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Microplastic Quantification on the Effect of Endoparasite Communities in Florida Seabirds

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NOVA SOUTHEASTERN UNIVERSITY

HALMOS COLLEGE OF ARTS AND SCIENCES

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Abstract

Microplastics are increasingly ubiquitous in marine ecosystems. Although there is an understanding that microplastics affect wildlife by altering critical biological processes, these distributions are poorly understood. Endoparasites are frequently trophically acquired and their intra-host community can serve as a proxy for an altered biological process. Coastal seabirds are known to ingest microplastic, but little is known about how this ingestion affects their endoparasite communities. This project aims to determine a better understanding of two main objectives: assessment of the presence of secondary ingestion of microplastics in coastal seabirds and a determination of the relationship between microplastics and endoparasite communities' structure and state of susceptibility. This project focused on three coastal marine bird species native to southeastern Florida: double-crested cormorant (*Phalacrocorax auratus*), brown pelican (*Pelecanus occidentalis),* and osprey (*Pandion haliaetus*). Endoparasite and microplastic samples were collected from carcasses from each bird species; brown pelican (n=14), double-crested cormorant (n=9), and osprey (n=3). Laboratory analysis included a collection of parasites from the bird's gastrointestinal tract (GI tract) using a dissecting microscope, storing the parasites in 70% ethanol, and staining and mounting them. Analysis also included the digestion of the GI tract, liver, and any liquids collected in 10% potassium hydroxide (KOH) in preparation for filtration powered by a Buchner funnel and vacuum flask. Visual analysis with a dissecting microscope allowed for the identification and quantification of microplastics that remained. The small sample size of the osprey species limited the information that could be collected. Correlation tests in pelicans and cormorants showed that microplastic abundance is not affecting endoparasite community structures, nor is it affecting total parasite abundance. There was a correlation between abundance of microplastics in the GI tract and in the liver in cormorants, but not in pelicans.

Keywords: persistent organic pollutants, feeding ecology, satiation, symbiosis, marine waste, potassium hydroxide, acanthocephalan, nematodes, kleptoparasitism, decantation

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Thanks to the President's Faculty Research & Development Grant program for granting us the funds to supply the necessary materials to proceed with the study. The funding was acquired by my committee before I became a part of the project but was well utilized and appreciated throughout the sample taking process.

I would like to thank my family and friends for supporting me through my academic journey. I could not have done it without your support and love.

To my parents, thank you for everything I have.

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Introduction

Marine Waste

Marine waste, persistent solid material abandoned in marine environments, is an ongoing threat to the survival of marine fauna. Marine waste is not only detrimental to the fauna that lives within it but impacts climate regulation globally. Anthropogenic waste varies in size, from large pieces of debris to small pieces of plastics unable to be seen with the naked eye (Rodrigues et al., 2018). The use of plastics has only increased due to their versatility, durability, and ease of production; unfortunately, the hazard and implications of using an abundance of plastics were not known until knowledge of its significance as an environmental hazard came to light, now showing that plastics encompass 80% of waste found on land, shorelines, and the ocean surface and floor (Clark, 2021; Seager et al., 2016). These plastics will never break down entirely and instead become smaller, in some ways more hazardous, pieces no larger than five millimeters (mm), referred to as microplastics. Microplastics are classified as primary or secondary; primary classification means that the plastic was manufactured to be minor, while secondary classification means that the plastic is degraded from larger plastics (Clark, 2021; Thompson et al., 2005). Microplastic particles differ in their size, shape, color, density, chemical makeup, and polymer type (Bergmann et al., 2015). Particles can take the form of granules, fragments, pellets, film, foam, and fibers (Gies et al., 2018; Ngo et al., 2019; Rodrigues et al., 2018). Due to their small size, acquiring information about them has proven difficult, limiting understanding and ability to prevent further damage due to microplastics (Bergmann et al., 2015).

Due to the small size of marine plastics, a wide range of species readily consume them. The extent of physical damage that microplastics can cause is still not fully understood. However, marine birds are primarily at risk due to their feeding habits, feeding at the ocean surface, at times in locations where flotsam and concentrated oceanic currents occur (Carlin et al., 2020; Moser & Lee, 1992). Ingestion of plastics can lead to physical damage to the gastrointestinal tract (GI tract), at times leading to starvation or the transfer of persistent organic pollutants (POPs) (Pierce et al., 2004). The extent to which this leads to mortality is still unclear, but plastic ingestion is responsible for blockage of the GI tract, perforations of the intestines, reduced food consumption, satiation leading to decreased feeding, and increased exposure to toxins (Young et al., 2009; Parker et al., 2020). Marine-adapted birds, or seabirds, are increasingly victims of plastic ingestion and the toxins they carry, reducing their body condition,

decreasing their reproduction rate, and increasing their mortality rate (Lavers et al., 2014; Spear et al., 1995).

Clark (2021) suggested that the physiology and feeding habits of seabirds makes it unlikely that the birds are ingesting microplastics directly; instead, he proposed that they are receiving plastics via "secondary exposure" from the fish on which they prey. This secondary exposure pathway has been detected in other large marine species such as sea turtles, via indirect ingestion (Clark, 2021; Nelms et al., 2015). Carlin et al. (2020) explored whether plastic intake varied among birds with differing feeding strategies and determined that terrestrial birds ingested much higher quantities of plastics, but they seemed to be doing so directly. Conversely, marine birds ingested smaller quantities of plastic but did so via indirect ingestion; this study suggested foraging strategies determined how microplastics were consumed, observing that predators that consumed prey whole had greater consumption of plastic through trophic transfer (Carlin et al., 2020).

Marine plastics are responsible for other morbidities aside from damage to the GI tract and the carrying of POPs. Studies are now suggesting that plastics may affect basic biological processes, such as the assembly and structuring of endoparasite communities (Carlin et al., 2020). Endoparasites are parasites that live within their host and depend on them for survival. Parasitism is a form of symbiosis, where a parasite species lives in association with its host(s) and derives some benefit while causing some harm to their host(s) (Rohde, 2013). Parasite communities provide invaluable information on feeding behavior, dietary information, and long-term movements of their hosts (Hernandez-Milian et al., 2019; Pennino et al., 2020). Various endoparasites can be found in birds, causing little to no distress to healthy individuals; some types include nematodes, trematodes, cestodes, acanthocephalans, and protozoa (Papini et al., 2012). Although some parasites penetrate their hosts, most are transmitted between hosts trophically, i.e., via ingestion; this is particularly true of bird endoparasites (Farrell et al., 2013). This form of transmission reveals host feeding preferences and identifies linkages within food webs, reflecting long-term trends in feeding ecology (Marcogliese & Cone, 1997; Farrell et al., 2013). It is not known how microplastics affect endoparasite communities in sea birds and if there could be a linkage between the presence of microplastics and composition and community structure of various endoparasites found in the GI tract of seabirds. Studies have been done on

the endoparasite microplastics relationship of other marine fauna such as seals, sardines, and anchovies (Hernandez-Milian et al., 2019; Pennino et al., 2020), although the studies found have mixed results. In grey seals, Hernandez-Milian et al. (2019) determined that although microplastics tended to accumulate in areas with high parasite load, there was no relationship between the number of parasites and number of microplastics found. On the other hand, in sardines and anchovies, Pennino et al. (2020) determined that parasite prevalence rate was positively correlated with microplastic abundance for both species. This study aimed to determine what the relationship would be in three different seabird species.

Study Species

The three seabird species studied during this project were the double-crested cormorant (*Phalacrocorax auritus*), brown pelican (*Pelecanus occidentalis*), and osprey (*Pandion haliaetus*). All species were collected from the Southeastern Florida region and typically inhabit the area year-round along the coastline. These species are generalists, mostly foraging on or near the ocean surface, feeding on fishes and invertebrates (Clark, 2021; Zaias et al., 2000); they usually feed in shallow water, catching prey via diving (Enstipp et al., 2006; Schreiber et al., 1975; Vana-Miller, 1987). That said, seabirds are known to be opportunistic, alternating between inshore, coastal, and offshore foraging areas in response to shifting prey abundance (Shealer, 2002). Common prey includes large and medium-sized fish such as the Atlantic needlefish, Scaled Sardine, and the Striped Mullet (Johnson et al., 2002). They typically live 20-40 years and are considered "Least Concern" by the International Union for the Conservation of Nature (*The IUCN Red List of Threatened Species*, 2023).

Double-crested cormorants are birds with a long black body and orange beak, typically weighing between 1.2-2.5kg, with a wingspan between 114-123cm as adults. The oldest documented double-crested cormorant lived to 22 years old, but typically these birds live only up to six years old (Hatch et al., 1999; Johnson et al., 2002; Blus et al., 1979). Nesting areas include atop trees or on the ground on rocks or reefs that lack vegetation. Females typically lay 1-2 broods per season, each brood producing 1-7 eggs (Hatch et al., 1999). Their primary feeding strategy is diving from the surface and chasing prey underwater, using their feet for momentum, typically eating around 0.45-0.68kg of fish a day (Nakama, 2018). Double-crested cormorants host a variety of endoparasites, including acanthocephalans, digeneans, cestodes, and nematodes

(Wagner et al., 2012). Nakama (2018) found 33 different species during their investigation, with a species richness (number of parasite species seen per individual bird) of 15 (9 digeneans, 1 cestode, 3 nematodes, and 2 acanthocephalans), and an 81.8% prevalence rate for the percent infection rate of each host species examined.

Brown pelicans are large birds with long, thick bills with gray expandable pouches to hold food. Adults have brownish-gray bodies and bright yellow and white heads. Adults typically weigh between 2.7-3.18kg with a typical wingspan of 200cm. The oldest documented living Brown pelican lived for 43 years, and usually, they live for longer than a decade (Anderson & Hickey, 1970). In Florida, these seabirds mostly nest in mangroves, brooding once per year with 2-4 eggs per brood (Sachs & Jodice, 2009). Their primary feeding strategy is to use a plunge-diving method, but they frequently participate in scoop-feeding or kleptoparasitism, robbing other animals of food, and typically consume about 1.8kg of fish a day (Clapp et al., 1982). Brown pelicans host a variety of endoparasite species, including acanthocephalans, digeneans, cestodes, and nematodes. Nakama (2018) identified 33 different species of endoparasites with species richness of 16 (9 digeneans, 1 cestode, 4 nematodes, and 2 acanthocephalans), and 75.8% of prevalence. Their broad foraging strategies in coastal and offshore areas are likely the reason for the brown pelican's extensive expose and occurrence of different taxa present in the study (Nakama, 2018).

Ospreys are large, distinctively shaped hawks with slender bodies and long narrow wings and legs. Ospreys are not considered true seabirds because they forge in marine and freshwater habitats, exposing them to endoparasites of seabirds and terrestrial birds (Forrester & Spalding, 2003). They fly with a marked kink in their wings, making an M-shape. Osprey adults typically weigh between 0.9-2.1kg, with a 127-180cm wingspan. The oldest documented living individual lived to 30 years of age, but typically they do not live past 7-10 years (Toschik et al., 2006). Their primary feeding strategy is a plunge-diving method, typically consuming about 0.3kg of fish a day. Nesting areas are typical atop large trees, and they continue to use the same nest in later years, adding to the depth each time. They brood only once per year, producing 1-4 eggs each time (Poole, 1982; Toschik et al., 2006). Ospreys hosts endoparasites, including trematodes, cestodes, nematodes, and acanthocephalans (Schmidt et al., 1985). Nakama (2018) distinguished

27 different species of endoparasites with species richness of 11 (8 digeneans, 1 cestode, 1 nematode, and 1 acanthocephalan), and 88.9% of prevalence.

Expectations

This study aimed to describe and quantify microplastics found in three species of Southeastern Florida seabirds and determine the relationship between microplastics and endoparasite community structure and composition. A previous study done by Clark (2021) quantified and differentiated microplastics from the same seabirds as this proposed project but did not address their effect on endoparasite communities. Microplastics are known to damage the GI tract of seabirds, cause starvation, and transfer persistent organic pollutants (Moore, 2008). Disruption of feeding habits and other biological processes could indicate further morbidities that may be seen when investigating vital endoparasite communities. Studying such changes could give insight into long-term trends in the diet and feeding ecology of the host birds (Sukhdeo & Hernandez, 2005). Endoparasites of birds are typically acquired tropically i.e., via ingestion of their previous host in a previous life cycle (Perrot-Minnot et al., 2023). This study was significant because it not only attempted to understand current patterns between microplastics and endoparasites but potentially allow for knowledge of the future effects of plastic pollution marine ecosystems, particularly in the region of study.

