Lipophilic antioxidants and lipid peroxidation in yellow perch subjected to various anthropogenic influences along the St. Lawrence River (QC, Canada)

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1. Introduction

For decades the yellow perch (\textit{Perca flavescens}), a common fish species throughout the St. Lawrence River, is considered an important component of recreational and commercial fishing. In the vast ecosystem of the St. Lawrence River, Leclerc et al. (2008) identified four distinct yellow perch populations based on a landscape genetics approach: Lake Saint-François (LSF), the north of Lake Saint-Louis (LSL), the south of LSL + the fluvial corridor downstream of Montreal, and a population composed of all individuals from Lake Saint-Pierre (LSP) and downstream to the freshwater limit (see Fig. 1). While the upstream yellow perch populations of LSF and LSL are abundant (Mailhot et al., 2015), the population of LSP has been declining for over 20 years despite the implementation of various management practices designed to protect this socio-economically important species.

In addition to pressure from commercial and sport fishing, the yellow perch is confronted with a series of stressors including the deterioration of habitats amenable to successful reproduction and growth, competition with exotic species and poor water quality (de la Chenelière et al., 2014). A recent paper by Giraudo et al. (2016) reported that yellow perch from LSP had higher liver metal content and greater liver damage compared to upstream populations.

A major feature of the yellow perch decline appears to be the low abundance of young fish (1 and 2 years), especially in the southern part of LSP (Mailhot et al., 2015). To address the problem of weak recruitment in LSP yellow perch, we selected biochemical markers associated with reproduction and early life stages. Retinoids (compounds related to vitamin A) were studied because they are essential for early development (Oliveira et al., 2013), growth and reproduction (Alsop et al., 2005, 2008). Furthermore, several ecotoxicological...
studies have linked contaminants (either urban, industrial or agricultural) to fish retinoid system disturbances (Alsop et al., 2003; Defo et al., 2012; Doyon et al., 1998; Zhang et al., 2002). The south of LSP is known to receive waste from agricultural watersheds (Richelieu, Yamaska and Saint-François) under wide-row cultivation, mainly maize and soy (Hudon et al., 2011; La Violette, 2004; Richard et al., 2011). Studies conducted in the Yamaska watershed showed a significant impact on the health of the bullfrog (Lithobates catesbeianus), namely growth retardation (Spear et al., 2009) and altered contents of retinoids. In males, total retinyl ester and retinol from the liver were decreased, as was retinol in plasma (Bérubé et al., 2005; Boily et al., 2005).

Retinoids are derived from food-related carotenoids. The following provitamin A carotenoids have been identified in fish: cryptoxanthin, α-carotene, β-carotene, astaxanthin, canthaxanthin, zeaxanthin, lutein and tunaxanthin (Kaisuyama and Matsuno, 1988; Matsuno, 1991). From a metabolic point of view, these precursors are oxidized in all-trans-retinaldehyde (RALD) or 3,4-dehydroretinaldehyde (DRALD) and mainly converted to all-trans-retinol (ROH) or 3,4-didehydro-all-trans-retinol (DROH) (Fig. S1, Supplementary material) (Novak et al., 2008; Shirakami et al., 2012; Comb, 2012c). In vertebrates, vitamin A is principally stored in the liver as retinyl esters (palmitate, linoleate, myristate, stearate, etc.). The hydrolyzed products of esters, DROH and ROH are then coupled with the retinol-binding protein and distributed in peripheral organs via the blood circulatory system (Alsop et al., 2005). In some fish (e.g. trout), the dehydro forms dominate (Alsop et al., 2005; Gesto et al., 2012).

Besides serving as precursors to vitamin A, carotenoids neutralize free radicals (see review by Kiokias and Gordon, 2004) that result from endogenous metabolism related to energy production (mitochondria and peroxisomes), inflammatory reactions, cytochrome P450 oxidative action and Fenton’s reaction (Ames et al., 1993; Fang et al., 2002; Girotti, 1998). For example, lycopene, a hydrocarbonated carotenoid, is excellent for capturing singlet oxygen (O₂), hydroxyl radicals (OH) and peroxyl radicals (ROO·) (Di Mascio et al., 1991).

