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Effects of biologically-active chemical mixtures on fish in a wastewater-impacted urban stream

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ABSTRACT

Stream flow in urban aquatic ecosystems often is maintained by water-reclamation plant (WRP) effluents that contain mixtures of natural and anthropogenic chemicals that persist through the treatment processes. In effluent-impacted streams, aquatic organisms such as fish are continuously exposed to biologically-active chemicals throughout their life cycles. The North Shore Channel of the Chicago River (Chicago, Illinois) is part of an urban ecosystem in which >80% of the annual flow consists of effluent from the North Side WRP. In this study, multiple samplings of the effluent and stream water were conducted and fish (largemouth bass and carp) were collected on 2 occasions from the North Shore Channel. Fish also were collected once from the Outer Chicago Harbor in Lake Michigan, a reference site not impacted by WRP discharges. Over 100 organic chemicals with differing behaviors and biological effects were measured, and 23 compounds were detected in all of the water samples analyzed. The most frequently detected and highest concentration (>100 μg/L) compounds were ethylenediaminetetraacetic acid and 4-nonylphenolmono-to-tetraethoxycarboxylic acids. Other biologically-active chemicals including bisphenol A, 4-nonylphenol, 4-nonylphenolmono-to-tetra-ethoxylates, 4-*tert*-octylphenol, and 4-*tert*-octylphenolmono-to-tetraethoxylates were detected at lower concentrations (<5 μg/L). The biogenic steroidal hormones 17β-estradiol, estrone, testosterone, 4-androstene-3,17-dione, and *cis*-androsterone were detected at even lower concentrations (<0.005 μg/L). There were slight differences in concentrations between the North Side WRP effluent and the North Shore Channel, indicating minimal in-stream attenuation. Fish populations are continuously exposed to mixtures of biologically-active chemicals because of the relative persistency of the chemicals with respect to stream hydraulic residence time, and the lack of a fresh water source for dilution. The majority of male fish exhibited vitellogenin induction, a physiological response consistent with exposure to estrogenic compounds. Tissue-level signs of reproductive disruption, such as ovatestis, were not observed.

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1. Introduction

Large metropolitan centers place great demands on urban stream ecosystems, which must perform a number of essential functions such as wastewater disposal, transportation, recreation, and drinking water supply. These demands compete with basic ecosystem services such as critical aquatic habitat, and understanding the relationships between hydrology, biology, and exposure to biologically-active chemicals, is imperative for sustainable management of these valuable resources. It is well established that contaminants make their way into sewage collection systems from domestic, commercial, and industrial use, and chemicals not removed by treatment in water-reclamation plants (WRPs) are discharged to the environment (Alder et al., 1990; Ahel et al., 1994a; Ternes et al., 1999; Barber et al., 2000). Once introduced into surface waters, contaminants can persist for considerable times and distances (Ahel et al., 1994b; Barber et al., 1996, 2006a,b; Ternes, 1998; Kolpin et al., 2002; Glassmeyer et al., 2005). Base flows in urban streams often are maintained entirely by WRP effluent, regardless of regional water availability (Brooks et al., 2005; Waiser et al., 2011). Because of the density of urban development along streams and the frequency of WRP discharges relative to freshwater inputs, there often is insufficient hydraulic residence time for significant in-stream attenuation to occur. As a consequence, the entire aquatic community can be exposed throughout life cycles and across generations to nearly undiluted WRP effluent and associated mixtures of biologically-active chemicals.

Among the most frequently detected compounds in wastewater-impacted streams are degradates of alkylphenolpolyethoxylate

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nonionic surfactants (used in domestic and commercial applications) including 4-nonylphenol and 4-*tert*-octylphenol (Kolpin et al., 2002; U.S. Environmental Protection Agency, 2005; Barber et al., 2000). Other frequently detected compounds include caffeine (Barber et al., 1996; Buerge et al., 2003), fragrances (Simonich et al., 2002), antimicrobials (Halden and Paull, 2005), prescription drugs (Tixier et al., 2003; Miao et al., 2004), organophosphate flame retardants (Marklund et al., 2005), aminocarboxylic complexing agents (Alder et al., 1990; Nowack, 2002), and triazole corrosion inhibitors (Giger et al., 2006).

Vertebrate animals, including fishes, have an endocrine system consisting of glands that secrete hormones that maintain organismal homeostasis by regulating developmental and reproductive functions (Norris and Carr, 2006). Some exogenous non-steroidal chemicals, such as 4-nonylphenol, 4-*tert*-octylphenol, and bisphenol A can mimic endogenous sex hormones such as 17β-estradiol. Fish residing in wastewater-impacted streams have been shown to exhibit evidence of endocrine disruption, including increased concentrations of plasma vitellogenin (an egg yolk precursor protein normally found in female fish) in male fish and disrupted gonadal morphology (Purdom et al., 1994; Jobling et al., 1998; Hemmer et al., 2002; Vajda et al., 2008). A dramatic example of the ecosystem effects of biologically-active chemicals is the dosing of a lake with environmentally relevant concentrations of the synthetic estrogen 17α-ethynylestradiol, which resulted in the rapid collapse of resident fish populations (Kidd et al., 2007).