The goal of this study was to address the knowledge gap surrounding the effect that microplastics have on endoparasite communities, and how parasite community structure varies due to toxic microplastics that only continue to accumulate in marine ecosystems. Similar research has been performed on other types of animals but does not determine what will be concluded during this experimentation. It was expected that endoparasite abundance is positively correlated with microplastic load and that birds with similar microplastic loads have structurally similar parasite communities. It was anticipated that microplastics will accumulate and be found in areas with high endoparasite populations and there will be a positive correlation between microplastics and endoparasite populations (Hernandez-Milian et al., 2019; Pennino et al., 2020).

Materials and Methods

Specimen Collection

All bird specimens were collected from rescue and rehabilitation centers in the South Florida region: the Florida Keys Wild Bird Center (FKWBC), the South Florida Wildlife Center (SFWC), the Key West Wildlife Center, and the Pelican Harbor Seabird Station. All specimens were donated by the centers gradually throughout the experiment and arrived at Nova Southeastern University (NSU) frozen. The specimen's cause of death varied individually: death upon arrival, euthanasia due to injury, or death during treatment. No species were killed for use in research, and all were donated after being collected opportunistically. All species were previously frozen after death and were given a unique identification number upon arrival at the lab. Before each examination, various metrics were collected for each individual, such as: sex, anticipated age (juvenile versus adult), body condition, mass, wingspan, wing cord, tail length, tarsus length, bill depth, bill from nostril length, bill from feathers length, and bill from base length (Labocha & Hayes, 2012; Senar & Pascual, 1997). After reviewing literature from both Clark (2021) and Nakama (2018), a minimum sample size of 20 birds per species was estimated to provide the best pool of species for accurate results. Due to a variety of unforeseen circumstances, the final sample size did not reflect 20 birds per species; brown pelican (BRPE) consisted of most of the samples taken (n=14), followed by the double-crested cormorant (DCCO) (n=9), and the osprey (OSPR) (n=3).

Parasite Collection

Birds were dissected using the following protocols: the sternum was opened, and the organs removed; the gastrointestinal tract (GI tract), including the esophagus, stomach, and intestines, were examined for parasites. Each organ was separated from the other and opened, removing any parasites and food using a stir-rinse-repeat cycle into glass jars filled with tap water. Decantation was done to separate the liquid from the solids in the mixture and allowed the debris to settle via gravity to the bottom of the glass, leaving the liquid to be safely removed. All organs of the GI tract and liver were collected for further examination of microplastics. All contents were reviewed for parasites under a dissecting microscope, and organs were placed between two glass plates before being inspected (McLaughlin, 2001).

Not all parasites observed were collected for further identification. During the dissection process parasites were identified individually under the microscope to the lowest taxonomic level and were counted out and recorded for later statistical analysis. Approximately 10-20 samples of

each type of parasite identified were then collected and properly stored. Platyhelminthes (digeneans and cestodes) were collected and stored in 70% ethanol before staining in acetocarmine, followed by dehydration in ethanol, clearing in clove oil, and mounting in Permount on glass slides. Nematodes were collected and stored in 70% ethanol/ 30% glycerol. They were progressively cleared in glycerol and semi-permanently mounted in glycerin jelly on glass slides. After mounting, all parasites were identified to the lowest possible taxonomic level using species identification from literature and taxonomic keys (Pritchard & Kruse, 1982). Upon identification, metrics for endoparasite abundance were determined, such as prevalence, mean abundance, component community species richness, and intracommunity species richness (Bush et al., 1997).

Microplastics Collection

As mentioned previously, the GI tract, liver, and decanted matter were processed for the examination of microplastics. During all microplastic processing procedures, a hairnet, cotton clothing, and nitrile gloves were worn to ensure no outside contamination; lab procedures were done under a workstation hood. All contents were processed at room temperature and immersed in 10% potassium hydroxide (KOH) to break down organic matter, allowing only the microplastics to remain; amount of KOH was used at a 3:1 ratio to the volume of material used (Abbasi et al., 2018; Karami et al., 2017). Contents were divided into glass mason jars with one jar containing a blank of potassium hydroxide, one for the liver, and one with the contents of the entire GI tract; the jars also contained the liquids collected from the decants associated with them. Once all decant liquid was collected, an average of 10 mason jars were required per bird for the organic material break down process. Blank samples, consisting solely of KOH, were provided to account for any outside containments and were added between each bird is processed. Jars were loosely covered with aluminum foil to allow expulsion of any gases associated with decomposition and prevent outside debris from entering.

Jars were left undisturbed, inside a workstation hood, for approximately two to four weeks, time-varying based on how quickly the organic matter was broken down. If organic material did not break down fully by the two-week mark, the contents of the jar were divided, and an additional portion of 10% KOH was added. In these cases, the jars were also placed on a hot plate with low heat and a magnetic stir bar for five minutes before being placed once again in

the workstation hood for additional time. Based on a previous study by Clark (2021), it was expected that there may be some difficulty in breaking down the fatty oils found in the seabirds, at which time solvalene, a commercial degreasing solvent, was required to allow for complete dissolution. The amount of solvalene added will vary by individual specimen; some may require more than others, depending on size and amount of matter remaining. During this study, solvalene was not used or required as most of the fatty components were removed during the initial dissection process. The remaining liquid was filtered through a one-micrometer (μm) filter on a Buchner funnel using a vacuum flask. Each mason jar took approximately two to four hours of filtration before being completed. Filters were transferred to a labeled peri dish and left to dry for 24-48 hours in an AirClean 600 workstation hood. Once filters are dry, they were analyzed under a dissecting microscope to identify the presence of microplastics better; size, color (light, mid, dark), and type of particle (fiber versus fragment) are the characteristics that were used to distinguish the pieces (Barrows et al., 2017; Battaglia et al., 2020). Blank samples were examined as a medium to determine the presence of contaminants in the study; it was expected that the blank would show no fibers or findings to showcase that the jars were not exposed to any outside particulates.

Statistical Analysis

Statistical analysis was done using the coding program R Studio. Descriptive statistics were used to determine various metric parameters for each individual bird and the microplastic particles found. A correlation test was done to determine if microplastic abundance affected types of parasites found. A correlation test was completed to determine the relationship between the total amount of microplastic and the total amount of parasites found in each individual bird species. A correlation test was also performed to determine the relationship between the total amount of microplastics found in the liver and the total microplastics found in the GI tract for each individual bird species.

Results

Specimen Collection

A total of 26 birds across the three different species being evaluated were collected and sampled throughout the course of this experiment. The brown pelican (BRPE) consisted of most

of the samples taken (n=14), followed by the double-crested cormorant (DCCO) (n=9), and the osprey (OSPR) (n=3). Most of the samples were collected from the Florida Keys Wild Bird Center (FKWBC) (n=16), while all the remaining samples were collected from the South Florida Wildlife Center (SFWC) (n=10).

Table 1. Sample Origin Location: This table distinguishes which wildlife rehabilitation center, Florida Keys Wild Bird Center (FKWBC) or South Florida Wildlife Center (SFWC), the different bird samples for double-crested cormorants (DCCO), brown pelican (BRPE), and osprey (OSPR) originated from.

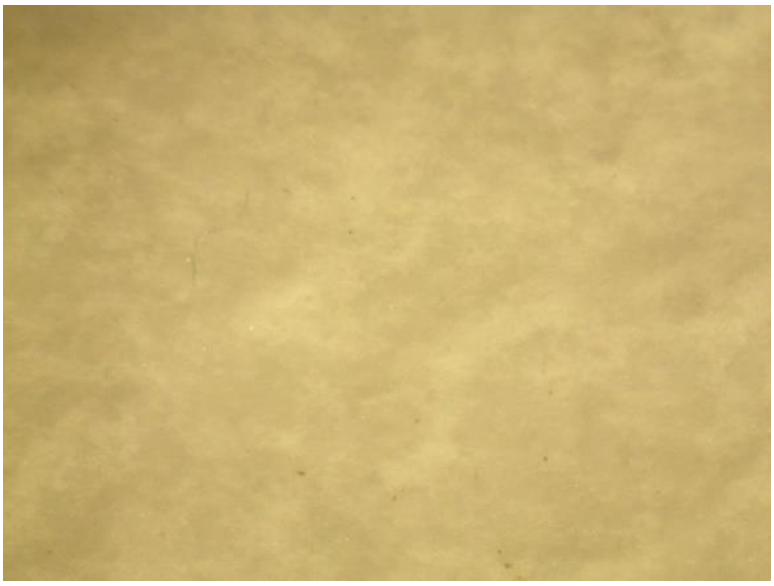
Species	Sample Number	Location
DCCO	3213	FKWBC
DCCO	3214	FKWBC
DCCO	3215	FKWBC
DCCO	3216	FKWBC
DCCO	3217	FKWBC
DCCO	3218	FKWBC
DCCO	3219	FKWBC
DCCO	3221	FKWBC
DCCO	1109	SFWC
OSPR	3129	FKWBC
OSPR	3130	FKWBC
OSPR	3131	FKWBC
BRPE	1121	SFWC
BRPE	1122	SFWC
BRPE	1123	SFWC
BRPE	1125	SFWC
BRPE	1126	SFWC
BRPE	1127	SFWC
BRPE	1128	SFWC
BRPE	1129	SFWC
BRPE	1130	SFWC
BRPE	3175	FKWBC
BRPE	3176	FKWBC
BRPE	3177	FKWBC
BRPE	3174	FKWBC
BRPE	3178	FKWBC

Morphometric Measurements

Brown pelicans had the greatest average total weight (2.6±0.7kg), followed by osprey (1.9±0.76kg), and the double-crested cormorants (1.0±0.3kg). Brown pelicans had the longest wingspan (180±20.5cm), followed by ospreys (148.7±10.3cm), and the double-crested cormorant (88.8±23.5cm). Brown pelicans had the longest tarsus length (12.4±8.7cm), followed by double-crested cormorant (8.0±2.7cm), and osprey (5.7±1cm).

Blank Filters

During the process to collect and identify the microplastic particles, a "blank" jar that consisted only of KOH was stored with all the others in the workstation hood; this blank was later filtered as the last sample in each specimen's jar collection, and observed to determine if contamination was present. Preventing contamination during the study was increasingly difficult and despite measures being taken to prevent it, contamination was still found on nearly all the blanks. Although contamination was not found in the form of microplastic beads or fibers as seen for the other filters, some type of particle was still seen and present.



Photograph 1. Brown Pelican 3174 Blank Filter: This is a photograph taken from the blank KOH sample for the brown pelican 3174 filter. It is an example of the small unidentified particles found in many of the blank samples.

Photograph 2. Double-crested Cormorant 3219 Blank Filter: This is a photograph taken from the blank KOH sample for the Double- crested Cormorant 3219 filter. It is an example of the small unidentified particles found in many of the blank samples.

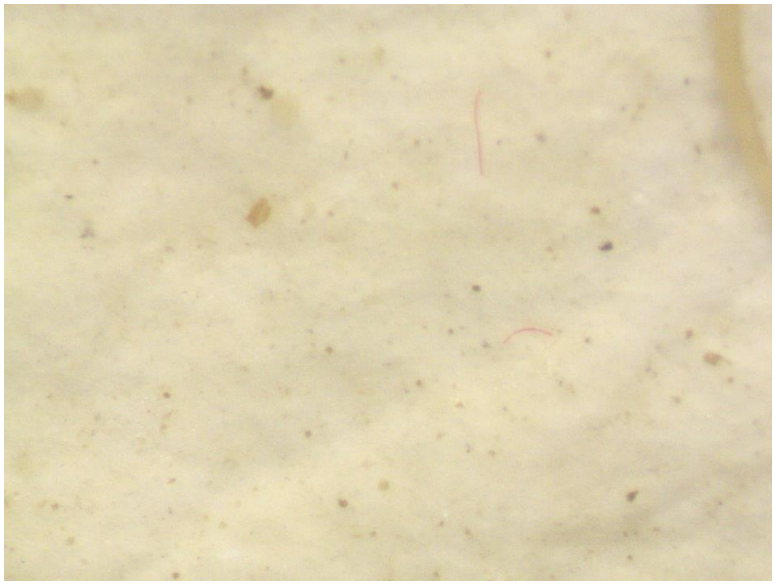
Microplastic Data

A total of 12,995 microplastic elements were counted across all three species of seabirds, located across the liver and gastrointestinal tract. The most abundant of the microplastics found were those of microplastic beads, with a total of 12,727 beads counted across all three species in both the liver and GI tract. The brown pelican consisted of the most microplastic beads across all samples with a total of 10,186 beads counted, 179 located in the liver, and 10,007 located in the GI tract. Followed by the double-crested cormorant with a total of 2,652 beads counted, 468 located in the liver, and 2,184 located in the GI tract. Osprey with a total of 157 beads counted, 16 located in the liver, and 141 located in the GI tract. The greatest number of microplastic beads were located in the GI tract for all three species.

