Environmental exposure of northern pike to a primary wastewater effluent: Impact on the lipidomic profile and lipid metabolism

Christine Dépatie[a], Magali Houde[b*], Jonathan Verreault[a]

[a] Centre de recherche en toxicologie de l'environnement (TOXEN), Département des sciences biologiques, Université du Québec à Montréal, C.P. 8888, Succursale Centre-ville, Montreal, QC, H3C 3P8, Canada

[b] Environment and Climate Change Canada, 105 McGill Street, Montreal, QC, H2Y 2E7, Canada

*Corresponding author:
Magali Houde
Environment and Climate Change Canada
105 McGill Street, Montreal, QC, H2Y 2E7, Canada
E-mail: magali.houde@canada.ca

Abstract

Lipids play important roles in growth, reproduction, locomotion, and migration of fish. Municipal effluents, which are complex mixtures of biological and chemical compounds including flame retardants, have been shown to alter lipid metabolism in environmentally and experimentally exposed fish. Down-regulation of several genes coding for fatty acid metabolism enzymes has previously been reported in male northern pike (*Esox lucius*) collected in the St. Lawrence River (QC, Canada) downstream of a major primary wastewater treatment plant (WWTP) point of discharge. The main objective of this study was to investigate the effects of exposure to the Montreal's WWTP effluent on the lipidomic profile (*i.e.*, fatty acids, acylcarnitines, and phospholipids) as well as the transcription of genes related to lipid metabolism in the liver of northern pike collected upstream and downstream of this WWTP effluent. Halogenated flame retardant concentrations were also determined in pike liver and used as markers of exposure to this effluent. Greater concentrations of saturated and monounsaturated lysophosphatidylcholines (LPCs) and lower concentrations of polyunsaturated LPCs were determined in the liver of pike collected downstream of the WWTP compared to those collected upstream. Lower mRNA levels of peroxisome proliferator-activated receptor alpha (*pparα*), a major regulator of lipid metabolism, were also measured in pike exposed to Montreal's WWTP effluent. In addition, the relative contributions (%) of LPC 18:2 and LPC14:0, compounds used as markers of obesity and inflammation, were significantly correlated with halogenated flame retardant concentrations and fish girth. Results of the present study suggest that chronic environmental exposure to a primary WWTP effluent

can modulate the transcription of genes related to lipid metabolism, and hence affect the hepatic phospholipid composition of pike from the St. Lawrence River.

Keywords: Fish; Metabolomics; Lipids; Halogenated flame retardants; Gene transcription.

1. Introduction

Lipids are part of the complex metabolome of fish tissues and play important roles in growth, reproduction, locomotion, and migration (Tocher, 2003). Investigating lipid composition in fish tissues can, therefore, provide valuable information on their health status. Neutral lipids including fatty acids are an essential source of energy for fish, and part of the constitution of phospholipids and polar lipids that play key roles in maintaining the structure of cell membranes (Tocher, 2003). The association of a fatty acid, coenzyme A (CoA) group and carnitine in fish cells forms the acylcarnitines. This complex allows fatty acids to cross the mitochondria membranes that are used for energy production in cell compartments (Schulz, 2002). Additionally, phospholipids are found under different forms in cells and can be grouped into phosphatidylcholines (PCs), phosphatidylethanolamines (PEs), phosphatidylinositols (PIs) and phosphatidylserines (PSs) according to their head group. Lysophospholipids in fish cells are derived from phospholipids and are mainly involved in inflammatory processes (Grzelczyk and Gendaszewska-Darmach, 2013).

Several enzymes and genes are involved in the metabolism of lipids in fish. Fatty acid synthase (FAS) plays a central role in the synthesis of fatty acids in the liver of fish, while acyl-CoA oxidase (ACOX) catalyzes their degradation in order to produce energy in the form of adenosine triphosphate (ATP) (Tocher, 2003). Phospholipids can be transformed from one form to another, for example by the action of phosphatidylethanolamine N-methyltransferase (PEMT) that catalyzes the transformation of PEs to PCs. The cleavage of phospholipids is catalyzed by phospholipase A2 (PLA2) leading to the release of fatty acids and lysophospholipids (Catalá, 2012). Both of these lipid groups can modulate

inflammatory processes depending on their degree of saturation (Grzelczyk and Gendaszewska-Darmach, 2013). Saturated lysophosphatidylcholines are known to be pro-inflammatory, whereas polyunsaturated lysophosphatylcholines are anti-inflammatory (Grzelczyk and Gendaszewska-Darmach, 2013). Moreover, fatty acids can be added to the lysophospholipids to form new phospholipids by the action of several isoforms of lysophosphatidylcholine acyltransferases (LPCAT) (Jackson et al., 2008). Finally, peroxisome proliferator-activated receptors (PPARs) are transcriptional regulators that maintain lipid homeostasis in cells mainly by modulating the activity of enzymes involved in lipid metabolism such as ACOX and LPCAT (Tocher, 2003). These processes must be tightly regulated in order to maintain a stable lipid composition in tissues. As such, lipid metabolites are closely related to pathogenesis such as inflammation, cancer and autoimmune disease (Santos and Preta, 2018). Thus, small changes in lipid composition within cells can have deleterious impact on the nutritional status and health of fish.

Municipal wastewater treatment plant (WWTP) effluents are composed of a variety of biological and chemical substances, and thus represent a significant source of environmental contaminants (Marcogliese et al., 2015). These include trace elements (Cantinho et al., 2015), halogenated flame retardants (HFRs) (Houde et al., 2014), pharmaceutical compounds as well as personal care products (Tran et al., 2018), which can be partly eliminated through wastewater treatment processes (e.g., sludge) or discharged into aquatic ecosystems via the effluents. Aquatic organisms in ecosystems receiving effluent waters can therefore be exposed to elevated concentrations of a myriad of environmental contaminants. Among these, tissue concentrations of polybromodiphenyl

ether (PBDE) flame retardants in perch (*Perca flavescens*) collected in the St. Lawrence River downstream of the Montreal's (Quebec, Canada) primary WWTP effluent point of discharge were found to be significantly greater compared to individuals collected upstream (Houde et al., 2014). PBDEs are added to plastics, textiles, upholstered furniture, and electronic and electric equipment to reduce their flammability (de Wit, 2002). All commercial mixtures of PBDEs (Penta-, Octa-, and Deca-BDE) are now listed as Persistent Organic Pollutants (POPs) under the Stockholm Convention (Stockholm Convention, 2009, 2017). However, PBDEs remain abundant in consumer products that are in-use and in landfills (waste phase), and will thus represent a continued source of environmental contamination for years to come (Abbasi et al., 2015). As a result of the growing demand for non-flammable products, alternative flame retardants are being marketed or produced in occasionally high volumes to replace PBDE mixtures and other restricted or banned HFRs (*e.g.*, hexabromocyclododecane) (Tongue et al., 2019). Among emerging HFRs, large volumes of Dechlorane Plus (DP) are produced or imported into the USA, reaching up to 450 tons/year (Sverko et al., 2011). Other Dechlorane (Dec)-related compounds including Dec-602 were measured at elevated concentrations in lake trout (*Salvelinus namaycush*) and whitefish (*Coregonus clupeaformis*) tissues from Lake Ontario (Canada), while DP, Dec-602, -604 and -604 Component B as well as Chlordene Plus were found at generally low concentrations in the liver of northern pike (*Esox lucius*) from the St. Lawrence River near Montreal (Houde et al., 2014). This suggests that certain Dechlorane-related compounds are bioaccumulative in the aquatic food web, and may also be of concern for fish health (Shen et al., 2014; Sverko et al., 2011).

There is increasing evidence that chronic exposure to complex mixtures of contaminants found in municipal effluents can alter lipid metabolism in fish. For example, a recent study from our research group reported a down-regulation of the *acox* gene in liver of male northern pike collected downstream of the Montreal's WWTP effluent point of discharge that was associated with a lower activity of the related enzyme (ACOX) as well as a down-regulation of the gene *fas* (Reinling et al., 2017). In this study, ACOX activity and *acox* transcription levels were also negatively correlated with PBDE concentrations in the liver of male pike (Reinling et al., 2017). Additionally, greater total lipid percentages were determined in the liver of female pike in the waters impacted by the WWTP effluent (Reinling et al., 2017). Compositional changes in fatty acids and phospholipids have also been reported in fish exposed to municipal effluents and individual contaminants in the environment as well as in the laboratory. As such, Sakalli et al. (2018) reported greater total lipid content and proportions of mono-unsaturated fatty acids, and lower proportions of saturated and polyunsaturated fatty acids in muscle of common carp (*Cyprinus carpio*) exposed for a year in the field to an urban effluent in Czech Republic. Ren et al. (2018) further measured lower concentrations of lysophospatidylcholines (LPCs) and higher concentrations of phosphatidycholines (PCs) in homogenate samples of zebrafish (*Danio rerio*) exposed to a low concentration (5 µg/L) of short-chain chlorinated paraffins. Moreover, Du et al. (2016) determined lower concentrations of acylcarnitines and linoleic acid and higher concentrations of arachidonic acid in the liver of zebrafish exposed for 7 days to 0.05 mg/L and 0.3 mg/L of the flame retardant triphenyl phosphate.