Because of the widespread occurrence of pharmaceutical and personal care products in the waters of the U.S. (Kolpin et al., 2002; Focazio et al., 2008) and their potential to accumulate in fish tissue (Brooks et al., 2005), the U.S. Environmental Protection Agency conducted a national survey of their occurrence in fish tissue from effluent-impacted streams (Ramirez et al., 2009). To better understand the relationships between sources of chemicals, exposure pathways, and potential ecological effects, the present study focuses on the North Shore Channel of the Chicago River (Chicago, IL), which in the national survey (Ramirez et al., 2009) had the greatest concentrations of pharmaceuticals and personal care products in wild-caught fish tissue. The North Side WRP effluent, which discharges to the North Shore Channel, was characterized for a range of biologically-active chemicals with differing environmental fates and biological effects. The attenuation capacity of the urban receiving stream was determined, and endocrine disruption in wild-caught fish was assessed.

2. Methods

2.1. Site descriptions

The North Side WRP is located in the Chicago, Illinois metropolitan area (Fig. 1A) and collects sewage from residential (>96%) and commercial/industrial (3.4%) sources. The WRP serves a population of 1.3 million people from an area of approximately 365 km^2, and treats an average of 11 m^3/s of sewage using activated sludge processes (Metropolitan Water Reclamation District, MWRD, 2010). Following treatment, the WRP effluent is discharged to the North Shore Channel of the North Branch of the Chicago River, where it contributes >80% of the annual stream flow (Fig. 1B). The only other flow into the North Shore Channel (apart from surface runoff during storm events) enters upstream from the North Side WRP via the Wilmette Pumping Station where Lake Michigan water is discretionally released to maintain minimum stream water quality (usually only during June–October).

2.2. Water and fish sampling

Grab-water samples were collected during September 2006 and March to April 2007 from the North Side WRP and the North Shore Channel at a site located 5.1 km downstream from the WRP outfall (Foster Avenue Bridge). Stream samples were collected from the center of the channel about 1 m below the water surface using a stainless steel bucket. Unfiltered water samples were collected in amber-glass bottles and stored at 4 °C. Samples for carboxylic acid compound analysis were preserved with 1% (v/v) formalin.

Fish were collected from the North Shore Channel between the North Side WRP outfall and the Foster Avenue Bridge site during September 2006 and March 2007 using a Smith-Root (Vancouver, WA) electro-shocking boat. Animal care and use protocols were

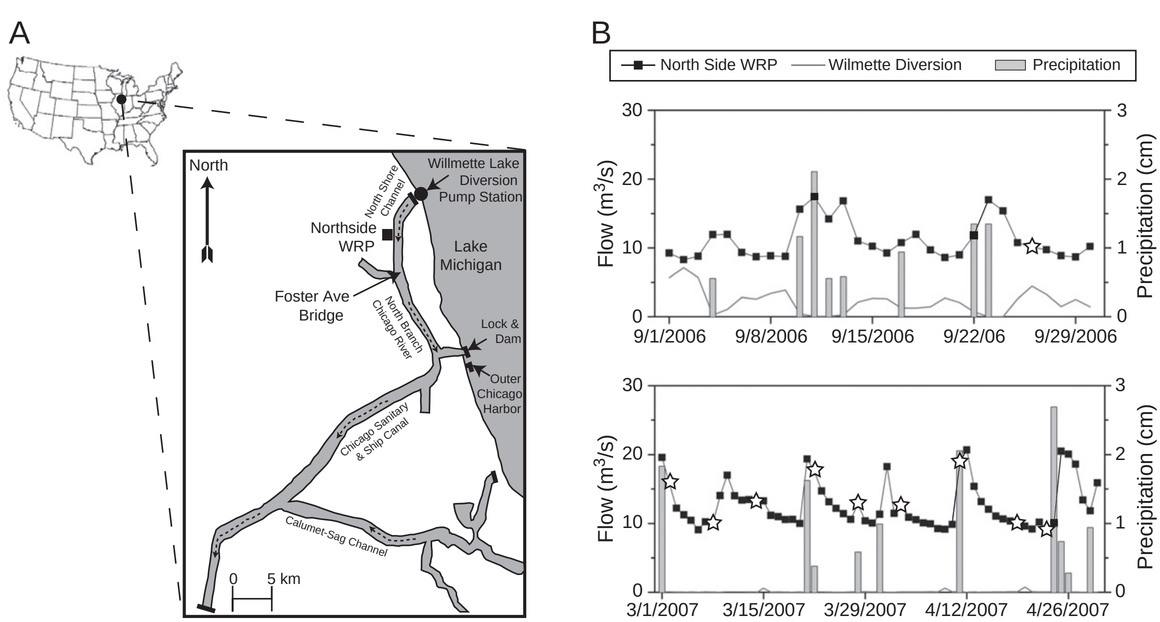

Fig. 1. (A) Map showing the Greater Chicago waterways, the North Side Water-Reclamation Plant (WRP) location, and the Foster Avenue Bridge North Shore Channel sampling location. (B) Flow from the North Side WRP and the Wilmette Pumping Station during the September 2006 and March–April 2007 sampling periods. [Open star symbols for the WRP flow indicate dates when water samples were collected; flow and precipitation data from Metropolitan Water Reclamation District (2010)].

approved by the St. Cloud State University Institutional Animal Care and Use Committee. During September 2006, fish were collected from a reference site located in the Outer Chicago Harbor of Lake Michigan, which is physically separated from the North Shore Channel by a lock and dam (Fig. 1A). Largemouth bass (*Micropterus salmoides*) were collected during September 2006, and largemouth bass and common carp (*Cyprinus carpio*) were collected during March 2007. Following anesthesia, blood was taken from the caudal vasculature, stored on ice during transport to the laboratory (<24 h), and centrifuged at 5000 rpm for 5 min to separate plasma. Plasma samples were stored at −80 °C. Following blood collection, liver and reproductive organ tissue was excised and processed for histological analysis.