Although there are various types of microplastics that could have been found during the experiment, microplastic beads, or fiber, were found across all three species with almost no other forms identified. In addition to microplastic beads, only one other form of microplastics was identified, microplastic strands. Across all species, a total of 268 microplastic strands were counted, located in both the liver and GI tract. Osprey had the largest amount of microplastic strands counted with a total of 113 strands counted, 10 located in the liver, and 103 located in the GI tract. Followed by double-crested cormorant with a total of 85 strands counted, 9 located in the liver, and 76 located in the GI tract. Brown pelican with a total of 70 strands counted, 7 located in the liver, and 63 located in the GI tract. The largest number of microplastic strands found were located in the GI tract for all three species.

The average number of microplastics found in brown pelicans was greater than the other species (727.6±1348.3), followed by double-crested cormorant (294.7±335.7), and the osprey (52.3±26.0).

Photograph 3. Brown Pelican 1131 GI Tract Filter: This is a photograph taken from one of the GI tract samples for the brown pelican 1131 filter. It is an example of microplastic beads and how they may appear under the microscope.



Photograph 4. Osprey 3129 GI Tract Filter: This is a photograph taken from one of the GI tract samples for the osprey 3129 filter. It is an example of microplastic fibers and how they may appear under the microscope, these fibers seen are of red coloration.

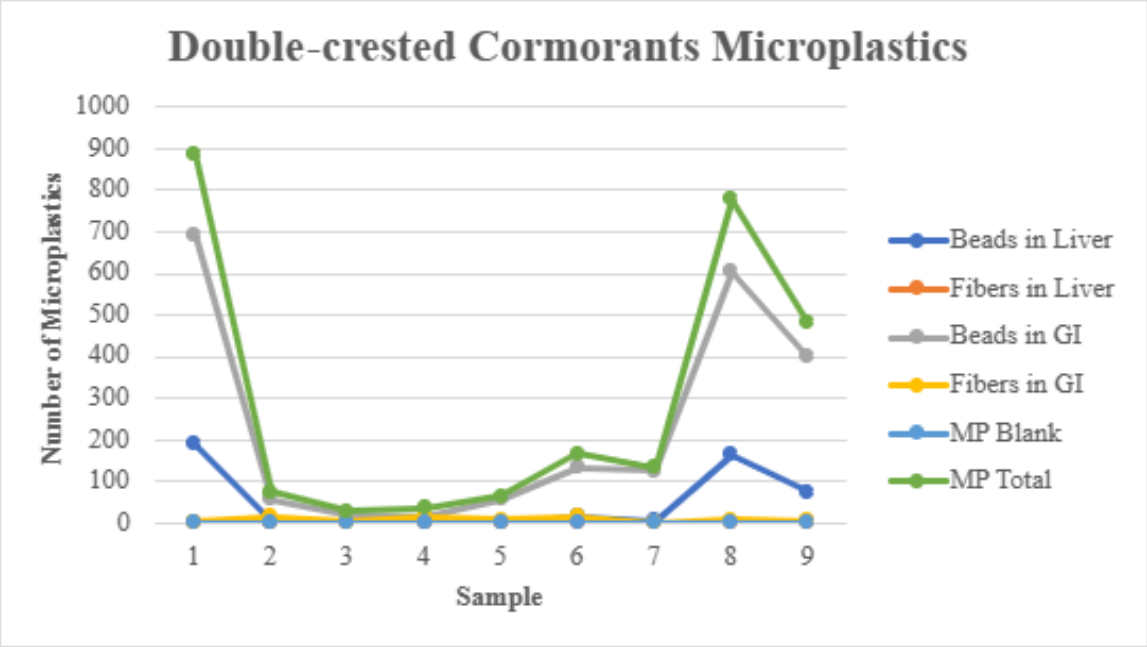


Figure 1. Microplastic Abundance Double-crested Cormorant: This scatterplot depicts how microplastic abundance varied based on location and type of microplastic detected. It also includes information for overall microplastic total abundance, and microplastic count found in the blank KOH samples. This scatterplot shows trends across the samples collected in double-crested cormorant (n=9).

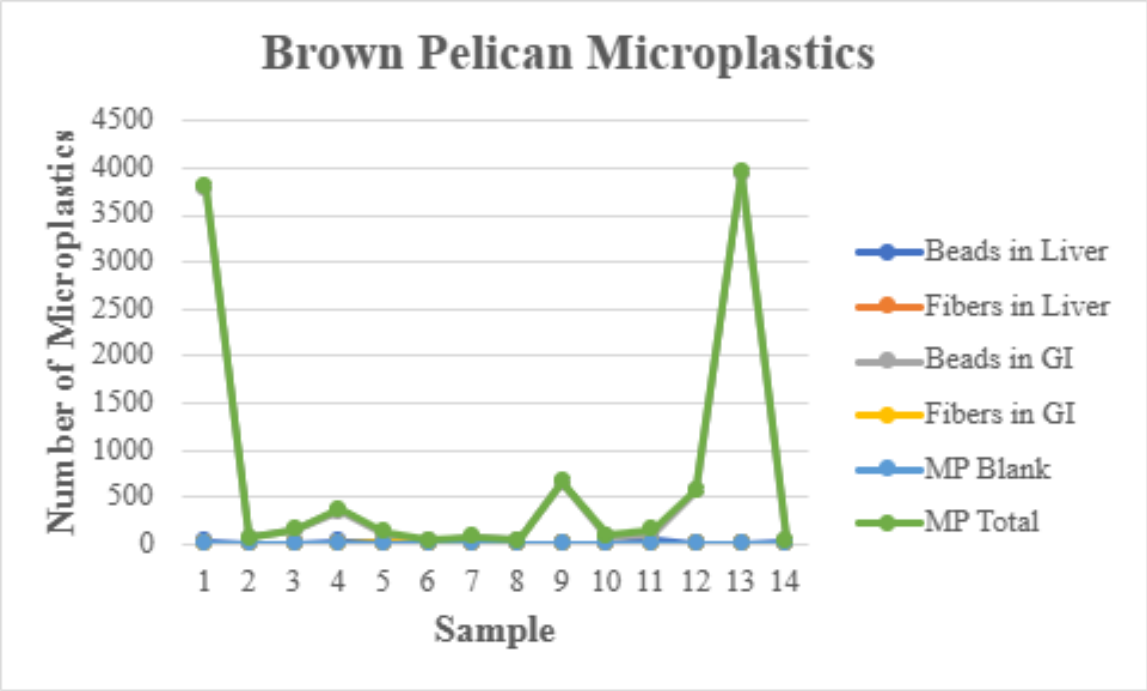


Figure 2. Microplastic Abundance Brown Pelican: This scatterplot depicts how microplastic abundance varied based on location and type of microplastic detected. It also includes information for overall microplastic total abundance, and microplastic count found in the blank KOH samples. This scatterplot shows trends across the samples collected in brown pelican (n=14).

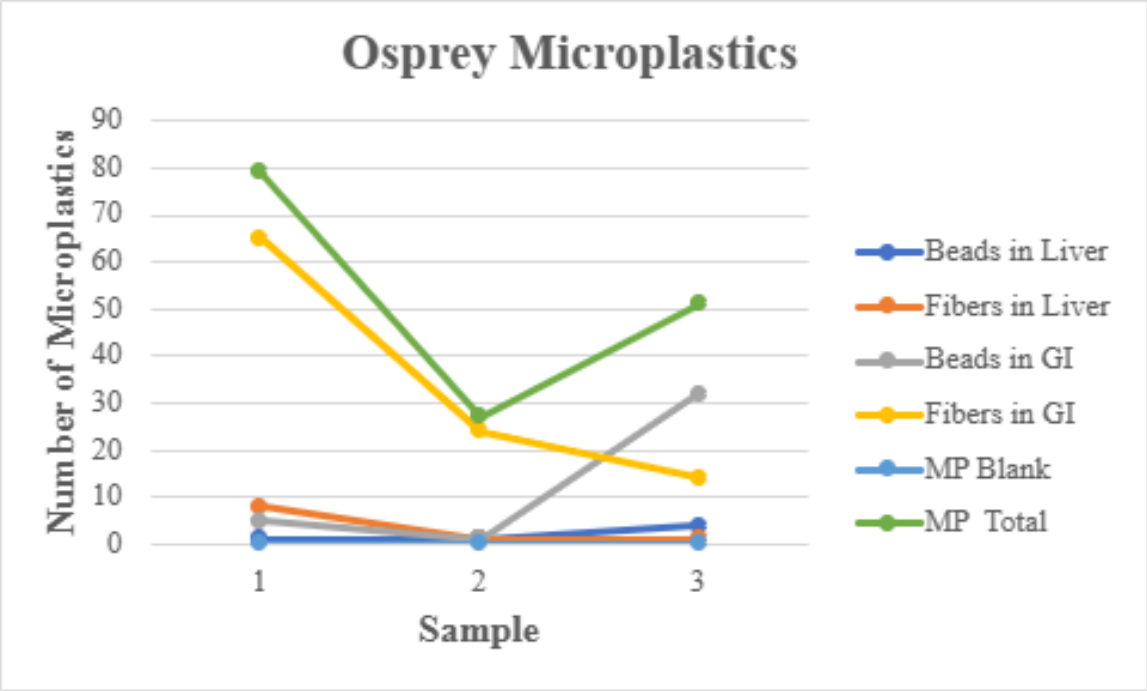


Figure 3. Microplastic Abundance Osprey: This scatterplot depicts how microplastic abundance varied based on location and type of microplastic detected. It also includes information for overall microplastic total abundance, and microplastic count found in the blank KOH samples. This scatterplot shows trends across the samples collected in ospreys (n=3).

Parasite Data

A total of 1,957 parasites were counted across all three species of seabirds, located in the gastrointestinal tract; no samples for parasites were collected from the liver. The brown pelican consisted of the most parasite samples across all species (n=825), followed by the double-crested cormorant (n=762), and the osprey (n=370). The average number of parasites found in osprey (123.3±193.7) was largest, followed by double-crested cormorant (84.7±36.7), and brown pelicans as the lowest (58.9±69.4).

Double-crested cormorants had the largest number of parasites counted in the esophagus (11.3±12.7), followed by brown pelican (5.8±11.8); there were no parasites detected in the esophagus of any osprey. Double-crested cormorants had the largest number of parasites counted in the stomach (68±39), followed by brown pelican (51.2±63.4), and osprey (12.7±9.5). Osprey had the largest number of parasites counted in the intestines (110.7±185.7), followed by double-crested cormorants (5.3±5.1) and brown pelican (1.9±2.5). Across all three species, four types of parasites were identified: nematodes, digeneans, acanthocephalans, and cestodes. Identification was completed under the microscope during the dissection of each specimen; at that time the parasites were counted out and recorded to distinguish between the tissue location across the parasite species.

Parasites were recorded across three main sections of the GI tract for the sampled specimens: the stomach, esophagus, and intestines. For double-crested cormorants averages were taken to distinguish the parasite species found across the various tissue samples. The stomach had on average 60.6 ± 36.1 nematodes, 0 ± 0 digeneans, 1.9 ± 5.7 acanthocephalans, and 5.6 ± 15.6 cestodes. The esophagus had on average 6.7 ± 10.7 nematodes, 0.11 ± 0.33 digeneans, 0.33 ± 1.0 acanthocephalans, and 4.2 ± 8.5 cestodes. The intestines had on average 3.3 ± 3.5 nematodes, 0 ± 0 digeneans, 1.7 ± 5 acanthocephalans, and 0.33 ± 1.0 cestodes. Across all tissue samples an average of 70.6 ± 40.1 nematodes, 0.11 ± 0.33 digeneans, 3.9 ± 7.7 acanthocephalans, and 10.1 ± 21.9 cestodes were detected.

For brown pelicans averages were taken to distinguish the parasite species found across the various tissue samples. The stomach had on average 39.8 ± 62.7 nematodes, 0.14 ± 0.53 digeneans, 1.3 ± 4.8 acanthocephalans, and 10 ± 18.2 cestodes. The esophagus had on average 4.2 ± 8.2 nematodes, 0 ± 0 digeneans, 0.36 ± 1.3 acanthocephalans, and 1.2 ± 4.3 cestodes. The

intestines had on average 0.93 ± 1.4 nematodes, 0 ± 0 digeneans, 0.43 ± 1.6 acanthocephalans, and 0.14 ± 0.53 cestodes. Across all tissue samples an average of 44.9 ± 66.9 nematodes, 0.14 ± 0.53 digeneans, 2.1 ± 7.8 acanthocephalans, and 11.4 ± 21.6 cestodes were detected.

