



## Concentration of organic contaminants in fish and their biological effects in a wastewater-dominated urban stream

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### ABSTRACT

Data are presented on the concentrations of alkylphenol and alkylphenol ethoxylates (APEs) and persistent organic compounds in largemouth bass collected from a waste-water dominated stream in downtown Chicago. The fish residue concentrations of APEs are compared to concentrations of the APEs in the water that were collected at weekly intervals over two months bracketing the fall (2006) and a spring (2007) fish collection. The concentrations of APEs were significantly higher in the spring-collected fish (5.42 µg/g) versus the fall (0.99 µg/g) and these differences were shared by differences in the water concentrations (spring – 11.47 versus fall – 3.44 µg/L). The differences in water concentration were negatively correlated with water temperatures observed over the two sampling times. Fish residue concentrations of persistent organic compounds (PCBs, PBDEs, toxaphene, and many legacy pesticides including the DDT family) did not vary from fall to spring. Some of these residue concentrations were comparable to the highest NPE (nonylphenol ethoxylate) homologue concentrations, e.g. NP1EO was 3.5 µg/g in the bass for the spring, the PBDE-congener 47 and p,p'-DDE averaged 1.0 µg/g and 0.5 µg/g, respectively, over both seasons. All the other persistent single-analyte concentrations were lower. Biological endpoints for endocrine effects measured in the same fish showed that there was an apparent positive correlation for physiological effects based on increased vitellogenin levels in males versus concentration of NPEs; however there were no observable histological differences in fall versus spring fish samples.

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### 1. Introduction

Wastewater-dominated urban streams have been cited as likely sites for the presence of endocrine active chemicals (EACs) (Jobling and Sumpter, 1993; Johnson and Sumpter, 2001; Kolpin et al., 2002; Sumpter and Johnson, 2005). In spite of much research on the presence and biological effects of EACs, it has been difficult to establish correlative relationships between the presence of EACs and adverse biological effects consistent with exposure to these compounds. Contributing to the current inability of linking EACs and effects in the environment are several factors. Many studies have focused on searching and assessing the most potent EAC compounds found in these waters, especially natural estrogens that survive passage through treatment systems and at levels that could affect aquatic

organisms (Johnson and Sumpter, 2001; Kolpin et al., 2002), and synthetic estrogens, such as 17- $\alpha$  ethinylestradiol, that are particularly potent. However, other suspected EACs were also found to be ubiquitous in these early studies, including high concentrations of alkylphenols downstream of wastewater treatment plants (Barber et al., 2000, 2007; Loyo-Rosales et al., 2007a). This was unexpected and only later found to be the result of the biodegradation of a then heavily used class of neutral surfactants, the alkylphenol ethoxylates (APEs). After further studies it is now recognized that in most systems these effluents contain a family of alkylphenolic compounds (alkylphenols (APs) and alkylphenol ethoxylates) that arise mostly from one parent group of surfactants, the nonylphenol ethoxylates (NPEs). The primary base structure in this group is p-nonylphenol (NP) that is linked through an ether bond to various chain lengths of repeating ethoxy units. Further studies of effluent-dominated streams have revealed that many additional compounds are common in these discharges (Kolpin et al., 2002) and may also act as EACs. Among these suspected EACs are bisphenol A, several pharmaceuticals, personal care products, and even the

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bioaccumulative chemicals that are routinely monitored in the Great Lakes such as polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs) and many organochlorine legacy pesticides (DDT, chlordane etc.). The study reported here focuses on these non-hormonal EACs, with particular emphasis on the alkylphenols and alkylphenol ethoxylates and the routinely monitored bioaccumulative chemicals, their presence as residues in the fish tissue, and their biological effects.

Numerous studies have been conducted to determine responses and subsequent effects on fish exposed to effluents containing natural estrogens and the family of alkylphenol ethoxylate (Barber et al., 2007; Bistodeau et al., 2006; Schoenfuss et al., 2008). Among the effects most commonly associated with exposure to EACs are the induction of the egg-yolk precursor protein vitellogenin and the presence of histopathological abnormalities in the gonads of male fish. Enhanced plasma concentrations of vitellogenin in male fish indicates recent (<30 days) (Hemmer et al., 2002; Hyndman et al., 2010) exposure to estrogenic compounds as the synthesis of this protein in liver hepatocytes is driven by elevated estrogen concentrations usually only found in mature female fish (Sumpter and Johnson, 2005). While elevated plasma vitellogenin concentrations in male fish indicate recent exposure to estrogenic EACs, histopathological changes to reproductive organs, especially in male fish, are likely indicative of longer-term or early life stage exposure to estrogenic EACs in the environment (Blazer et al., 2007; Hinck et al., 2009b; Jobling et al., 1998; Kidd et al., 2007). In this study, we quantified plasma vitellogenin concentrations and assessed histological preparations of reproductive tissues for indication of abnormalities in wild largemouth bass (*Micropterus salmoides*) collected from a waste-water dominated urban stream.

It continues to be a problem to establish clear proof for a link between suspected EACs in aquatic environments and biological effects in fish under environmental conditions. Typically the approach has been to use the concentrations of the expected agent(s) in the water contacting the fish and then attempt to associate certain fish EAC responses to these compounds. With this technique, however, questions arise about actually proving whether accumulation has occurred and what the duration of the exposures were. These questions are especially difficult to answer using water analyses even with flow-weighted continuous collections. However for accumulated compounds like persistent pollutants and APEs, fish residue values can be measured and effectively used to supplant these water measurements for determining exposures. Therefore relying on tissue concentrations was the tool employed here to attempt to establish correlation to EAC effects in these fish.

In terms of accumulation, APEs are considered “pseudo-persistent” (Schultz et al., 2010) and even though moderate depuration by the fish has been observed (Spehar et al., 2010b), considerable evidence for their elevated concentrations in fish has been documented (Blackburn et al., 1999; Rice et al., 2003; Snyder et al., 2001). A convenient method for assessing aquatic uptake of APEs is to use bioaccumulation factors (BAFs or BCF) (Staples et al., 1998).

A central objective of this study was to attempt to test the hypothesis that high concentrations of non-steroidal EACs in fish can induce vitellogenin synthesis and alter gonadal histology in resident fish. In this study, we attempted to also include many of the persistent organic pollutants among the monitored compounds along with establishing levels of the alkylphenols and alkylphenol ethoxylates in fish tissue. Furthermore, data are presented for weekly grab samples of water showing how the concentrations of alkylphenols and alkylphenol ethoxylates vary over time in the water column. Finally, these data are correlated with observations of biological effects in the analyzed fish.

## 2. Methods

### 2.1. Site description

The North Shore Channel flows from the North Chicago shoreline of Lake Michigan, Wilmette Harbor, to the North Branch of the

Chicago River just downstream of the Foster Avenue Bridge a distance of about 8 km, (Fig. 1). The major flow in this channel (>80% during the summer and >95% in winter) is contributed by the North Side Water Reclamation Plant which is located about 4 km downstream of Wilmette pumping station which is used frequently from April to October to augment flow to the Channel. The North Side Water Reclamation Plant collects sewage from residential, >96%, and commercial/industrial, 3.4%, sources from an area of approximately 777 km<sup>2</sup>, serves a population of 1.3 million people, and treats an average of 10.8 m<sup>3</sup> s<sup>-1</sup> of sewage using activated sludge processes (Metropolitan Water Reclamation District, MWRD, 2012). The section of the channel between the North Side Water Reclamation Plant and Foster Avenue Bridge was extensively sampled for both water and fish between September 2006 and March 2007. This site was also selected as one of 5 effluent-dominated streams that were sampled in the Environmental Protection Agency's Pilot Study of Pharmaceuticals and Personal Care Products in Fish Tissue, <http://water.epa.gov/scitech/swguidance/ppcp/fish-tissue.cfm>.

