PRESENCE OF PLASTICS IN THE GASTROINTESTINAL TRACT OF SHARKS IN TEXAS BAYS

by

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DEDICATION

I would like to dedicate this to all the wonderful, supportive friends and family that helped me throughout my life.

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LIST OF ABBREVIATIONS

Abbreviation	Description
ANOVA	Analysis of variance
ATR	Attenuated total reflection
BPA	Bisphenol A
Cd	Cadmium
Co	Cobalt
Cr	Chromium
Cu	Copper
FTIR	Fourier transform infrared spectroscopy
Hg	Mercury
KOH	Potassium hydroxide
Ni	Nickel
Pb	Lead
PCB	Polychlorinated biphenyl
PE	Polyethylene
POP	Persistent organic pollutant
PS	Polystyrene
PVC	Polyvinyl chloride
TPWD	Texas Parks and Wildlife Department
YOY	Young-of-the-Year

ABSTRACT

Plastics in the marine environment have become an important topic in the recent decade due to its ubiquitous presence, long lasting impacts, and detrimental health effects. Plastics have been observed affecting marine organisms by causing gastrointestinal or respiratory blockages or tears. Other consequences of plastic ingestion include exposure to endocrine disrupting compounds, persistent organic pollutants, and toxic trace metals. Few studies have investigated the presence of plastics in shark gastrointestinal tracts, and nothing is known for sharks in Texas bays. This study assessed the presence, abundance, and type of plastics present in the gastrointestinal tracts (cardiac stomach, pyloric stomach, and spiral valve intestine) of three shark species [blacktip shark (*Carcharhinus limbatus*), bonnethead shark (*Sphyrna tiburo)*, and bull shark (*Carcharhinus leucas*)] in four bays (Sabine Lake, Aransas Bay, Corpus Christi Bay, and Lower Laguna Madre) along the Texas coast using a stereomicroscope and Fourier transform infrared spectroscopy (FTIR). Young-of-the-year, juvenile, and adult individuals were examined in this study. Of the total number of sharks examined (n = 240) only eight individuals (two blacktip sharks, two bonnethead sharks, and four bull sharks) were found to contain plastic pieces, and a total of nine pieces of plastic were found. Each plastic piece was found in the cardiac stomach. Monofilament fishing line and hooks were commonly found, meaning that fishing practices, not plastic ingestion through diet is the main problem. The most frequently found polymers were polyethylene (PE) and nylon 6. Due to the low number of plastics found in these sharks, plastics do not appear to pose a threat to these species in these bay systems, however, future studies should include smaller fibers which were omitted from this study and investigate nanoplastics which could cross the gastrointestinal tract lining and be remobilized around the body.

I. INTRODUCTION

Marine Plastics Overview

It is estimated that by 2025, the mass ratio of plastics to fish in the ocean will be 1:3, and by 2050 the amount of plastics in the marine environment will equal or surpass fish (Jovanovic, 2017). Plastic items >5 mm (macroplastics), are responsible for millions of metric tons of waste in marine ecosystems (Ghaffar et al., 2022). These items, composed of long-chain polymers, include plastics such as polyvinyl chloride (PVC) and polyethylene (PE) and their numbers have increased exponentially in global marine ecosystems since 1980 (Bellou et al., 2021). After their introduction to the marine environment, macroplastics break down into progressively smaller fragments, and as a result remain in the ecosystem for hundreds to thousands of years, becoming micro- (<5 mm) and nano-plastic (<1 µm) particles (Barnes et al., 2009). Microplastics are a globally pervasive pollutant that entered the marine environment at a rapid rate, similar to their level of production since the 1950s (Moore, 2008). Between 1970 and 2003, plastics became the fastest growing portion of municipal waste generated in the U.S., and marine litter is now composed of up to 95% plastic in some areas (Moore, 2008; Meyer et al., 2023).

Microplastics are categorized into two types - primary and secondary. Primary microplastics are small plastics that are produced at <5 mm in size. One form is pre-production resin pellets (nurdles) that are used to manufacture plastic products, as well as microbeads used in cosmetic products, glitter for cosmetics and confetti, and powder for industrial uses (Moore, 2008). Secondary plastics are fragments of plastics <5 mm in size generated when macroplastics break down in the environment by UV exposure, oxidation, hydrolysis, or mechanical processes such as wave action (Moore, 2008). These plastics can be classified as fragments - pieces broken from larger objects, fibers - threadlike strands of plastics, film - sheet like pieces of plastic such as cellophane, and pellets - nurdles and pre-production resin beads as well as Styrofoam (Moore, 2008; Andrady, 2011).

Nanoplastics, (particles <1 μm) can also be formed by either primary or secondary means, but the resulting products frequently possess vastly different properties than microplastics. They are often highly heterogeneous in size and have a much greater tendency to further degrade into smaller particles when compared to microplastics (Gigualt et al., 2021). Although nanoplastic research is an emerging field, it is known that their small size and unique properties, such as interaction with light, bioavailability, and transport properties pose a threat to biological organisms (Hazeem et al., 2020; Allen, et al., 2022). Nanoplastic particles can both quickly access the bloodstream through ingestion and cross the blood-brain barrier; they have been found in the egg yolk, gut, swim bladder, eyes, and brain of fish under laboratory conditions, resulting in DNA damage, cytotoxicity, and developmental issues (Zhang et al., 2020; Gupta et al., 2022).

About 80% of plastic waste found in the marine environment comes from land-based sources including beach litter and inland waste that enters rivers, while the fishing industry accounts for roughly 18% of marine plastic debris (Andrady, 2011). The most common plastic polymer in production and observed in the marine environment is PE, at ~30% and ~60%, respectively (Andrady, 2011; Nerland et al., 2014; Schwarz et al, 2019).

Impacts of Plastics on Fish Health

Microplastics have been found in the gastrointestinal tract of marine teleost (bony) fishes worldwide, including in species that are commercially and recreationally fished for human consumption. For example, microplastics were found in the guts of herring (*Clupea sp.*), whiting

(*Merlangius merlangus*), horse mackerel (*Trachurus trachurus*), haddock (*Melanogrammus aeglefinus*), and cod (*Gadus morhua*) in the North Sea (Foekema et al., 2013). A review summarizing previous studies on plastic ingestion by fish has found plastics present in fishes from the North Pacific Gyre, English Channel, Baltic Sea, Gulf of Mexico, Australia, Southern Ocean, South Africa, Tokyo Bay, North Atlantic Ocean, Norwegian coast, Coast of Portugal, Adriatic Sea, and Mediterranean Sea (Jovanovic, 2017). In the northwestern Gulf of Mexico, a study found plastic pieces present in fishes collected from Texas harbors, bays, and offshore habitats (Phillips and Bonner, 2015).

Plastics are ingested by fish through either direct consumption of plastic fragments or when a fish consumes a lower trophic level prey item that has consumed and accumulated plastics in their gastrointestinal tract or gills. Ingestion through either means can cause damage to the fish in various ways. Plastics can accumulate in the gastrointestinal tract of these organisms causing blockages resulting in a false sense of fullness that can lead to starvation, or lesions and tears that can prove to be fatal (Alimba and Faggio, 2019). Alternately, nanoplastics may be absorbed through the gastrointestinal lining, threatening survival by relocating to other areas of the body (Jovanovic, 2017; Mallik et al., 2021).

The effects of plastic exposure have been observed in teleost fishes, which may be similar in elasmobranchs (sharks, skates, and rays). Microplastics can affect teleost fish by altering their behavior, causing histopathological alterations in the intestine, changes in lipid metabolism, penetration of intestinal lining, mechanical injuries, and ulcerations in the gastrointestinal tract, and microplastics can also become translocated into the liver (Jovanovic, 2017; Pannetier et al., 2020; Zhang et al., 2020). A lab-based study that exposed estuarine barehead glassfish (*Ambassis dussumieri)* to a mixture of PE, PVC, and polystyrene (PS) particles, observed a decrease in growth and survival rates in the presence of plastics (Naidoo and Glassom, 2019). Research on plastic toxicity in teleost fish has shown that exposure to PE and PVC caused decreased reproductive output of 31.1% and 50%, respectively, compared to control groups in the freshwater zebrafish (*Danio rerio*) (Cormier et al., 2021).

Microplastics also contain biologically disruptive compounds from the manufacturing process such as bisphenol A (BPA), phthalates, coupling agents, plasticizers, lubricants, and flame retardants that may leach out once ingested (Moore, 2008; Jovanovic, 2017). Microplastics can also sorb harmful compounds found in the environment including persistent organic pollutants (POPs), polychlorinated biphenyls (PCBs), and other hydrophobic pollutants, as well as trace metals such as cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), mercury (Hg), nickel (Ni), and lead (Pb) (Moore, 2008; Andrady, 2011; Holmes et al., 2014). Due to the hydrophobic nature of these compounds and affinity of trace metals to plastics, they are readily bound to microplastics at a level that is several orders of magnitude higher than what is found in seawater (Andrady, 2011). Upon ingestion, these microplastics can act as a vector for harmful contaminants to enter the marine food web, and bioaccumulate in organisms resulting in genotoxic, neurotoxic, and endocrine disruptive effects as well as reduced reproductive success (Andrady, 2011; Cormier et al., 2021).

Plastics in Sharks

The presence of plastics in sharks and resulting health effects are difficult to determine because sharks are challenging to study in a laboratory setting due to their large size, long lifespans, amount of space required to hold sharks in captivity, and are not easy to handle. However, research on the presence of microplastics in sharks have been increasing in the recent decades. Microplastics have been globally found in coastal, pelagic, and deep-water shark species. Microplastics have been found in three deep-water elasmobranchs in the Tyrrhenian Sea: the blackmouth catshark (*Galeus melastomus*; n = 32), lesser-spotted dogfish (*Scyliorhinus canicula*; n = 30), and velvet belly dogfish (*Etmopterus spinax*; n = 34) with a total of 512 plastics identified among all species (Valente et al., 2019). The diet of the velvet belly dogfish is mostly pelagic while the blackmouth catshark and lesser-spotted dogfish are mainly benthic feeders, but all species were found to contain microplastics (Valente et al., 2019). Studies conducted in the North Atlantic and South Pacific found plastic present in stomachs of the surface-dwelling blue shark (*Prionace glauca*; n = 96) with three plastic pieces found, and shortfin mako shark (*Isurus oxyrinchus*; n = 396) with 12 plastic pieces identified (Fernandez and Anastasopoulou, 2019; Mucientes and Queiroz, 2019). Another study in the northeast coast of Taiwan found plastics present in the stomachs of shortfin mako sharks (n = 20) with two plastic pieces found (Hsu et al., 2021). A study investigating microplastics in demersal sharks in the northeast Atlantic also found plastic particles in the small-spotted catshark (*Scyliorhinus canicula*; n = 12), starry smooth-hound (*Mustelus asterias*; n = 12), spiny dogfish (*Squalus acanthias*; n = 12), and bull huss (*Scyliorhinus stellaris*; n = 10) with a total of 379 plastics found in the gastrointestinal tract of these four species (Parton et al., 2020).

Ingestion of microplastics is not exclusive to predatory shark species, having been overserved in filter feeding shark species as well. Whale sharks (*Rhinocodon typus*) and basking sharks (*Cetorhinus maximus*) are filter feeding shark species that primarily consume plankton. Information regarding microplastic consumption on filter feeding elasmobranch species is scarce due to the protected status of these species, but occasional strandings can provide valuable information on these species to researchers. A stranded whale shark in the Philippines yielded an opportunity for researchers, finding plastics in both the gastrointestinal tract and gills (Abreo et al., 2019). Another instance of a stranded juvenile whale shark located on the Brazilian coast of Bahia in 2008 revealed plastic debris located in the stomach of the individual, with speculation that the plastic could have been contributing to the stranding (Sampaio et al., 2018). Theoretical estimates regarding rates of microplastic ingestion by filter feeding elasmobranchs have been made as well by comparing microplastic numbers in ocean water samples where these species frequent to the amount of water these species filter hourly (Germanov et al., 2019). Using this method, whale sharks theoretically ingest 22.8 to 136.9 microplastics per hour depending on study location (Germanov et al., 2019).

Plastics in Texas Marine Fishes

Few studies have investigated plastics in the gastrointestinal tract of marine teleost fishes in Texas bays (Phillips and Bonner, 2015; Peters et al., 2017; Hajovsky, 2019; DuBois et al., 2021), even though some parts of the coast are heavily industrialized and urbanized. To my knowledge, there are currently no published studies that have examined the presence of plastics in the gastrointestinal tracts of sharks in Texas bays. Thus, this thesis research is warranted to fill this information gap because sharks are important species for the marine ecosystem. Many shark species are keystone species that regulate the populations of other organisms at lower trophic levels which humans rely on for food and as an economic resource (Leigh et al., 2017). Additionally, sharks can serve as an indicator species for contaminant levels present within both the marine environment and lower trophic levels. While filter-feeding sharks can provide a direct indication of the environmental levels of plastics, predatory sharks can provide important information about the bioaccumulation of plastics and toxic contaminants derived from predation of species in lower trophic levels. This is an important factor in assessing human health risk, as many of the fish sharks choose to prey upon are commercially sold for human consumption (Fossi et al., 2014; Goyanna et al., 2023).

