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

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ABSTRACT

In Lake Saint-Pierre (LSP), the last great widening of the St. Lawrence River (province of Québec, Canada), the yellow perch has been experiencing a significant decline since the mid-1990s. The combined effect of several stressors (deterioration of habitats appropriate for reproduction and growth, invasive species and poor water quality) seems to exert considerable influence on the yellow perch population in LSP, characterized by low recruitment. To better understand possible stressor effects at the biochemical level, LSP yellow perch were compared with other sites along a gradient of increasing human influences from upstream to downstream along the St. Lawrence River. Morphometry (size, weight, circumference and Fulton's condition factor) and biomarkers associated to the peroxidation of lipids, lipophilic antioxidants (α-tocopherol and carotenoids), along with retinoids (vitamins A1and A2) and proteins were compared between sites at the larval, juvenile and adult stages. Fulton's condition factor was similar between sites for juveniles but was significantly lower in LSP adults, suggesting a weakened physiological condition. In most contaminated sites as LSP, lipid peroxidation tended to be higher in juveniles and adults whereas the lipophilic antioxidant lycopene and proteins content were lower. Retinyl esters were significantly lower for LSP fish compared to other sites, not only in larvae but also in the livers of juveniles and adults. These results are consistent with possible altered metabolism in the retinoid system of LSP yellow perch. The overall results reflect the "pressure" gradient tested, where the yellow perch from the most affected sites located downstream had impaired physiological and biochemical conditions compared to the upstream sectors.

1. Introduction

For decades the yellow perch (*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

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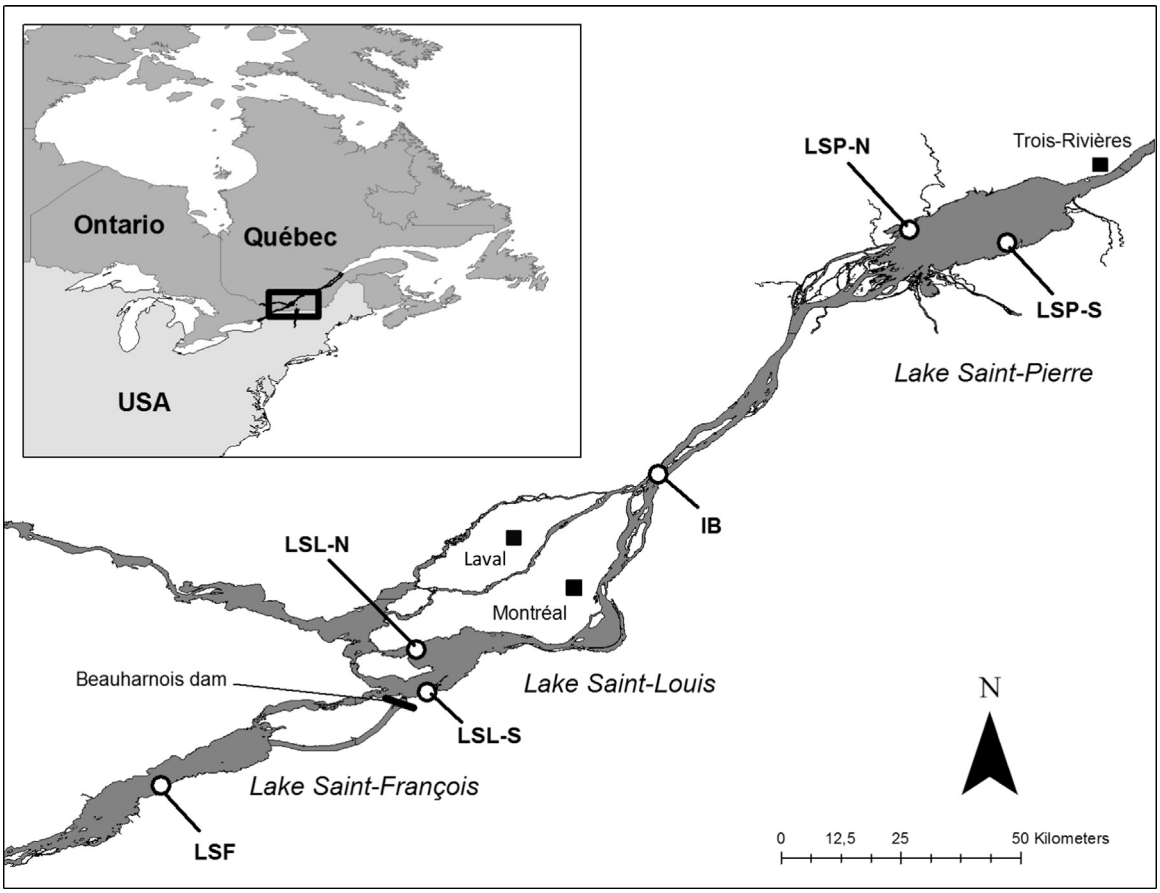

Fig. 1. Location of yellow perch sampling sites (open circles) along the St. Lawrence River. *LSF*, Lake Saint-François; *LSL-N*, Lake Saint-Louis north; *LSL-S*, Lake Saint-Louis south; *IB*, Ile Beauregard; *LSP-N*, Lake Saint-Pierre north; *LSP-S*, Lake Saint-Pierre south.

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, canthaxathin, 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 ($^{\cdot}O_2$), hydroxyl radicals ($^{\cdot}OH$) and peroxyl radicals ($ROO^{\cdot}$) (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 ($ROO^{\cdot}$), particularly lipid peroxyl radicals ($LOO^{\cdot}$), which can metabolize lipids (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,