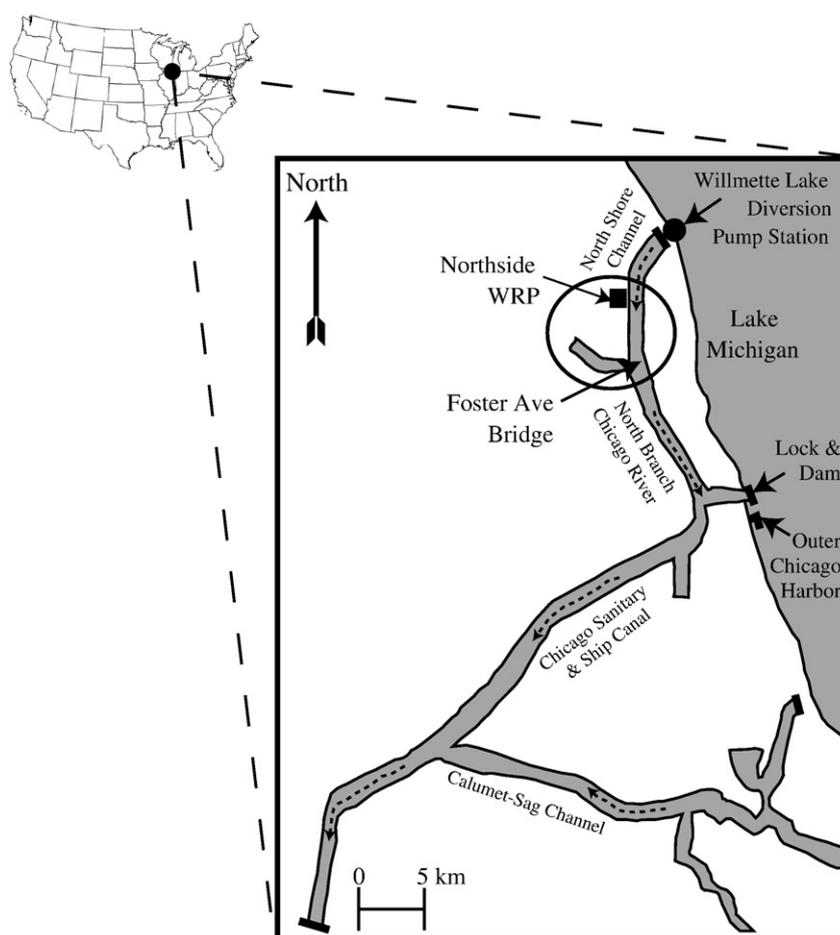
### 2.2. Sample collection

#### 2.2.1. Fish sampling

Largemouth bass were collected by electro-shocking (Smith-Root electro-shocking boat, Vancouver, WA) from the North Shore Channel by personnel of the Metropolitan Water Reclamation District of Greater Chicago at two different times once in the fall (September, 28, 2006) and once in the spring (March 3, 2007). Largemouth bass were chosen for this study as they represent a native and a sustained fish population in the Chicago River system. Animal care and use protocols were approved by the St. Cloud State University Institutional Animal Care and Use Committee. As a long-lived piscivore species, largemouth bass are also most likely to exhibit signs of prolonged exposure to EACs in the North Shore Channel. In the fall, six fish were sampled in the region just before the confluence of the North Shore Channel with the North Chicago River [between Foster Avenue Bridge and the outfall of the North Side Water Reclamation Plant] (Fig. 1) a distance of ~5 km. Three reference fish were collected from the Outer Chicago Harbor of Lake Michigan (separated by a lock-and-dam structure from the Chicago River system with minimal water exchange between the two systems). For the spring collection, five largemouth bass along with several additional largemouth bass that were prepared as fillet composites were collected from the same section of the North Shore Channel, while 3 reference fish were obtained from Braidwood cooling pond located south of Joliet IL ~141 km away, at the Braidwood Power Plant. For all sites, fish were sacrificed by a sharp blow to the head immediately prior to the collection of whole blood samples from the caudal vasculature using a syringe and heparinized vacutainers (VWR). Vacutainers were stored on ice and within 24 h returned to the laboratory where each sample was centrifuged for 5 min at 5900 g and 4 °C. Plasma samples were stored at -80 °C until analysis. Following blood collection, a midsagittal incision was made in the abdominal region of each fish using cleaned stainless steel instruments. Several small tissue samples (~3 mm<sup>3</sup>) were collected, secured inside histoscreen-cassettes (Thermo-Scientific, Waltham, MA), and immersed in 10% buffered formalin until further processing. The remainder of each fish was wrapped in aluminum foil placed on ice and frozen for later homogenization in order to prepare them for analysis of accumulated organics (alkylphenols and alkylphenol ethoxylates and selected persistent organic pollutants monitored in the Great Lakes Program monitoring program (Great Lakes Monitoring Program, 2010)).

#### 2.2.2. Water sampling

Grab water samples were collected from the North Shore Channel, primarily at the Foster Ave. bridge location before the two fish sampling periods and then continued at weekly intervals for about 1 month after the fish sampling. Water samples were also collected as effluent discharging into the North Shore Channel at the North



**Fig. 1.** Map showing the North Shore Channel where the fish and water samples were collected and the North Side Water Reclamation Plant (WRP) where the effluent was sampled. The area studied is marked with a black circle.

Side Water Reclamation Plant starting in the fall on 9/14/2006, two weeks before fish collection, and in the spring, sampling commenced on 2/26/2007, 5 days before the fish were obtained. The stream samples were collected from the center of the channel about 1 m below the water surface using a stainless steel bucket lowered from the bridge. The bucket and glass sampling bottles were rinsed three times with field-collected water prior to their use. These unfiltered water samples were stored at 4 °C after being preserved either with a sulfuric acid addition (2 ml of 50% sulfuric acid added to 1-l) for APEs in the low ethoximer (EO) category (AP, and AP 1, 2 and 3 EOs) or with formaldehyde addition (7 ml of 37% formalin solution added to 250 ml of sample) for the higher ethoximer analyses (AP 2 to 18 EOs). These storage and preservation treatments were done to minimize degradation prior to extraction and/or analysis. The stream sampling was performed by USEPA Region V staff and the North Side Water Reclamation Plant effluent samples were collected by the staff of the Metropolitan Water Reclamation District of Greater Chicago, Chicago, IL, USA.

### 2.3. Biological analysis

Plasma vitellogenin and histopathology were evaluated on all of the collected fish at the St. Cloud State University Aquatic Toxicology Laboratory (St. Cloud, MN). Plasma vitellogenin in largemouth bass was measured using indirect enzyme-linked immunosorbent assay (ELISA) protocols (Denslow et al., 1999; Sepúlveda et al., 2002). Standard vitellogenin from rainbow trout (*Oncorhynchus mykiss*) at varying concentrations or plasma from largemouth bass at three different dilutions was coated onto micro titer wells. After incubation at 25 °C for 2 h, the

wells were washed three times using an automated plate washer (Awareness Technology, Palm City, FL), a monoclonal striped bass (*Morone saxatilis*) antibody was added to the wells at a 1:100 dilution, and the wells were incubated for 2 h at room temperature. After incubation, wells were triple washed and an anti-mouse IgG horseradish peroxidase, conjugated antibody was added to the wells at a 1:2000 dilution, and the wells were incubated at 25 °C for 1 h. After incubation, the wells were washed again, tetramethylbenzidine was added as a substrate, and the wells were incubated for 15 min. Absorbance was read at 620 nm using a Thermo Multiscan plate reader.

