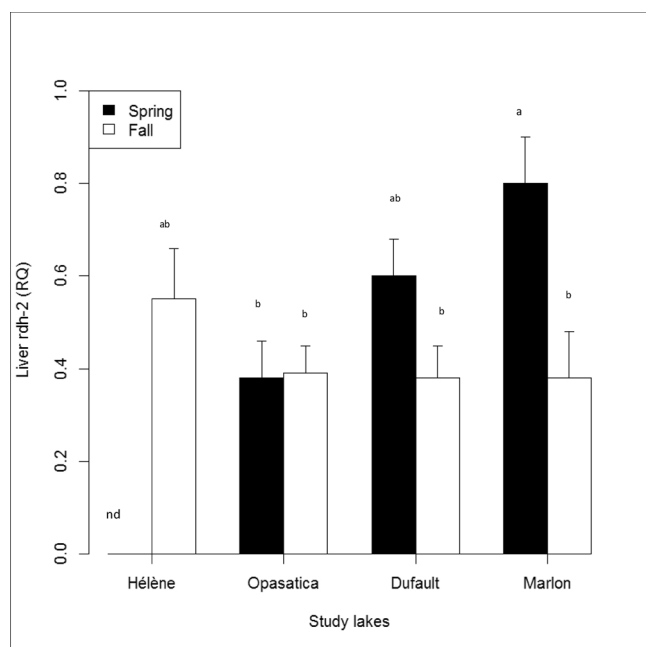
Fig. 4. Liver gene transcription levels (mean ± _SE) of *rdh-2* analysed in indigenous yellow perch sampled in four lakes from the Rouyn-Noranda region in the spring and in the fall 2012. Means designated with different letters are significantly different (p-value < 0.05; N = 9/lake). nd: not determined.

seasonal decrease was not significant (Table 3). A negative relationship was found between muscle dehydroretinyl stearate concentrations and kidney Cd concentrations in the spring, and with Zn concentrations in the fall (Table 4).

Unlike for liver, there was no obvious relationship between metal contamination and the percentage of muscle-free dehydroretinol in either season (Fig. 3). Season only affected the percentage of muscle-free dehydroretinol in fish from Dufault and Opasatica Lakes, for which values were higher in the fall compared to the spring (Fig. 3). In the fall, we found a negative correlation between the percentage of muscle-free dehydroretinol and kidney Zn concentrations (Table 4).

3.4. Relationship between metal concentrations and tissue oxidative stress responses

3.4.1. Liver

Liver *cat* transcription levels in this study were not influenced by metal contamination and did not vary seasonally (Table 5). Liver CAT activity was also generally comparable among lakes and between seasons. However, significantly higher values were measured in fish from Dufault in the spring compared to other lakes, but CAT activity in these fish decreased twofold in the fall. The lowest liver CAT activity recorded was for fish from Hélène Lake in the fall (Table 5). We observed a negative correlation between liver CAT activity and kidney Zn concentrations in the fall only (Table 4).

Yellow perch from Marlon Lake expressed higher liver *Cu/Zn sod-1* transcription levels in the spring compared to fish from other lakes, but these values decreased to levels comparable with those measured in fish from other lakes in the fall (Table 5). Liver SOD activity was lower in fish from both metal-contaminated lakes than in Opasatica samples in the spring, while in the fall, it was only lower in fish from Dufault Lake compared to Opasatica fish. No significant seasonal variation in yellow perch liver SOD activity was observed (Table 5).

Liver *g6pdh* transcription levels were higher in fish from metal-contaminated lakes compared to fish from Opasatica Lake in the spring (Table 5). There were no seasonal variations in *g6pdh* transcription levels except in samples from Opasatica Lake where this value was six-fold higher in the fall than in the spring (Table 5). A strong and positive relationship was found between liver *g6pdh* transcription levels and kidney Cd concentrations in spring captures (Table 4). In the spring, fish living in metal-contaminated lakes displayed higher liver G6PDH activity than fish from Opasatica Lake but in contrast, in the fall, fish from Hélène Lake expressed higher activities than those measured in fish from the other lakes (Table 5). Like liver *g6pdh* transcription levels, higher values of liver G6PDH activity were observed in fish from Opasatica Lake in the fall compared to the spring. The opposite was observed for fish from Dufault Lake, with lower values in the fall (Table 5). Liver G6PDH activity was positively correlated with kidney Cu concentrations in the fall (Table 4).

