4-19-2021

Microplastic Quantification of the Proventriculus and Gizzard of Florida Seabirds

Jonathan J. Clark
Nova Southeastern University

Capstone of
Jonathan J. Clark

Submitted in Partial Fulfillment of the Requirements for the Degree of

Master of Science
Marine Science

Nova Southeastern University
Halmos College of Arts and Sciences

April 2021

Approved:
Capstone Committee

Committee Chair: David Kerstetter, Ph.D.

Committee Member: Abigail Renegar, Ph.D.

Nova Southeastern University

Halmos College of Arts and Sciences

Microplastic Quantification of the Proventriculus and Gizzard of Florida Seabirds

By

Jonathan J Clark

A Capstone

Submitted to the Faculty of

Nova Southeastern University

Halmos College of Arts and Sciences

In partial fulfillment of the requirements for the degree

of Master of Science with a specialty in:

Marine Biology

April 29, 2021

TABLE OF CONTENTS

List of Tables

List of Figures

Abstract

Plastic pollution is a global problem that exists in even the most remote locations. Plastic biodegrades slowly but breaks apart into progressively smaller pieces relatively quickly. Plastic pieces <5 mm in size are termed ‘microplastics’ and are of interest due to their ability to associate with harmful chemicals known as persistent organic pollutants (POPs). Through microplastics, POPs can enter the food web and have the potential to biomagnify in higher trophic levels. Once ingested, microplastics and their associated POPs can reduce body condition, alter reproduction rates, and increase mortality. Marine birds are at a higher risk of ingesting microplastics because they feed at the ocean’s surface where buoyant microplastics commonly accumulate. However, very little is known about the prevalence of microplastics and coastal seabirds. This study quantifies the amount of microplastics found in four species of seabirds based on quantity, size, type (fiber or fragment), and color (light, mid, or dark). Bird specimens were obtained from local wildlife rescue centers, and a total of 643 microplastic particles were identified, with 43 of the 44 study specimens found to contain microplastics (97.7% frequency). The ‘fiber’ type and the ‘mid’ color tone were the most common microplastics. There were no significant differences between species for particle sizes, but Brown Pelicans contained significantly more particles than the other three species. These results highlight the prevalence of plastic pollution in these mesopredators, but more work is needed to further determine microplastic patterns between taxa and foraging environments.

Keywords: microplastic, pollution, seabird, brown pelican, laughing gull

Introduction

Plastic pollution is an ever-growing problem facing all species, and the United Nations Environment Programme (UNEP) lists environmental plastic waste as a critical problem (UNEP, 2016). Plastic makes up about 80% of the waste found on land, shorelines, the ocean surface, and the ocean floor (Barnes et al., 2009). Environmental plastic wastes exist even in extremely remote environments such as the Arctic Sea, Antarctic Sea, and the Sonoran Desert (Barnes et al., 2010; Zarfl & Matthies, 2010; Zylstra, 2013). The use of plastics has increased significantly in the last century due to its light weight, ease of production, and durability. Unfortunately, these same characteristics make it a significant environmental hazard because plastics do not biodegrade rapidly, especially in the ocean.

The persistence of plastics was highlighted when an albatross's stomach was found to contain plastic originating from a plane shot down 60 years before and almost 10,000 kilometers away (Weiss et al., 2006). Most plastic is buoyant and only subject to abrasion or photodegradation, and these two processes act to reduce the plastic into smaller and smaller pieces (Barnes et al., 2009). These small plastic pieces have become of interest in recent years and been described in the scientific literature as "microplastics" or plastic particles <5 mm. Currently, microplastics are classified as either primary or secondary, the difference being their source. Primary microplastics are manufactured to be small, such as industrial pellets and abrasive microbeads used in soaps and cosmetics. Secondary microplastics are particles that have degraded from larger plastic items ones as mentioned previously. Examples of this secondary microplastic type are far ranging but include fibers shed from clothing during laundering and photodegraded monofilament fishing line and disposable shopping bags.

One of the biggest dangers posed by microplastics is their near invisibility to the naked eye. Because of their small size, microplastics can easily be taken up by a range of organisms, starting with zooplankton all the way up to marine birds (Cole et al., 2013; Provencher et al., 2014; Zhao et al. , 2016). Plastic bags and six-pack rings can easily be seen and disposed of by those with the proper motivation, but not their degraded counterparts. Microplastics can persist virtually undetected in the environment and wreak just as much havoc as macroplastics (>5 mm). While the extent of the damage that microplastics cause in the environment is still being studied, it is known that microplastics can act as vectors for harmful chemicals known as persistent organic pollutants (POPs) and transport them into the food web with the potential to biomagnify

in higher trophic levels (Teuten et al., 2007). In 2015, the U.S. Congress enacted the Microbead-Free Waters Act of 2015, which prohibited the addition of microbeads in the manufacture of personal care products, such as skin cleansers and toothpaste, by 2017 and the sale of the products in the United States by 2018 (Pub. L. 114-114).

Marine Birds

Marine birds are at a particular risk of ingesting microplastics because many of them feed on prey at the ocean's surface, where buoyant plastic pieces commonly accumulate (Moser & Lee, 1992). Plastics can physically damage the gastrointestinal tract of birds and can even cause starvation in large quantities (Pierce et al., 2004). Another danger of ingested microplastics is their affinity for transporting persistent organic pollutants (POPs) due to their shared hydrophobicity (Moore, 2008). Seabirds have been shown to assimilate POPs from plastic sources into their tissues (Ryan et al., 1988; Tanaka et al., 2013). Once ingested, microplastics and their associated POPs can reduce body condition, alter reproduction rates, and increase mortality in seabirds (Lavers et al., 2014; Spear et al., 1995).

Study Species

The four seabird species chosen for this study are the Brown Pelican (*Pelecanus occidentalis*), the Royal Tern (*Thalasseus maximus*), the Laughing Gull (*Leucophaeus atricilla*), and the Double-crested Cormorant (*Phalacrocorax auratus*). Although a part of these species' populations seasonally migrates, most of these birds commonly inhabit Florida year-round and all are found along the coastline. These species are generalists, but they mostly forage for prey on or near the ocean surface including fishes and invertebrates (Aygen & Emslie, 2006; Johnson et al., 2002; Pierotti & Annett, 1990; Shields, 2014).

Though the Brown Pelican weighs 3.5 – 4.5 kg and has a wingspan of ~2 m, it is the smallest member of species *Pelecanidae*. These birds are found throughout the Pacific, Atlantic, and Gulf Coasts of both North and South America. The Atlantic Brown Pelican subspecies *P. o. carolinensis* (used in this study) has a large range along the Atlantic coast extending from Venezuela to Nova Scotia. They have relatively long lifespans with one individual found 31 years after it was originally tagged. They are very social species, often found to congregate in large flocks as well as nesting in large groups. Breeding usually begins between ages 3 – 5,

where a male will bring materials for a female to create a nest either on the ground or on the tops of trees. Both parents alternate incubating the 2 – 3 eggs laid by the female. The diet of *P. occidentalis* consists almost primarily of smaller fish that tend to conglomerate near the surface: menhaden, mullet, herring, sheepshead, pigfish, etc. (Shields, 2014). Two studies have found that breeding pelicans in South Carolina and western Florida coast (respectively) feed almost exclusively on menhaden, with silversides, mullet and herring accounting for the remaining small percentage (Blus et al., 1979; Fogarty et. Al, 1981). *P. occidentalis* use their sharp eyesight to spot potential prey while gliding over the ocean's surface. Once the prey is spotted, the bird will plunge headfirst into the water and envelope their target in their large beak and attached pouch – a pouch than can hold three times the volume of the stomach. Once caught, the pelican will let the water drain out of its pouch and swallow the captured fish. The biggest threat to *P. occidentalis* is humans. They were hunted for their feathers to near extinction in the early 20th century and then again faced extinction after the advent of Dichlorodiphenyltrichloroethane (DDT) in the 1940s. DDT entered the *P. occidentalis's* system through secondary ingestion of prey fish exposed to the pesticide and caused their eggshells to be so thin that they broke during incubation (Blus et al., 1979). Due to the banning of DDT by the EPA in 1972, and the protections conferred from being listed as "Threatened" under the Endangered Species Act of 1973, *P. occidentalis* population levels have risen to the point where they are now classified as "Least Concern" by the IUCN (IUCN, 2021).

The Royal Tern is another social, coastal bird identified by a bright orange bill and sharp black crest ranging in weight from 390-430 g and in wingspan from 100-110 cm. This species is found along both coasts of North and South America as well as the islands of the Caribbean. They have been found to live up to ~30 years (Buckley, 2002) . Royal Terns nest on isolated beaches and islands in colonies and usually only produce one egg per clutch. Males will feed females during incubation and both parents alternate foraging and feeding their young after hatching (Buckley & Buckley, 1972). *T. maximus* is an opportunistic, "single-load" species – they only capture one prey item at a time – so they require prey of appropriate size and quality to be close to the colony (<50 miles) (McLeay et al., 2009). *T. maximus* flies over the water and plunge-dives into the surface so their prey tends to be small, surface-schooling fishes (e.g., menhaden, anchovy, drums) and crabs (Liechty et al., 2016). Other than during the egg stage, *T. maximus* does not experience much predation. The only danger to *T. maximus* populations are

human related effects: beach/colony disturbance, erosion, and rising sea levels; they also are classified as “Least Concern” by the IUCN (Buckley, 2002: IUCN, 2021).

The Laughing Gull, identified by its distinctive black hood, can be found throughout the eastern coast of North America and both coasts of Central America including the Northern Caribbean Islands. Their weight ranges from 203-371 g and wingspan ranges from 92-120 cm. The oldest known individual lived ~22 years. They dominate beaches, mangroves, saltmarshes, and virtually all other habitats along the coasts. They nest in saltmarshes and islands usually higher than ground level to avoid destruction by the tides or storms. *L. atricilla* is found to be monogamous and both male and females build the nest. The female will hatch 2-4 eggs either synchronously or asynchronously; reproductive success for *L. atricilla* increases if they hatch their brood asynchronously, as they focus more on feeding the elder chicks as opposed to trying to feed all at once (Hahn, 1981). They are highly generalized eaters and have been found to consume both terrestrial and marine prey opportunistically. These include invertebrates (earthworms, insects, snails, crabs) as well as fish, berries, and human food given as handouts (González-Medina et al., 2020). The biggest threat to this species is also humans; destruction of nests, loss of coastline, and the use of pesticides are some potential dangers to *L. atricilla* (White et al., 1979). Ironically, the fish offal discarded from fishing boats may have helped the *L. atricilla* population to rise in recent years to a “Least Concern” level as classified by the IUCN (Burger, 2015; IUCN, 2021).

The Double-crested Cormorant is identified by its long black body and orange beak, weighing in around 1.2–2.5 kg with a wingspan between 114-123 cm. It is widely distributed throughout most of North America, including both coastlines. The oldest recorded individual lived to at least 22 years. They are found wherever large enough bodies of water to support their prey fish exist. They are easy to spot as they commonly rest upon high structures with their wings spread after hunting for prey by diving from the surface of the water to capture prey. Prey consists of almost entirely marine and freshwater fishes (up to 250 different prey species have been reported) found on the surface, including: yellow perch, alewife, bass, shad, carp, cod, pollock, etc. (Hatch et al., 1999; Johnson et al., 2002). Nests can be found atop trees, or on the ground in rocks and reef without vegetation. Both mates build the nest, and the female will lay a 1-2 broods per season of 1-7 eggs each. Both birds in the pair participate in the incubation process. The biggest threat to population levels of *P. auratus* is anthropogenic, as the

introduction of DDT caused thinning of *P. auratus* eggshells and decimated wild populations (Weseloh et al., 1995). Since the DDT ban, the U.S. population has significantly rebounded and now enjoy a "Least Concern" status as classified by the IUCN (Hatch et al., 1999; IUCN, 2021).

Microplastic pollution has gained awareness over the last 50 years, but there is still much work to be done, especially considering the lack of data on microplastics in coastal seabirds. Previous work has shown that seabirds will commonly ingest plastic (Amélineau et al., 2016; Zhao et al., 2016; Zhu et al., 2019), but there are currently no quantitative studies published on microplastic ingestion in the seabirds of the Florida coastline. This study aims to provide data for that deficit and to help us understand how common plastic is in the environment, both on macroscopic and microscopic scales. The first goal was to discern if there are differences in the rates of occurrence for microplastic ingestion between spices, and the second was to determine if there are differences in the types (shape and color) of microplastic ingested between species.

Materials and Methods

Collection

All bird specimens were collected from two rescue and rehabilitation centers in South Florida: the Florida Keys Wild Bird Center (FKWBC) in Tavernier and South Florida Wildlife Center (SFWC) in Fort Lauderdale. The specimens were pronounced dead upon arrival (DOA), euthanasia upon admittance (EOA) due to traumatic impairment or physical condition, or they died receiving treatment at the wildlife centers. Bird specimens were collected from these wildlife centers under FFWCC permits LSSC-12-00075 and LSSC-18-00062, USFWS permit MB8290-A-0, and a USFWS LOA to D.W. Kerstetter.

