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Environmental Toxicology

BIOACCUMULATION OF HUMAN PHARMACEUTICALS IN FISH ACROSS HABITATS OF A TIDALLY INFLUENCED URBAN BAYOU

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Abstract

Though pharmaceuticals and other contaminants of emerging concern are

increasingly observed in inland water bodies, the occurrence and bioaccumulation of pharmaceuticals in estuaries and coastal ecosystems are poorly understood. In the present study, bioaccumulation of select pharmaceuticals and other contaminants of emerging concern was examined in fish from Buffalo Bayou, a tidally influenced urban ecosystem that receives effluent from a major (~200 million gallons per day) municipal wastewater treatment plant in Houston, Texas, USA. Using isotope dilution liquid chromatography–tandem mass spectrometry, various target analytes were observed in effluent, surface water, and multiple fish species. The trophic position of each species was determined using stable isotope analysis. Fish tissue levels of diphenhydramine, which represented the only pharmaceutical detected in all fish species, did not significantly differ between freshwater and marine fish predominantly inhabiting benthic habitats; however, saltwater fish with pelagic habitat preferences significantly accumulated diphenhydramine to the highest levels observed in the present study. Consistent with previous observations from an effluent-dependent freshwater river, diphenhydramine did not display trophic magnification, which suggests site-specific, pH-influenced inhalational uptake to a greater extent than dietary exposure in this tidally influenced urban ecosystem. The findings highlight the importance of understanding differential bioaccumulation and risks of ionizable contaminants of emerging concern in habitats of urbanizing coastal systems.

Keywords: Tidally influenced, Water quality, Urban ecosystem, Bioaccumulation, Estuary, Urbanization, Ionizable contaminant, Contaminant of emerging concern

All Supplemental Data may be found in the online version of this article.

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INTRODUCTION

Human pharmaceuticals and other contaminants of emerging concern have increasingly been observed in inland surface waters and have been found to accumulate in aquatic organisms [1,2]. However, the occurrence of many contaminants of emerging concern in coastal and estuarine systems remains poorly understood [3–7]. During dry months, the instream base flows of urban streams are dominated by or even dependent on effluent discharges from wastewater treatment plants (WWTPs), which represents a worst-case scenario for potential ecological effects of contaminants of emerging concern in inland and coastal systems [8], by resulting in increased effective exposure duration to aquatic life [9]. This consideration is particularly critical for fast-growing urbanized coastal systems, where the quantity and quality of instream flows to bays and estuaries are increasingly influenced by population growth and climate variability [8]. In fact, understanding the influences of anthropogenic contaminants on marine life was recently identified as an important global ocean research priority [10]. Estuaries of the Gulf of Mexico in Texas, USA, are particularly relevant for investigations of such emerging water quality concerns as a result of pronounced urbanization and annual rainfall gradients (W.C. Scott et al., unpublished manuscript).

Bioaccumulation of human pharmaceuticals has been observed in aquatic organisms globally [11–16]. Physicochemical properties of pharmaceuticals and

other contaminants of emerging concern dictate an advancement of historical approaches to define bioaccumulation, hazards, and risks [15]. For example, bioaccumulation of pharmaceuticals in fish may be elevated because of limited intrinsic clearance [17], particularly when organisms are continuously exposed to WWTP effluent discharges in urban aquatic systems [18]. Further, because approximately 70% of human pharmaceuticals are ionizable weak bases, understanding pH influences on the bioavailability and toxicity of ionizable pharmaceuticals was recently identified as a major research need [19,20].

Determination of basal energy sources is more difficult in dynamic estuarine systems because aquatic communities include species with diverse physiological tolerance to salinity gradients and large diel variability in water chemistry (e.g., salinity, pH) [21]. For example, the migratory fish *Paralichthys lethostigma* (southern flounder) exhibits seasonal migration in and out of estuaries [22], resulting in variability of energy sources at different life stages. Though many estuarine fish are not considered migratory, they move vertically to seek better diet sources and habitats [23]. Many fish species move among shallower and deeper water to spawn [24]. Vertical movement also affects respiration, which may influence the passive diffusion of environmental contaminants across fish gills [25].

Unfortunately, an understanding of ionizable pharmaceutical and other contaminant of emerging concern bioaccumulation in fish occupying different habitats in urban estuaries is lacking, but such an understanding is necessary to reduce uncertainty during environmental risk assessment and management. In tidally influenced systems, traditional water chemistry parameters such as pH vary

across habitats and salinity gradients and thus may differentially influence the spatial bioaccumulation of ionizable contaminants. In the present study, we examined the occurrence of select pharmaceuticals in effluent, surface water, and bottom water and various aquatic species from a tidally influenced urban bayou in Houston, Texas, the fourth largest city in the United States. We then employed stable isotope analysis to identify the trophic position of organisms within this complex food web. Finally, we identified whether bioaccumulation of several pharmaceuticals by fish differed across habitats in an estuary receiving discharge from one of the largest WWTPs in the southern United States.