Shark Gastrointestinal Tracts

The gastrointestinal tract of sharks is classified into three sections: the cardiac stomach, the pyloric stomach, and the spiral intestine (Figure 1). The cardiac stomach is the first stomach that prey enters upon ingestion. Comprised of thick folds, it is both muscular and stretchy, allowing large quantities of food to be consumed, agitated, and broken down by digestive fluids and enzymes (Crow et al., 1990). The pyloric stomach which is involved in water, urea, and ion transport (Crow et al., 1990; Liew et al., 2013) resembles a stout tube; it can be traced from the posterior end of the cardiac stomach to the anterior end of the spiral intestine and is as long or longer than the cardiac stomach. This portion of the stomach terminates in the pyloric valve, a muscular sphincter which allows for the passage of partially digested material into the intestines (Crow et al., 1990). The spiral intestine extends posteriorly from the pyloric stomach, ending at the rectum (Crow et al., 1990). The spiral intestine contains internal tissue folds to provide a large surface area to slow the passage of food and aid in nutrient absorption (Crow et al., 1990; Leigh et al., 2017). The fact that shark spiral intestines contain these tissue folds may increase the likelihood of plastics becoming lodged in this section of gastrointestinal tract.

Species Investigated

This study investigated the presence of plastic in the gastrointestinal tract of blacktip sharks (*Carcharhinus limbatus*), bonnethead sharks (*Sphyrna tiburo*), and bull sharks

(*Carcharhinus leucas*) in Texas bays. Most individuals were classified as young-of-the-year (YOY) or juvenile, however some adults were included. Texas bays are an important nursery habitat for these shark species, which have varying diets, thus offering the opportunity to estimate levels of microplastic consumption by a vast array of species at lower trophic levels (Cortez et al., 1996; Bethea et al., 2007; Froeschke et al., 2010; Matich et al., 2020; Matich et al., 2021).

The blacktip shark is a cosmopolitan elasmobranch species found globally in tropical and subtropical marine waters (Castro, 1996). This species has been found entangled in plastic debris as well as ingesting plastic particles (Cliff et al., 2002). Males typically mature at a length of 140 cm to 150 cm total length (TL) and age of about 4.5 years, while females mature at about 155 cm TL and age about 5.7 years (Castro, 1996; Carlson et al., 2006). Blacktip sharks are a placental viviparous species with a gestation period of 12 months, litter sizes ranging from 1 to 13 pups, and average length at birth ranging from 55 cm to 60 cm TL (Castro, 1996; Capape et al., 2004; Carlson et al., 2006). Prior work has shown some variation in the size classes of juvenile blacktip shark in Texas bays due to temporal differences in birth, but it has been determined that individuals less than 71 cm TL are YOY, while individuals ranging from 71 cm to 110 cm TL are considered juveniles (Matich et al., 2021). A study conducted on blacktip shark diet from San Antonio Bay and Galveston Bay, Texas found that this species feeds on Atlantic croaker (*Micropogonias undulatus*), sand seatrout (*Cynoscion arenarius*), ariidae, tarpon (*Megalops atlanticus*), flounder (*Paralichthys spp*), clupeids, white and striped mullet (*Mugil spp*), and red drum (*Sciaenops ocellatus*) (Matich et al., 2020).

Bonnethead sharks inhabit nearshore tropical and subtropical marine waters of the western Atlantic Ocean from North Carolina to southern Brazil, including the Gulf of Mexico and Caribbean Sea, and the eastern Pacific Ocean from southern California to Ecuador (De Acevedo et al., 2020). There are currently no published studies regarding plastic entanglement or ingestion by bonnethead sharks. Age and size at which this species matures ranges greatly depending on latitudinal distribution, males have been observed reaching sexual maturity at 1.6 to 3.0+ years, and between 65 cm to 83 cm TL, and females can mature from 2.9 years to 4.0+ years of age and between 77 cm to 94 cm TL (Lombardi-Carlson et al., 2003). Bonnethead sharks are a placental viviparous species with a rapid gestation period of 4.5 to 5 months, litter sizes ranging from 1 to 13 pups, and average length at birth ranging from 21.5 cm to 34.7 cm TL (Manire et al., 1995; Lombardi-Carlson et al., 2003; De Acevedo et al., 2020; Palacios-Hernandez et al., 2020). In the northern Gulf of Mexico, bonnethead sharks smaller than 50 cm TL are considered YOY and individuals ranging from 50 cm to 70 cm TL are considered juveniles (Carlson and Parsons, 1997). The diet of bonnethead sharks primarily consists of crustaceans, particularly blue crabs (*Callinectes sapidus*) but also includes seagrass, mollusks, cephalopods, teleost fish, and other small organisms (Cortez et al., 1996; Bethea et al., 2007). A dietary study on individuals collected from Matagorda Bay and Galveston Bay, Texas found their prey consisted of more than 90% crustaceans (Harrington et al., 2016).

Bull sharks are a coastal, estuarine, riverine, and lacustrine species that can be found globally in tropical and subtropical waters (Cruz-Martinez et al., 2004). There is currently no literature available on the ingestion of plastics in bull sharks, but it has been observed that this species is susceptible to entanglement in plastic debris (Cliff et al., 2002; Brunnschweiler and Marosi, 2020). The age and length at which male bull sharks reach sexual maturity is 9 to 10 years and 190 cm to 234 cm TL, while females tend to reach sexual maturity at an age of 10 years and a TL of 204 cm to 257 cm (Cruz-Martinez et al., 2004; Pirog et al., 2019). Bull sharks are a placental viviparous species with a gestation period of 12 months, a litter size ranging from 5 to 14 pups, and average size at birth ranging from 60 cm to 80 cm TL (Chen et al., 2014; Pirog et al., 2019). Previous work conducted on bull sharks in Texas classified individuals less than 90 cm TL as YOY and individuals between 90 cm and 160 cm TL as juvenile (Froeschke et al., 2010). A study of juvenile bull shark stomach contents collected in San Antonio Bay, Texas found a diet consisting of white and brown shrimp (*Litopenaeus spp*), striped and white mullet, and sheepshead minnows (*Cyprinodon variegatus*) (Matich et al., 2021).

Study Area

This thesis examined plastics in the gastrointestinal tracts of sharks from Sabine Lake on the upper Texas coast, Aransas Bay and Corpus Christi Bay on the mid-coast, and the Lower Laguna Madre on the lower Texas coast (Figure 2). These locations were chosen because they provide spatial variability in human population and industry that contribute to plastic pollution to determine if sharks in one region are at a greater risk of plastic exposure than other areas, as well as variation in freshwater inflows from rivers, as rivers may act as a source for plastic pollution to enter these bay systems (Shruti et al., 2021).

Coastal bays and estuaries have long been recognized as important nursery areas for a variety of species, including sharks (Froeschke et al., 2010). A study utilizing long-term fisheries independent gill net survey data collected from 1975 to 2006 showed that the central region of the Texas coast contains the most important habitat for these three shark species (Froeschke et al., 2010). The same study also explained that juvenile sharks of all three species were frequently captured in this area, suggesting the Texas coast may be an important nursery area (Froeschke et al., 2010).

Sabine Lake is a shallow estuary located on the border of southeast Texas and southwest Louisiana averaging 1.8 m in depth and an average salinity of 2.3 ppt (Ravichandran et al., 1995). Two rivers flow into Sabine Lake- the Neches River which flows through larger cities such as Beaumont and Port Arthur, as well as smaller towns and cities upstream, and the Sabine River which flows through the town of Orange, and other smaller towns; both rivers could potentially transport plastics into Sabine Lake (He et al., 2021; Kunz et. al, 2023).

Aransas Bay, located mid-way along the Texas Coast, is about 14 miles long and 4 miles wide, with its greatest water depth of about 4 m and a salinity ranging from 6.2 ppt in Copano Bay that feeds into Aransas Bay, to 33.5 ppt in Aransas Bay (Norris, 1953; Froeschke et al., 2013). Aransas Bay is bordered on its southeast side by San Jose Island and Copano Bay and the Texas mainland on its northwest. The Aransas and Mission Rivers flow into Copano Bay, which then flows into Aransas Bay. The Mission River flows along the outskirts of the town, Refugio, which may contribute to plastics entering Aransas Bay. The main cities located directly along Aransas Bay are Rockport and Fulton, each being a tourist destination for anglers and beachgoers that add to the plastic litter in this marine environment.

Corpus Christi Bay is also located mid-way along the Texas Coast and is south of Aransas Bay, bordered on its southeast side by Mustang Island and the Texas mainland on its north and west. Corpus Christi Bay is a flat, shallow bay with depth ranging from 3 to 4 m, and a salinity ranging from 26 ppt to 37 ppt (Applebaum et al., 2005; Islam et al., 2014). The Nueces River empties into Nueces Bay, which then flows into Corpus Christi Bay. The Nueces River and Nueces Bay flow past industrial areas that likely contribute to the presence of plastics in Corpus Christi Bay. On the west side of Corpus Christi Bay is the large city of Corpus Christi, a large destination for beachgoers and anglers that contribute plastic litter to the area that can enter the bay.

The Lower Laguna Madre, the most southern collection site in this study, is located near the Texas and Mexico border at Port Isabel, extending north to the Port Mansfield Channel. This large, hypersaline, shallow bay system has an average depth of < 1 m and an average salinity > 35 ppt, reaching 55 ppt in some areas (McMahan, 1968; Mitchell, 1992). South Padre Island borders the eastern side of the bay, and the Texas mainland on the western border. There are no freshwater drainages of significance flowing into the Lower Laguna Madre, and few large cities are located along this bay, the exceptions being the city of South Padre Island and Port Isabel at the bay's southern border, and Port Mansfield on its northern border (McMahan, 1968). While it has been shown that transport of trace elements can be detected from estuaries of the Rio Grande, these appear to contribute little to the overall quality of the marine environment of the Lower Laguna Madre (Whelan et al., 2005).

Study Objectives

The overall goal of this study was to determine the presence, abundance, and type of plastics in the gastrointestinal tract of YOY, juvenile, and adult sharks in four bay systems along the Texas coast. This goal can be broken down into five objectives:

1. Investigate the intra- and interspecies variability in the prevalence of plastics within and among sites, with the prediction that bull sharks from Corpus Christi Bay will have the highest frequency of plastics.

   Rationale: Direct and indirect human interactions due to recreational and commercial fishing, a large human population center (Corpus Christi) and industry, combined with a high density of immature bull sharks as a result of the nursery habitat created by the Nueces River estuary provides an increased likelihood of finding plastics in the gastrointestinal tract of this species (Charles, 2005; Froeschke et al., 2010; Landon et al., 2012; Laurrabaquio-A et al., 2019; Tinhan and Wells, 2021).

2. Determine which section of gastrointestinal tract (cardiac stomach, pyloric stomach, spiral valve intestine) will have the most plastics present among all species, with the prediction that the most plastics will be found in the spiral valve intestine.

   Rationale: The presence of internal tissue folds in the spiral valve intestine could increase the likelihood of plastic pieces becoming lodged (Crow et al., 1990; Leigh et al., 2017).

3. Determine if there is more plastics present in YOY, juvenile, or adult sharks, with the prediction that there will be more plastics found in juveniles.

   Rationale: Texas bays provide ideal nursery habitats for immature sharks and are in close proximity to human activity, whereas adult sharks tend to frequent these areas less. Both YOY and juvenile sharks tend to stay within the bays, however, the diet of juvenile sharks increases the chances of plastic ingestion due to consuming a wider variety of larger prey items (Pawar et al., 2016; Laurrabaquio-A et al., 2019).

4. Determine the most frequently found type, color, and size of plastics present in sharks, with the prediction that colorless (clear) fragments <5 mm will be the most frequently found.

   Rationale: Most of the plastic in the marine environment is categorized as clear or colorless, thus increasing the potential of ingestion regardless of the origin of introduction to the local environment (Moore, 2008; Barnes et al., 2009; Meyer et al., 2023).

5. Determine the polymer composition of plastics found in the greatest abundance, with the prediction that polyethylene will be the most frequently found.

   Rationale: Polyethylene is the most commonly produced plastic and represents a majority of plastic in the marine environment (Andrady, 2011; Bellou et al., 2021; Shruti et al., 2021).

II. METHODS

Sample Collection

Texas State University partnered with the Texas Parks and Wildlife Department (TPWD) Coastal Fisheries Division to collect sharks. Young-of-the-year, juvenile, and adult sharks were collected by TPWD during 10-week gill net abundance surveys in fall 2020, spring 2021, fall 2021, and spring 2022. These nets were set in various locations throughout each bay (Figure 2) for between 9 and 14 hours. Upon retrieval of the nets, sharks that were deceased were collected and stored at -20°C at TPWD offices located along these bays [Port Arthur (Sabine Lake), Rockport (Aransas Bay and Corpus Christi Bay), and Brownsville (Lower Laguna Madre)] until they were transported to Texas State University and stored at -20°C until further processing.