There was no significant variation among lakes or between seasons in the transcription levels of liver *mgst-3* (Table 5). Likewise, for liver GST activity, there were no consistent inter-lake variations within a

Table 5

Liver gene transcription level and enzyme activities in wild yellow perch sampled in spring and fall 2012 in the lakes of the Rouyn-Noranda region. Values are mean ± SE (6 ≤ N ≤ 9 /lake). Means within columns designated with different letters are significantly different (p-value < 0.05).

Lakes	Gene transcription levels[+]				Enzyme activities			
	cat	*Cu/Zn sod-1*	*g6pdh*	*mgst-3*	CAT[*]	SOD[*]	G6PDH[×]	GST[×]
Spring								
Opasatica	0.43 ± 0.08[a]	0.64 ± 0.15[b]	0.19 ± 0.04[c]	0.67 ± 0.14[a]	0.24 ± 0.05[b]	51.4 ± 9.4[a]	0.016 ± 0.004[c]	0.035 ± 0.001[b]
Dufault	0.44 ± 0.10[a]	0.54 ± 0.10[b]	0.55 ± 0.08[b]	0.52 ± 0.12[a]	0.60 ± 0.11[a]	33.5 ± 3.7[c]	0.054 ± 0.005[a]	0.030 ± 0.001[b]
Marlon	0.68 ± 0.15[a]	0.88 ± 0.14[a]	1.26 ± 0.21[ab]	0.99 ± 0.14[a]	0.26 ± 0.02[b]	35.7 ± 4.2[bc]	0.047 ± 0.007[ab]	0.037 ± 0.003[ab]
Fall								
Hélène	0.38 ± 0.07[a]	0.55 ± 0.08[b]	1.50 ± 0.30[a]	0.60 ± 0.12[a]	0.12 ± 0.03[c]	45.3 ± 4.6[abc]	0.056 ± 0.007[a]	0.049 ± 0.008[a]
Opasatica	0.44 ± 0.08[a]	0.57 ± 0.07[b]	1.21 ± 0.16[ab]	0.63 ± 0.11[a]	0.21 ± 0.02[b]	49.3 ± 2.9[ab]	0.039 ± 0.005[b]	0.058 ± 0.005[a]
Dufault	0.52 ± 0.12[a]	0.49 ± 0.08[b]	0.59 ± 0.11[b]	0.85 ± 0.20[a]	0.28 ± 0.02[b]	34.1 ± 2.9[c]	0.037 ± 0.003[b]	0.045 ± 0.003[a]
Marlon	0.51 ± 0.13[a]	0.54 ± 0.10[b]	0.93 ± 0.14[b]	0.83 ± 0.16[a]	0.21 ± 0.02[b]	43.8 ± 4.1[abc]	0.035 ± 0.003[b]	0.045 ± 0.005[a]

[*] Expressed in U/mg protein.

[×] Expressed in U/g protein.

[+] Expressed in relative quantification (RQ).

season. However, an increase in the activity of this enzyme was observed in fall captures compared to the spring samples for fish from Opasatica and Dufault Lakes (Table 5). Liver GST activity was positively correlated with kidney Cu concentrations in the spring (Table 4).

3.4.2. Muscle

In muscle, the relations between tissue metal concentrations and oxidative stress responses could only be examined for enzyme activities. Compared to Opasatica Lake, muscle CAT activity increased in fish from Marlon Lake in the spring. However, in the fall, no significant differences in this activity were reported in sampled populations (Table 6). Inter-seasonal differences in muscle CAT activity were observed in the contaminated Marlon Lake where this activity was higher in the spring than in the fall. In contrast, in fish captured in the cleaner Opasatica Lake, muscle CAT activity was four times lower in the spring captures than in the fall captures (Table 6).

There were no inter-lake differences in muscle SOD activity in the spring, whereas in the fall, muscle SOD activity was lower in fish living in metal-contaminated lakes compared to those from Hélène Lake (Table 6). There were also no inter-seasonal differences in the activity of this enzyme except in Hélène Lake where it was twice as high in the fall as in the spring (Table 6). A strong but negative correlation was found between muscle SOD activity and kidney Cd concentrations in the fall (Table 4). However, the correlation was positive between this parameter and kidney Zn concentrations in the same season. There was no significant relationship between muscle SOD activity and any kidney

Table 6

Muscle enzyme activities of wild yellow perch sampled in four lakes in the spring and in the fall 2012 in the region of Rouyn-Noranda. Values are mean ± SE (6 ≤ N ≤ 8/lake). Means within columns designated with different letters are significantly different (p-value < 0.05).