MATERIALS AND METHODS

Study site

Buffalo Bayou (Supplemental Data, Figure S1) starts in Fort Bend County, Texas, USA, and flows to the Houston Ship Channel, then to Galveston Bay, and finally to the Gulf of Mexico **<ZAQ;1>**(www.buffalobayou.org). Buffalo Bayou was selected for study because this intensively urban watershed receives effluent discharge, in addition to stormwater runoff, from a major WWTP in the city of Houston, Texas. We sampled downstream of the 69th Street WWTP because this facility is the largest (~200 million gallons per day) in the states of Texas, New Mexico, Louisiana, Arkansas, and Oklahoma. During an initial study, we previously observed a number of pharmaceuticals and other contaminants of emerging concern in the surface waters of Houston [26]. Prior to the present study, the presence of pharmaceuticals in water or aquatic life of estuaries and coastal habitats in Texas was unknown.

Field sampling

Based on our previous observations of salinity differences with depth in Buffalo Bayou (W.C. Scott et al., unpublished manuscript), samples of effluent, surface water (0.3 m from surface), and bottom water (0.3 m from sediment–water interface) and biological samples were collected during a sampling event on 15 September 2013. Sample collection followed Texas Commission on Environmental Quality methods by boat electrofishing, minnow trapping, and cast netting for 14 fish species, including freshwater fish *Lepisosteus oculatus* (spotted gar; family Lepisosteidae), *Micropterus salmoides* (largemouth bass; family Centrarchidae), *Lepomis megalotis* (longear sunfish; family Centrarchidae), *Dorosoma cepedianum* (gizzard shad; family Clupeidae), *Menidia beryllina* (inland silverside; family Atherinopsidae), *Hypostomus plecostomus* (armored catfish; family Loricariidae), *Ictiobus bubalus* (buffalo fish; family Catostomidae), *Ictalurus punctatus* (channel catfish; family Ictaluridae), marine fish *Mugil curema* (white mullet; family Mugilidae), *Mugil cephalus* (striped mullet; family Mugilidae), *Dormitator maculatus* (fat sleeper; family Eleotridae), *Brevoortia patronus* (Atlantic menhaden; family Clupeidae), *Micropogonias undulatus* (Atlantic croaker; family Sciaenidae), and *Fundulus grandis* (gulf killifish; family Fundulidae). Fish were collected within a 200-m radius of the effluent discharge; specific boat electrofishing locations within this area were determined by salinity influences on electrofishing effectiveness. Fish length and weight were measured on-site immediately after anesthetization using MS-222. All samples were transported to the lab on ice and then stored at –20 °C until further analyses.

During the sampling event, duplicate effluent surface water and bottom water samples, approximately 50 m downstream from the discharge, were collected in 4-L prerinsed amber glass bottles, transported on ice to the lab, and stored for less than 48 h at 4 °C prior to filtration and extraction.

Water chemistry parameters and pharmaceutical analysis

During fish sample collection, diel measurement of routine water chemistry parameters (pH, salinity, dissolved oxygen, temperature) was performed for both surface water (0.3 m from the surface) and bottom water (0.3 m from sediment–water interface) locations over a 24-h period using calibrated multiparameter datasondes (YSI 600 XLM, 6920; YSI Instruments). Surface water and bottom water samples were also collected for target pharmaceutical and effluent tracer determination. Twenty-three target analytes, which were also selected in a previous study [27], were examined based on previous reported occurrences of these pharmaceuticals and other effluent tracers in aquatic ecosystems [13,27]. All target analytes and their corresponding isotopically labeled analogs were purchased from various vendors and used as received (see Du et al. [27] and Supplemental Data, Table S1). Detailed description of sample extraction and analysis was previously provided by Du et al. [13], in which isotope dilution was used to compensate for any inherent matrix interference with isotopically labeled internal standards for each corresponding target analyte. Briefly, full-body homogenates were subsampled for 1.0 g of tissue and extracted with 8 mL of a 1:1 mixture of 0.1 M aqueous acetic acid and methanol in a 20-mL borosilicate glass vial (Wheaton; VWR Scientific). A mixture of isotopically labeled standards

(corresponding to deuterated analogs of each target analyte) was added to each sample prior to extraction. Samples were equilibrated by gentle end-over-end inversion for 20 min at 25 ± 0.1 °C, then centrifuged at 16 000 rpm for 45 min. The supernatant was collected, evaporated over a gentle stream of nitrogen, and reconstituted in 1 mL 95:5 0.1 % (v/v) aqueous formic acid-methanol prior to analysis. Two different previously published methodologies were applied for water extraction [28,29]. Similarly, the tissue sample extraction protocol generally followed previously developed methodologies [13,30]. As noted previously by our research team, tissue levels of target analytes were not lipid-normalized because it is not appropriate to do so for ionizable pharmaceuticals [11].