Vitamin E derivatives, nonenzymatic antioxidants also related to the diet, include all elements of tocopherols and tocotrienols (Cuvelier et al., 2003). Their antioxidant role is mainly to “trap” peroxyl radicals (ROO·), particularly lipid peroxyl radicals (LOO·) (Brigelius-Flohe and Traber, 1999; Girotti, 1998; Liebler et al., 1996). Although several tocopherol isomers have been identified in vertebrates (α, γ and δ) (Cuvelier et al., 2003), a positive discrimination favors the α-tocopherol form (90%) through a specific transfer protein or “α-tocopherol transfer protein” (Combs, 2012a). Lipoproteins ensure the distribution of α-tocopherol, which is mainly stored in the cell membranes of the liver but also in muscle and adipose tissue (Combs, 2012a).

Several pesticides found in agricultural watersheds, including LSP (Giroux et al., 2016), have been shown to increase ROS production in fish such as atrazine (Nwani et al., 2010), glyphosate (Sinhorin et al., 2014) and neonicotinoids (Ge et al., 2015) just to name a few. Excessive ROS production affects biological components such as lipids, DNA and proteins (see review by Valavanidis et al., 2006). In our previous investigation of yellow perch, values of peroxidation of lipids (in muscle) and retinoids content (in liver) were associated with the transcription of genes involved in the oxidative stress responses (cat and sod3) and the transport of cell retinoids (rbp2) (Bruneau et al., 2016). However these results did not take into account the development stages of the fish as all individuals were part of a single group without distinguishing juveniles from adults. In the present study, therefore, we re-examined this single group of fish by sorting juveniles and adults and by adding the larval stage. Morphometric data (weight, length,
circumference and Fulton’s condition factor) for juveniles and adults were examined to characterize growth. Biochemical parameters (carotenoids, retinoids, α-tocopherol and peroxidation of lipids) were analyzed for the three life stages. Our objective was to investigate several biomarkers at different life stages, germane to the question of low recruitment, and gain a better understanding of multiple stressors at the biochemical level in the yellow perch living along a gradient of anthropogenic influences.

2. Materials and methods

2.1. Study sites

Yellow perch populations were studied at six sites on the St. Lawrence River along a west-east gradient of increasing anthropogenic influences (see Fig. 1). Lake Saint-François (LSF) was considered to have the lowest degree of stressors, followed by Lake Saint-Louis (LSL) with a moderate degree and the downstream LSP with the highest degree of environmental stressors. The LSF site is characterized by physicochemical properties and resources of a major water supply (98%) from the Great Lakes (La Violette, 2004). The LSL watershed is dominated by urban and industrial activities; the north shore is largely supplied by the Ottawa River while the south shore receives inputs from Saint-Louis and Châteauguay tributaries (La Violette, 2004). The Île Beauregard (IB) site is located downstream the city of Montreal (see Fig. 1) and receives discharges from the wastewater treatment plant, characterized by various industrial and urban contaminants such as metals (Giraudo et al., 2016), polychlorinated biphenyls (PCBs), polybrominated biphenyl ethers (PBDEs), antibiotics as well as pharmaceutical products (Houde et al., 2014; Marcogliese et al., 2015; Lajeunesse et al., 2008). The IB site was of interest in this study because it has recently been recognized that the ecosystem in this area is experiencing anthropogenic pressure of urban origin (Houde et al., 2014; Marcogliese et al., 2015). Intensive crops of corn and soy are present in the floodplain of LSP leading to the loss of valuable natural wetlands and affecting water quality (de la Chenelière et al., 2014). To the south, agricultural activities predominating result in significant inputs of pesticides (Giroux et al., 2016). Although the north shore is also subject to contributions of agricultural contaminants, water quality is largely influenced by residual pollution from the cities of Montreal and Laval, as well as urban and industrial exploitation in adjacent territories (Hudson and Carignan, 2008; La Violette, 2004).

In this study, the northern and southern shores of LSL (LSL-N and LSL-S) and LSP (LSP-N and LSP-S) were considered distinct sites due to the presence of the navigation channel, which has limited mixing of water masses from the two sides since its construction in 1850 (Frenette et al., 2003). A single sampling site was used at LSF and IB, less influenced by the navigation channel.

