High microplastic and anthropogenic particle contamination in the gastrointestinal tracts of tiger sharks (Galeocerdo cuvier) caught in the western North Atlantic Ocean

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A B S T R A C T

Few studies have documented microplastics (<5 mm) in shark gastrointestinal (GI) tracts. Here, we report microplastic contamination in the tiger shark (Galeocerdo cuvier), an apex predator and generalist feeder, at several different life stages. We examined seven stomachs and one spiral valve from eight individuals captured off the United States Atlantic and Gulf of Mexico coasts (eastern US) and conducted a literature review of publications reporting anthropogenic debris ingestion in elasmobranchs. Specimens were chemically digested in potassium hydroxide (KOH) and density separated using calcium chloride (CaCl₂) before quantifying and categorizing suspected anthropogenic particles (>45 μm) by size, morphology, and colour. Anthropogenic particles were found in the stomachs and spiral valve of all sharks. A total of 3151 anthropogenic particles were observed across all stomachs with 1603 anthropogenic particles observed in a single specimen. A subset of suspected anthropogenic particles (14%) were chemically identified using Raman spectroscopy and μ-Fourier Transform Infrared spectroscopy to confirm anthropogenic origin. Overall, >95% of particles analyzed via spectroscopy were confirmed anthropogenic, with 45% confirmed as microplastics. Of the microplastics, polypropylene (32%) was the most common polymer. Diverse microparticle morphologies were found, with fragments (57%) and fibers (41%) most frequently observed. The high occurrence and abundance of anthropogenic particle contamination in tiger sharks is likely due to their generalist feeding strategy and high trophic position compared to other marine species. The literature review resulted in 32 studies published through 2022. Several methodologies were employed, and varying amounts of contamination were reported, but none reported contamination as high as detected in our study. Anthropogenic particle ingestion studies should continue in the tiger shark, in addition to other elasmobranch species, to further understand the effects of anthropogenic activities and associated pollution on these predators.

1. Introduction

Plastic pollution is ubiquitous across global oceans (Akdogan & Guven, 2019) and in consumers across trophic positions (Carbery et al., 2018). Microplastics (<5 mm), which result from degradation of meso- and macroplastics (>5 mm) over time, have been found in a variety of marine taxa, including marine invertebrates (e.g., zooplankton), fish, seabirds, and marine mammals (reviewed in Ugwu et al., 2021). Microparticles have been observed in many species of marine fish (Lusher et al., 2013; Neves et al., 2015; Peters et al., 2017; etc.), which are often prey items for sharks and other top predators. Microparticles may be consumed indirectly by top predators when ingesting contaminated prey (e.g., trophic transfer; Benjamin et al., 2014; Nelms et al., 2018) and/or ‘food falls’ (i.e., trash from human activity sinking in the water column) associated with anthropogenic activities (Cartes et al., 2016), or by direct uptake and ingestion. Plastics of all size classes, including microplastics, have been observed in the gastrointestinal (GI) tracts of sharks (Cliff et al., 2002; Leclerc et al., 2012; Benjamin et al., 2014; López-López et al., 2018; Smith, 2018; Maes et al., 2020; Mancia et al., 2020, etc.) and other elasmobranchs (Anastasopoulou et al., 2013; Neves et al., 2015; López-López et al., 2018).

The tiger shark (Galeocerdo cuvier) is an apex predator residing in tropical and temperate marine habitats that consumes a wide spectrum of food items compared to other sharks (Papastamatiou et al., 2006; Dicken et al., 2017). Tiger shark diets are known to comprise teleost fishes, sea...
snakes, sea turtles and other elasmobranchs including smaller sharks (Heithaus, 2001). Several indigestible items, including plastics, have been observed in tiger shark GI tracts off the coast of South Africa (Cliff et al., 2002), Australia (Simpfendorfer et al., 2001) and Hawaii (Lowe et al., 1996). Examples of indigestible items include cellophane, tin cans, clothing, and plastic bags (Lowe et al., 1996). Tiger sharks are highly likely to encounter microplastics in the ecosystem via exposure to contaminated water and prey species through direct or indirect consumption (Lowe et al., 1996; Simpfendorfer et al., 2001; Cliff et al., 2002). Considering its position as Near Threatened globally (UCN, 2023), in addition to its susceptibility to ingesting plastics due to its high trophic position, indiscriminate feeding strategy, and associations with high anthropogenic activity (Parton et al., 2019), the tiger shark is an ideal species to study for the consumption of microplastics.