Lakes	Enzyme activities		
	CAT[*]	SOD[*]	GST[×]
Spring			
Hélène	0.0033 ± 0.0004[ab]	4.15 ± 0.17[b]	0.013 ± 0.001[c]
Opasatica	0.0007 ± 0.0001[c]	3.58 ± 0.64[b]	0.028 ± 0.005[a]
Dufault	0.0023 ± 0.0002[bc]	5.80 ± 1.02[ab]	0.020 ± 0.004[b]
Marlon	0.0039 ± 0.0005[a]	6.57 ± 1.36[ab]	0.010 ± 0.002[c]
Fall			
Hélène	0.0021 ± 0.0004[bc]	7.86 ± 0.78[a]	0.012 ± 0.001[c]
Opasatica	0.0030 ± 0.0006[ab]	5.59 ± 0.89[ab]	0.013 ± 0.001[c]
Dufault	0.0033 ± 0.0004[ab]	3.94 ± 0.32[b]	0.015 ± 0.001[bc]
Marlon	0.0020 ± 0.0003[bc]	3.76 ± 0.65[b]	0.015 ± 0.001[bc]

[*] Expressed in U/mg protein.

[×] Expressed in U/g protein.

metal concentrations in the spring captures.

Muscle GST activity was higher in fish from Opasatica Lake than in those from other lakes in the spring, but no differences among fish from the different lakes were apparent in the fall (Table 6). There were no seasonal variations in muscle GST activity except in Opasatica Lake where this activity in the fall was half that in the spring (Table 6).

4. Discussion

4.1. Seasonal variations in kidney metal concentrations and influence of contamination

Since livers were small and needed for biochemical and molecular analyses, metal concentrations were analysed in kidney tissue. Strong relationships between liver and kidney metal concentrations (particularly Cd) have been reported in previous studies for yellow perch and demonstrated that renal metal concentrations are a suitable surrogate for estimating prior metal exposure (Couture et al., 2008a; Kraemer et al., 2005a,b). The sampling region is known to present broad gradients of Cd, Cu and Zn in water, sediment and prey (Campbell et al., 2008; Croteau et al., 2003). However, a metal contamination gradient was only observed in kidney Cd concentrations, in both seasons, with a 60-fold difference between the least and the most contaminated fish in spring and a 32-fold difference in the fall. One of the advantages of our study area is that it is situated in a relatively underpopulated area of northern Canada and there are very few sources of pollutants other than metal mining and smelting activities. There are differences in water quality among the lakes (e.g., pH and dissolved organic carbon concentrations – see Mueller et al., 2012a,b) but these are natural variations. A gradient in kidney Cd concentration was also reported for yellow perch in previous investigations carried out in this region (Couture et al., 2008a; Levesque et al., 2002). Being essential metals, kidney Cu and Zn are likely better regulated than Cd through homeostatic control mechanisms (Quintaneiro et al., 2015; Viarengo et al., 1990) supporting the absence of tissue concentration gradients among lakes for these metals, particularly in the spring. We observed lower kidney Zn concentrations in metal-contaminated fish compared to those living in cleaner lakes, in the fall. Zinc is involved in physiological processes that could explain the lower values in contaminated fish.

Kidney Cu and Cd concentrations were higher in the spring captures than in fish sampled in the fall, except in Marlon and Opasatica lakes for Cd. This result is in agreement with a previous study reporting that yellow perch living in metal-contaminated lakes, in the Sudbury region, displayed higher liver Cu concentration in the spring than in the fall (Eastwood and Couture, 2002). Although we did not measure aqueous metal concentrations and other parameters that influence the metal

bioavailability, such as total dissolved carbon, our results suggest the presence of seasonal variations in those parameters in lakes studied (Couture et al., 2008a). This hypothesis is supported by the findings of Kraemer et al. (2005a), who noted that in metal-contaminated lakes, total dissolved Cd concentrations were 2 to 3.5- fold higher in the spring than in the fall, although no seasonal variations in dissolved organic carbon were observed. Other factors, including an increase in metal bioavailability due to variations in pH, snowmelt events or lake turnover, that influence water quality parameters, might have contributed to the higher kidney metal levels observed in the spring, especially in Dufault Lake fish.