All tissue samples were analyzed using liquid chromatography–tandem mass spectrometry following a previously reported method, in which instrumentation parameters, separation strategy, detection of target analytes, calibration method, and method detection limits were specified in detail [13]. Similarly, effluent and surface water sample analyses also followed a recently reported method by our research team [31]. Method detection limits for each analyte represented the lowest concentrations that were reported with 99% confidence that the concentration was different from 0 in a given matrix. One method blank sample and a pair of matrix spikes were also analyzed in each analytical sample batch. Matrix spike samples were spiked with 100 μg/kg of all target analytes. Recoveries were within 20% of the spiking concentration.

Stable isotope analysis

Stable isotopes ($\delta^{15}N$, $\delta^{13}C$) were determined in the Stable Isotope Core

Laboratory at Baylor University using a dual-inlet gas-source Stable Isotope Mass Spectrometer (Thermo-Electron) and an Elemental Analyzer (Costech). Whole biological tissue samples were dried to constant weight (for 24 h at 95 °C in a drying oven) and crushed to a fine powder using a mortar and pestle. Dried, crushed samples were weighed to approximately 1 mg and wrapped in Sn capsules prior to instrumental analysis. Data were calibrated using internationally recognized standards USGS-40 and USGS-41 with analytical precision of ±0.02%. Isotopic ratios were calculated using the Equation 1

$$\delta X\ (‰) = (R_{sample}/R_{standard} - 1) \times 1000 \qquad (1)$$<**ZAQ;2**>

where the heavier isotope X is ^{15}N or ^{13}C, R_{sample} is the ratio of heavy to light isotope in the analyzed sample, and $R_{standard}$ is the ratio of heavy to light isotope in the standards [32]. Trophic position was determined using Equation 2 [32,33]

$$TP_{consumer} = ([\delta^{15}N_{consumer} - \delta^{15}N_{secondary\ consumer}]/\Delta^{15}N) + 3 \qquad (2)$$

where $TP_{consumer}$ is the trophic position and $\delta^{15}N_{consumer}$ is the stable isotope abundance of the study organism. Also, $\delta^{15}N_{secondary\ consumer}$ is the baseline of the trophic structure, in this case calculated from the gizzard shad (*D. cepedianum*), which possessed the lowest $\delta^{15}N$ of (13.13 ± 0.74‰ [mean ± standard deviation], *n* = 3) and occupied trophic position 3. Gizzard shad is a schooling fish and commonly inhabits freshwater, brackish, and marine environments [34]. In the

larval stage it feeds on zooplankton, and as an adult it filter-feeds on small invertebrates and phytoplankton, which indicates that it is a secondary consumer (presuming primary producers are algae and primary consumers are zooplankton) [35]. A $\Delta^{15}N$ enrichment factor of 3.4‰ was chosen for the present study [32,33].

Statistical analysis

Differences in diphenhydramine concentration in fish with differential habitat and salinity preferences were evaluated using randomized/permutation analysis of variance because of unequal sample size, followed by Tukey's honestly significant difference (using the library agricolae), using R **<ZAQ;3>**[36].

RESULTS AND DISCUSSION

Water chemistry parameters and pharmaceuticals in effluent, surface water, and bottom water

Buffalo Bayou experienced a median salinity of 2.80 ppt (parts per thousand; **<ZAQ;4>**range, 2.88 ppt) at the surface, but the salinity of bottom waters was much greater with a 24-h median of 10.58 ppt (range, 3.07), which reflects the pronounced chemocline between pelagic and benthic habitats that existed in this tidally influenced system (Figure 1). Similar differences were observed between surface and bottom waters for other water quality parameters. For example, median surface and bottom pH values were 7.00 and 6.63, with 24-h ranges (minimum–maximum) of 0.49 and 0.31, respectively (Figure 1). In fact, a significant relationship ($p < 0.05$) was observed between pH and salinity in surface, but not bottom, waters (Figure 1). Similarly, Buffalo Bayou exhibited a higher median dissolved oxygen concentration of 4.21 mg/L (range 2.44 mg/L) in surface

waters compared with hypoxic conditions observed in bottom waters with a median of 1.08 mg/L (range, 0.70 mg/L). A significant relationship was observed ($p < 0.05$) between pH and dissolved oxygen at both depths. Temperature was relatively high and consistent over the 24-h period. Median surface water temperature was 30.30 °C with a range of 1.27 °C, whereas bottom water had a median of 30.33 °C with a smaller range of just 0.14 °C over the 24-h period.