The purpose of this study was to determine whether opportunistically collected tiger shark ingest microplastics and other anthropogenic particles (hereinafter referred to as anthropogenic particles). Anthropogenic particle ingestion in tiger sharks was compared to contamination in other sharks and elasmobranchs as reported in the literature. Given its feeding ecology, the tiger shark may be an indicator species of microplastic accumulation or exposure as a large marine predator. Moreover, if tiger sharks are heavily contaminated with anthropogenic particles, further research should investigate the potential long-term effects of ingestion in this species.

2. Methods

2.1. Quality assurance and quality control

Throughout collection of specimens and analyses, measures were taken to reduce the potential for procedural contamination, as recommended by Munno et al. (2023). During specimen collection, samples were exposed to air for as little time as possible and kept in sealed containers following collection. In the laboratory, materials were thoroughly rinsed with reverse osmosis water (RO) a minimum of three times prior to use. All materials were covered with lids and/or aluminum foil when not in use to reduce potential airborne contamination. A white cotton lab coat was worn throughout sample processing and quantification. The laboratory is equipped with a HEPA filter to reduce airborne contamination. One laboratory blank was acquired for every two specimens processed, and laboratory blanks were carried through the entire process from sample extraction to quantification and chemical identification to estimate the procedural contamination. Laboratory blanks were processed in the same manner as the shark specimens, using the same materials and containers and the same approximate volumes of added solutions. A representative subsample of all suspected anthropogenic particles was chemically identified using spectroscopy. Blank contamination is reported, but final data is not blank- or spectroscopy-corrected.

2.2. Description of environmental samples

Tiger shark stomachs and one spiral valve were opportunistically collected from eight individuals captured in the western North Atlantic Ocean. Seven stomachs (five from Alabama’s Gulf coast, one from near Port Royal, South Carolina, and one from Long Island, New York) and one spiral valve (from Hilton Head, South Carolina) were collected (Table 1). Stomachs and the spiral valve were dissected whole after securing zip ties on both the cranial and caudal end of the stomach or spiral valve. Specimens were then stored intact in large plastic bags and frozen prior to transportation to the University of Toronto, Canada. Stretch total, fork length, and sex were recorded at the time of sampling.

3. Sample processing

At the University of Toronto, stomachs and the spiral valve were weighed and externally rinsed thoroughly with RO water to remove any potential contamination that may have come from the sample bag. They were then placed in 19 L polypropylene plastic bins for digestion immediately following rinsing. The wet weight (ww) of the tissue was measured using a precision balance (Sartorius Entris® II - Essential Line, Model 3202i-1x). If the mass exceeded the maximum capacity of the balance (3200 g) and the mass could not be measured, the tissue ww was recorded as >3200 g. Specimens and laboratory blanks were fully submerged in 1 μm filtered 20% potassium hydroxide (KOH), with the specimens and KOH solution combined ranging in volume from approximately 1.3 L–12 L. Specimens and blanks were incubated in solution at 45–55 °C for 5–7 days, or until completely digested. Digested samples were rinsed through a 25 μm mesh stainless steel sieve and soaked in Contrad® liquid detergent for up to 24 h. Samples were sieved again and placed in a 1.4 g/mL CaCl2 density separation. Floating particles were sieved into three size fractions (>355 μm, 125–355 μm, 45–125 μm). The sieved floating particles for the two larger size fractions (>355 μm, 125–355 μm) were rinsed into glass jars, and the smallest size fraction (45–125 μm) was vacuum filtered onto 20 μm polycarbonate filters. While processing specimens, some suspected anthropogenic particles (i.e., not yet confirmed by chemical identification) were visually observed in the lower portion of the density separation. To better quantify all anthropogenic particles, particles remaining in the lower (dense) portion of the density separation were also sieved and stored in glass jars for analysis with the two larger size fractions (>355 μm, 125–355 μm). However, the lower portion of the density separation contained a large amount of sediment and so it was not feasible to examine filters for the smallest size fraction (45–125 μm). Therefore, with the exclusion of the lower portion from quantification, the abundance of anthropogenic particles that were quantified in this study likely underestimates total anthropogenic particle contamination. Four laboratory blanks were run in parallel with specimens to estimate procedural contamination and cross-contamination.

