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Ontogenetic dietary shifts and bioaccumulation of diphenhydramine in *Mugil cephalus* from an urban estuary

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ABSTRACT

Though bioaccumulation of pharmaceuticals has received attention in inland waters, studies of pharmaceutical bioaccumulation in estuarine and marine systems are limited. Further, an understanding of pharmaceutical bioaccumulation across size classes of organisms displaying ontogenetic feeding shifts is lacking. We selected the striped mullet, *Mugil cephalus,* a euryhaline and eurythermal species that experiences dietary shifts with age, to identify whether a model base, diphenhydramine, accumulated in a tidally influenced urban bayou. We further determined whether diphenhydramine accumulation differed among size classes of striped mullet over a two year study period. Stable isotope analysis identified that ontogenetic feeding shifts of *M. cephalus* occurred from juveniles to adults. However, bioaccumulation of diphenhydramine did not significantly increase across age classes of *M. cephalus* but corresponded to surface water levels of the pharmaceutical, which suggests inhalational uptake to diphenhydramine was more important for bioaccumulation than dietary exposure in this urban estuary.

1. Introduction

Reports of human pharmaceuticals accumulating in aquatic biota from inland surface waters have increased in recent years, particularly from rapidly urbanizing regions (Brooks et al., 2005; Du et al., 2012, 2014a; Kolpin et al., 2002; Ramirez et al., 2007, 2009). Though there is increasing information for freshwater, there remains a poor understanding of the occurrence, bioaccumulation and risks of human pharmaceuticals in coastal systems (Alvarez et al., 2014; Daughton and Brooks, 2011; Du et al., 2016; Gaw et al., 2014; Jiang et al., 2014; Lazarus et al., 2015; Maruya et al., 2012; Meador et al., 2016). Coastal waters receive freshwater inflows, which are influenced by watershed practices, including discharge from municipal and industrial wastewater treatment plants (WWTPs), and runoff from stormwater in agricultural and urban areas. Instream flows of many freshwater streams in semi-arid regions of the world are dominated by or even dependent on effluent discharge (Brooks et al., 2006). These urban systems likely represent worst-case scenarios for potential ecological effects of pharmaceuticals and other consumer chemicals because effective exposure duration increases with limited dilution of continuous chemicals introduction (Ankley et al., 2007). Such exposure scenarios for consumer chemicals are also critically important to understand and manage in rapidly urbanizing coastal systems (Brooks et al., 2006). In fact, a recent global horizon scanning workshop identified developing an understanding of the bioaccumulation and risk associated with pharmaceuticals and personal care products (PPCPs) in wildlife among the top questions necessary to understand risks of PPCPs in the environment (Boxall et al., 2012; Rudd et al., 2014). Coastal contamination from urban areas was also identified as a priority research need for marine science (Rudd, 2014).

Our recent research observed accumulation of a calcium channel blocker, diltiazem, in plasma of multiple fish species exceeding human therapeutic plasma doses (Scott et al., 2016). We also

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identified bioaccumulation of several other pharmaceuticals in fish from four estuaries of the Gulf of Mexico in Texas, USA, with differential land use and urbanization features (Du et al., 2016). Whether these observations extend across life history stages of fish or other aquatic life is poorly understood. Further, influences of dietary exposure on bioaccumulation of ionizable contaminants in species displaying ontogenetic feeding shifts across their life histories are not known. In the current study, we selected the striped mullet, *Mugil cephalus,* to explore whether accumulation of an ionizable pharmaceutical differs among life history stages. Ontogenetic shifts in diet specifically occur in smaller *M. cephalus* (1–100 mm; Akin and Winemiller, 2006; Eggold and Motta, 1992), but have received limited study in larger individuals.

The striped mullet is an estuarine species with a wide distribution in tropical, subtropical, and temporal coastal waters in all major oceans between the latitudes of 42° N and 42° S (Thompson, 1966). In many coastal populations, *M. cephalus* lay eggs near shore in the marine environment where these eggs remain suspended until hatch (Strydom and d'Hotman, 2005). After a month at sea in the surf zone, early juveniles transition to coastal estuaries where juvenile and part of the sub-adult life stages are lived (Hsu et al., 2009; Lawson and Abayomi, 2010) before returning to the ocean as adults to spawn. A euryhaline (Cardona, 2006) and eurythermal teleost (Marais, 1978), *M. cephalus* may represent a 'sentinel' species to monitor environmental changes (Whitfield et al., 2012). Herein, an understanding of exposure and accumulation of most contaminants of emerging concern (CECs), including pharmaceuticals, is unknown as organisms grow, but necessary to reduce uncertainty during environmental hazard and risk assessment.