Liver and reproductive organs were processed for histological analysis following a one-week fixation period. Briefly, tissue samples were dehydrated through a series of ethanol and xylene baths in a Leica Jung TP 1050 automated tissue processor (Wetzlar, Germany), and embedded in paraffin using a Tissue Tek Cryo Console (Torrance, CA). Tissue sections were cut at 5 µm (approximately 50 µm between sections) on a Reichert Jung 2030 microtome (Wetzlar, Germany). At least six sections from each organ (right/left gonad, liver) were stained with a Leica Autostainer XL using hematoxylin and eosin staining protocols modified after Gabe (1976) and Carson (1996). Histological sections of the livers were assessed for the abundance of hepatocyte vacuoles and gonads were assessed for sex, maturity (spermatogenic stage), and pathological changes. Liver endpoints were chosen to provide insight into the energetic condition of the fish since hepatocyte vacuoles increase in prominence in energetically stable fish and may decrease as a result of environmental stress including, but not limited to exposure to pollutants. Furthermore the liver is the most prominent detoxifying organ of chordates and the site of vitellogenin production. Gonadal (testis and ovary) endpoints were selected to allow histological identification of

sex and the assessment of the state of spermatogenesis which may be altered by exposure to EACs. In addition, the condition of intersex (ovarian tissues located in male reproductive organs) has been linked prominently with exposure to estrogenic EACs (Blazer et al., 2007; Hinck et al., 2007; Jobling et al., 1998).

#### 2.4. Chemical analyses

##### 2.4.1. Preparation of fish for chemical analyses

The whole fish were individually ground using a Robot Coupe RS1 6V® Chopper following the method of Hesselberg (1997) and Datta et al. (2002). Once the fish were reduced to a smooth uniform paste, they were weighed and placed in pre-cleaned 350 ml I-Chem glass jars (two jars per sample). These samples were then refrozen and kept at  $-15\text{ }^{\circ}\text{C}$  until the day they were prepped for APEs extraction or until they were sent on dry ice to Clarkson Center for the Environment, Clarkson University, Potsdam, NY for their analysis of PBDEs, PCBs, toxaphenes and other organochlorine compounds (OCs).

##### 2.4.2. Alkylphenols and alkylphenol ethoxylates in fish

For alkylphenol and alkylphenol ethoxylate analyses the fish extracts were prepared using a modification of the method of Mao et al. (2006). Five grams of each homogenized sample was amended with isotopically-labeled APE internal standards and sequentially mixed for 2 min with 30 ml of acetonitrile using a model Polytron® PT 10-35 (Kinematica AG) homogenizer. The resulting organic extract was filtered through baked  $\text{Na}_2\text{SO}_4$ . These initial fish extracts were each evaporated by rotary evaporator using a  $35\text{ }^{\circ}\text{C}$  water bath to dryness, then redissolved in 10 ml of hexane. These extracts were shaken sequentially, three times starting with two 15-ml portions of acetonitrile, and finally with 30 ml of acetonitrile. This acetonitrile was previously prepared by saturating it with hexane for 5 min (ACN: hexane 50:50 v/v). The combined acetonitrile extracts were then evaporated just to dryness using a rotary evaporator held at  $35\text{ }^{\circ}\text{C}$  as before. These residues were next dissolved in 5 ml of hexane and cleaned up using a 10% water-deactivated florisil column (250-mm length  $\times$  11-mm internal diameter) after pre-rinsing it with 50 ml of hexane. The optimum florisil deactivation level was previously determined by tests using 8, 10, and 12% water. The cleanup step involved addition of the 5 ml extract to the top of each column that was layered with 1-cm of  $\text{Na}_2\text{SO}_4$ . The column was eluted with 40 ml of hexane:acetone (90:10, v/v). The collected eluates (50 ml) were evaporated to dryness in a  $35\text{ }^{\circ}\text{C}$  water bath using a rotary evaporator, transferred in acetonitrile to a 10-ml test tube and finally blown down to 1.5 ml using a stream of nitrogen. These final extracts were analyzed using LC/MS–MS methods as described by Loyo-Rosales et al. (2007b). Isotopically labeled APE internal standards [ $^{13}\text{C}_6$ ]-mixtures of  $^{13}\text{C}_6$ -NP and  $^{13}\text{C}_6$ -NP(1.6)EO were used for quantitation based on the method of isotope dilution mass spectrometry (Loyo-Rosales et al., 2007a). This method allows correction for matrix interferences. For each batch of fish samples, e.g. the fall-collected fish batch and the spring-collected fish batch, one spike, one blank and one replicate was prepared and analyzed for quality-control purposes.

The alkylphenols and alkylphenol ethoxylates in the extracts were chromatographically separated using a mixed-mode MSpak GF-310 4D liquid chromatography column,  $4.6\times 150\text{ mm}$  (Shodex, Shoko Co., Tokyo, Japan) and detection was done in multiple-reaction monitoring mode using electrospray positive for the APEOs and electrospray negative for the APs. The full method is described in Loyo-Rosales et al. (2003).

##### 2.4.3. Alkylphenols and alkylphenol ethoxylates in water

The water samples analyses for the NP, NP1EO, and NP2EO were carried out by gas chromatography mass spectrometry after solvent extraction of 1-l samples using a CRL (USEPA-Chicago Regional Laboratory) method based on ASTM D7065 (ASTM, 2003) as modified for low-level analysis using large volume injection as discussed in CRL's

SOP MS004 (Chicago Regional Laboratory-, 2007) "Short chain alkylphenol ethoxylates and bisphenol A". Briefly the acid stabilized water samples are solvent extracted using methylene chloride and the resulting extracts were concentrated to 0.5 ml and analyzed by GC/MS with electron impact and selected ion monitoring to identify 12 NP isomers and 10 each NP1EO and NP2EO isomers (e.g., branched chain homologues of the alkyl chain carbon group (9 carbons), none of which is straight chain). These do not represent identified isomers, only those separated by the DB-5MS column and identified by ion chromatographs when 135, 149, 163 m/z peaks are selected and when the use of secondary peaks (121, 107, 220 m/z) are there to verify the identifications. These same sets of ions were used for the 1 and 2EO but increased by 44 mass units to represent the addition of the  $-\text{CH}_2-\text{CH}_2-\text{O}^+$  (ethoxylate) unit ( $28 + 16 = 44$ ). The specific instrument used for the GC analyses was an Agilent 6890 GC coupled to an Agilent 5973 Mass Selection Detector operated in single ion detection mode. The compounds were separated using a DB5MS J&W  $30\text{-m}\times 0.25\text{-mm}$  i.d. film thickness of  $0.25\text{ }\mu\text{m}$  (5% phenyl) methylpolysiloxane coating. Quantitation was accomplished by matching ion chromatograms for standards and samples and the minimum number of peaks for each group, NP, NP1EO and NP2EO was, respectively 10, 8 and 8. Total average for each analyte group was equal to the sum of each of these selected peaks. NP was quantitated against the internal standard, acenaphthene- $\text{d}_{10}$ ; and NP1EO and NP2EO analyte groups were quantitated using phenanthrene- $\text{d}_{10}$ . Octylphenol was also quantitated using acenaphthene- $\text{d}_{10}$  as well as n-NP and n-NP1EO, which were added as surrogate standards to verify performance.

Analyses for longer chain APEOs (NPnEO,  $3\leq n\leq 18$ ) and octylphenol polyethoxylates (OPnEO,  $2\leq n\leq 12$ ) were also carried out using liquid chromatography mass spectrometry after solvent extraction using CRL's SOP MS006 V1 (Nagarnaik et al., 2010). However, none of these compounds was detected above the method detection limits that were based on direct injection of collected 250 ml water samples (i.e. without preconcentration).