For osprey averages were taken to distinguish the parasite species found across the various tissue samples. The stomach had on average 3.3 ± 3.5 nematodes, 5.0 ± 8.7 digeneans, 1.0 ± 1.7 acanthocephalans, and 3.3 ± 5.8 cestodes. The esophagus had on average 0 ± 0 nematodes, 0 ± 0 digeneans, 0 ± 0 acanthocephalans, and 0 ± 0 cestodes. The intestines had on average 0 ± 0 nematodes, 108.3 ± 187.6 digeneans, 2.3 ± 4.0 acanthocephalans, and 0 ± 0 cestodes. Across all tissue samples an average of 3.3 ± 3.5 nematodes, 113.3 ± 196.3 digeneans, 3.3 ± 5.8 acanthocephalans, and 3.3 ± 5.8 cestodes were detected.

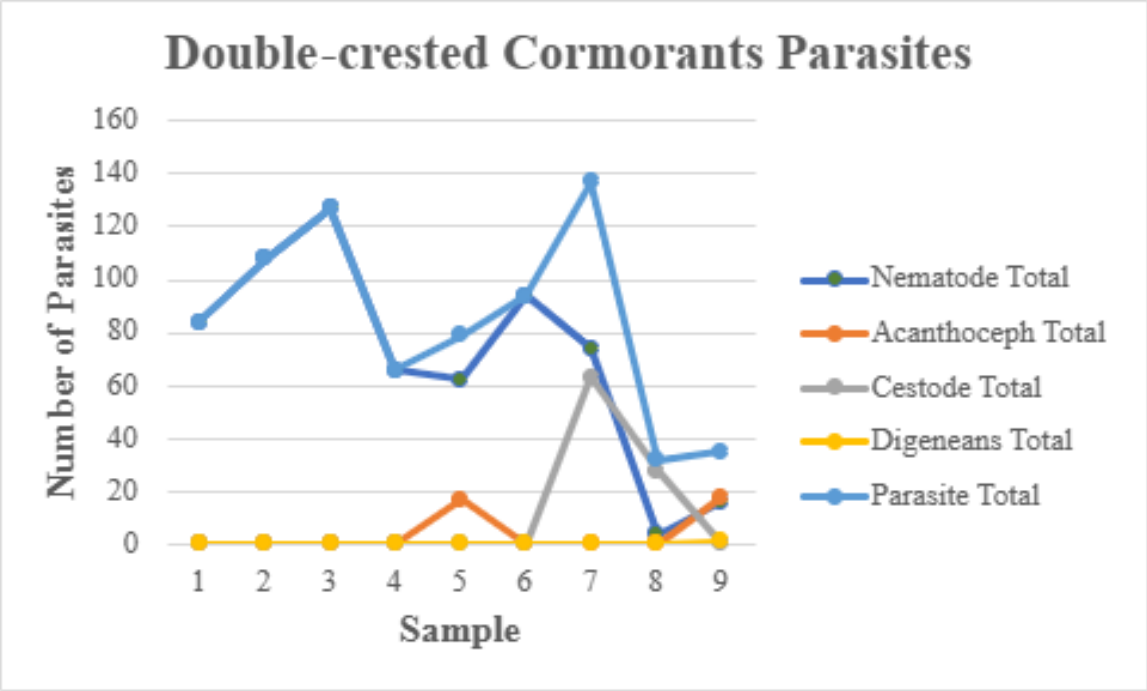


Figure 4. Parasite Abundance Double-crested Cormorant: This scatterplot depicts the totals for types of parasites detected as well as parasite total overall across the various samples. Double-crested cormorant (n=9) samples are depicted.

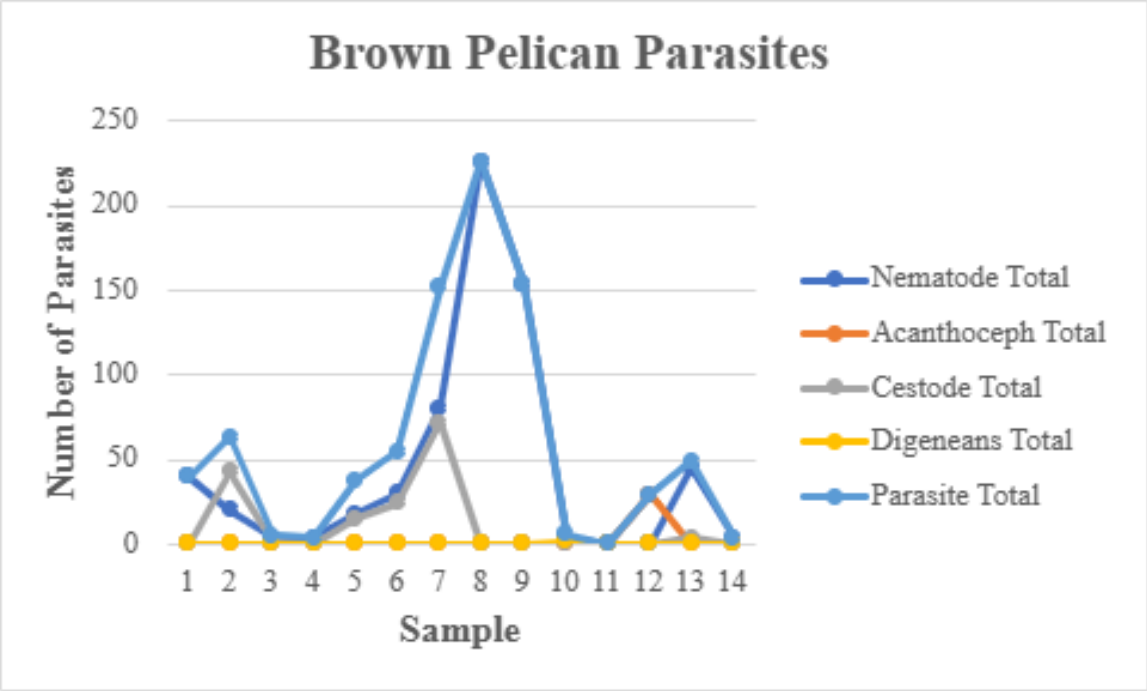


Figure 5. Parasite Abundance Brown Pelicans: This scatterplot depicts the totals for types of parasites detected as well as parasite total overall across the various samples. Brown pelicans (n=14) samples are depicted.

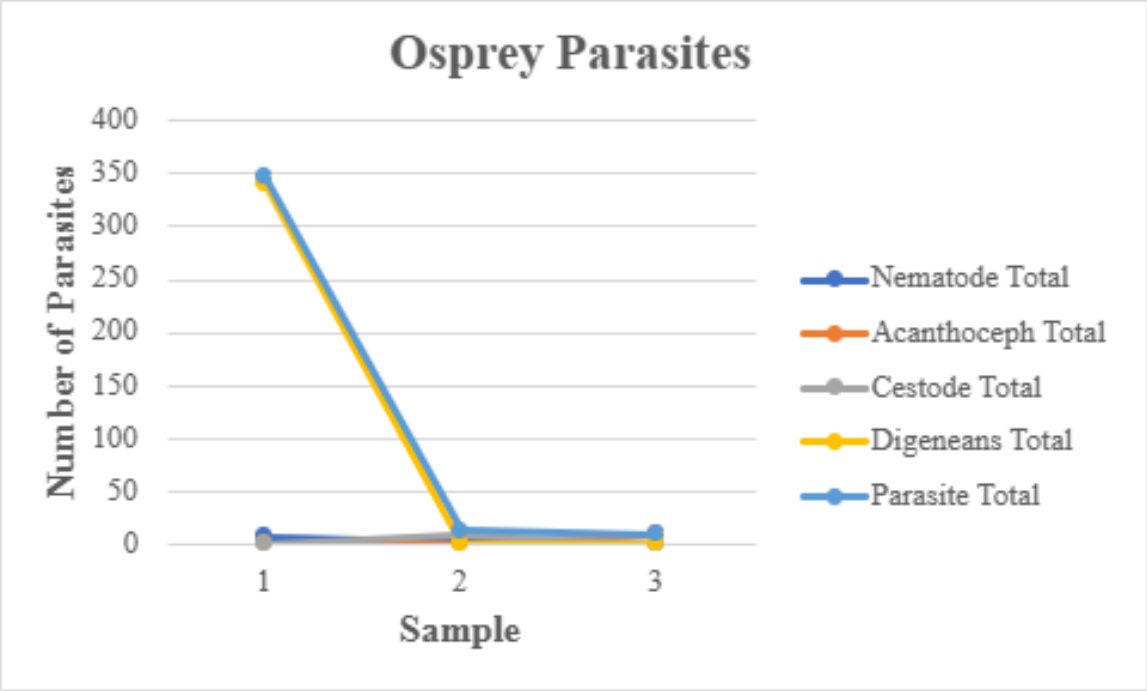


Figure 6. Parasite Abundance Osprey: This scatterplot depicts the totals for types of parasites detected as well as parasite total overall across the various samples. Osprey (n=3) samples are depicted.

Descriptive Statistics: Osprey

Unfortunately, the sample size of the osprey was not significant enough to consider important questions such as the correlation between microplastic total versus parasite total, and microplastic in liver total versus microplastic in GI tract total. Due to the small sample size sourced for the osprey species (n=3), descriptive statistics were the only statistical analysis able to be completed to interpret the data. The values for total amount of microplastics found in each individual bird were run to determine different parameters and understand the values found. The mean of the microplastic total was 52.3, median of 51, and range of 52. The interquartile range is 26 and when compared to the range indicates platykurtic, or negative kurtosis, a measurement of spread observations. The standard deviation for microplastic total was 26.0, with a range of variance of 677.3, and standard error of 15.0. The high variance for microplastic total indicates the data is very spread out from the mean and from one another; this is quantified by the high standard deviation indicated. Confidence interval for microplastic total was estimated at 52.3, [-12.3, 117].

The values for total amount of parasites found in each individual bird were run to determine different parameters and understand the values found. The mean of the parasite total was 123.3, median of 13, and range of 337. The interquartile range is 168.5 and when compared to the range indicates platykurtic, or negative kurtosis, a measurement of spread observations. The standard deviation for parasite total was 193.7, with a range of variance of 37522.3, and standard error of 111.8. Confidence interval for parasite total was estimated at 123.3, [-357.9, 604.5]. The high variance for parasite total indicates the data is very spread out from the mean and from one another; this is quantified by the high standard deviation indicated.

Microplastic Total vs Parasite Species

Sample size for the double-crested cormorants (DCCO) and the brown pelicans (BRPE) was substantial enough to perform a variety of statistical analysis to quantify the data. A correlation test was performed to determine the relationship between microplastic total and the various species of parasites that were present.

In double-crested cormorants (DCCO) correlation tests were completed to determine the relationship between microplastic total versus the four various species of parasites detected was

completed. All variables run during these correlation tests were first tested for normality and when parametric assumptions were not met for any variable, the data was transformed using the square root of the data, providing a clearer picture to interpret. Using the transformed data, variables total microplastics and total nematode count were found to be significantly negatively correlated when using a Pearson's correlation test ($t= -2.073$, $df= 7$, $p= 0.077$, $r= -0.617$). For digenean total, transformed variables total microplastics and total digenean count were negatively correlated when tested using a Spearman's correlation test ($S= 87.137$, $p= 0.476$, $r= 0.274$). For cestode total, transformed variables total microplastics and total cestode count were insignificantly correlated when tested using a Spearman's correlation test ($S= 87.137$, $p= 0.476$, $r= 0.274$). For acanthocephalan total, transformed variables total microplastics and total acanthocephalan count were insignificantly correlated when tested using a Spearman's correlation test ($S= 114.52$, $p= 0.907$, $r= 0.046$).

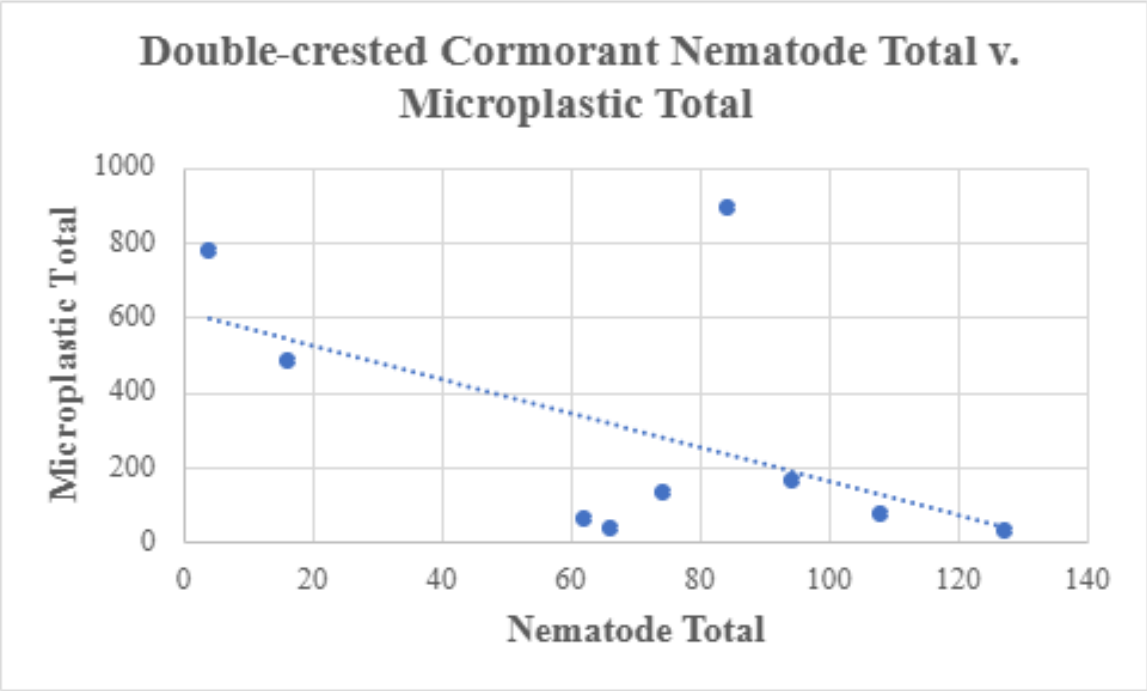


Figure 7. Double-crested Cormorant Nematode Total v. Microplastic Total: This scatterplot depicts how the total number of nematodes compares to the total number of microplastics found in double-crested cormorants. This relationship was insignificantly correlated when using a Pearson's correlation test (t= -2.073, df= 7, p= 0.077, r= -0.617).