No pharmaceuticals or effluent tracers were detected in the method blank samples; however, various target analytes were observed in duplicate samples of the effluent discharge and surface and bottom waters (Table 1). Target analytes were generally higher in effluent than surface or bottom water samples (Table 1). Concentrations of select detected compounds, such as sucralose, erythromycin, atenolol, and trimethoprim, remained relatively consistent regardless of surface water or bottom water. The effluent tracers sucralose and caffeine were detected in effluent at the highest levels (3900 ng L^{-1} and 24 000 ng L^{-1}, respectively) among all target analytes (Table 1). It may be that sucralose provides a more representative indication of effluent discharge than some other effluent tracers. Though the scope of surface water sampling in the present study was limited to 1 season and we did not attempt to quantify effluent dilution in this tidally influenced bayou, similar levels of sucralose were observed in surface water and effluent, which had similar salinities, relative to bottom waters characterized by markedly elevated salinity, while caffeine levels in surface water were an order of magnitude lower than in the effluent discharge (Table 1). A related study by our team observed similar patterns between surface and bottom observations and

salinity across seasons in Buffalo Bayou and several other tidally influenced urban estuaries of Texas [37]. Future studies are necessary to understand tidal influences on exposure and risks of pharmaceuticals and other consumer products in such hydrologically dynamic systems.

Trophic positions of fish in Buffalo Bayou

To understand biomagnification of ionizable environmental contaminants in the Buffalo Bayou food web, it was important to define the food web structure and to understand energy flow among organisms before developing a relationship among contaminants and trophic positions. Stable $\delta^{13}C$ ratios (Table 2) were used to examine the flow of energy and contributions to higher trophic positions. Typically, the source of energy from a higher trophic position is derived from the group below with a similar $\delta^{13}C$ signature [32,33,38]. In the present study, however, $\delta^{13}C$ varied extensively among various fish species with a range of 23.18‰ to 28.33‰. Numerous overlapping standard deviations (Figure 1) made it difficult to separate individual food chains to fully characterize the food web of such a dynamic tidally influenced urban bayou. Further, because we focused on fish and did not examine invertebrates and primary producers, it was not possible to construct a complete food web.

Site fidelity is likely another factor that influenced stable isotope signatures observed and modeling efforts in the present study. Although nitrogen stable isotope ratios vary from upstream to downstream in a river, nitrogen cycling and anthropogenic introduction of nitrogen from WWTPs and runoff also influence nitrogen isotope signatures of aquatic systems [39]. Therefore, when organisms

migrate or routinely move across large spatial ranges, isotope signatures are affected by both sampling area and conditions of other locations visited. For example, in the present study, *M. curema* and *M. cephalus* [23] are considered migratory species, which move within and among estuaries periodically with daily tides for feeding and reproduction. However, the present study included sampling at only 1 location in Buffalo Bayou, which was tidally influenced.

Accumulation of pharmaceuticals in fish

We further examined whole tissue concentrations of target analytes in all fish species. Weight, length, and composite groupings are provided in Table 2. Although a number of compounds were not detected, 2 pharmaceuticals, the antibiotic erythromycin and the first-generation antihistamine diphenhydramine, were consistently detected in multiple species from Buffalo Bayou (Tables 3 and 4). The antibiotic trimethoprim and the antiepileptic carbamazepine were occasionally detected, with all observations occurring below their corresponding method detection limits. Among detected pharmaceuticals, erythromycin was detected at the highest level (6.2 µg/kg) in *H. plecostomus*. Typically, fewer target analytes with a lower detection frequency were observed in fish from Buffalo Bayou relative to our recent study in Chesapeake Bay [7]. Both of these urban estuarine studies demonstrate fewer pharmaceuticals at lower levels in fish tissues than another recent study that focused on an effluent-dependent inland river [27]. Diphenhydramine was the only target pharmaceutical detected in all biological samples. Levels of diphenhydramine in fish were generally consistent with previous studies investigating bioaccumulation of pharmaceuticals in fish collected

from inland aquatic systems in the United States, though occurrence patterns were slightly different between the present study and other inland studies at different geographical locations. No selective serotonin reuptake inhibitors were observed in fish in the present study (Tables 3 and 4); however, some selective serotonin reuptake inhibitors are known to accumulate in fish and other aquatic species in inland waters [11–13,27,40].