2.2. Capture of fish and tissue sampling

From April to June 2013, yellow perch were caught by beach seine or hoop nets. Blood and tissue collections were performed in the field following the anesthesia with a clove oil solution (250 mg/L). For all individuals, morphometric measurements, weight (g), length (mm) and body circumference (mm) were noted and blood (300–800 µL) was collected from the caudal vein using heparinized syringes and needles and transferred in 1.5 mL vials. After 10 min on ice, vials were centrifuged for 3 min at 5000 × g. Plasma was transferred to a 0.5 mL vial, frozen on dry ice and stored at −80 °C until analysis was performed. Whole gonads, samples of liver (0.5–1.0 g) and trunk muscle (0.1–0.5 g) were collected from each individual. The tissue was immediately frozen on dry ice and stored at −80 °C until biochemical analysis. The sex of the fish was determined following histological examination of the gonads by the Centre Québécois sur la santé des animaux sauvages (CQSAS) at the University of Montreal’s Faculty of Veterinary Medicine. Estimation of the age was performed by the Ministère des Forêts, de la Faune et des Parcs (MFFP) by counting the number of growth annuli in operculum, based on Deschamps (2013). According to Mailhot et al. (2015), fish of age 1+ and 2+ were classified as juveniles and fish of age 3+ and older as adults. The Fulton’s condition factor (K = 105 × weight (g)/size (mm)3) was computed following the method described by Barnham and Baxter (2003).

Yellow perch larvae were collected at the LSL and LSP sites using push nets (two-meter-long plankton-type net; 500-mm mesh) as described in Bertolo et al. (2012). After capture, whole larvae were immediately frozen on dry ice and stored at −80 °C.

Sampling protocols were approved by Environment and Climate Change Canada’s Animal Care Committee working under the Canadian Council on Animal Care.

2.3. Lipid peroxidation

The lipid peroxidation was evaluated in the muscle of juveniles and adults, and in whole larvae by thiorbarbituric acid reactive substances (TBARS) based on Okawa et al. (1979) method using 1,1,3,3,5,5-tetramethoxypropane solution (Sigma-Aldrich Ltd., Oakville, ON, Canada) for the standard curve instead of malonaldehyde (MDA). The muscle (250 mg) was homogenized in a 2-mL PBS buffer using a Kinematica Polytron PT 1600E Benchtop Homogenizer (Kinematica AG, Bohemia, NY, USA). The homogenate (1.5 mL) was centrifuged at 4000 × g for 10 min at 4 °C. For the larvae, the homogenate was prepared with 180 mg in 3 mL of PBS containing 0.1% of ascorbic acid and was centrifuged at 1000 × g for 10 min at 4 °C. The supernatant (200 µL) of each sample was transferred to a glass tube. Subsequent steps were performed under the exact conditions described in Hedrei Helmer et al. (2014). The TBARS content was determined by means of a fluorescence reading using i-control 1.9 Microplate Reader Software (Tecan, Morrisville, NC, USA) at an excitation of 532 nm and an emission of 553 nm for a total volume of 200 µL of supernatant/well, using a 96-wells, quartz microplate (Hellma Canada Ltd., Markham, ON, Canada). TBARS data are expressed as nmol/g of tissue for larvae and as pmol/mg of protein for juveniles and adults.

2.4. Protein content determination

The protein concentration in each muscle sample of juveniles and adults (supernatants obtained following centrifugation of homogenates, 4000 × g for 10 min at 4 °C) was measured using the Pierce BCA Protein Assay Kit (ThermoScientific™ Pierce™ BCA protein Assay, Fisher Scientific, Ottawa, ON, Canada).

2.5. Alpha-tocopherol, carotenoids and retinoids (liver and whole larvae)

The methods for analyzing α-tocopherol, carotenoids and retinoids were based on Spear and Moon (1986), Doyon et al. (1998) and Hedrei Helmer et al. (2014) and carried out under yellow light to prevent isomerization of the compounds. All compounds were identified with commercial standards of carotenoids (DHI, Hørsholm, Denmark), retinoids and α-tocopherol (Sigma-Aldrich Ltd., Oakville, ON, Canada). DROH and all-trans-3,4-didehydroretinyl palmitate (DPAL) were synthesized as described in Boily et al. (2005) and Bérubé et al. (2005).

Briefly, 200 mg of the liver was dehydrated with 2 g of anhydrous sodium sulfate (Na2SO4) (Anachemia Science, Lachine, QC, Canada) using a mortar and pestle. The resulting powder was then transferred into a screw-cap borosilicate tube into which 5 mL of hexane containing 0.1% of butylated hydroxytoluene (BHT) (Sigma-Aldrich Ltd., Oakville, ON, Canada) was added. Tubes were mixed for 15 min and centrifuged at 1625 × g for 5 min. An aliquot of 0.5 mL of supernatant was taken and evaporated to dryness in an vacufuge (Eppendorf™, Fisher Scientific, Ottawa, ON, Canada) at 45 °C for 10 min. The pellet was
K (Fulton’s condition factor) was compared between sites by ANOVA (\(F\)) followed by HSD Tukey-Kramer’s test and between juveniles and adults by Mann-Whitney (\(U\)) test: * \(p < 0.05\).