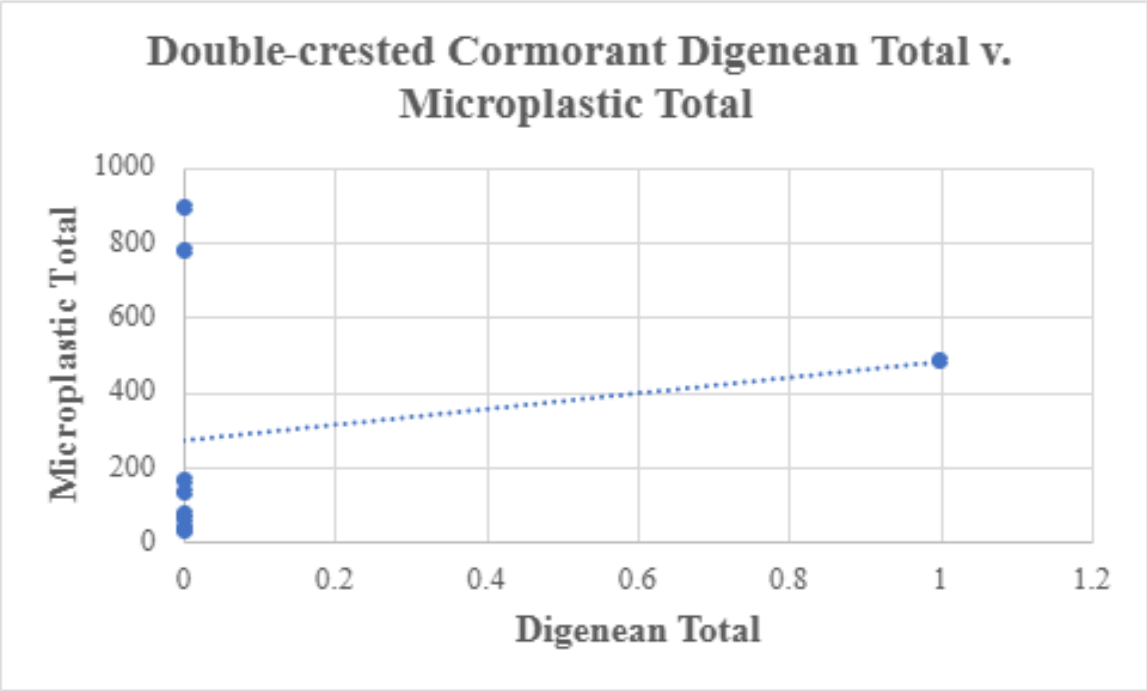


Figure 8. Double-crested Cormorant Digenean Total v. Microplastic Total: This scatterplot depicts how the total number of digenean compares to the total number of microplastics found in double-crested cormorants. This relationship was insignificantly correlated when tested using a Spearman's correlation test (S= 87.137, p= 0.476, r= 0.275).

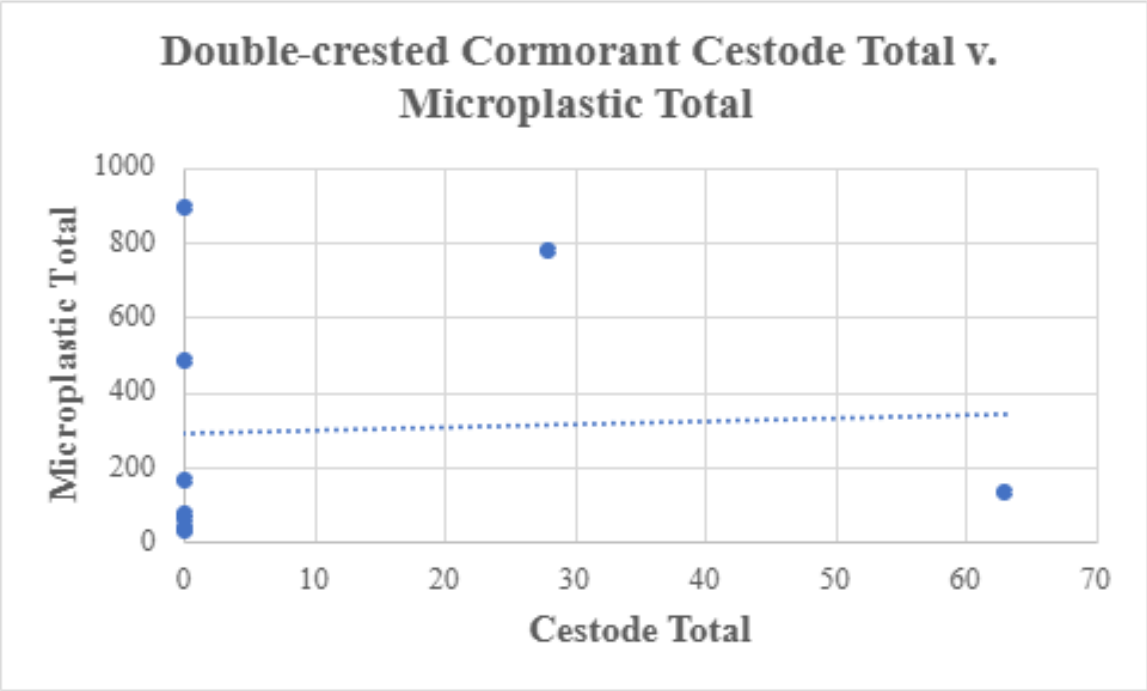


Figure 9. Double-crested Cormorant Cestode Total v. Microplastic Total: This scatterplot depicts how the total number of cestode compares to the total number of microplastics found in double-crested cormorants. This relationship was insignificantly correlated when tested using a Spearman's correlation test (S= 87.137, p= 0.476, r= 0.274).

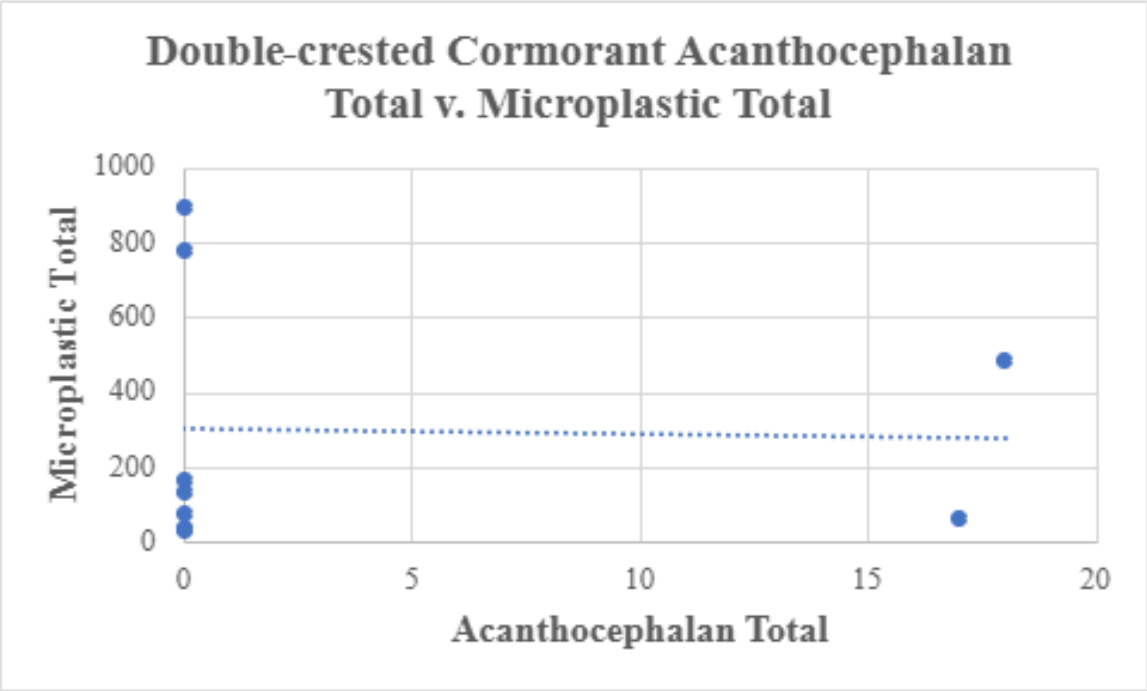


Figure 10. Double-crested Cormorants Acanthocephalan Total v. Microplastic Total: This scatterplot depicts how the total number of acanthocephalan compares to the total number of microplastics found in double-crested cormorants. This relationship was insignificantly correlated when tested using a Spearman's correlation test (S= 114.52, p= 0.907, r= 0.046).

In brown pelicans (BRPE) correlation tests were completed to determine the relationship between microplastic total versus the four various species of parasites detected was completed. All variables run during these correlation tests were first tested for normality and when parametric assumptions were not met for any variable, the data was transformed using the square root of the data, providing a clearer picture to interpret. For nematode total, transformed variables total microplastics and total nematode count were insignificantly correlated when tested using a Spearman's correlation test ($S= 496.55$, $p= 0.756$, $r= -0.091$). For digenean total, transformed variables total microplastics and total digenean count were insignificantly correlated when tested using a Spearman's correlation test ($S= 502.01$, $p= 0.725$, $r= -0.103$). For cestode total, transformed variables total microplastics and total cestode count were insignificantly correlated when tested using a Spearman's correlation test ($S= 596.35$, $p= 0.280$, $r= -0.311$). For acanthocephalan total, transformed variables total microplastics and total acanthocephalan count were insignificantly correlated when tested using a Spearman's correlation test ($S= 345.31$, $p= 0.405$, $r= 0.241$).

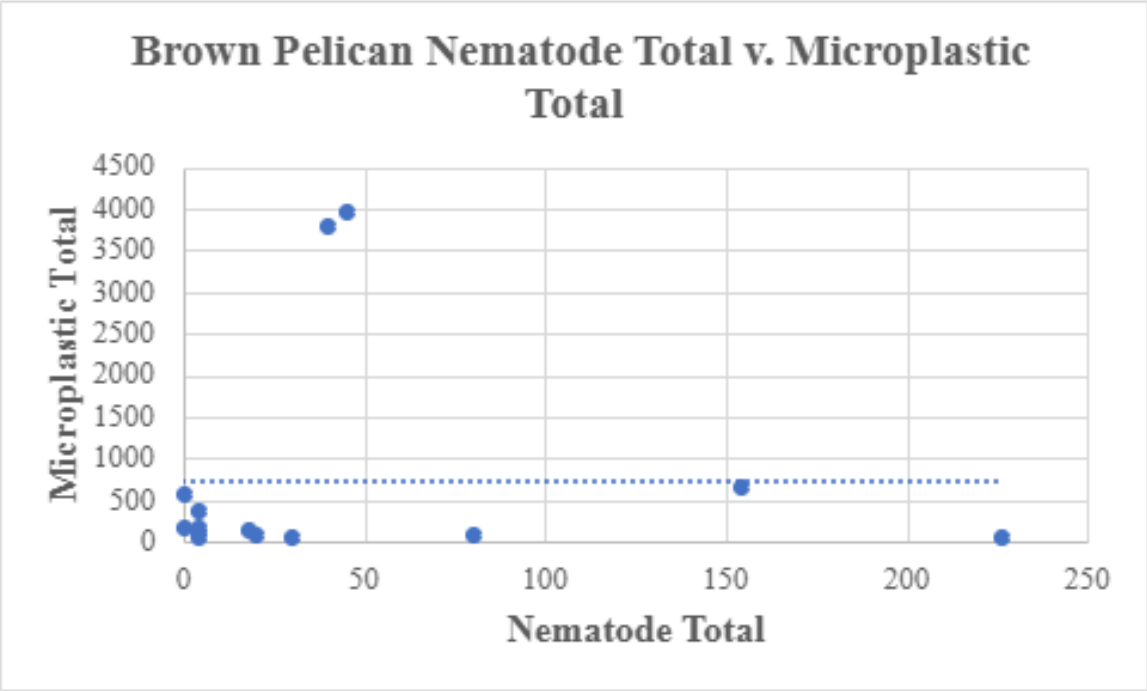


Figure 11. Brown Pelican Nematode Total v. Microplastic Total: This scatterplot depicts how the total number of nematodes compares to the total number of microplastics found in brown pelicans. This relationship was insignificantly correlated when tested using a Spearman's correlation test (S= 496.55, p= 0.756, r= -0.091).