##### 2.4.4. PBDEs, PCBs, DDTs, toxaphene and chlorinated pesticide in fish

Congener-specific PCB analyses were conducted based on dual-capillary column procedures previously described; (Chiarenzelli et al., 2001; Pagano et al., 1999; Stewart et al., 2008). Briefly, analytical instruments were recalibrated every five samples, with a system blank, instrument blank, and mid-level calibration check solutions analyzed during each analytical run. Two Agilent Model 7890A GCs with micro electron capture detectors (ECD –  $\text{Ni}^{63}$ ) and auto samplers were used for data acquisition. The primary analytical column was a 60-m DB-XLB capillary column with  $0.25\text{-mm}$  i.d. and  $0.25\text{-}\mu\text{m}$  film thickness. The calibration standard used was a 1:1:1:1 mixture of congener mixture sets (C-CSQ-SET 1–5;  $10\text{ }\mu\text{g}/\mu\text{l}$  per individual congener, AccuStandard, Inc., New Haven, CT) based on the work of George Frame et al. (1996). This analytical setup allowed for the analysis of 122 chromatographic zones of 155 PCB congeners/co-eluters. PCB analyses were confirmed utilizing an Agilent Ultra II, 25-m column with  $0.22\text{-mm}$  i.d. and  $0.33\text{-}\mu\text{m}$  film thickness. Congener determination, assignments and accuracy of quantitation were verified for both GC-ECD analytical systems utilizing nine PCB congener mixtures (C-CSQ-SET; AccuStandard, Inc., New Haven, CT) (Frame et al., 1996). Chromatographic data was collected and processed by use of the Agilent ChemStation software and Microsoft Excel spreadsheet procedures. The Agilent software system generated the identity and amount of each PCB congener, confirmed by operator reprocessing of each chromatographic run. Coeluting congeners were assumed to be in equal proportions for all spreadsheet calculations (Pagano et al., 1995). Select organochlorine pesticides were measured based on Method 8081A (Organochlorines Pesticides by Gas Chromatography) described in USEPA protocols (USEPA, 1996). Single instrument/column detection was used for quantitation (DB-XLB, see conditions above) and based on retention time matching with standards. The calibration

standard is a 100 pg/μl composite mixture (Single-Column Analytes Mix, M-8081-SC, AccuStandard, Inc. of USEPA 8081A standard analytes). Polybrominated diphenyl ethers (PBDEs) were coanalyzed with the OCs on the DB-XLB column setup. The 5-component PBDE calibration standard used was a 50 pg/μl composite mixture (BDE-USE, AccuStandard, Inc.). All PBDE congeners were confirmed with pure PBDE congener standards (BDE-MXE–27 components) purchased from Wellington Laboratories (Guelph, ON, Canada) by mass spectrometric confirmation. Analytical confirmation of PCBs, OCs, PBDEs, and any potential co-eluting contaminants are routinely determined utilizing an Agilent 7890/5975C Inert XL EI/CI MSD with PTV injector and auto sampler.

Toxaphene analyses were performed using a GC/MS–MS method as described by Xia et al. (2009). Briefly the method involves quantitation of toxaphene in the extracts using MS–MS capability provided by an ion trap (PolarisQ-ThermoFinnigan, San Jose, CA, USA) instrument. The method provided selective identification of 125 → 89 m/z transition pairs that was shown by Xia et al. (2009) to be selective for chloroborane compounds important to the toxaphene mixture. This method also avoided interferences from other compounds likely to be present in the extracts. The gas chromatographic column separation was performed using a 60-m×0.25-mm×0.25-μm J&W DB-XLB capillary column. Quantitative analysis of total toxaphene in the samples extracts and the NIST SRM 1946 standard reference material were performed by integrating the total area of all visible signals of the 125 → 89 m/z chromatograms. The results were quantitated against eight commercially available toxaphene congeners and a technical toxaphene mixture. These were also used for spike recovery determinations.

## 2.5. Quality assurance/quality control (QA/QC)

### 2.5.1. Alkylphenols and alkylphenol ethoxylates in fish

Method detection limits for the APEs were as follows (units, ng g<sup>-1</sup> wet wt.): NP and OP–21.6 and 42.8, respectively; NP1EO and OP1EO – 36.6 and 28.5; NP2EO and OP2EO – 8.0 and 4.5; NP3EO and OP3EO – 5.1 and 7.8. The recoveries of these analytes varied between 77.9 and 98.8% for the NPs and 73.4 and 111% for the OPs. Reproducibility for replicates samples were all less than 20% relative standard deviation.

### 2.5.2. QA/QC for alkylphenols and alkylphenol ethoxylates in water

QA performance for the water extractions was nearly all within the acceptance criteria set forth in the Chicago Regional Laboratory criteria. For the Fall collections recovery of matrix spikes and lab control spikes varied from 62% to 80% for NP, NP1 and 2EOs while relative percent differences for duplicates were all 22% or lower. The Spring QA/QC performance was within guidance for percent recoveries for all analytes; however, the relative percent difference for NP1EO matrix spikes for one duplicate pair was higher than the acceptance of 30%. Surrogate data were compiled using normal nonyl substituted forms of the NPs, e.g., n-NP, n-NP1EO and n-NP2EO added to all of the samples to monitor recovery. For the fall sample extracts, all surrogate values were between 76 and 88%; whereas in the spring all the values varied between 62 and 88%. Blank results were all below method detection limits.

### 2.5.3. QA/QC for PBDEs, PCBs, toxaphenes and chlorinated pesticides in fish

Laboratory Quality Assurance/Quality Control for all these analytes were carried out in accordance with the quality criteria outlined in the Great Lakes Fish Monitoring and Surveillance Program (GLFMSP)–Quality Documentation (Holsen et al., 2008). The program consists of replicate analyses, blind duplicate analyses, surrogate analyte recoveries, NIST SRM 1946, matrix spikes/matrix spike duplicates and, method, reagent and system blanks at intervals prescribed in the project quality-assurance plan (QAP). Complete GLFMSP quality documentation can be accessed at: <http://www.epa.gov/greatlakes/monitoring/fish/index.html>. All of the toxaphene analyses met the GLFMSP QA/QC requirements except that the blanks were higher relative to the expected blank value of 7.68 ng/g which might impact the control fish values, but had minimal impacts on

the exposed fish. Also for the toxaphene QA/QC, the surrogate recoveries of 2,3,4,5,6 pentachloro-p-terphenyl (PCT\_5) were between 82 and 118% and the SRM recoveries seemed typical of data reported by others, and duplicate performance was acceptable.

### 2.5.4. QA/QC for plasma vitellogenin analysis in fish

Plasma samples were analyzed in triplicate to determine variation in test results. Coefficients of variation were calculated to assess variation in analytical results for the vitellogenin data. The coefficients of variation were within 10%, which is the St. Cloud State University standard for variation. Multiple sections of testes and livers were taken from each fish in order to obtain a representative sample of these organs for histological characterization. In the laboratory, three slides of the testes (right and left testis analyzed separately) and livers were made and assessed (HLS) without knowledge of the treatment according to U.S. Environmental Protection Agency protocols (USEPA, 2006).

## 2.6. Data analysis

Statistics were calculated using GraphPad (GraphPad Software Inc. San Diego, CA). Fish bioaccumulation factors (BAFs) for the nonylphenol and nonylphenol ethoxylates were computed using the concentrations for NPEs in water and the fish (Staples et al., 1998). Using a procedure proposed by Gobas and Morrison (2000), the concentration in the fish on a wet weight basis was divided by the concentration of the analyte dissolved in the water (CW) (since these compounds are predominantly in the dissolved state, e.g., log K<sub>ow</sub> < 5). The selected exposure concentrations for the water were the averages of the water concentrations in samples obtained in the weeks prior to fish sampling since these would represent the likeliest exposure doses leading to the uptake concentrations that were observed.

