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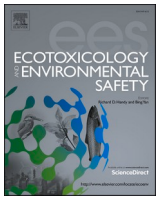

Time-dependent biological responses of juvenile yellow perch (*Perca flavescens*) exposed *in situ* to a major urban effluent

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ABSTRACT

Municipal wastewater treatment plant (WWTP) effluents are significant sources of organic and inorganic pollutants to aquatic ecosystems. Several studies have shown that the health of aquatic organisms can be adversely impacted following exposure to these complex chemical mixtures. The objective of this study was to examine the effects of in situ exposure in the St. Lawrence River (QC, Canada) of juvenile yellow perch (*Perca flavescens*) to a major WWTP effluent. Perch were caged at a reference site in the St. Lawrence River and downstream of a WWTP effluent-influenced site for one, three, and six weeks. Fish kept in controlled laboratory setting were also examined at the beginning of the experiment to evaluate the potential effect of caging on fish. Liver metabolites and gill oxidative stress biomarkers as well as body condition of perch were investigated at four time points (zero, one, three, and six weeks). Nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) stable isotopes as well as tissue concentrations of halogenated flame retardants and trace metals were also analyzed. Results indicated that body condition of perch caged in the effluent increased after three and six weeks of exposure compared to that of reference fish. Perch caged at the WWTP effluent-influenced site also had higher muscle $\delta^{13}C$ and slightly depleted muscle $\delta^{15}N$ after three and six weeks of exposure, suggesting differences in sewage-derived nutrient assimilation between sites. Concentrations of Σ_{34} polybrominated diphenyl ether (PBDE) were 2-fold greater in perch exposed downstream of the WWTP compared to those caged at the reference site. Metal concentrations in kidney of perch after three weeks of exposure were significantly lower at the effluent-influenced site. Kidney concentrations of Cd, Cu, Se, As, Zn and Fe were, however, higher after six weeks of exposure, supporting that metal accumulation is time- and element-specific. The metabolomes of perch from the effluent-influenced and reference sites were similar, but were distinct from the laboratory control fish, suggesting a caging effect on fish. Seven liver metabolites (glucose, malate, fumarate, glutamate, creatinine, histamine, and oxypurinol) were significantly more abundant in perch from cages than in the laboratory control perch. The combination of metabolomics and physiological variables provides a powerful tool to improve our understanding of the mechanisms of action of complex environmental pollutant mixtures in wild fish.

1. Introduction

Municipal wastewater treatment plants (WWTP) represent important sources of environmental contamination to aquatic ecosystems. Depending on waste sources, seasonal conditions and plant treatment processes, the effluent end product composition consists of a complex mixture of biological and chemical compounds. These include a range of organic and inorganic contaminants such as polycyclic aromatic hydrocarbons (PAH), pharmaceuticals, personal care products, flame retardants, and trace elements (Chen et al., 2006; Lajeunesse et al., 2012; Sehonova et al., 2018; Vieno et al., 2005). Studies have shown that exposure to treated effluents may impair plasma cortisol (Ings et al.,

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2011), reproduction (Petrovici et al., 2020; Tetreault et al., 2012; Vajda et al., 2008), immune functions (Burgos-Aceves et al., 2016; Gagné et al., 2013; Houde et al., 2014; Ings et al., 2011), gluconeogenesis (Houde et al., 2014; Ings et al., 2012a), and energy metabolism (Burgos-Aceves et al., 2021; Dépatie et al., 2020; Reinling et al., 2017) in fish residing in receiving waters. The glycolytic capacity of rainbow trout (*Oncorhynchus mykiss*) chronically exposed to municipal wastewater effluents was also shown to be disrupted (Ings et al., 2012a, 2012b) along with cellular changes in the same rainbow trout individuals (Ings et al., 2011). Furthermore, effects on the lipidomic profile and lipid metabolism were reported in northern pike (*Esox lucius*) environmentally exposed to a primary treated wastewater effluent (Dépatie et al., 2020).

Toxicological studies have traditionally focused on the acute impact of single chemicals on aquatic organisms in controlled laboratory settings. However, wild fish are continuously exposed to complex mixtures of contaminants and other natural stressors (e.g., microorganisms, water temperature fluctuations, etc.) in the environment. In contrast to laboratory studies, caging experiments in the field enable exposure of organisms in more realistic environmental and ecological conditions; in situ caging studies have been successfully used to investigate the impacts of environmental contaminants such as metals, PAHs and polychlorinated biphenyls (Beyer et al., 1996; Chesman et al., 2007; Goksøyr et al., 1994) and wastewater effluents (Vincze et al., 2015; Tetreault et al., 2021) on freshwater fish.

Yellow perch (*Perca flavescens*) are indigenous to North America and are of great commercial and recreational importance in the St. Lawrence River, Canada (Mailhot et al., 2015). This species can live in polluted waters (Defo et al., 2012, 2015; Giguère et al., 2005) and tolerate mesotrophic conditions (Eaton et al., 1992). Cumulative effects of anthropogenic stressors have been reported to affect yellow perch inhabiting the St. Lawrence River, from transcriptomic and cellular responses to population level effects (Bruneau et al., 2016; Defo et al., 2018a; Landry et al., 2017). Metal concentrations in yellow perch tissues have also been correlated with biological responses such as retinoid metabolic imbalance (Defo et al., 2012, 2014), impairment of redox homeostasis capacities (Bruneau et al., 2016; Landry et al., 2020), as well as poor health condition (Giraudo et al., 2016).

Traditional biomarkers, such as gene transcription or enzyme activity have predominantly been used to assess the health of fish populations in natural environments (Defo et al., 2015; Giuliani et al., 2013; Houde et al., 2014). However, these targeted techniques are limiting the assessment of responses to specific biological pathways. Since high-throughput technologies such as transcriptomics, proteomics and metabolomics are used as a screening tool, they can help to better elucidate the toxicological effects and associated mechanisms of environmental stressors in aquatic organisms (De Kock et al., 2020; Sun et al., 2019) as well as help identifying novel biomarkers.

Metabolomics, which measures numerous low molecular weight metabolites (e.g., amino acids and simple sugars) was shown to be a highly relevant method to assess the effects of specific environmental conditions and exposure duration on aquatic organisms (Bundy et al., 2008; Cappello, 2021). While providing sensitive insights into biological impacts of contaminant exposure to fish, metabolomic approaches can be used as a screening tool to detect subtle changes in tissue metabolite profiles associated with chemical pollution (Long et al., 2020; Samuelsson and Larsson, 2008). For example, exposure to the pesticide and disinfectant pentachlorophenol was reported to alter the metabolite profile of marine blue mussels (*Mytilus edulis*) resulting in energy availability disturbance for growth, reproduction, and survival (Hines et al., 2010). Moreover, imbalance in muscle metabolite abundance such as lactate, phosphocreatine, alanine or histidine, was observed in fish exposed to crude oil in the laboratory (Van Scoy et al., 2012, 2010). In the same manner, under field conditions, changes in metabolite profiles were also observed in fish, including marine golden grey mullet (*Liza aurata*) exposed to mercury (Cappello et al., 2016) and to complex chemical mixtures including wastewater effluent (Skelton et al., 2014; Southam et al., 2014). These results suggest that environmental contaminants may impact fish health at multiple levels, including osmoregulation, energy metabolism, and antioxidant protection (Alimba and Faggio, 2019; Blahova et al., 2020; Brandão et al., 2015; Burgos-Aceves et al., 2018; Faria et al., 2021; Plhalova et al., 2018; Prokić et al., 2019; Sehonova et al., 2019).

Fish metabolic responses to environmental stressors may also be time-dependent. In order to maintain homeostasis, adaptive response may be compensated over short exposure periods, however, additional adaptive responses involving different metabolic pathways may be activated as the duration of exposure increases. For instance, an increase in the percentage of free dehydroretinol and the transcription level of retinol dehydrogenase-2 in liver of perch caged for four weeks in a metal impacted lake have been observed (Defo et al., 2015). Furthermore, under salinity stress, a number of differentially transcribed genes linked to fitness traits were shown to increase with exposure duration in European flounder (*Platichthys flesus*), indicating a response to local changes in environmental conditions (Larsen et al., 2007).