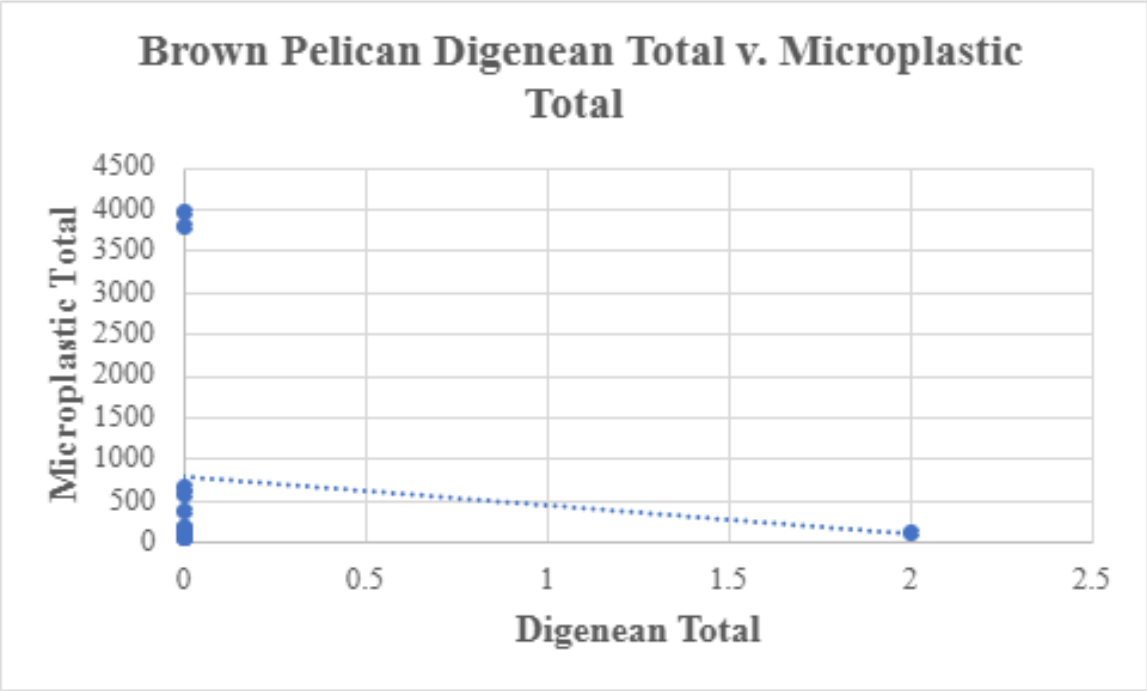


Figure 12. Brown Pelican Digenean Total v. Microplastic Total: This scatterplot depicts how the total number of digeneans compares to the total number of microplastics found in brown pelicans. This relationship was insignificantly correlated when tested using a Spearman's correlation test (S= 502.01, p= 0.725, r= -0.103).

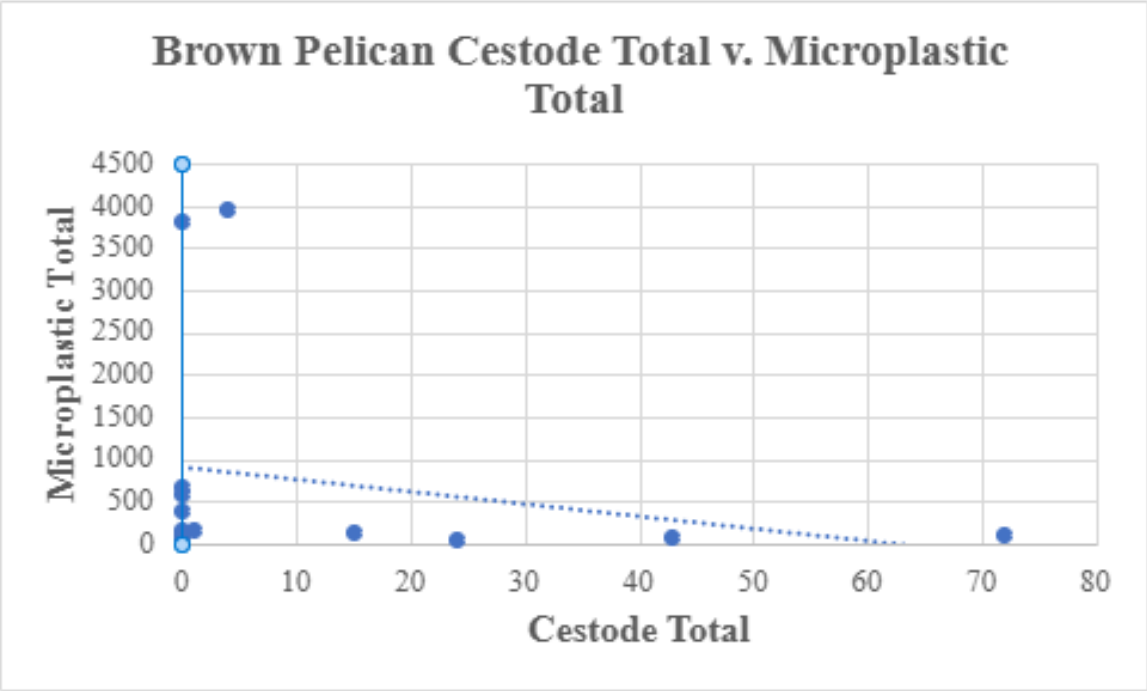


Figure 13. Brown Pelican Cestode Total v. Microplastic Total: This scatterplot depicts how the total number of cestode compares to the total number of microplastics found in brown pelicans. This relationship was insignificantly correlated when tested using a Spearman's correlation test (S= 596.35, p= 0.280, r= -0.311).

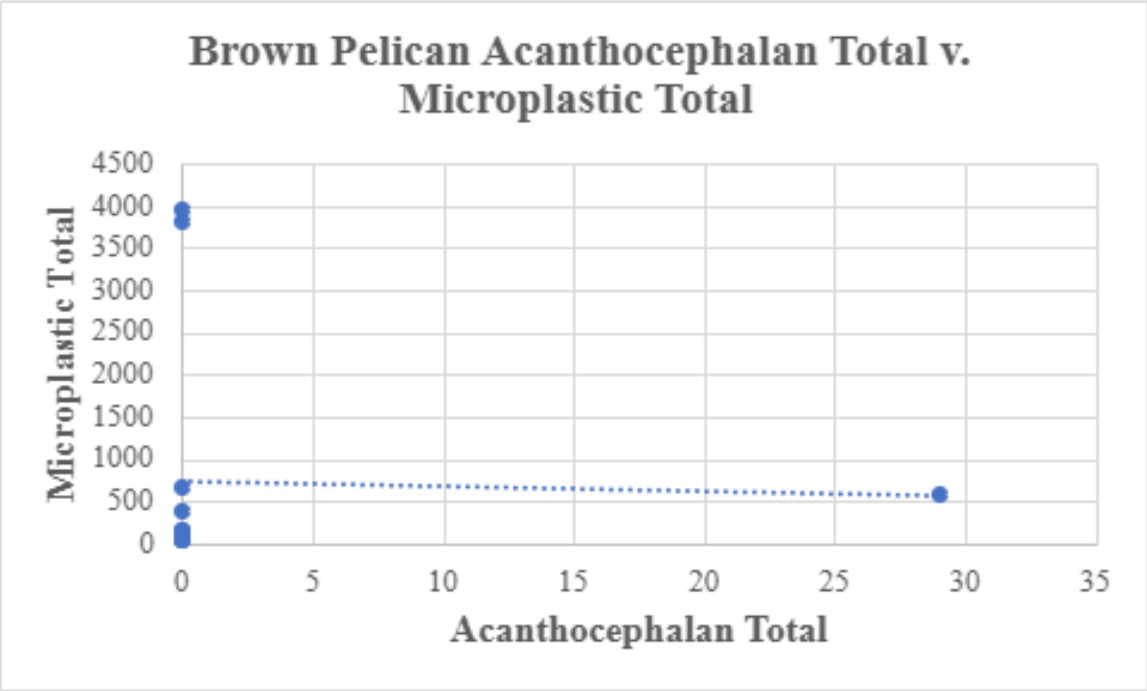


Figure 14. Brown Pelican Acanthocephalans Total v. Microplastic Total: This scatterplot depicts how the total number of acanthocephalans compares to the total number of microplastics found in brown pelicans. This relationship was insignificantly correlated when tested using a Spearman's correlation test (S= 345.31, p= 0.405, r= 0.241).

Microplastic Total vs Parasite Total

A correlation test was performed to determine the relationship between the total number of microplastics and the total number of parasites per individual bird species. Variables total microplastics and total parasites were first tested for normality and when parametric assumptions were not met for either species, the data was transformed using the square root of the data, providing a clearer picture to interpret. Using the transformed data, variables total microplastics and total parasites were found to be insignificantly negatively correlated in double-crested cormorants using a Pearson's correlation test ($t= -1.932$, $df= 7$, $p= 0.095$, $r= -0.590$). Brown pelican transformed variables total microplastics and total parasites were insignificantly correlated when tested using a Spearman's correlation test ($S= 541.19$, $p= 0.517$, $r= -0.189$). The negative correlation for both species indicates that total microplastics and total parasites are not trending in the same direction and cannot be used to predict one another.

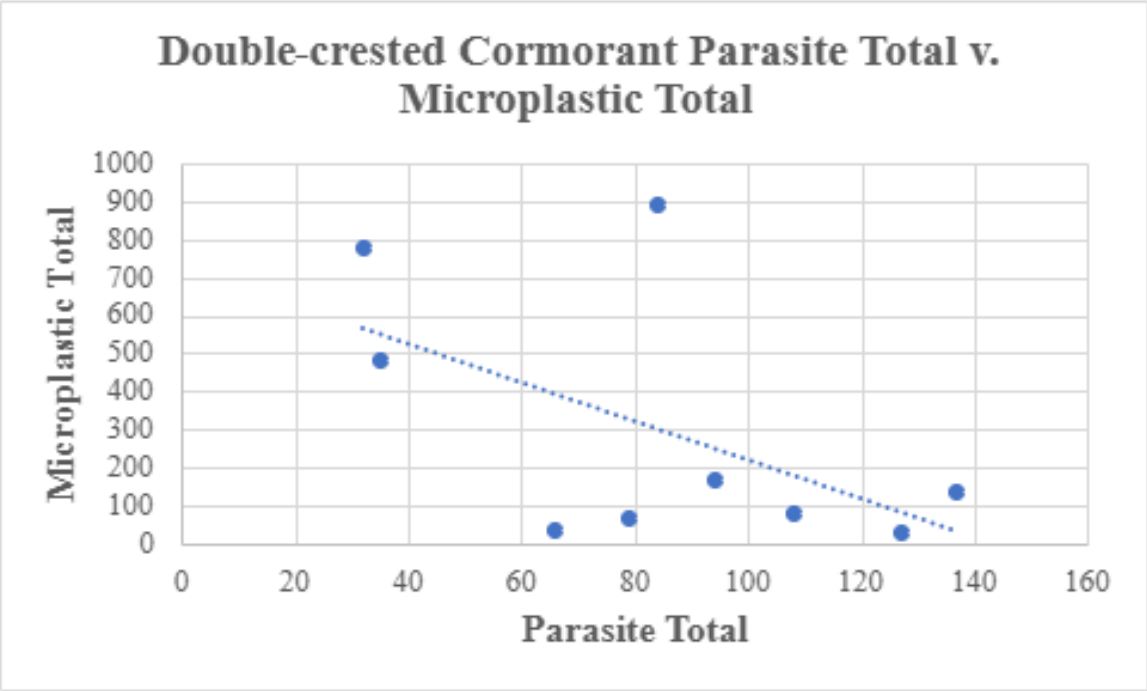


Figure 15. Double-crested Cormorant Parasite Total v. Microplastic Total: This scatterplot depicts how parasite total compared against microplastic total in double-crested cormorants. This relationship was insignificantly negatively correlated (t= -1.932, df= 7, p= 0.095, r= -0.590).