2.3. Chemical analysis

Water quality parameters, including temperature, 5-day biological oxygen demand, ammonium, and nitrite + nitrate were measured following standard procedures (MWRD, 2010). Total organic carbon (TOC) was analyzed by persulfate/ultraviolet light oxidation (Barber et al., 2001). Unfiltered, formalin preserved water samples for carboxylic acid compound analyses were evaporated (EVAP) to dryness and derivatized with acetyl chloride:propanol (Barber et al., 2000). Alkylphenols and other neutral organic compounds were isolated from unfiltered water samples using continuous liquid–liquid extraction (CLLE) with methylene chloride at pH 2 following ionic strength adjustment with sodium chloride (Barber et al., 2000). Steroidal hormones were isolated from unfiltered water samples by octadecylsilica solid-phase extraction (C18-SPE) followed by elution with methanol (Barber et al., 2003). The methanol extracts were taken to dryness, dissolved in dichloromethane:diethylether:methanol, passed through pre-conditioned Florisil, and evaporated to dryness. Hydroxyl and keto functional groups were derivatized with N-methyl-N-trimethylsilyltrifluoroacetamide. Glass-fiber filtered (1-μm) samples were extracted using polystyrene divinylbenzene solid-phase-extraction (SDB-SPE) and eluted with methylene chloride (Zaugg et al., 2002). Pharmaceutical compounds were isolated from filtered water samples using hydrophilic–lipophilic-balance solid-phase-extraction (HLB-SPE), eluted with methanol/trifluoroacetic acid, evaporated to dryness, and reconstituted in acetonitrile/water buffered with ammonium formate/formic acid (Cahill et al., 2004).

The EVAP, CLLE, and SDB-SPE extracts were analyzed by gas chromatography/mass spectrometry (GC/MS) in full scan and selected ion monitoring (SIM) modes. Compound identification was based on SIM data using retention times (±0.05 min), diagnostic ions, and ion ratios (±20%) determined from standards. The derivatized C18-SPE samples were analyzed by gas chromatography/tandem mass spectrometry. For each compound, the most abundant ion in the electron impact mass spectra was selected as a precursor and appropriate conditions were selected to maximize signal for three precursor-product transitions. The HLB-SPE samples were analyzed by liquid chromatography/mass spectrometry (LC/MS) in the SIM mode.

2.4. Biological analysis

After the liver and reproductive organs were excised from each fish, several small tissue samples (~3 mm^3) were collected and immersed in 10% buffered formalin until further processing. After a 1-week fixation period, tissue samples were dehydrated through a series of ethanol and xylene baths and embedded in paraffin. Tissue sections were cut at 5-μm thickness (~50 μm between sections) on a microtome. At least 6 sections from each organ (right/left gonad, liver) were stained using standard haematoxylin and eosin techniques (Gabe, 1976; Carson, 1996) similar to methods used in other histopathological studies (Kidd et al., 2007; Vajda et al., 2008). Histological sections of the gonads and livers were assessed for maturity, sex, and pathological changes.

Plasma vitellogenin in largemouth bass was measured using indirect enzyme-linked immunosorbent assay (ELISA) protocols modified from the methods of Denslow et al. (1999) and Sepulveda et al. (2002). Standard vitellogenin from rainbow trout (*Oncorhynchus mykiss*) at varying concentrations or plasma from largemouth bass at 3 dilutions were coated onto microtiter wells, a monoclonal striped bass (*Morone saxatilis*) antibody was added, followed by an anti-mouse IgG horseradish peroxidase conjugated antibody and tetramethylbenzidine. Absorbance was read at 620 nm. Carp plasma samples were analyzed using a commercially available carp vitellogenin ELISA kit (Biosense Laboratories, Bergen, Norway).

3. Results

3.1. Sampling conditions

During September 2006, the North Side WRP discharge averaged 11.1 m^3/s and the Wilmette Pumping Station discharge averaged 2.2 m^3/s (MWRD, 2010). On the sampling date, the North Side WRP effluent was diluted by Lake Michigan water which contributed 44% of the North Shore Channel flow (Fig. 1B). Eight rainfall events in September 2006 added approximately 8.6 cm of precipitation. During March to April 2007, the North Side WRP discharge averaged 12.7 m^3/s and contributed >99% of the North Shore Channel flow (no contribution from the Wilmette Pumping Station). Rainfall events added a total of 12.1 cm of precipitation over the 2-month sampling period.

3.2. Chemical occurrence

A spectrum of >100 organic compounds, including a number of biologically-active chemicals, were measured in the North Side WRP effluent and the North Shore Channel, of which 23 were detected in all samples analyzed (Fig. 2). Quality assurance results and summation of the concentrations and frequency of detections for all of the compounds detected are presented in Appendix A (Tables A-1 and A-2). The high frequency of detection is due to the targeted nature of the compounds, their widespread occurrence in WRP effluents, and the wastewater-impacted nature of the stream.