Such bioaccumulation differences between previous inland studies and the present study likely resulted from several factors. Though the sampling location of the present study was downstream of a large (~200 million gallons per day) WWTP effluent discharge, effluent exposure was decreased by elevated dilution in Buffalo Bayou compared with our previous findings in effluent-dependent wadeable streams [2,27,30,40]. In addition, surface and bottom water salinity and pH vary spatially (upstream, downstream, and vertically) and temporally (W.C. Scott et al., unpublished manuscript), which strongly affects uptake of ionizable pharmaceuticals (discussed below) across fish gills [27,41,42]. It also may be that bioaccumulation differences among some compounds reported in Tables 3 and 4 resulted from fish species predominantly occupying freshwater or saltwater habitats. As noted above, available data for comparison are lacking as investigations of pharmaceutical bioaccumulation in urbanized coastal or estuarine areas are limited. However, Maruya et al. [14] reported occurrence of select contaminants of emerging concern in marine mussels from urban coastal ecosystems in California, USA. Carbamazepine, diphenhydramine, and erythromycin were detected in fish from the present study. These 3

pharmaceuticals, though not frequently detected (<20%) by Maruya et al., were also observed in mussels along the urban California coast [14]. Additionally, the antidepressant sertraline was frequently (64%) identified in marine mussels relative to other pharmaceuticals [12]. Though we did not observe sertraline or other antidepressants in estuarine fish of the present study, these contaminants were consistently detected during our recent research with bivalves in an effluent-dependent freshwater stream [27].

To determine whether bioaccumulation differed because of habitat or salinity preferences, fish with more than 3 individuals collected from Buffalo Bayou were partitioned to 4 groups based on predominant habitat (benthic, pelagic) and salinity (freshwater, marine, or brackish) preferences. We then performed a 2-way analysis of variance using diphenhydramine as the dependent variable and the 2 categories as the predictor variables, including their interactions. Figure 2**<ZAQ;5>** presents a box plot with a data overlay of diphenhydramine for the 4 group combinations. Diphenhydramine levels in fish did not significantly ($p > 0.05$) differ between benthic freshwater and benthic saltwater fish, but both groups accumulated diphenhydramine to significantly ($p < 0.05$) higher levels than pelagic fish. However, saltwater fish with pelagic habitat preferences accumulated diphenhydramine to the highest levels observed ($p < 0.05$; Figure 3) in this tidally influenced urban ecosystem.

We then investigated whether diphenhydramine accumulated differentially in fish across various trophic positions in Buffalo Bayou. Trophic positions of fish collected from Buffalo Bayou were determined; $\delta^{15}N$ and calculated trophic

positions of fish are provided in Table 1. As noted above, *D. cepedianum* (gizzard shad), which possessed the lowest $\delta^{15}N$ (13.13 ± 0.74‰ [mean ± standard deviation], $n = 3$), is a secondary consumer and was placed at the bottom of the fish trophic structure. The range of $\delta^{15}N$ for this fish species was 13.13‰ to 19.29‰, resulting in a range of trophic positions of 3.00 to 4.87. The $\delta^{15}N$ values of *M. beryllina* and *F. grandis* were higher than those for most fish species, which apparently resulted from diverse energy sources, including municipal effluent. Because *F. grandis* typically displays higher site fidelity than the schooling gizzard shad, which travels relatively larger distances to forage, an elevated trophic position of *F. grandis* appears to have been influenced by anthropogenic N from effluent discharge. Future studies are necessary to understand trophic relationships and energy flow within these complex urban systems.

Linear regression revealed no significant relationship ($p > 0.05$) between contaminants in fish and trophic position, likely resulting from the relatively homogenous distribution of trophic positions in Figure 3. Du et al. [27] presented a novel study of diphenhydramine in various components of the aquatic food web of a freshwater stream (North Bosque River, TX, USA), which resulted in a trophic magnification factor for diphenhydramine of 0.38. A trophic magnification factor <1 indicates that a contaminant is being diluted with increased trophic positions, which is also known as “trophic dilution” [43]. Differential biotransformation rates of environmental contaminants, such as pharmaceuticals in the present study, between predators and organisms at lower trophic positions introduce additional complexity when assessing trophic transfer of chemicals [43]. Our research group

also recently reported that rainbow trout exhibited limited in vitro biotransformation of diphenhydramine and other pharmaceuticals commonly detected in fish tissue [17]. Similar studies investigating comparative biotransformation of pharmaceuticals and other contaminants of emerging concern in estuarine and marine organisms are unavailable.