Few fish caging studies evaluating changes in metabolite profiles have been conducted in situ or under natural conditions. The objectives of this study were to: i) examine the time-associated effects of in situ WWTP effluent exposure on juvenile perch at multiple biological levels (i.e., metabolomic, biochemical, and organismal), ii) determine whether stable isotope signature can be used as proxy of effluent exposure and assimilation of sewage-derived nutrients, and iii) examine if contaminants (metals and halogenated flame retardants) accumulated in tissues of perch exposed to this effluent are related to metabolomic and biochemical responses as well as body condition. To this end, young-of-the-year yellow perch were caged for one, three, and six weeks at a reference site and at an effluent-influenced site located downstream of Montreal's WWTP effluent point of discharge in the St. Lawrence River (SLR; Quebec, Canada).

2. Materials and methods

2.1. Fish husbandry, caging and sampling

Commercially purchased young-of-the-year yellow perch (Station Piscicole Trois-Lacs, QC, Canada) were acclimatized in the Environment and Climate Change Canada's laboratory (Montreal, QC) for two months prior to caging. Upon reception, yellow perch (n = 400; mean weight: 0.85 g) were kept in a 400 L tank containing 350 L of dechlorinated water with a photoperiod set at 16 h light: 8 h dark. During three consecutive days, fish were treated with NaCl (1%) for 1 h in order to reduce the stress induced under transport and to prevent development of diseases from the hatchery. For the first 24 h, fish were not fed and were acclimatized at 15 °C by changing half of the water twice. Throughout the acclimatization period, perch were maintained at the same temperature in order to limit fish growth. Perch were fed daily with Gemma 0.8 (Nutreco, Guelph, ON, Canada) at a ratio of 3% body wet weight (w. w.) that was gradually replaced with frozen brine shrimps (*Artemia salina)* up to 5% of body weight equivalent. In order to prevent mortality due to bacterial disease during the acclimatization period, fish were treated with Aquaflor (15 mg/kg) during 10 consecutive days, which consisted of mixing 250 μL Aquaflor dissolved in vegetable oil with diet before fish feeding.

The walls (5 mm mesh size) of cages (diameter: 1.3 m, volume: 2 m^3) were designed to allow free movement of water and prey in the cages. About 240 fish were distributed in six mesh cages suspended in the water column at a depth of 1.3 m from the surface. Initially, each cage containing 37–40 planktivorous yellow perch (mean body mass ± SEM: 2.71 ± 0.21 g; length: 6.70 ± 0.18 cm) were placed at two sites in the St. Lawrence River. Three enclosures were installed at Canards Island (reference site; 45°42'0.7''N and 73°27'50.6''O) and three cages at Robinet Island (45°44'29.1''N and 73°25'38.6''O) the WWTP-influenced site located downstream of Montreal's WWTP effluent

point of discharge (Fig. 1).

Fish were caged at each site for one, three, and six weeks from September 11th to October 23rd, 2018. Water temperature, dissolved oxygen, conductivity and pH were recorded during the week of deployment, twice a week during the exposure, and at the time of cage retrieval. Moreover, fish in cages were checked for mortality twice a week. During the exposure period, cages were checked twice a week in order to remove algae and other materials susceptible to clogging the cage netting.

At the end of each of the three exposure periods, a sub-group of fish was sampled following the protocol described in Defo et al. (2015) with minor modifications. Briefly, on each sampling day, 6–7 fish/cage/site were transferred to water tanks on a boat, immediately transported ashore, and euthanized in 100 mg/L phosphate buffered MS-222 solution. The total length and weight of fish were recorded and tissue samples were collected on site.

During fish dissection, whole liver of four fish/condition was removed and immediately transferred into a tube on dry ice for metabolomic analyses. The whole kidney and gill as well as part of muscle tissue samples were also removed and transferred into tubes on dry ice for trace metal, enzyme activity and stable isotope analyses, respectively. Fish carcasses excluding liver, kidney and part of muscle were placed in aluminum foil, and frozen on dry ice for flame retardant analyses. Upon return to the laboratory, all tissues were stored at − 80 °C. Furthermore, 20 fish were kept in the laboratory and dissected at the beginning of the experiment (T_0). The same tissues were collected and measurements performed in order to have a baseline of the WWTP effects. All fish husbandry was performed in compliance with Environment and Climate Change Canada's Animal Care Committee, which is accredited by the Canadian Council on Animal Care (Ottawa, ON, Canada).

2.2. Fish condition index

A size-weight relationship was assessed using the Fulton condition index (K) and used as proxy of body condition of yellow perch. This index was used to evaluate the body condition of fish post-caging and calculated as K= [(Weight/Length^3) x 1000] (Ricker, 1975), where "Weight" is the fish weight (g) and "Length" is the total fish length (cm) reported at the end of each exposure period.

2.3. Stable isotope analyses

Based on a method developed elsewhere and applied without modification (Drimmie and Hemmskerk, 2005), stable isotope analysis was performed at the Environmental Isotope Lab, University of Waterloo (ON, Canada). Briefly, dorsal muscle tissue of caged yellow perch was dried, ground into a fine powder, weighed (0.35 ± 0.05 mg) into tin cups, and analyzed for stable carbon (δ^{13}C) and nitrogen (δ^{15}N) isotopes and elemental composition (%) using a Delta Plus XL continuous flow stable isotope ratio mass spectrometer (Thermo Finnigan, Bremen, Germany) coupled to a Carlo Erba Elemental Analyzer (Thermo Scientific, Milan, Italy). Abundance of stable isotopes was expressed in delta (δ) annotation as the deviation from standards in parts per thousand (‰)