Specific compounds with the highest concentrations (Fig. 2) were the nonionic surfactant degradates 4-nonylphenolmono-to-tetraethoxy-carboxylic acids (ΣNPEC) and the metal complexing agent ethylenediaminetetraacetic acid (EDTA). Other frequently detected compounds included: the biogenic sterols coprostanol and cholesterol; the nonionic surfactant degradates 4-nonylphenol, 4-nonylphenolmono-to-tetraethoxylates (ΣNPEO), 4-*tert*-octylphenol, and 4-*tert*-octylphenolmono-to-tetraethoxylates (ΣOPEO); the plasticizer tris-2-butoxyethyl phosphate; the polycyclic musk fragrances hexahydrohexamethylcyclopentabenzopyran (HHCB) and acetylhexamethyltetrahydronaphthalene (AHTN); and the metal complexing agent nitrilotriacetic acid. The natural estrogens (17β-estradiol and estrone) and androgens (testosterone, 4-androstene-3,17-dione, and *cis*-androsterone) were detected at much lower concentrations.

During the study period, dissolved oxygen concentrations in the North Shore Channel averaged 6.9, 8.0, and 7.2 mg/L for September 2006, March 2007, and April 2007, respectively, and were adequate for fish survival (MWRD, 2010). Basic water quality data including temperature, 5-day biological oxygen demand, ammonium, and nitrite + nitrate were variable in the WRP effluent (Fig. 3A and B). Concentrations of total organic carbon (data not shown), which is a measure of the bulk organic characteristics of the water, averaged 6.9 ± 0.9 mg/L in the North Side WRP effluent, and there was no significant difference (paired *t*-test, $p = 0.05$) between the effluent and North Shore Channel.

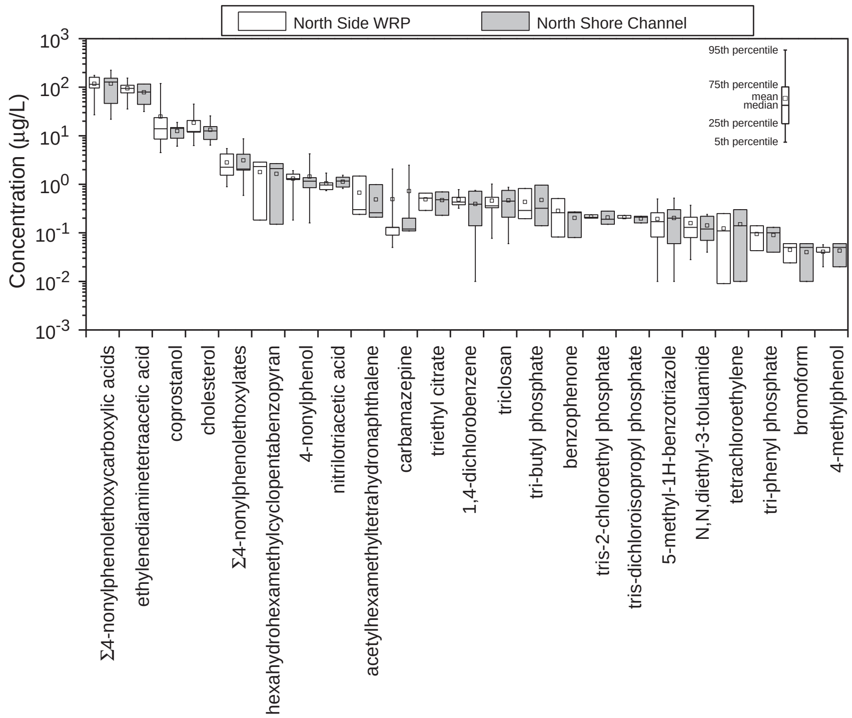

Fig. 2. Box plots summarizing results for the 23 organic contaminants that were detected in 100% of the North Side Water-Reclamation Plant (WRP) effluent and North Shore Channel samples analyzed [n = 3–10 sampling events].

During the March to April sampling, concentrations of EDTA and ΣNPEC (Fig. 3C) were relatively constant in the North Side WRP effluent and the North Shore Channel. Exceptions were the March 2 and March 22 samplings which had lower concentrations, reflecting storm events (Fig. 1B). Concentrations of ΣNPEO decreased from March to April, whereas 4-nonylphenol remained relatively constant (Fig. 3D). The plasticizer bisphenol A and the antimicrobial triclosan showed variable concentrations (Fig. 3E). Concentrations of 4-*tert*-octylphenol and ΣOPEO (Fig. 3F) had similar trends as the 4-nonylphenol compounds. The estrogens 17β-estradiol and estrone (Fig. 3G) and the androgens *cis*-androsterone and 4-androstene-3,17-dione (Fig. 3H) generally decreased from March to April. Concentrations of most contaminants in the North Shore Channel were similar to concentrations in the WRP effluent, reflecting the effluent-impacted nature of the stream. These results indicate continuous and relatively stable exposure of the aquatic ecosystem to a mixture of biologically-active chemicals.

3.3. Biological findings

In September 2006, 8 mature largemouth bass were caught in the North Shore Channel (1000 ± 74 g; mean mass ± standard error) and 9 mature largemouth bass (860 ± 78 g) were collected from the Outer Chicago Harbor (Table 1). In March 2007, 9 mature largemouth bass (950 ± 64 g) and 14 mature common carp (3400 ± 620 g) were collected in the North Shore Channel. Fish were not collected from the Outer Chicago Harbor site during March because water temperatures were too cold for the fish to be at the same stage of sexual maturity. There were no significant differences in fish weights between sites or sampling dates (Mann Whitney *U* test, $p = 0.05$).