Laboratory/Bird Processing

None of these species were captured, they were collected opportunistically from agencies, so sample size was dependent on the number available. Upon arrival at NSU, they were assigned a unique identification number and then placed in a laboratory freezer (~$^{-}$20°C) until processing. At least 2 days before dissection, the specimens were removed from the freezer and placed in a laboratory refrigerator (~ 4°C) for thawing. Prior to dissection, the standard morphometric measurements were taken: weight, wing chord length, tarsus length, and cause of death (if identifiable) in accordance with Labocha & Hayes (2012). Tarsus length is a sufficient predictor of body size and was used as such in this study (Senar & Pascual, 1997).

For each specimen, the proventriculus and gizzard were removed, dissected, and any macroscopic pieces of plastic or other debris removed. Those organs were immersed in a potassium hydroxide solution (10% KOH) at a 3:1 potassium hydroxide to organ volume ratio and kept at room temperature for two to three weeks to allow the organic matter to dissolve completely. All specimens during the dissolution process were kept in glass mason jars sealed with aluminum foil. Once completely dissolved, the remaining liquid was subjected to a vacuum filtration through a filter (Whatman brand, Grade GF/B Glass Microfiber filters) with a 1 µm pore size to ensure all particles were filtered out and to acquire the very small pieces. Materials were filtered via a Buchner funnel paired with a vacuum flask.

A total of 14 of the higher fat samples (usually Brown Pelican and Double-crested Cormorant) did not dissolve completely with 10% KOH alone, even when additional solution was added (Table 1) (Figure 10). This necessitated a trial-and-error process of adding additional solvents/emulsifiers to dissolve the thick fat layers. Standard dish soap (Dawn brand), standard degreaser (Zep brand Purple Degreaser, professional strength), and 100% acetone were added sequentially to try and break up the fat, all to no avail. Finally, the industrial-strength degreaser Solvalene (Superior Industries, Inc.; Chattanooga, TN) succeeded. About 15-20% Solvalene per total specimen volume and more than one day on a hot stir plate was required to emulsify the fat into particles small enough to pass through the filter (Solvalene was added in ~10 ml increments until all the fat was visually dissolved). All additional liquids (including Solvalene) were verified by the manufacturers to not dissolve or affect plastics.

After passing the completely dissolved solution through the filter, filters were kept in covered petri dishes and left to dry for at least 48 hours before microscopic visual examination. One procedural blank of filtered DI water was ran through the filter for microscopic examination as well.

Table 1. Bird specimens that necessitated Solvalene. Solvalene commercial degreasing solvent was required for the full dissolution of 15 bird specimen gastrointestinal tracts. The amount of Solvalene added varied by individual specimen. “Specimen Code” refers to the internal laboratory specimen identification number.

Species	Specimen Code	Solvalene (mL)
Brown pelican	BRPE 3164	500
Brown pelican	BRPE 3163	50
Brown pelican	BRPE 3173	50
Brown pelican	BRPE 3169	750
Brown pelican	BRPE 3167	250
Brown pelican	BRPE 3171	100
Brown pelican	BRPE 3170	100
Brown pelican	BRPE 3162	100
Brown pelican	BRPE 3172	100
Brown pelican	BRPE 3165	100
Double-crested cormorant	DCCO 3207	100
Double-crested cormorant	DCCO 3210	50
Double-crested cormorant	DCCO 3209	50
Double-crested cormorant	DCCO 3211	250
Laughing gull	LAGU 3167	30

Microscopic Examination

Microplastic processing techniques used were the same as described in Zhao et al. (2016) and Provencher et al. (2019). All filters were examined under a dissecting microscope at 2x power, and photos were taken of every identified microplastic item (AmScope 3.5x-180x trinocular stereomicroscope with LED ring light, AmScope 10 MP camera). These photos were later used to determine the size of the items as outlined below. The quantity, color, size, and shape of plastic particles were documented according to the three-step procedure outlined by Zhao et al. (2016) and following the guide for identifying microplastics from Barrows (2017).

- Step one: identify man-made particles via the following criteria:
    1. no cellular or organic structures are visible;
    2. fibers should be equally thick, not tapered at the end and should have a three-dimensional bend;
    3. fibers are not segmented, nor do they appear as twisted flat ribbons;
    4. colored items are clear and homogeneously colored;
    5. potential microscopic anthropogenic litter that is transparent or whitish is examined with extra care and under higher magnification;
    6. particles should not be lustrous; and
    7. fibers were bendable or soft.
- Step two: identify characteristics. Individual plastic pieces will be classified into two groups: fragments and fibers. Colors will be classified as light, mid, or dark and the longest dimension of each particle will be measured in mm.
- Step three: confirmation of unknown pieces via melting test. Any pieces that are of unknown composition will be subjected to a heated insect pin and, if plastic, the piece will melt like plastic and smell of melting plastic.

All the processing occurred in a clean fume hood, with the proper clothing worn (hat/hair net, and gloves), and filtered DI water was used to minimize potential contamination from airborne microfiber pollution (Hidalgo-Ruz et al.,2012; Provencher et al., 2017; Zhao et al., 2016).

Statistical Analysis

RStudio (version 1.3.1073, 2020) was used for statistical analysis. Data was assessed for normality and homoscedasticity assumptions. Evaluation of the hypotheses were run accordingly in parametric (ANOVA) or non-parametric (Kruskal-Wallis) analyses.

Standard descriptive statistics were used to describe the standard morphometric data for the bird specimens (weight, wing chord length, and tarsus length), as well as the size (mm) of all the microplastics found, in total and for all species. A negative binomial test was used to evaluate differences in the rates of microplastic ingestion between species. A one-way ANOVA was used to evaluate differences between species and size of ingested microplastics. Two negative binomial tests were used to evaluate differences in the (1) types and (2) colors of microplastics ingested between species.

Results

Specimen Collection

A total of 44 birds belonging to three different taxonomic families were collected and processed. The Laughing Gull had the highest number of specimens (n=13), followed by Brown Pelicans (n=12), then Double-crested Cormorants (n=10), and finally Royal Tern (n=9). Most of the specimens were from the Florida Keys Wild Bird Center (n=43), with one Royal Tern coming from the South Florida Wildlife Center (n=1).

Morphometric Measurements

Brown Pelicans had the greatest weight (2182.5 ±518.6g), followed by Double-crested Cormorants (1040 ±467.14g), then Laughing Gull (243.85 ±49.92g), and finally Royal Tern (225.56 ±43.33g) (Table 2). Brown Pelicans had the greatest wing chord length (313.08 ±40.19mm), followed by Double-crested Cormorants (156.1 ±13.85mm), then the Laughing Gull (128.23 ±64.91mm), and finally Royal Tern (124.44 ±7.99mm) (Table 2). Brown Pelicans had the greatest tarsus length (75.58 ±4.87mm) and therefore the greatest body size (Senar & Pascual, 1997). Double-crested Cormorants are next (59.5 ±3.21mm), then Laughing Gull (54.46 ±3.45mm), and finally Royal Tern (34.89 ±4.81mm) (Table 2).

Microplastic Data

A total of 643 microplastic particles was found across all specimens in varying shape, size and color as seen in Fig. 1. 43 of the 44 study specimens contained microplastics (97.7% frequency). The mean length for the particles found was 1.145 (±1.23 mm), the median 0.74mm, with 95% of the particles found being smaller than 3mm (Fig. 2). In terms of fragment vs fiber, the 'fiber' type was by far the most abundant, accounting for 72% of the particles found; fragment accounted for the remaining 28% (Fig. 3). Out of the three colors used to classify the particles, the 'mid' color type was the most frequent (61%), followed by 'dark' (21%) and then 'light' (19%) (Fig. 4).

Brown Pelicans had the highest average microplastic content of all four species with 29.9 particles/bird (±20.1 particles). The number of particles ranged from 6 – 66 among the samples. The mean length of the particles for this species was 1.168 (±1.2 mm). The 'fiber' (77.2%) type was more abundant type of microplastic than 'fragment' (22.8%). The 'mid' color was the most abundant (66.3%), followed by 'light' (17.3%), and then finally 'dark' (16.4%).

Laughing Gulls had the lowest average microplastic content with 7.6 particles/bird (±4.6 particles). The number of particles ranged from 2 – 18 among the sample. The mean length of the particles was 1.248 (±1.44mm). The 'fiber' type (78.8%) was more abundant than the 'fragment' type (21.2%). The 'mid' color was the most abundant (60.6%), followed by 'dark' (28.3%), and then finally 'light' (11.1%).

Double-crested Cormorants had an average microplastic content of 9.6 particles/bird (±8.1 particles). The number of particles ranged from 0 – 28 among the sample. The mean length of the particles was 1.116 (±1.31 mm). The 'fiber' type (53.1%) was more abundant than the 'fragment' type (46.9%). The 'mid' color was the most abundant (47.9%), followed by 'light' (27.1%), and then finally 'dark' (25%).

Table 2. **Results of morphometric measurements.** The weight, wing chord length, and tarsus length measurements of all four study species. Results are reported in mean (with standard deviation) and range for each measurement taken. Brown Pelican consistently ranked in as the largest of all measurements, followed by Double-crested Cormorants, then Laughing Gull and finally Royal Tern.

	Weight (g)		**Wing Chord Length (mm)**		**Tarsus Length (mm)**	
Species (n)	Mean (±SD)	Range	Mean (±SD)	Range	Mean (±SD)	Range
Laughing Gull (13)	243.85 (±49.92)	200 - 300	128.23 (±64.91)	101 - 344	54.46 (±3.45)	49 - 60
Royal Tern (9)	225.56 (±43.33)	200 - 300	124.44 (±7.99)	113 - 137	34.89 (±4.81)	31 - 46
Brown Pelican (12)	2182.5 (±518.6)	1300 - 2800	313.08 (±40.19)	235 - 354	75.58 (±4.87)	69 - 84
Double-crested Cormorant (10)	1040 (±467.14)	500 - 2000	156.1 (±13.85)	140 -180	59.5 (±3.21)	54 - 64

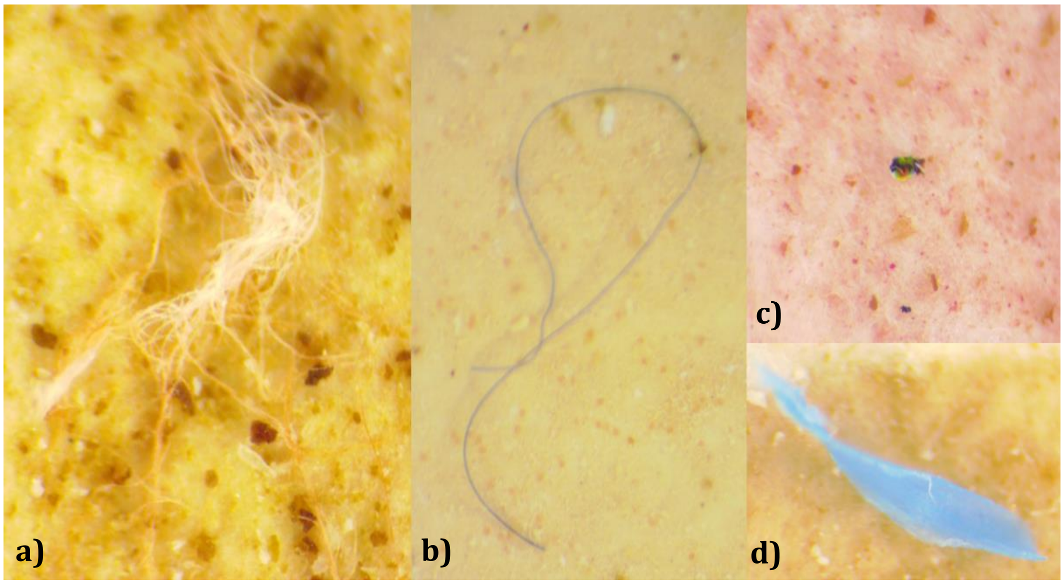

Figure 1. **Photographs of microplastic particles found.** Examples of found microplastics from coastal seabird gastrointestinal tracts: a) light fiber (Brown Pelican, 3167), b) mid fiber (Brown Pelican, 3172), c) mid fragment (Laughing Gull, 3169), d) mid fragment (Double-crested Cormorant, 3202).

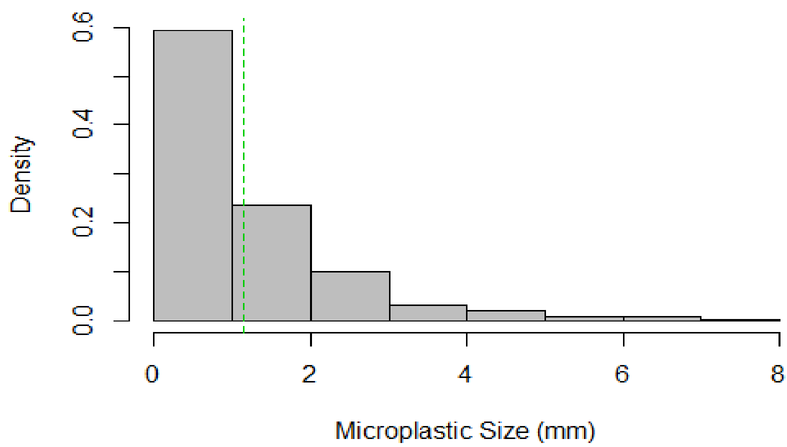

Figure 2. Microplastic size histogram. Histogram representing the size of all microplastics found from gastrointestinal tracts of 44 coastal seabirds from southeast Florida. The dashed line represents the mean of the sample (n=643 total microplastic fibers and fragments).