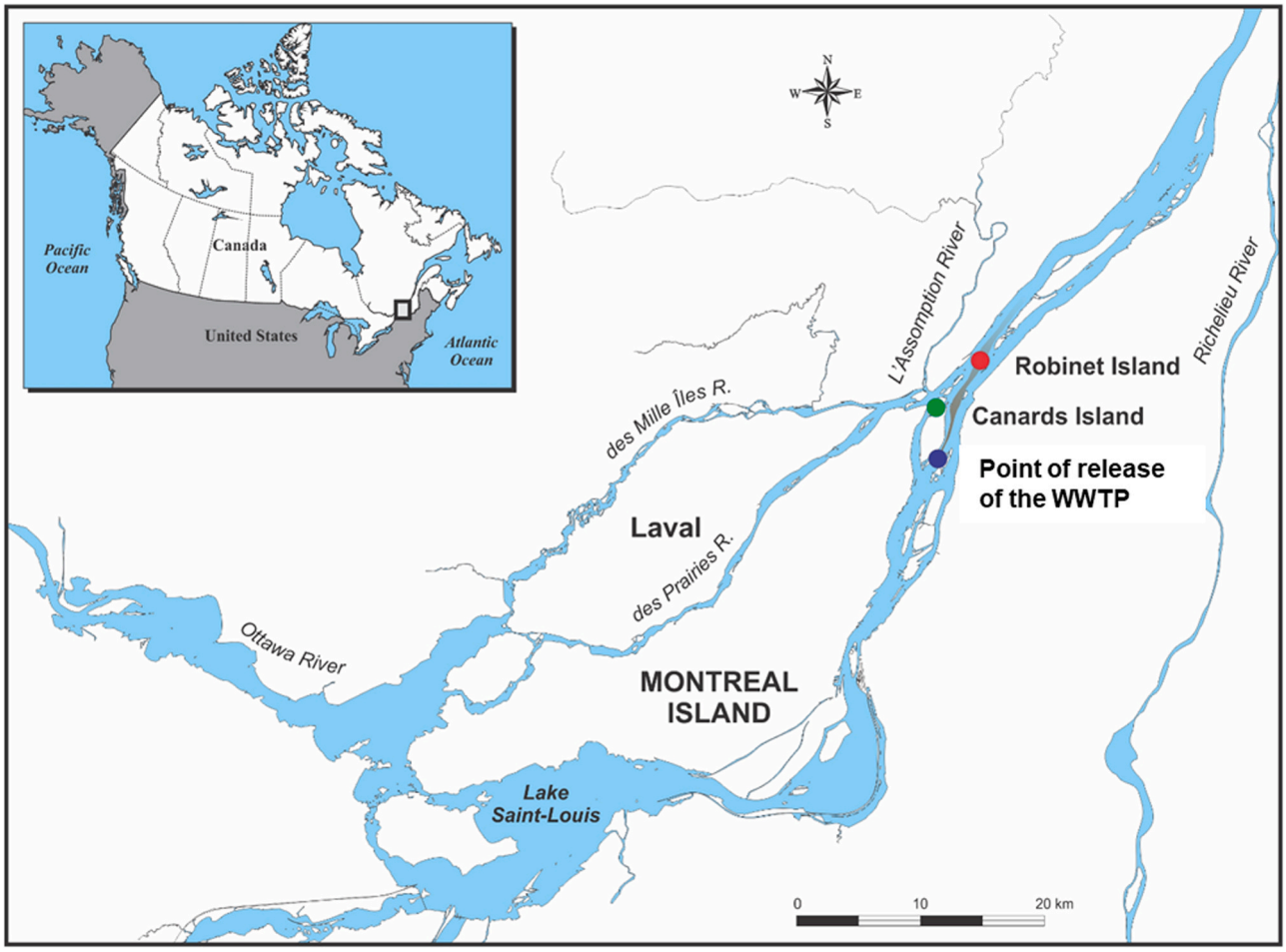

Fig. 1. The cage locations of yellow perch (*Perca flavescens*) deployed in the St. Lawrence River (Quebec, Canada) at Canards Island (reference site, green) and at Robinet Island (red), an impacted site located downstream of Montreal's primary wastewater treatment plant (WWTP, blue) effluent point of discharge. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

using the equation: δ sample ‰ = [($R_{sample}/R_{standard}$) - 1] x 1000, where R is the ratio of heavy to light isotope ($^{15}N/^{14}N$ or $^{13}C/^{12}C$) in the sample and standard.

2.4. Chemical analyses

2.4.1. Metals

Metal concentrations (Cu, Zn, Fe, Se, As, Cd, Ni, and Al) were assessed in yellow perch kidney following the methods described by Pierron et al. (2011) without modification. In brief, lyophilized samples were digested in trace metal grade nitric acid (HNO_3) at room temperature. Five to six samples of certified reference materials from the National Research Council of Canada (TORT and DOLT) were also analyzed in order to monitor for analytical accuracy and recovery. To control for potential contamination during digestion and analytical procedures, blanks (trace metal grade HNO_3) were subjected to the same treatment. Metal concentrations were measured by inductively coupled plasma-atomic emission spectrometry (ICP-AES; Varian Vista XP Axial CCD Simultaneous ICP-AES, Agilent Technologies, Santa Clara, CA, USA) and when the metal concentration detection limit was too low, inductively coupled plasma-mass spectrometry (ICP-MS; Model x-7, Thermo Elemental, Winsford, England, UK) was used. Internal standards (yttrium for ICP-AES and rhodium/rhenium for ICP-MS) were all within 10% of nominal values. Recoveries (mean ± SEM) of metals in TORT and DOLT were 88 ± 7% and 70 ± 5% for Ni, 96 ± 7% and 78 ± 11% for Cu, 108 ± 8% and 84 ± 12% for Zn, 110 ± 8% and 63 ± 8% for As, 100 ± 8% and 71 ± 10% for Se, 95 ± 7% and 71 ± 10% for Cd, and 92 ± 7% and 76 ± 12% for Fe, respectively. Aluminum was not detected in any certified materials.

2.4.2. Halogenated flame retardants

Yellow perch whole body homogenate samples (0.5–1 g) that excluded liver, gills and part of muscle were analyzed at the Université du Québec à Montréal for 34 polybrominated diphenyl ether (PBDE) congeners and 12 other flame retardants including pentabromoethylbenzene (PBEB), hexabromobenzene (HBB), octabromo-1,3,3-trimethyl-1-phenylindan (OBIND), decabromodiphenyl ethane (DBDPE) and dechlorane (Dec)-related compounds (Dec-602, −603, −604, Dec-604 CB), Chlordene Plus (CP) as well as isomers of Dechlorane Plus (*syn*- and *anti*-DP). Sample extraction and clean-up procedures were carried out following methods detailed in Houde et al. (2014) without modification. The total lipid content in homogenate samples was determined gravimetrically, and the results were expressed as percentages to the original wet mass (Reinling et al., 2017). Identification and quantification of PBDEs and other flame retardants were conducted using a gas chromatograph (GC) coupled to a single quadrupole mass spectrometer (MS) (Agilent Technologies 5975C Series, Palo Alto, CA, USA) operating in electron capture negative ionization mode (GC/MS-ECNI). GC separation of target analytes was achieved on a DB-5 HT capillary column (15 m × 0.25 mm i.d. × 0.10 μm film thickness; J & W Scientific).

Quality control and assurance procedures included analysis of procedural method blanks, duplicate samples and standard reference materials (SRM) (NIST 1947; Lake Michigan fish tissue; NIST, Gaithersburg, MD, USA) for each batch of ten samples. Fish homogenate sample concentrations were blank-corrected for BDE-7, − 10, − 17, − 28/PBT, − 47, and − 49. Mean (± SEM) recoveries of spiked internal standards in perch, blank and NIST 1947 samples were as follows: BDE-30 (108 ± 5.6%), BDE-156 (102 ± 3.5%), ^{13}C-BDE-209 (35 ± 13.8%), and ^{13}C-*anti*-DP (101 ± 0.5%). Concentrations of the six PBDE congeners in NIST 1947 (n = 2) showed less than 11.6% variation from the certified values. Method limits of detection (MLODs; defined as signal to noise ratio S/N = 3) and method limits of quantification (MLOQs; minimum amount of analyte producing a peak with S/N = 10) were based on replicate analyses (n = 8) of matrix samples spiked at a concentration of 3–5 times the estimated detection limit. Non-detected values were replaced with values using NDExpo, version 1.0 (http://expostats.ca/site/app-local/NDExpo/). The Expostats toolkit implements robust regression on order statistics (ROS), which is a semi-parametric method to estimate censored data with assumption of log-normal distribution (Lavoué et al., 2019).

2.5. Metabolomic analyses

Yellow perch liver metabolomic analyses were performed according to Izral et al. (2018) with minor modifications. For metabolite extraction, two livers were pooled together to obtain 20 mg; the minimum wet mass required for metabolomic analyses. A total of 26 samples were analyzed using a dual-phase methanol/chloroform/water procedure with a final solvent ratio of 2:2:1.8 (Viant, 2007). The polar fraction was evaporated until dryness using a Savant Speedvac vacuum concentrator (Thermo Scientific). Prior to nuclear magnetic resonance (NMR) analysis, polar samples were suspended in 500 μL pH 7.0 solution of 100 mM sodium phosphate buffer in 90% water and 10% deuterium oxide, containing 3 mM sodium azide and 1 mM of sodium 3-trimethylsilyl-2, 2,3,3-d 4-propionate (TMSP) that acted as internal chemical shift standard. Once re-suspended, samples were vortexed for 60 s, centrifuged for 5 min at 14,000 × *g* at 4 °C, and transferred to 5-mm NMR glass precision tubes for analysis.