The majority of male fish collected in the North Shore Channel during both sampling events had detectable plasma vitellogenin concentrations (Table 1) albeit at lower values than female conspecifics at the same site (averaging 5–10% of female values). In contrast, none of the male largemouth bass from the Outer Chicago Harbor had measurable plasma vitellogenin concentrations. All mature fish from both sites were gametogenic with males actively producing spermatozoa and females producing vitellogenic oocytes. No histopathological changes (ovatestis, atretic testis, or atretic ovaries) were observed in reproductive tissues in fish from both sites.

4. Discussion

The North Side WRP effluent and the North Shore Channel water contained mixtures of biologically-active chemicals. The high concentrations of EDTA and ΣNPEC reflect their large consumption rates, recalcitrance to removal during treatment, and high water solubility (Alder et al., 1990; Ahel et al., 1994a; Nowack, 2002) and were similar to reported values (Field and Reed, 1996; Barber et al., 2000). Due to high loading rates, compounds such as caffeine (Buerge et al., 2003), 4-nonylphenol and ΣNPEO (Ahel et al., 1994a), and triclosan (Halden and Paull, 2005) are widely present in WRP effluents despite removal during wastewater treatment (Castiglioni et al., 2006; Ying et al., 2009). Concentrations of other contaminants in the North Side WRP effluent and the North Shore Channel were similar to reported values, including HHCB and AHTN (Simonich et al., 2002), organophosphorous compounds (Marklund et al., 2005), 5-methyl-1H-benzotriazole (Giger et al., 2006), carbamazepine (Tixier et al., 2003), and sulfamethoxazole (Miao et al., 2004).

There were no statistical differences (paired *t*-test, $p = 0.05$) in concentrations of contaminants detected in the North Shore Channel compared to the North Side WRP effluent (with the exception of caffeine, cholesterol, and 17β-estradiol; Appendix A, Table A-1), indicating minimal in-stream dilution or transformation. In-stream removal for caffeine primarily occurs by biodegradation (Buerge et al., 2003; Bradley et al., 2007), whereas coprostanol is removed primarily by sorption (Writer et al., 1995). Increased water temperatures can enhance microbial transformation rates, consistent with observed decreases in concentrations of estrogens and androgens (Fig. 3G and H). Biotransformation is a primary removal mechanism for these compounds (Layton et al., 2000; Bradley et al., 2009). The trend of decreasing hormone concentrations from March to April in the North Side WRP effluent also was observed in the North Shore