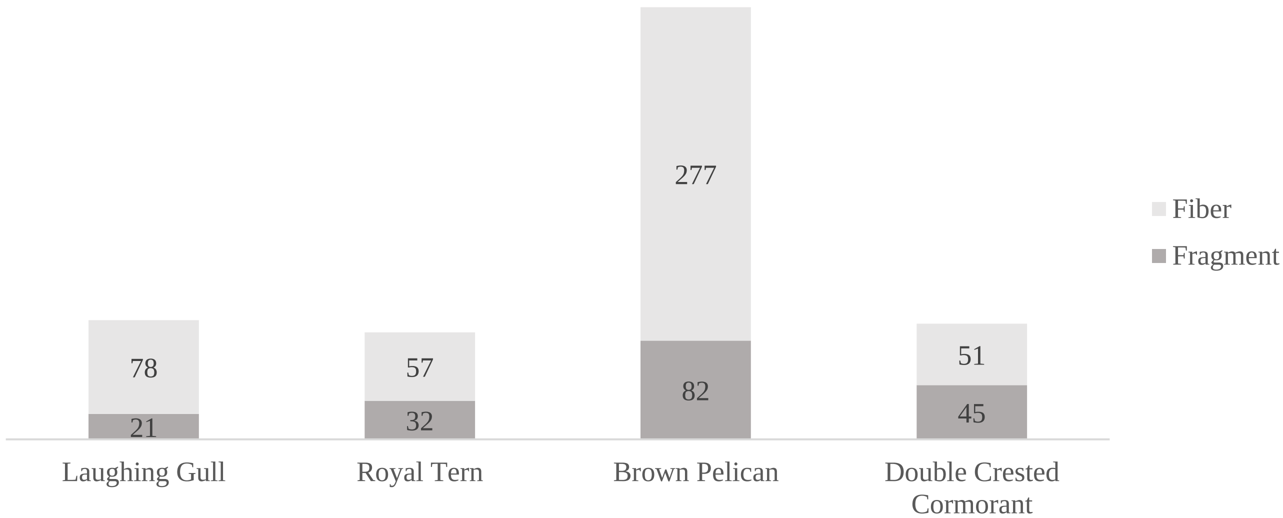

Figure 3. Type of microplastic chart. Depicting how the total number of fibers and fragments compared to one another within a species as well as across the four species. Fiber is shown to be the more ingested type. The numbers in the chart are the n values for that group.

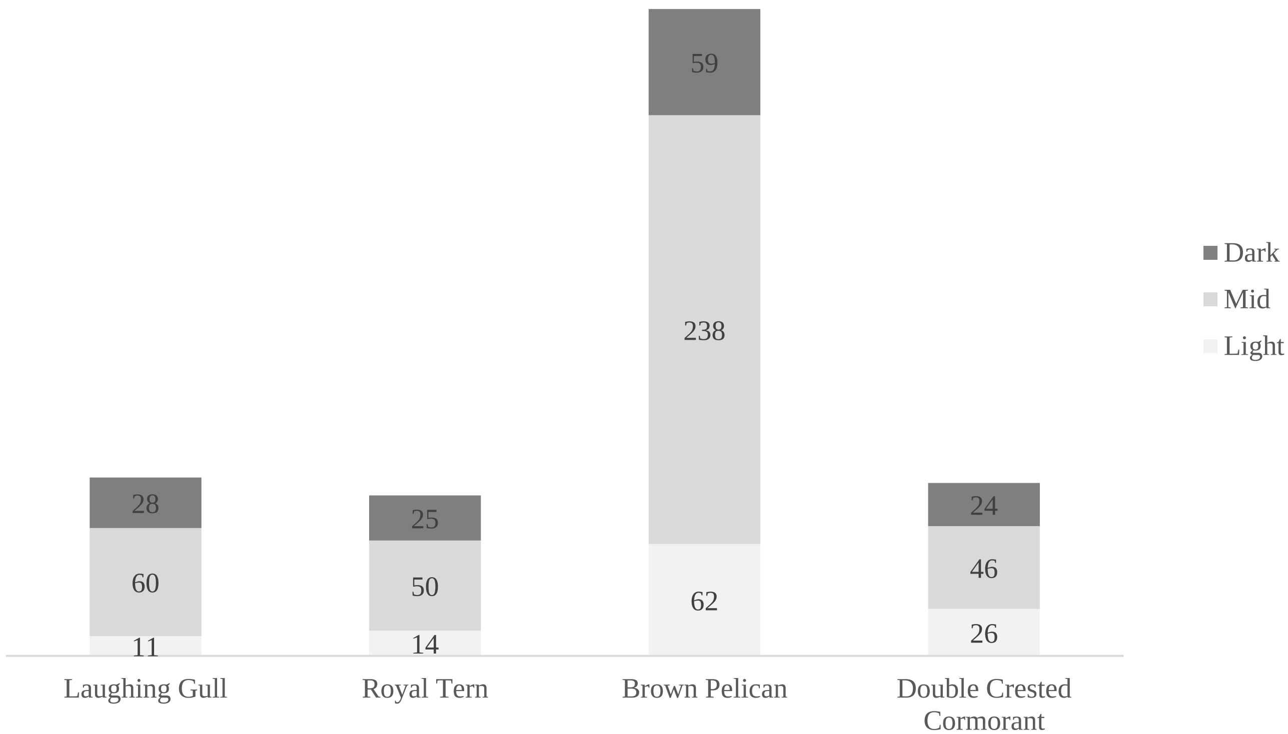

Figure 4. Color of microplastic chart. Depicting how the the total number of each color type compared to one another within a species as well as across the four species. The ‘mid’ color type is shown to be ingested significantly more than ‘light’ or ‘dark’. The numbers in the chart are the n values for that group.

Royal Terns had an average microplastic content of 9.9 particles/bird (±4.5 particles). The number of particles ranged from 5 – 17 among the sample. The mean length of the particles was 0.958 (±0.952mm). The 'fiber' type (64%) was more abundant than the 'fragment' type (36%). The 'mid' color was the most abundant (56.2%), followed by 'dark' (28.1%), and then finally 'light' (15.7%).

The procedural blank did produce three microplastic particles. Two 'mid' fibers and one 'dark' fragment with an average size of 0.817 mm were found on the blank filter.

Species vs Size

A one-way ANOVA test initially tried to evaluate if the different species ingest different sizes of microplastics, but the data was not normal even after a logarithmic and square root transformations, so a non-parametric Kruskal-Wallis test was used. There was not a significant difference between size of microplastics and species (p-value = 0.2578, Kruskal-Wallis chi-squared = 4.0343, df = 3) (Figure 5). In other words, all species contain similarly sized microplastics.

Number of Microplastics per Species

A negative binomial test was run to ascertain if there were differences in the rate of ingestion of microplastics (number of particles/bird) between species. It was found that there were significant differences in the rates of occurrence for microplastic ingestion between species ($p = 1.358e^{-8}$, $R^2 = 0.457$). As shown in Figure 6, Brown Pelican contained significantly more microplastics than the other examined species, followed by Double-crested Cormorants and Royal Terns, with Laughing Gulls containing the least amount.

Type and Color Differences Between Species

Two tests were run for each separate predictor (independent) variable: 1) type (fiber/fragment) and 2) color. Species was the other predictor variable in both tests.

1) Type: Species had a significant effect on the total number of microplastics ingested (all p values <<< 0.05). Type did have a significant effect on the total number of microplastics ingested because there were significant differences between the two levels of the

independent variable fragment and fiber ($p = 1.9e^{-6}$, $z = -4.76$). Fiber was the type more frequently ingested as shown in Figure 7.

2) Color: As before, species did have a significant effect on the total number of microplastics ingested (all p values $<<< 0.05$). The ‘mid' color was the only color to have a significant effect on total number of microplastics and was the only color to be significantly different between species ($p = 1.1e^{-6}$, $z = 4.87$) (Figure 8).

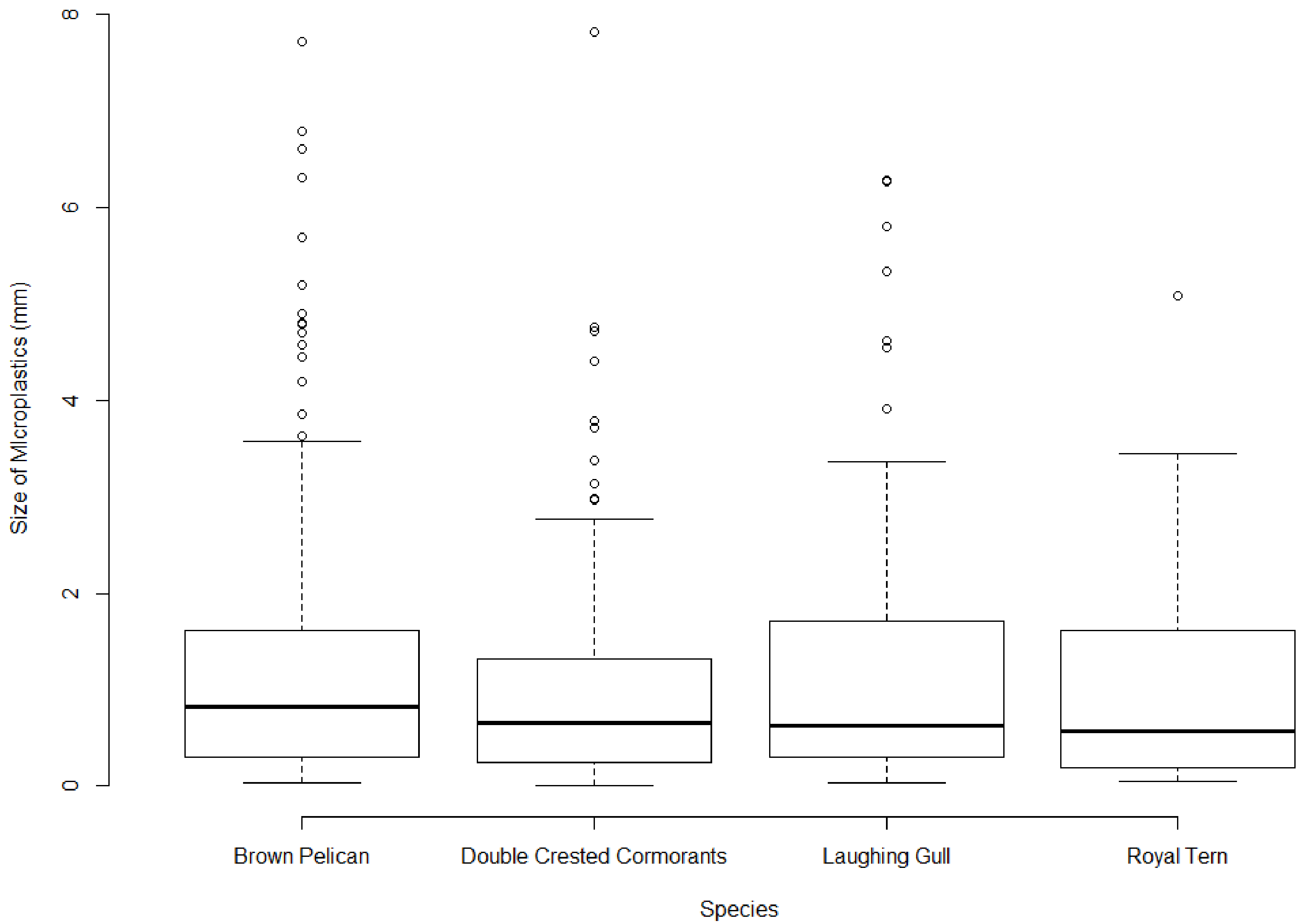

Figure 5. **Microplastic size comparison.** This chart compares the size of microplastics (mm) ingested between each of the 4 study species, showing that size was not significantly different between species. The points above each plot represent outliers. Brown Pelican (n=359), Double-crested Cormorant (n=96), Laughing Gull (n=99), Royal Tern (n=89).

