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Urbanization is a major influence on microplastic ingestion by sunfish in the Brazos River Basin, Central Texas, USA☆

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A B S T R A C T

Microplastics, degraded and weathered polymer-based particles, and manufactured products ranging between 50 and 5000 μm in size, are found within marine, freshwater, and estuarine environments. While numerous peer-reviewed papers have quantified the ingestion of microplastics by marine vertebrates, relatively few studies have focused on microplastic ingestion by freshwater organisms. This study documents microplastic and manufactured fiber ingestion by bluegill (*Lepomis macrochirus*) and longear (*Lepomis megalotis*) sunfish (Centrarchidae) from the Brazos River Basin, between Lake Whitney and Marlin, Texas, USA. Fourteen sample sites were studied and categorized into urban, downstream, and upstream areas. A total of 436 sunfish were collected, and 196 (45%) stomachs contained microplastics. Four percent (4%) of items sampled were debris on the macro size scale (i.e. >5 mm) and consisted of masses of plastic, metal, Styrofoam, or fishing material, while 96% of items sampled were in the form of microplastic threads. Fish length was statistically correlated to the number of microplastics detected ($p = 0.019$). Fish collected from urban sites displayed the highest mean number of microplastics ingested, followed by downstream and upstream sites. Microplastics were associated with the ingestion of other debris items (e.g. sand and wood) and correlated to the ingestion of fish eggs, earthworms, and mollusks, suggesting that sunfish incidentally ingest microplastics during their normal feeding methods. The high frequency of microplastic ingestion suggest that further research is needed to determine the residence time of microplastics within the stomach and gut, potential for food web transfer, and adverse effects on wildlife and ecosystemic health.

1. Introduction

The United States discards plastic waste at a rate of 29.6 million tons per year (U.S. EPA, 2015). Plastic is a versatile, lightweight, and strong material composed of various elements, such as carbon, hydrogen, oxygen, nitrogen, chlorine, and sulfur and is ideal for a variety of applications in many industries (Andrady, 2011). Early reports of plastic waste in marine systems occurred in the 1960's (Harper and Fowler, 1987; Kenyon and Kridler, 1969) and plastic has now been reported in both freshwater and deep ocean environments (Dris et al., 2015a; Galgani, 2015). A rough estimate predicts that 70–80% of plastic-based marine litter originates from inland sources and is transported by rivers to oceans (Wagner et al., 2014). Potential sources include wastewater treatment plants, cargo shipping, human litter from beaches, and fisheries (Wagner et al., 2014). While most marine studies assign inland waters as the most realistic sources, the proportional contribution of various point and non-point sources have not been established at either the regional level for the Gulf of Mexico or for marine systems as a whole (Thiel et al., 2013).

Primary microplastics are plastics manufactured at a microscopic scale (i.e. <5 mm) and used in products such as facial cleansers, boat cleaners, and drug vectors. Secondary microplastics form from the prolonged mechanical, photolytic, or chemical degradation of primary macroplastics and often result in fragmented pieces or fibers (Mathalon and Hill, 2014). To date, sources of microplastics in freshwater systems have not been fully characterized. Due to the variation in physicochemical properties for the different types of plastics (e.g. specific gravity, molecular weight, functional groups), the difficulties of developing accurate detection and quantification methods, and the variation in transport

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pathways; the relative availability of microplastics in freshwater is largely unknown (Eerkes-Medrano et al., 2015).

Studies of plastic contamination have reported microplastics within freshwater rivers (Dris et al., 2015b; Klein et al., 2015; Moore et al., 2011), lakes (Eriksen et al., 2013; Free et al., 2014), and shoreline sediments (Zbyszewski and Corcoran, 2011). Field studies on microplastic interaction with fish are mainly the result of marine system studies and indicate an occurrence of ingestion ranging from 2.6% to 36.5% (Foekema et al., 2013; Lusher et al., 2013; Ramos et al., 2012; Romeo et al., 2015). Freshwater system studies inclusive of microplastic ingestion by fish are limited. One study by Sanchez et al. (2014) reports a 12% occurrence of microplastic ingestion within wild gudgeons (*Gobio gobio*) from French rivers.

This study defines microplastics as plastics, artificial polymers (e.g. polyester or Nylon), and manufactured products, that range in size from 50 to 5000 μm (Masura et al., 2015). The aim of this work is two-fold; first, to examine a sentinel taxon (i.e. the sunfish Centrarchidae) for microplastic ingestion; and second, to evaluate the influence of urbanization on microplastic ingestion. This research compared the frequency of microplastic ingestion by two species of sunfish, bluegill (*Lepomis macrochirus*) and longear (*Lepomis megalotis*), collected from 14 geographic sites representing upstream, downstream, and urban areas within the Central Brazos River Basin, Texas.