Dissection Process

All sharks were thawed for 24 to 48 hours prior to dissection. Morphometric measurements including TL (cm) to identify the life stage of the individual, weight (kg), and sex were taken prior to dissection (Table 1). The ventral side of the body from the cloaca to the heart was cut open to access the gastrointestinal tract which was then removed. Cotton twine was used to tie the cardiac stomach, pyloric stomach, and spiral valve intestine at their anterior and posterior ends to keep contents in situ (Figure 1). The gastrointestinal tract was then placed in a labeled plastic bag and held at -20°C until further processing.

Dietary Analysis and Plastic Extraction

Each gastrointestinal tract was thawed at 4°C for 24 to 48 hours prior to processing. Each of the three sections of gastrointestinal tract were separated using stainless steel scissors rinsed with Milli-Q water (MilliporeSigma, Burlington, MA), and the length of each section (cm) recorded (Appendix A). To determine the weight of the contents in each section of the gastrointestinal tract, each section was weighed before and after the contents of each section were emptied into beakers or petri dishes (Appendix A).

Contents of the cardiac stomach were examined to note the diet of each shark species. Pyloric stomach contents were also initially examined for diet, but contents were too digested to identify, so only cardiac stomach contents were used. Prey items that had been ingested and found intact were recorded to species, while prey items that could not be identified to species were listed to taxa.

Contents of each section were visually inspected for suspected plastics using a stereomicroscope (Nikon SMZ 745 10X magnification; Nikon Corporation, Tokyo, Japan) or magnifying glass on a stand (AORAEM 2.5X-10X magnification; Haifeihua Trading Limited, Shenzhen, China). Initially, the contents of the gastrointestinal tract were chemically digested to dissolve organic matter while leaving plastics intact, making it easier to identify suspected plastic particles. This digestion process was done when the gastrointestinal tract was very large or very full of material. To dissolve the organic materials from the gastrointestinal tract contents, the digestion procedures from Foekema et al. (2013), Deahut et al (2016), and Atamanalp et al. (2022), were adopted: beakers containing either the contents of the cardiac stomach, pyloric stomach, or spiral valve intestine were filled with a 9% or 18% potassium hydroxide (KOH; Sigma-Aldrich reagent grade 90% flakes; Sigma-Aldrich, St. Louis, MO) solution at least 3-times the volume of the organic material in the beakers, covered with aluminum foil, and heated at 60°C for 72 hours on hot plates that were placed in a fume hood (Figure 3). Some of the beakers containing the contents of the cardiac stomach followed the same procedure but were filled with a pre-made stock solution of 30% hydrogen peroxide (Fisher BioReagents 30% Hydrogen Peroxide in Water; Thermo Fisher Scientific, Waltham, MA.) (Foekema et al., 2013; Dehaut et al., 2016; Atamanalp et al., 2022). Following KOH or hydrogen peroxide digestion, contents of the beakers were incrementally placed into petri dishes and visually inspected for the presence of plastics under stereomicroscope or magnifying glass (Foekema et al., 2013). A 72-hour digestion process, in combination with visual inspection was initially used but produced little to no discernible digestion of organic matter. Potassium hydroxide digestions did not break down organic material in the sample enough to be a viable option (Figure 3). While hydrogen peroxide digestions bleached the organic material a white color, making visual inspection and plastic extraction difficult. After multiple failed digestion attempts, a visual inspection of gastrointestinal tract contents diluted with Milli-Q water was used. Suspected plastics were extracted from petri dishes with stainless steel tweezers or fine tipped paint brushes and stored in labeled glass vials for further processing.

Plastic Data Collection

Suspected plastics were extracted, photographed, and categorized by type (fragment, fiber, film, or pellet), size (length and width in mm), clarity (opaque, translucent, or transparent), and color during visual inspection under a stereomicroscope (Leicia S9D 10X magnification; Leica Biosystems, Wetzlar, Germany) with mounted digital camera (Leica Flexacam C3; Leica Microsystems, Wetzlar, Germany), and measuring software (Leica Emspira-Flexacam). Monofilament fishing line fibers too large to be measured via microscope had length measurements taken with the use of a ruler to the nearest millimeter. The weight of each suspected plastic was obtained using a microbalance (Mettler-Toledo MX5; Mettler-Toledo, Columbus, OH). Small textile fibers were omitted from this study because samples were exposed to open air and could be contaminated by airborne fibers, however, if balls of fibers or large fibers were found they would be included.

Fourier transform infrared spectroscopy (FTIR) was used to analyze the suspected plastics using a polymer identification technique at the Shared Research Operations Center located at Texas State University, as described in Foekema et al., (2013), Roch and Brinker (2017) and Bessa et al., (2018). The FTIR spectrometer used was a Bruker Alpha II (Bruker Corporation, Billerica, MA) coupled with a Platinum-ATR module using a wavenumber range of 400 to 4000 cm^{-1}. The attenuated total reflection (ATR) module with diamond/germanium windows were cleaned and a background scan was performed prior to suspected plastics being scanned (Ibor et al., 2023). The FTIR produced an absorbance spectrum of each suspected plastic using 20 digitally combined scans using OPUS software, and polymer identification was completed using Open Specy online spectra library (https://openanalysis.org/openspecy/) (Roch and Brinker, 2017).

Statistical Analysis

To determine if there was a significant difference in the presence of plastics found in the cardiac stomach among life stages (YOY, juvenile, adult), a one-way analysis of variance (ANOVA) was performed, with the independent variable being life stage and the dependent variable being the number of individuals with plastic present in the gastrointestinal tract. The assumption of normality was analyzed using a Shapiro-Wilk test and equal variance using a Levine’s test. All statistical analysis was done using SigmaPlot version 14 (Systat Software Inc., San Jose, CA) and the alpha was set at 0.05.

III. RESULTS

Of the total number of sharks investigated in this study (n = 240), blacktip sharks accounted for 25% (n = 60) of individuals examined, bonnethead sharks for 36% (n = 87), and bull sharks for 39% (n = 93) (Table 1). When broken down by location, 20% (n = 49) of the sharks were from Sabine Lake, 26% (n = 62) from Aransas Bay, 32% (n =76) from Corpus Christi Bay, and 22% (n = 53) from the Lower Laguna Madre (Table 1).

Dietary Habits

Based on occurrence, results of the dietary analysis showed that for each species, their diet was similar across all locations (Table 2). Across all sites, prey items identified to species that were found in blacktip shark cardiac stomachs included gulf menhaden (*Brevoortia patronus*), hardhead catfish (*Arius felis*), striped mullet (*Mugil Cephalus*), sheepshead (*Archosargus probatocephalus*), and brief squid (*Loligo brevis*). From inspection of the cardiac stomach in bonnethead shark in all sampling locations, the species found to be ingested were blue crab (*Callinectes sapidus*), mantis shrimp (*Squilla empusa*), brief squid, and shrimp eel (*Ophichthus gomesii*). Bull shark cardiac stomach contents from all sampling locations contained Atlantic croaker, gulf menhaden, gulf toadfish (*Opsanus beta*), hardhead catfish, largemouth bass (*Micropterus salmoides*), striped mullet, pinfish (*Lagodon rhomboids*), sheepshead, spotted seatrout (*Cynoscion nebulosus*), blue crab, and southern stingray (*Hypanus americanus*).

Across all sites, blacktip sharks and bull sharks mainly ingested fish, whereas bonnethead sharks predominantly consumed crabs (Table 2). With respect to the number of sharks that contained identifiable prey items in their cardiac stomach at individual locations, in Sabine Lake where only bull sharks were examined, fish of any species were the main prey item consumed with 95% of individuals containing fish in their cardiac stomach. Aransas Bay blacktip shark consumed only fish (100% of individuals), while bonnethead sharks consumed mainly crab (100% of individuals), and bull sharks consumed mainly fish (96% of individuals). Corpus Christi Bay blacktip sharks also consumed mostly fish (94% of individuals), bonnethead sharks appeared to favor seagrass (83% of individuals), and bull sharks mostly consumed fish (100% of individuals). Lower Laguna Madre blacktip sharks and bonnethead sharks consumed mostly fish (100% of individuals and seagrass (100% of individuals), respectively (Table 2).

Presence and Type of Plastics Found

For all bays combined, eight sharks (3.33% of total sharks examined) had identifiable plastic in their gastrointestinal tract (two blacktip sharks, two bonnethead sharks, and four bull sharks (Table 3). The location with the highest number of sharks that contained plastic was Aransas Bay ($n = 3$), followed by Corpus Christi Bay ($n = 2$) and Sabine Lake ($n = 2$), and the Lower Laguna Madre ($n = 1$) (Table 3).

All pieces of identified plastic ($n = 9$) were found in the cardiac stomach. Seven of the eight sharks were found to contain one piece of plastic in their cardiac stomach, except for one bonnethead shark from Corpus Christi Bay that contained two pieces of plastic (Table 3). For all species and sites combined, there was no significant difference in the number of sharks found with plastic in their cardiac stomach and life stage ($p > 0.05$); the highest occurrence of plastics was found in juveniles ($n = 4$), followed by YOY ($n = 3$), and adult ($n = 1$) (Table 4).

Plastics were classified by physical characteristics (fragment, film, fiber, or pellet), color, and clarity (transparent or opaque) (Figure 4-6; Table 5). Fibrous samples, including monofilament fishing line, were most common at a prevalence rate of 77.8% ($n = 7$) and the rest were fragments (22.2%; n = 2). Most plastic pieces were either colorless (clear) or green in color, both representing 33.3% of the total (n = 3 for each), followed by black (22.2%; n = 2), and yellow (11.2%; n = 1). Transparent pieces of plastic were most common at 66.67% (n = 6) and the remaining 33.33% were opaque (n = 3).

Samples identified in all species across all sites in this study were then further classified by polymer types using FTIR (Figures 7-10; Table 5). Polyethylene made up made up 33.3% (n = 3) of the plastic pieces found, nylon 6 also made up 33.3% (n = 3) of plastic pieces found, polypropylene made up 22.2% (n = 2), and poly(vinylidene fluoride) made up 11.2% (n = 1) (Figure 11; Table 5).

IV. DISCUSSION

This study was conducted to supplement current knowledge regarding the presence and abundance of plastics in Texas bays, as well as fill a knowledge gap by determining the prevalence and abundance of plastic in the gastrointestinal tract of three common shark species. This is unique, as prior studies conducted in Texas have only considered lower trophic level fishes that are typically regarded as prey items (Phillips and Bonner, 2015; Wang et al., 2020; Geist et al., 2021). The three shark species investigated here were chosen to compare and contrast gastrointestinal plastic accumulation by sharks among different bays, taking into consideration typical levels of potential plastic exposure due to human interaction, notably through recreational and commercial fishing. The three shark species investigated in this study encompassed an array of different dietary habits and behavioral patterns with respect to food consumption, such as feeding depth and method of prey capture (Harrington et al., 2016; Gardiner et al., 2017; Matich et al., 2020; Matich et al., 2021 Moyer et al, 2021). While studies have been conducted regarding the presence of plastics in sharks from other coastal areas throughout North America and Asia (Jovanovic, 2017; Parton et al., 2020), this is the first reported study for bays located specifically along the coastal region of Texas within the northern Gulf of Mexico. These areas are key in providing local and global revenue in the form of recreational and commercial fishing, creating a bustling economy that attracts both tourism and industry (Shepard et al. 2013; Murawski et al., 2019; Sammiappan et al., 2019; Swinea et al., 2021). As such, the presence of plastic in Texas bays and its marine life requires monitoring. Assessing the levels of plastics in sharks can help to better understand the composition and distribution of these pollutants within Texas bay ecosystems.

Prevalence of Plastics

While it was predicted that bull sharks from Corpus Christi Bay would have the greatest number of individuals containing plastic in their gastrointestinal tract (objective 1), due to the low sample size of sharks with plastic in their gastrointestinal tract this objective was unable to be addressed. However, across all sharks and locations sampled, bull sharks did contain the highest number of plastic pieces, with a rate of 4.08% (n = 2) in Sabine Lake and Aransas Bay in 5.72% (n = 2). Bonnethead and blacktip sharks followed with plastic pieces each. Bonnetheads with plastics were identified from Aransas Bay 5.56% (n = 1) and Corpus Christi Bay 2.78% (n = 1), while blacktips were found in Corpus Christi Bay 3.23% (n = 1) and Lower Laguna Madre 5.00% (n = 1). The size of particles assessed may have contributed to the low number of identified pieces. Including smaller, finer items such as clothing or textile fibers might increase overall plastic counts per sample. However, these measurements are similar to other studies; an examination of blue sharks from the South Pacific Ocean (n = 136) found one piece of plastic each in three individuals (2.2%), and a study of the small-spotted catshark (*Scyliorhinus canicula*) (n = 200) found 13 individuals (6.5%) containing plastics (Fernandez and Anastasopoulou, 2019; Morgan et al., 2021). Other studies involving filter feeding species such as whale sharks show a theorized ingestion rate of 22.8 to 136.9 microplastics per hour depending on study location (Germanov et al., 2019). Unfortunately, in this study due to the low number of plastics identified in all three shark species across sites, statistical analysis was unable to be performed to determine differences in frequency regarding either specific species or sites. Further studies including small fibers and nanoplastics may provide additional perspective with respect to the prevalence of plastics amongst shark species at different sites within Texas bays.