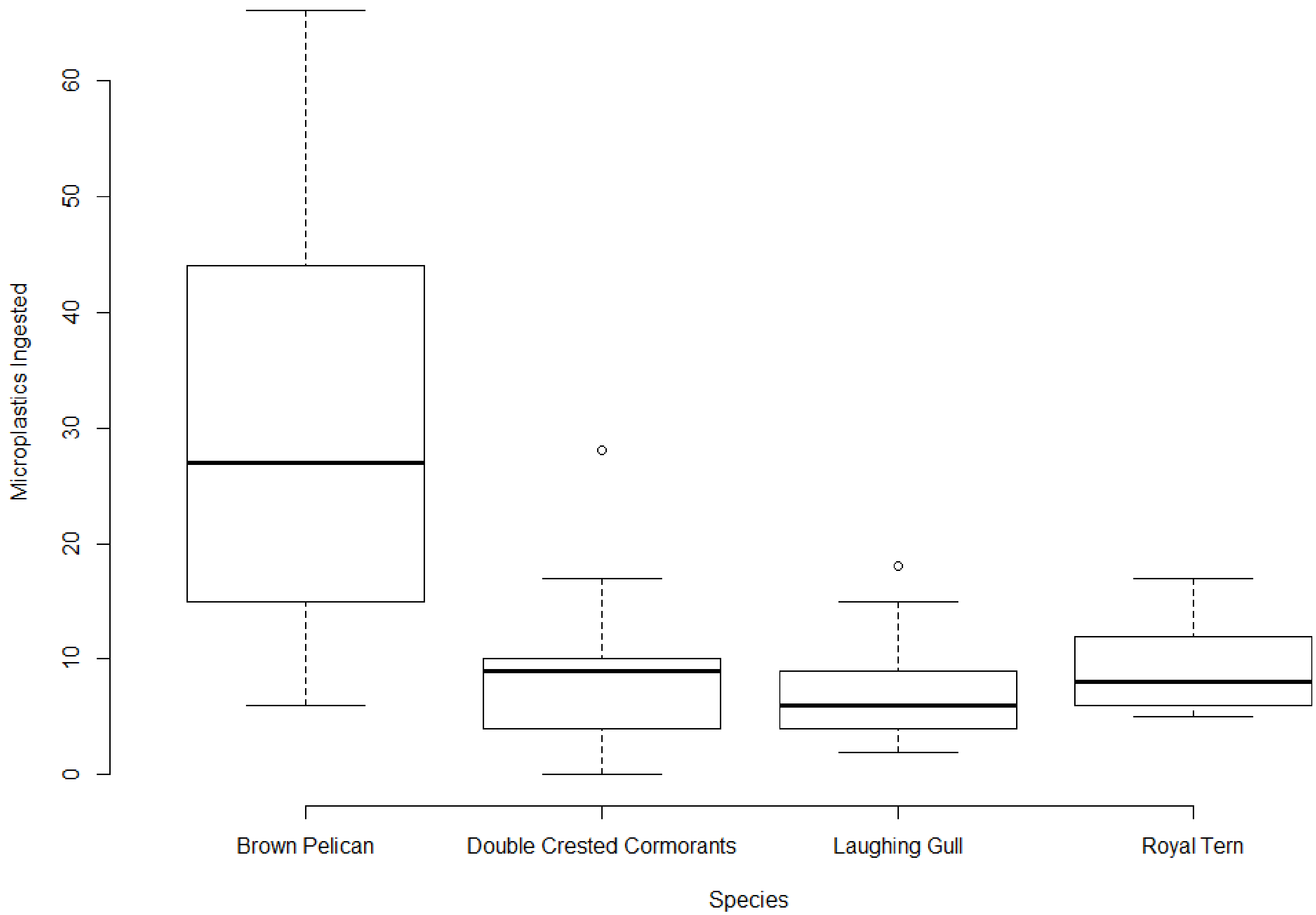

Figure 6. Total microplastics ingested. Comparison of the total number of microplastics ingested in each of the 4 study species, showing that BRPE was the only species with significantly more microplastic particles. The points above each plot represent outliers. Brown Pelican (n=359), Double-crested Cormorant (n=96), Laughing Gull (n=99), Royal Tern (n=89).

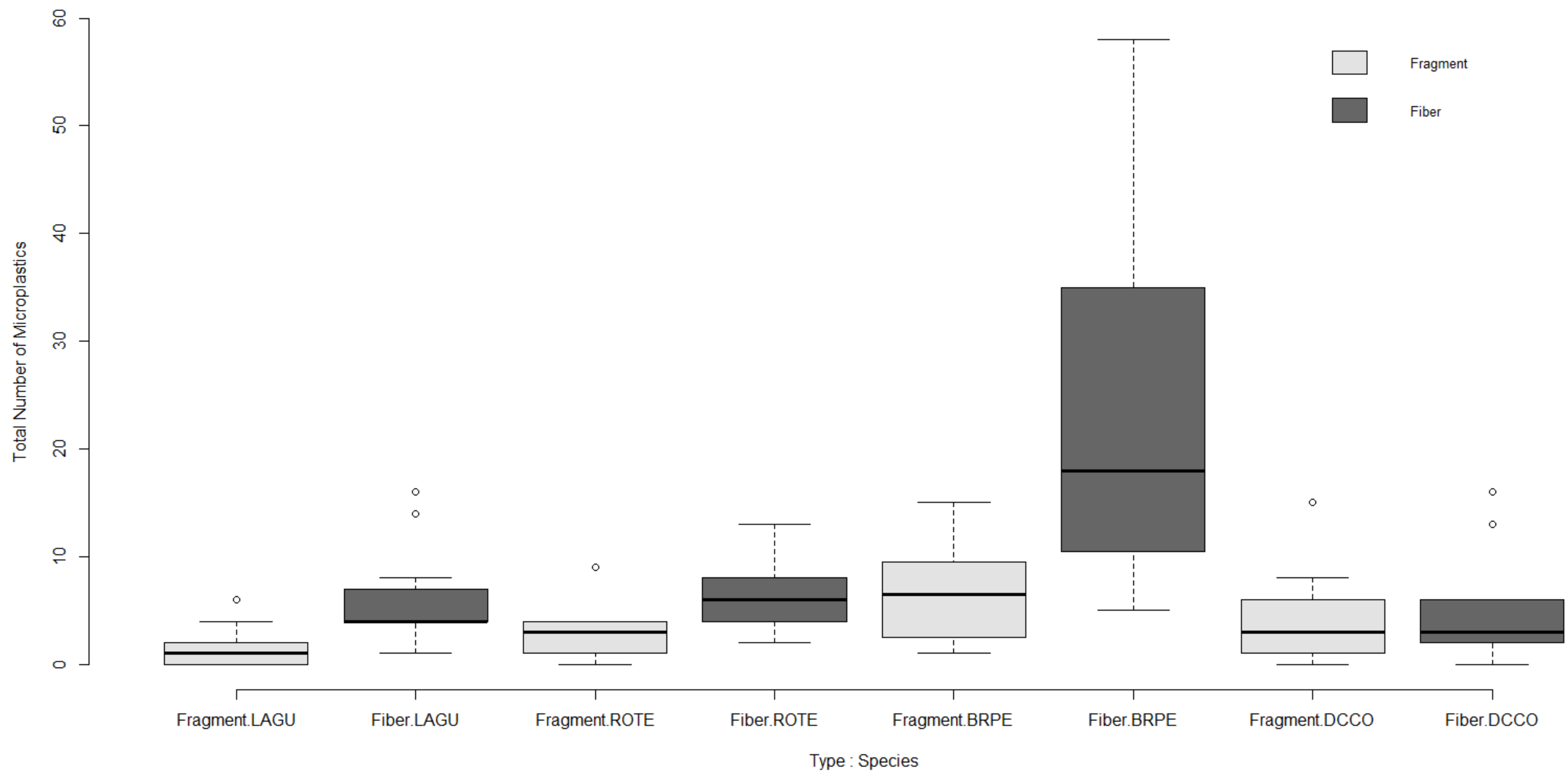

Figure 7. Fiber vs fragment breakdown. Comparison of microplastic type (fiber vs fragment) within and across the four study species. ‘Fiber’ was the type to be significantly ingest more frequently than fragment. LAGU = Laughing Gull, ROTE = Royal Tern, BRPE = Brown Pelican, DCCO = Double-crested Cormorant. The points above each plot represent outliers, n values for each boxplot from left to right are: 21, 78, 32, 57, 82, 277, 45, 51.

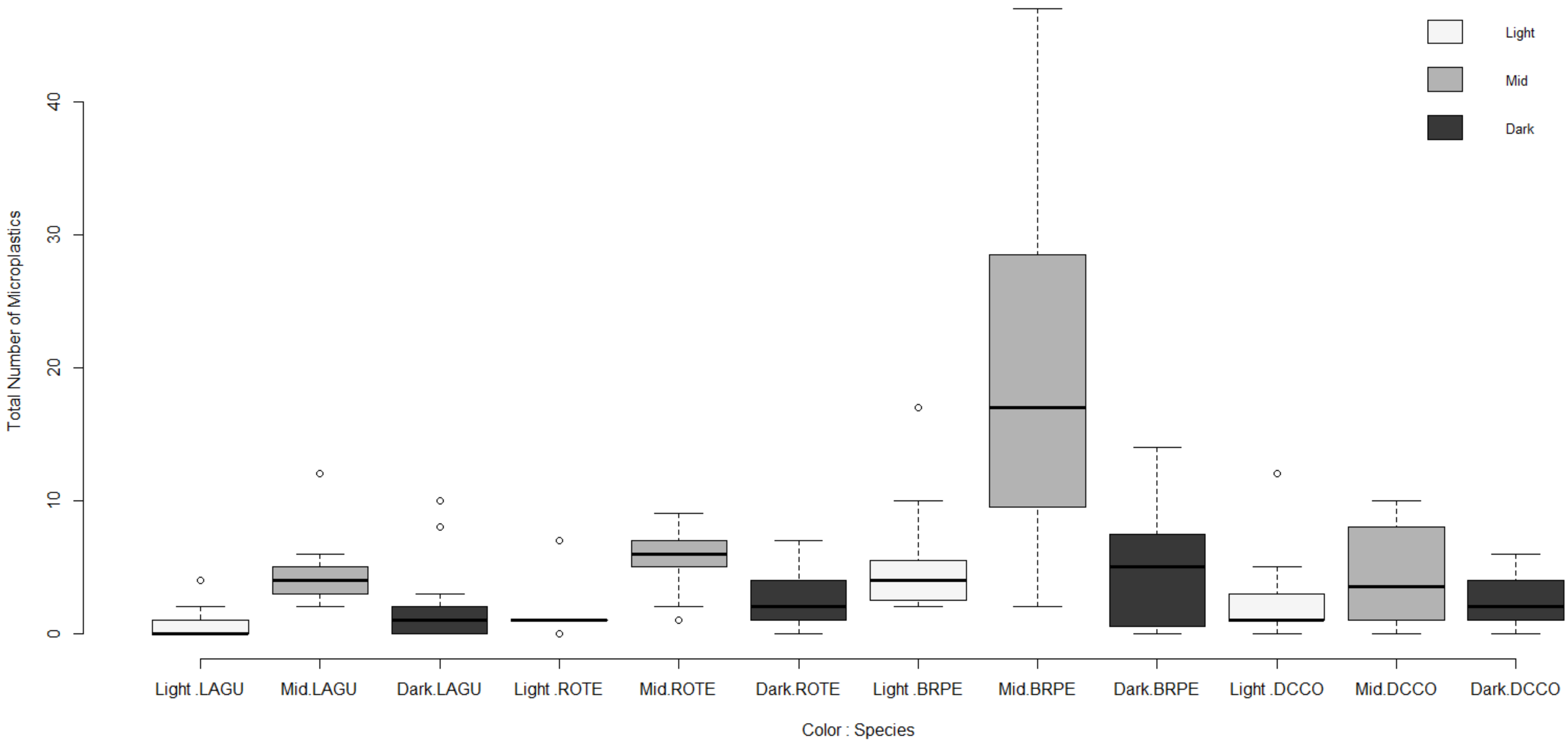

Figure 8. Color breakdown. Comparison of the three color classification groups (light, mid, and dark) of microplastics ingested within and across the four study species. The 'mid' color was ingested significantly more than 'light' or 'dark'. LAGU = Laughing Gull, ROTE = Royal Tern, BRPE = Brown Pelican, DCCO = Double-crested Cormorant. The points above each plot represent outliers, n values for each boxplot from left to right are: 11, 60, 28, 14, 50, 25, 62, 238, 59, 26, 46, 24.

Figure 9. Prey fish in stomach. A prey fish (most likely a stripped mullet) found in the stomach of specimen BRPE 3165 during dissection of gastrointestinal tract. Photo taken on July 15, 2020.

Figure 10. High fat layer. This is an example of an undissolved high fat layer that remained after dissection and the normal 2-week dissolution period. Solvalene was added, which allowed the contents to dissolve completely. BRPE 3169. Photo taken October 19, 2020.

Discussion

Morphometric measurements

Though this study may not represent a true random sample of the study species (as the birds were collected opportunistically from wildlife centers), it is the first of its kind to evaluate microplastic content for these four species in North America (*P. occidentalis, T. maximus, L. atricilla*, and *P. auratus*). All specimens were consistent with their expected morphometrics; Brown Pelicans had the widest range in weight, wing chord length, and tarsus length of any of the four species. This can be attributed to the fact that, of the four species, Brown Pelicans had the highest number of juveniles sampled. Double-crested Cormorants were consistently recorded as the second largest of the sampled bird specimens for all morphometric measurements. Laughing Gull was third largest, followed closely by Royal Tern as the smallest of the four.