Both aqueous and dietary sources influence Cd accumulation in yellow perch living in contaminated environments (Couture et al., 2008a). In contrast to fish from Dufault Lake, the seasonal differences observed in kidney Cd concentrations in Marlon Lake fish, which increased from spring to fall, suggest that their feeding rate could be higher in the fall than in the spring in this lake. This is unlikely true since no variation in Kn, a metric reflecting recent feeding activity (Lambert and Dutil, 1997), was found in fish from this lake. Moreover, fall samples also displayed lower corrected pyloric cæca wet weights than did the spring captures. Taken together, these findings suggest that although the feeding rate is lower in the fall than in the spring, the important feeding activities in the spring (and summer) led to an important renal accumulation of Cd, such that the kidney Cd concentrations always remained higher in Marlon Lake fish than in the fish from the other lakes.

4.2. Relationship between metal concentrations and tissue retinoid metabolism

Unsurprisingly, all-trans-3,4-didehydroretinoids (vitamin A$_2$) were the predominant forms found in yellow perch tissues. In the liver, retinoids were mainly stored in the form of dehydroretinyl palmitate and secondarily in the forms of dehydroretinyl myristate, dehydroretinyl stearate and other unidentified forms. In our previous field study in the same region (Defo et al., 2012), we reported substantially higher liver retinoid concentrations in adult yellow perch living in Cd-contaminated lakes. The present results confirm for juveniles the metabolic imbalance of retinoids in fish chronically exposed to elevated Cd concentrations and provide valuable information supporting the idea that wild fish inhabiting contaminated lakes in the Rouyn-Noranda region are impacted by metals, especially Cd (Campbell et al., 2008; Levesque et al., 2002; Pierron et al., 2009, 2011). We interpret the increase liver dehydroretinol, as well as dehydroretinyl palmitate and total esters of vitamin A in fish from metal-impacted lakes, as an adaptive response to higher metal concentrations to fight against Cd-induced oxidative stress (Defo et al., 2012).

Importantly, seasonal variations in the concentrations of all forms of liver retinoids analysed were observed in fish from contaminated lakes, being higher in spring than in fall. This study is to our knowledge the first field investigation directly linking temporal fluctuations in tissue metal and retinoid concentrations in wild yellow perch. In fact, a significant and positive correlation between kidney Cd concentrations and the concentrations of liver dehydroretinol, dehydroretinyl palmitate and total esters of vitamin A was observed in the spring. This finding agrees well with our previous work where we reported such relationships between kidney Cd concentrations and the concentrations of liver retinoids (Defo et al., 2012). Retinoids are naturally occurring compounds derived from carotenoid metabolism or directly accumulated from food (D'Ambrosio et al., 2011; Fernández and Gisbert, 2011). We hypothesize that yellow perch prey types may be richer in dehydroretinyl esters or in their precursor, β-carotene, in the spring than in the fall. In our study, the heavier pyloric cæca in spring than in fall captures suggests that in metal-contaminated lakes, food availability is greater in the spring than in the fall. A food web simplification (Iles and Rasmussen, 2005) coupled to a loss in yellow perch prey diversity

(Kövecses et al., 2005) were reported in metal-contaminated lakes in the Rouyn-Noranda region. Seasonal changes in perch liver retinoid concentrations could also be due to the consumption of prey rich in vitamin A after spawning in early spring and a redistribution of retinoids stored in their livers for gonad maturation in the fall.

In this study, we indirectly examined the influence of metal contamination on the activity of enzymes involved in retinoid metabolism by calculating the percentage of liver free dehydroretinol. This parameter has been reported to be a biomarker of pollutant exposure and can be considered as a surrogate for retinoid enzyme activities, such as retinyl ester hydrolase (REH) and lecithin retinyl acyl transferase (LRAT) (Boily et al., 2005; Defo et al., 2012). Although higher liver retinoid concentrations were reported in metal-contaminated fish, a spring to fall decrease in the percentage of liver free dehydroretinol was observed in those fish compared to their homologs from clean lakes. This result suggests that enzymes involved in retinoid storage (LRAT) could be more active in the spring than in the fall, or that retinoid homeostasis enzymes and/or binding proteins are disturbed by metals. Although the present result agrees with our previous finding, where we observed lower percentages of free dehydroretinol in the livers of adult yellow perch inhabiting Cd-impacted lakes in the Rouyn-Noranda region compared to fish from clean lakes (Defo et al., 2012), it contrasts with our laboratory investigation where we reported an increase in the percentage of free retinol and in the activities of enzymes involved in retinoid metabolism in the livers of yellow perch exposed to environmentally relevant concentrations of Cd for six weeks (Defo et al., 2014a). Wild fish sampled in impacted areas are progeny from parent fish permanently exposed to metals. They may be less reactive to metal-induced adverse effects compared to offspring from previously unexposed fish, such as those that were used in our earlier lab exposure. Fish life history characteristics could explain the discrepancy between these studies.