Based on the observations of the present study and consistent with our recent observations in inland waters, diphenhydramine bioaccumulation by fish was more likely influenced by inhalational, rather than dietary, uptake. Surface water pH strongly influences bioavailability, bioaccumulation, and toxicity of ionizable contaminants [44–46]. In addition to pH, respiratory uptake of ionizable organic chemicals may be influenced by diel fluctuation of salinity in tidally influenced systems. Relative to bottom water, lower salinity in the surface water typically corresponded to significantly higher pH values (Figure 1), which favors formation of the neutral species of diphenhydramine that readily partitions across fish gills [42]. Thus, diel pH variability in Buffalo Bayou surface waters, but not bottom waters (Figure 1), modified bioavailability and thus potentially resulted in elevated bioaccumulation of ionizable weak bases by pelagic fish preferentially inhabiting brackish and marine waters during the present study (Figure 2). Clearly, more research is needed to understand how habitat preference influences bioaccumulation in tidally influenced urban systems.

Uptake of ionizable organic acids by fish gills was previously examined by Erickson et al. [41,47], who developed a model to predict pH-specific uptake by rainbow trout. A recent study by our research team extended this effort to examine

pH influences on whole-body bioaccumulation of the ionizable weak base diphenhydramine in the fathead minnow [42]. Nichols et al. [42]**<ZAQ;6>** observed the apparent volume of distribution (V_d) for diphenhydramine in fathead minnows (3 L/kg) to be almost identical to that in humans (3–8 L/kg), which further highlights the potential for biological "read-across" with human pharmacological and toxicological data [17,44,48–51]. Such observations further indicate that V_d, in addition to clearance, is more relevant to predict bioaccumulation of ionizable contaminants than traditional log octanol–water partition coefficient–based modeling efforts with lipid normalization for nonionizable chemicals. Whether such observations extend to the estuarine and marine organisms examined in the present study is unknown, but it is decidedly necessary to advance an understanding of bioaccumulation dynamics and ionizable contaminant risks in urban coastal ecosystems. In the present study, we examined a limited number of contaminants of emerging concern, which represent a small fraction of the exposome of aquatic life residing in this urban coastal system, which is strongly influenced by the city of Houston, Texas, the fourth largest and one of the fastest-growing metropolitan areas in the United States. Unfortunately, an understanding of differential risks from many contaminants of emerging concern, including most ionizable compounds, is currently lacking for wildlife in these urban coastal systems.

SUPPLEMENTAL DATA

Figure S1. (39 KB DOC).

Acknowledgment—B. Du and S.P. Haddad contributed equally to the present study.

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Data availability—Contact Bryan Brooks (Bryan_Brooks@Baylor.edu) for data from the present study.

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Figure 1. Significant relationship ($p < 0.05$) between 24-h pH and salinity in surface (**A**; 0.3 m from surface) but not bottom (**B**; 0.3 m from sediment water interface) water in Buffalo Bayou, Harris County, Texas, USA.

Figure 2. Whole-body concentration of diphenhydramine in fish across habitat and salinity preferences in Buffalo Bayou, Harris County, Texas, USA. A box plot with data overlay of diphenhydramine is presented for 4 group combinations, including pelagic freshwater, benthic freshwater, pelagic salt water, and benthic salt water. The size of the circles represents the trophic position of the individual fish in each group, with sizes of the circles being proportional to their trophic positions. Numbers under the box plot are the number of fish species in each group. Because of unequal sample size, randomized/permutation analysis of variance was performed. No significant ($p > 0.05$) relationships were observed for diphenhydramine concentration between benthic freshwater fish and benthic saltwater fish, but both groups significantly ($p < 0.05$) differed from pelagic fish regardless of whether they reside predominantly in freshwater or salt water.

Figure 3. Geometric mean (± standard deviation) of $\delta^{13}C$ and $\delta^{15}N$ for fish species collected from Buffalo Bayou, Harris County, Texas, USA.

<<ENOTE>> AQ1: Is this website necessary? If so, it will need to be converted to a reference (number 26), and all subsequent references will need to be renumbered. If not, please delete.

<<ENOTE>> AQ2: Please add brackets to clarify: "([R_{sample}/$R_{standard}$] – 1)" or "(R_{sample}/[$R_{standard}$ – 1])"?

<<ENOTE>> AQ3: This software was added to the reference list as #36, and all subsequent references were renumbered accordingly. Please check for accuracy.

<<ENOTE>> AQ4: Please check throughout. Unclear how a "range" is a single value.

<<ENOTE>> AQ5: check Figures 2 and 3 were cited out of order (3 was cited first). The two figures have been swapped, so the original Figure 2 is now Figure 3 and vice versa.

<<ENOTE>> AQ6: Addition of “[42]” correct here? If not, please insert correct citation.

<<ENOTE>> AQ7: Please check Reference 36 (added) for accuracy.