Microplastic Data

A total of 643 microplastic pieces were found across all specimens sampled, with an average of 14.6 particles/bird. Only one bird specimen out of the 44 sampled did not contain any microplastics (97.7% found with microplastics). The order of magnitude for the average number of particles per specimen is consistent with other studies' results on marine/coastal bird microplastic content. A study by Moser & Lee (1992) in the western North Atlantic found an average of 4.3 particles per bird of their 20 different study species with a 55% frequency. Amélineau et al. (2016) conducted a study between 2005 and 2014 on the zooplanktivorous diving seabird, the Little Auks (*Alle alle*), reporting an average of 9.5 microplastic items per bird with a frequency of 20.4%. Zhao et al. (2016) reported an average of 10.6 ± 6.4 items/bird of their study of terrestrial Chinese birds, with a frequency of 94.1%.

Though the average number of microplastic particles per bird remained consistent, the frequency has steadily increased over the years in these four avian studies. This can easily be attributed to the ever-increasing production and existence of plastic since the 1940s. Barnes et al. (2009) found clear evidence for a decrease in the average size of plastic litter and an increase in the fragmentation of plastic leading to a much higher particle availability in the Northern Hemisphere. Thompson et al. (2004) argued that microplastic concentration in the environment

has consistently increased over the decades as a direct result of plastic's persistence and proclivity to fragment, .

Species vs Size

No significant relationship was found between species and particle size; all four study species contained similarly sized particles, which is consistent with other seabird microplastic studies. The results of this study showed a mean of 1.145 ±1.23 mm and a median of 0.74 mm for all particles. Amélineau et al. (2016) reported a median microplastic size of 0.77 mm, Zhao et al. (2016) reported a mean of 1.6 ±1.2 mm, and Zhu et al. (2019) a mean of 2.38 ±0.21 mm (if only 'mean' or 'median' is reported here it is because the author did not include the other value in their study). The small size of particles found in this study, in comparison with the other studies, suggests that these birds are not directly ingesting microplastics, i.e., the birds are not mistaking them for prey and must be ingesting them another way. Zhao et al. (2016) suggests three possible routes: (1) accidental ingestion while foraging for food, (2) fragmentation of macroplastic particles in the GI tract of birds, and (3) secondary ingestion for carnivorous birds. This third assertion seems highly likely for this study as described below.

Number of Microplastics per Species

Brown Pelicans contained significantly more microplastics than any of the other species, but they are also the largest of the four, so they hunt more and larger prey than the others. As stated previously, it is unlikely they are mistaking the small microplastic items for prey. Instead, this could be explained via microplastic biomagnification. Biomagnification is a "sequence of processes by which higher concentrations of a substance are attained in organisms at higher trophic levels." (Nordberg et al., 2009). As stated previously, the Brown Pelican's diet consists mostly of menhaden with mullet, silversides, herring, and Sailfin Molly making up the remainder (Blus et al., 1979; Fogarty et al., 1981). Menhaden gastrointestinal (GI) tracts have been found to contain microplastics, and one study found that, in mullet, microplastics can pass from the GI tract to the liver where they are retained; which was the first study to show microplastic translocation and retention in marine vertebrates (Avio et al., 2015; Parker et al., 2020). Two of the prey species for Brown Pelicans (mullet and menhaden) have been shown to contain microplastic particles, suggesting the significantly higher microplastics in Brown Pelicans could be attributed to secondary ingestion. Upon dissection of specimen BRPE 3165, a prey fish (most

likely a striped mullet, *Mugil cephalus*) was found in the bird's stomach and could, itself, contain some microplastic particles (Figure 9). Future studies could further investigate this idea by subjecting prey fish found in stomachs to the same microplastic identification techniques used here. Furthermore, tertiary ingestion may contribute to the high particle numbers as both mullet and menhaden feed on detritus as well as zooplankton, which has also been found to ingest microplastics (Cole et al., 2013). The Brown Pelican is the largest species of this study; therefore, they eat more bulk total prey than the other four, so it is logical to conclude biomagnification is responsible for their significantly higher quantity of microplastics. Previous work has shown the ability for microplastic particles to be ingested by lower trophic level organisms and then transferred up to their predators, where they are retained in tissues (Eriksson and Burton, 2003; Farrell and Nelson, 2013; Tosetto et al., 2017). No published studies have specifically explored a relationship between microplastics and biomagnification in Brown Pelicans, but it could be an avenue for further research.

Type and Color Differences Between Species

Species type did have a significant effect on the total number of microplastics. The 'fiber' classification of microplastic was significantly more frequent than 'fragment'. This result is in parallel with other microplastic bird studies: 92.3% fiber and 7.7% fragment in Zhao et al. (2016), 97.2% filament/fiber and 2.8% fragment in Amélineau et al. (2016), and 89.2% thread/fiber and 10.8% other in Zhu et al. (2019). Among the microplastic types, the fiber type seems to dominate. Fibers are used frequently in furniture, clothing, and hygiene products; global fiber production reached 111 million metric tons in 2020, with polyester fibers accounting for 52.2% of the total (Preferred Fiber & Materials Market Report 2020). Fragments, though less common, are still dangerous to animal life. They can cause chemical toxicity due to their association with POPs and physical damage such as blockage, inflammation, or cellular necrosis in the GI tract (Rochman, 2015).

The 'mid' color was the most frequent color type of the three (61%) and the only one to be significantly different between species. The other studies concur with this result; Zhao et al. (2016) reported 81.6% of the 'mid' color type and Zhu et al. (2019) reported 91.1% of the blue/mid color type. This result is likely due to their easy detection against a low color

background, frequency in the environment, or lower trophic organisms mistaking them for food (Zhao et al., 2014; Zhu et al., 2019).

Conclusion

This is the first study to quantify microplastics in these four specific Florida marine seabird species. Ultimately, 97.7% of the specimens contained a total of 643 particles, with 14.6 particles per bird. 'Fiber' was the most abundant type, and 'mid' the most abundant color found. Brown Pelicans ingested significantly more microplastics than any of the other three species. This finding in Brown Pelicans could be due to their highly carnivorous diet and potential for microplastics to increase via biomagnification. More studies should further assess this hypothesis before any solid conclusions can be drawn.

However, the present study has some limitations. Some microplastic particles were identified on the blank filter despite following all precautions suggested by Provencher et al. (2019). One explanation for this is that microplastics are found in the air in both indoor and outdoor environments (Gasperi et al., 2018). This highlights the biggest issue when it comes to plastic pollution: its ubiquitous existence. Further precautions should be implemented in future studies to help prevent contamination. Additionally, microplastic particles could be further identified via Fourier-transform infrared spectroscopy (FTIR) to identify the exact type of plastic, but this process is costly and time consuming. Despite this, significant gaps in knowledge, data, and procedure exist for any microplastic study, especially on marine birds, so studies like this are imperative to establish some baseline data on the ever-increasing threats posed by plastic pollution. One threat is the potential association of microplastics with persistent organic pollutants (POPs), because it is known that plastic can accumulate large amounts of toxic chemicals and one study has already demonstrated that plastic-derived chemicals transfer to biological tissues from the GI tract in birds (Mato et al., 2001; Tanaka et al., 2013).

All of the plastic that has ever been introduced to the environment remains in existence today, as large items or fragmented microplastics (Thompson et al., 2005). Plastic is projected to persist for hundreds if not thousands of years (Barnes et al., 2009) and will continue to fragment into progressively smaller pieces, becoming easier for organisms to ingest, uptake, and

potentially biomagnify. This problem will not disappear anytime soon, and more research is needed to understand the full effects that plastics have on the world's ocean ecosystems.

Literature Cited

Aygen, D., & Emslie, S. D. (2006). Royal Tern (*Sterna maxima*) chick diet at Fisherman Island National Wildlife Refuge, Virginia. *Waterbirds*, 29(3), 395-400.

Amélineau, F., Bonnet, D., Heitz, O., Mortreux, V., Harding, A. M. A., Karnovsky, N., . . . Grémillet, D. (2016). Microplastic pollution in the Greenland Sea: Background levels and selective contamination of planktivorous diving seabirds. *Environmental Pollution, 219*, 1131-1139. doi:https://doi.org/10.1016/j.envpol.2016.09.017

Avio, C. G., Gorbi, S., & Regoli, F. (2015). Experimental development of a new protocol for extraction and characterization of microplastics in fish tissues: First observations in commercial species from Adriatic Sea. *Marine Environmental Research, 111*, 18-26. doi:https://doi.org/10.1016/j.marenvres.2015.06.014

Barnes, D. K. A., Galgani, F., Thompson, R. C., & Barlaz, M. (2009). Accumulation and fragmentation of plastic debris in global environments. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences*, *364*(1526), 1985-1998. doi:10.1098/rstb.2008.0205

Barnes, D. K. A., Walters, A., & Gonçalves, L. (2010). Macroplastics at sea around Antarctica. *Marine Environmental Research*, *70*(2), 250-252. doi:https://doi.org/10.1016/j.marenvres.2010.05.006

Barrows, A. P. W., Neumann, C.A., Pieper, C., Berger, M.L., Shaw, S.D. (2017). Guide to Microplastics Identification, A Comprehensive Methods Guide for Microplastics Identification and Quantification in the Laboratory. *Marine & Environmental Research Institute, Blue Hill, ME.*

Blus, L. J., Lamont, T. G., & Neely, B. S., Jr. (1979). Effects of organochlorine residues on eggshell thickness, reproduction, and population status of brown pelicans (Pelecanus occidentalis) in South Carolina and Florida, 1969-76. *Pesticide Monitoring Journal, 12*(4), 172-184.

Buckley, F. G., & Buckley, P. A. (1972). The breeding ecology of Royal Terns, *Sterna (Thalasseus) maxima*. *Ibis, 114*(3), 344-359. doi:https://doi.org/10.1111/j.1474-919X.1972.tb00832.x

Buckley, P. A. (2002). Royal Tern (Thalasseus maximus), version 2.0. *In The Birds of North America.* Retrieved from https://www.allaboutbirds.org/guide/Royal_Tern/

Burger, J. (2015). Laughing Gull (Leucophaeus atricilla), version 2.0. *The Birds of North America.* Retrieved from https://www.allaboutbirds.org/guide/Laughing_Gull

Cole, M., Lindeque, P., Fileman, E., Halsband, C., Goodhead, R., Moger, J., & Galloway, T. S. (2013). Microplastic ingestion by zooplankton. *Environmental Science & Technology, 47*(12), 6646-6655.

Eriksson, C. & Burton, H. (2003) Origins and biological accumulation of small plastic particles in fur seals from Macquarie Island. Ambio. 2003 Sep;32(6):380-4. doi: 10.1579/0044-7447-32.6.380. PMID: 14627365.

Farrell, P., & Nelson, K.(2013). "Trophic level transfer of microplastic: Mytilus edulis (L.) to Carcinus maenas (L.)." *Environmental Pollution* 177 (2013): 1-3.

Foekema, E. M., De Gruijter, C., Mergia, M. T., van Franeker, J. A., Murk, A. J., & Koelmans, A. A. (2013). Plastic in North Sea Fish. Environmental Science & Technology, 47(15), 8818-8824. doi:10.1021/es400931b

Fogarty, M. J., Nesbitt, S. A., & Gilbert, C.R. (1981). Diet of nestling brown pelicans in Florida. Florida Field Naturalist 9:38-40.

Gasperi, J., Wright, S.L., Dris, R., Collard, F., Mandin,C., Guerrouache, M., Langlois, V., Kelly, F.J., & Tassin, B.(2018). "Microplastics in air: are we breathing it in?" Current Opinion in Environmental Science & Health 1 (2018): 1-5.

González-Medina, E., Castillo-Guerrero, A., Masero, J., & Fernández, G. (2020). Too salty for you? Changes of diet in the Laughing gull nestlings during the growing period. *Journal of Avian Biology, 51*, e02432. doi:10.1111/jav.02432

Hahn, D. C. (1981). Asynchronous hatching in the laughing gull: Cutting losses and reducing rivalry. *Animal Behaviour, 29*(2), 421-427. doi:https://doi.org/10.1016/S0003-3472(81)80101-7

Hatch, J. J., Weseloh, D., & Dorr, B. (1999). *Double-crested cormorant*: American Ornithologists' Union.

Hidalgo-Ruz, V., Gutow, L., Thompson, R. C., & Thiel, M. (2012). Microplastics in the Marine Environment: A Review of the Methods Used for Identification and Quantification. *Environmental Science & Technology*, *46*(6), 3060-3075. doi:10.1021/es2031505

IUCN 2021. The IUCN Red List of Threatened Species. Version 2021-1. https://www.iucnredlist.org. Downloaded on [25-3-2021].

Johnson, J. H., Ross, R. M., & McCullough, R. D. (2002). Little Galloo Island, Lake Ontario: a review of nine years of double-crested cormorant diet and fish consumption information. *Journal of Great Lakes Research, 28*(2), 182-192.