Plastics in Gastrointestinal Tract Sections

The second objective determined which section of the gastrointestinal tract (cardiac stomach, pyloric stomach, spiral valve intestine) had the greatest number of plastic pieces. Despite predicting plastic would be located in the spiral valve intestine, it was instead noted that all pieces of plastic identified were found in the cardiac stomach. This supports findings from similar studies describing the potential for starvation to occur as a result of inability to feed due to incurred stomach abrasions, the creation of an obstruction to further digestion, or in some cases death (Alomar et al. 2017; Sampaio et al., 2018). Other studies involving the presence of plastics in the cardiac stomach of marine tertiary consumers, including sharks, show resulting behavioral changes in speed, motility, feeding, and predator-prey interactions (Tuuri and Leterme, 2023).

In this study, the gastrointestinal tracts of the 93 bull sharks sampled yielded three specimens with one piece of plastic each in the cardiac region, or 4.30% of the total caught across all locations. Bonnethead shark plastic distribution was slightly different; out of 87 caught, two sharks (2.30% of the total) contained plastic, with one individual containing one piece and the other two pieces. The 60 black tip sharks caught included 2 specimens (3.33% of the total) with one piece of plastic each in their cardiac stomachs. Similar studies have reported comparable results, such as one involving blue sharks and shortfin mako sharks in the South Pacific, that found hooks and line within the cardiac portion of the stomach in each of the sampled populations at rates of 4.82% and 1.76%, respectively (Mucientes and Queiroz, 2019). However, another study from the Mediterranean Sea identified higher rates of occurrence, with plastics found in 21 out of 125 (16.8%) sampled individual blackmouth catshark (*Galeus melastomis*) (Alomar et al., 2017). Diet, feeding, and predatory habits of shark species, along with immediate stressors in the environment that result in reflexive expulsion of debris can all affect these trends (Joyce et al., 2002; Christie, 2012; Maes, 2020).

Further research, however, has found plastics both in the cardiac stomach and the spiral valve intestine, and one study performed in the northeast Atlantic Ocean found that microplastics were present only in the spiral valve intestine at a rate of 100% in 56 porbeagle sharks (*Lamna nassus*) sampled (Maes et al., 2020; Morgan et al., 2021). The absence of plastic observed in this region in the current study could be a potential result of the lack of direct plastic consumption or ingestion of plastic-containing prey, or feeding frequency which can affect the presence and identification of plastics in particular samples (Joyce et al., 2002; Amundsen and Hernandez, 2019). Alternatively, some of the spiral valve intestines in this study may have become prolapsed (expelled from the body becoming inside out) prior to processing. The Carcharhinidae and Sphyrnidae families of sharks are able to expel nondigestible materials from their intestine by means other than defecating via intestinal eversion, where the intestine is protruded outside the body via the cloaca (Copeia et al., 1990; Christie, 2012; Rangel et al., 2021). This might also explain discrepancies in observed hook identification, providing the debris was able to pass into the spiral intestine without perforation of any portion of the gastrointestinal wall (Joyce et al., 2002; Mucientes et al., 2019). Categorization and analysis of this category of plastics as addressed in the discussions for objectives 4 and 5 suggest a possibility of a correlation between Texas sport fishing and the accumulation of plastic, hooks, and line found in this region which could be either actively or passively ingested by sharks.

Finally, no plastics were found in the pyloric stomachs of sharks in this study, potentially due to the shape and physical features of this section. The pyloric stomach is a stout tube; it contains no tissue folds, wrinkles, or physical features to increase surface area for nutrient absorption where plastic particles could become lodged (Crow et al., 1990).

Plastics in Life Stages

The third objective of this study examined the number of plastic pieces found at each life stage among all shark species, with the prediction that there would be more plastics found in juveniles. In many coastal species, both YOY and juvenile sharks tend to spend the majority of their time in shark nursery environments frequently located in proximity to estuary sites within bays, which provide both shelter and higher levels of available prey (Froeschke et al., 2010; Heupel et al., 2011). Not surprisingly, these sites also provide prime fishing grounds for both recreational anglers and commercial fishermen (Stickney et al, 2011). Direct or indirect ingestion of plastics produced by the activity in these environments will affect sharks in various developmental stages in different ways based on their unique behaviors and characteristics. YOY sharks, as categorized by species specific length parameters in this study, generally include all neonates up to one year of age exhibiting limited movement around and within the nursery site and naivety with respect to prey capture techniques (Kinney et al., 2009; Heupel et al., 2011). They also experience high levels of competition for food after birth and primarily consume small prey composed of teleosts and invertebrates due to their size (Bethea et al., 2006). The combination of high population density and limited diet of small prey makes them less likely to ingest plastics due to fishing activity in Texas bays, despite being localized in areas of high angler activity. Juvenile sharks on the other hand tend to have an increased range, while maintaining relative site fidelity to the nursery grounds (Bethea et. al., 2015). They also have a higher survival rate due to less competition for food sources, as their diet begins to include larger prey such as clupeids and scaeinids (Gallucci et al., 2006; Bethea et al., 2006). This puts them at an increased likelihood of being affected by human activity in bays frequented by commercial and recreational fishing. Adult sharks have much larger ranges throughout the bay system and stray further away from the coast, in part due to a lower threat of predation in adult sharks and an increased variety in the types of prey consumed (Bethea et al., 2006; Bethea et al., 2015). While they can return to the estuary-based bays to mate and pup, their increased range of movement makes encounters with human bay traffic and the potential debris caused by constant fishing activity less likely (Speed et al., 2010).

Plastics were found in sharks at each life stage (YOY, juvenile, adult) in this study. Sharks at the YOY developmental stage contained 37% ($n = 3$) of plastics found, with all three specimens identified as YOY bull sharks, containing a plastic fiber, plastic fragment, and line attached to a hook, respectively. Juveniles in this study were found to contain 50% ($n = 4$) of plastics, including one bull shark containing a hook with fishing line, two blacktips in one of which was found a plastic fiber and the other a hook and line, and one bonnethead which contained two individual pieces of plastic fibers. Adults contained 13 % ($n = 1$) of the pieces, with one specimen found to contain a plastic fragment. Though juveniles did contain more plastics than the other two life stages examined in this study, it was not a statistically significant difference ($p > 0.05$). In addition, there did not appear to be an obvious correlation between life stage and size of plastic pieces observed. In contrast with the assumption that perhaps smaller YOY individuals might be less likely to ingest larger particles, this was not representative of the results. There is very little research assessing the presence of plastics in sharks at differing life stages, although Bernardini et al. (2018) found that the incidence of marine litter ingestion in juvenile blue sharks were statistically higher compared to adults. The general trend that once sexually mature, adult sharks tend to leave bay nursery areas or be present in relatively low numbers may have contributed to the fact that only one adult shark was found to contain plastic in this study (Huepel et al., 2007). The characteristics of the plastic fragment do not make the source immediately clear, and due to their range; it is possible it was ingested at a previous location. The increased frequency of plastics and hooks found in younger specimens, particularly juveniles, does suggest their proximity to coastal fishing areas can result in an increased likelihood of plastic ingestion as a direct result of local fishing activity and practices.

Categorization of Plastics Found

Findings regarding the prediction presented in the fourth objective were mixed. All three species investigated in this study showed plastic in their gastrointestinal tract 3.33% (n = 8). A total of seven plastics were identified as fibers (78%), followed by fragments 22% (n = 2). Of the seven fibers encountered, four were identified as monofilament fishing line. The fact that fibers were found in the highest numbers is consistent with other studies investigating the prevalence of plastics in sharks (Valente et al., 2019; Mancia et al., 2020).

Clear and green plastics each comprised 33% (n = 3) of plastics found. However, clear plastics were found in a greater number of sites sampled (n = 3), including Sabine Lake, Aransas Bay, and Lower Laguna Madre. Meanwhile, green plastics were found only in two sites - Aransas Bay and Corpus Christi Bay, though they represented the same total of pieces found (n = 3). Additionally, the prediction of finding most fragments <5 mm in dimension was not supported, as all identified plastic pieces exceeded this value and are therefore categorized as mesoplastic (5 – 50 mm).

Recent studies suggest that clear items contribute a large portion, up to 47% of overall ocean debris (Marti et al. 2020, Thushari et al. 2023). In addition, previous work investigating the presence of plastics in shark gastrointestinal tracts have had similar results to those found here, as the most identified color of plastic particles have been clear (Alomar and Duedro, 2017; Sabrana et al., 2022). The size of plastics in this study was larger than predicted, as the smallest piece of plastic found was a fiber that measured 0.47 mm wide and 6.93 mm long. It has been shown from previous studies that the size of plastics ingested by sharks can vary greatly from larger plastic shopping bags to microplastic particles (Bernardini et al., 2018; Fernandez and Anastasopoulou, 2019). The diversity of the plastic size found in this study does support these previous investigations.

Plastic Polymers

Objective 5 predicted that the most frequently found type of plastic polymer would be PE. The results of the study confirmed this prediction, as the most commonly identified plastic polymers were PE and nylon 6, each constituting 33% (n = 3) of the plastics found in the gastrointestinal tract of sharks in Texas bays. Though PE and nylon 6 were found at the same frequency in this study, PE is the most commonly produced polymer type, and this trend is reflected by what is typically found in the marine environment (Andrady, 2011; Nerland et al., 2014; Schwarz et al, 2019). Previous studies investigating the presence of plastics in shark species have found that PE was the dominant type of polymer identified (Alomar and Deudero, 2017; Bernardini et al., 2018; Fernandez and Anastasopoulou, 2019; Huang et al., 2022; Sbrana et al., 2022). The nylon 6 fragments found in this study came from monofilament fishing line, indicating sportfishing practices play a large role in plastic ingestion as well. This is supported by previous work that has found fishing hooks and lines retained in the mouth and stomach of shark species in the Pacific Ocean (Mucientes and Queiroz, 2019).

Health Implications of Plastic Ingestion in Sharks

Out of all sharks examined in this study (n = 240), eight sharks had plastic in their gastrointestinal tract. All the plastics identified in this study, including polyethylene, nylon 6, polypropylene, and poly (vinylidene chloride) have been implicated in negative impacts on shark health (Wright et al., 2013; Mancia et al., 2020). In addition, plastics have the potential to cause visible bodily damage, such as that incurred via entanglement in fishing nets and lines and intestinal perforation by hooks attached to line (Porcher et al, 2022). While outside the scope of this study, hooks and monofilament fishing line were found outside of the gastrointestinal tract, freely moving about the abdominal cavity, lodged in the liver, or in the process of being pushed from the cardiac stomach (Figures 12-15). This has been observed in other cases where hooks and monofilament fishing line have been found in the liver of a sandbar shark (*Carcharhinus plumbeus*) as well as the spiral valve intestine of a gray nurse shark (*Carcharhinus taurus*), causing them to experience chronic weight loss or death (Lecu et al., 2011; Otway et al., 2021). Due to the number of hooks and monofilament fishing lines found, angling practices in the bay systems focused on in this study seem to be responsible for more harm than the direct ingestion of plastics or plastics found in prey items.

Improvements to Plastic Extraction Procedure and Limitations of Study

Because plastics in the marine environment is a relatively new area of study, there is not a standardized protocol for the extraction of plastics. Some methods use hydrogen peroxide, acidic solutions, or alkaline solutions to digest organic material, others use hypersaline solutions to extract plastics, (Avio et al., 2015; Dehaut et al., 2016; Atamanalp et al., 2022). A standardized protocol should be established for future studies. The methods used in this study utilized an alkaline solution of KOH or hydrogen peroxide that was incubated at 60°C for 72 hours to digest organic material leaving plastics intact with minimal results. The KOH solution did not digest very much organic material from the samples, and the hydrogen peroxide bleached the organic material white making it difficult to sort through and extract plastic pieces.

Cross contamination is another challenge to this study. The possibility that small airborne textile fibers may land in the exposed petri dishes containing the contents of the gastrointestinal tracts caused us to omit these types of materials from the study. However, larger fibers that were clearly too large to be airborne were included in this study.