Metabolomic analyses of extracted liver tissue samples were performed using a Bruker Advance III HD 500 MHz NMR spectrometer (Agilent Technologies, Palo Alto, CA, USA) set at 500.27 MHz with a 5-mm Bruker TCI cryoprobe. For each liver sample, an excitation sculpting with gradients pulse sequence (Hwang and Shaka, 1995) with a 60° pulse was used to suppress the large water resonance in the spectral data. Data were acquired with an acquisition time of 1.7 s, a 2-s relaxation delay, a 9615.39 Hz spectral width, and 128 scans at 298 K. Each spectra was referenced to the TMSP peak, phased manually, baseline corrected using a polynomial baseline correction, with line broadening of 0.3, and zero filled to 32,768 data points using TopSpin software (v 3.5.5).

Prior to statistical analyses, binned spectral data were filtered using interquartile range, normalized by sum, and autoscaled for statistical analyses. Prometab within MATLAB was used for the conversion of spectra data into a working format (Viant, 2003). The 1D spectra were reduced from 0.80 to 10.00 ppm wide bins to 0.02 ppm after removing the residual water peak (4.7–4.9 ppm), and the total spectral area was integrated and normalized to the TMSP peak area.

2.6. Oxidative stress analyses

Gill tissues of caged yellow perch were assessed for the oxidative stress endpoints glutathione peroxidase (GPx) and glutathione reductase (GR) using the methods outlined in details elsewhere (Massarsky et al., 2013) without modification. The oxidative stress analyses were performed at Environment and Climate Change Canada (Burlington, ON, Canada).

2.7. Data analyses

Metal and halogenated flame retardant concentrations, enzyme activity, stable isotope values, and Fulton condition index were compared between treatment sites (Canard Island and Robinet Island) and exposure periods (zero, one, three, and six weeks) using two-way analysis of variance (ANOVA) after ensuring that the assumptions of normality and homoscedasticity of the error term were verified. When normality was not achieved, even after data transformation (e.g., log), non-parametric paired Wilcoxon tests were applied. Tukey's HSD *post-hoc* test was performed to test differences among conditions. Results were expressed as mean ± SEM and statistical analyses were performed using JMP 16.0 software (SAS Institute Inc.). Probability of $p \leq 0.05$ indicated a statistically significant difference between exposure periods and sites.

In order to assess changes in liver metabolomes among fish exposure conditions, the program MetaboAnalyst 4.0 (Chong et al., 2019) was used for data analysis. We used principal component analysis to visually inspect differences in metabolomes between laboratory and each in situ reared perch condition. Due to the unbalanced design with only one sampling of lab-reared perch, an initial one-way ANOVA was performed in MetaboAnalyst to identify chemical shifts (ppm) that most differentiated between lab-reared and caged perch located at a reference site and downstream of the Montreal's WWTP. The ANOVA with a false discovery rate (FDR; correction $p \leq 0.05$) (Benjamini and Hochberg, 1995) was used to determine important bins. An ANOVA-simultaneous component analysis (ASCA) test was then used to confirm the effects of sewage effluent and exposure periods on the metabolome of caged perch. The ASCA can identify major patterns of two given factors (sewage effluent exposure and exposure period) and their interaction (Smilde et al., 2005). Similar to ANOVA, ASCA can also identify FDR-corrected chemical shifts that differentiate the metabolomes among the exposure conditions.

3. Results and discussion

3.1. Effects of effluent exposure on fish mortality, body condition and stable isotopes

The results of water temperature, dissolved oxygen, conductivity, and pH measurements recorded during the exposure period are presented in Table S1. No changes in water temperature and pH were observed among sampling conditions in the field as a function of the exposure period (Table S1). However, dissolved oxygen was found to be greater in water collected at the reference site, and water conductivity was higher at the effluent-impacted site compared to the reference site (Table S1).

For all sites and exposure periods, low mortality rates of yellow perch were recorded during the experiment (0–5.4%); the highest percentage of mortality was observed in cages located at the reference site (Table 1). Cage mortality mainly occurred during the first days of the experiment and was likely due to stress associated with transport of fish to the site and captivity in the field. For all three exposure periods, caged perch had a lower body condition index compared to fish raised in the lab (Table 1), suggesting potential stress induced by caging or field transplantation itself, or greater energy expenditure due to continuous current at these sites. This observation supported those of other studies that found that confinement-related stress had negative effects on fish (Borcier et al., 2019; Catteau et al., 2019). For instance, compared to fish kept in situ during the same period of time, a proteomic disruption of heat stress related pathways, which is a marker of exposure to stressful conditions, was reported in caged European flounder (Borcier et al., 2019). However, in our study, after three and six weeks of exposure, the Fulton condition index (K) was higher in yellow perch exposed to the municipal WWTP effluent compared to fish from the reference site (Table 1). These results may be explained by greater food availability in the municipal wastewater impacted site compared to the reference site (DeBruyn et al., 2003). These findings may be an explanation to the low mortality rate of perch observed in the municipal wastewater impacted site (Table 1).

Muscle tissue $\delta^{13}C$ and $\delta^{15}N$ signatures were used to verify the exposure of perch to the effluent and sewage-derived nutrients. No difference in $\delta^{13}C$ values was found for caged fish among the different sites and exposure periods (Table 1). However, compared to the reference fish, lower $\delta^{15}N$ values were found after three and six weeks in fish caged downstream of the WWTP (Table 1). The difference in $\delta^{15}N$ signatures between these sites suggests potential effects of the effluent on yellow perch diet. This result is consistent with that of DeBruyn et al. (2003), who reported a muscle $\delta^{15}N$ shift in primary and secondary consumers such as perch when exposed to this municipal effluent in the St. Lawrence River. Moreover, a study carried out at two WWTP discharging effluents into the Grand River (southern Ontario, Canada) has reported a depletion of $\delta^{15}N$ in the muscle of rainbow darter (*Etheostoma caeruleum*), which correlated with ammonia levels in receiving waters (Hicks et al., 2017). The reduction in $\delta^{15}N$ in the diet of perch residing in effluent-impacted areas could be related to larger proportions of microorganisms consuming nutrients, thus resulting in lower tissue $\delta^{15}N$ values (Robinson et al., 2016). Information on the water chemistry of the effluent could not be collected during the course of the study. However, water chemistry of this effluent has been characterized in a previous study (Marcogliese et al., 2015).

The Montreal's WWTP discharges between 2.5 and 7.6 million m^3 of water daily into the St. Lawrence River depending on weather conditions (Marcogliese et al., 2009), thus representing the largest amount of treated water discharged into this aquatic ecosystem (Marcogliese et al., 2015). Although treated, the Montreal's WWTP effluent contains contaminants at levels that may be harmful to fish and other organisms (Marcogliese et al., 2015). Compared to the influent, the current primary wastewater treatment provides significant reduction (20–60%) in suspended solid matter, phosphorus and metal concentrations (Cyr et al., 2015). In contrast, emerging contaminants including pharmaceutical products are minimally reduced in the effluent compared to concentrations in the influent (Cyr et al., 2015). Organic matter under dissolved and particulate forms also represents a major fraction of effluents in aquatic environments (Michael-Kordatou et al., 2015). It is possible that there was more organic matter in the cages installed in the effluent plume than in those from the reference site, as suggested by the presence of more biofilm and algae on cages (M. Defo; personal observations). Moreover, fish caged at the effluent impacted site were generally heavier (Table 1), but had lower tissue $\delta^{15}N$ than those caged at the reference site (Table 1). In future experiments, evaluating prey items in the vicinity of the cages and stomach content of fish would help understanding these differences between fish caged at reference sites and downstream of WWTP effluents.

Table 1
Mortality, body condition, total length, weight and stable isotopes of yellow perch following exposure to the WWTP effluent in the St. Lawrence River. Results are expressed as mean ± SEM. Different letters indicate a statistically significant difference ($p \leq 0.05$) among treatments.