Montreal's primary WWTP treats the wastewaters of more than 4 million inhabitants and has been found to be a significant source of HFR exposure for fish residing in its plume in the St. Lawrence River (Houde et al., 2014; Reinling et al., 2017). As such, northern pike exposed to this WWTP effluent were found to accumulate high tissue concentrations of PBDEs and low to moderate concentrations of emerging HFRs including hexabromobenzene (HBB), DP isomers, and Dec-related compounds (Houde et al., 2014; Reinling et al., 2017). As a result, HFRs (mainly PBDEs) were suggested as good markers of effluent exposure for northern pike and other top predator fish in this freshwater ecosystem including the muskellunge (*Esox masquinongy*). Despite that several other classes of contaminants have been reported in this urban effluent, HFRs remain the best studied chemicals and several exposure-related effects have been reported for fish species from this area including the northern pike (Houde et al., 2014; Reinling et al., 2017). The objective of the present study was to examine the effects of a chronic environmental exposure of St. Lawrence River northern pike to the Montreal's WWTP effluent on the hepatic lipid composition and the transcription of genes related to lipid metabolism. Targeted lipidomics was used to determine the concentrations of three lipid classes (i.e., fatty acid, acylcarnitines, and phospholipids) in pike liver collected upstream and downstream of the effluent point of discharge in the river. The mRNA levels of genes involved in the metabolism of lipids (*pla2*, *lpcat*, *pemt*, and *ppars*) were also measured in pike liver. The lipidome and mRNA levels were additionally related to the concentrations of PBDEs and selected emerging HFRs in pike liver. This study provides insight into WWTP effluent exposure-related effects on lipid composition and metabolism of a top predator fish at the molecular and cellular level, and could ultimately lead to the

development of novel biomarkers in fish species exposed to complex environmental contaminant mixtures.

2. Materials and methods

2.1 Field sampling

Northern pike were sampled from late May to early June 2014 and 2015 using a beach seine. Sampling was carried out after spawning to minimize variations in liver lipid content. Pike were collected 4 km upstream (Ile de Boucherville; 9 males and 17 females) and 4 km downstream (Ilet Vert; 6 males and 18 females) of the point of discharge of the Montreal's primary WWTP effluent in the St. Lawrence River (QC, Canada). Fish were euthanized in clove oil (250 mg/L), measured (length and circumference) and weighed (± 0.01 g). The Fulton's condition index was determined using the following equation: [body mass (g)/total length (cm3)] x 1000 and used as a proxy of body condition. Several liver aliquots (15 mg) were collected in tubes and kept in dry ice in the field, and stored at -80°C in the laboratory for lipid and gene transcription analyses (section 2.4). Remaining liver was kept in a tube on ice packs in the field, and then stored at -20°C in the laboratory for contaminant analysis (section 2.2). Cleithra were collected for age determination that was conducted at the Ministère des Forêts, de la Faune et des Parcs (Trois-Rivières, QC, Canada) by counting the number of growth annuli following methods by Le Cren (1947). Gonads were collected and preserved in formalin for sex determination at the University of Montreal (Saint-Hyacinthe, QC, Canada) according to methods described by Blazer et al. (2002). Protocols for handling and sampling of fish were approved by Environment and Climate Change

Canada's Animal Care Committee in accordance with guidelines of the Canadian Council on Animal Care (Ottawa, ON, Canada).

2.2 Chemical analysis

HFR concentrations were analyzed in pike liver samples according to methods described by Reinling et al. (2017) without modification. Briefly, sample aliquots (0.75-1.0 g) were ground with diatomaceous earth, spiked with 100 µL of a 200 ppb internal standard solution (BDE-30, BDE-156, ^{13}C-BDE-209, and ^{13}C-*syn*-DP), and extracted with dichloromethane:*n*-hexanes (50:50 volume ratio). Identification and quantification of analytes were performed using a gas chromatograph (GC) coupled to a single quadrupole mass spectrometer (MS) (Agilent Technologies 5975C Series, Palo Alto, CA) operating in electron capture negative ionization mode (GC/MS-ECNI). Quality control procedures including blanks, repeated analyses of fish samples and standard reference material (SRM 1947, Lake Michigan Fish Tissue; National Institute of Standards and Technology, Gaithersburg, MD, USA) were analyzed in each batch of ten samples. Concentrations of the six PBDE congeners in SRM 1947 (n = 4) showed less than 18.5% 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. Mean (± SEM) recoveries for spiked internal standards in pike liver, blank and SRM 1947 samples were as follows: BDE-30 (96 ± 1.0%), BDE-156 (100 ± 1.6%), ^{13}C-BDE-209 (59 ± 1.5%), and ^{13}C-*syn*-DP (96 ± 1.2%). A complete list of congeners/compounds analyzed can be found in Table S1.

205 *2.3 Lipid compound analysis*

206 Targeted lipidomic analyses were performed by SGS AXYS Analytical Services Ltd
207 (Sydney, BC, Canada) according to methods described by Benskin et al. (2014) without
208 modification. A total of 122 lipid compounds were analyzed in pike liver (see complete list
209 in Table S2). Briefly, liver samples were homogenized and subjected to three sequential
210 extractions, each using 6 mL/g of methanol in a bead blender. The resulting extracts were
211 pooled and plated on 96-well plates with a mixture of labeled surrogates and authentic
212 standards for quantification. Lipid metabolites were analyzed using a high performance
213 liquid chromatograph (HPLC) (Agilent 1100) coupled to a API4000 triple quadrupole MS
214 (Applied Biosystems/Sciex, Concord, ON, Canada). Ten saturated and unsaturated fatty
215 acids were quantified by isotopic dilution and analyzed by HPLC-MS/MS. Eight
216 acylcarnitines, 11 sphingomyelins, 84 phosphatidylcholines, and 9
217 lysophosphatidylcholines were analyzed by flow-injection tandem MS (FI-MS/MS) and
218 quantified by single-point calibration. Recovery percentages of internal standards varied
219 between 78% and 128% for fatty acids (FA 14:0, FA 20:4, FA 20:5, and FA 22:6), between
220 82% and 136% for LPC 18:0, and between 77% and 114% for PC 36:2. The contribution
221 percentages of individual lipids were calculated as the concentrations of a given lipid
222 compound divided by the sum of the concentrations of all lipid compounds within the same
223 functional family. The percentages of individual lipids were used in statistical analyses to
224 evaluate their relative contributions in liver rather than comparing raw concentrations in
225 order to minimize the effect related to the large inter-individual differences in lipid
226 concentrations. The ratios between LPC and PC concentrations were also used in the

227 analyses to explore the potential effects of this urban effluent exposure on PC remodeling
228 into LPCs because of the enzymatic linkages between these two phospholipid groups.
229
230 *2.4 Transcription analyses*
231 *2.4.1 RNA extraction and reverse transcription*
232 The targeted genes were related to enzymes that are involved in the synthesis, catabolism
233 and remodeling of lipids (Table S3). Transcription analyses were conducted using methods
234 described by Reinling et al. (2017) without modification. Briefly, RNA extraction was
235 performed using the RNeasy plus mini kit (Qiagen, Toronto, ON, Canada) following the
236 manufacturer's instructions. The concentration and purity of the RNA was estimated with
237 NanoDrop 1000 (Thermo Fisher Scientific, ON, Canada). The integrity of the RNA was
238 verified with the Experion™ Automated Electrophoresis System (Bio-Rad, Mississauga,
239 ON, Canada) using the Experion™ RNA StdSens Analysis Kit (Bio-Rad). Reverse
240 transcription was performed with the QuantiTect® reverse transcription kit (QIAGEN)
241 according to the manufacturer's instructions for a total cDNA volume of 20 µL. The cDNA
242 samples were stored at -80°C until real-time PCR analysis (section 2.4.3).
243
244 *2.4.2 Primer design*
245 Expressed gene sequences for *Esox lucius* were selected in the GenBank database (Table
246 1). All primers were designed by the authors using Primer-BLAST from NCBI (Rozen and
247 Skaletsky, 2000). Oligo Analyzer (Integrated DNA Technologies, Coralville, IA, USA)
248 was used to examine the presence of secondary structures. For each gene, two pairs of
249 primers were evaluated, except for the reference genes, which have previously been

validated by Reinling et al. (2017). Primers were manufactured by Integrated DNA Technologies (Coralville, IA, USA). A calibration curve was performed for each primer pair (starting cDNA concentration: 2 ng; 8 serial dilutions; 2- or 3-fold) to estimate PCR efficiency (values were between 90% and 114%) and limits of detection. A description of the function of these genes can be found in Table S3.

255 **Table 1.** Genes, symbols and primers used for RT-qPCR analysis in northern pike liver.