Due to complications with chemical digestion and the large number of gastrointestinal tracts to inspect in this study, prey items found in gastrointestinal tracts were not digested. An unpublished study conducted by a student of Jeremy Conkle at Texas A&M Corpus Christi examined the presence of plastic in some of these prey species along the Texas coast and found no plastic in their gastrointestinal tracts. Because plastics were not found in the prey items and a low prevalence of plastics in sharks in the current study it is indicative of low amounts of plastics in prey items in Texas bays. Previously published studies have found high numbers of plastic in the stomachs of similar prey species, such as striped mullet and European flounder (*Platichthys flesus*) from a South African estuary and the North Sea at rates of 73% (n = 70) and 5.5% (n = 290), respectively, indicating that plastic is consumed by these species (Naidoo et al., 2016; Rummel et al., 2016).

Conclusion

This was the first study investigating the presence of plastic in sharks from Texas bays. Out of the sample size (n = 240), eight sharks representing three different species from four coastal bays were found to contain plastics, five of which were monofilament fishing line. Due to the prevalence of fishing related items found, this indicates that angling practices in these bay systems present more of an issue to the health of these species than secondary routes of plastic ingestion.

Table 1. Total length (TL; mean ± standard deviation), weight (mean ± standard deviation), sample size (n), and percent and sample size of individuals broken down by life stage (YOY = young-of-the-year, J = juvenile, A = adult) and sex [% and n female (F)] for each investigated species from each collection location (SL = Sabine Lake, AB = Aransas Bay, CCB = Corpus Christi Bay, LLM = Lower Laguna Madre). Minimum and maximum body lengths and weights are in parentheses.

Site	Species	n	TL (cm)	Weight (kg)	% YOY (n)	% J (n)	% A (n)	% F (n)
SL	Bull	49	89.6 ± 11.2 (64.4 - 115.0)	6.2 ± 2.5 (1.9 - 13.1)	59 (29)	41 (20)	0 (0)	53 (26)
AB	Blacktip	9	62 ± 11.5 (49.4 - 78.0)	1.8 ± 1.1 (0.7 - 3.6)	67 (6)	33 (3)	0 (0)	44 (4)
	Bonnethead	18	75.18 ± 13.98 (46.0 - 94.0)	2.09 ± 1.03 (0.4 - 3.7)	11 (2)	17 (3)	72 (13)	50 (9)
	Bull	35	90.7 ± 15.8 (76.5 - 148.5)	6.9 ± 4.8 (2.6 - 27.0)	69 (24)	31 (11)	0 (0)	40 (14)
CCB	Blacktip	31	82.2 ± 15.7 (61.4 - 134.7)	4 ± 2.8 (1.5 - 15.0)	13 (4)	81 (25)	6 (2)	48 (15)
	Bonnethead	36	61.8 ± 10.8 (43.6 - 77.9)	1.1 ± 0.5 (0.4 - 2.0)	17 (6)	55 (20)	28 (10)	61 (22)
	Bull	9	91.4 ± 9.5 (80.0 - 113.5)	6.9 ± 2.5 (4.9 - 13.2)	56 (5)	44 (4)	0 (0)	56 (5)
LLM	Blacktip	20	71.9 ± 8.6 (51.5 - 86.8)	2.5 ± 1 (1.9 - 4.3)	40 (8)	60 (12)	0 (0)	70 (14)
	Bonnethead	33	70.5 ± 10.3 (49.9 - 105.0)	1.8 ± 0.8 (0.5 - 5.0)	3 (1)	42 (14)	55 (18)	73 (24)

Table 2. Cardiac stomach contents of each species from each collection location (SL = Sabine Lake, AB = Aransas Bay, CCB = Corpus Christi Bay, LLM = Lower Laguna Madre) with sample size (n), number of individuals with food in their cardiac stomach, the identified prey items, and the percentage and number of individual sharks with those prey items in their cardiac stomach.

Site	Species	n	Number with food	Prey	% (n)
SL	Bull	49	22	Fish	95 (21)
				Crab	5 (1)
AB	Blacktip	9	5	Fish	100 (5)
	Bonnethead	18	18	Crab	100 (18)
				Shrimp	22 (4)
				Mantis Shrimp	39 (7)
				Squid	17 (3)
				Sea Grass	83 (15)
	Bull	35	23	Fish	96 (22)
				Seagrass	13 (3)
				Stingray	9 (2)
CCB	Blacktip	31	16	Fish	94 (15)
				Crab	6 (1)
				Shrimp	12 (2)
				Squid	6 (1)
	Bonnethead	36	36	Fish	3 (1)
				Crab	75 (27)
				Shrimp	19 (7)
				Mantis shrimp	36 (13)
				Squid	14 (5)
				Seagrass	83 (30)
				Polychaetae worm	3 (1)
	Bull	9	5	Fish	100 (5)
				Crab	20 (1)
LLM	Blacktip	20	6	Fish	100 (6)
				Crab	17 (1)
	Bonnethead	33	33	Fish	6 (2)
				Crab	97 (32)
				Shrimp	27 (9)
				Seagrass	100 (33)
				Eel	9 (3)

Table 3. Presence of plastic in each section (cardiac stomach, pyloric stomach, and spiral valve intestine) of the gastrointestinal tract for each investigated species and collection location (SL = Sabine Lake, AB = Aransas Bay, CCB = Corpus Christi Bay, LLM = Lower Laguna Madre). For each location and species, the sample size (n) is provided, along with the percentage and number of individuals that had plastic in their gastrointestinal tract, whether plastic was present in each section (Y = yes, N = no), and the number of pieces of plastics found throughout the whole gastrointestinal tract.

Site	Species	n	% with plastic (n)	Cardiac	Pyloric	Intestine	Number of pieces
SL	Bull	49	4.08 (2)	Y	N	N	2
AB	Blacktip	9	0 (0)	N	N	N	
	Bonnethead	18	5.56 (1)	Y	N	N	1
	Bull	35	5.72 (2)	Y	N	N	2
CCB	Blacktip	31	3.23 (1)	Y	N	N	1
	Bonnethead	36	2.78 (1)	Y	N	N	2
	Bull	9	0 (0)	N	N	N	
LLM	Blacktip	20	5 (1)	Y	N	N	1
	Bonnethead	33	0 (0)	N	N	N	

Table 4. Number of plastic pieces found in each section (cardiac stomach, pyloric stomach, spiral valve intestine) of the gastrointestinal tract for each shark that had plastic present. The collection location (SL = Sabine Lake, AB = Aransas Bay, CCB = Corpus Christi Bay, LLM = Lower Laguna Madre), species, life stage (YOY = young-of-the-year, J = juvenile, A = adult), and size (length x width) and weight of each plastic piece is provided. For the bonnethead shark from Corpus Christi Bay, the letters A and B denote the two separate pieces of plastic that were found in one individual.

			Number of plastics found				
Site	**Species**	**Life Stage**	**Cardiac**	**Pyloric**	**Intestine**	**L x W (mm)**	**Weight (mg)**
SL	Bull	YOY	1	0	0	45.9 x 84.3	70.1
	Bull	YOY	1	0	0	4.34 x 11.32	29.0
AB	Bonnethead	A	1	0	0	4.55 x 6.76	7.7
	Bull	J	1	0	0	29.5 x 21.0	902.0
	Bull	YOY	1	0	0	95.9 x 35.0	2224.0
CCB	Blacktip	J	1	0	0	0.47 x 6.93	0.2
	Bonnethead	J	2	0	0	A = 64.2 x 1.6	A = 6.0
						B = 19.2 x 1.6	B = 1.4
LLM	Blacktip	J	1	0	0	69.3 x 28.0	2883.0

Table 5. Description (type, color, clarity, and polymer type) of plastic pieces found in species from each sampling site (SL = Sabine Lake, AB = Aransas Bay, CCB = Corpus Christi Bay, LLM = Lower Laguna Madre).

Site	Species	Type	Color	Clarity	Polymer type
SL	Bull	Fiber	Clear	Transparent	Nylon 6
	Bull	Fragment	Black	Opaque	Polyethylene
AB	Bonnethead	Fragment	Yellow	Opaque	Polyethylene
	Bull	Fiber	Clear	Transparent	Nylon 6
	Bull	Fiber	Green	Transparent	Nylon 6
CCB	Blacktip	Fiber	Black	Opaque	Polyethylene
	Bonnethead	Fiber	Green	Transparent	Polypropylene
		Fiber	Green	Transparent	Polypropylene
LLM	Blacktip	Fiber	Clear	Transparent	Poly(vinylidene fluoride)

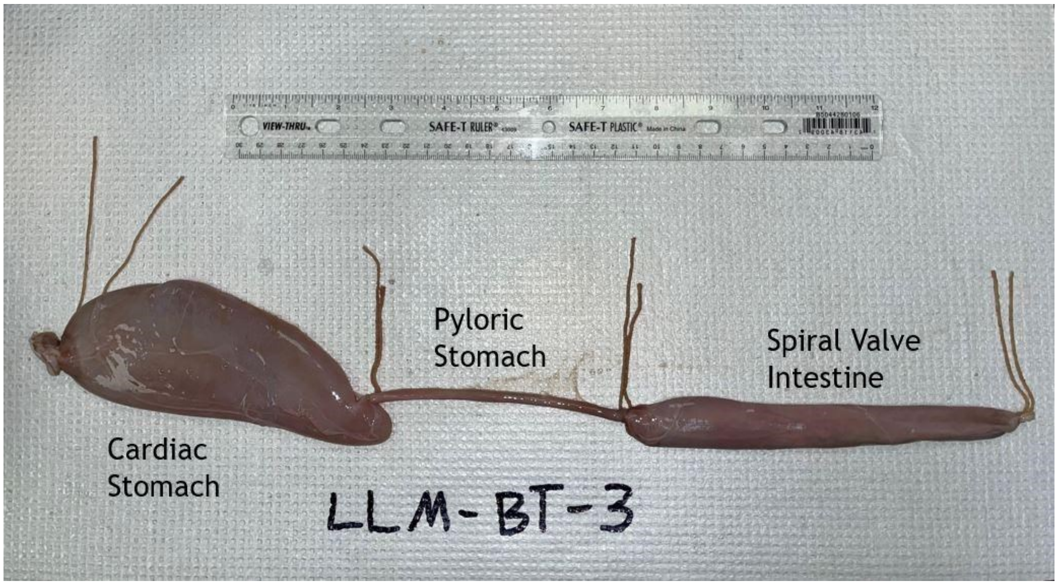

Figure 1. Gastrointestinal tract of a blacktip shark. Cotton twine separates each section.

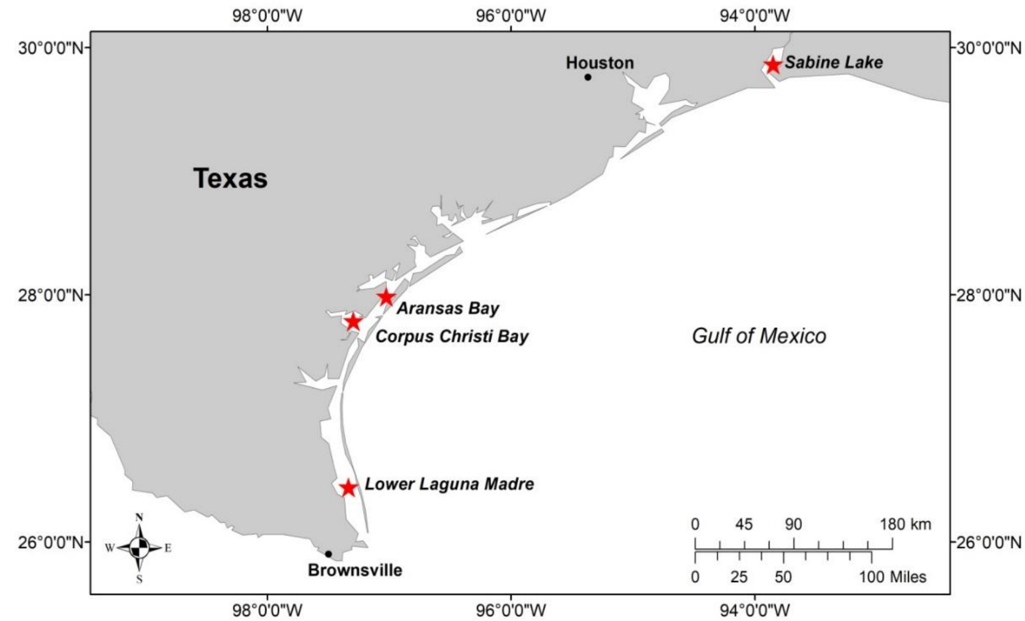

Figure 2. Shark sampling locations along the upper (Sabine Lake), middle (Aransas Bay and Corpus Christi Bay), and lower (Lower Laguna Madre) Texas coast. Bull sharks were collected from Sabine Lake; bull sharks, blacktip sharks, and bonnethead sharks were collected from Aransas Bay and Corpus Christi Bay; and blacktip sharks and bonnethead sharks were collected from the Lower Laguna Madre.