Exposure site	Laboratory	Reference site (Canards Island)			Effluent-Impacted site (Robinet Island)		
Exposure time (weeks)	0	1	3	6	1	3	6
Exposure condition	T0	T1	T2	T3	T1	T2	T3
Number of fish (N)*	20	37	40	38	40	38	39
Percentage (%) of mortality#	0	5.4	5	0	0	2.6	0
Fulton condition index (K)	8.69 ± 0.14^{a}	7.67 ± 0.06^{bc}	7.68 ± 0.20^{cd}	6.59 ± 0.14^{e}	7.46 ± 0.12^{cd}	8.03 ± 0.13^{b}	7.33 ± 0.10^{d}
Fish length (cm)	6.70 ± 0.18^{a}	6.25 ± 0.11^{abc}	6.32 ± 0.10^{abc}	6.10 ± 0.10^{bc}	5.97 ± 0.15^{c}	6.27 ± 0.11^{abc}	6.55 ± 0.13^{ab}
Fish weight (g)	2.71 ± 0.21^{a}	1.92 ± 0.10^{bc}	1.97 ± 0.10^{bc}	1.52 ± 0.07^{c}	1.65 ± 0.11^{bc}	2.00 ± 0.10^{bc}	2.11 ± 0.12^{b}
$\delta^{15}N$ (‰)	11.22 ± 0.08^{ac}	11.37 ± 0.05^{b}	11.24 ± 0.06^{ab}	11.29 ± 0.09^{ab}	11.24 ± 0.07^{ab}	10.71 ± 0.10^{c}	10.17 ± 0.22^{c}
$\delta^{13}C$ (‰)	-20.72 ± 0.08^{a}	-20.58 ± 0.04^{bc}	-20.62 ± 0.05^{bc}	-20.64 ± 0.07^{bc}	-20.79 ± 0.07^{ab}	-20.62 ± 0.02^{ac}	-20.56 ± 0.06^{c}

*Number of juvenile fish per cage at the beginning of the experiment. # Percentageof fish mortality per cage at the end of the experiment. T0, T1, T2, and T3 refer to laboratory (control) and one, three and six weeks exposure period in the field, respectively.

3.2. Concentrations of contaminants

3.2.1. Halogenated flame retardants

Concentrations and limits of detection of halogenated flame retardants analyzed in perch whole body homogenates are presented in Table 2. BDE-47, − 49, − 99, and − 100 were the most abundant congeners in fish and their mean contributions to the sum of 34 PBDEs (Σ_{34}PBDE) were 47%, 15%, 10%, and 8%, respectively. Pentabromoethylbenzene (PBEB) and HBB were also quantified in most whole body homogenate, although at very low concentrations compared to the major PBDEs (<1 ng/g ww; Table 2).

In the reference site, Σ_{34}PBDE concentrations were not different among sample periods, however, in the effluent impacted site, a significant difference was found between week three and six (Table 2). Concentrations of Σ_{34}PBDE were approximately 2-fold greater in fish exposed downstream of the WWTP compared to fish caged at the reference site (range of means: 1.6–3.1 ng/g ww and 2.8–6.2 ng/g ww, respectively, from one week to six weeks). In contrast, PBEB and HBB concentrations were not different between the sites. These results corroborate those of Houde et al. (2014) who also found greater PBDE concentrations in perch caught downstream of the Montreal's wastewater treatment facility around the same area where the present cages were deployed, although no other flame retardants were detected in these perch whole body homogenates. Concentrations of Σ_{34}PBDE in the present study were, however, 260-fold lower than levels reported for wild yellow perch (Houde et al., 2014). Moreover, elevated Σ_{34}PBDE concentrations in female (167 ± 29.3 ng/g ww) and male (393 ± 110 ng/g ww) adult northern pike, a top predator, environmentally exposed to the same urban effluent were also reported (Reinling et al., 2017). Consistent with our findings, Σ_{34}PBDE levels in liver of northern pike caught downstream of Montreal's WWTP were approximately 5-fold greater than in fish collected upstream (Dépatie et al., 2020). These differences in PBDE concentrations between studies could be attributed to fish age, tissues analyzed, and/or fish ecology and diet.

Furthermore, at both sites, a 2-fold increase in Σ_{34}PBDE concentrations was observed over the exposure period in perch, from one to six weeks (Table 2). Moreover, Σ_{34}PBDE concentrations were also greater in field-exposed fish than in laboratory perch; Σ_{34}PBDE levels were 1.2-, 1.7-, 2.5- time greater in fish caged at the reference site after one, three and six weeks, respectively, and Σ_{34}PBDE levels were 2.2-, 3.2- and 5.0-time greater in fish caged downstream to the WWTP effluent after the same periods compared to the T0 fish (Table 2).

3.2.2. Metal concentrations

After one week of exposure, the sum of kidney metal concentrations was higher in caged perch than in lab control fish (Fig. S1). After three weeks of exposure, kidney concentrations of Cd, Cu, and Zn were significantly lower (approximately 1.5-fold) in the kidney of perch at the effluent influenced site compared to the reference site, whereas no such difference was observed for the metalloids As and Se (Fig. 2), suggesting time-dependent and element-specific metal accumulation in perch. However, after six weeks of exposure, a significant increase in kidney concentrations of all metals examined was observed in fish caged at the effluent impacted sites compared to those caged at the reference site (Fig. 2). These changes could be related to temporal variability in effluent metal composition or parameters influencing metal bioavailability including organic carbon content between week three and six. Metal concentrations in perch kidney can also reflect the metal loadings in water and sediment at the two sites. Gagnon et al. (2006) reported major differences in aqueous and particulate metal concentrations among sites located downstream and upstream of the dispersion plume of this effluent. Specifically, dissolved and particulate metal concentrations including Cd, Ag, Cr, Cu, Ni, and Zn were typically higher in sites located downstream of Montreal's WWTP (8 km) compared to an upstream site (Gagnon et al., 2006; Gillis, 2012). Concentrations of dissolved organic carbon and suspended particulate matter between the sites were not measured in the present study, although it has been well demonstrated that metal bioavailability and bioaccumulation strongly depend on physical and chemical characteristics of water such as concentrations of suspended particulate matter and dissolved organic carbon in wastewater effluents (Gagnon et al., 2006). Similarly, concentrations of a suite of metals (Zn, Pb, Al, Rb, Mg, Na, Ca, Sr, and V) were also reported to be greater in whole body homogenates of wild yellow perch environmentally exposed in the St. Lawrence River to this primary WWTP effluent, compared to those caught in an upstream site (Houde et al., 2014). In contrast, a study reported that gill concentrations of Ag, Cd, Cr, Ni, and Pb in the mussel *Elliptio complanata* caged 8 km downstream of Montreal's WWTP were significantly lower than in those caged at a reference site; the metal concentrations were, however, higher in mussels caged 12 km downstream of the effluent plume (Gagnon et al., 2006). The discrepancies between studies could be related to the different accumulation/elimination between species and/or to the influence of other unidentified sources of metals to the ecosystem downstream of the effluent outfall.

Table 2
Concentrations (mean ± SEM; ng/g ww, n = 3/condition) of halogenated flame retardants quantified in whole body homogenates of yellow perch sampled after one, three, and six weeks of exposure to a WWTP effluent in the St. Lawrence River. Values are means ± SEM. Different letters indicate significant differences among treatments ($p \leq 0.05$).