Gene names	Symbols	Species	Accession numbers	Primers	Sequences
Target genes					
Peroxisome proliferator activated receptor alpha	*pparα*	*Esox lucius*	XM_010883898.2	Forward	CTGAGCCCACGGATTTG
				Reverse	GTTGAGCTACGGTGATAGTG
Peroxisome proliferator activated receptor delta	*pparδ*	*E. lucius*	XM_010893343.2	Forward	GCACCCTAATCAGCCTTAC
				Reverse	CACAGGCATGAGGTAGTTG
Lysophosphatidylcholine acyltransferase 3	*lpcat3*	*E. lucius*	XM_010900842.3	Forward	TATGGGCTATCCTCTGGTG
				Reverse	GGAGGTACGGCAACATTAG
Lysophosphatidylcholine acyltransferase 4	*lpcat4*	*E. lucius*	XM_010903766.3	Forward	AGAAAGCCAGAGGTGGAG
				Reverse	GTCAGTCAGTCCCAGTAGAG
Phosphatidylethanolamine N-methyltransferase	*pemt*	*E. lucius*	XM_010863691.2	Forward	CAGGACGGACTGTGTTTG
				Reverse	TGATCCCACTCCCTTGAC
Phospholipase A2 (group IV)	*pla2*	*E. lucius*	XM_020049184.1	Forward	GCTCAGTGTCCCTTTCAC
				Reverse	CCAGCATTCCCAGTTCTC
Reference genes					
β2 microglobulin precursor	*b2mg*	*E. lucius*	BT079123.1	Forward	TGTTGCCCTTGTTTTCTGCGTGGT
				Reverse	TGGCCGTATTTGCCAGGGTTGC
Ribosomal protein S20	*rps20*	*Salmo salar*	BT060032.1	Forward	CCCCTGTTGAGGCTGAGGTTGC
				Reverse	TTGGTGGGCATACGGACTGGT
Peptidyl-prolyl cis-trans isomerase	*ppia*	*E. lucius*	BT080015.1	Forward	TGCTAAAACTGCTTGGCTGGATGG
				Reverse	TCGCTTTCGTCTTGCCGCTG

Tubulin α chain	*tba*	*E. lucius*	BT079618.1	Forward	GGTCACTACACCATCGGCAAGGA
				Reverse	ACAGGCGTTCCATCAGCAGGGA
Ubiquitin-like protein 4A	*ubl4a*	*E. lucius*	BT079424.1	Forward	CAGCAGGCGAAAGGAGTGGAGT
				Reverse	TGCCAGGACGGTGGACAAAGT

256

2.4.3 Real-time quantitative PCR analysis

The qPCR analyses were conducted on a real-time PCR thermal cycler (CFX96 Touch™ Real-Time PCR Bio-Rad Detection System) according to methods described by Reinling et al. (2017). For each pair of primers selected for the targeted genes, a calibration curve was established to obtain the PCR efficiency and limits of detection. Data analysis was performed using the CFX Manager™ software (Bio-Rad).

2.5 Statistical analysis

Shapiro Wilk and NVC tests were used to verify the normality and homoscedasticity of variables. Student and Mann-Whitney tests were then performed to investigate the differences in Σ_{34}PBDE and Σ_{7}Dec concentrations in northern pike liver between sites (upstream and downstream) and sexes. Student and Mann-Whitney tests were also performed to investigate differences in summed concentrations of fatty acids (Σ_{2}SFA, Σ_{2}MUFA, Σ_{6}PUFA), acylcarnitines (Σ_{8}ACYL), LPCs (Σ_{9}LPC), PCs (Σ_{12}sfaPC, Σ_{13}mufaPC, and Σ_{49}pufaPC) and sphingomyelins (Σ_{11}SM) in northern pike liver between sites and sexes. The differences in relative abundance (%) of individual compounds and gene mRNA levels between sexes and sites were also assessed using the Student and Mann-Whitney tests. Spearman correlation coefficients were used to examine the strength of the relationships between Σ_{34}PBDE and Σ_{7}Dec concentrations, relative abundance (%) of individual lipid compounds, gene transcription levels, and fish girth. Statistical analyses were performed using R version 3.2.1 (R Core Team, 2015, Vienna, Austria) with a significance threshold set to 0.05.

3. Results

3.1 HFR concentrations

Σ_{34}PBDE concentrations were 4.5-fold greater in liver of pike collected downstream of the WWTP effluent point of discharge compared to those collected upstream (Table 2). This site-specific difference was also observed when males (n = 15) and females (n = 35) were tested separately (male: t (8.67) = -3.5, p = 0.008; female: t (32) = -5.27, p < 0.001). Moreover, liver Σ_{34}PBDE concentrations were 2-fold greater in male pike (209 ± 59 ng/g ww; mean ± SEM) compared to females (104 ± 19 ng/g ww) (t (27.98) = -2.34, p = 0.03). Comparatively, concentrations of Σ_{7}Dec were slightly greater in pike collected downstream, although not significantly relative to those in pike collected upstream (Table 2). The congener/compound concentrations and profiles of HFRs in pike liver in the present study have been comprehensively described in Reinling et al. (2017) in which the same individuals were used, and thus will not be further described here.

Table 2. Mean (± SEM) concentrations of major halogenated flame retardants (ng/g ww) and lipid classes (μg/g ww) in liver of St. Lawrence River northern pike collected upstream and downstream of the Montreal's WWTP effluent discharge point. Asterisks (*) indicate a significant difference between sites ($p \leq 0.05$). The full list of halogenated flame retardants and lipid compounds can be found in Tables S1 and S2.

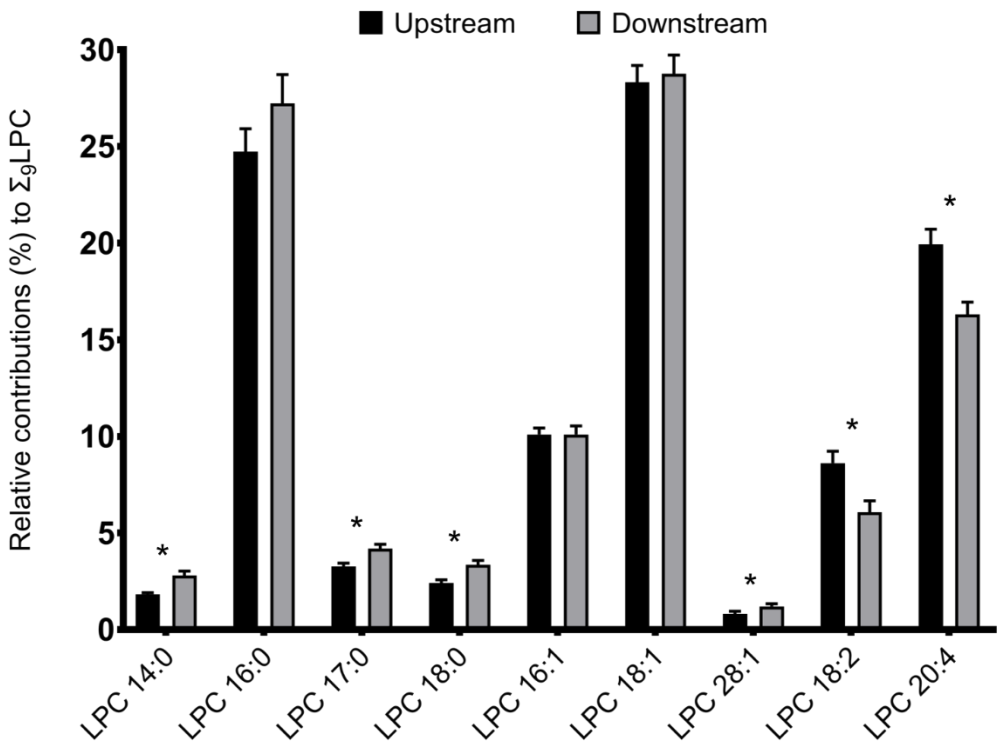
	Upstream (*n* = 25)	**Downstream (*n* = 25)**
Σ_{34}PBDE	55 ± 9	218 ± 38 *
Σ_{7}Dec	1.2 ± 0.2	2.5 ± 0.5
Σ_{2}SFA	484 ± 33	415 ± 31
Σ_{2}MUFA	1,131 ± 9	1,091 ± 126
Σ_{6}PUFA	819 ± 56	778 ± 97
Σ_{8}ACYL	9 ± 0.6	8 ± 0.9
Σ_{9}LPC	269 ± 20	211 ± 21 *
Σ_{12}sfaPC	7,361 ± 434	7,521 ± 619
Σ_{13}mufaPC	435 ± 19	481 ± 21
Σ_{49}pufaPC	2,447 ± 80	2,488 ± 95
Σ_{11}SM	369 ± 16	364 ± 21

PBDE: polybrominated diphenyl ethers; Dec: dechloranes; SFA: saturated fatty acids; MUFA: mono-unsaturated fatty acids; PUFA: polyunsaturated fatty acids; ACYL: acylcarnitines; LPC: lysophosphatidylcholines; sfaPC: phosphatidylcholine with saturated fatty acids; mufaPC: phosphatidylcholine with mono-unsaturated fatty acids; pufaPC: phosphatidylcholine with polyunsaturated fatty acids; SM: sphingomyelins.

3.2 Lipid compound profiles

The liver concentrations of the majority of lipids were found to be similar between male and female pike; sexes were therefore combined in subsequent analyses (Table S4). This absence of differences between males and females in lipid concentrations must however be interpreted with caution given the large difference in sample sizes between males and females for both sites (upstream: 17 females and 9 males; downstream: 18 females and 6 males). Among all lipid classes determined in pike liver, only Σ_9LPC concentrations were significantly different between the two sites. Specifically, Σ_9LPC concentrations in individuals collected downstream of the WWTP effluent discharge point were 1.3-fold lower relative to those collected upstream ($t_{(47,9)} = 2.05$, $p = 0.05$; Table 2). Concentrations of several individual compounds within each of these lipid classes were also significantly different between the two sites, and detailed results can be found in Table S5.