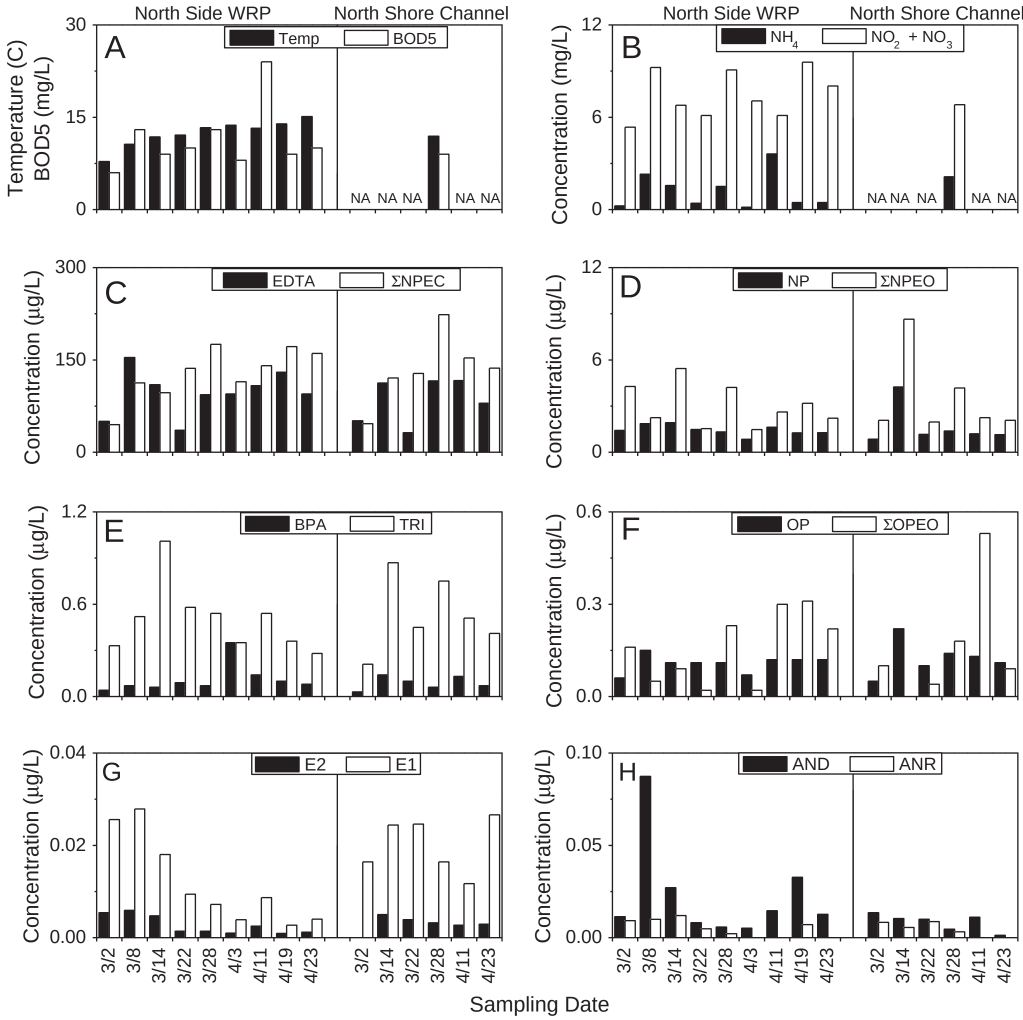

Fig. 3. Basic water-quality constituents and biologically-active chemicals in the North Side Water-Reclamation Plant (WRP) effluent and the North Shore Channel on various sampling dates during 2007. (A) temperature (Temp) and 5-day biological oxygen demand (BOD5), (B) ammonium (NH_4) and nitrite + nitrate ($NO_2 + NO_3$), (C) ethylenediaminetetraacetic acid (EDTA) and 4-nonylphenolmono-to-tetraethoxycarboxylic acids (ΣNPEC); (D) 4-nonylphenol (NP) and 4-nonylphenolmono-to-tetraethoxylates (ΣNPEO); (E) bisphenol A (BPA) and triclosan (TRI); (F) 4-*tert*-octylphenol (OP) and 4-*tert*-octylphenolmono-to-tetraethoxylates (ΣOPEO), (G) 17β-estradiol (E2) and estrone (E1); and (H) 4-androstene-3,17-dione (AND) and *cis*-androsterone (ANR).

Channel, with the exception of estrone (a transformation product of 17β-estradiol), which decreased in the WRP effluent but increased in the stream. These results illustrate the dynamic nature of source and treatment variables, and the relationships between concentration, temperature, microbial degradation, and sorption. The influence of stream physical factors, such as sediment resuspension and combined sewer overflows, adds to the complexity of environmental fate and effects.

In the National fish survey (Ramirez et al., 2009) the pharmaceutical compounds carbamazepine, diltiazem, and diphenhydramine were detected in filet tissue from largemouth bass collected from the North Shore Channel. In the present study, these compounds were detected in the North Side WRP effluent and North Shore Channel water. Although caffeine, sulfamethoxazole, and trimethoprim were detected in the WRP effluent and stream water, they were not detected in the fish tissue (Ramirez et al., 2009), suggesting

Table 1
Plasma vitellogenin results for fish collected from the North Shore Channel and the Outer Chicago Harbor of Lake Michigan [n, sample size; vitellogenin reported as mean ± standard error; frequency, percentage of fish in sample expressing vitellogenin above the detection limit of 0.25 µg/mL].

	Male Fish			Female Fish		
	n	Vitellogenin	Frequency	n	Vitellogenin	Frequency
		µg/mL	%		µg/mL	%
September 26/27, 2006						
North Shore Channel—largemouth bass	5	0.23 ± 0.10	60	3	4.4 ± 2.5	100
Outer Chicago Harbor—largemouth bass	4	0	0	5	13 ± 3.9	100
March 28, 2007						
North Shore Channel—largemouth bass	1	3.3	100	8	7.5 ± 1.8	88
North Shore Channel—common carp	9	38 ± 17	78	5	39,000 ± 9300	100

Table 2
Relationships between concentrations of biologically-active chemicals measured in North Shore Channel water during this study, and filet tissue concentrations measured in largemouth bass collected from the North Shore Channel during an earlier study (Ramirez et al., 2009). Also presented are aquatic organism/water bioaccumulation factors (BAF) reported in the literature. [C_w, concentration in water, C_t, concentration in fish tissue; K_{ow}, octanol/water partition coefficient; NA, not available].

Compound	Log K_{ow}	C_t[a] µg/kg	C_w µg/L	Measured C_t/C_w	Reported BAF
Diphenhydramine	3.7[a]	1.4	0.02	70	NA
Diltiazem	3.6[a]	0.13	0.03	4.3	3.2[b]
Carbamazepine	2.7[a]	2.3	0.73	3.2	2.2–13[c]
Triclosan	5.2[a]	<38	0.46	<82	2000–5,200[d]
Hexahydrohexamethylcyclopentabenzopyran	6.2[a]	1300	1.6	810	20–620[e]
Acetylhexamethyltetrahydronaphthalene	6.4[a]	160	0.49	330	40–670[e]
Caffeine	−0.13[a]	<3.9	0.05	<78	NA
Sulfamethoxazole	0.89[a]	<2.3	0.07	<33	3.2[b]
Trimethoprim	0.79[a]	<2.2	0.03	<73	3.2[b]
N,N-Diethyl-*m*-toluamide	1.2[a]	<5.1	0.14	<37	NA
4-Nonylphenol	3.8–4.8[f]	<9.7	1.4	<6.9	280[g], 150[h]
4-*tert*-Octylphenol		<8.2	0.12	<68	NA

[a] Ramirez et al., 2009.
[b] Schwab et al., 2005.
[c] Vernouillet et al., 2010.
[d] Orvos et al., 2002.
[e] Gatermann et al., 2002.
[f] U.S Environmental Protection Agency, 2005.
[g] Mitchelmore and Rice, 2006.
[h] Clifford Rice, U.S. Department of Agriculture, unpublished data.

differences in uptake and accumulation relative to the other pharmaceutical compounds. The fragrances HHCB and AHTN were the most abundant contaminants detected in fish tissue collected from the North Shore Channel, and also were detected at high concentrations in the WRP effluent and stream water.