In the present study, we examined whether a model ionizable base, diphenhydramine (DPH), was accumulated by *M. cephalus* from a tidally influenced urban bayou, which receives municipal effluent from Houston, Texas, USA. We then determined whether DPH accumulation differed with size of *M. cephalus* over a two year study period. Stable isotope analysis was employed to identify if ontogenetic feeding shifts of *M. cephalus* occurred with age.

2. Methods and materials

2.1. Study site

Buffalo Bayou (Fig. 1) begins in Fort Bend County, Texas, flows to the Houston Ship Channel, and then on to Galveston Bay, a critically important commercial fishery and port in the Gulf of Mexico. Buffalo Bayou was selected for study because this intensively urbanized watershed is the receiving system for appreciable effluent discharge and stormwater runoff from the City of Houston, Texas, the fourth largest city and one of the fastest growing urban regions in the USA. During an initial study, we observed a number of pharmaceuticals and other CECs in the surface waters of Houston (Watkins et al., 2014). We sampled downstream of the 69th Street WWTP, which is the largest WWTP (~200 Million Gallons Daily) in the US Environmental Protection Agency (EPA) Region 6 states of Texas, New Mexico, Louisiana, Arkansas and Oklahoma.

2.2. Field sampling

Surface water and biological samples were collected on two different sampling events in October 2012 and September 2013. September and October are considered by the Texas Commission on Environmental Quality as important periods for monitoring surface water quality because these months represent a time of the year when rainfall, and thus instream dilution, is typically lowest, and subsequent exposure to aquatic contaminants is expected to be highest (TCEQ, 2012). Sample collection followed Texas Commission on Environmental Quality methods by boat electrofishing, minnow trapping, and cast netting (TCEQ, 2012). Specific boat electrofishing locations within a 200 m radius of the discharge were determined by salinity influences on electrofishing. Fish length and weight were measured on site immediately after anesthetization using MS-222. All samples were transported to the lab on ice and stored at −20 °C until further analyses. During each sampling event, duplicate surface water samples were collected ~50 m downstream of the discharge in 4-L pre-rinsed amber glass bottles, transported on ice to the lab, and stored for less than 48 h at 4 °C in the dark prior to filtration and extraction.

2.3. Pharmaceutical analysis in water and fish tissue

Analytical methods for surface water and tissue followed previously reported procedures by our research team (Du et al., 2012, 2014a, 2014b, 2016), which were adapted from earlier reported methods (Lajeunesse et al., 2008; Ramirez et al., 2007; Vanderford and Snyder, 2006). Information for other pharmaceutical occurrence in water and bioaccumulation for other fish species from Buffalo Bayou can be found elsewhere (Du et al., 2016). Isotope dilution was used to compensate for matrix interference with an isotopically-labeled internal standard for the target analyte (Du et al., 2014a, 2016).

All tissue samples were analyzed using liquid chromatography-tandem mass spectrometry (LC-MS/MS) following a previously reported method, in which instrumentation parameters, separation strategy, detection of the target analyte, calibration method, and method detection limits (MDLs) were detailed (Du et al., 2012). MDLs for the analyte represented the lowest concentration that was reported with 99% confidence that the concentration was different from zero 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 the target analyte. All matrix spike recoveries were within 80–120% of this spiking value.

2.4. 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, Waltham, Massachusetts, USA) and an Elemental Analyzer (Costech, Valencia, CA, USA). Whole biological tissue samples were dried 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 the instrumental analysis. Data was calibrated using internationally recognized standards USGS-40 and USGS-41 with analytical precision of ±0.02%. Isotopic ratios are calculated using the following equation:

$$\delta X(\%) = \left(R_{sample} \Big/ R_{standard} - 1\right) \times 1000 \quad (1)$$

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 (Jardine et al., 2006).

2.5. Statistical analysis

Individual *M. cephalus* were partitioned to size classes from <149 mm (<12 month old juveniles), 150–249 mm (~12–24 month old juveniles), 250–349 mm (~2 year old juveniles to adults), or > 350 mm (adults). Because the maximum size of *M. cephalus* at