<<ENOTE>> AQ8: ET&C does not divide tables into parts (such as “A” and “B”). Table 3B was converted to Table 4. The text was updated accordingly.

Table 1. Mean (duplicate) levels of human pharmaceuticals and effluent tracers in effluent, surface water, and bottom water samples from Buffalo Bayou, Harris County, Texas, USA

Analyte	MDL (ng L^{-1})	Effluent (ng L^{-1})	Surface (ng L^{-1})	Bottom (ng L^{-1})
Acetaminophen	2.9	42	110	51
Atenolol	4.3	260	99	98
Carbamazepine	0.53	250	140	130
Caffeine	4.5	24 000	2900	560
Diclofenac	2.8	770	620	440
Diltiazem	0.24	44	12	4.7
Diphenhydramine	0.22	200	42	35
Erythromycin	8.6	53	19	19
Gemfibrozil	2.1	380	120	86
Sucralose	36	3900	3900	2300
Sulfamethoxazole	1.3	670	260	220
Trimethoprim	1.3	81	26	24

MDL = method detection limit.

Table 2. Sample number and mean (±standard deviation) length, weight, stable isotopes, and trophic position of fish predominantly associated with freshwater and saltwater pelagic and benthic habitats collected from Buffalo Bayou, Harris County, Texas, USA

Species		*n*	Length		Isotope (‰)		Trophic
Common name	Scientific name	(composite)	(cm)	Weight (g)	$\delta^{13}C$	$\delta^{15}N$	position
Freshwater pelagic							
Spotted gar	*Lepisosteus oculatus*	6	62 ± 4.7	978 ± 299	–25.71 ± 0.73	19.00 ± 1.23	4.78 ± 0.37
Largemouth bass	*Micropterus salmoides*	14	27 ± 7.2	358 ± 243	–27.26 ± 1.29	19.07 ± 1.40	4.80 ± 0.43
Longear sunfish	*Lepomis megalotis*	22	9.7 ± 1.5	21 ± 11	–24.91 ± 2.82	16.51 ± 2.11	4.02 ± 0.64
Gizzard shad	*Dorosoma cepedianum*	3	NM	NM	–27.16 ± 1.31	13.13 ± 0.74	3.00
Inland silverside	*Menidia beryllina*	15 (2)	NM	NM	–24.79 ± 0.46	19.29 ± 1.02	4.87 ± 0.31
Freshwater benthic							
Armored catfish	*Hypostomus plecostomus*	6	41 ± 1.8	639 ± 70	–24.60 ± 2.94	15.85 ± 1.53	3.82 ± 0.46
Smallmouth buffalo	*Ictiobus bubalus*	3	28 ± 1.3	342 ± 68	–28.33 ± 0.46	14.38 ± 0.68	3.38 ± 0.21
Channel catfish	*Ictalurus punctatus*	6	46 ± 7.0	910 ± 480	–25.00 ± 0.83	14.90 ± 1.62	3.53 ± 0.49
Saltwater pelagic							
Atlantic menhaden	*Brevoortia tyrannus*	20 (2)	NM	NM	–26.83 ± 1.57	16.80 ± 1.26	4.11 ± 0.38
Gulf killifish	*Fundulus grandis*	22 (2)	NM	NM	–23.72 ± 0.33	17.77 ± 1.22	4.41 ± 0.37
Saltwater benthic							

Stripped mullet	*Mugil cephalus*	17	15 ± 4.3	57 ± 47	–23.18 ± 2.85	15.24 ± 1.62	3.64 ± 0.49
White mullet	*Mugil curema*	26	14 ± 7.0	65 ± 167	–24.04 ± 2.05	14.57 ± 1.63	3.44 ± 0.49
Atlantic croaker	*Micropogonias undulatus*	12	NM	NM	–25.61 ± 1.24	17.06 ± 1.98	4.19 ± 0.60
Fat sleeper	*Dormitator maculatus*	2	32	290	–25.42 ± 0.62	16.69 ± 0.56	4.05

N

Table 3. Mean (±SD) of target analytes in fish ($\mu g\,kg^{-1}$) from Buffalo Bayou, Harris County, Texas, USA