<table>
<thead>
<tr>
<th>Stage/Parameters</th>
<th>Sampling sites</th>
<th>Low</th>
<th>Moderate</th>
<th>Statistical Models</th>
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<tbody>
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<td>LSF</td>
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<td>LSL-S</td>
<td>IB</td>
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<td>n=4</td>
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<td>n=29</td>
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<td>(H(3,36)=25.4;)</td>
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<td>Weight (g)</td>
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<td>22.9 ± 5.0b</td>
<td>26.4 ± 3.4d</td>
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<td>0.14 ± 0.14ab</td>
<td>0.18 ± 0.10b</td>
<td>0.24 ± 0.08b</td>
</tr>
<tr>
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<td>n=3</td>
<td>n=0</td>
</tr>
<tr>
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<td>3F/0M</td>
<td>2F/0M (2U)</td>
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<td>1.7 ± 0.5</td>
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<td>1.21 ± 0.28a</td>
<td>1.18 ± 0.11a</td>
<td>1.07 ± 0.09a</td>
</tr>
</tbody>
</table>

— Indicates that no measure has been reported.

Age and morphometric parameters (length, weight and circumference) were compared between sites by Kruskal-Wallis (\(H\)) analysis followed by Dunn’s test. Means not sharing the same letters are significantly different (\(p<0.05\)).

K (Fulton’s condition factor) was compared between sites by ANOVA (\(F\)) followed by HSD Tukey-Kramer’s test and between juveniles and adults by Mann-Whitney (\(U\)) test: * \(p < 0.05\).

\( * \): Female, M: Male and U: undefined.

was used to return to 100% solvent A. The flow rate was 1 mL/min for the total 29-min elution time. Under these conditions, four endogenous retinoids were detected: DROH (6.5 min), ROH (7.3 min), DPAL (17.2 min) and all-trans retinyl palmitate (PAL, 18.8 min).

2.6. Plasma retinoids

Extraction and quantification of plasma retinoids were performed according to Bérubé et al. (2005), except that combined organic phases (2.8 mL) were evaporated to dryness in a vacufuge at 45 °C for 15 min. The retinoids were dissolved in 100 µL of acetonitrile and vortexed for 30 s. A volume of 80 µL was injected into an HPLC system equipped with a Waters 481 detector set at 350 nm. Peaks were separated with an ACN analytical C18 column to 46 × 250 mm, 5 µm particles (Canadian Life Science). Under these conditions, two retinoids were detected: DROH (14.3 min) and ROH (19.8 min). The analytic method allows the detection of 13-cis-retinoic acid (18.1 min), 9-cis-retinoic acid (20.3 min), all-trans-retinoid acid (21.5 min) and RALD (23.6 min) although none of these compounds were detected in whole larvae. In juveniles and adults, the compounds 13-cis-retinoic acid and 9-cis-retinoic acid were detected in the plasma of several individuals but not in sufficient numbers to be included in the statistical treatment.

2.7. Statistical analysis

Data analyses were conducted using JMP Pro 11.0.0 software (SAS Institute Inc., Cary, NC, USA). No influence of sex was found on any studied parameters; the data for males and females were then pooled. Independent statistical analysis was performed for the three development stages of yellow perch: larval, juvenile and adult. The means of morphometric parameters, carotenoid, retinoid, \(\alpha\)-tocopherol, TBARS and protein were compared between sites using ANOVA (\(F\)). When the model was significant (\(p<0.05\)), pairwise comparison tests were conducted using HSD Tukey-Kramer test. In the event of a skewed distribution of data, the non-parametric Kruskal-Wallis test (\(H\)) (\(\alpha=0.05\)) was used and when significant, was followed by Dunn's
pairwise comparison test ($\alpha = 0.05$). Spearman ($\rho$) correlations were performed to explore relationships between parameters. The means of Fulton’s condition factor and biochemical biomarkers were compared between juveniles and adults using the non-parametric Mann-Whitney U test.