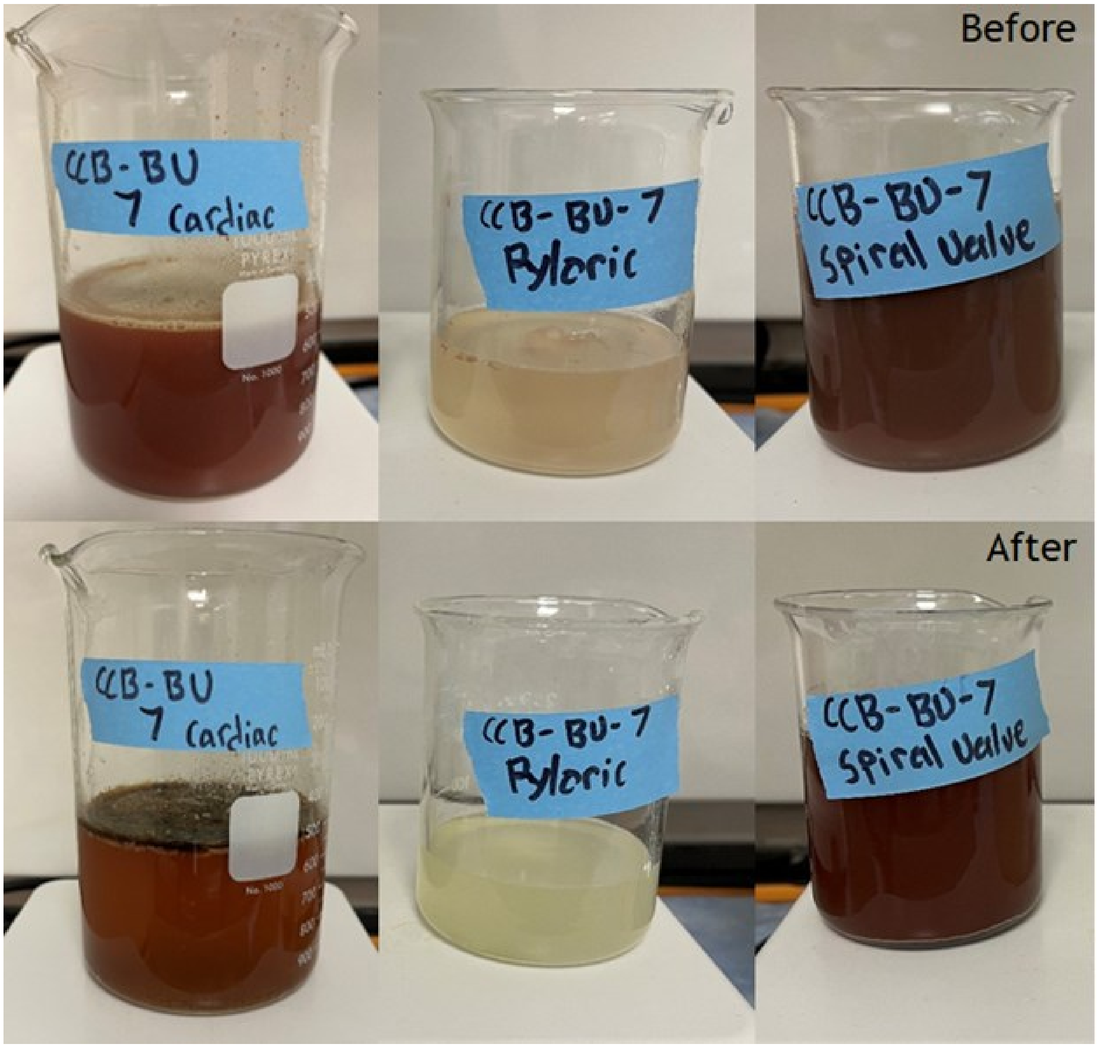

Figure 3. Contents of the cardiac stomach, pyloric stomach, and spiral valve intestine of a bull shark before (top row) and after (bottom row) digestion in 18% KOH at 60°C for 72 hours.

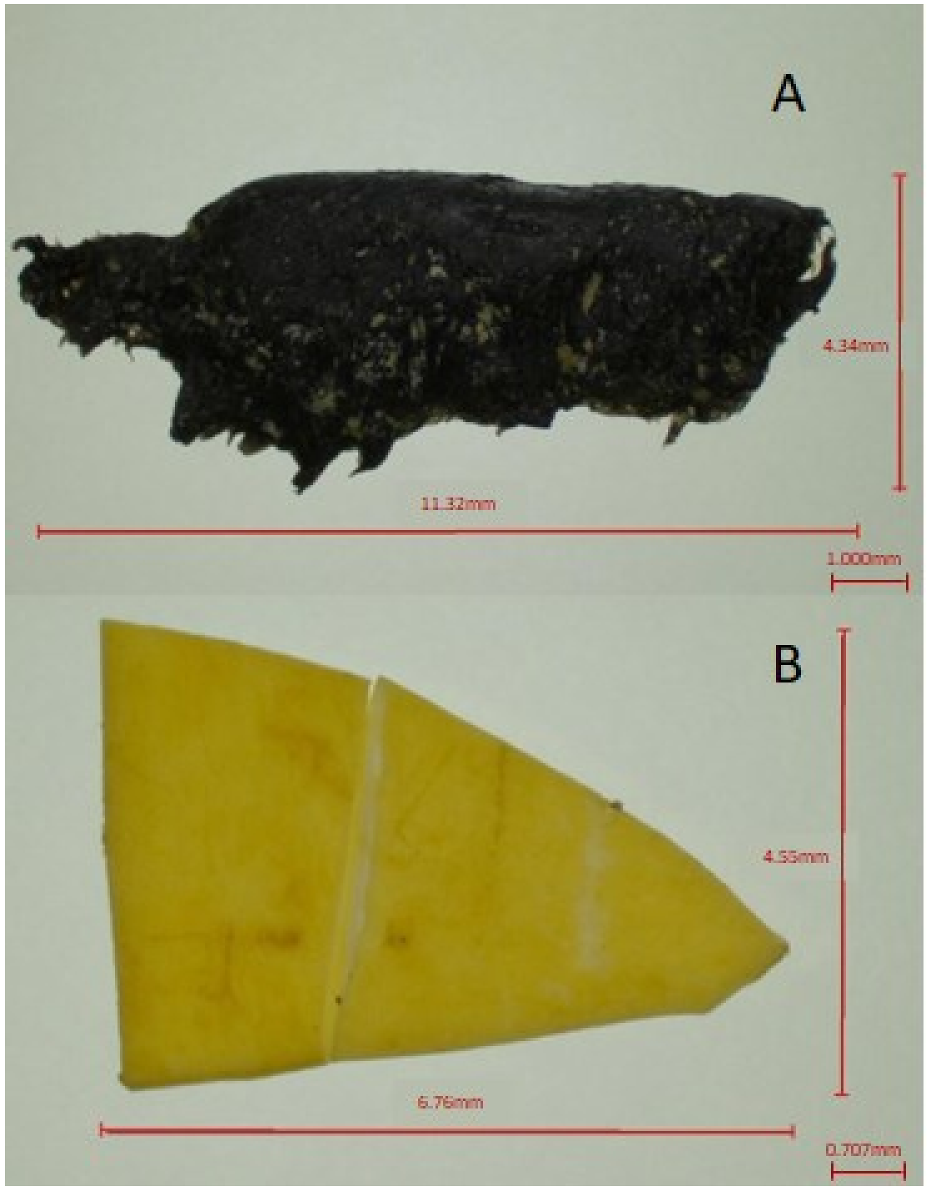

Figure 4. Fragments of plastic that were extracted from a bull shark in Sabine Lake (A) and a bonnethead shark in Aransas Bay (B).

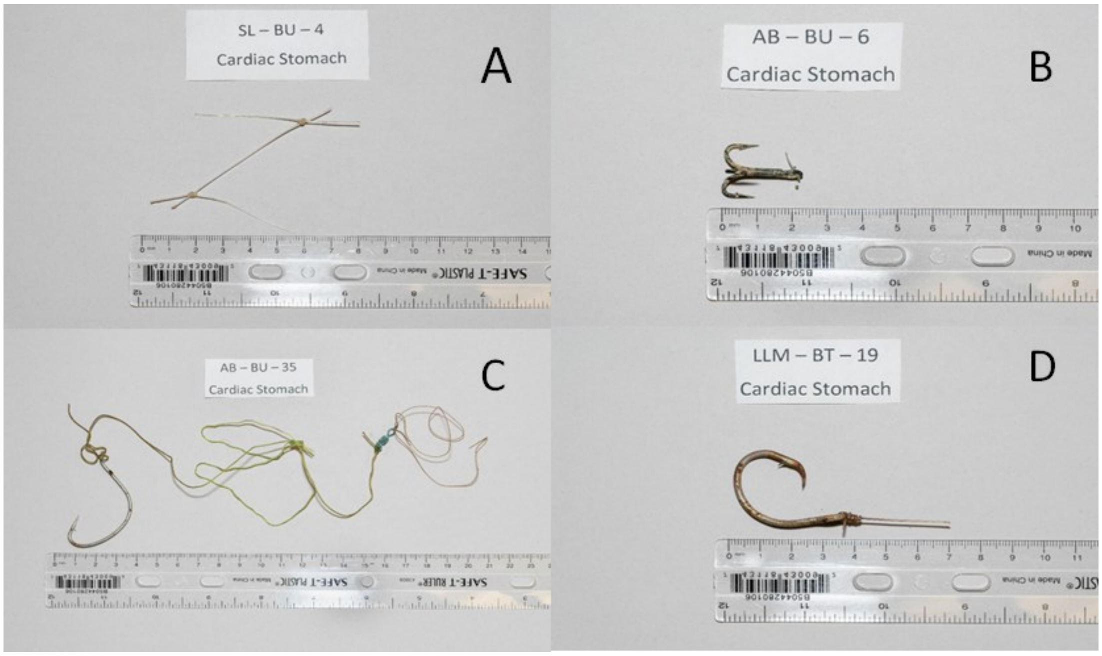

Figure 5. Monofilament fishing line (fibers) extracted from a bull shark in Sabine Lake (A) and Aransas Bay (B, C), and a bonnethead shark in the Lower Laguna Madre (D).

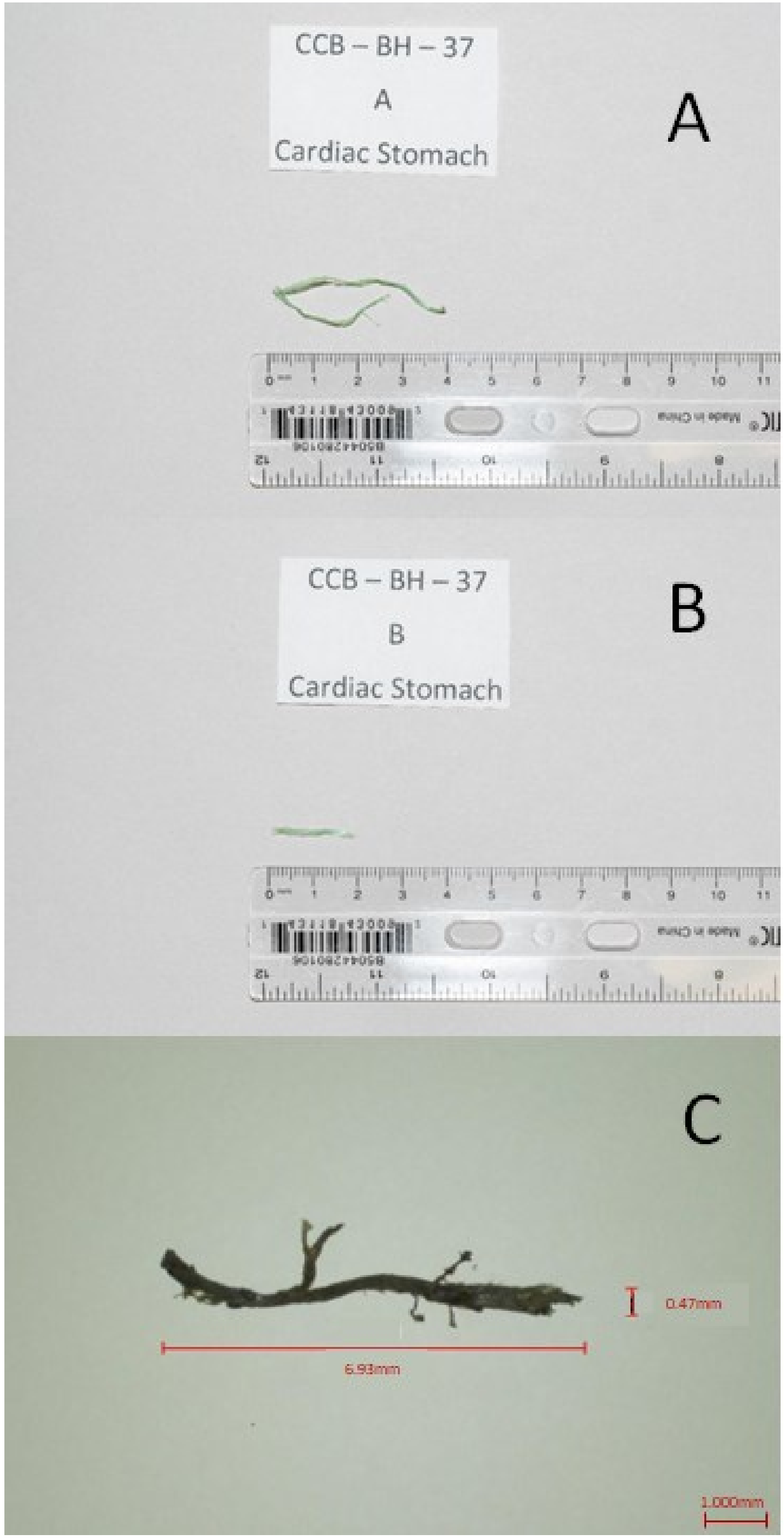

Figure 6. Plastic fibers extracted from one bonnethead shark in Corpus Christi Bay (A, B) and one blacktip shark in Corpus Christi Bay (C).