Three individual saturated and one monounsaturated LPCs showed greater percent contributions to Σ_9LPC concentrations in pike collected at the downstream site compared to those collected upstream (LPC 14:0, W = 119, $p = 0.001$; LPC 17:0, W = 145, $p = 0.008$; LPC 18:0, W = 144, $p = 0.007$; LPC 28:1, W = 164, $p = 0.03$) (Fig. 1). In contrast, two polyunsaturated LPCs had lower percent contributions to Σ_9LPC in pike collected downstream of the WWTP effluent discharge point (LPC 18:2, W = 364, $p = 0.03$; LPC 20:4, W = 421, $p < 0.001$) (Fig. 1).



329

330 **Figure 1.** Mean (± SEM) relative contributions (%) of individual LPC compounds to
331 Σ_9LPC concentrations (ng/g ww) in the liver of northern pike collected upstream (n = 25)
332 and downstream (n = 25) of the WWTP effluent discharge point (Mann-Whitney U test; *:
333 $p \leq 0.05$). The relative percent contributions of each LPC to Σ_9LPC concentrations can be
334 found in Table S6.

335

336 *3.3 Gene transcription*

337 Peroxisome proliferator-activated receptor alpha (*ppar*α) mRNA levels were 4-fold lower
338 in liver of pike collected downstream of the WWTP effluent discharge point compared to
339 those collected upstream (W = 286, p = 0.04) (Fig. 2, Table S7). No significant difference
340 was found for other genes in pike liver between the two sites (Fig. 2). Moreover, mRNA
341 levels of the majority of the genes were found to be similar between male and female pike
342 (Table S8).

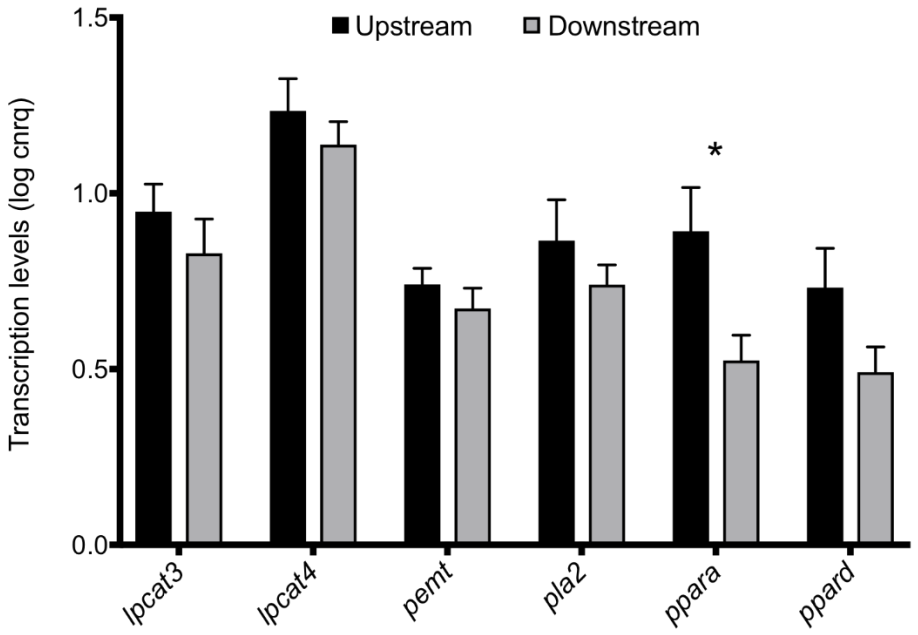
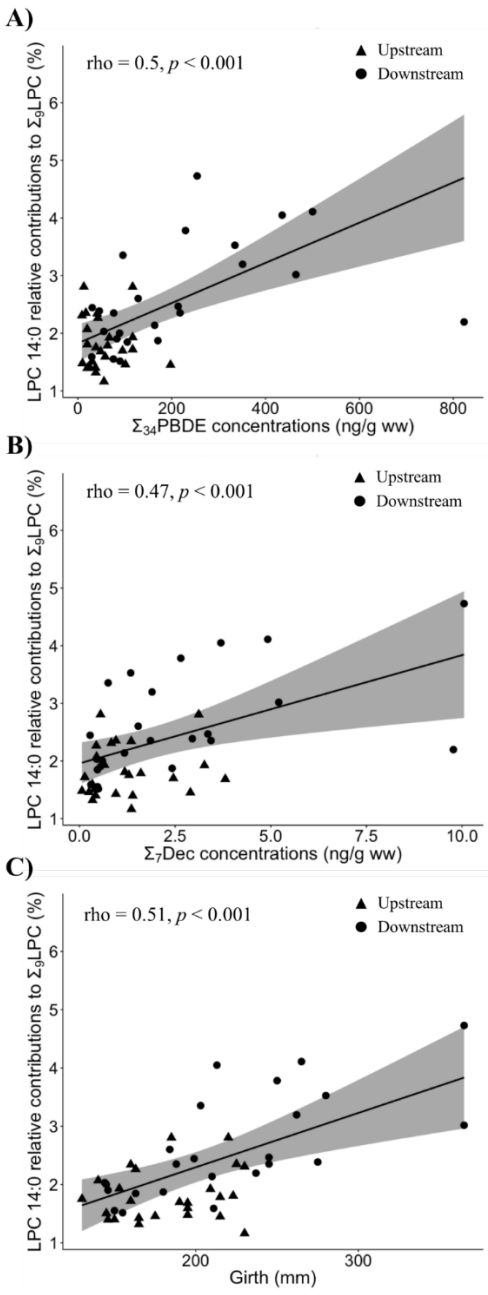


Figure 2. Liver transcription levels (mean log calibrated normalized relative quantity (cnrq) ± SEM) of genes coding for enzymes involved in lipid metabolism in northern pike collected upstream and downstream of Montreal's WWTP effluent point of discharge (Mann-Whitney *U* Test; *: $p \leq 0.05$).

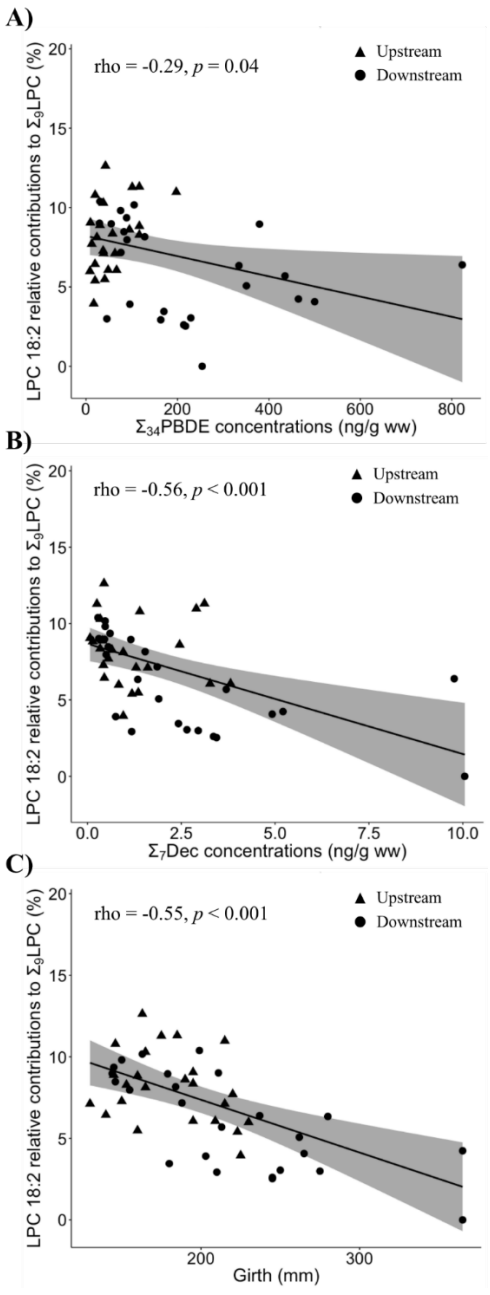
3.4 Correlations between contaminants and lipidome

Pike from both sites had similar condition index, therefore, fish girth was used to characterize the potential links between modulation of lipid profiles and the fish body condition. The relative percent contributions of LPC 14:0 were positively correlated with Σ_{34}PBDE (rho = 0.5, $p < 0.001$) and Σ_{7}Dec (rho = 0.47, $p < 0.001$) concentrations in the liver of pike as well as with fish girth (rho = 0.51, $p < 0.001$) (Fig. 3). Moreover, the contributions of LPC 18:2 were negatively correlated with concentrations of Σ_{34}PBDE (rho = -0.29, $p = 0.04$) and Σ_{7}Dec (rho = -0.56, $p < 0.001$) as well as fish girth (rho = -0.55, $p < 0.001$). Also, ratios of pufaLPC to pufaPC concentrations were negatively correlated with those of Σ_{34}PBDE (rho = -0.38, $p = 0.007$) (Fig. 5).



359

360 **Figure 3.** Correlations between the relative percent (%) contributions of liver LPC 14:0 to
361 Σ_9LPC concentrations (ng/g ww) and A) Σ_{34}PBDE concentrations in liver (ng/g ww), B)
362 Σ_7Dec concentrations in liver (ng/g ww), and C) fish girth (mm) of northern pike collected
363 upstream and downstream (n = 50) of the WWTP effluent discharge point. Grey fields
364 represent 95% confidence interval.