## 3. Results and discussion

### 3.1. Alkylphenols and alkylphenol ethoxylates in fish

Of the alkylphenols and alkylphenol ethoxylates that were sought (octylphenol and nonylphenol forms) only nonylphenols (NPs), NP, NP1EO, NP2EO, and NP3EO were consistently detected in the fish at concentrations above the methods detection limit. Among these, the ethoxylates, NP1EO and NP2EO, were the highest alkylphenols detected (Table 1 and Fig. 2). The concentrations in the spring-collected fish were statistically higher (student *t*-test comparisons) than the fish collected in the fall, both for total NPEs and for the individual homologues. Octylphenol and octylphenol ethoxylates were only detected in a few fish above the method detection limits but like the NPs the ethoxylated 1 and 2 forms of this family were highest and also at higher concentrations in fish collected in the spring versus the fall (Table 2). Due to the small number of individual fish collected (6 in the fall and 5 in the spring) no meaningful statistical analysis based on either size or sex of the collected fish was possible. Three fillet composites were provided to us for quality-assurance purposes (Leanne Stahl, USEPA personal communication) and analyzed using our analytical methods. These fish were collected at the same time, spring 2007, and at the same location as the fish that were used as whole fish in this study. These fish were part of a US-sponsored National Pilot Study of pharmaceutical and personal care products in fish and analyses from the fall collection have been reported elsewhere (Ramirez et al., 2009). Nonylphenol and nonylphenol ethoxylates residues in these fillet samples were all much lower than the values in the whole fish samples analyzed in this study and location (i.e. 4.8 times lower). However, for the octylphenol and octylphenol ethoxylates the average ratio of fillet vs. whole concentrations was only 1.4 times lower. These differences are noteworthy and may indicate an important difference for these two groups of APEs since they associate differently with muscle tissues versus whole fish.

**Table 1**  
Concentrations of contaminant mixtures and vitellogenin found in fish collected from an effluent-dominated waterway, North Shore Channel (NSC) of the Chicago River, compared to fish collected from clean reference sites near Chicago IL in the fall and spring of 2006/2007.

Fish	Sex <sup>a</sup>	Vitellogenin $\mu\text{g ml}^{-1}$	Location	NPEs-total $\mu\text{g g}^{-1}$ fresh wt.	PBDE-total	PCB-total	DDTs-total	Toxaphene-total
Fall-1	M	1.7	NSC	0.98	1.27	2.69	1.04	0.47
Fall-2	F	0.4	NSC	1.34	2.36	2.8	1.42	0.45
Fall-3	M	<0.1	NSC	1.02	1.08	0.9	0.55	0.25
Fall-4	F	0.3	NSC	0.53	1.04	2.29	1.51	0.32
Fall-5	F	0.91	NSC	1.13	0.92	1.77	0.69	0.62
Fall-6	M	<0.1	NSC	0.91	1.48	4.73	1.35	0.45
Average Fall NSC				0.99	1.36	2.53	1.09	0.43
Ref F 1	F	<0.1	Ref. <sup>b</sup> Chicago Harbor	0.04	0.02	0.48	0.12	0.05
Ref F 2	F	15.3	Ref. Chicago Harbor	0.04	0.06	0.65	0.12	0.06
Ref F 3	M	<0.1	Ref. Chicago Harbor	0.03	0.02	0.5	0.08	0.04
Spring-1	F	5.76	NSC	4.01	1.31	2.25	0.84	0.48
Spring-2	F	7.47	NSC	8.28	0.85	1.85	0.68	0.3
Spring-3	M	3.29	NSC	4.95	1.77	2.32	1.05	0.45
Spring-4	F	2.71	NSC	4.41	1.27	2.98	1	0.69
Spring-5	F	<0.1	NSC	5.46	n.a. <sup>c</sup>	n.a.	n.a.	n.a.
Average Spring NSC				5.42	1.30	2.35	0.89	0.48
Ref S 1	M	<0.1	Ref. Braidwood	0.04	0	0.01	0.01	0.04
Ref S 2	M	0.39	Ref. Braidwood	0.03	0	0.02	0.01	0.03
Ref S 3	M	<0.1	Ref. Braidwood	0.11	0	0.02	0.03	0.04

<sup>a</sup> M = male and F = female.

<sup>b</sup> Ref. represent the reference fish.

<sup>c</sup> n.a. = not analyzed.

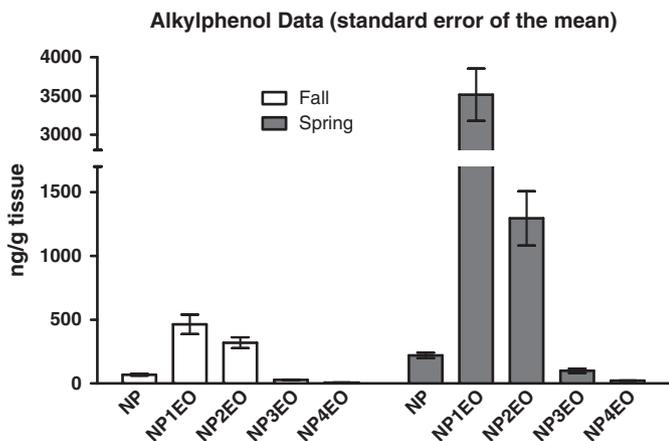
### 3.2. Alkylphenols in water

The nonylphenol and nonylphenol ethoxylates measured in the water are shown as averages in Table 3 (stream water and effluent water samples) and these are plotted as individual sample results for their effluent concentrations in Fig. 3. The data in Table 3 confirm that the higher levels of NP and NP-ethoxylates measured in the fish in the spring versus the fall were also present in the channel water and that these constituents were likewise present in the effluent from the water reclamation plant loading into the North Shore Channel. No significant differences were found between NP and NP-ethoxylates concentrations measured in the North Shore Channel versus those measured directly in the effluent outfall. Some reductions in this downstream water were expected (Ahel et al., 1994; Loyo-Rosales et al., 2007b; Maruyama et al., 2000), however the transit time in the channel may be too short to observe any reduction or the prevalence of effluent in this waterway may delay biodegradation activity in the channel. When the individual effluent concentrations

were plotted versus time of collection (Fig. 3) there is a clear inverse relationship with increase in temperature. Rearranging the data by plotting individual NPEs versus temperature without regard to date, allowed linear regression coefficients to be plotted (Fig. 4). The respective values for these regression coefficients were as follows: NP = 0.85, NP1EO = 0.74; and NP2EO = 0.51. Similar temperature effects have been observed in effluents from other wastewater treatment plants (WWTPs) (Ahel et al., 1994; Loyo-Rosales et al., 2007b) and in effluent-impacted rivers (Maruyama et al., 2000). This temperature effect appears to be an important factor leading to the large differences between lower APE concentrations in the fall versus the higher spring-collected water concentrations and offers a likely explanation for higher concentrations of these compounds to occur in fish collected in the spring versus those collected in the fall. However before one ascribes water temperature as the only factor leading to differences in water concentrations, it is also important to recognize the complexity of the flows in the North Shore Channel over these periods. Actually in the spring there were more periods where channel flow augmentation from the Wilmette pumping station were needed to maintain adequate channel flows for flushing out the system versus the fall. Therefore this should have resulted in more diluted concentrations of APE in the channel at this time due to addition of this cleaner non-effluent water and the opposite was observed for water concentrations of APEs.