In contrast to the decrease in the percentage of liver dehydroretinol, an increase in liver *rdh-2* transcription level was observed in the spring in fish originating from the metal-contaminated Marlon Lake, probably in order to stimulate the activity of retinoid metabolism enzymes such as REH or LRAT. In contrast, Pierron et al. (2011) found a negative correlation between liver transcription levels of genes encoding for the enzymes involved in retinoid metabolism and liver Cd concentrations in juvenile wild yellow perch chronically exposed to metals. Both studies took place in the same season (spring) and the same lakes were sampled. However, tissue Cd concentrations were at least two-fold higher in Pierron et al. (2011) than in the present study. The discrepancies between these investigations could be related to the sampling years, 2009 and 2012 respectively. Taken together, these studies suggest that in the context of chronic exposure to a cocktail of metals in the field, variations in the transcription levels of genes involved in retinoid metabolism are a function of metal concentrations accumulated in fish tissues.

Depending on cell physiological needs, retinoids stored in the liver mainly in their esterified form can be hydrolysed (Branchaud et al., 1995) and transported in their free form (retinol) to the target tissues, such as muscle, by binding with retinol binding proteins (RBP) and transthyretin (TTR). Under our experimental conditions, dehydroretinyl stearate was the only ester found in yellow perch muscle. Compared to the liver concentrations, the levels in the muscle were much lower. Fish from contaminated lakes displayed higher muscle dehydroretinol but lower dehydroretinyl stearate than those from clean lakes, suggesting hydrolysis of the esterified forms into the free form, probably to fight against oxidative stress engendered by Cd exposure (Defo et al., 2012, 2014b). This assumption is further supported by the higher percentage of muscle dehydroretinol in these fish. We confirmed this hypothesis experimentally in an earlier study where an increase in the percentage of muscle dehydroretinol was observed in perch exposed to Cd for six weeks at a concentration similar to that measured in water (0,8 µg/L) of contaminated lakes in the Rouyn -Noranda region (Defo et al., 2014a).

4.3. Relationship between tissue metal concentrations and oxidative stress responses

4.3.1. Liver

We tested the hypothesis that seasonal variations in tissue metal concentrations alter the normal oxidative stress response at the gene transcription and enzyme activity levels in wild fish. The liver plays a key role in the fight against chemically-induced oxidative stress (Benedetti et al., 2007). Liver CAT activity increased with increasing metal contamination (e.g., Dufault Lake) in the spring and fall, whereas the transcription level of the corresponding gene remained unchanged, suggesting that the response of wild yellow perch to metal exposure mainly occurs at the post-transcriptional or catalytic levels. The present result somewhat differs from a previous laboratory study reporting that an increase in liver CAT activity was associated with an upregulation of the expression of *cat* gene in fish chronically exposed to an environmentally relevant concentration of waterborne Cd (Defo et al., 2014a). Catalase belongs to the first line of oxidative stress defense (Yonar, 2013) and its stimulation either at the molecular or biochemical levels contributes to the clean-up of cellular ROS generated by metals (Webster et al., 2013). In contrast to CAT activity, SOD activity decreased with increasing metal contamination in the spring captures. Unsurprisingly, the opposite response between the two biomarkers reflects a strong negative correlation between the activities of SOD and CAT indicating that an increase in SOD activity will be followed by a decrease in CAT activity (Cao et al., 2012; Qu et al., 2014) Indeed, an increase in SOD activity would result in an accumulation of hydrogen peroxide (H_2O_2), which is used as substrate for CAT activity. In contrast, the ability to degrade H_2O_2 by CAT increases when the activity of SOD is low (Qu et al., 2014). There is a synergy or cooperation between the two metabolic pathways (Asagba et al., 2008): SOD catalyses the dismutation of superoxide into O_2 and H_2O_2 while CAT degrades H_2O_2 in H_2O and O_2. Since SOD activity normally increases with ROS production (Qu et al., 2014), the decrease observed in the activity of SOD with increasing metal contamination in the spring captures can also be explained by an imbalance between ROS generation and SOD production, the latter being slower than the former (Qu et al., 2014). Although SOD activity was lower in fish from the metal-contaminated lakes, the transcription level of *Cu/Zn sod-1* was only higher in fish from the contaminated Marlon Lake compared to those in fish from the clean Opasatica Lake in spring. The higher *Cu/Zn sod-1* transcription level likely aimed at maintaining the pool of enzymes involved in liver ROS reduction. Such a negative effect of metal contamination on liver SOD activity was also reported in a study in which yellow perch were chronically exposed to a combination of heat and Cd stress (Grasset et al., 2016). In another study at the transcriptional level, Pierron et al. (2009) reported a decrease in liver *sod* transcription levels in yellow perch living from Cd-impacted lakes, a finding opposite to our observations. Although both studies took place in the same region, sampling occurred in August 2006 (summer) in Pierron et al. (2009), whereas in the present study fish were collected in May (spring) 2012. Additionally, the impacted lakes sampled were different and tissue metal (Cd and Cu) concentrations were much higher in their study compared to ours.