Under a dissecting microscope (Olympus SZ61 stereo microscope, 6.7X – 45X magnification), suspected anthropogenic particles from all

Table 1

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Date sampled</th>
<th>Location</th>
<th>Sex</th>
<th>PCL (mm)</th>
<th>FL (mm)</th>
<th>ST (mm)</th>
<th>Tissue Sampled</th>
<th>Tissue ww (g)</th>
<th>Total N Anthropogenic Particles</th>
</tr>
</thead>
<tbody>
<tr>
<td>GC-01</td>
<td>2019-09-26</td>
<td>Gulf of Mexico, AL</td>
<td>F</td>
<td>630</td>
<td>690</td>
<td>935</td>
<td>stomach</td>
<td>279</td>
<td>355</td>
</tr>
<tr>
<td>GC-02</td>
<td>2019-06-22</td>
<td>Port Royal, SC</td>
<td>F</td>
<td>2150</td>
<td>2390</td>
<td>2860</td>
<td>stomach</td>
<td>&gt;3200*</td>
<td>1603</td>
</tr>
<tr>
<td>GC-03</td>
<td>2019-09-16</td>
<td>Gulf of Mexico, AL</td>
<td>M</td>
<td>985</td>
<td>1095</td>
<td>1440</td>
<td>stomach</td>
<td>&gt;3200*</td>
<td>151</td>
</tr>
<tr>
<td>GC-04</td>
<td>2019-06-25</td>
<td>Gulf of Mexico, AL</td>
<td>M</td>
<td>610</td>
<td>675</td>
<td>925</td>
<td>stomach</td>
<td>&gt;3200*</td>
<td>260</td>
</tr>
<tr>
<td>GC-06</td>
<td>2019-08-28</td>
<td>Gulf of Mexico, AL</td>
<td>M</td>
<td>745</td>
<td>825</td>
<td>1125</td>
<td>stomach</td>
<td>406.2</td>
<td>211</td>
</tr>
<tr>
<td>GC-07</td>
<td>2019-06-06</td>
<td>Gulf of Mexico, AL</td>
<td>M</td>
<td>1085</td>
<td>1190</td>
<td>1540</td>
<td>spiral valve</td>
<td>274</td>
<td></td>
</tr>
</tbody>
</table>

*-* indicates data not recorded during sampling.
**+** indicates the mass was greater than the maximum capacity of the precision balance.
size fractions were quantified visually and described by colour and morphology according to a visual identification key (adapted from Munno et al., 2021). Possible morphologies include fiber, fiber bundle, fragment, film, foam, sphere, pellet, and rubber. A subset of suspected anthropogenic particles (ten particles of each colour-morphology combination within each size fraction) were taken from each specimen and mounted on double-sided tape on transparent film for chemical identification (see below). For each specimen, a minimum of ten suspected non-anthropogenic particles were also extracted (across all size fractions) to estimate rates of false negative visual identification. All extracted particles were photographed and measured. Measurements were taken in two dimensions, with the longest dimension representing the length, and the widest dimension perpendicular to the length.