### 3.3. Tissue residues of persistent organic pollutants

Several persistent and bioaccumulative pollutants were measured in the same fish assayed for NPEs. Table 1 presents concentration values for those with the highest totals approaching those for the total of all NPEs. For initial evaluation the relative fish concentrations of the individual groups identified were considered. Clearly the average total NPE concentration for the spring-collected fish (5.42  $\mu\text{g/g}$ ) was the highest of all the measured analyte groups but this concentration was only 0.99  $\mu\text{g/g}$  in fall-collected fish. Next in concentration were total PCB and total PBDE which were also above 1  $\mu\text{g/g}$  (ppm) and both were higher than the NPE total for the fall-collected fish. All of the total values for the persistent organic pollutants generally compare with literature values for urban effluent-dominated streams



**Fig. 2.** Nonylphenol (NP) and nonylphenol 1 to 4 ethoxylate (NP(1–4)EO) concentrations found in fish collected in the Fall of 2006 and Spring of 2007. Concentrations represent mean  $\pm$  standard error of the mean of all fish captured for each season.

**Table 2**

Average octylphenol (OP) and octylphenol ethoxylates residues in whole large-mouth bass ( $n = 6$  for the fall and  $n = 5$  for the spring) collected from the North Shore Channel downstream of the North Side Water Reclamation Plant. Gray highlighted data were below detection limit of the method.

Collection season	OP	OP1EO	OP2EO	OP3EO
	(ng/g wet wt.)			
Fall	5.2	10.1	4.6	1.5
Spring	10.1	70.9	14.5	1.6

(Eljarrat et al., 2007; Hinck et al., 2007; Pinkney and McGowan, 2006; Rice et al., 2002); except for total PBDE concentration which was higher than others normally observe. La Guardia and associates (Chen et al., 2011; La Guardia et al., 2007) did report one location downstream of a manufacturing facility in North Carolina where even higher concentrations of PBDE in fish were observed. It is not immediately apparent why these high PBDE concentrations were observed in fish from the North Shore Channel; however the pattern is similar to the commercial PBDE product, Penta (D-71), which suggests that little alteration was taking place in this system.

### 3.4. Fish residue concentrations (accumulation factors)

Bioaccumulation of contaminants by fish from water is considered a critical stage leading to their effects on the fish and to eventual exposure to humans that may consume these fish. When both water concentrations and fish residue values are measured it is possible to estimate field-related bioaccumulation potentials. Since accumulation is not instantaneous, then water concentrations values should be integrated over time. Average APE water concentrations occurring prior to the fish samplings were used to make these calculations for the nonylphenol and nonylphenol ethoxylates in this study. For the fall period, only NP water concentrations prior to fish sampling were higher than the method detection limits, thus only a BAF for NP was possible. It was determined to be 145; while for the spring-collected fish, 4 water sample results prior to the fish collection were averaged resulting in the following BAFs: NP-147; NP1EO-517, NP2EO-360 and NP3EO-49. Since aquatic exposures appeared to be fairly uniform, at least on a weekly basis, then uptake and depuration was likely to be a continuous process even though uptake and depuration are reported to be fairly fast (3 to 10 days) for NP (Lewis and Lech, 1996; Spehar et al., 2010a) and for OP (Pedersen and Hill, 2002). These calculated BAF values are comparable to those reported for carp (280, 1700 and 693, respectively, for NP, NP1EO and NP2EO), which is a bottom dwelling fish (Mitchellmore and Rice, 2006). Carp could be expected to have higher BAFs than bass since sediment has been acknowledged as an additional source for contaminant buildup for these bottom dwelling fish (David et al., 2009).

All of the residue concentrations of the persistent contaminants (Table 1 and Figs. 5, 6, 7 and 8) were also acquired through accumulation from the water. It is therefore important to consider the uptake potentials of all of the compounds in order to address the broader topics that concern their major differences, e.g. relative degradability and partitioning properties. All of the APEs have  $\log K_{ow}$  values in the range of 4.2 to 4.5 (Loyo-Rosales et al., 2007a; Patrolecco et al., 2006) while the majority of the more persistent compounds have  $\log K_{ow}$  values in the 6 to 7 range except for toxaphenes which range from 2.9 to 5.4. Thus for all these compounds, their high  $\log K_{ow}$  values would predict likely uptake from the water with APEs being perhaps less sorptive. In addition to partitioning properties of these compounds they also have a similarity in that they appear to remain at fairly uniform concentrations in the water for long enough periods for them to be accumulated, e.g. they have lengthy exposure durations. For persistent compounds, like PBDEs and the other non-APE compounds studied here, resistance to chemical transformation is an important property causing this continued low-level concentration in the water phase which is also likely maintained by releases from sedimentary reservoirs. However APEs actually have a propensity to degrade in the aquatic environment and this is actually what makes them a problem pollutant (Montgomery-Brown and Reinhard, 2003). For example, when APEs are in their polymeric (typically NPE-9 form), a form used most heavily in commercial products, they are considered “moderately or slightly toxic” (Staples et al., 1998). However, the products of degradation, especially the terminal alkylphenols (nonyl or octyl-phenol) are described by Staples using USEPA criteria as “highly to very highly toxic” and furthermore they accumulate in sediment plants and fish, they are recalcitrant, they are endocrine active, and they are ubiquitous downstream from waste treatment operations (Ying et al., 2002).

Also for APEs it appears that their variable release as degradates from polymeric APEs that load into waste water treatment facilities can explain the variable concentrations patterns observed in this study. However, for the more persistent pollutant, their overall resistance to degradability probably explains why there was little variability in fish residue concentrations for these compounds between the fall and spring collected fish.

### 3.5. Comparisons of fish residues of NPEs with other fish residue analytes

As already mentioned above, all of the persistent contaminants were measured as mixtures which is also the case for the APEs. However, since effect-based interpretation of these findings was an objective of this study, it is important to understand that chemical-based studies assume that compound evaluations should equate to compound-specific interpretation as much as the analytical data will allow. In order to accomplish this, the mixed compound data were segregated down to their individual compounds as much as our analyses would allow. The

**Table 3**

Average nonylphenol (NP) and nonylphenol 1–3 ethoxylate (NP(1–3)EO) analyzed in the water samples. Concentrations in the stream represent average concentrations analyzed in the water samples collected from the North shore channel ( $n = 21$  per season) and concentrations in the effluent represent the average concentrations analyzed in effluent samples collected in North Side Water Reclamation Plant ( $n = 6$  per season).

Compound	Fall <sup>a</sup>		Spring		Stream <sup>b</sup>	Effluent
	Stream ( $\mu\text{g L}^{-1}$ )	Effluent ( $\mu\text{g L}^{-1}$ )	Stream ( $\mu\text{g L}^{-1}$ )	Effluent ( $\mu\text{g L}^{-1}$ )	Factor increase (spring/fall)	Factor increase (spring/fall)
NP	0.55	0.31	1.01	1.38	1.8	4.5
NP1EO	1.16	0.83	5.80	6.47	5.0	7.8
NP2EO	1.73	1.44	2.92	3.12	1.7	2.2
NP3EO	U	U	1.74	1.49	n.c.	n.c.
NPE <sub>t</sub>	3.44	2.58	11.5	12.5	3.3	4.8

<sup>a</sup> U indicate concentration undetected.

<sup>b</sup> n.c. means not calculated.

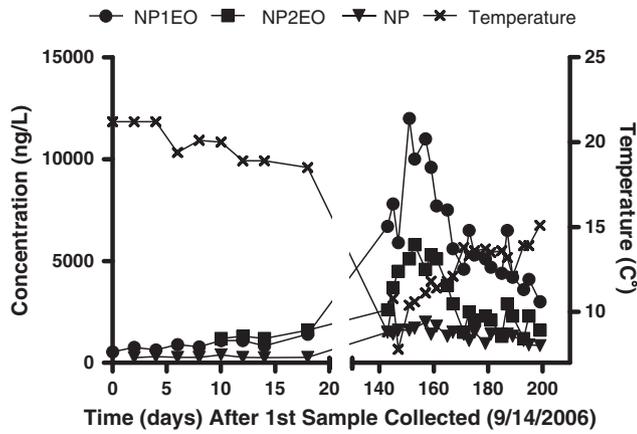


Fig. 3. Effluent concentrations of nonylphenol (NP) and nonylphenol 1 to 2 ethoxylate (NP1–2 EO), and temperature in effluent versus days after 1st sample collection at the North Side Water Reclamation Plant inclusive of a fall 2006 collection interval (0 to 20 days) followed by a spring 2007 collection (140 to 200 days).