Kidney Cd accumulation was associated with an induction of the liver *g6pdh* gene and a corresponding increase in G6PDH enzyme activity, in the spring. This enzyme is involved in the pentose phosphate pathway and has recently been used as an oxidative stress-related biomarker in a study evaluating the impacts of environmental stressors on fish (Regoli, 2011). It regulates the glutathione response to chemical exposure through its involvement in NADPH biosynthesis, which in turn serves as a reducing equivalent in the activity of glutathione reductase (Regoli, 2011). The catalytic response typically follows the transcriptomic response (Nikinmaa and Rytkönen, 2011). Thus, the synchronous responses obtained at the molecular and biochemical levels reflect a fight against metal-induced oxidative stress and suggest

that in an environment contaminated by a cocktail of metals, the regulation of the G6PDH metabolic pathway occurs at both levels of biological organisation. In support of this hypothesis, liver transcription levels of *g6pdh* were increased in clean yellow perch transplanted into a metal-contaminated lake (high Cd concentration) and this up-regulation was accompanied by an increase in G6PDH activity (Defo et al., 2015). Furthermore, six weeks of exposure of yellow perch to Cd in the laboratory led to an increase of the liver *g6pdh* transcription level followed by a corresponding increase in enzyme activity (Defo et al., 2014a).

Unlike in spring, in the fall, neither liver *g6pdh* transcription levels nor G6PDH enzyme activity were affected by metal contamination. For liver G6PDH activity, this is in agreement with an earlier report for yellow perch from the same region (Levesque et al., 2002). In our study, seasonal variations in G6PDH were only observed in fish from Dufault Lake and occurred solely at the catalytic level. A lower activity of liver G6PDH was reported in yellow perch inhabiting metal-contaminated lakes, including Dufault Lake, in summer (Levesque et al., 2002).

4.3.2. Muscle

Among all the oxidative stress-related biomarkers analysed in muscle, only SOD activity responded to elevated metal exposure, with a slight decrease in metal-exposed fish compared to cleaner fish, leading to a significant negative relationship with kidney Cd in the fall. Accumulation of most metals in fish muscle is much lower than in the liver (Pyle et al., 2005; Rajotte and Couture, 2002), the latter tissue being involved in metal storage and detoxification. Our results support the contention that metal accumulation in this tissue is likely insufficient to induce an antioxidant response.

5. Conclusion

Our study examined the relations between seasonal variations in tissue metal concentrations and oxidative stress response biomarkers in wild juvenile yellow perch and constitutes a first report linking seasonal changes in tissue metal concentrations and biomarkers of retinoid metabolism and oxidative stress response in fish chronically exposed to sublethal metal concentrations. Our results clearly show that within a season, liver dehydroretinol, dehydroretinyl palmitate and total vitamin A ester concentrations were higher in fish sampled in contaminated lakes, presumably reflecting an organismal response to oxidative stress principally engendered by Cd in the system studied. Fish living in metal-impacted lakes generally displayed higher liver retinoid metabolite concentrations, higher percentages of free dehydroretinol and higher epidermal retinol dehydrogenase 2 transcription levels, in the spring than in the fall. Our data suggest that metal impacts on oxidative stress response biomarkers are exacerbated in the spring compared to the fall, probably reflecting seasonal differences in ROS generation. This study highlights the importance of considering seasonal variations in the assessment of environmental risks posed by metal contamination on the physiology of wild fish.

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