### 3.1. Chemical identification

The subset of suspected anthropogenic particles was chemically identified to measure the accuracy of our visual identification using attenuated total reflectance - Fourier Transform infrared (ATR-FTIR) and Raman spectroscopy. We selected this method to be representative of broad material type categories (e.g., anthropogenic particles versus non-anthropogenic particles, as recommended by De Frond et al. (2023)). At minimum, five particles were randomly selected from each colour-morphology combination (e.g., black fiber, red fragment, etc.) for chemical identification from each specimen. If fewer than five particles in each color-morphology combination were present, all particles were chemically identified. If greater than 50 particles in each colour-morphology were present, 10% of the particles were chemically identified up to a maximum of 20 particles per colour-morphology. The subsampled particles were proportionally representative of each size fraction, with at least one particle from each size fraction analyzed per specimen whenever possible. For laboratory blanks, all particles were chemically identified. All suspected non-anthropogenic particles removed from specimens and blanks were also chemically identified. For particles larger than ~300 μm, ATR-FTIR with a diamond internal reflection (Bruker Ltd., Milton, ON, CA) operating with OPUS – TOUCH software (Bruker Ltd., version 7.8.44) was used. Infrared spectra were collected at a resolution of 4 cm⁻¹ between 4000 and 400 cm⁻¹. Spectra were averaged over 24 scans, and background scans were recorded (24:1 scans). Particle identification was based on existing spectral library databases (Bruker Ltd.; Primpke et al., 2018). If spectra could not be obtained from the examined particle, or if no spectral reference existed, the particles were re-examined using Raman spectroscopy. For several black rubbery fragments, µ-FTIR was used. Spectra were collected with a Nicolet i10 infrared microscope (Thermo Fisher Scientific – ATR mode; 15X objective, 0.7 numerical aperture), using a germanium ATR crystal and a cooled mercury cadmium telluride single point detector. For each particle, 32 co-added scans and one background spectrum of the crystal were recorded. Spectral resolution was 4 cm⁻¹, and the spectral range was used 4000–675 cm⁻¹. Resulting spectra were matched to reference materials using the FloPP and FloPP-E libraries (De Frond et al., 2021) and commercial libraries using the OMNIC Picta software (version 9.11.706 – Thermo Fisher Scientific). A combination of Hit Quality Index (HQI) and visual confirmation were used to assign material type matches. Most particles were small (<300 μm) and had to be analyzed using Raman spectroscopy (Horiba Raman XploRA PLUS confocal microscope, Piscataway, NJ, USA) operating with LabSpec6 software (version 6.5.1.24) and equipped with a charge coupled device detector (−60 °C, 1024 × 256 pixels). Raman spectra were obtained using a 100X LWD objective (NA = 0.8) resulting in laser powers of 11.2 mW and 20.2 mW at 100% filter for the 532 nm and 785 nm lasers, respectively. Spectral resolution ranged from 1.3 cm⁻¹ (785 nm excitation laser, 600 grooves/mm) to 3.3 cm⁻¹ (532 nm excitation laser, 1200 grooves/mm). Spectra acquired via Raman spectroscopy were assigned database matches using the Wiley KnowItAll and ID Expert spectral matching software (KnowItAll Informatics System, 2022; Analytical Edition) from the KnowItAll Raman Spectral Library as well as the Spectral Library of Plastic Particles (SLoPP and SLoPP-E) (Munno et al., 2020). Microparticles were sorted into material types based on polymer identity and groupings (Munno et al., 2020) using the same categorization scheme described in Munno et al. (2021). Manual and automated software baseline correction was applied (e.g., baseline, vertical clipping, intensity distortion, horizontal offset, vertical offset, Raman intensity distortion). Visual confirmation of similar peak alignment and intensity and HQI score were used to assign spectral matches. Anthropogenic particles were assigned polymer identities based on spectroscopy database matches.

### 3.2. Data analysis

Summary statistics, including the mean, standard deviation, median and interquartile range, were calculated for all stomach samples and by anthropogenic particle characteristics, excluding the single spiral valve. The contents of the spiral valve were reported separately as it is a different organ and not comparable to the abundances observed in the stomachs. Anthropogenic particle characteristics of interest include size fraction, morphology, colour, and material type. Anthropogenic particle abundance in the stomachs and total length in millimeters (mm) of each shark were log transformed and plotted to explore the relationship between size and particle abundance (R software version 4.2.1; R Core Team, 2021). Statistical correlation was not performed as the sample size of stomachs was small (n = 7).

### 4. Literature review

To contextualize our findings with previous reports, we conducted an unsystematic literature search to extract published data on plastic contamination in elasmobranchs. Reports were selected based on some mention of plastic or anthropogenic material ingestion (e.g., “indigestible items”, “garbage”, “anthropogenic debris”) for any species of elasmobranch (subclass Elasmobranchii) published before the conclusion of 2022. No range was selected for geographic location, sample size, or species. Data for location, species and sample size were extracted from each source and organized into a summary table. Taxon and trophic level were identified for each species from FishBase (Froese & Pauly, 2021). The tissues examined, number of individuals ingesting anthropogenic items and the abundance of anthropogenic items/particles were also extracted. If not already expressed as such, the proportion of individuals with ingested anthropogenic particles/items were converted to a frequency of occurrence (%). Based on the methods reported and/or limits of detection, the minimum particle size for each publication was classified as either microscopic (<5 mm) or non-microscopic (≥5 mm). Anthropogenic abundance was expressed as a total observed across all samples, the range of abundance observed within individual samples, and a mean abundance. If the data were not reported in these forms (i.e., total, range and mean) in the literature, the rows were reported as “—” to indicate that the values are not reported within the original publication. If no anthropogenic particles were detected, abundances are reported as “n.d.” – not detected. When possible, the anthropogenic particle morphologies/products, colours, and polymer/material types were included. When reported, the methods of particle extraction, quantification, and chemical verification were included in the table. Particle extraction methods were simplified by extraction type (e.g., alkaline digestion, enzymatic digestion) based on the methods reported.