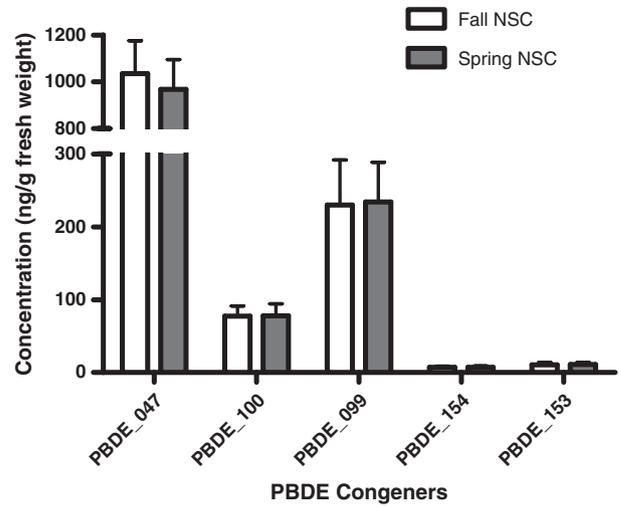


Fig. 5. Polybrominated (PBDE), congener concentrations in the fish collected from the North Shore Channel (NSC) in the Fall (2006) versus the Spring (2007).

compositional patterns for each of the groups are presented for all of the identified components (Fig. 2 (NPE homologues), Fig. 5 (PBDE congeners), Fig. 6 (DDT-residues) Fig. 7 (PCB congeners) and Fig. 8 (miscellaneous organochlorine pollutants)). In the subsequent discussion a concentration ranking was utilized in order to prioritize possible effects. In this ranking, the PCB group is notably absent since the individual congener concentrations were relatively low due to the fact that the major congeners in this mixture number more than 18 and this reduced the highest single amount to less than 170 ng/g (Fig. 7). A similar situation applies to the toxaphene mixture which had mixed total amounts of 426 ng/g fall, and 482 ng/g spring, but because of its mixed isomer content being much like PCB, it would result in even lower unit isomer amounts. Using this reduced number of identified analyte groups to rank concentrations and thus potential effect, the highest NPE homologue mixture concentration was NP1EO which was 3,500 ng/g in the spring-collected bass and 460 ng/g in the fall; and next in concentrations was PBDE-congener # 47 at 1000 ng/g (average of all fish) and p,p'-DDE at 500 ng/g, again average of all fish. It is also important to realize that using concentration to distinguish the homologue-specific nonylphenol data (Fig. 2) does not actually represent the true isomer-specific concentrations of these compounds. Each homologue group consists of a relatively similar family of branched chain 9-carbon isomers. Based on current literature, there are somewhere upwards of 18 to 22 major isomers present in the commercial products (Bhatt et al.,

1992; Wheeler et al., 1997). However, it is still unclear how many of these may occur in environmental samples even though researchers have started looking into this question more recently (Dembitsky et al., 2002; Eganhouse et al., 2009; Guenther et al., 2002; Ieda et al., 2005). Therefore attempting to define compound-specific information on the NPEs requires dividing the concentrations listed by us for each of the NP-homologues into their specific isomer groups. The exact chemical forms that are important for toxicity in these isomeric groups is also complicated by the fact that there are also numerous enantiomeric forms of many of them (Eganhouse et al., 2009; Guenther et al., 2006; Zhang et al., 2009); however, this topic is beyond the scope of this study. It should be mentioned, however, that this is not true of the octylphenol (OP) group of homologues which are 99.2% composed of 4-(1,1,3,3-Tetramethylbutyl)phenol (Brooke et al., 2005). Finally then, considering thus that each NPE homologue group may have as many as 18 to 22 individual isomers then this would result in perhaps around 1/22nd of the total concentration for each group displayed in Fig. 3. This would result in perhaps a maximum of 160 ng/g for each NP1EO isomer in the spring and 21 ng/g in the fall (this assumes that all the peaks in the isomer mix are equal which is probably unlikely). Thus for comparison purposes and basing this solely on individual analyte data, it appears that PBDE-47 followed by p,p'-DDE were possibly higher in concentration in these fish than any one of the NPE isomers.

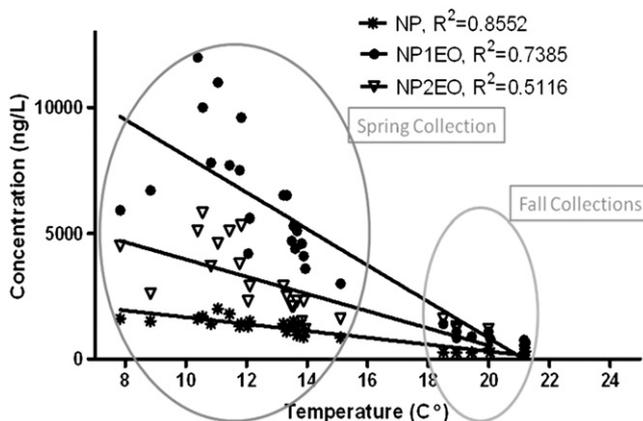


Fig. 4. NPE concentration in effluent samples discharging from the Northside Water Reclamation Plant versus effluent water temperatures.

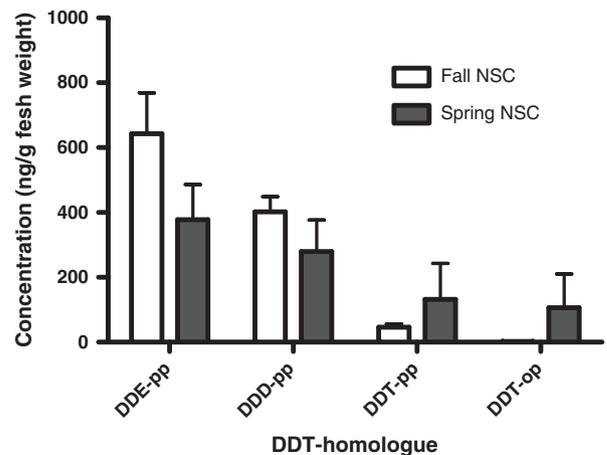


Fig. 6. Individual component concentrations of DDT residues in fish collected from the North Shore Channel in the Fall of 2006 and Spring of 2007.