	DIP		ERY		ACE		ATE		CAR		CAF	
Organism	Mean (±SD)	Frequency	Mean (±SD)	Frequency	Mean (±SD)	Frequency	Mean (±SD)	Frequency	Mean (±SD)	Frequency	Mean (±SD)	Frequency
Lepisosteus oculatus	0.20 ± 0.08	6/6	<MDL	6/6	ND	0/6	ND	0/6	ND	0/6	ND	0/6
Micropterus salmoides	0.30 ± 0.15	14/14	<MDL	14/14	ND	0/14	ND	0/14	ND	0/14	ND	0/14
Lepomis megalotis	0.38 ± 0.22	22/22	1.6 ± 0.49	22/22	ND	0/22	ND	0/22	ND	0/22	ND	0/22
Dorosoma cepedianum	0.11 ± 0.09	3/3	<MDL	3/3	ND	0/3	ND	0/3	ND	0/3	ND	0/3
Menidia beryllina	0.32 ± 0.10	15/15	1.6 ± 0.59	15/15	ND	0/15	ND	0/15	ND	0/15	ND	0/15
Hypostomus plecostomus	1.1 ± 0.45	6/6	4.0 ± 1.1	6/6	ND	0/6	ND	0/6	<MDL	6/6	ND	0/6
Ictiobus bubalus	0.36 ± 0.33	3/3	3.5 ± 0.2	3/3	ND	0/3	ND	0/3	ND	0/3	ND	0/3
Ictalurus punctatus	0.60 ± 0.51	6/6	3.2 ± 0.21	6/6	ND	0/6	ND	0/6	ND	0/6	ND	0/6
Brevoortia tyrannus	1.5 ± 1.1	20/20	3.9 ± 0.34	20/20	ND	0/20	ND	0/20	ND	0/20	ND	0/20
Fundulus grandis	0.74 ± 0.35	22/22	2.7 ± 1.0	22/22	ND	0/22	ND	0/22	ND	0/22	ND	0/22
Mugil cephalus	0.98 ± 0.53	17/17	2.7 ± 1.0	17/17	ND	0/17	ND	0/17	ND	0/17	ND	0/17
Mugil curema	0.67 ± 0.32	27/27	1.9 ± 0.8	27/27	ND	0/27	ND	0/27	ND	0/27	ND	0/27
Micropogonias	0.66 ± 0.33	12/12	3.7 ± 0.34	12/12	ND	0/12	ND	0/12	ND	0/12	ND	0/12

undulates												
Dormitator maculatus	0.48	2/2	3.8	2/2	ND	0/2	ND	0/2	ND	0/2	ND	0/2
MDL	0.11		2.9		4.9		1.9		0.59		5.5	

SD = standard deviation; DIP = diphenhydramine; ERY = erythromycin; ACE = acetaminophen; ATE = atenolol; CAR = carbamazepine; CAF = caffeine; MDL = method detection limit; ND = not detected..

M

Table <**ZAQ;8**>4. Mean (±SD) of target analytes in fish (µg kg^{-1}) from Buffalo Bayou, Harris County, Texas, USA

	DIC		DIL		GEM		SUL		TRI	
Organism	Mean (±SD)	Frequency	Mean (±SD)	Frequency	Mean (±SD)	Frequency	Mean (±SD)	Frequency	Mean (±SD)	Frequency
Lepisosteus oculatus	ND	0/6	ND	0/6	ND	0/6	ND	0/6	ND	0/6
Micropterus salmoides	ND	0/14	ND	0/14	ND	0/14	ND	0/14	ND	0/14
Lepomis megalotis	ND	0/22	ND	0/22	ND	0/22	ND	0/22	ND	0/22
Dorosoma cepedianum	ND	0/3	ND	0/3	ND	0/3	ND	0/3	ND	0/3
Menidia beryllina	ND	0/15	ND	0/15	ND	0/15	ND	0/15	ND	0/15
Hypostomus plecostomus	ND	0/6	ND	0/6	ND	0/6	ND	0/6	ND	0/6
Ictiobus bubalus	ND	0/3	ND	0/3	ND	0/3	ND	0/3	ND	0/3
Ictalurus punctatus	ND	0/6	ND	0/6	ND	0/6	ND	0/6	ND	0/6
Brevoortia tyrannus	ND	0/20	ND	0/20	ND	0/20	ND	0/20	<MDL	2/20
Fundulus grandis	ND	0/22	ND	0/22	ND	0/22	ND	0/22	ND	0/22
Mugil cephalus	ND	0/17	ND	0/17	ND	0/17	ND	0/17	ND	0/17
Mugil curema	ND	0/27	ND	0/27	ND	0/27	ND	0/27	ND	0/27
Micropogonias undulates	ND	0/12	ND	0/12	ND	0/12	ND	0/12	ND	0/12
Dormitator maculatus	ND	0/2	ND	0/2	ND	0/2	ND	0/2	ND	0/2

MDL	2.7	0.11	5.1	3.7	1.8

SD = standard deviation; DIC = diclofenac; DIL = diltiazem; GEM = gemfibrozil; SUL = sulfamethoxazole; TRI = trimethoprim; ND = not detected; MDL = method detection limit.

= not measured.