365

366 **Figure 4.** Correlations between the relative percent (%) contributions of liver LPC 18:2 to
367 Σ_9LPC concentrations (ng/g ww) and A) Σ_{34}PBDE concentrations in liver (ng/g ww), B)
368 Σ_7Dec concentrations in liver (ng/g ww), and C) fish girth (mm) of northern pike collected
369 in the St. Lawrence River upstream and downstream (n = 50) of the Montreal's WWTP
370 effluent discharge point. Grey fields represent 95% confidence interval.

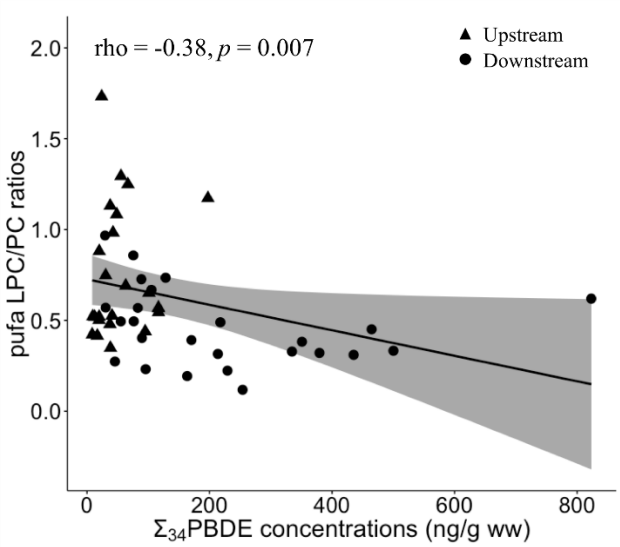


Figure 5. Correlation between polyunsaturated Σ_2LPC/Σ_{49}PC concentration ratios (ng/g ww) and Σ_{34}PBDE concentrations (ng/g ww) in liver of northern pike collected upstream and downstream (n = 50) of the Montreal's WWTP effluent discharge point. Grey field represents 95% confidence interval.

4. Discussion

4.1 HFR concentrations

Concentrations of Σ_{34}PBDE were found to be 4.5-fold greater in liver of pike collected downstream of the WWTP effluent point of discharge compared to pike collected upstream. The most abundant PBDE congeners in pike were BDE-47, -99 and -100 as previously reported by Reinling et al. (2017). These three PBDE congeners were also reported to be the most abundant in homogenate samples of yellow perch collected from the area impacted by this same municipal effluent (Houde et al., 2014) as well as in hornyhead turbot (*Pleuronichthys verticalis*), common carp (*Cyprinus carpio*), crucian carp (*Carassius auratus*), leather catfish (*Silurus meridionalis*), and java tilapia (*Tilapia nilotica*) collected near major WWTP effluents in southern California, USA (Maruya et al.,

2012) and Gaobeidian, China (Wang et al., 2007). As reported by Reinling et al. (2017), Dec-604 CB was generally found at greater concentrations than Dec-604 in liver of pike at the downstream site; similar results were also found in lake trout and whitefish collected from Lake Ontario, Canada (Shen et al., 2014). Results in pike confirmed that PBDEs can be used as chemical tracers of exposure to the Montreal's WWTP effluent in fish collected from this sector of the St. Lawrence River.

4.2 Lipid profiles and gene transcription

We had predicted that higher concentrations of PUFAs and lower concentrations of acylcarnitines would be found in pike collected downstream of the WWTP as suggested by the lower transcription of *acox* determined in a previous study from our laboratory in pike from this same site (Reinling et al., 2017). ACOX catalyzes the oxidation of long and polyunsaturated fatty acids in the peroxisome prior to their passage through the mitochondria in the form of acylcarnitines to enter in the β-oxidation reaction (Tocher, 2003). The under-transcription of *acox* in the liver of pike sampled downstream of Montreal's WWTP effluent point of discharge could have increased the concentrations of fatty acids including PUFAs, and in turn reduced the formation of acylcarnitines. However, present results indicated no significant differences for the sum of SFA, MUFA, PUFA and acylcarnitine concentrations in liver of pike between the upstream and downstream sites. These results differed from other fish studies in which exposure to effluent and/or individual contaminants have been shown to alter fatty acid metabolism. For example, lower concentrations of SFAs and PUFAs, and higher concentrations of MUFAs were measured in muscle of common carp exposed for a year to an urban effluent in Vodňany

411 (Czech Republic) compared to fish collected from a reference site (Sakalli et al., 2018).
412 Similarly, lower concentrations of PUFAs and higher concentrations of MUFAs were
413 determined in muscle of brown trout (*Salmo trutta*) exposed in the wild for 30, 90 or 180
414 days to the South Bohemia's effluent (Czech Republic) (Giang et al., 2018). Moreover,
415 lower concentrations of acylcarnitines were measured in the liver of zebrafish exposed to
416 0.05 mg/L and 0.3 mg/L of the flame retardant triphenyl phosphate for 7 days (Du et al.,
417 2016). These results suggest that the complex chemical composition of WWTP effluents
418 may have contrasting effects on fatty acid and acylcarnitine profiles in fish tissues
419 (Samuelsson et al., 2011).
420
421 In the present study, no significant difference was found in pike between the two sampling
422 sites for the summed liver concentrations of PCs with saturated (sfaPCs), monounsaturated
423 (mufaPCs), and polyunsaturated fatty acids (pufaPCs). Nevertheless, significant site-
424 specific differences in concentrations of more than 20 individual PC compounds were
425 observed in these fish. Previous studies have shown that the tissue composition of PCs can
426 be altered following exposure to a range of individual contaminants and/or mixtures. For
427 instance, greater concentrations of sfaPCs and mufaPCs, and lower concentrations of
428 pufaPCs were measured in the muscle of yellow perch exposed to cadmium at different
429 water temperatures compared to control fish (Fadhloui and Couture, 2016). Melvin et al.
430 (2019) further reported perturbation of phospholipid composition in fish exposed to metals
431 and metalloids (*e.g.*, lead, cadmium, manganese, chromium, arsenic, etc.) in the field.
432 These authors noted higher relative abundance of PCs in homogenate samples of
433 mosquitofish (*Gambusia holbrooki*) collected at a metalloid-contaminated wetland

compared to fish collected at a reference site. Finally, Dimastrogiovanni et al. (2015) measured higher concentrations of pufaPC compounds in a rainbow trout liver cell line dosed with two plasticizers for 24 to 72 hours (5 uM of bis-(2-ethylhexyl)-phthalate (DEHP) and 20uM of 4-nonylphenol (4-NP)).

The only lipid class for which the sum concentrations in pike differed between the two sites was LPCs, in which lower concentrations were measured in the liver of fish collected downstream of the effluent point of discharge in this river. Moreover, significant differences in the relative contributions of six individual LPC compounds (LPC 14:0, LPC 17:0, LPC 18:0, LPC 28:1, LPC 18:2, and LPC 20:4) were determined between the upstream and downstream sites. Results from the present study were not consistent with those reported in plasma of roach (*Rutilus rutilus*) experimentally exposed to a WWTP effluent (Al-Sahli et al., 2012). These authors found greater concentrations of LPC 16:1 and LPC 18:2 in roach, whereas results of the present study showed no difference in LPC 16:1 contributions between pike sampled upstream and downstream, and lower contributions of LPC 18:2 in pike collected downstream.

Given the possible impacts of effluent exposure on the transcription of genes related to lipid metabolism in pike liver as suggested by Reinling et al. (2017) and the metabolic linkages between fatty acids and phospholipids, a difference in mRNA levels of *pparα, pparδ, pla2, pemt, lpcat3 and lpcat 4* was expected. However, only lower *pparα* mRNA levels were measured in the liver of pike collected from the effluent. A study reported lower transcription of *pparα* in liver of juvenile salmon exposed to water concentrations of the

two organophosphate ester flame retardants (2-chloroethyl) phosphate (TCEP) and tris (2-butoxyethyl) phosphate (TBOEP) (Arukwe et al., 2016). In addition to measuring under-transcription of *pparα*, these authors determined under-transcription of *pparδ* in juvenile salmon (Arukwe et al., 2016). In contrast, present results showed that mRNA levels of *pparδ* did not differ between pike collected upstream and downstream of the WWTP. Furthermore, Maradonna et al. (2015) measured higher mRNA levels of *pparα*, *ppar*δ and *pla2* in sea bream exposed to three plasticizers (nonylpnenol (NP), 4-tert-octylphenol (t-OP), and bisphenol A (BPA)). Gene transcription only represents a single step in gene expression that allows the mRNA levels of genes to be translated into proteins (Nikinmaa and Rytkönen, 2011). Therefore, it can be suggested that exposure to this urban effluent did not induce measurable effects at the mRNA level of a gene coding for the enzyme, but might have effects on its activity. Also, it is possible that some effects occurred at the gene transcription level, but that the fish compensated for these changes by inactivating other regulating genes. As such, fish can be fairly tolerant to the harmful effects of contaminants through cellular repair for example, and may adapt up to a certain concentration beyond which the effects are too important to return to a normal state (Ankley et al., 2007; Ekman et al., 2008). The under-transcription of hepatic *pparα* in the present study was also consistent with the under-transcribed *acox* previously measured in pike from the same site by Reinling et al. (2017). Moreover, PPAR*α* and ACOX are known to represent good markers of fatty acid metabolism in environmental monitoring of effects elicited by organic contaminants in fish (Olivares-Rubio and Vega-Lopez, 2016).