Labocha, M. K., & Hayes, J. P. (2012). Morphometric indices of body condition in birds: a review. *Journal of Ornithology, 153*(1), 1-22. doi:10.1007/s10336-011-0706-1

Lavers, J. L., Bond, A. L., & Hutton, I. (2014). Plastic ingestion by Flesh-footed Shearwaters (Puffinus carneipes): Implications for fledgling body condition and the accumulation of plastic-derived chemicals. *Environmental Pollution, 187*, 124-129. doi:https://doi.org/10.1016/j.envpol.2013.12.020

Liechty, J. S., Fontenot, Q. C., & Pierce, A. R. (2016). Diet composition of Royal Tern (Thalasseus maximus) and Sandwich Tern (Thalasseus sandvicensis) at Isles Dernieres Barrier Island Refuge, Louisiana, USA. *Waterbirds, 39*(1), 58-68.

Mato, Y., Isobe, T., Takada, H., Kanehiro, H., Ohtake, C., & Kaminuma, T. (2001). Plastic resin pellets as a transport medium for toxic chemicals in the marine environment. *Environmental Science & Technology, 35*(2), 318-324.

McLeay, L. J., Page, B., Goldsworthy, S. D., Ward, T. M., & Paton, D. C. (2009). Size matters: variation in the diet of chick and adult crested terns. *Marine Biology, 156*(9), 1765-1780. doi:10.1007/s00227-009-1211-4

Moore, C. J. (2008). Synthetic polymers in the marine environment: A rapidly increasing, long-term threat. *Environmental Research, 108*(2), 131-139. doi:https://doi.org/10.1016/j.envres.2008.07.025

Moser, M. L., & Lee, D. S. (1992). A fourteen-year survey of plastic ingestion by western North Atlantic seabirds. *Colonial Waterbirds*, 83-94.

Nordberg, M., Templeton, D.M., Andersen, O., and Duffus, J.H. (2009). Glossary of terms used in Ecotoxicology. International Union of Pure and Applied Chemistry. 81(5): 829–970. doi:10.1351/ PAC-REC-08-07-09.

Parker, B. W., Beckingham, B. A., Ingram, B. C., Ballenger, J. C., Weinstein, J. E., & Sancho, G. (2020). Microplastic and tire wear particle occurrence in fishes from an urban estuary: Influence of feeding characteristics on exposure risk. *Marine Pollution Bulletin, 160*, 111539. doi:https://doi.org/10.1016/j.marpolbul.2020.111539

Pierce, K. E., Harris, R. J., Larned, L., & Pokras, M. (2004). Obstruction and starvation associated with plastic ingestion in a Northern Gannet Morus bassanus and a Greater Shearwater Puffinus gravis. *Marine Ornithology, 32*, 187-189.

Pierotti, R., & Annett, C. A. (1990). Diet and reproductive output in seabirds. *BioScience, 40*(8), 568-574.

Preferred Fiber & Materials Market Report 2020. (2020). Retrieved from https://textileexchange.org/wp-content/uploads/2020/06/Textile-Exchange_Preferred-Fiber-Material-Market-Report_2020.pdf

Provencher, J. F., Bond, A. L., Avery-Gomm, S., Borrelle, S. B., Bravo Rebolledo, E. L., Hammer, S., . . . van Franeker, J. A. (2017). Quantifying ingested debris in marine megafauna: a review and recommendations for standardization. *Analytical Methods, 9*(9), 1454-1469. doi:10.1039/C6AY02419J

Provencher, J. F., Bond, A. L., Hedd, A., Montevecchi, W. A., Muzaffar, S. B., Courchesne, S. J., . . . Mallory, M. L. (2014). Prevalence of marine debris in marine birds from the North Atlantic. *Marine Pollution Bulletin, 84*(1), 411-417. doi:https://doi.org/10.1016/j.marpolbul.2014.04.044

Provencher, J. F., Borrelle, S. B., Bond, A. L., Lavers, J. L., van Franeker, J. A., Kühn, S., . . . Mallory, M. L. (2019). Recommended best practices for plastic and litter ingestion studies in marine birds: Collection, processing, and reporting. *FACETS, 4*(1), 111-130. doi:10.1139/facets-2018-0043

Rochman, C. M. (2015). The Complex Mixture, Fate and Toxicity of Chemicals Associated with Plastic Debris in the Marine Environment. In M. Bergmann, L. Gutow, & M. Klages (Eds.), *Marine Anthropogenic Litter* (pp. 117-140). Cham: Springer International Publishing.

Ryan, P. G., Connell, A. D., & Gardner, B. D. (1988). Plastic ingestion and PCBs in seabirds: Is there a relationship? *Marine Pollution Bulletin, 19*(4), 174-176. doi:https://doi.org/10.1016/0025-326X(88)90674-1

Senar, J. C., & Pascual, J. (1997). Keel and tarsus length may provide a good predictor of avian body size. *Ardea, 85*.

Shields, M. (2014). Brown Pelican (Pelecanus occidentalis), version 2.0. *The birds of North America. Cornell Lab of Ornithology, Ithaca, New York, USA.[online] URL: https://birdsna. org/Species-Account/bna/species/brnpel/introduction*.

Spear, L. B., Ainley, D. G., & Ribic, C. A. (1995). Incidence of plastic in seabirds from the tropical pacific, 1984–1991: Relation with distribution of species, sex, age, season, year and body weight. *Marine Environmental Research, 40*(2), 123-146. doi:https://doi.org/10.1016/0141-1136(94)00140-K

Tanaka, K., Takada, H., Yamashita, R., Mizukawa, K., Fukuwaka, M.-a., & Watanuki, Y. (2013). Accumulation of plastic-derived chemicals in tissues of seabirds ingesting marine plastics. *Marine Pollution Bulletin, 69*(1), 219-222. doi:https://doi.org/10.1016/j.marpolbul.2012.12.010

Teuten, E. L., Rowland, S. J., Galloway, T. S., & Thompson, R. C. (2007). Potential for plastics to transport hydrophobic contaminants. *Environmental Science & Technology, 41*(22), 7759-7764.

Thompson, R., Moore, C., Andrady, A., Gregory, M., Takada, H. & Weisberg, S. (2005). New directions in plastic debris. Science 310, 1117

Thompson, R. C., Olsen, Y., Mitchell, R. P., Davis, A., Rowland, S. J., John, A. W. G., . . . Russell, A. E. (2004). Lost at Sea: Where Is All the Plastic? *Science, 304*(5672), 838. doi:10.1126/science.1094559

Tosetto, L., Williamson J.E., & Brown C. (2017). "Trophic transfer of microplastics does not affect fish personality." *Animal Behaviour* 123 (2017): 159-167.

UNEP (2016). Global Gender and Environment Outlook The Critical Issues. United Nations Environment Programme, Nairobi, Kenya.

Wabnitz C, N. W. (2010). Plastic Pollution: An ocean emergency. *Marine Turtle Newsletter, 129*, 1-4.

Weiss, K. R., McFarling, U., & Loomis, R. (2006, 08/05/2006). Plague of plastic chokes the seas. *Los Angeles Times, 2(2),* 5. Retrieved from http://www.latimes.com/news/local/oceans/la-me-ocean2aug02,0,71579,full.story

Weseloh, D. V., Hamr, P., Bishop, C. A., & Norstrom, R. J. (1995). Organochlorine Contaminant Levels in Waterbird Species from Hamilton Harbour, Lake Ontario: an IJC Area of Concern. *Journal of Great Lakes Research, 21*(1), 121-137. doi:https://doi.org/10.1016/S0380-1330(95)71026-7

White, D. H., King, K. A., Mitchell, C. A., Hill, E. F., & Lamont, T. G. (1979). Parathion causes secondary poisoning in a laughing gull breeding colony. *Bulletin of Environmental Contamination and Toxicology, 23*(1), 281-284. doi:10.1007/BF01769956

Zarfl, C., & Matthies, M. (2010). Are marine plastic particles transport vectors for organic pollutants to the Arctic? *Marine Pollution Bulletin, 60*(10), 1810-1814. doi:https://doi.org/10.1016/j.marpolbul.2010.05.026

Zhao, S., Zhu, L., & Li, D. (2016). Microscopic anthropogenic litter in terrestrial birds from Shanghai, China: Not only plastics but also natural fibers. *Science of The Total Environment, 550,* 1110-1115. doi:https://doi.org/10.1016/j.scitotenv.2016.01.112

Zhao, S., Zhu, L., Wang, T., & Li, D. (2014). Suspended microplastics in the surface water of the Yangtze Estuary System, China: First observations on occurrence, distribution. *Marine Pollution Bulletin, 86*(1), 562-568. doi:https://doi.org/10.1016/j.marpolbul.2014.06.032

Zhu, C., Li, D., Sun, Y., Zheng, X., Peng, X., Zheng, K., . . . Mai, B. (2019). Plastic debris in marine birds from an island located in the South China Sea. *Marine Pollution Bulletin, 149*, 110566. doi:https://doi.org/10.1016/j.marpolbul.2019.110566

Zylstra, E. R. (2013). Accumulation of wind-dispersed trash in desert environments. *Journal of Arid Environments, 89*, 13-15. doi:https://doi.org/10.1016/j.jaridenv.2012.10.004

Appendix Table 1a. Brown Pelican (BRPE) microplastic data

Species	Specimen	Plastic Item	Color	Type	Size (mm)
BRPE	3173	1	Light	Fiber	2.14
BRPE	3173	2	Dark	Fragment	0.17
BRPE	3173	3	Mid	Fiber	1.92
BRPE	3173	4	Mid	Fiber	0.34
BRPE	3173	5	Dark	Fragment	0.15
BRPE	3173	6	Mid	Fragment	0.12
BRPE	3173	7	Dark	Fragment	0.24
BRPE	3173	8	Light	Fiber	3.85
BRPE	3173	9	Light	Fiber	0.8
BRPE	3173	10	Mid	Fiber	0.15
BRPE	3173	11	Mid	Fiber	0.22
BRPE	3173	12	Mid	Fiber	1
BRPE	3173	13	Mid	Fiber	0.71
BRPE	3173	14	Mid	Fragment	0.13
BRPE	3173	15	Mid	Fiber	3.17
BRPE	3173	16	Mid	Fiber	0.17
BRPE	3173	17	Mid	Fragment	0.05
BRPE	3173	18	Dark	Fragment	0.13
BRPE	3173	19	Dark	Fiber	1.28
BRPE	3173	20	Mid	Fragment	0.1
BRPE	3173	21	Mid	Fiber	0.25
BRPE	3173	22	Mid	Fiber	1.17
BRPE	3173	23	Dark	Fiber	0.49
BRPE	3173	24	Dark	Fragment	0.18
BRPE	3167	25	Mid	Fiber	1.13
BRPE	3167	26	Mid	Fragment	0.56
BRPE	3167	27	Mid	Fragment	0.15
BRPE	3167	28	Light	Fragment	0.43
BRPE	3167	29	Mid	Fiber	1.27
BRPE	3167	30	Light	Fiber	0.87
BRPE	3167	31	Mid	Fiber	1.27
BRPE	3167	32	Mid	Fiber	0.31
BRPE	3167	33	Mid	Fiber	1.16
BRPE	3167	34	Mid	Fiber	2.33
BRPE	3167	35	Light	Fiber	3.15
BRPE	3167	36	Light	Fragment	0.38
BRPE	3167	37	Light	Fragment	0.63
BRPE	3167	38	Mid	Fiber	1.52
BRPE	3167	39	Mid	Fiber	0.88
BRPE	3167	40	Mid	Fiber	0.88
BRPE	3167	41	Mid	Fragment	0.22
BRPE	3167	42	Light	Fragment	0.54
BRPE	3167	43	Mid	Fiber	3.32
BRPE	3167	44	Mid	Fiber	0.33
BRPE	3167	45	Light	Fiber	0.49
BRPE	3167	46	Light	Fiber	1.64
BRPE	3167	47	Light	Fragment	0.18
BRPE	3167	48	Mid	Fiber	1.28
BRPE	3167	49	Mid	Fiber	1.39
BRPE	3167	50	Light	Fragment	2.97
BRPE	3167	51	Mid	Fiber	1.08
BRPE	3167	52	Mid	Fragment	0.23
BRPE	3167	53	Mid	Fiber	0.68
BRPE	3167	54	Mid	Fiber	0.15
BRPE	3164	55	Mid	Fragment	0.13
BRPE	3164	56	Dark	Fiber	0.57
BRPE	3164	57	Dark	Fragment	0.13
BRPE	3164	58	Mid	Fragment	0.04
BRPE	3164	59	Mid	Fragment	0.11
BRPE	3164	60	Mid	Fiber	1.37
BRPE	3164	61	Mid	Fiber	0.13
BRPE	3164	62	Dark	Fiber	0.15
BRPE	3164	63	Mid	Fiber	1.24
BRPE	3164	64	Mid	Fiber	1.12
BRPE	3164	65	Mid	Fragment	0.06
BRPE	3164	66	Mid	Fiber	6.31
BRPE	3164	67	Mid	Fragment	0.14
BRPE	3164	68	Mid	Fragment	0.26
BRPE	3164	69	Dark	Fiber	0.36
BRPE	3164	70	Mid	Fragment	0.14
BRPE	3164	71	Mid	Fiber	3.04
BRPE	3164	72	Mid	Fragment	0.09
BRPE	3164	73	Mid	Fiber	1.51
BRPE	3164	74	Mid	Fiber	1.49
BRPE	3164	75	Light	Fiber	1.05
BRPE	3164	76	Mid	Fragment	0.44
BRPE	3164	77	Mid	Fragment	0.24
BRPE	3164	78	Mid	Fragment	0.07
BRPE	3164	79	Light	Fiber	3
BRPE	3164	80	Mid	Fiber	0.28
BRPE	3164	81	Mid	Fiber	1.04
BRPE	3164	82	Mid	Fragment	0.13
BRPE	3164	83	Mid	Fiber	3.15
BRPE	3164	84	Light	Fiber	2
BRPE	3164	85	Mid	Fiber	0.94
BRPE	3164	86	Mid	Fragment	0.09
BRPE	3164	87	Dark	Fragment	0.14
BRPE	3164	88	Dark	Fiber	0.73
BRPE	3164	89	Light	Fiber	1.07