The lipophobic (octanol/water partition coefficient, log K_{ow}) characteristics of the compounds varied (Table 2). Although the ionization potentials of the various compounds also vary and can influence bioavailability and partitioning, further discussion is beyond the scope of this study. Generally, compounds detected in the fish tissue (Ramirez et al., 2009) had log K_{ow} values greater than 2.5 and elevated concentrations in the North Shore Channel water (Table 2). Other compounds detected at elevated concentrations in the stream water but not in the fish tissue had log K_{ow} values <2.0. Compounds that were measured but not detected in the water and effluent also were not detected in the fish tissue. Although concentrations of the pharmaceutical compounds in the fish tissue did not correlate with lipid content, HHCB and AHTN were highly correlated with lipid content (Ramirez et al., 2009). Partitioning of these compounds into fish tissue in other studies has been correlated with lipid content in a manner similar to lipophobic organochlorine compounds (Wan et al., 2007). The generalized tissue/water concentration ratios for largemouth bass from the North Shore Channel were in the range of reported bioaccumulation factors for HHCB and AHTN (Table 2), although the measured bioaccumulation factors can be substantially less than values predicted from log K_{ow} and lipid content, indicating the compounds are readily metabolized by fish (Gatermann et al., 2002). The least lipophilic compound detected in the fish tissue samples was carbamazepine, and although the water concentration was similar to HHCB, carbamazapine had a much lower tissue/water concentration ratio, consistent with studies showing little bioaccumulation across multiple trophic levels (Vernouillet et al., 2010).

Based on log K_{ow} values and measured stream concentrations, 4-nonylphenol and 4-*tert*-octylphenol should be present at intermediate tissue concentrations between carbamazepine and HHCB; however, 4-nonylphenol and 4-*tert*-octylphenol were not detected in largemouth bass tissue from the North Shore Channel (Ramirez et al., 2009). In contrast, concentrations of 3800 ng/g for 4-nonylphenol and 43 ng/g for 4-*tert*-octylphenol were detected in carp (whole-fish tissue) collected from the North Shore Channel in August 1999 (Rice et al., 2003a). Another study on the bio-uptake of 4-nonylphenol also reported elevated concentrations in fish tissue collected downstream from a WRP (Rice et al., 2003b; Mitchelmore and Rice, 2006). Additional sampling of largemouth bass from the North Shore Channel in September 2007 and March 2008 conducted as part of this study provided bioaccumulation factors of 145 and 147, respectively (Clifford Rice, U.S. Department of Agriculture, unpublished data). These values are comparable to the bioaccumulation factor of 280 reported for carp (Mitchelmore and Rice, 2006), a bottom dwelling fish that could be expected to produce higher bioaccumulation factors since sediments and biofilms downstream from WRP discharges can have elevated concentrations of 4-nonylphenol (Writer et al., 2011). These diverse bio-uptake results show the complexity in evaluating the effects of environmental exposure to chemical mixtures, interspecies differences in lipid content and life history, differing analytical methods, and measuring concentrations in different tissue compartments (whole fish, filet, and liver).

In addition to 4-nonylphenol and 4-*tert*-octylphenol compounds, other potential endocrine disrupting chemicals including bisphenol A, 17β-estradiol, and estrone were present in the North Shore Channel at concentrations in the range of values reported to cause biological effects (Van den Belt et al., 2004; Terasaki et al., 2005; Bistodeau et al., 2006; Schoenfuss et al., 2008). Potential exposure to mixtures of estrogenic chemicals can be integrated into an index, the 17β-estradiol equivalency quotient (EEQ), by multiplying measured concentrations by their potency relative to 17β-estradiol, and summing the equivalencies (Vajda et al., 2008). The average EEQ for the sampling events in the North Shore Channel was 0.009 µg/L 17β-estradiol equivalents and the maximum EEQ was 0.02 µg/L (Appendix A, Table A-3), which are in the range of concentrations shown to induce plasma vitellogenin in male fish in laboratory studies (Bistodeau et al., 2006; Barber et al., 2007; Hyndman et al., 2010). The average EEQ value was in the range predicted to result in a "medium" endocrine-disruption effect (Sumpter et al., 2006) and the maximum EEQ value falls into the "high" effect class. Although 80% of the EEQ in the North Shore Channel was attributed to 17β-estradiol, estrone, and estriol, it is important to consider the mixture of chemicals that occur when evaluating potential endocrine disruption risk in wastewater-impacted ecosystems.