The transcription of genes was used in this study to investigate the potential linkages between lipid composition and transcription levels of genes coding for enzymes implicated in lipid metabolism. For instance, PPAR*α* is known as a key factor involved in hepatic lipid metabolism in fish and other animals (Olivares-Rubio and Vega-Lopez, 2016; Takahashi et al., 2015) by regulating the transcription of genes involved in the β-oxidation of fatty acids and lipogenesis (Rakhshandehroo et al., 2010). The activation of *ppar*α can also mediate changes in the composition of LPC compounds in liver of animals as it was reported in mouse hepatocytes (Takahashi et al., 2015). In the Takahashi et al. (2015) study, higher concentrations of LPC 16:0 and LPC 18:2 in liver of mice were associated with an activation of *ppar*α following a bezafribrate (i.e., drug used for the control of cholesterol and triglyceride levels) treatment. Moreover, Zhao et al. (2008) reported that LPCAT3, the enzyme catalyzing the transformation of LPCs to PCs, was regulated by PPAR*α* agonists such as fenofibrate, a drug also used to control cholesterol and triglycerides. Considering the functional links between LPC metabolism and PPAR*α*, the under-transcription of *ppar*α observed in the present study could provide mechanistic insights onto the changes in LPC composition in liver of pike exposed to the Montreal's primary effluent.

4.3 Associations between HFR concentrations and lipidome

The relative contributions of LPC 14:0 increased with the Σ_{34}PBDE and Σ_{7}Dec concentrations in pike, contrary to the relative contributions of LPC 18:2 that decreased with concentrations of both these contaminant classes. PBDEs as well as other chemicals found in the Montreal's effluent (*e.g.*, pharmaceutical compounds, PCBs, plasticizers, metals, etc.) could have the potential to modulate lipid metabolism. For example, Wen et

al. (2019) reported that exposure to BDE-99 increased adipogenesis via transcriptional regulation of PPARγ. Liu et al. (2014) further observed a disruption of LPC homeostasis mediated by *pparα* action in mice treated with gemfibrozil, a drug used for the treatment of hypertriglyceridemia. Moreover, negative correlations between *pla2* expression levels and PCB concentrations (CB-170, -180, and -171) were measured in the liver of largescale suckers (*Catostomus macrocheilus*) collected at three sites with different levels of urbanization and contaminant exposure in the lower Columbia River, USA (Christiansen et al., 2014). Lower abundance of pufaLPCs and higher abundance of sfaLPCs could be explained by the under-transcription of *pparα* in the liver of pike sampled in the plume of this urban effluent, which could have contributed to a reduction of the abundance of LPC 18:2 and increase of LPC 14:0 mediated by a possible under-regulation of *pla2* (Takahashi et al., 2015). In spite of the fact that no correlation was observed between *pparα* mRNA levels and PBDE or Dec concentrations in pike (data not shown), several other contaminants found in WWTP effluents have been shown to modulate *pparα* expression in fish (Olivares-Rubio and Vega-Lopez, 2016). These results could help explain the positive and negative correlations observed between the relative contributions of some LPC compounds and HFR concentrations in pike liver.

Moreover, correlations between the concentration ratios of polyunsaturated LPCs/PCs and PBDE concentrations in pike liver were consistent with the initial hypothesis of effluent exposure-related effects on the remodeling of phospholipids by the action of PLA2. Transcription of *pla2* belonging to group IV, a cytosolic form of PLA2, was not different in the liver of pike between the upstream and downstream sites. However, there are

multiple groups of PLA2 enzymes, and each of them catalyzes the transformation of PCs to LPCs in different cell compartments and with different specificity for PC compounds according to their degree of saturation (Astudillo et al., 2019; Mouchlis and Dennis, 2019). Thus, it could be suggested that other groups of PLA2 are implicated in the modulation of LPC levels via the action of *pparα*. Measurement of the transcription of other groups of PLA2 would be required in future studies in order to investigate their potential role in the modulation of LPC profiles in pike exposed to Montreal's WWTP effluent.

Additionally, correlations between LPC 14:0 and LPC 18:2, LPC/PC level ratios, and HFR concentrations could also be the result of oxidative stress. Contaminants generally found in effluents including cadmium, diclofenac and acetaminophen (non-steroidal anti-inflammatory drugs) and BDE-99 have previously been identified as oxidative stress inducers in fish tissues and cell lines as they may increase the formation of reactive oxygen species (ROS) (Espinosa-Ruiz et al., 2019; Lu et al., 2018; Nava-Álvarez et al., 2014). Phospholipids containing highly unsaturated PUFAs are the most sensitive to the action of ROS, and can thus undergo lipid peroxidation (Catalá, 2012). Oxidized phospholipids can be cleaved by PLA2 to remove the oxidized segment and release fatty acids and lysophospholipids such as LPCs (Catalá, 2012). It can therefore be suggested that the modulation of LPC composition and LPC/PC ratios in the liver of pike collected in the plume of this effluent could be linked to the direct effect of ROS in fish tissues resulting from effluent exposure. Moreover, as LPCs are closely linked to inflammatory processes (Grzelczyk and Gendaszewska-Darmach, 2013), it may be considered that their abundance

547 in the liver of pike is perturbed by inflammatory inducers including cadmium, a metal
548 found in this effluent and the St. Lawrence River (Gobeil et al., 2005; Lu et al., 2018).
549 The design of this study therefore allowed us to address the potential linkages between
550 effluent exposure and lipid metabolism perturbation, but not to determine the underlying
551 mechanisms of toxicity that led to the observed effects.
552
553 Interestingly, LPC 14:0 and LPC 18:2 are known markers of metabolic disorders such as
554 obesity, insulin resistance and dyslipidemia in other animals (Del Bas *et al.*, 2016;
555 Rauschert et al., 2016; Suárez-García et al., 2017; Wang-Sattler et *al.*, 2012). In the present
556 study, the relative abundance of LPC 14:0 and 18:2 were positively and negatively
557 associated, respectively, with pike girth; concentrations of these LPCs were also linked
558 with waist circumference in human (Rauschert et al., 2016). Moreover, HFRs are known
559 to exhibit endocrine disruptive properties and have been associated with obesogenic effects
560 in murine and human adipocytes via the action with PPARs (Darbre et al., 2017; Wen et
561 al., 2019). Considering these effects and the associations between PPARs and changes in
562 LPC composition in pike exposed to this urban effluent, investigating linkages in
563 mechanisms of action between concentrations of these lipid compounds and HFRs, and
564 metabolic disorders in fish exposed to urban effluents may be of interest in future studies.
565
566 **5. Conclusions**
567 Results from the present study suggest that phospholipid metabolism may be disrupted in
568 northern pike exposed to a major primary WWTP effluent, a known important source of
569 HFRs to the St. Lawrence River. LPCs containing polyunsaturated fatty acids represented

570 a lower proportion of all LPCs determined in the liver of pike collected downstream of the
571 point of discharge of this effluent. In contrast, LPCs containing saturated and mono-
572 unsaturated fatty acids represented a greater proportion of all LPCs found in the
573 downstream fish. Moreover, lower hepatic levels of *pparα* mRNA were measured in pike
574 collected from the effluent-impacted site. The results also showed that the relative
575 abundance of LPC 14:0 and LPC 18:2 were correlated with the concentrations of PBDEs
576 and Dechlorane-related compounds as well as with pike girth. The levels of different LPCs
577 that varied with HFR concentrations was potentially due to the modulation of the activity
578 or expression of the PLA2 enzyme and related to the under-expression of *pparα* in pike
579 collected downstream of the point of discharge of this effluent. Differences in LPC
580 abundance between pike collected upstream and downstream of the effluent could also be
581 due to an increase in oxidative stress and inflammatory response in fish collected
582 downstream. Indeed, oxidative stress and inflammation can induce changes in
583 phospholipid profiles by remodeling via the action of PLA2. This study allowed a better
584 understanding of the impacts of an environmental exposure to a WWTP effluent from a
585 highly urbanized and densely populated region on the composition of lipids and
586 transcription of lipid-related genes. Evaluating the transcription of several other PLA2
587 groups in future studies may contribute to better understand the underlying mechanisms of
588 action related to the alteration of phospholipid metabolism in fish exposed to urban
589 effluents. Moreover, investigating potential linkages between the concentrations of LPCs
590 (*e.g.*, LPC 14:0 and LPC 18:2) and markers of oxidative stress and inflammatory responses
591 in fish exposed to municipal effluents could be of added value to better understand the
592 health implications this exposure may have on predatory fish.

6. Acknowledgments

This study was funded primarily by Environment and Climate Change Canada (to M.H.) and supports the St. Lawrence Action Plan. Supplemental funding was provided by the Natural Sciences and Engineering Research Council of Canada (to J.V.). The authors would like to thank C. Beauvais, G. Cottin, M. Douville, M. Pilote, M. Lépine and A. Bernier-Graveline for assistance with field sampling, L. Wang for assistance with chemical analyses as well as M. Giraudo for assistance with primer design and RT-qPCR analyses. The authors declare they have no conflict of interest.