2. Methods

2.1. The study region and selection of sampling sites

The Brazos River watershed originates in the Texas panhandle and reaches the Gulf of Mexico at Freeport, Texas, southwest of Houston (Fig. 1). Three major tributaries: the Salt Fork, the Double Mountain Fork, and the Clear Fork of the Brazos River, converge west of Dallas-Fort Worth to form the Brazos River Basin. The basin has a contributing drainage area of approximately 109,000 km^2 (Brazos River Basin and Bay Expert Science Team BBEST, 2012). In order to provide an array of conditions relative to the position of urban areas and the structure of reservoirs, sampling sites incorporated a variety of areas along the Brazos River. The immediate land use around the sample areas includes natural forested riverbanks and wetlands, mowed lawns, docks with boat ramps, marinas, paved roads, and parking areas (Table 1). True color remote sensed imagery was utilized from Google Earth to create 40,000 m^2 land plots (200 × 200 m) and a set of three 1000 m transect lines associated with each sample location. Land plots centered on the sampling site and the nearest point on the river, and extended directly landward. Transects were placed 100 m apart, centered on the sampling site, and extended directly landward. Land use, associated with sample site, was categorized into the following divisions: park, road, development, dock, pasture, plow field, forest/wetland, and manmade structure.

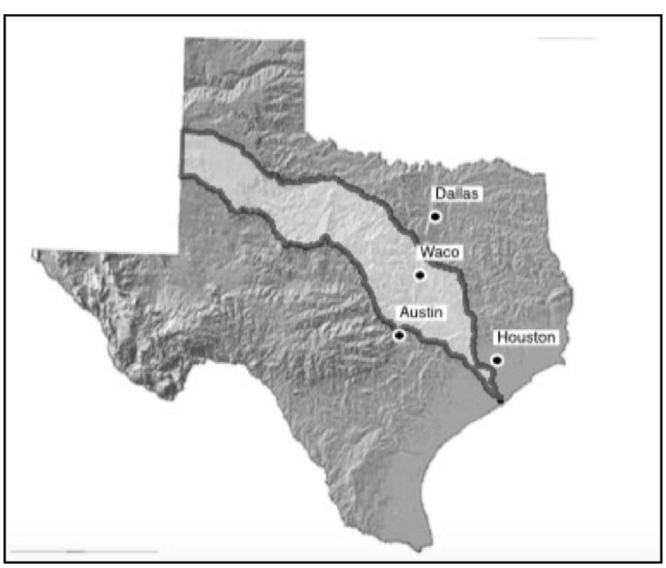

Fig. 1. Map of the Brazos River Basin. Major cities are noted within the figure. Map shows the topography across the state of Texas. Dark line represents the boundary of the Brazos River Basin.

2.2. Species examined in the study

The Brazos River Basin sustains a variety of sunfish species, such as bluegill (*L. macrochirus*), longear (*L. megalotis*), green (*L. cyanellus*), and redear (*L. microlophus*) (Armstrong, 1998). Bluegill and longear sunfish served as the study specimens and are found throughout Central Texas freshwater systems and reside within streams, ponds, and reservoirs (Texas Parks and Wildlife, 2015). Both study species forage throughout the entirety of the water column and utilize methods, such as suction feeding, to capture prey (Mecozzi, 2008; Rider and Margraf, 1998). Bluegill and longear sunfish were chosen as the study specimens because of their abundance throughout the study area, accessibility for collection, and position within the food chain. Sunfish, as a sentinel species, can be used as an indication of ecosystem health and offer insight into the potential impacts of microplastic ingestion on other organisms.

2.3. Sample collection and laboratory analysis

Between 21 March 2014 and 25 July 2014, 318 bluegill and 118 longear sunfish were collected from 14 sample locations using hook-and-line and cast nets (RS-750 Series, Fitec, Memphis, TN). In the field, samples were taken directly from a riverbank or dock (Table 1), thus samples were collected in shallow water generally between 50 cm and 5 m in depth. Upon capture, fish were immediately euthanized via pithing and cutting through the spinal column. Animal use was in accordance with the American Veterinary Medical Association guidelines on euthanasia and was approved by the Baylor University Institutional Animal Care and Use Committee. Sunfish were placed into sealed freezer bags, labeled with location, temperature, date, time, and capture method, and transferred in to a −4 °C freezer for storage. In the laboratory, each fish sample was defrosted, weighed, measured, and grouped into a length class based on three major size categories: ≤10 cm (n = 91), 10.1–13.9 cm (n = 203), and ≥14.0 cm (n = 142). Stomach contents were also removed, weighed, and stored in glass vials containing 70% ethanol.

Stomach contents were washed with distilled deionized water through four filters, 1000 μm, 243 μm, 118 μm, and 53 μm (Wildco Supply Company, Yulee, FL). This process resulted in the separation of individual ingested items into unique size populations. During this process, each filter was visually inspected for laboratory dust or filter particle contamination under a dissecting microscope. Resultant stomach contents were examined using a stereomicroscope with 10× oculars (Motic, DMW 143, VWR). Contents were separated and categorized as organic (i.e. biological) or inorganic (i.e. manufactured). Items determined to be inorganic and on the micro scale consisted of a variety of materials. These materials were collectively classified as microplastic and manufactured materials because of the variation in functional groups and physiochemical properties, and included items such as woven or dyed natural to reconstituted materials, manufactured materials coated in plastic,