Due to the persistence of the chemicals in the North Shore Channel relative to stream hydraulic residence times, fish populations in the wastewater-impacted stream are continuously exposed to endocrine-disrupting chemicals. The majority of male largemouth bass and carp caught in the North Shore Channel had elevated plasma vitellogenin concentrations indicating exposure to estrogens (Table 1), whereas none of the male largemouth bass from the Outer Chicago Harbor had detectable plasma vitellogenin. Evidence of endocrine disruption also was observed in carp collected from the Cuyahoga River downstream from the Akron WRP, where decreased plasma vitellogenin concentrations in female fish and increased concentrations in male fish were correlated with increasing tissue concentrations of 4-nonylphenol (Mitchelmore and Rice, 2006). In contrast to other locations such as Boulder Creek, Colorado, where a similar set of chemicals were measured and population-level reproductive disruption (skewed sex ratios, ovatestis, and vitellogenin induction) was observed in white sucker (*Catostomus commersoni*) populations downstream from a WRP (Vajda et al., 2008), no histopathological changes were observed in fish from the North Shore Channel. The wastewater-impacted reach in Boulder Creek (30–75% effluent) had EEQ values ranging from 0.003 to 0.011 μg/L 17β-estradiol equivalents, near values measured in the North Shore Channel. Controlled on-site, continuous-flow exposure experiments using fathead minnows (*Pimephales promelas*) showed a positive dose–response relationship between a number of endocrine disruption biomarkers (plasma vitellogenin concentrations, gonad histology, and secondary sexual characteristics) and the fraction of Boulder WRP effluent contributing to flow (Vajda et al., 2011). Induction of vitellogenin in male largemouth bass and carp from the North Shore Channel without indications of histopathological changes, suggests inter-species differences in sensitivity to endocrine disrupting chemicals because it is likely that the fish have been continuously exposed to WRP effluent their entire life-cycle (>5 yr at the mean collected size).

Although the link between androgen exposure and biological effects is not as well documented as with estrogen exposure (Kolodziej et al., 2004; Soto et al., 2004), androgens also affect the endocrine system. The testosterone metabolites 4-androstene-3,17-dione and *cis*-androsterone were observed at the same frequency and concentrations as 17β-estradiol and estrone, whereas the parent androgen, testosterone, was not detected as frequently. Although total androgen concentrations (0.015 μg/L) in the North Shore Channel were similar to the total estrogen concentrations (0.024 μg/L), the only biological data consistent with androgen exposure was the lower plasma vitellogenin concentrations observed in female fish from the North Shore Channel relative to the Outer Chicago Harbor (Table 1). Statistical significance could not be resolved due to small sample size. The lower vitellogenin concentrations may reflect the reproductive biology of female largemouth bass which spawn only once in the spring (in contrast to carp, which spawn repeatedly throughout the summer), and females in the North Shore Channel may have completed their spawning activity earlier than conspecific females in Lake Michigan, which warms up more slowly in the spring, thus delaying spawning activity. Plasma vitellogenin concentrations among female largemouth bass from the North Shore Channel were within reported values (Sepulveda et al., 2002). Laboratory studies using the androgenic chemical 17β-trenbolone (Ankley et al., 2003) found masculinization of female fathead minnows, including reduction in plasma vitellogenin. Reduction in vitellogenin biosynthesis in female fathead minnows exposed to androgens is linearly related to fecundity, and model predictions suggest that moderate decreases in vitellogenesis can decrease fish population size (Miller et al., 2007). However, in the present study, female largemouth bass and carp produced vitellogenin at concentrations orders-of-magnitude greater than male conspecifics (independent of exposure history) and had vitellogenic oocytes indicating advanced gametogenesis and reproductive viability.

Exposure of organisms to biologically-active chemicals in urban streams is not limited to vertebrates, and in some instances there is greater sensitivity in invertebrates, microbes, and plants than in fish (Brooks et al., 2003; Waiser et al., 2011), which can alter the entire community structure (Slye et al., 2011). Taken together with alterations in the physical and chemical environments of wastewater-impacted streams, changes in invertebrate community structure may persist into vertebrate populations (Brooks et al., 2006). Effects on aquatic vertebrates may differ between species based on life history. For example, carp, which forage in the sediments of the river bed encounter different exposure scenarios than largemouth bass, which are open-water predators. The pseudo-persistence of biologically-active chemicals measured in this study, their unabated presence across several kilometers of river reach, and their likely effects across the entire aquatic organismal community deserve greater study to allow for better understanding of urban stream ecosystems.

5. Conclusions

This study characterized the occurrence of a variety of organic contaminants, including biologically-active chemicals, in a wastewater-impacted urban stream ecosystem. Evidence of endocrine disruption consistent with exposure to estrogenic chemicals was observed in wild-caught fish. However, tissue-level endocrine disruption was not observed in any of the male or female fish. Because of the complex chemical mixtures present, it is important to consider other possible effects in addition to endocrine disruption. Chemicals such as antidepressants can bioaccumulate (Brooks et al., 2005; Schultz et al., 2010) and impact other organismal responses such as predator avoidance behavior (Painter et al., 2009; Schultz et al., 2011). The adverse outcome pathways framework (Ankley et al., 2010) provides a linkage between molecular initiating events and effects at a level of biological organization relevant to risk assessment. Although the limited sampling in this study precludes consideration of population-level effects on fish in the North Shore Channel, the data indicate exposure to chemicals resulting in induction of biomolecular responses with potential for ecological risk. Further research is needed to assess the relationships between exposures to mixtures of biologically-active chemicals and population-level effects.

With projected human population growth in the 21st Century (United Nations, 2008) and anticipated changes in climate and rainfall patterns (United Nations, 2001) there will be increased urbanization pressures on aquatic ecosystems (World Health Organization, 2000). As wastewater-impacted urban streams become more pervasive, it will become increasingly important to understand differences in the biological responses of urbanized fish populations relative to those in less impacted non-urban aquatic environments.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at doi:10.1016/j.scitotenv.2011.06.039.

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