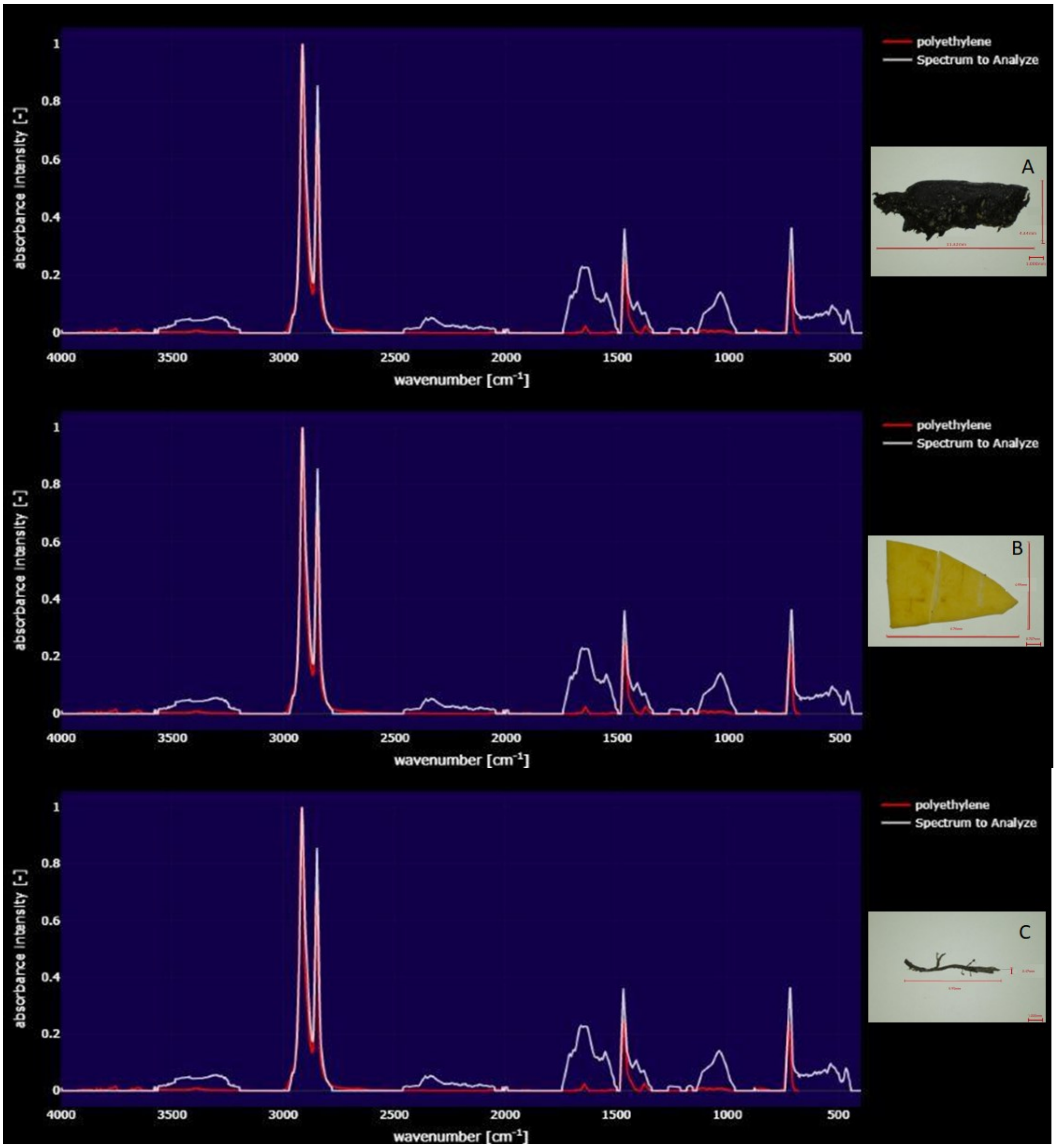

Figure 7. FTIR spectrum readout of a suspected plastic (white line) compared to polyethylene (red line) spectra readout from Open Specy online spectra library along with corresponding plastic that was analyzed.

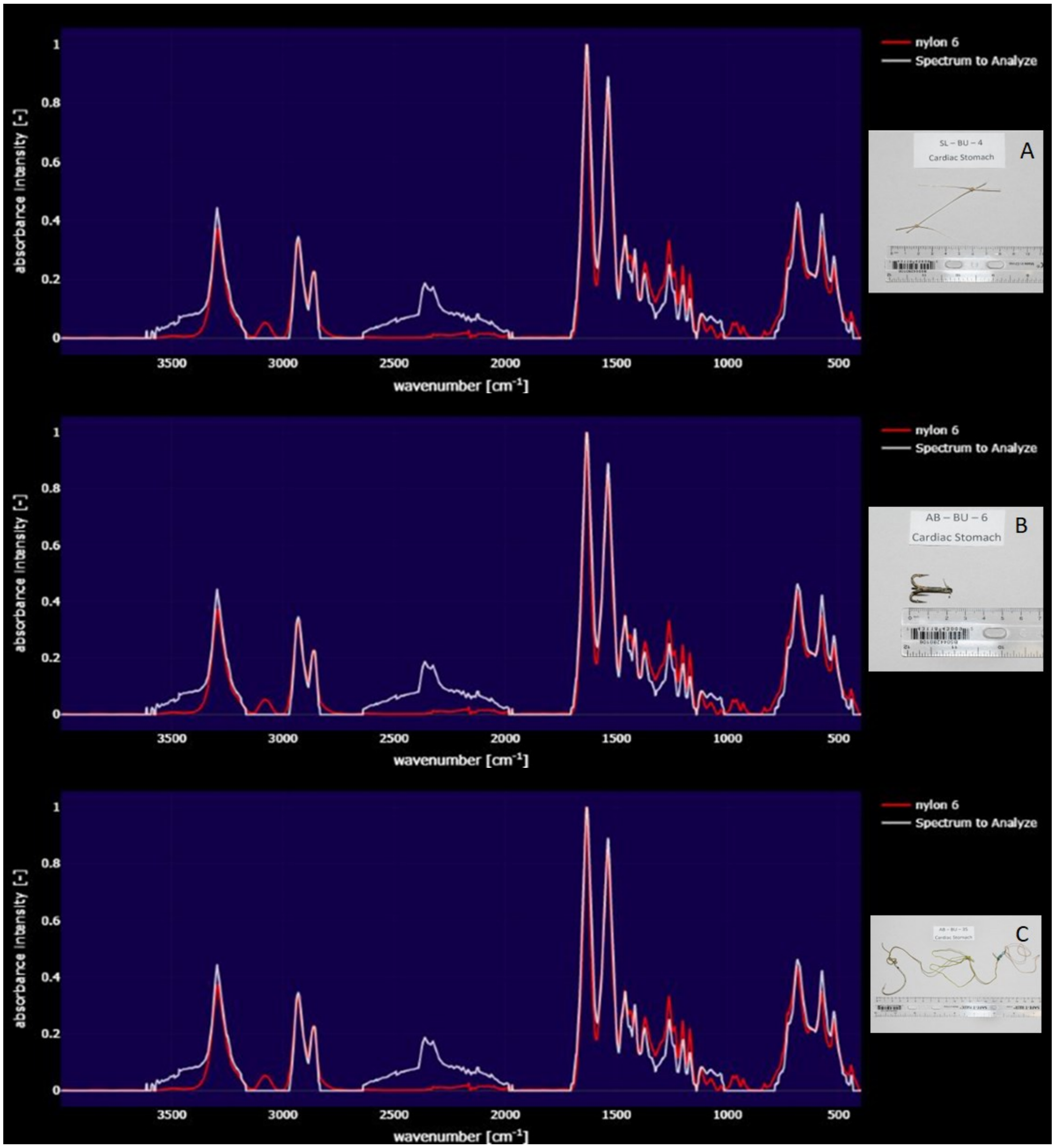

Figure 8. FTIR spectrum readout of a suspected plastic (white line) compared to nylon 6 (red line) spectra readout from Open Specy online spectra library along with corresponding plastic that was analyzed.

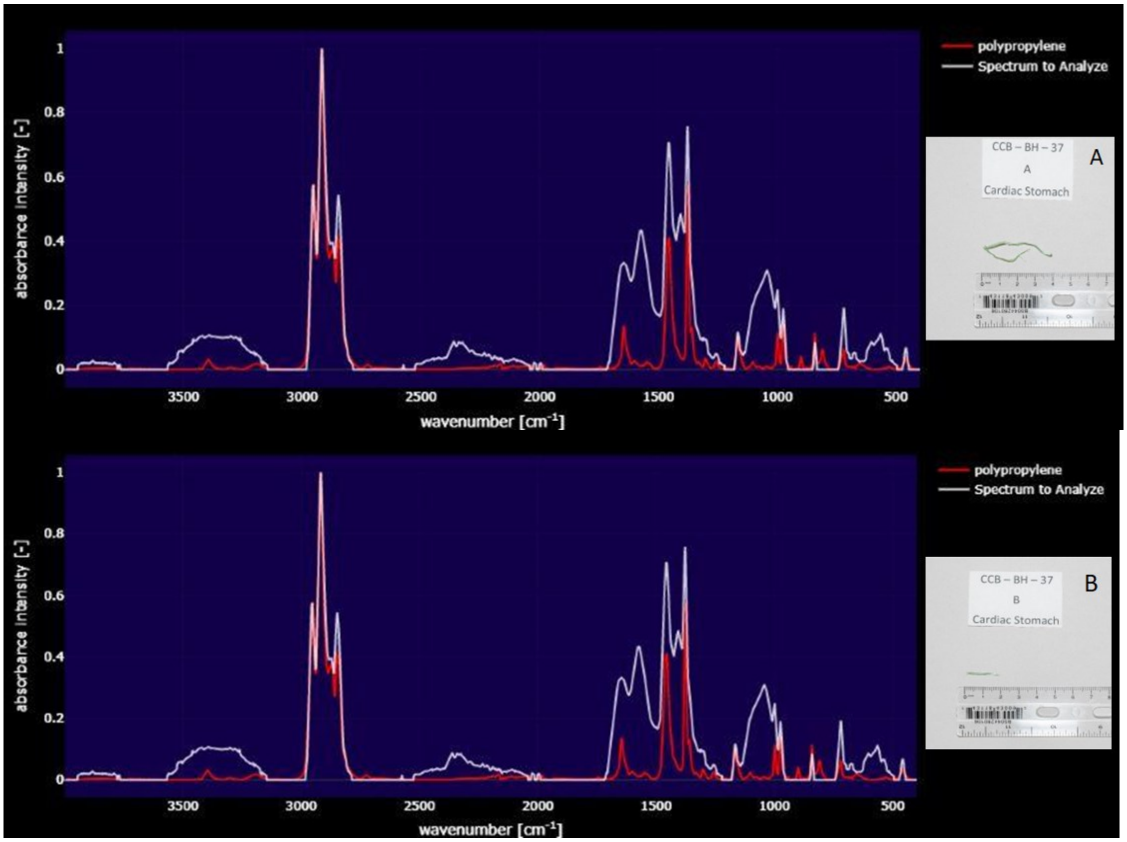

Figure 9. FTIR spectrum readout of a suspected plastic (white line) compared to polypropylene (red line) spectra readout from Open Specy online spectra library along with corresponding plastic that was analyzed.