3. Results and discussion

3.1. Morphometric parameters

The morphometry of the yellow perch larvae varied between sites. The weight of larvae from the LSP-N and LSP-S was higher than at the LSL-N site, whereas larvae from the LSP-S were longer than LSL-N (Table 1). For juvenile perch, age, weight and Fulton’s condition factor were similar between sites. Only the circumference for the adult group, although a significant difference of lipids in the muscle of sea bream (Sparus aurata), 10-200 pmole/mg protein were relatively low compared to those measured in the muscle of sea bream (Sparus aurata), 460-990 pmole/mg of protein (Avci et al., 2005). TBARS content in juveniles from LSL-S was significantly higher than in adults ($U_{(1,15)} = 5.92; p < 0.05$). According to Jentzsch et al. (1996), unsaturated lipids would be more appropriate than proteins to normalize data pertaining to lipid peroxidation. In fact, these authors found a strong positive relationship between TBARS and triglycerides values ($r = 0.75; p = 0.003$), suggesting that a high level of triglycerides increases MDA production, resulting in higher TBARS levels.

Several contaminants such as organochlorines (Balk et al., 2011) and metals (Authman et al., 2015) have been identified to alter lipid metabolism or decrease lipid level in fish. These contaminants are present in the St. Lawrence River ecosystem, as evidenced by recent publications (Houde et al., 2014; Giroux et al., 2016; Giraud et al., 2016). Moreover, in the publication by Bruneau et al. (2016) from the same perch sampling, the transcription of some genes linked to lipid metabolism were downregulated: high density lipoprotein-binding protein, hdlbp and lipid sensors acting as transcription factors, PPARY and PPARÎ¿. Although these measurements were carried out without taking into account the stage of development, they suggest an alteration of lipid metabolism in yellow perch, and, consequently, effects on TBARS content.

Like lipids, proteins play an important role in the growth and reproduction of fish (Tocher, 2003). When comparing the concentration of proteins in muscle of the yellow perch, we found significant differences. In juveniles, protein content tended to decrease according to the upstream-downstream gradient with significant differences between LSF compared to LSL-S and LSP (N and S) (Fig. 2-A). In adults, protein values were higher at IB site compared to LSL-S and LSP (N and S) (Fig. 2-B).

Excluding IB site, a downward trend as a function of the gradient was also observed for adults (Fig. 2-B). Some authors reported that contamination or malnutrition, by increasing metabolism, had a detrimental effect on protein levels (Gisbert et al., 2008; Ullah et al., 2014; 2015). Furthermore, this condition can be accentuated by the fact that the yellow perch is not known as a “fatty” fish. The so-called ‘fatty’ fish have high concentrations of lipids in the muscles and the breakdown of the proteins in this tissue occurs after a long period of starving. In contrast, the main source of fat for the ‘non-fatty’ fish is found in the liver. These fish tend to maintain carbohydrate stores (by active gluconeogenesis) at the expense of proteins in the periphery (Moon and Johnston, 1980).

However, adults from the IB site remained an exception. While living in a highly contaminated environment, they had the highest protein concentration compared to all others with significant differences with LSL-S and LSP (N and S) (Fig. 2-B). Additional studies targeting nutritional status and energy metabolism would be helpful to better explain this result.

The TBARS test is often used in combination with the measurement of enzymatic antioxidants (superoxide dismutase, SOD, catalase, CAT, glutathione peroxidase, GPx), which form a significant line of defense against ROS (Di Mascio et al., 1991; Girotti, 1998). In fish exposed to deltamethrin and endosulfan, a significant increase in TBARS and a decrease in antioxidant enzymatic activities were reported (Pandey et al., 2001; Sayeed et al., 2003; Yonar and Sakin, 2011). Increase in lipid peroxidation and decreased CAT, SOD and GPx activities were also other species have been obtained from controlled laboratory studies, where exposure to contaminants and special diets were tested. However, for mere comparison, we refer to the study of Richetti et al. (2011) in which the larvae of zebrasfish (Danio rerio) were exposed to various metals. In this experiment, the TBARS values for the control group was about 1 nmol/g of tissue, which is comparable to what we have obtained for yellow perch larvae (Table 2). The TBARS levels estimated for juveniles and adults (10–80 pmole/mg prot) were respectively 0.05). According to Jentzsch et al. (1996), unsaturated lipids would be more appropriate than proteins to normalize data pertaining to lipid peroxidation. In fact, these authors found a strong positive relationship between TBARS and triglycerides values ($r = 0.75; p = 0.003$), suggesting that a high level of triglycerides increases MDA production, resulting in higher TBARS levels.

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Means were compared between sites by Kruskal-Wallis (\(p \leq 0.05\)). Boxes not sharing the same letters are significantly different (\(p \leq 0.05\)).

DROH (3,4-didehydro-trans-retinol), ROH (all-trans-retinol), DPAL (3,4-didehydroretinyl palmitate), PAL (Retinyl palmitate).