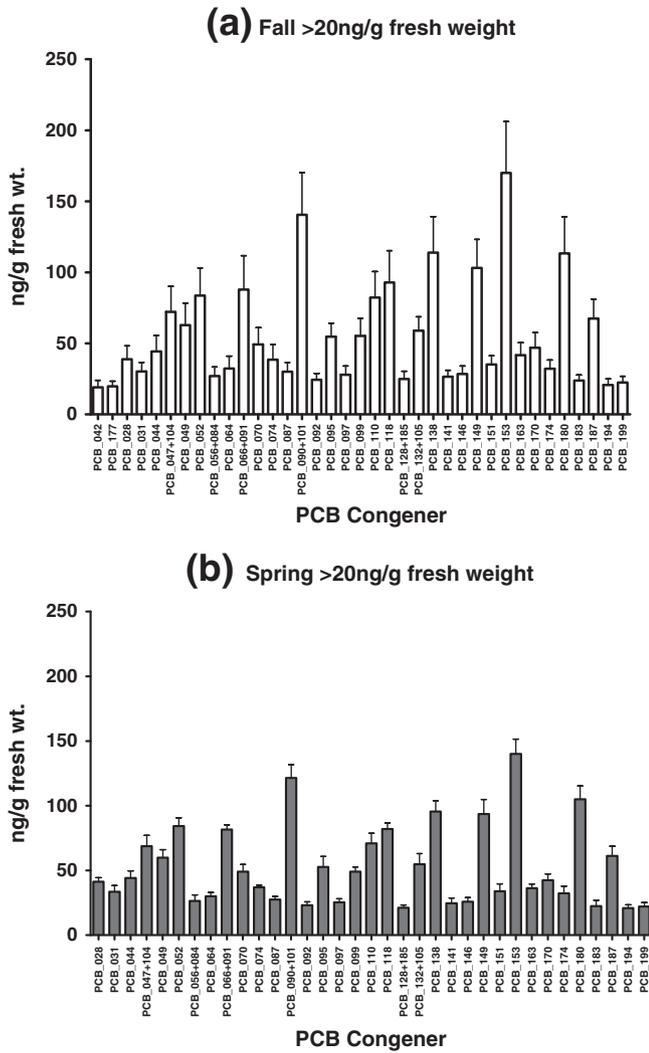


Fig. 7. Average PCB congener concentrations in largemouth bass collected from the North Shore Channel in the Fall of 2006 (a) and Spring of 2007(b) that were greater than 20 ng/g fresh.

3.6. Endocrine active contaminants and biological effects

It was decided to narrow the focus of the pollutants that were measured in the fish tissues to those that are suspected EACs. All of the alkylphenols and alkylphenol ethoxylates belong to this group, as do certain PCBs, PBDEs, and selected members of the organochlorines. Notable of these persistent pollutants are the PCB congeners, 95, 99, 101 and 153, which are EACs with known estrogenic and anti-androgenic capacity (Fossi and Marsili, 2003). These authors also listed pp-DDT and pp-DDE as active among those compounds for which data are available. Considering this type of emphasis for the results presented in this study, only pp-DDE and perhaps PCB congener 153 stand out while many of the alkylphenols and alkylphenol ethoxylates also take on a more significant role as EACs. Toxaphene is listed as a suspected EAC by some authors (PAN (Pesticide Action Network) Pesticide Database- <http://www.pesticideinfo.org/>) as are some of the PBDEs isomers; but insufficient data are available at this time to judge their impact on the exposed fish.

Although the extensive tissue analysis limited the absolute number of fish that could be analyzed, attempting to relate tissue concentrations of EACs with plasma vitellogenin induction in male fish and histopathological findings in fish of both sexes is valuable considering the current lack of such data. All largemouth bass (male and female) collected in the North Shore Channel during both collection events (n = 11) contained higher concentrations of suspected EACs (particularly the alkylphenols and alkylphenol ethoxylates) in their tissues than all reference fish (n=6). Two of the four male largemouth bass collected in the North Shore Channel contained plasma vitellogenin at concentrations (1.7 µg/ml and 3.29 µg/ml) comparable to mean plasma vitellogenin measured in the collected female fish (6.67 ± 1.71 µg/ml, mean ± standard error). Interestingly, plasma vitellogenin concentrations in fish collected in this study matched reported values from other field studies (Sepulveda et al., 2002). Although the small sample size did not allow for a meaningful statistical analysis of the relationship between fish tissue EAC concentrations and vitellogenin concentrations in male or female fish, the induction of plasma vitellogenin in 50% of male largemouth bass in this study, and in 67% of largemouth bass and 78% of carp in a companion study (Barber et al., 2011) in the North Shore Channel suggest that the EACs measured in this study elicit a physiological response in these fish. An analysis of the liver and reproductive tissues of all collected largemouth bass in this study (n = 17) did not find any consistent

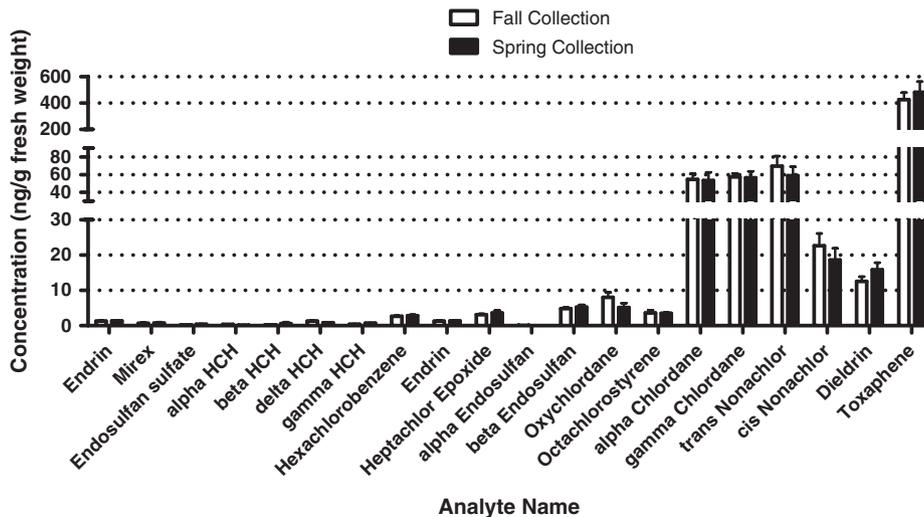


Fig. 8. Individual average organochlorine pesticide concentrations in fall and spring-collected fish plus the pesticide mixture, toxaphene. These are the less abundant pesticides and industrial pollutants.

structural alteration to either tissue. Liver hepatocyte vacuolization, an indicator of pollutant stress, did not differ significantly among sites or sex. Maturity of reproductive organs also did not differ between sites suggesting that all fish were in comparable reproductive condition. None of the fish was found to contain any ovarian tissues in the testis (“intersex”) and no incident of atretic ovarian tissues was noted. The lack of histopathological changes, and especially the lack of intersex in male fish is contrary to findings by Hinck et al. (2009a) who reported widespread occurrence of intersex in male bass in effluent dominated systems across the US. However, in the analysis by Hinck et al. (2009a) larger numbers of testis sections were evaluated increasing the likelihood of detecting ovarian tissues. These authors also noted that the occurrence of intersex was not readily predicted by plasma vitellogenin concentrations or the presence of specific chemical contaminants (Hinck et al., 2009a). The lack of intersex in the current study may not be surprising as the North Shore Channel represents a man-made, effluent dominated environment for almost 100 years, suggesting that any sustained fish population (such as the largemouth bass) would have adapted to the presence of estrogenic EACs through processes of natural selection, thus providing some resilience to structural alterations caused by the continuous presence of these compounds in their habitat (reviewed by Klerks and Weis (1987), and Wymore et al. (2011)). In contrast, sensitivity to vitellogenin induction would have remained high, as adequate vitellogenin production is necessary to allow for reproduction and reduced sensitivity of estrogen receptors would likely affect vitellogenin production and reproductive outcome (Miller et al., 2007).

#### 4. Conclusions

Fish in urban aquatic ecosystems are exposed to complex mixtures of EACs including a range of non-steroidal compounds such as alkylphenol ethoxylates. The alkylphenols and alkylphenol ethoxylates compounds were observed in water samples and fish tissue as seasonably variable values (higher in early spring colder water and lower in the late autumn warmer waters and at predictable bioaccumulation ratios). The EAC-active mixture of chemicals that were measured appeared to affect physiological processes in resident fish communities without altering the anatomy of the exposed organism or resulting in fish population declines. The more abundant persistent compound groups (DDTs, PCBs, PBDEs, toxaphenes) that were measured in the fish tissues showed no significant seasonal variation and were present at concentrations typical of effluent-dominated streams except for the PBDEs which were higher than normal.

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