### 5. Results

#### 5.1. Quality assurance and quality control

Procedural contamination detected in laboratory blanks was low relative to specimens (Fig. S1). A total of 39 anthropogenic particles were detected and chemically identified across four laboratory blanks (mean ±SD) = 10 ± 2; median = 10). Most particles were detected in
the 45–125 μm size fraction (64%) (Fig. S1A). All particles were categorized as fibers, and clear was the most common colour (38%; Figure S1B and Figure S1C). Most of the particles in the blanks (51%) were anthropogenic cellulose (Fig. S2A). Only six particles were identified as plastic, consisting of polyester/polyethylene terephthalate (67%), acrylic (17%) and polypropylene (17%) (Fig. S2B). Nine particles were cellulose and two could not be identified (Fig. S2A). Because procedural contamination was low relative to particle abundances, blank correction was not applied to the data.

5.2. Particle abundance

A total of 3151 anthropogenic particles were quantified across seven tiger shark stomachs, and an additional 274 anthropogenic particles were measured in the spiral valve of one tiger shark (GC-71) (Table 1, Table S1). Anthropogenic particle abundances range from 151 to 1603 particles per stomach (median = 260) (Table 1, Fig. 1). The mean ± SD number of stomach anthropogenic particles was 450 ± 513 (Table S2). Excluding one individual shark with the most contaminated stomach (GC-03), the range in anthropogenic particle abundances was 151–355 (median = 250) (Table 1, Fig. 1). No visual relationship was observed between tiger shark length (stretch total) and anthropogenic particle abundance (Fig. 2).

5.3. Anthropogenic particle characteristics

In tiger shark stomachs, most particles were found in the two largest size fractions (Fig. 3), with 25% of all particles >355 μm (mean ± SD, 106 ± 69; median = 84) and 58% of particles in the 125–355 μm size fraction (234 ± 410; median = 107) (Fig. S1A). The smallest size fraction (45–125 μm) represented 17% of all particles (88 ± 50; median = 57); however, this size fraction only included the floating particles from the density separation and likely underestimates total anthropogenic particle contamination.

All anthropogenic particle morphologies, with the exception of pellets, were observed in the stomachs and spiral valve (Fig. 3B; Fig. 1B). Most anthropogenic particles observed in the stomachs were fragments (57%) and fibers (41%) (Fig. S3B). Tiger shark stomachs had a mean ± SD of 255 ± 508 (median = 57) fragments, and 183 ± 44 fibers (median = 178) (Table S2). The remaining particles consist of relatively small proportions of fiber bundles, films, foams, and spheres (0–2%). The anthropogenic particles in the spiral valve consisted mostly of fibers (76%) (Fig. 3B). Black, rubbery fragments (classified as “fragments”) were detected in five of seven stomachs and in the spiral valve. These rubbery fragments made up 3% of the total fragments in the stomachs and 8% of the fragments in the spiral valve. Two white/clear gel-like fragments were also detected in stomach specimens. Most anthropogenic particles in specimen GC-03, the most contaminated stomach, were fragments (n = 1404) (Fig. 3B). Of these fragments, 28 were black rubbery fragments. The most common colours in stomach samples were blue (49%), clear (30%) and black (11%) (Fig. S4). Similarly, spiral valve particles were also predominantly blue (46%), black (23%) and clear (15%).

5.4. Chemical identification

For the subset of particles that were chemically identified in stomachs (n = 452), most particles identified were confirmed anthropogenic (88%; Fig. 4A, Fig. S5), with a small percentage (3%) of the particles having a confirmed natural (non-anthropogenic) origin. Of the confirmed anthropogenic particles, 45% were plastic and 36% were anthropogenic cellulose. An additional 0.2% were anthropogenic synthetic and 7% were anthropogenic unknown (i.e., where anthropogenic dyes or additives were detected but the underlying material type could not be determined as described in Munno et al. (2021)). For all confirmed plastic particles, polypropylene (29%) and acrylic (23%) were most common (Fig. 4B). For the subset of particles extracted from stomachs that were suspected to be natural (n = 75), only nine were confirmed to be anthropogenic (12%) (Fig. S6).