--- Indicates that no measure has been reported.

Means were compared between sites by Kruskal-Wallis (\(H\)) analysis followed by Dunn’s test (\(p \leq 0.05\)).

Means not sharing the same letters are significantly different (\(p \leq 0.05\)).

3.3. Alpha-tocopherol, carotenoids and retinoids

Alpha-tocopherol and carotenoids are the main lipophilic non-enzymatic antioxidants involved in the protection of lipids against 
ROS (Di Mascio et al., 1991; Cavelier et al., 2003). They are recognized as good chemical biomarkers of the state of oxidative stress and, to some extend, as good indicators of the nutritional quality (Burton, 1994; Brigelius-Flohé and Traber, 1999; Gardès-Albert et al., 2003). In LSP larvae (N and S) lower \(\alpha\)-tocopherol values were observed compared to LSL-N (Table 2). In juveniles, hepatic \(\alpha\)-tocopherol levels were similar between sites (Table 3), while those measured in adults at IB site were significantly lower than those measured at LSL-S (Table 3). The concentration of \(\alpha\)-tocopherol may vary depending on the type of tissue. In a study by Machlin et al. (1978) on guinea pigs (Cavia porcellus), \(\alpha\)-tocopherol was sequestered in adipocytes and was not mobilized in cases of food deficiency, resulting in a significant decrease of this vitamin in plasma, as well as in liver and heart tissue. Furthermore, a study of the gilthead sea bream by Mourente et al. (2000) demonstrated the importance of the polyunsaturated fatty acids (PUFA)/\(\alpha\)-tocopherol ratio in predicting the potential of lipid peroxidation. Animals exposed to a PUFA-enriched diet with low \(\alpha\)-tocopherol showed high levels of TBARS.

To our knowledge, this was the first time that carotenoids were measured in yellow perch larvae. This early stage of development was characterized by the dominance of \(\alpha\)-carotene, representing 40.32 \pm 6.25% of total carotenoids. Recognized as an important precursor to retinoids (Combs, 2012c; Alsop et al., 2005; Moren et al., 2002), \(\alpha\)-carotene mean values were similar between sites (Table 2). However, \(\beta\)-carotene mean values were similar between sites (Table 2), while those measured in adults at IB site were significantly lower than those measured at LSL-S (Table 3). The concentration of \(\alpha\)-tocopherol may vary depending on the type of tissue.
lower in individuals collected in LSP-N compared to LSL-N (Table 3). In adults, zeaxanthin and cryptoxanthin values were also significantly lower in the LSP-N sample compared to the LSL-S sample (Table 3). The α-carotene levels were significantly higher in the adults from the LSP-N and LSL-S sites compared to the IB site, whereas the β-carotene concentrations were lower at the LSP-N site than at the LSP-S and LSL-S sites compared to the IB site, whereas the β-carotene concentrations were lower in the LSP-N sample compared to the LSL-S sample (Table 3). The same carotenoids were detected in all stages of development, higher concentrations of carotenoids and fucoxanthin were observed in larvae (Tables 2 and 3). This result is not surprising given that these carotenoids are abundant in phytoplankton and zooplankton (Takaichi, 2011; Pandolfi et al., 2000) which constitute the diet of larvae and, to a lesser extent, that of juveniles. In addition, some carotenoids were higher in juveniles compared to adults: fucoxanthin (LSP-N: \( U_{4;19} = 5.49; p \leq 0.05 \)) and lutein (LSP-N: \( U_{4;19} = 3.59; p \leq 0.05 \)) (Table 3).