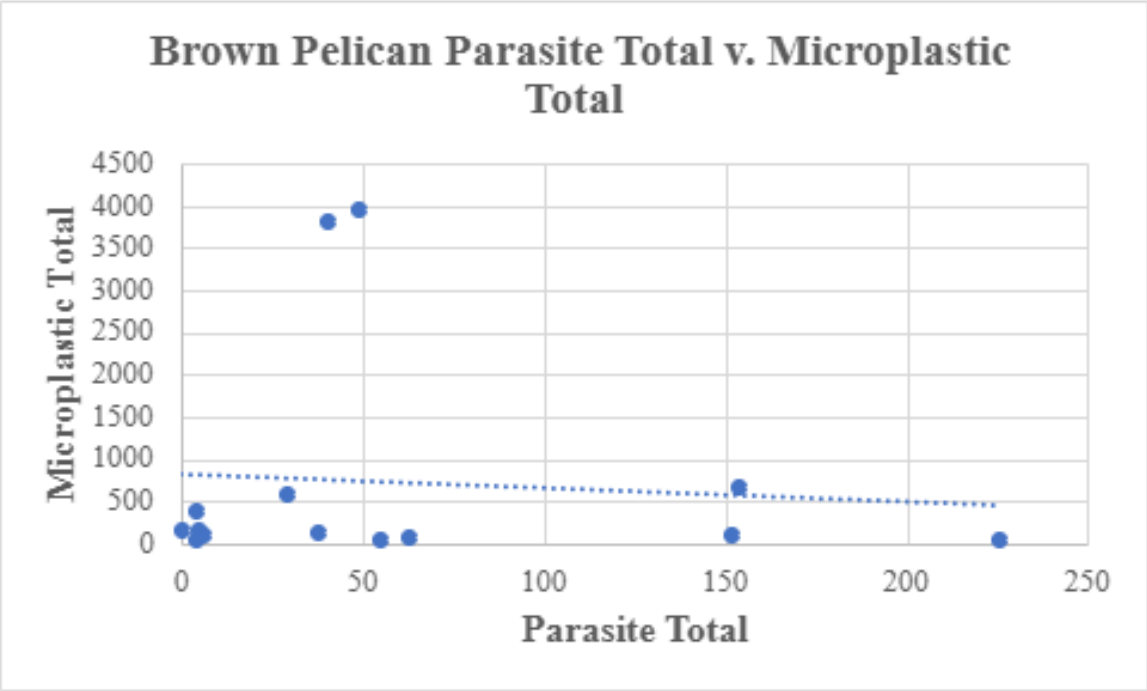


Figure 16. Brown Pelican Parasite Total v. Microplastic Total: This scatterplot depicts how parasite total compared against microplastic total in brown pelicans. This relationship was insignificantly correlated (S= 541.19, p= 0.517, r= -0.190).

Microplastic in Liver Total vs Microplastic in GI Tract Total

A correlation test was performed to determine the relationship between total microplastics found in the liver and total microplastics found in the GI tract for each individual species. The variables were first tested for normality and when parametric assumptions were not met for either species, the data was transformed using the square root of the data, providing a clearer picture to interpret. For double-crested cormorant, variables total microplastics in liver and total microplastics in GI tract were significantly positively correlated when tested using a Pearson's correlation test ($t= 9.242$, $df= 7$, $p= 3.589e-05$, $r= 0.961$). Brown pelican, variables total microplastics in liver and total microplastics in GI tract were negatively correlated when tested using a Spearman's correlation test ($S=316.08$, $p= 0.289$, $r= 0.305$). The positive correlation for both species indicates that variables total microplastics in liver and total microplastics in GI tract can be used to predict patterns in one another because they are trending in the same direction.

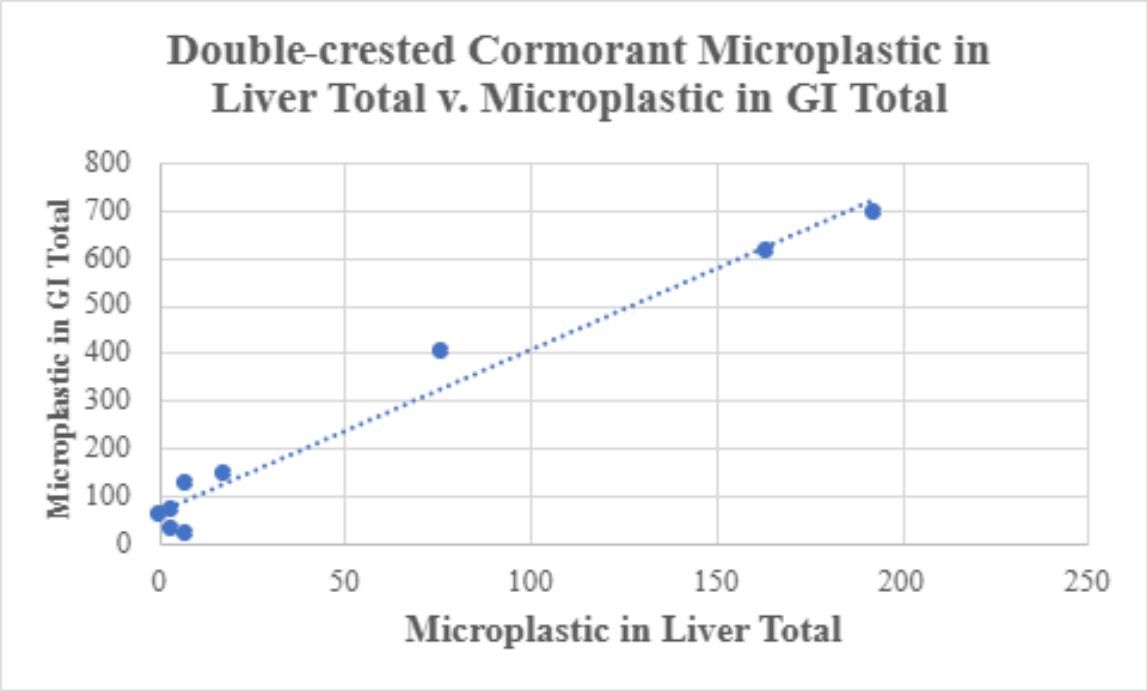


Figure 17. Double-crested Cormorant Microplastic in Liver Total v. Microplastic in GI Total: This plot depicts how microplastic total in the liver compared against microplastic total in the GI tract in the double-crested cormorant. This relationship was significantly positively correlated (t= 9.242, df= 7, p= 3.589e-05, r= 0.961).

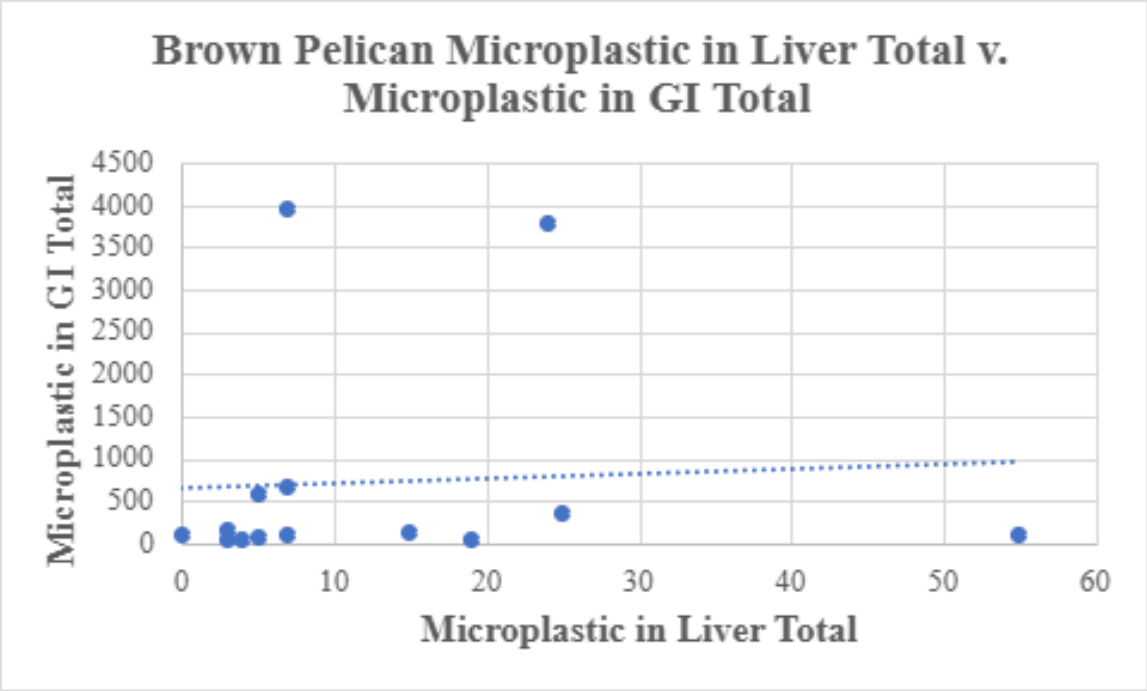


Figure 18. Brown Pelican Microplastic in Liver Total v. Microplastic in GI Total: This plot depicts how microplastic total in the liver compared against microplastic total in the GI tract in brown pelicans. The relationship was insignificantly correlated (S=316.08, p= 0.289, r= 0.305).

Discussion

Of the 26 birds sampled throughout this study, a total of 14 brown pelicans, 9 double-crested cormorants, and 3 ospreys were observed. The aim of this study was to understand the relationship between microplastic quantities and endoparasite communities; correlation tests run using R studio provided some clarity on this relationship. It was expected that microplastic exposure would result in increased susceptibility to parasites, and community structure would be influenced by microplastic load. The small sample size for ospreys resulted in an inability to test these relationships and descriptive statistics were preformed instead. It was determined that microplastic abundance is not affecting endoparasite community structure; there was no correlation found for any of the parasite types determined in either species. Microplastic abundance is not affecting susceptibility to parasites; there was no correlation found to determined that an increase in microplastics was resulting in an increase in parasite abundance. There was correlation found between the abundance of microplastics in the liver and that in the GI tract for double-crested cormorants but not for brown pelicans.

Microplastics

Although this study is the first of its kind, there have been other notable studies that allow for the opportunity to cross-reference the information discovered from these samples. Kappos (2022), studied the microplastic quantities in the prey fish species of the birds in this study, including the striped mullet, and scaled sardine. Kappos (2022) found on average 8.57 pieces of microplastics in each fish. Comparatively, this study found an average of 52.3 pieces in each osprey, 294.7 in double-crested cormorant individuals, and 727.6 pieces in brown pelican individuals. On average ospreys consume 0.3kg of fish a day, double-crested cormorants consume 0.45-0.68kg a day, and brown pelicans consume 1.8kg a day (Enstipp et al., 2006; Schreiber et al., 1975; Vana-Miller, 1987). This not only supports the theory of secondary ingestion as the method of microplastic consumption but also explains the quantity of particles found and the variance between the different bird species (Carlin et al., 2020; Nelms et al., 2015).

Clark (2021) quantified microplastic abundance in seabirds in double-crested cormorants and brown pelicans. The study found the average number of pieces of microplastics per individual was 9.6 pieces in double-crested cormorants and 29.9 in brown pelicans. This study

found an average of 52.3 pieces in each osprey, 294.7 in double-crested cormorant individuals, and 727.6 pieces in brown pelican individuals. The averages per individual were incredibly different between the two studies and the contribution could be a result of a variety of different factors. Additionally, Clark (2021) found the majority of microplastics were found in the form of fibers, while this study had a significantly larger abundance of beads discovered, almost to an unprecedented amount. Further samples could be taken to help determine the cause; tap water, DI water, and ocean water samples could all possibly shed some light on the differentiation. Microplastic particles were not found in the KOH blank samples collected for this study, removing the possibility that the microplastic particles were found due to contamination from outside sources.

Microplastic abundance continues to increase as the years progress as seen when comparing frequency seen in this study and that of studies conducted in previous years (Barnes et al., 2009; Thompson et al., 2004). Not only are microplastic concentrations increasing but they continue to fragment into smaller pieces, attributing to the higher particle count. It is unsurprising that when comparing this information to that seen in Clark (2021), this study found a larger average microplastic count per sample. This study also found that 100% of the samples contained microplastics to some degree, compared to 97.7% containing pieces in Clark (2021).