For the spiral valve, 145 particles were chemically identified and ~90% were confirmed anthropogenic. The majority of particles (48%) were anthropogenic cellulose. Of the particles identified as plastic...
(27%), polypropylene (54%) and polyester/polyethylene terephthalate (18%) were most common (Fig. 4B). A small percentage were natural (5%) or unidentified (6%). From the spiral valve, only one of ten particles that were suspected to be natural was confirmed to be anthropogenic cellulose and none were confirmed to be plastic (Fig. S6).

6. Literature review

Thirty-two reports of anthropogenic item/plastic ingestion in elasmobranchs were identified in the literature from 1996 to 2022 (Table S3). Reports span several geographic locations, including the coast of eastern North America, the Gulf of California, the Hawaiian Islands, the North Sea, Iberian Sea, Tyrrenhian Sea, Mediterranean Sea, the South African coast, the coasts of India and the Philippines, the United Kingdom, Norway, and Australia (Table S3). Taxa include sharks, rays, and skates encompassing 43 species. Trophic position (±SE) of species ranged from 2.92 (Peel et al., 2019) to 4.9 ± 0.5 (Froese & Pauly, 2021) (Table S3). Sample sizes for individual species range from one to 9981 individuals, though some sample sizes were not reported. The tissues examined include the gut (when localized tissue was not specified), stomach, stomach contents (excluding stomach lining), intestine/spiral valve, entire GI tract (esophagus to anus), GI tract contents (excluding GI tract lining), vomit, and fecal matter (excrated) (Table S3). Anthropogenic particle/item sizes range from microscopic (8 μm to <5 mm) to non-microscopic (5 mm to 258 mm), though actual sizes were not consistently reported. Anthropogenic particle/item abundances range from 0 to 770/individual; however, abundances were often not reported or reported with varying units (Table S3). Several item/particle morphologies were reported (Table S3). Fibers and fragments were common among microscopic particles, and plastic bags were common among non-microscopic items.

7. Discussion

Based on the current body of literature from our review, anthropogenic particle contamination in tiger sharks is high relative to other elasmobranchs. Microplastic and other anthropogenic particle ingestion occurred consistently in this study with 100% of specimens containing microplastics and other anthropogenic particles, whereas most other studies report relatively low rates of contamination. While we have analyzed specimens from only eight individuals, the frequency of occurrence and relatively high abundance of anthropogenic particle contamination leads us to believe that the detection of anthropogenic particles in the tiger shark is likely to occur consistently. Six previous studies have observed ubiquitous anthropogenic particle contamination in sharks, reporting microplastics in 100% of brown bay (Raja miraletus) (Capillo et al., 2020), whale shark (Rhincodon typus) (Cardelli et al., 2021), reef manta ray (Mobula alfredi) (Germanov et al., 2019), Atlantic sharpnose shark (Rhizoprionodon terraenovae) (Pullen, 2019; Sittlinger, 2022) and porbeagle shark (Lamna nasus) (Maes et al., 2020). The mean number of anthropogenic particles we observed in tiger shark stomachs (450 ± 513 particles/stomach) was more than four times the next highest reported mean abundance in an elasmobranch (57.9 ± 11.7 particles; Pullen, 2019). While the range in abundance observed in our study varied greatly, the median (260 particles) was still considerably higher than values reported for other elasmobranchs. The high anthropogenic particle contamination observed in tiger sharks may be linked to feeding ecology, geography, proximity to areas of anthropogenic activity, and methodology.

The most likely reason we observed higher anthropogenic particle contamination in the tiger shark relative to other elasmobranchs is their notorious diet. For example, tiger shark feeding strategy likely leads to increased anthropogenic particle ingestion. The tiger shark is a generalist feeder and is known to consume a wide array of food items (Papastamatiou et al., 2006; Randall, 1992; Lowe et al., 1996; Dicken et al., 2017), including garbage. Ingestion may occur through direct consumption of plastic in seawater or indirect consumption from contaminated prey (e.g., trophic transfer). The tiger shark is an apex predator and consumes more types and sizes of organisms than most other shark species (Randall, 1992; Dicken et al., 2017), creating many opportunities for trophic transfer of microplastics through contaminated prey. Fish, sea turtles and sea birds, known prey items, have been heavily contaminated with microplastics (Duncan et al., 2019; Ugwu et al., 2021). In addition to trophic transfer of microplastics through prey, the tiger shark is known to directly consume larger anthropogenic items, such as plastic bags (Lowe et al., 1996), which likely contributes to their high level of contamination.