Lycopene was the major form of carotenoids in juveniles and adults, with 61.98 ± 5.58% and 52.87 ± 6.07% respectively (Table 3). While it is not recognized as a provitamin A carotenoid, lycopene is known for its strong antioxidant potential, nearly twice that of β-carotene and 100 times that of α-tocopherol (Di Mascio et al., 1991). For example, after exposing carp (Cyprinus carpio) to sub-lethal doses of deltamethrin with or without lycopene, Yonar and Sakin (2011) observed an improved redox balance, reduced lipid peroxidation and increased enzymatic activity in fish receiving the lycopene supplement. These effects were recorded in the liver, kidney, gills and blood. In human beings, the health benefits of lycopene are well known, particularly in protecting against atherosclerosis, certain types of cancer and UV damage (Combs, 2012b; Matos et al., 2000; Rao and Rao, 2007). Moreover, Matos et al. (2000) highlighted the role of lycopene in preserving the redox balance in CV1-P monkey cells exposed to ferric nitroliotriacetic acid (Fe-NTA), plus ascorbate with or without lycopene. Cells supplemented with lycopene experienced reduced lipid peroxidation (77%) and DNA damage (86%) compared to cells without lycopene, indicating the carotenoid’s effectiveness in protecting against free radical attacks. In our study, the comparative analysis of lycopene between sites revealed that fish from LSP had lower values. Significant differences were observed in juveniles between LSP-N and LSL-N and in adults between LSP-N and LSP-S (Table 3). No significant relationship could be found between lycopene and TBARS. However, these two analyses were conducted in different tissues: TBARS was measured in the muscle, while lycopene was measured in the liver. At this point, we may only suppose that the lipophilic antioxidant compounds, stored in the liver, are mobilized and distributed in all tissues including the muscle. It would therefore be appropriate to add liver TBARS measurement to our future studies in order to test the relationship between TBARS, lycopene and α-tocopherol.

In the yellow perch species, the dominant molecular form of retinoids was a dehydro type not only in adults, but also in larvae and juveniles. The characteristic dehydroretinyl compounds (DROH, vitamin A2) compared to retinol (ROH, vitamin A1) lies in the double bond in the 3,4-position of the β-ionone ring. A few studies have demonstrated that vitamin A1 or A2 production in fish could be influenced by the source of carotenoids used as precursors. In tilapia (Tilapia nilotica), xanthophylls provitamin A such as zeaxanthin and lutein were predominantly metabolized to DROH, while with α-carotene and β-carotene, DROH formation was subject to the prior influence (77%) and DNA damage (86%) compared to cells without lycopene, indicating the carotenoid’s effectiveness in protecting against free radical attacks. In our study, the comparative analysis of lycopene between sites revealed that fish from LSP had lower values. Significant differences were observed in juveniles between LSP-N and LSL-N and in adults between LSP-N and LSP-S (Table 3). No significant relationship could be found between lycopene and TBARS. However, these two analyses were conducted in different tissues: TBARS was measured in the muscle, while lycopene was measured in the liver. At this point, we may only suppose that the lipophilic antioxidant compounds, stored in the liver, are mobilized and distributed in all tissues including the muscle. It would therefore be appropriate to add liver TBARS measurement to our future studies in order to test the relationship between TBARS, lycopene and α-tocopherol.

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it could be hypothesized that xanthophylls, not carotenoids, served as provitamins A. In vertebrates, vitamin A surplus from the diet is esterified and stored in the liver, kidneys and other fatty organs. In whole larvae, DROH levels were significantly higher at the LSP-N and LSL-S sites than at the LSL-N site (Table 2). In parallel, DPAL concentrations were also significantly lower in the LSP-N and LSP-S larvae than in LSL-N larvae (Table 2). Similar results were also observed for PAL (Table 2). Overall, it seemed that in sites experiencing greater anthropogenic influences, retinoid stock (DPAL) was highly mobilized, generating high concentrations of DROH, particularly for larvae from the LSP-N and LSP-S sites (Table 2). These results could indicate an imbalance of LRAT (DROH esterification) and HER (DPAL, hydrolisis) enzyme activities (see Fig. S1 for details). In a study on the bullfrog, Boily et al. (2009) demonstrated that frogs exposed to agricultural contamination had a lower hepatic PAL/ROH ratio. Assays of liver microsomes from these individuals revealed that ester hydrolysis activity (HER) was much greater than esterification activity (LRAT).

A variety of xenobiotics (pesticides, dioxins and PCBs) have also been associated with an imbalance of LRAT/HER activities in several vertebrate species, including fish (see review by Novak et al., 2008). Given that the development and growth of yellow perch larvae in LSP takes place in water masses where agricultural contaminants are ubiquitous, and in the absence of other explanatory factors, it is plausible to suggest that some contaminants may have interfered with retinoid-specific enzymes and led to altered concentrations of retinoids in larvae. For now, we do not know the consequences of a high DROH concentration in larvae. The fact that individual analyses of liver and plasma could not be performed for these small fish limits our understanding of retinoid metabolism for this stage of development. However, we know that an unesterified DROH surplus can follow the metabolic pathway leading to the retinoid acid form, a molecule known to disrupt gene regulation and cause defects during embryonic development in vertebrates (Combs, 2012c) including fish (Lall and Lewis-McCrea, 2007).