Endoparasite Communities

Nakama (2018) sampled the species of birds in this study to determine the endoparasite community breakdown. Mirroring this study, a breakdown of nematodes, cestodes, digeneans, and acanthocephalans were found in the seabirds. Nakama (2018) analyzed liver samples in addition to the GI tract which could explain the difference in endoparasite community structure that was concluded in that study. Nakama determined a larger distribution of digeneans in all bird species, as opposed to digeneans only having a larger abundance in ospreys in this study. Nematodes were found in larger abundance in double-crested cormorants and brown pelicans in this study. The presence of the types of parasites detected suggests that the seabirds are opportunistically feeding in both marine and freshwater environments (Enstipp et al., 2006; Schreiber et al., 1975; Vana-Miller, 1987). Although this is to be expected from ospreys, as they are not true seabirds, it suggests that double-crested cormorants and brown pelicans are as well (Withers et al., 2004; Pettex et al., 2012; Lamb, 2016). The difference in community structure

seen could be a result of Nakama (2018) also considering liver samples whereas this study chose to omit sampling parasites in the liver.

Relationship between microplastics and endoparasites

The relationship between microplastic abundance and endoparasite communities has been questioned in previous studies, albeit very few. Hernandez-Milian et al., (2019) wanted to determine the relationship in the grey seal and highlighted two key points; microplastics tended to accumulate in areas where more parasites were aggregated, however a significant relationship was not found. As samples in the study contained some degree of microplastics and provided valuable insight to coastal food webs. Alternatively Pennino et al., (2020), studied the relationship in sardines and anchovies and determined that parasite prevalence was positively related to microplastic abundance in both species. In sardines, individuales with lower body condition had higher microplastic ingestion, and in achovies this was true for individuals with higher gonadosamatic indices and smaller size. These contraditing results could ultimately indicate that the relationship between microplastics and endoparasites is determined by the species at present. Hernandez-Milian et al., (2019) determined that grey seals are capable of processing microplastics and digesting them, releasing them back to the environment; this contradicts the information that we have regarding the inability for seabirds to fully process microplastics, instead suffering various ailments and in some cases death. Further studies on a wider variety of marine fauna could provide more clear insight to determine if there is a relationship only in some species, or if other factors should be considered.

Limitations

This study is the first to consider how microplastic abundance is affecting endoparasite communities in Florida seabirds. Although some studies have been done on the relationship between microplastics and endoparasites in other marine fauna (Hernandez-Milian et al., 2019; Pennino et al., 2020), this is the first completed on brown pelicans, double-crested cormorants, and ospreys. Originally it was decided that a sample size of 20 per bird species would provide the most reliable results and mimic studies such as Clark (2020), Nakama (2018), and Kappos (2022) which had a similar scientific design. Unfortunately, during the two-year sampling period, only 26 total birds were sampled to completion due to two main reasons. During the sampling period a large-scale outbreak of bird flu, that affected all three species, was reported in

the south Florida region where samples were being collected from. Due to this outbreak, receiving samples from the various wildlife centers became difficult as any bird testing positive or suspected of being infected could not be used for the study.

The largest limiting factor of the study was the unprecedented amount of time required to filter through the mason jars that contained the organic material and the microplastics. Collection of the liver, GI tract, and decanted material resulted in an average of ten mason jars per bird required for the process of breaking down organic material; the anticipated two-week requirement during this period was surpassed and, in most cases, required up to four weeks' time. If organic material did not break down fully by the two-week mark, the contents of the jar were divided, and an additional portion of 10% KOH was added. In these cases, the jars were also placed on a hot plate with low heat and a magnetic stir bar for five minutes before being placed once again in the workstation hood for additional time. The extended time and jars required resulted in periods of time where no additional birds could be worked up due to limited space in the workstation hood to properly store all the jars. Despite further breaking down the material, running the remaining liquid through the filtration system took approximately 2-4 hours per jar, significantly reducing the speed at which samples could be inspected for microplastics. Even with the organic material broken down, it took significant time for the remaining liquid to complete filtration through the one-micrometer filter, at times resulting in multiple sessions of filtration per jar, increasing the risk of contamination. Once the filters were dried, multiple photographs were taken of the filters that were later used to count the number of microplastics. Each individual microplastic needed to be hand counted, not only was this a time-consuming process because some filters contained hundreds of particles, but human error may have occurred altering the number of particles detected.

Further Studies

Despite the information gained from this experimentation, there were several limitations to consider during the implementation of further studies. Despite significant precautions taken, some particles and contamination were detected on the blank potassium hydroxide petri dishes that accompanied the remaining dishes during the filtering and drying process. The AirClean 600 workstation hood that the mason jars and petri dishes were kept was a shared workspace; ideally the mason jars and petri dishes should be left in isolation to reduce contamination as the

aluminum foil placed loosely over the jars did not provide adequate coverage. The use of aluminum foil could also be improved due to how easily the material was broken down with any contact to KOH; it is possible that some of the contaminate particles found were from the aluminum foil itself. Using a small watch glass over the jar could be a sufficient alternative. Additionally, the method of breaking down the organic matter using potassium hydroxide should be further improved upon; using a 3:1 ratio of potassium hydroxide required a much larger time frame for digestion than previously anticipated. Potassium hydroxide has been noted to break down some forms of biodegradable plastics, cellulose acetate from cigarette filters, and single use polyethylene sheets (Kühn et al., 2017), and therefore could not be used at a 1:1 ratio, but alternative ratios, or chemicals such a hydrogen peroxide, should be explored to improve filtration technique. The limited sample size severally restricted the information that was taken away from the study; overall, a larger sample size would have provided more clarification and likely, a different result.

Conclusion

Marine waste continues to be an ongoing threat to marine fauna, especially with the knowledge that microplastics are located even in the organs of seabirds and the species in which they feed (Carlin et al., 2020; Clark, 2021; Kappos, 2022). Over time, they continue to degrade into smaller pieces, finding their way into all the samples seen in this study. Microplastic beads were significantly more abundant through the samples but microplastic fibers were found in no shortage as well. The parasite community structure and types seen were reflections of what was anticipated for these opportunistic seabird species. Nematodes, cestodes, digeneans, and acanthocephalans seen suggest that the seabirds are feeding in both saltwater and freshwater environments. Microplastic abundance did not make the specimen more susceptible to parasites, nor did they influence endoparasite community structure as anticipated. Further studies with a larger sample size may reflect different results and should be conducted in the future.

The persistence of methods to reduce anthropogenic waste will help greatly in the cause for climate regulation but does not solve the detrimental issue of microplastics altogether without further research. This study can provide a baseline to compare how microplastic abundance and their effect on parasites has changed over time. Additionally, it provides information on changes in the scientific design that would be required to make the most of the information available.

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Appendix Table 1a. *Double-crested Cormorants morphometric measurments*

Sample Number	Bill from Base (cm)	Bill from Feathers (cm)	Bill from Nostrils (cm)	Bill Depth (cm)	Tarsus Length (cm)	Tail Length (cm)	Wing Cord (cm)	Wing Span (cm)	Total Weight (kg)
3213	6.5	5.5	4.5	1.1	10.0	15	2.9	106.0	1.4
3214	6.5	5.4	4.2	1.4	8.0	14.3	2.7	92.0	0.7
3215	6.3	5.4	4.8	1.7	9.2	17	3.0	50.0	0.7
3216	6.7	5.6	3.8	1.6	9.4	15.5	2.9	102.0	0.7
3217	6.5	5.5	5.0	1.5	9.4	14.4	2.8	100.0	1.0
3218	7.0	6.3	4.4	2.0	9.0	16.5	3.2	112.0	1.3
3219	7.2	6.5	5.4	2.0	9.0	15	3.0	92.0	1.5
3221	7.0	6.0	5.0	2.0	9.0	14	3.0	98.0	1.3
1109	5.7	5.0	4.8	2.0	8.0	12	2.8	48.0	0.8

Appendix Table 1b. *Double-crested Cormorants microplastic data*

Sample Number	Beads in the Liver	Fibers in the Liver	Beads in the GI Tract	Fibers in the GI Tract	Liver Total	GI Tract Total	Total Microplastics
3213	189	3	694	3	192	697	889
3214	3	0	58	15	3	73	76
3215	2	5	19	3	7	22	29
3216	3	0	16	17	3	33	36
3217	0	0	57	8	0	65	65
3218	16	1	134	15	17	149	166
3219	7	0	126	0	7	126	133
3221	163	0	605	9	163	614	777
1109	76	0	399	6	76	405	481

Appendix Table 1c. *Double-crested Cormorants parasite data*

Sample Number	Nematode Total	Acanthocephalan Total	Cestode Total	Digenean Total	Parasite Total
3213	84	0	0	0	84
3214	108	0	0	0	108
3215	127	0	0	0	127
3216	66	0	0	0	66
3217	62	17	0	0	79
3218	94	0	0	0	94
3219	74	0	63	0	137
3221	4	0	28	0	32
1109	16	18	0	1	35

Appendix Table 2a. *Brown Pelican morphometric measurments*

Sample Number	Bill from Base (cm)	Bill from Feathers (cm)	Bill from Nostrils (cm)	Bill Depth (cm)	Tarsus Length (cm)	Tail Length (cm)	Wing Cord (cm)	Wing Span (cm)	Total Weight (kg)
1121	32.0	41.0	31.0	3.5	12.0	22	50.0	219.0	4.2
1122	40.0	35.0	33.0	3.0	14.0	11	50.0	180.0	3.6
1123	36.0	29.0	25.0	2.5	11.0	20	52.0	190.0	2.7
1125	42.0	36.0	33.0	3.0	12.0	19	53.0	190.0	2.5
1126	35.0	31.0	28.5	3.0	12.0	19	50.0	160.0	1.6
1127	35.0	30.0	28.0	3.0	12.0	19	50.0	180.0	1.7
1128	35.0	31.0	30.0	4.5	13.0	17	52.0	180.0	2.9
1129	35.0	34.0	33.0	4.0	13.0	12	52.0	180.0	2.2
1130	36.0	33.0	30.0	5.0	12.0	12	16.0	170.0	2.8
3175	35.0	32.0	31.0	5.5	13.0	17	53.0	182.0	2.4
3176	33.0	32.0	29.0	5.0	12.0	14	54.0	170.0	2.1
3177	33.0	29.0	25.0	5.0	12.0	16	46.0	130.0	2.3
3174	35.0	32.0	30.0	4.6	13.9	14.3	56.0	212.0	3.0
3178	36.0	32.0	29.0	4.0	11.5	12.2	56.0	176.0	2.9

Appendix Table 2b. *Brown Pelican microplastic data*

Sample Number	Beads in the Liver	Fibers in the Liver	Beads in the GI Tract	Fibers in the GI Tract	Liver Total	GI Tract Total	Total Microplastics
1121	24	0	3771	0	24	3771	3795
1122	5	0	62	0	5	62	67
1123	3	0	143	6	3	149	152
1125	24	1	349	3	25	352	377
1126	14	1	110	12	15	122	137
1127	2	2	36	2	4	38	42
1128	0	0	65	18	0	83	83
1129	2	1	39	0	3	39	42
1130	6	1	649	8	7	657	664
3175	6	1	84	5	7	89	96
3176	55	0	96	3	55	99	154
3177	5	0	568	3	5	571	576
3174	7	0	3946	1	7	3947	3954
3178	19	0	26	2	19	28	47

Appendix Table 2c. *Brown Pelican parasite data*

Sample Number	Nematode Total	Acanthocephalan Total	Cestode Total	Digenean Total	Parasite Total
1121	40	0	0	0	40
1122	20	0	43	0	63
1123	4	0	1	0	5
1125	4	0	0	0	4
1126	18	0	15	0	38
1127	30	0	24	0	55
1128	80	0	72	0	152
1129	226	0	0	0	226
1130	154	0	0	0	154
3175	4	0	0	2	6
3176	0	0	0	0	0
3177	0	29	0	0	29
3174	45	0	4	0	49
3178	4	0	0	0	4

Appendix Table 3a. *Osprey morphometric measurments*

Sample Number	Bill from Base (cm)	Bill from Feathers (cm)	Bill from Nostrils (cm)	Bill Depth (cm)	Tarsus Length (cm)	Tail Length (cm)	Wing Cord (cm)	Wing Span (cm)	Total Weight (kg)
3129	4.5	5.0	3.5	2.0	4.5	19.5	45.0	146.0	2.8
3130	5.0	4.5	2.6	2.0	6.0	22	53.0	140.0	1.4
3131	5.5	4.5	3.0	2.0	6.5	21.5	51.0	160.0	1.6

Appendix Table 3b. *Osprey microplastic data*

Sample Number	Beads in the Liver	Fibers in the Liver	Beads in the GI Tract	Fibers in the GI Tract	Liver Total	GI Tract Total	Total Microplastics
3129	1	8	5	65	9	70	79
3130	1	1	1	24	2	25	27
3131	4	1	32	14	5	46	51

Appendix Table 3c. *Osprey parasite data*

Sample Number	Nematode Total	Acanthocephalan Total	Cestode Total	Digenean Total	Parasite Total
3129	7	0	0	340	347
3130	3	0	10	0	13
3131	0	10	0	0	10