			Laboratory	Reference site (Canards Island)			Effluent-impacted site (Robinet Island)		
Exposure condition	MLOD[♭]	MLOQ[♯]	T0*	T1	T2	T3	T1	T2	T3
Total lipid content (%)			5.00 ± 0.47^{a}	3.47 ± 0.15^{ab}	2.58 ± 0.34^{b}	2.64 ± 0.52^{b}	4.01 ± 0.44^{ab}	2.95 ± 0.60^{ab}	2.83 ± 0.52^{ab}
Σ_{34}PBDE[♪]	0.01–0.09	0.01–0.76	1.26 ± 0.08^{d}	1.57 ± 0.24^{cd}	2.20 ± 0.26^{cd}	3.12 ± 0.64^{bc}	2.75 ± 0.17^{bcd}	4.01 ± 0.33^{b}	6.19 ± 0.29^{a}
Σ_{7}Dec	0.01–0.14	0.01–0.47	< 0.075	< 0.075	< 0.075	< 0.075–0.38	< 0.075	< 0.075	< 0.075–0.28
PBEB	0.01	0.01	0.020 ± 0.010^{a}	0.010 ± 0.003^{a}	0.050 ± 0.020^{a}	0.03 ± 0.007^{a}	0.020 ± 0.005^{a}	0.02 ± 0.007^{a}	0.03 ± 0.009^{a}
HBB	0.01	0.01	0.060 ± 0.006^{b}	0.100 ± 0.020^{b}	0.160 ± 0.050^{ab}	0.290 ± 0.050^{a}	0.090 ± 0.026^{b}	0.200 ± 0.050^{ab}	0.17 ± 0.017^{ab}
OBIND	0.06	0.19	< 0.06	< 0.06	< 0.06	< 0.06	< 0.06	< 0.06	< 0.06
DBDPE	0.36	1.21	< 0.36	< 0.36	< 0.36	< 0.36	< 0.36	< 0.36	< 0.36
DBCD	0.20	0.60	< 0.20	< 0.20	< 0.20	< 0.20	< 0.20	< 0.20	< 0.20

*T0, T1, T2, and T3 refer to laboratory (control) and one-, three- and six-week exposure period in thefield, respectively.Polybrominated diphenyl ether (PBDE); dechlorane(Dec); pentabromoethylbenzene (PBEB); hexabromobenzene (HBB); octabromo-1,3,3-trimethyl-1-phenylindan(OBIND); decabromodiphenylethane (DBDPE). ♪ Σ34PBDE: sum of BDE-7, −10, −15, −17, −28/PBT, −47, −49,−66, −71, −77, −85, −99, −100, −119, −126, −138, −139, −140, −153, −154/BB-153,−171, −180, −183/Dec-604, −191, −196, −197/-204, −201, −203,−205, −206, −207,−208, and −209. Σ7Dec: sum of Dec-602, −603, −604, −604 CB, chlordene plus (CP), syn-DP/BEHTBP, and anti-DP. ♭ Method limits of detection (MLODs; defined as signalto noise ratio (S/N) = 3). ♯Method limits of quantification (MLOQs; minimum amountof analyte producing a peak with S/N = 10). BDE-15, -71, -119, -138, -139, -140, -171, -180, -184, -191, -201, -205, -206, -207, -208, -209, OBIND, DBDPE, Dec-601,-602,-603, -604CB, Cplus, and syn-DP were not detected in any of the samples analyzed.

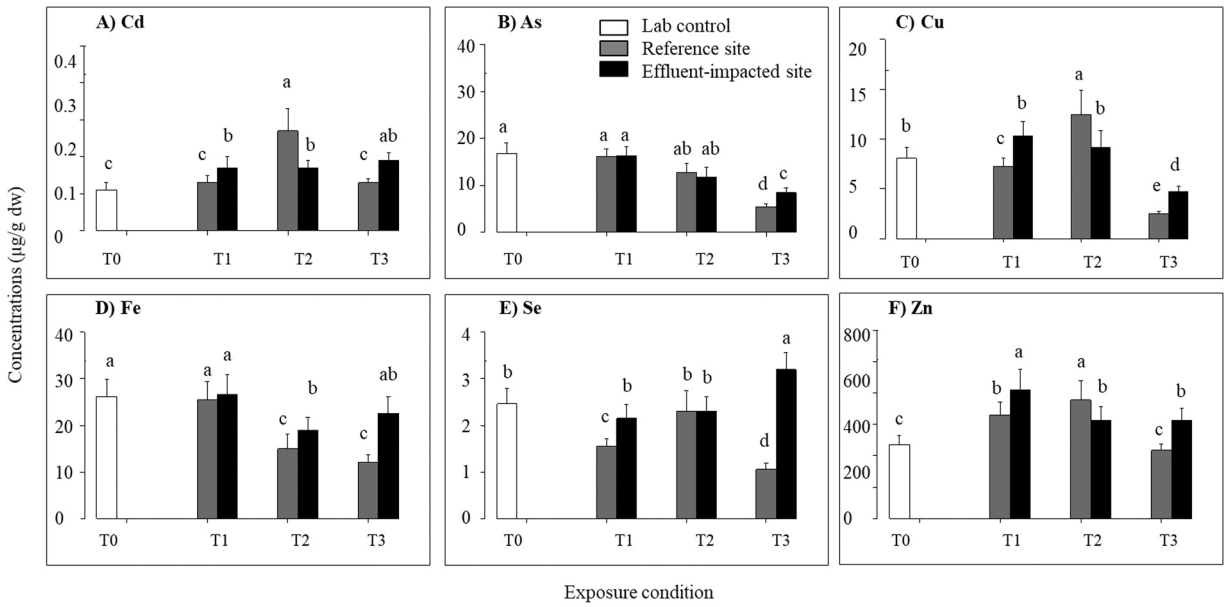

Fig. 2. Concentrations (µg/g dw) of A) cadmium (Cd), B) arsenic (As), C) copper (Cu), D) iron (Fe), E) selenium (Se), and F) zinc (Zn) in kidney of yellow perch (*Perca flavescens*) sampled in the laboratory (Lab control) and after one, three, and six weeks of in situ exposure to a municipal effluent or at a reference site in the St. Lawrence River. Values are means ± SEM. Different letters indicate significant differences among treatments ($p \leq 0.05$). T0, T1, T2, and T3 refer to laboratory (control) and one, three and six week exposure period in the field, respectively.

3.3. Effects of effluent exposure on metabolomic profiles

Most of the variation explained in the yellow perch liver metabolome (51%) was along the first principal component axis (PC1) and was associated with differences in exposure period of yellow perch as the metabolomic profiles progressed from week one through week six (Fig. 3). However, the yellow perch liver metabolome collected at week six most resembled that of the laboratory-raised fish (Fig. 3). In addition, there was some apparent separation in the liver metabolome between reference and effluent-exposed yellow perch at week six (Fig. 3), although a much larger sample size would be required to statistically confirm this pattern. The second principal component axis (PC2) explained an additional 28% of the variation in the yellow perch liver metabolome, although it did not improve the discrimination of treatments according to exposure condition or duration. The results from the present study indicated that the liver metabolome of young-of-the-year perch exposed to a WWTP effluent was not altered regardless of the duration of the exposure.

Seven metabolites (glucose, malate, fumarate, glutamate, creatinine, histamine, and oxypurinol) were on average 2.7-fold more abundant in liver of perch exposed in cages compared to those held in the laboratory, with the exception of glucose that was 3.5-fold more abundant (Table 3). In contrast, liver glycogen was 2.7-fold less abundant in caged relative to laboratory perch (Table 3). A majority of these metabolites are associated with energy metabolism and thus body maintenance and growth of caged yellow perch from week one through week six relative to the laboratory-raised perch. These results are somewhat consistent with the significant changes in body weight observed between the caged fish groups and the laboratory-exposed group (Table 1). When considering metabolite abundance over the exposure duration, glucose, malate, fumarate, creatinine, histamine, oxypurinol and glutamate significantly increased in caged perch compared to laboratory reared fish, whereas glycogen, a metabolite that is broken down in the liver into glucose and can be used to fuel maintenance and growth, significantly decreased.