As observed in larvae, liver vitamin A was found to be lower among juveniles and adults caught from LSP (Fig. 3-A and -B). Specifically, DPAL (Fig. 3-A and -B) and PAL (Table 3) levels were significantly lower in juveniles and adults compared to the LSF site. The DPAL content at LSP-N was also significantly lower than at the LSL-N site. In adults, the DPAL (Fig. 3-B) and PAL (Table 3) levels followed the same trend at LSL and LSP, with low levels at LSP. A significant difference was observed only between the LSP and IB sites; fish caught at IB had higher DPAL concentrations than fish from the LSP sites (north and south) (Fig. 3-B). When testing DROH or ROH (liver and plasma) in juveniles and adults, no significant differences between sites were found in the liver or plasma (Fig. 3-A, -B and Table 3). However, retinol was significantly higher in juveniles than in adults at LSP-N (U(1,44) = 4.40; \( p \leq 0.05 \)). Overall, retinoid results suggest an over-mobilization of the hepatic reserve to maintain basal concentrations of the essential vitamin A. Payne et al. (1998) demonstrated a potential link between oxidative stress and a lessening of liver esters. The authors have studied the effects of high iron contamination in lake trout (Salvelinus namaycush) (Wabush Lake) versus a reference lake (Lake Shabogomo), both located in Labrador (Newfoundland, Canada). Fish caught in Wabush Lake had low liver concentrations of PAL and ROH, as well as damage from DNA. Although iron is known to promote oxidative stress, certain pesticides (atrazine, glyphosate, neonicotinoids, etc.) can also have this effect in fish (Ge et al., 2015; Nwani et al., 2010; Sinhorin et al., 2014). Retinoids may then be altered by non-enzymatic oxidation reactions and the induction of key enzymes involved in xenobiotic detoxification. In a study published in 2002, Zhang et al. sampled silver carp (Hypophthalmichthys molitrix) in a lake contaminated with POPs (PCBs, PAHs, etc.) and measured ethoxyresorufin-O-deethylase activity (EROD) and liver retinoids. EROD activity was negatively related to both ROH and PAL, suggesting that liver retinoid content could be influenced by the induction of CYP450 enzymes following exposure to organochlorinated compounds (OCs). Considering the strong anthropogenic pressures on the IB site, which is located downstream from Montreal and Laval cities, we expected results similar to those observed at LSP, which is also submitted to anthropogenic activities. However, the type of contamination differed between these two sectors. The IB site was characterized by urban and industrial xenobiotics (PBDEs, pharmaceuticals, PCBs, etc.) (Houde et al., 2014; Marcogliese et al., 2015; Lajeunesse et al., 2008), while agricultural contaminants (pesticides and fertilizers) dominated at the LSP (Giroux, 2015; Hudon and Carignan, 2008; La Violette, 2004).

Since the diet changes during the development of the yellow perch, it was expected that retinoids and carotenoids varied in terms of compounds and concentrations. After the disappearance of the yolk sac, the larvae feed primarily on zooplankton and gradually include large benthic invertebrates in their diet up to the juvenile stage (Brown et al., 2009). The diet of adults is more diversified, consisting mainly of invertebrates and small fish. These factors could explain, at least in part, the significant differences between carotenoids and retinoids found in our study. However, ecosystem quality is a key component of available resources, and all disturbances (contaminants, resource exploitation, etc.) of the ecosystem are likely to modulate the habitat structure and consequently the environmental carrying capacity (Hudon et al., 2011; Massicotte et al., 2015).
4. Conclusion

The main objective of this research was to compare yellow perch populations in the St. Lawrence River and evaluate a gradient of anthropogenic disturbances in relation to morphological and biochemical parameters. This study allowed confirmation of a relationship between biological responses and the degree of perturbation, particularly at the LSP site, which is greatly impacted by chemical contamination and human activities (Giroux, 2015; Hudon and Carignan, 2008; La Violette, 2004). This site differed from the upstream sites with respect to the condition factor (general index of physiology) in adults. The peroxidation of lipids as TBARS could be improved by the analysis of unsaturated lipids. The results also highlighted that lycopenes could play a major role as an antioxidant in juveniles and adults of this species. Finally, for the three stages of development (larval, juvenile and adult), alteration of retinoids was observed, particularly at sites associated with greatest anthropogenic stress. Considering that retinoids are essential for reproduction, embryonic development and growth, the retinoid imbalances reported here may be part of the underlying mechanisms responsible for weak recruitment in the LSP yellow perch.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ececoenv.2017.01.051.

References