The tiger shark can reach large body sizes (Randall, 1992), and higher plastic contamination may be expected in larger animals. In other fish species, anthropogenic particle abundance increases with increasing body size as measured by total length (Munno et al., 2021; Mellwraith et al., 2021). The prevalence of indigestible items, including anthropogenic items, has been observed to be higher in larger sharks (Lowe et al., 1996; Dicken et al., 2017). While we did not observe a clear relationship to total length in our study, our largest shark was the most contaminated (GC-03). However, with the tiger shark being such a generalist feeder, clear relationships between length and contamination may be obscured, particularly if sharks exhibit individual prey preferences (e.g., Matich et al., 2011). Future studies should aim to include larger sample sizes, if possible, to better analyze potential relationships between anthropogenic particle abundance and body size. However, we acknowledge that it is challenging
to acquire shark specimens opportunistically and laborious to process and quantify specimens containing such high abundances of anthropogenic particles.

Tiger shark biogeography and home range also may contribute to the relatively high anthropogenic particle contamination observed. The tiger shark is a circumglobal species that has a large home range over long temporal scales, where individuals are documented to travel thousands of kilometers in a matter of months (Heithaus et al., 2007). Tiger sharks also use a variety of depths from shallow, coastal habitats to deep, pelagic waters (Heithaus et al., 2007). When tiger sharks use coastal waters (Heithaus et al., 2007), they may be subjected to nearby anthropogenic activities (Dulvy et al., 2014), including anthropogenic debris from urbanization (Dulvy et al., 2014) and fishing activity (Randall, 1992). The broad home range of tiger sharks likely puts them at risk for exposure to a variety of types of anthropogenic debris originating from several sources. In our study, we observed a variety of anthropogenic particle morphologies, colours, and polymer types. Anthropogenic particles are derived from a diverse contaminant suite (Rochman et al., 2019), and this is reflected in the diversity of particles observed here. Fragments were most common and are a broad morphology including fragments of potential commercial origin (Helm, 2017), secondary microplastics resulting from the breakdown of larger items (Rochman et al., 2019), and road runoff (Mani et al., 2015). Black rubbery fragments were observed in several shark stomachs and are likely the result of tire wear (Sommer et al., 2018; Sutton et al., 2019). Notably, tire wear particles were found in high abundances in

Fig. 4. A) The overall distribution of anthropogenic particles by material type, excluding GC-71 (spiral valve), and B) the distribution of polymer types for materials categorized as ‘plastic’ for each stomach and spiral valve and the total distribution for all stomachs and spiral valve.
Charleston Harbor Estuary tributaries (Leads & Weinstein, 2019), relatively near the locations where some of our sharks were caught. Fibers were observed in high proportions and are likely from textiles (Rochman et al., 2019), wastewater (Gribić et al., 2020), and/or fishing gear (Randall, 1992; Rochman et al., 2019; Parton et al., 2019). The range of polymers detected in our study also indicates many potential sources of exposure, especially considering the many aggregations of anthropogenic debris a single shark may encounter across its broad home range. Another reason for higher anthropogenic particle contamination in our study may be due to the evolution of methods and greater attention paid to smaller plastic debris over time. Methodology for sample processing to extract anthropogenic particles has advanced greatly over recent years (Lusher et al., 2020b), allowing the detection of increasingly smaller particles and chemical digestion of complex matrices. For example, most of the studies (23 of 32) in our literature review did not remove organic material during sample processing. The presence of organic material increases the difficulty in detecting anthropogenic particles/items (Lusher et al., 2020b). Studies where organic material was not digested likely faced challenges in quantifying anthropogenic particles, especially within the microscopic size range. Ten of 32 studies in our literature review relied on visual identification alone to detect anthropogenic particles/items, three of which reported particles within the microscopic size range. However, visual identification alone is not sufficient for detecting most microplastics (Lusher et al., 2020a).