Species	Specimen	Plastic Item	Color	Type	Size (mm)
BRPE	3164	90	Dark	Fiber	0.59
BRPE	3164	91	Mid	Fiber	3.53
BRPE	3171	92	Mid	Fiber	1.1
BRPE	3171	93	Mid	Fiber	2.66
BRPE	3171	94	Mid	Fiber	0.73
BRPE	3171	95	Dark	Fiber	0.64
BRPE	3171	96	Dark	Fragment	0.34
BRPE	3171	97	Mid	Fiber	4.9
BRPE	3171	98	Mid	Fiber	5.68
BRPE	3171	99	Light	Fiber	1.46
BRPE	3171	100	Light	Fiber	0.87
BRPE	3171	101	Light	Fiber	1.83
BRPE	3171	102	Light	Fiber	0.92
BRPE	3171	103	Mid	Fiber	1.69
BRPE	3171	104	Mid	Fiber	1.94
BRPE	3171	105	Mid	Fiber	0.78
BRPE	3171	106	Light	Fiber	3.41
BRPE	3171	107	Light	Fiber	1.72
BRPE	3171	108	Mid	Fiber	0.38
BRPE	3171	109	Mid	Fragment	0.19
BRPE	3165	110	Mid	Fiber	0.2
BRPE	3165	111	Mid	Fragment	0.06
BRPE	3165	112	Mid	Fiber	0.4
BRPE	3165	113	Light	Fiber	1.66
BRPE	3165	114	Mid	Fiber	1.82
BRPE	3165	115	Mid	Fiber	0.16
BRPE	3165	116	Mid	Fiber	1.12
BRPE	3165	117	Mid	Fiber	0.32
BRPE	3165	118	Mid	Fiber	1.96
BRPE	3165	119	Mid	Fiber	0.32
BRPE	3165	120	Mid	Fiber	0.49
BRPE	3165	121	Mid	Fiber	0.5
BRPE	3165	122	Mid	Fiber	1.39
BRPE	3165	123	Mid	Fiber	1.47
BRPE	3165	124	Mid	Fiber	0.97
BRPE	3165	125	Mid	Fiber	0.21
BRPE	3165	126	Mid	Fiber	4.78
BRPE	3165	127	Light	Fiber	2.42
BRPE	3165	128	Mid	Fiber	1.42
BRPE	3165	129	Mid	Fragment	0.25
BRPE	3165	130	Mid	Fiber	0.19
BRPE	3165	131	Mid	Fiber	0.19
BRPE	3165	132	Mid	Fiber	0.28
BRPE	3165	133	Mid	Fragment	0.12
BRPE	3165	134	Mid	Fiber	2.11
BRPE	3165	135	Mid	Fiber	1.37
BRPE	3165	136	Mid	Fragment	0.15
BRPE	3165	137	Mid	Fiber	2.26
BRPE	3165	138	Dark	Fiber	1.2
BRPE	3165	139	Dark	Fiber	0.9
BRPE	3165	140	Mid	Fiber	2.53
BRPE	3165	141	Mid	Fiber	0.21
BRPE	3165	142	Mid	Fiber	0.25
BRPE	3165	143	Mid	Fiber	6.6
BRPE	3165	144	Mid	Fiber	1.61
BRPE	3165	145	Dark	Fiber	1.85
BRPE	3165	146	Mid	Fragment	0.2
BRPE	3165	147	Mid	Fiber	0.4
BRPE	3165	148	Light	Fiber	1.43
BRPE	3165	149	Light	Fiber	0.54
BRPE	3165	150	Mid	Fragment	0.17
BRPE	3165	151	Dark	Fiber	1.61
BRPE	3165	152	Mid	Fiber	1.01
BRPE	3165	153	Dark	Fiber	1.17
BRPE	3165	154	Mid	Fiber	1.5
BRPE	3165	155	Mid	Fragment	0.26
BRPE	3165	156	Dark	Fiber	3.43
BRPE	3165	157	Dark	Fiber	0.59
BRPE	3165	158	Mid	Fiber	0.63
BRPE	3165	159	Mid	Fiber	0.63
BRPE	3165	160	Dark	Fiber	1.61
BRPE	3170	161	Mid	Fiber	4.7
BRPE	3170	162	Light	Fiber	1.58
BRPE	3170	163	Light	Fiber	1.86
BRPE	3170	164	Light	Fragment	0.43
BRPE	3170	165	Mid	Fiber	0.28
BRPE	3170	166	Mid	Fiber	2.73
BRPE	3163	167	Mid	Fiber	1.14
BRPE	3163	168	Mid	Fiber	0.47
BRPE	3163	169	Mid	Fragment	0.18
BRPE	3163	170	Light	Fiber	0.98
BRPE	3163	171	Dark	Fiber	0.37
BRPE	3163	172	Mid	Fragment	0.16
BRPE	3163	173	Mid	Fragment	0.22
BRPE	3163	174	Mid	Fiber	0.61
BRPE	3163	175	Mid	Fragment	0.6
BRPE	3163	176	Mid	Fiber	1.72
BRPE	3163	177	Mid	Fiber	3.63
BRPE	3163	178	Mid	Fiber	1.25
BRPE	3163	179	Mid	Fiber	0.69

Species	Specimen	Plastic Item	Color	Type	Size (mm)
BRPE	3163	180	Mid	Fragment	0.08
BRPE	3163	181	Light	Fiber	2.31
BRPE	3163	182	Mid	Fragment	0.07
BRPE	3163	183	Mid	Fiber	0.34
BRPE	3166	184	Mid	Fiber	0.73
BRPE	3166	185	Light	Fragment	0.14
BRPE	3166	186	Light	Fragment	0.17
BRPE	3166	187	Mid	Fiber	0.6
BRPE	3166	188	Mid	Fiber	1.14
BRPE	3166	189	Mid	Fiber	2.22
BRPE	3166	190	Mid	Fiber	2.76
BRPE	3166	191	Mid	Fiber	2.94
BRPE	3166	192	Light	Fiber	3.57
BRPE	3166	193	Light	Fragment	1.28
BRPE	3166	194	Mid	Fiber	2.82
BRPE	3166	195	Mid	Fiber	2
BRPE	3166	196	Mid	Fiber	0.4
BRPE	3172	197	Mid	Fiber	0.67
BRPE	3172	198	Mid	Fiber	0.45
BRPE	3172	199	Mid	Fiber	0.31
BRPE	3172	200	Mid	Fiber	1.18
BRPE	3172	201	Mid	Fiber	1.13
BRPE	3172	202	Mid	Fiber	6.79
BRPE	3172	203	Mid	Fragment	0.1
BRPE	3172	204	Dark	Fiber	0.49
BRPE	3172	205	Dark	Fiber	0.33
BRPE	3172	206	Dark	Fiber	4.19
BRPE	3172	207	Dark	Fiber	0.43
BRPE	3172	208	Mid	Fiber	0.69
BRPE	3172	209	Mid	Fiber	1.17
BRPE	3172	210	Mid	Fiber	0.82
BRPE	3172	211	Dark	Fiber	0.21
BRPE	3172	212	Mid	Fiber	0.93
BRPE	3172	213	Mid	Fiber	0.21
BRPE	3172	214	Mid	Fragment	0.09
BRPE	3172	215	Mid	Fiber	1.97
BRPE	3172	216	Light	Fiber	0.94
BRPE	3172	217	Mid	Fiber	7.72
BRPE	3172	218	Dark	Fiber	1.13
BRPE	3172	219	Mid	Fiber	2.38
BRPE	3172	220	Dark	Fiber	0.36
BRPE	3172	221	Light	Fiber	0.51
BRPE	3172	222	Mid	Fiber	3.49
BRPE	3172	223	Mid	Fragment	0.19
BRPE	3172	224	Mid	Fiber	0.94
BRPE	3172	225	Mid	Fragment	0.19
BRPE	3172	226	Mid	Fiber	1.77
BRPE	3172	227	Mid	Fragment	0.2
BRPE	3172	228	Mid	Fiber	1.05
BRPE	3172	229	Dark	Fiber	1.7
BRPE	3172	230	Dark	Fiber	0.67
BRPE	3172	231	Dark	Fiber	0.2
BRPE	3168	232	Mid	Fiber	1.81
BRPE	3168	233	Light	Fiber	1.14
BRPE	3168	234	Light	Fiber	5.19
BRPE	3168	235	Dark	Fiber	0.55
BRPE	3168	236	Dark	Fiber	0.21
BRPE	3168	237	Mid	Fiber	0.73
BRPE	3168	238	Dark	Fragment	0.09
BRPE	3162	239	Mid	Fiber	1.23
BRPE	3162	240	Light	Fiber	1.29
BRPE	3162	241	Light	Fragment	0.63
BRPE	3162	242	Light	Fragment	0.51
BRPE	3162	243	Light	Fragment	0.56
BRPE	3162	244	Mid	Fragment	0.19
BRPE	3162	245	Light	Fragment	0.27
BRPE	3162	246	Mid	Fiber	2.22
BRPE	3162	247	Mid	Fiber	0.89
BRPE	3162	248	Mid	Fiber	4.8
BRPE	3162	249	Mid	Fiber	2.6
BRPE	3162	250	Mid	Fiber	0.51
BRPE	3162	251	Mid	Fragment	0.28
BRPE	3162	252	Light	Fiber	2.01
BRPE	3162	253	Mid	Fiber	2.79
BRPE	3162	254	Mid	Fragment	0.66
BRPE	3162	255	Light	Fiber	2.15
BRPE	3162	256	Light	Fiber	1.76
BRPE	3162	257	Mid	Fiber	1.97
BRPE	3162	258	Mid	Fiber	2.58
BRPE	3162	259	Mid	Fiber	4.57
BRPE	3162	260	Mid	Fragment	0.17
BRPE	3162	261	Mid	Fiber	0.37
BRPE	3162	262	Light	Fiber	1.08
BRPE	3162	263	Light	Fiber	0.49
BRPE	3162	264	Dark	Fiber	0.15
BRPE	3162	265	Mid	Fiber	2.22
BRPE	3162	266	Mid	Fiber	1.02
BRPE	3162	267	Light	Fiber	0.29
BRPE	3162	268	Light	Fiber	0.22
BRPE	3162	269	Dark	Fiber	0.38