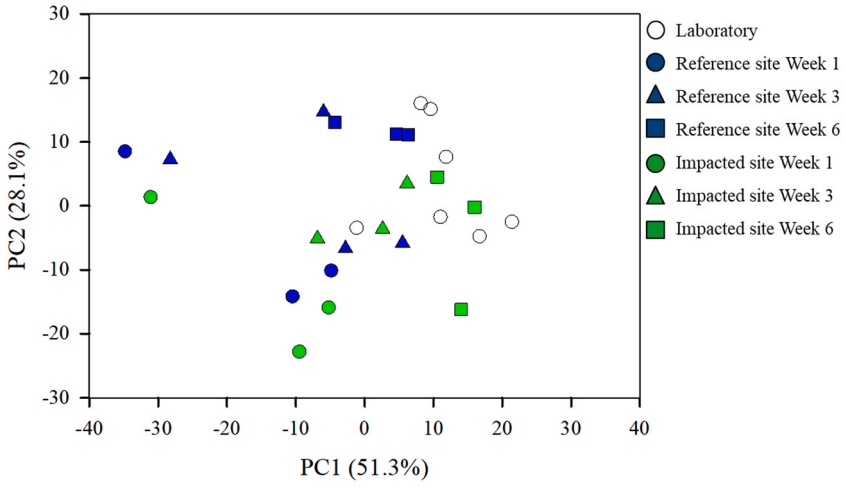

Fig. 3. Principal component scores of yellow perch sampled in the laboratory (controls) and after one, three, and six weeks of in situ exposure to an effluent-impacted site and at a reference site in the St. Lawrence River.

Table 3
Normalized metabolite abundance (mean ± SEM) of juvenile yellow perch (*Perca flavescens*) raised in the laboratory compared to those reared in the St. Lawrence River that were either exposed to the municipal WWTP effluent (Robinet Island) or at a reference site (Canard Island). The metabolite abundance represents the average over time. Different letters indicate significant differences among the treatments based on the false discovery rate.

Metabolite	Treatment				
	Laboratory	Reference site (Canards Island)	Effluent-impacted site (Robinet Island)	F value	False Discovery Rate
Glutamate	-1.42 ± 0.08^{b}	0.31 ± 0.32^{a}	0.50 ± 0.24^{a}	9.76	0.017
Malate	-1.02 ± 0.09^{c}	0.02 ± 0.24^{b}	0.77 ± 0.31^{a}	11.85	0.010
Creatinine	-1.27 ± 0.17^{b}	0.39 ± 0.23^{a}	0.55 ± 0.22^{a}	18.69	0.003
Glucose	-1.17 ± 0.20^{b}	0.51 ± 0.30^{a}	0.34 ± 0.17^{a}	12.94	0.009
Fumarate	-1.01 ± 0.07^{b}	0.48 ± 0.37^{a}	0.26 ± 0.18^{a}	7.67	0.033
Histamine	-1.32 ± 0.14^{b}	0.49 ± 0.24^{a}	0.48 ± 0.18^{a}	22.84	0.001
Oxypurinol	-1.23 ± 0.06^{b}	0.37 ± 0.28^{a}	0.54 ± 0.21^{a}	15.98	0.003
Glycogen	1.12 ± 0.16^{a}	-0.47 ± 0.26^{b}	-0.35 ± 0.28^{b}	18.05	0.002

The decrease in liver glycogen and the increase in tricarboxylic acid cycle (TCA) metabolites, both involved in energy production and growth, suggests a transplanting, acclimation and/or caging effect as body condition of caged fish was lower compared with laboratory fish (Table 1). This further suggests an increased reliance on internal energy reserves from liver glycogen and possibly body lipids, as those were also lower in caged fish compared to laboratory fish (Table 2). Worthwhile mentioning was the increase in glutamate, which has several metabolic functions beside energy metabolism. Specifically, glutamate is a precursor in the production of glutathione (Newsholme et al., 2003); hence an increase in glutamate could also explain the increase in gill GPx and GR activity measured in caged perch compared to laboratory fish (Fig. 4). In fact, compared to control fish, an increase of glutamate was observed in liver of zebrafish exposed to WWTP effluents, and this observation appeared to coincide with a decrease in glutamine and glutathione concentrations (Zhen et al., 2018). This result suggested a disturbance of the glutamate metabolism pathway by WWTP effluent exposure in zebrafish liver.

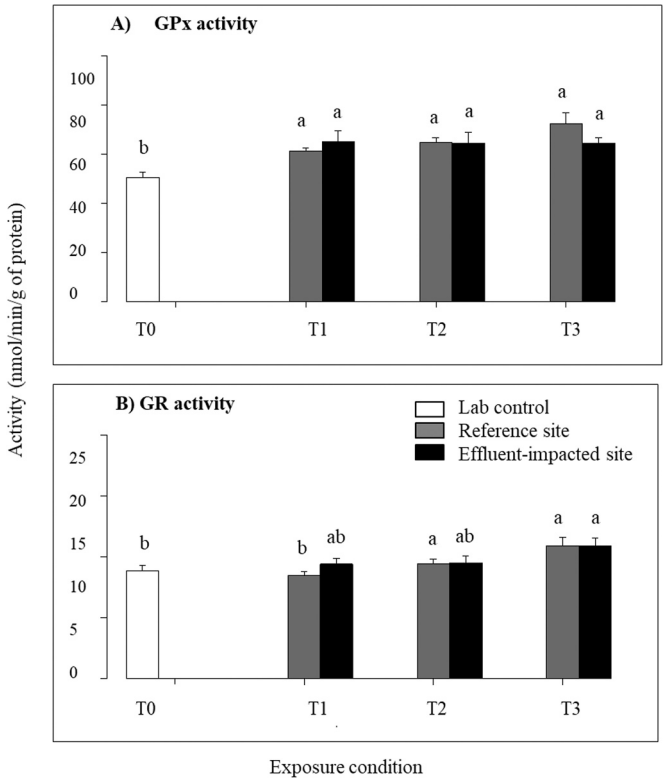

Fig. 4. A) Gill glutathione peroxidase (GPx) and B) glutathione reductase (GR) activity in yellow perch sampled after one, three and six weeks exposure to the municipal WWTP effluent (Robinet Island) or at a reference site (Canard Island). Values presented are means ± SEM. Different letters and uppercase/lowercase indicate significant differences among treatments ($p \leq 0.05$). T0, T1, T2, and T3 refer to laboratory (control) and one, three, and six weeks exposure period in the field, respectively.

Beside the caging effect, the effluent exposure effects on yellow perch liver metabolomic profiles were also observed between sites. Among the eight liver metabolites that were found to vary as a function of site exposure, only malate was found to be 38.5-fold more abundant in fish caged at the effluent impacted site compared to those caged at the reference site (Table 3). Malate belongs to the five TCA intermediates whose concentrations can affect the metabolite fluxes into and out the TCA cycle, and thus the aerobic ATP production (Gibala et al., 2000). This metabolite is dehydrogenated to oxaloacetate, which is one the most critical metabolite that controls the rate of ATP production in aerobic cells (Bendahan et al., 2002). Although we are not aware of any studies that specifically addressed the potential linkages between malate disturbance and effluent exposure in fish, Dépatie et al. (2020) reported associations between municipal WWTP effluent exposure and perturbation of lipid metabolism in the liver of northern pike naturally exposed in the St. Lawrence River. Indeed, compared to pike collected upstream of the WWTP, greater concentrations of saturated and monounsaturated lysophosphatidylcholines and lower levels of polyunsaturated lysophosphatidylcholines were reported in pike liver sampled downstream of the WWTP (Dépatie et al., 2020). Furthermore, a down-regulation of genes coding for lipid metabolism, including peroxisome proliferator-activated receptor alpha (*pparα*) (Dépatie et al., 2020) and acyl-coA oxidase (*acox*) (Reinling et al., 2017) were also measured in pike collected downstream of a major primary WWTP in the St. Lawrence River compared to those living upstream in this same river.