References

Abbasi, G., Buser, A. M., Soehl, A., Murray, M. W., Diamond, M. L., 2015. Stocks and flows of PBDEs in products from use to waste in the U.S. and Canada from 1970 to 2020. Environ. Sci. Technol. 49, 1521−1528. doi: 10.1021/es504007v

Al-Salhi, R., Abdul-Sada, A., Lange, A., Tyler, C. R., Hill, E. M., 2012. The xenometabolome and novel contaminant markers in fish exposed to a wastewater treatment works effluent. Environ. Sci. Technol. 46, 9080-9088. doi:10.1021/es3014453

Ankley, G. T., Jensen, K. M., Kahl, M. D., Makynen, E. A., Blake, L. S., Greene, K. J., Johnson, R.D., Villeneuve, D. L., 2007. Ketoconazole in the fathead minnow (*Pimephales promelas*): reproductive toxicity and biological compensation. Environ. Toxicol. Chem. 26, 1214-1223. doi:10.1897/06-428R.1

Arukwe, A., Cangialosi, M. V., Letcher, R. J., Rocha, E., Mortensen, A. S., 2013. Changes in morphometry and association between whole-body fatty acids and steroid hormone profiles in relation to bioaccumulation patterns in salmon larvae exposed to perfluorooctane sulfonic or perfluorooctane carboxylic acids. Aquatic Toxicol. 130-131, 219-230. https://doi.org/10.1016/j.aquatox.2012.12.026

Astudillo, A. M., Balboa, M. A., Balsinde, J., 2019. Selectivity of phospholipid hydrolysis by phospholipase A2 enzymes in activated cells leading to polyunsaturated fatty acid

624 mobilization. BBA – Mol. Cell Biol. Lipids, 1864, 772-783.
625 https://doi.org/10.1016/j.bbalip.2018.07.002
626
627 Benskin, J. P., Ikonomou, M. G., Liu, J., Veldhoen, N., Dubetz, C., Helbing, C. C.,
628 Cosgrove, J. R., 2014. Distinctive metabolite profiles in in-migrating Sockeye salmon
629 suggest sex-linked endocrine perturbation. Environ. Sci. Technol. 48, 11670-11678.
630 doi:10.1021/es503266x
631
632 Blazer, VS., 2002. Histopathological assessment of gonadal tissue in wild fishes. Fish
633 Physiol. Biochem. 26, 85-101. doi:10.1023/A:1023332216713
634
635 Cantinho, P., Matos, M., Trancoso, M., Santos, M., 2016. Behaviour and fate of metals in
636 urban wastewater treatment plants: a review. Intern. J. Environ. Sci. Technol. 13, 359–386.
637 doi:10.1007/s13762-015-0887-x
638
639 Catalá, A., 2012. Lipid peroxidation modifies the picture of membranes from the “Fluid
640 Mosaic Model” to the “Lipid Whisker Model”. Biochimie. 94, 101-109.
641 https://doi.org/10.1016/j.biochi.2011.09.025
642
643 Christiansen, H. E., Mehinto, A. C., Yu, F., Perry, R. W., Denslow, N. D., Maule, A. G.,
644 Mesa, M. G., 2014. Correlation of gene expression and contaminant concentrations in wild
645 largescale suckers: A field-based study. Sci. Total Environ. 484, 379-389.
646 http://dx.doi.org/10.1016/j.scitotenv.2013.08.034

Darbre, P. D., 2017. Endocrine disruptors and obesity. Cur. Obesity Rep. 6, 18-27. doi:10.1007/s13679-017-0240-4

De Wit, C. A., 2002. An overview of brominated flame retardants in the environment. Chemosphere. 46, 583-624. https://doi.org/10.1016/S0045-6535(01)00225-9

Del Bas, J. M., Caimari, A., Rodriguez-Naranjo, M. I., Childs, C. E., Chavez, C. P., West, A. L., Miles, E.A., Arola, L., Calder, P. C., 2016. Impairment of lysophospholipid metabolism in obesity: altered plasma profile and desensitization to the modulatory properties of n-3 polyunsaturated fatty acids in a randomized controlled trial. Amer. J. Clinic. Nut. 104, 266–79. doi:10.3945/ajcn.116.130872.

Dimastrogiovanni, G., Córdoba, M., Navarro, I., Jáuregui, O., Porte, C., 2015. Alteration of cellular lipids and lipid metabolism markers in RTL-W1 cells exposed to model endocrine disrupters. Aquatic Toxicol. 165, 277-285. doi: 10.1016/j.aquatox.2015.06.005

Du, Z., Zhang, Y., Wang, G., Peng, J., Wang, Z., Gao, S., 2016. TPhP exposure disturbs carbohydrate metabolism, lipid metabolism, and the DNA damage repair system in zebrafish liver. Science Rep. 6, 6136. doi:10.1038/srep21827

Ekman, D.R., Teng, Q., Villeneuve, D.L., Kahl, M.D., Jensen, K.M., Durhan, E.J.,Ankley, G.T., Collette, T.W., 2008. Investigating compensation and recovery of fathead minnow

(*Pimephales promelas*) exposed to 17α-ehynylestradiol with metabolite profiling. Environ. Sci. Technol. 42, 4188-4194. doi:10.1021/es8000618

Espinosa-Ruiz, C., Manuguerra, S., Cuesta, A., Esteban, M. A., Santulli, A., Messina, C. M., 2019. Sub-lethal doses of polybrominated diphenyl ethers affect some biomarkers involved in energy balance and cell cycle, via oxidative stress in the marine fish cell line SAF-1. Aquatic Toxicol. 210, 1-10. https://doi.org/10.1016/j.aquatox.2019.02.014

Fadhlaoui, M., Couture, P., 2016. Combined effects of temperature and metal exposure on the fatty acid composition of cell membranes, antioxidant enzyme activities and lipid peroxidation in yellow perch (*Perca flavescens*). Aquatic Toxicol. 180, 45-55. http://dx.doi.org/10.1016/j.aquatox.2016.09.005

Giang, P. T., Sakalli, S., Fedorova, G., Tilami, S. K., Bakal, T., Najmanova, L., Burkina, V., 2018. Biomarker response, health indicators, and intestinal microbiome composition in wild brown trout (*Salmo trutta m. fario L.*) exposed to a sewage treatment plant effluent-dominated stream. Sci. Total Environ. 625, 1494-1509. https://doi.org/10.1016/j.scitotenv.2018.01.020

Gobeil, C., Rondeau, B., Beaudin, L., 2005. Contribution of municipal effluents to metal fluxes in the St. Lawrence River. Environ. Sci. Technol. 39, 456-464. doi:10.1021/es049335x

692 Grzelczyk, A., Gendaszewska-Darmach, E., 2013. Novel bioactive glycerol-based
693 lysophospholipids: New data - New insight into their function. Biochimie 95, 667-679.
694 https://doi.org/10.1016/j.biochi.2012.10.009
695
696 Houde, M., Giraudo, M., Douville, M., Bougas, B., Couture, P., Lair, S., Spencer, C., De
697 Silva, A.O., Bernatchez, L., Gagnon, C., 2014. A multi-level biological approach to
698 evaluate impacts of a major municipal effluent in wild St. Lawrence River yellow perch
699 (*Perca flavescens*). Sci. Total Environ. 497-498, 307-318.
700 https://doi.org/10.1016/j.scitotenv.2014.07.059
701
702 Jackson, S. K., Abate, W., Tonks, A. J., 2008. Lysophospholipid acyltransferases: Novel
703 potential regulators of the inflammatory response and target for new drug discovery.
704 Pharmacol. Therap. 119, 104-114. doi:10.1016/j.pharmthera.2008.04.001
705
706 Le Cren, E. D., 1947. The determination of the age and growth of the perch (*Perca*
707 *fluviatilis*) from opercular bone. J. Anim. Ecol. 16, 188-204. doi: 10.2307/1494
708
709 Liu, A., Krausz. K. W., Fang, Z.-Z., Brocker, C., Qu, A., Gonzalez, F. J., 2014. Gemfibrozil
710 disrupts lysophosphatidylcholine and bile acid homeostasis via PPARα and its relevance
711 to hepatotoxicity. Arch. Toxicol. 88, 983-996. doi:10.1007/s00204-013-1188-0
712

713 Lu, K., Qiao, R., An, H., Zhang, Y., 2018. Influence of microplastics on the accumulation
714 and chronic toxic effects of cadmium in zebrafish (*Danio rerio*). Chemosphere. 202,
715 514e520. https://doi.org/10.1016/j.chemosphere.2018.03.145
716
717 Maradonna, F., Nozzi, V., Santangeli, S., Traversi, I., Gallo, P., Fattore, E., Mita, D.G.,
718 Mandich, A., Carnevali, O., 2015. Xenobiotic-contaminated diets affect hepatic lipid
719 metabolism: Implications for liver steatosis in *Sparus aurata* juveniles. Aquatic Toxicol.
720 167, 257-264. http://dx.doi.org/10.1016/j.aquatox.2015.08.006
721
722 Marcogliese, D. J., Blaise, C., Cyr, D., de Lafontaine, Y., Fournier, M., Gagné, F., Gagnon,
723 C., Hudon, C., 2015. Effects of a major municipal effluent on the St. Lawrence River: A
724 case study. Ambio. 44, 257-274. doi:10.1007/s13280-014-0577-9
725
726 Maruya, K. A., Vidal-Dorsch, D. E., Bay, S. M., Kwon, J. W., Xia, K., Armbrust, K. L.,
727 2012. Organic contaminants of emerging concern in sediments and flatfish collected near
728 outfalls discharging treated wastewater effluent to the Southern California Bight.
729 Environm. Toxicol. Chem. 31, 2683-2688. doi: 10.1002/etc.2003
730
731 Melvin, S. D., Lanctôt, C. M., Doriean, N. J. C., Bennett, W. W., Carroll, A. R., 2019.
732 NMR-based lipidomics of fish from a metal(loid) contaminated wetland show differences
733 consistent with effects on cellular membranes and energy storage. Sci. Total Environ. 654,
734 284-291. https://doi.org/10.1016/j.scitotenv.2018.11.113
735

