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Uptake of human pharmaceuticals in bull sharks (*Carcharhinus leucas*) inhabiting a wastewater-impacted river

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HIGHLIGHTS

- Bull sharks are one of few shark species that reside in sewage-impacted habitats.
- Bull sharks residing in wastewater-impacted rivers accumulate human drugs.
- EE2 and SSRI levels are low in bull shark plasma, but effects remain unknown.

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ABSTRACT

The presence of human pharmaceuticals in sewage-impacted ecosystems is a growing concern that poses health risks to aquatic wildlife. Despite this, few studies have investigated the uptake of active pharmaceutical ingredients (APIs) in aquatic organisms. In this study, the uptake of 9 APIs from human drugs was examined and compared in neonate bull sharks (*Carcharhinus leucas*) residing in pristine (Myakka River) and wastewater-impacted (Caloosahatchee River) tributaries of Florida's Charlotte Harbor estuary. The synthetic estrogen used in human contraceptives (17α-ethynylestradiol) and 6 of the selective serotonin/norepinephrine reuptake inhibitors (citalopram, fluoxetine, fluvoxamine, paroxetine, sertraline, venlafaxine) used in human antidepressants were observed at detectable and, in some cases, quantifiable levels in plasma of Caloosahatchee River sharks. Comparatively, only venlafaxine was detected in the plasma of a single Myakka River shark at a level below the limit of quantitation. These results suggest that sharks residing in wastewater-impacted habitats accumulate APIs, a factor that may pose special risks to *C. leucas* since it is one of few shark species to regularly occupy freshwater systems. Further research is needed to determine if the low levels of API uptake observed in Caloosahatchee River bull sharks pose health risks to these animals.

1. Introduction

Due to their widespread use, continuous introduction into sewage-impacted water systems, and ability to alter animal physiology, there is growing concern regarding the presence of active pharmaceutical ingredients (APIs) in aquatic ecosystems. Despite this, only a few published studies have investigated tissue concentrations of APIs in aquatic organisms, particularly freshwater fish (Daughton and Brooks, 2011). Because of this, there is a poor understanding of the ecologically relevant levels of exposure to and accumulation of APIs, information that is essential for assessing the risks that these compounds pose to the health and stability of aquatic wildlife populations. The need for such information has become even more critical in light of recent studies that have demonstrated that exposure to some APIs at levels comparable with those observed in sewage-impacted sites may be capable of altering a number of important physiological processes such as development, reproduction, and nervous system function (Corcoran et al., 2010; Lajeunesse et al., 2011; Mennigen et al., 2011; Thomas and Klaper, 2012).

Compared to bony fish, sharks have received little attention with regards to API exposure. This is perhaps not unexpected as very few of the approximately 400 extant species of sharks regularly occur in freshwater habitats and are potentially exposed to greater than trace levels of human sewage. However, one shark species – the bull shark (*Carcharhinus leucas*) – has been shown to frequent some rivers highly impacted by sewage effluent, placing them at special risk from exposure to APIs and other wastewater-related contaminants. For example, previous studies have demonstrated that neonate bull sharks use the Caloosahatchee River, a wastewater-impacted tributary of Florida's Charlotte Harbor estuary, as nursery habitat for the first two years of their life (Heupel et al., 2010). Furthermore, despite their small size in comparison with adult conspecifics, juvenile *C. leucas* are generally

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viewed as terminal consumers in freshwater systems such as the Caloosahatchee River (Ortega et al., 2009) and are therefore likely to accumulate sizeable quantities of environmental pollutants that are prone to biomagnification in aquatic ecosystems. Given these factors, *C. leucas* is more likely to be regularly exposed to not insignificant quantities of wastewater-related pollutants than most coastal shark species and is a logical choice of species to focus on in any studies on API exposure in sharks and their relatives.

With these points in mind, the objective of this study was to examine API uptake in bull sharks residing in sewage-impacted habitats, focusing on neonate *C. leucas* from wastewater-receiving waters of the Caloosahatchee River. Because of their high rate of use, previous detection in environmental samples and biota from sewage-impacted habitats (Kolpin et al., 2002; Fick et al., 2010; Daughton and Brooks, 2011; Du et al., 2012), and potential for inducing effects in aquatic wildlife at ecologically relevant levels of exposure (Corcoran et al., 2010; Mennigen et al., 2011), the synthetic estrogen in human contraceptives (17α-ethynylestradiol, EE2) and the active components in the human antidepressants known as selective serotonin and serotonin/norepineprhine reuptake inhibitors (SSRIs/SNRIs) were selected as target APIs. The popular cholesterol-lowering agent atorvastatin calcium and impotence drug sildenafil citrate were also selected for investigation, one of the rare instances either has been surveyed in aquatic wildlife.

2. Materials and Methods

2.1. Study Sites and Animal Sampling

We tested the hypothesis that residence in wastewater-impacted habitats would result in measurable accumulation of human pharmaceuticals in neonate bull sharks by comparing plasma concentrations of EE2, SSRIs/SNRIs, atorvastatin calcium, and sildenafil citrate in *C. leucas* sampled from the sewage-impacted Caloosahatchee River with those measured in sharks from the lower Myakka River, a separate tributary of the Charlotte Harbor estuary that is minimally impacted by wastewater effluent and was used as a reference location for this study (Fig. 1). At the time that this study was conducted, a total of six sewage treatment facilities were permitted to discharge directly into the Caloosahatchee River, and the total permitted discharge of these facilities was approximately 44 million gallons per day (mgd) of secondary- to tertiary-level treated effluent (South

Fig. 1. Location of the Caloosahatchee River and Myakka River, the two southwest Florida rivers used as sampling sites in the present study.