When comparing only the metabolomes of caged perch at the reference and effluent-exposed sites, results from the two-way ASCA analysis revealed no site difference ($p = 0.13$), although differences among sampling weeks ($p < 0.01$) were observed in the PCA. There was, however, no interaction between exposure and week of sampling ($p = 0.75$), suggesting that the liver metabolome of reference and effluent-exposed fish responded similarly over time. The only metabolite that varied significantly among time periods was glucose, which increased in abundance from week one to week six for both cage sites. This result is consistent with the above analysis, suggesting that the primary change in liver metabolomes was associated with energy mobilization in caged yellow perch as opposed to contaminant exposure. Despite higher concentrations of metals in kidney and Σ_{34}PBDE in whole body homogenates in effluent-exposed perch at week six, liver metabolome profiles were similar between the wastewater effluent and reference sites. This is potentially related to exposure duration (only six weeks), contaminant dilution and it bioavailability, and tissue-specific metabolomic responses. Williams et al. (2014) also used a transcriptomics and NMR-based metabolomics approach, and detected liver alterations in the transcriptome (e.g., perturbation of immune response and apoptotic pathways) in European flounder exposed for seven months to estuarine sediment contaminants including PAHs,

polychlorinated biphenyls, and metals. Moreover, Meador et al. (2020) reported that several pathways involving amino acid metabolism, nucleotides, steroids, fatty acids, and the Krebs cycle were altered in plasma metabolomes of juvenile Chinook salmon (*Oncorhynchus tshawytscha*) after four weeks exposure to a wastewater effluent-receiving estuary containing many contaminants of emerging concern. These chemicals included pharmaceuticals (antibiotics, metabolism-regulating drugs, and neurological medicines), personal care products as well as industrial compounds such as PBDEs, perfluoroalkyl substances (PFASs), alkylphenols, bisphenol A, and phthalates (Meador et al., 2020).

Although there was some apparent separation of week six liver metabolomes between effluent-exposed and laboratory control perch, a larger sample size would be required to confirm this observation. Also, a longer exposure period may be needed in systems with high dilution of contaminants related to fish growth as observed in the St. Lawrence River. In addition, fish studies have reported tissue-specific metabolomic responses to contaminants or wastewater effluent (Izral et al., 2020; Li et al., 2014; Lin et al., 2009), which may explain why no differences in liver metabolomes were found despite the increased concentrations of contaminants in tissues. Our study has provided a good insight into the utility of metabolomics to discriminate between laboratory control fish and field caged perch. We are not aware of studies reporting the transplanting or caging effects on fish metabolomics. However, liver transcriptional responses of juvenile yellow perch have been reported to be affected by caging itself (Bougas et al., 2016).

3.4. Oxidative stress responses

Environmental exposure to the effluent did not lead to a significant change in yellow perch gill glutathione peroxidase (GPx) or glutathione reductase (GR) activity at the end of the 6-week exposure compared to reference groups (Fig. 4 A and B). Glutathione reductase and GPx are antioxidant enzymes that contribute to the clean-up of cellular free radicals, including reactive oxygen species (ROS). While GR is involved in cellular glutathione pool reduction (Mourente et al., 2002), GPx participates to the H_2O_2 and lipid hydroperoxydes scavenging (Winston and Di Giulio, 1991). In the present study, the contaminant concentrations or duration of exposure may not have been sufficient to trigger redox reactions that, in turn, would have generated free radicals in the gills of these perch. It is also possible that metal-exposed perch have developed different non-enzymatic defense strategies such as an increase in metallothionein levels (Giguère et al., 2005) to cope with oxy-radical production. However, positive relationships between sum of kidney concentrations of essential metals (Cu, Fe, Se, and Fe) and gill GPX activity were observed ($\rho = 0.24$; $p = 0.033$) (Table S2), suggesting a potential impact of these inorganic contaminants on oxidative stress responses in yellow perch. Significant positive relationships were also found between gill GPX activity and kidney Zn and Se concentrations (Table S2). As evidenced by a reduction in GR activity, lipid peroxidation and glutathione concentrations, limited oxidative stress in liver of perch caught in metal impacted lakes were associated with metal bioaccumulation compared to fish living in a less metal impacted environment (Giguère et al., 2005). Compared to the reference group, a decrease in GR activity coupled to reduced thiol levels was previously observed in gills of yellow perch exposed to complex mixtures of contaminants in the St. Lawrence River (Dautremepuits et al., 2009). Interestingly, no change in either gill oxidative stress related biomarkers was observed in the present study, suggesting the prevalence of non-enzymatic mechanisms in the biological defenses against environmental chemical pollution.

Gill tissue was used in the present study as liver samples of young-of-the-year perch were very small and used entirely for metabolomic analysis. Although in this study no change was observed, gills have previously been used to assess oxidative stress responses in fish. For example, an increase in activity for glutathione-S-transferase (GST) and superoxide dismutase (SOD) was observed in gills of rainbow trout and fathead minnow (*Pimephales promelas*) exposed in situ to municipal wastewater (Tetreault et al., 2021). Catalase activity in gills was also significantly reduced in wastewater exposed of fathead minnows (Tetreault et al., 2021). However, a more comprehensive tissue comparison would give a more complete picture to assess oxidative stress responses in WWTP effluent exposed fish.

Although cages used in this experiment were designed to minimize stress associated with confinement, results indicated that transplanting or caging had an effect on fish oxidative stress and metabolomic profiles. Indeed, after six weeks of caging, a significant increase in gill GPx and GR activities was observed relative to laboratory fish (Fig. 4). Moreover, juvenile perch were also exposed during caging to natural stressors (e.g., changing water temperatures and dissolved oxygen levels, organic matter, and microorganisms) and environmental contaminants other than metals and halogenated flame retardants, which may have affected the biological responses of fish. In addition to these multiple stressors, we hypothesize that a lack of food in the cages may also have increased their foraging activity with a possible consequence of increasing energy expenditure, which may in part explain the lower body condition of caged fish compared to laboratory control fish.

4. Conclusions

Results from the present study suggest that over a relatively long period of time, this major primary WWTP effluent may represent a significant source of exposure and accumulation of PBDEs and metals (e.g., Cd, Cu, Se, As, Zn, and Fe) for fish inhabiting the St. Lawrence River near the densely populated Montreal area. The results also showed that fish caged in the effluent had greater body condition compared to the reference site. Based on fish tissue $\delta^{15}N$ signature, the results suggested that over six weeks of exposure, yellow perch diet was affected by exposure to the effluent. Moreover, effluent exposure did not lead to significant changes in gill oxidative stress, suggesting that perch may have developed adaptive strategies to cope with effluent-induced oxidative stress or that the exposure was insufficiently high to elicit biological responses. Differences in malate abundance observed between perch caged at the reference site and downstream of the effluent could be explained by the need for fish to produce more energy in order to compensate for the stress elicited by effluent exposure. Furthermore, liver metabolomic (glucose, malate, fumarate, glutamate, creatinine, histamine, oxypurinol, and glycogen) responses of caged perch were significantly different from the laboratory-reared fish, suggesting an acclimation, transplanting or caging effect. Overall, this study highlights the relevance of evaluating the multi-level biological effects of WWTP effluent exposure in representative environmental conditions, and improves our understanding of the modes of action of complex environmental pollutant mixtures in wild fish.

CRediT authorship contribution statement

Michel A. Defo: Conceptualization, Methodology, Visualization, Validation, Formal analysis, Investigation, Data curation, Writing - original draft, Writing - review & editing, **Laurie Mercier:** Investigation; Methodology; writing review. **Conrad Beauvais:** Investigation; Methodology; writing review. **Robert B. Brua:** Formal analysis; Data curation; Investigation; Methodology; Visualization, original draft, Funding acquisition. **Gerald Tétreault:** Conceptualization, Formal analysis, Data curation; Writing - review & editing, Funding acquisition. **Anthony Fontaine:** Formal analysis; writing review. **Patrice Couture:** Conceptualization, Resources, Writing - review & editing, Funding acquisition. **Jonathan Verreault:** Conceptualization, Writing - review & editing, Funding acquisition. **Magali Houde:** Conceptualization, Methodology, Resources, Writing - review & editing, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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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.ecoenv.2021.112483.

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