736 Mouchlis, V. D., Dennis, E. A., 2019. Phospholipase A2 catalysis and lipid mediator
737 lipidomics. BBA – Mol. Cell Biol. Lipids. 1864, 766-771.
738 https://doi.org/10.1016/j.bbalip.2018.08.010
739
740 Nava-Álvarez, R., Razo-Estrada, A. C., García-Medina, S., Gómez-Olivan, L. M., Galar-
741 Martínez, M., 2014. Oxidative stress induced by mixture of diclofenac and acetaminophen
742 on common carp (*Cyprinus carpio*). Water Air Soil Poll. 225, 1-9. doi:10.1007/s11270-
743 014-1873-5
744
745 Nikinmaa, M., Rytkönen, K. T., 2011. Functional genomics in aquatic toxicology - do not
746 forget the function. Aquatic Toxicol. 105, 16-24.
747 https://doi.org/10.1016/j.aquatox.2011.05.019
748
749 Olivares-Rubio, H. F., Vega-Lopez, A., 2016. Fatty acid metabolism in fish species as a
750 biomarker for environmental monitoring. Environ. Poll. 218, 297-312.
751 http://dx.doi.org/10.1016/j.envpol.2016.07.005
752
753 Rakhshandehroo, M., Knoch, B., Müller, M., Kersten, S., 2010. Peroxisome proliferator-
754 activated receptor alpha target genes. PPAR Res. 2010, 1-20. doi:10.1155/2010/612089
755
756 Rauschert, S., Uhl, O., Koletzko, B., Kirchberg, F., Mori, T. A., Huang, R.C., beilin, L.J.,
757 Hellmuth, C., Oddy, W. H., 2016. Lipidomics reveals associations of phospholipids with

758 obesity and insulin resistance in young adults. J. Clin. Endocrinol. Metabol. 101, 871-87.
759 doi:10.1210/jc.2015-3525
760
761 Reinling, J., Houde, M., Verreault, J., 2017. Environmental exposure to a major urban
762 wastewater effluent: Effects on the energy metabolism of northern pike. Aquatic Toxicol.
763 191, 131-140. http://dx.doi.org/10.1016/j.aquatox.2017.08.003
764
765 Ren, X., Zhang, H., Geng, N., Xing, L., Zhao, Y., Wang, F., Chen, J., 2018. Developmental
766 and metabolic responses of zebrafish (*Danio rerio*) embryos and larvae to short-chain
767 chlorinated paraffins (SCCPs) exposure. Sci. Total Environ. 622-623, 214-221.
768 http://dx.doi.org/10.1016/j.scitotenv.2017.11.304
769
770 Rozen, S., Skaletsky, H., 2000. Primer3 on the WWW for general users and for biologist
771 programmers. Methods Mol. Biol., 132, 365-386. doi:10.1385/1-59259-192-2:365
772
773 Sakalli, S., Giang, P. T., Burkina, V., Zamaratskaia, G., Rasmussen, M. K., Bakal, T.,
774 Tilami, S.K., Sampels, S., Kolarova, J., Grabic, R., Turek, J., Randak, T., Zlabek, V., 2018.
775 The effects of sewage treatment plant effluents on hepatic and intestinal biomarkers in
776 common carp (*Cyprinus carpio*). Sci. Total Environ. 635, 1160-1169.
777 https://doi.org/10.1016/j.scitotenv.2018.04.188
778

779 Samuelsson, L. M., Björlenius, B., Förlin, L., Larsson, D. G. J., 2011. Reproducible 1H
780 NMR-based metabolomic responses in fish exposed to different sewage effluents in two
781 separate studies. Environ. Sci. Technol. 45, 1703−1710. doi: 10.1021/es104111x
782
783 Santos, A. L., Preta, G., 2018. Lipids in the cell: organisation regulates function. Cell. Mol.
784 Life Sci. 75, 1909–1927. https://doi.org/10.1007/s00018-018-2765-4
785
786 Schulz, H., 2002. Oxidation of fatty acid in eukaryotes. In D. E. Vance and J. E. Vance,
787 Biochemistry of Lipids, Lipoproteins and Membranes (p. 127-150), 4th Ed. Elsevier
788 Science B.V.
789
790 Shen, L., Jobst, K. J., Reiner, E. J., Helm, P. A., McCrindle, R., Taguchi, V. Y., Marvin,
791 C.H., Backus, S., MacPherson, K.A., Brindle, I. D., 2014. Identification and occurrence of
792 analogues of Dechlorane 604 in Lake Ontario sediment and their accumulation in fish.
793 Environ. Sci. Technol. 48, 11170-11177. dx.doi.org/10.1021/es503089c
794
795 Stockholm Convention, 2009. Report of the conference of the parties of the Stockholm
796 convention on persistent organic pollutants on the work of its fourth meeting**.** Conference
797 of the Parties of the Stockholm Convention on Persistent Organic Pollutants Fourth
798 meeting.
799

800 Stockholm Convention, 2017. The 16 New POPs - An introduction to the chemicals added
801 to the Stockholm Convention as Persistent Organic Pollutants by the Conference of the
802 Parties. Geneva, Switzerland.
803
804 Suárez-García, S., Caimari, A., del Bas, J. M., Suárez, M., Arola, L., 2017. Serum
805 lysophospholipid levels are altered in dyslipidemic hamsters. Scientific Rep. 7, 10431.
806 doi:10.1038/s41598-017-10651-0
807
808 Sverko, E., Tomy, G. T., Reiner, E. J., Li, Y.-F., McCarry, B. E., Arnot, J. A., Law, R.J.,
809 Hites, R. A., 2011. Dechlorane Plus and related compounds in the environment: A review.
810 Environ. Sci. Technol. 45, 5088-5098. dx.doi.org/10.1021/es2003028
811
812 Takahashi, H., Goto, T., Yamazaki, Y., Kamakari, K., Hirata, M., Suzuki, H., Shibata, D.,
813 Nakata, R., Inoue, H., Takahashi, N., Kawada, T., 2015. Metabolomics reveal 1-palmitoyl
814 lysophosphatidylcholine production by peroxisome proliferator-activated receptor. J. Lipid
815 Res. 56, 254-265. doi:10.1194/jlr.M052464
816
817 Tocher, D. R., 2003. Metabolism and functions of lipids and fatty acids in teleost fish. Rev.
818 Fish. Sci. 11, 107–184. doi:10.1080/713610925
819
820 Tongue, A. D. W., Reynolds, S. J., Fernie, K. J., Harrad, S., 2019. Flame retardant
821 concentrations and profiles in wild birds associated with landfill: A critical review.
822 Environ. Poll. 248, 646e658.

Tran, N. H., Reinhard, M., Gin, K. Y.-H., 2018. Occurrence and fate of emerging contaminants in municipal wastewater treatment plants from different geographical regions-a review. Water Res. 133, 182e207. https://doi.org/10.1016/j.watres.2017.12.029

Wang, Y., Li, X., Li, A., Wang, T., 2007. Effect of municipal sewage treatment plant effluent on bioaccumulation of polychlorinated biphenyls and polybrominated diphenyl ethers in the recipient water. Environ. Sci. Technol. 41, 6026-6032. doi:10.1021/es070913u

Wang-Sattler, R., Yu, Z., Herder, C., Messias, A. C., Floegel, A., He, Y., Heim, K., Campillos, M., Holzapel, C., Thorand, B., Grallert, H., Xu, T., Bader, E., Huth, C., Mittelstrass, K., Döring, A., Meisinger, C., Gieger, C., Prehn, C., Roemisch-Margl, W., Carstensen, M., Xie, L., Yamanaka-Okumura, H., Xing, G., Ceglarek, U., Thiery, J., Giani, G., Lickert, H., Lin, X., Li, Y., Boeing, H., Joost, H.-G., de Angelis, M.H., Rathmann, W., Suhre, K., Prokisch, H., Peters, A., Meitinger, T., Roden, M., Wichmann, H.-E., Pischon, T., Adamski, J., Illig, T., 2012. Novel biomarkers for pre-diabetes identified by metabolomics. Mol. Syst. Biol. 8, 615. doi:10.1038/msb.2012.43

Wen, Q., Xie, X., Zhao, C., Ren, Q., Zhang, X., Wei, D., Emanuelli, B., Du, Y., 2019. The brominated flame retardant PBDE 99 promotes adipogenesis via regulating mitotic clonal expansion and PPARγ expression. Sci. Total Environ. 670, 67-77. https://doi.org/10.1016/j.scitotenv.2019.03.201

Zhao, Y., Chen, Y.-Q., Bonacci, T. M., Bredt, D. S., Li, S., Bensch, W. R., Moller, D.E., Kowala, M., Konrad, R.J., Cao, G., 2008. Identification and characterization of a major liver lysophosphatidylcholine acyltransferase. J. Biol. Chem. 283, 8258-8265. doi: 10.1074/jbc.M710422200