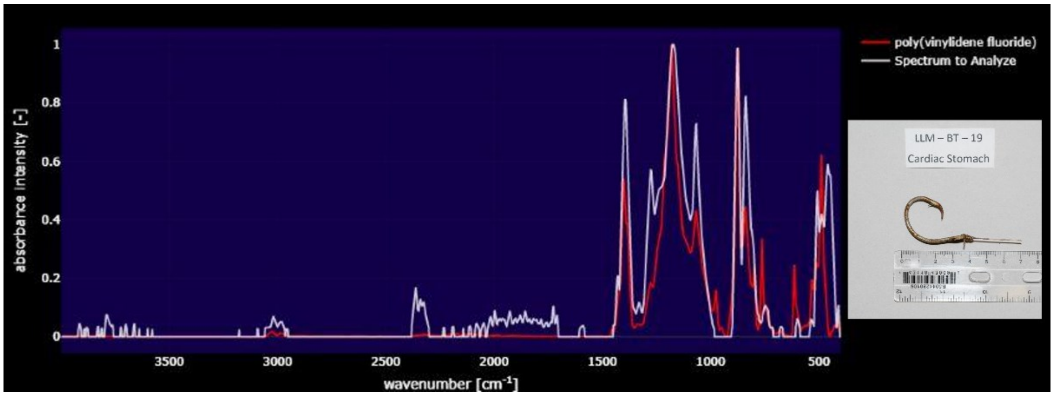

Figure 10. FTIR spectrum readout of a suspected plastic (white line) compared to poly(vinylidene fluoride) (red line) spectra readout from Open Specy online spectra library along with corresponding plastic that was analyzed.

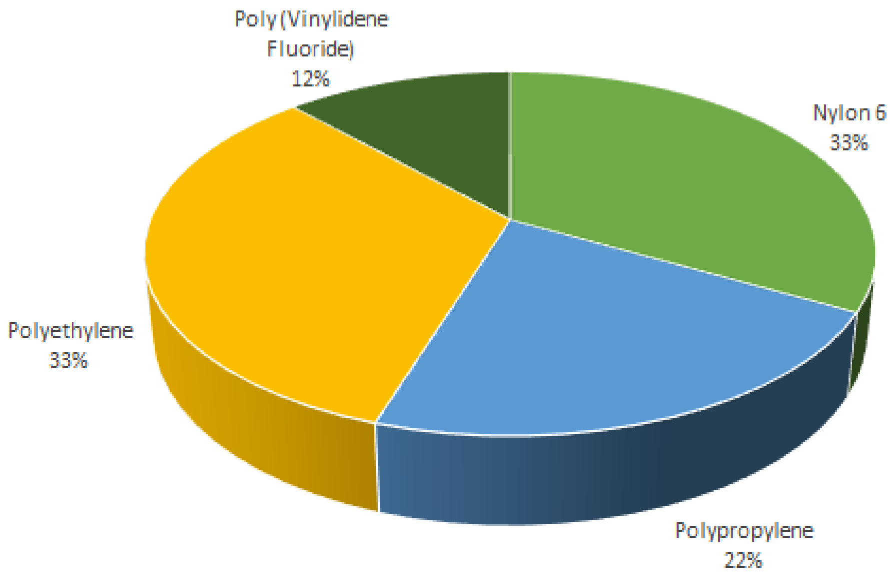

Figure 11. Percentages of polymer types found within shark gastrointestinal tracts.

Figure 12. Fishing hook in the process of passing through the cardiac stomach into the body cavity in a blacktip shark.

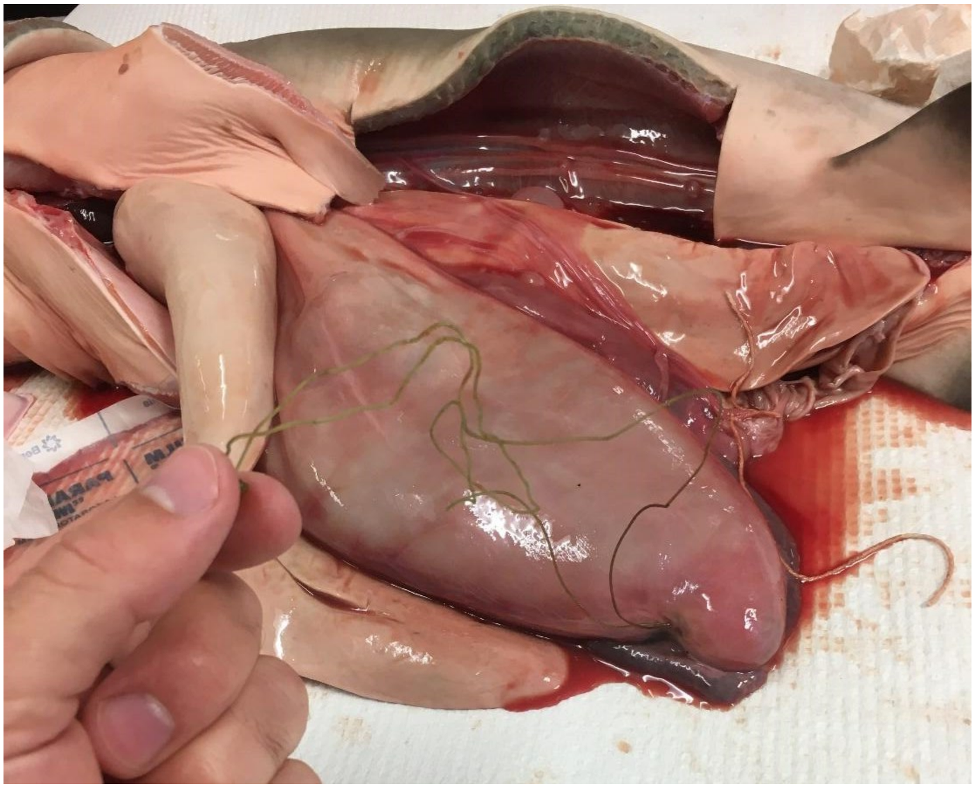

Figure 13. Monofilament fishing line passing through the cardiac stomach into the body cavity in a bull shark.

Figure 14. Fishing hook that passed through the wall of the cardiac stomach of a bull shark and was loose in the body cavity.

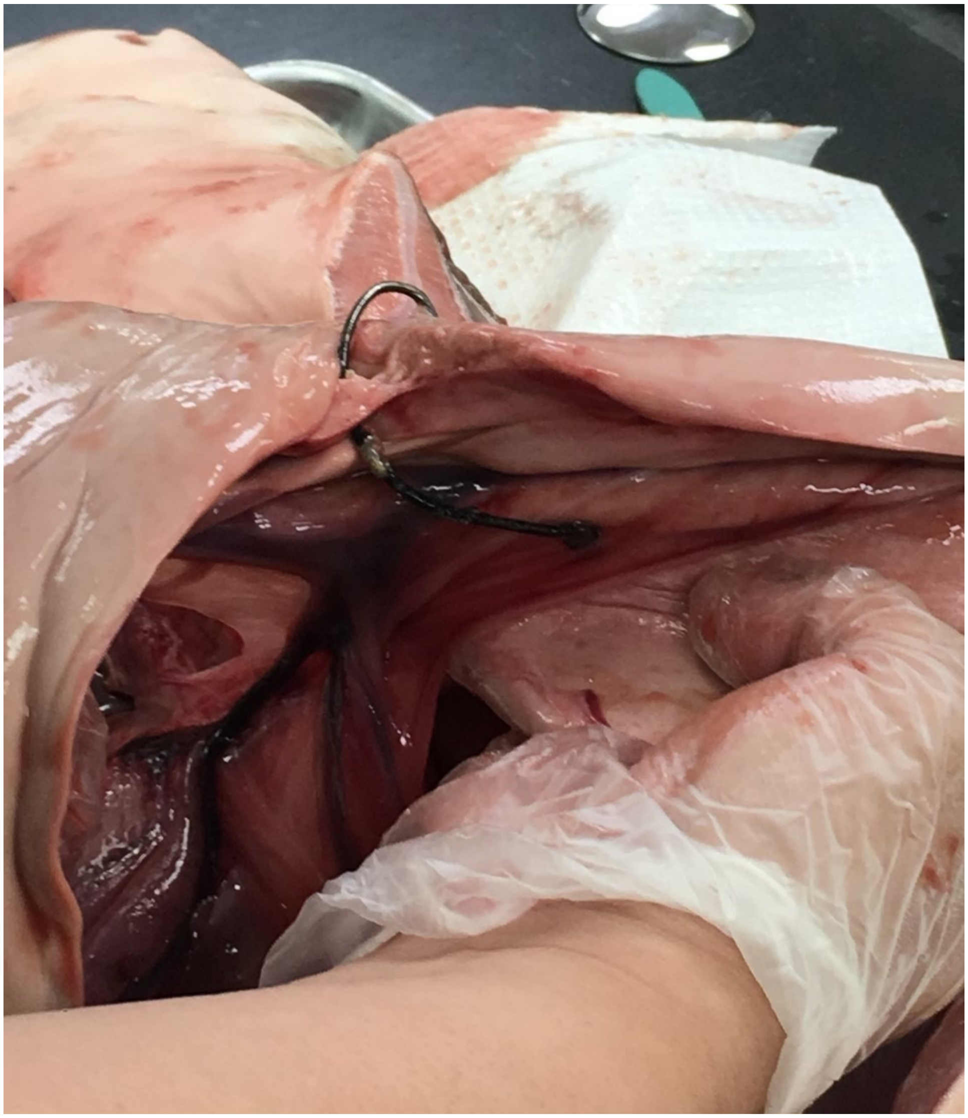

Figure 15. Fishing hook that passed through the wall of the cardiac stomach of a bull shark into the body cavity and then became and had became hooked in the liver.

APPENDIX SECTION

Appendix A. Length of each section of gastrointestinal tract (cardiac stomach, pyloric stomach, and spiral valve intestine) and the weight of the contents of each section for sharks collected from each location (SL = Sabine Lake, AB = Aransas Bay, CCB = Corpus Christi Bay, LLM = Lower Laguna Madre). Values are mean ± standard deviation and the minimum and maximum values are in parentheses. n = sample size.

			Section length (cm)			Content weight (g)		
Site	**Species**	**n**	**Cardiac**	**Pyloric**	**Intestine**	**Cardiac**	**Pyloric**	**Intestine**
SL	Bull	49	27.6 ± 3.5	21.8 ± 3.5	34.6 ± 5.5	209.8 ± 180.9	1.4 ± 1.0	49.3 ± 25.7
			(19.4 - 35.0)	(13.8 - 29.4)	(19.7 - 44.8)	(8.0 - 852.3)	(0.2 - 5.3)	(7.5 - 103.2)
AB	Blacktip	9	16.5 ± 4.4	11.7 ± 3.5	19.9 ± 5.2	28.3 ± 59.2	0.3 ± 0.2	13.0 ± 8.9
			(9.5 - 23.5)	(7.1 - 17.8)	(12.9 - 26.4)	(1.6 - 185.7)	(0.1 - 0.5)	(2.8 - 25.5)
	Bonnethead	18	22.5 ± 5.0	16.9 ± 3.8	17.4 ± 3.0	83.2 ± 51.2	3.0 ± 1.8	5.5 ± 3.9
			(11.8 - 29.4)	(9.3 - 21.7)	(10.0 - 21.9)	(5.7 - 179.9)	(0.4 - 6.6)	(1.4 - 15.2)
	Bull	35	28.5 ± 4.9	21.7 ± 4.5	35.1 ± 6.0	236.9 ± 242.1	1.1 ± 0.7	43.0 ± 22.7
			(20.6 - 45.0)	(15.8 - 38.7)	(26.0 - 56.0)	(21.3 - 1118.6)	(0.2 - 3.9)	(18.3 - 126.1)
CCB	Blacktip	31	21.1 ± 4.5	16.8 ± 3.7	25.5 ± 5.7	61.0 ± 92.0	0.7 ± 1.3	21.8 ± 17.6
			(15.5 - 37.1)	(11.0 - 25.0)	(18.5 - 41.2)	(1.9 - 756.0)	(0.1 - 5.6)	(0.9 - 72.8)
	Bonnethead	36	19.6 ± 3.9	14.0 ± 2.6	14.4 ± 3.0	39.0 ± 29.8	2.1 ± 1.3	3.4 ± 2.3
			(13.5 - 29.5)	(9.2 - 18.0)	(10.0 - 20.6)	(4.0 - 133.8)	(0.2 - 5.7)	(0.7 - 11.0)
	Bull	9	27.7 ± 3.7	21.9 ± 3.1	33.7 ± 4.5	133.3 ± 87.5	0.8 ± 0.5	35.2 ± 6.3
			(23.7 - 35.4)	(18.6 - 28.7)	(30.4 - 45.0)	(13.6 - 284.9)	(0.2 - 1.6)	(27.5 - 41.1)
LLM	Blacktip	20	18.9 ± 2.3	14.0 ± 1.8	21.3 ± 2.5	33.8 ± 28.1	0.3 ± 0.2	16.2 ± 9.2
			(13.3 - 22.2)	(9.5 - 16.5)	(15.5 - 27.2)	(1.4 - 104.8)	(0.1 - 0.8)	(3.1 - 45.0)
	Bonnethead	33	21.5 ± 3.3	15.9 ± 2.9	16.2 ± 2.9	56.0 ± 30.9	2.7 ± 1.0	4.9 ± 3.3
			(15.0 - 28.2)	(10.0 - 22.8)	(10.6 - 22.8)	(4.8 - 128.0)	(0.6 - 4.7)	(0.1 - 11.0)

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