Literature in the field of microplastic ingestion is trending toward better detection of minimum particle sizes (Gouin, 2020) and anthropogenic particle counts typically increase with decreasing particle sizes (Koelmans et al., 2022). It is difficult to compare anthropogenic particle contamination by ingestion among studies with varying minimum particle sizes. This variation in minimum particle sizes in literature (as reported in our literature review) likely contributes to the lack of anthropogenic particle contamination observed in many samples where only non-microscopic anthropogenic particles were considered (eight of 32 studies). Of the remaining studies, eight of 24 studies only detected particles >1 mm, and an additional three studies did not report any means of quantifying the minimum limits of detection beyond reporting the use of microscopes. The high microplastic ingestion we observed in the tiger shark stomachs and spiral valve may be the result of lower detection limits. Despite the improved methodology we utilized, anthropogenic particle contamination was likely an underestimate of the total particle contamination in the 45-125 μm size fraction as only the floating portion of the density separation was quantified, and we observed anthropogenic particles in the lower portion of the density separation for larger size fractions. To capture a larger portion of denser particles, use of a denser salt solution for density separations may improve detection ability (Lusher et al., 2020b). The use of improved methodologies for detecting microscopic anthropogenic particles is recommended in future studies to quantify particles more accurately.

Based on our results, future studies should consider any potential health and/or physiological risk to individual tiger sharks. Effects of anthropogenic particle ingestion vary across aquatic species and life stages (Foley et al., 2018; Bucci et al., 2020), and there is scant data on how this is reflected in elasmobranch species. To the best of our knowledge, this is the first study to quantify and characterize microplastics and other anthropogenic particles in the tiger shark. Our findings are valuable to the scientific community because the tiger shark is a circumboreal species and apex predator. The relatively high contaminant levels we observed suggest high exposure to microplastics and other anthropogenic particles in this near threatened species. This warrants further understanding about risk, i.e., exposure and effect in this species and other elasmobranchs. Since the tiger shark has a long life span (Randall, 1992; Wosnick et al., 2020), if anthropogenic particles are retained, they may persist in the body of sharks for a long time. Retained and/or accumulated anthropogenic particles/items may result in false satiation (Koelmans et al., 2022), potentially interfering with the urge to feed. However, sharks are also able to evert their stomachs (Brunschweiler et al., 2005), which may provide a mechanism by which they could rid themselves of accumulated particles. Nevertheless, if microplastic particles translocate beyond the gut, they may lead to other mechanisms of toxicity (McIvor et al., 2021; Mehinto et al., 2022). To avoid sampling sharks for this purpose only, fishing tournaments can be used as opportunities to collect stomachs and other organs. Moreover, if translocation occurs, non-lethal methods of sampling to detect microplastics should be investigated (e.g., sampling in blood) to increase sample availability for monitoring and reduce potential harm to this near-threatened species. More work is needed to understand the contamination and biological fate of microplastics in elasmobranchs. Given the feeding behaviour, biogeography, and relatively long life span of the tiger shark, this species may be considered to monitor microplastics in the future.

8. Conclusions

This study presents relatively high contamination of microplastics and other anthropogenic particles in the GI tracts of tiger sharks that exceed levels of contamination observed in other elasmobranchs. Currently, it is unknown whether these ingested and/or retained microplastics cause harm in the tiger shark, and the severity of effects they may have. Future work is needed to understand the potential effects of microplastic ingestion in the tiger shark and other elasmobranchs, as well as the fate and transport (i.e., toxicokinetics) of microplastics in individual animals.

CRediT authorship contribution statement

**Keenan Munno**: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing – original draft, Writing – review & editing. **Lisa Hoopes**: Conceptualization, Funding acquisition, Investigation, Methodology, Resources, Supervision, Writing – review & editing, Data curation, Project administration. **Kady Lyons**: Conceptualization, Data curation, Investigation, Methodology, Project administration, Resources, Writing – original draft, Writing – review & editing. **Marcus Drymon**: Investigation, Methodology, Writing – review & editing, Resources. **Bryan Frazier**: Investigation, Methodology, Resources, Writing – review & editing. **Chelsea M. Rochman**: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Raw data are available at https://doi.org/10.5683/SP3/EKQIMX.

Acknowledgements

The authors thank L. Natanson (NOAA/NMFS) for sampling collection and donation and A. Galloway (SCDNR) for assistance in fishing expeditions.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2023.123185.