Species	Specimen	Plastic Item	Color	Type	Size (mm)
BRPE	3162	270	Mid	Fragment	0.1
BRPE	3162	271	Mid	Fiber	0.66
BRPE	3162	272	Mid	Fiber	0.94
BRPE	3162	273	Light	Fiber	1.18
BRPE	3162	274	Mid	Fiber	0.25
BRPE	3162	275	Dark	Fragment	0.13
BRPE	3162	276	Dark	Fragment	0.2
BRPE	3162	277	Mid	Fragment	0.12
BRPE	3162	278	Light	Fiber	1.28
BRPE	3162	279	Light	Fragment	0.37
BRPE	3162	280	Light	Fiber	1.61
BRPE	3162	281	Mid	Fiber	1.34
BRPE	3162	282	Mid	Fiber	1.06
BRPE	3162	283	Mid	Fiber	2.16
BRPE	3162	284	Mid	Fragment	0.15
BRPE	3162	285	Light	Fiber	1.58
BRPE	3162	286	Dark	Fragment	0.17
BRPE	3162	287	Dark	Fiber	0.32
BRPE	3162	288	Mid	Fiber	1.77
BRPE	3162	289	Mid	Fiber	2.38
BRPE	3162	290	Mid	Fiber	1.25
BRPE	3162	291	Dark	Fiber	0.35
BRPE	3162	292	Mid	Fiber	1.17
BRPE	3162	293	Mid	Fiber	1.52
BRPE	3169	294	Mid	Fiber	0.78
BRPE	3169	295	Mid	Fragment	0.17
BRPE	3169	296	Mid	Fiber	1.29
BRPE	3169	297	Mid	Fiber	1.19
BRPE	3169	298	Mid	Fiber	0.74
BRPE	3169	299	Mid	Fiber	0.97
BRPE	3169	300	Mid	Fiber	0.46
BRPE	3169	301	Mid	Fragment	0.44
BRPE	3169	302	Mid	Fiber	2.47
BRPE	3169	303	Mid	Fiber	2.19
BRPE	3169	304	Mid	Fragment	0.09
BRPE	3169	305	Mid	Fiber	0.85
BRPE	3169	306	Mid	Fiber	1.17
BRPE	3169	307	Mid	Fiber	0.61
BRPE	3169	308	Mid	Fiber	0.28
BRPE	3169	309	Mid	Fiber	1.08
BRPE	3169	310	Dark	Fiber	1.62
BRPE	3169	311	Mid	Fragment	0.13
BRPE	3169	312	Dark	Fiber	0.88
BRPE	3169	313	Light	Fiber	0.82
BRPE	3169	314	Mid	Fiber	1.77
BRPE	3169	315	Mid	Fiber	1.3
BRPE	3169	316	Mid	Fiber	0.83
BRPE	3169	317	Mid	Fiber	1.62
BRPE	3169	318	Mid	Fiber	0.83
BRPE	3169	319	Mid	Fiber	1.51
BRPE	3169	320	Mid	Fiber	2.65
BRPE	3169	321	Mid	Fiber	1.06
BRPE	3169	322	Mid	Fiber	0.73
BRPE	3169	323	Mid	Fiber	0.59
BRPE	3169	324	Mid	Fiber	1.16
BRPE	3169	325	Light	Fiber	2.55
BRPE	3169	326	Mid	Fiber	0.71
BRPE	3169	327	Mid	Fiber	1.64
BRPE	3169	328	Mid	Fiber	2.23
BRPE	3169	329	Mid	Fiber	1.19
BRPE	3169	330	Mid	Fiber	1.76
BRPE	3169	331	Mid	Fragment	0.34
BRPE	3169	332	Dark	Fiber	0.96
BRPE	3169	333	Mid	Fiber	4.45
BRPE	3169	334	Mid	Fiber	0.47
BRPE	3169	335	Mid	Fiber	0.66
BRPE	3169	336	Dark	Fiber	0.23
BRPE	3169	337	Dark	Fiber	0.88
BRPE	3169	338	Mid	Fiber	1.7
BRPE	3169	339	Mid	Fiber	0.88
BRPE	3169	340	Mid	Fiber	0.76
BRPE	3169	341	Mid	Fiber	0.79
BRPE	3169	342	Dark	Fragment	0.32
BRPE	3169	343	Dark	Fiber	0.47
BRPE	3169	344	Dark	Fiber	0.72
BRPE	3169	345	Mid	Fiber	0.64
BRPE	3169	346	Mid	Fiber	0.48
BRPE	3169	347	Light	Fiber	3.31
BRPE	3169	348	Mid	Fiber	1.3
BRPE	3169	349	Mid	Fragment	0.81
BRPE	3169	350	Dark	Fiber	2.1
BRPE	3169	351	Light	Fiber	1.86
BRPE	3169	352	Light	Fiber	1.45
BRPE	3169	353	Dark	Fiber	0.5
BRPE	3169	354	Dark	Fiber	0.22
BRPE	3169	355	Dark	Fiber	1.58
BRPE	3169	356	Dark	Fiber	1.96
BRPE	3169	357	Dark	Fiber	2.72
BRPE	3169	358	Mid	Fragment	0.13
BRPE	3169	359	Mid	Fiber	1.45

Appendix Table 1b. Laughing Gull (LAGU) microplastic data

Species	Specimen	Plastic Item	Color	Type	Size (mm)
LAGU	3161	1	Light	Fiber	0.93
LAGU	3161	2	Mid	Fiber	1.21
LAGU	3161	3	Mid	Fiber	3.91
LAGU	3161	4	Mid	Fiber	0.5
LAGU	3161	5	Mid	Fiber	1.8
LAGU	3161	6	Light	Fiber	0.84
LAGU	3161	7	Mid	Fragment	0.04
LAGU	3161	8	Mid	Fiber	0.63
LAGU	3161	9	Light	Fiber	1.8
LAGU	3161	10	Mid	Fiber	1.69
LAGU	3161	11	Mid	Fragment	0.06
LAGU	3161	12	Light	Fiber	5.34
LAGU	3161	13	Mid	Fiber	0.74
LAGU	3161	14	Mid	Fiber	4.62
LAGU	3161	15	Dark	Fiber	4.55
LAGU	3161	16	Dark	Fiber	2.27
LAGU	3161	17	Mid	Fiber	0.89
LAGU	3161	18	Mid	Fiber	0.42
LAGU	3160	19	Mid	Fiber	1.07
LAGU	3160	20	Mid	Fiber	3.23
LAGU	3160	21	Dark	Fiber	1.49
LAGU	3160	22	Mid	Fiber	1.64
LAGU	3160	23	Dark	Fragment	0.18
LAGU	3160	24	Dark	Fiber	0.31
LAGU	3160	25	Dark	Fragment	0.15
LAGU	3160	26	Dark	Fiber	0.57
LAGU	3160	27	Dark	Fragment	0.15
LAGU	3160	28	Dark	Fiber	1.73
LAGU	3160	29	Dark	Fragment	0.1
LAGU	3164	30	Light	Fiber	5.8
LAGU	3164	31	Light	Fiber	0.62
LAGU	3164	32	Mid	Fragment	0.13
LAGU	3164	33	Mid	Fragment	0.23
LAGU	3164	34	Mid	Fiber	1.28
LAGU	3164	35	Mid	Fiber	1.02
LAGU	3167	36	Mid	Fiber	0.35
LAGU	3167	37	Mid	Fiber	2.94
LAGU	3167	38	Mid	Fiber	0.34
LAGU	3167	39	Mid	Fiber	2.27
LAGU	3172	40	Dark	Fragment	0.14
LAGU	3172	41	Light	Fiber	2.91
LAGU	3172	42	Light	Fiber	2.52
LAGU	3172	43	Mid	Fiber	0.42
LAGU	3172	44	Mid	Fiber	0.45
LAGU	3172	45	Mid	Fiber	1.86
LAGU	3172	46	Mid	Fragment	0.08
LAGU	3171	47	Mid	Fiber	0.46
LAGU	3171	48	Mid	Fiber	1.09
LAGU	3171	49	Mid	Fiber	2
LAGU	3171	50	Mid	Fiber	0.94
LAGU	3171	51	Dark	Fiber	1.88
LAGU	3171	52	Dark	Fiber	1.15
LAGU	3171	53	Light	Fiber	6.28
LAGU	3171	54	Mid	Fiber	0.67
LAGU	3163	55	Mid	Fiber	0.95
LAGU	3163	56	Dark	Fiber	0.33
LAGU	3163	57	Dark	Fiber	0.29
LAGU	3163	58	Dark	Fiber	0.3
LAGU	3163	59	Light	Fragment	2.89
LAGU	3163	60	Mid	Fiber	0.23
LAGU	3163	61	Dark	Fiber	0.5
LAGU	3163	62	Dark	Fiber	1.18
LAGU	3163	63	Dark	Fiber	0.16
LAGU	3163	64	Dark	Fiber	2.6
LAGU	3163	65	Dark	Fiber	0.5
LAGU	3163	66	Dark	Fiber	0.46
LAGU	3163	67	Mid	Fiber	0.38
LAGU	3163	68	Dark	Fiber	0.59
LAGU	3163	69	Mid	Fiber	0.95
LAGU	3165	70	Mid	Fragment	0.35
LAGU	3165	71	Mid	Fragment	0.13
LAGU	3165	72	Dark	Fiber	2.55
LAGU	3165	73	Mid	Fragment	0.2
LAGU	3165	74	Dark	Fiber	0.88
LAGU	3165	75	Dark	Fiber	4.54
LAGU	3165	76	Mid	Fragment	0.2
LAGU	3165	77	Mid	Fragment	0.11
LAGU	3165	78	Mid	Fragment	0.24
LAGU	3169	79	Dark	Fiber	0.48
LAGU	3169	80	Mid	Fiber	6.27
LAGU	3169	81	Mid	Fragment	0.08
LAGU	3169	82	Mid	Fiber	0.12
LAGU	3169	83	Mid	Fiber	0.57
LAGU	3162	84	Mid	Fiber	0.86
LAGU	3162	85	Mid	Fragment	0.49
LAGU	3162	86	Dark	Fiber	3.36
LAGU	3162	87	Mid	Fragment	0.14
LAGU	3168	88	Mid	Fragment	0.13
LAGU	3168	89	Mid	Fiber	0.3
LAGU	3166	90	Mid	Fiber	1.24
LAGU	3166	91	Mid	Fiber	1.26
LAGU	3166	92	Mid	Fiber	0.59
LAGU	3166	93	Mid	Fiber	0.5
LAGU	3166	94	Mid	Fiber	0.4
LAGU	3166	95	Mid	Fiber	1.38
LAGU	3170	96	Light	Fiber	2.18
LAGU	3170	97	Mid	Fiber	0.21
LAGU	3170	98	Mid	Fiber	0.5
LAGU	3170	99	Mid	Fiber	0.28

Appendix Table 1c. Double-crested Cormorant (DCCO) microplastic data

Species	Specimen	Plastic Item	Color	Type	Size (mm)	Species	Specimen	Plastic Item	Color	Type	Size (mm)
DCCO	3211	1	Mid	Fiber	0.75	DCCO	3209	49	Mid	Fiber	1.26
DCCO	3211	2	Dark	Fragment	0.25	DCCO	3209	50	Dark	Fragment	0.37
DCCO	3211	3	Mid	Fragment	0.23	DCCO	3209	51	Dark	Fragment	0.19
DCCO	3211	4	Mid	Fragment	0.24	DCCO	3209	52	Mid	Fragment	0.21
DCCO	3211	5	Mid	Fragment	0.26	DCCO	3209	53	Dark	Fragment	0.25
DCCO	3211	6	Light	Fragment	0.36	DCCO	3209	54	Dark	Fragment	0.23
DCCO	3211	7	Mid	Fiber	1.13	DCCO	3209	55	Light	Fiber	2.19
DCCO	3211	8	Mid	Fiber	0.45	DCCO	3209	56	Mid	Fragment	0.25
DCCO	3211	9	Mid	Fragment	0.1	DCCO	3203	57	Mid	Fiber	2.77
DCCO	3211	10	Mid	Fiber	1.32	DCCO	3203	58	Mid	Fiber	1.27
DCCO	3202	11	Light	Fragment	0.56	DCCO	3203	59	Mid	Fiber	0.37
DCCO	3202	12	Light	Fragment	0.76	DCCO	3203	60	Light	Fiber	3.79
DCCO	3202	13	Mid	Fragment	0.86	DCCO	3203	61	Light	Fiber	3.13
DCCO	3202	14	Dark	Fragment	0.44	DCCO	3203	62	Mid	Fragment	0.58
DCCO	3202	15	Mid	Fiber	0.78	DCCO	3203	63	Mid	Fragment	0.08
DCCO	3202	16	Dark	Fragment	0.83	DCCO	3203	64	Mid	Fragment	0.12
DCCO	3202	17	Mid	Fiber	0.95	DCCO	3203	65	Mid	Fiber	2.13
DCCO	3202	18	Mid	Fiber	0.33	DCCO	3203	66	Mid	Fragment	0.13
DCCO	3202	19	Dark	Fiber	0.13	DCCO	3207	67	Mid	Fiber	0.88
DCCO	3202	20	Light	Fragment	1.05	DCCO	3207	68	Mid	Fiber	0.95
DCCO	3202	21	Light	Fragment	0.4	DCCO	3207	69	Mid	Fiber	0.17
DCCO	3202	22	Light	Fragment	0.3	DCCO	3207	70	Mid	Fiber	2.13
DCCO	3202	23	Mid	Fiber	2.37	DCCO	3207	71	Mid	Fiber	0.75
DCCO	3202	24	Mid	Fiber	0.95	DCCO	3207	72	Mid	Fiber	1.74
DCCO	3202	25	Light	Fiber	0.65	DCCO	3207	73	Mid	Fiber	2.97
DCCO	3202	26	Light	Fragment	0.59	DCCO	3207	74	Dark	Fiber	0.3
DCCO	3202	27	Dark	Fiber	1.98	DCCO	3207	75	Light	Fiber	1.09
DCCO	3202	28	Mid	Fragment	0.12	DCCO	3207	76	Light	Fiber	3.38
DCCO	3202	29	Mid	Fragment	2.98	DCCO	3207	77	Mid	Fiber	0.85
DCCO	3202	30	Mid	Fiber	1.99	DCCO	3207	78	Light	Fiber	1.32
DCCO	3202	31	Light	Fragment	0.67	DCCO	3207	79	Light	Fiber	1.29
DCCO	3202	32	Mid	Fiber	1.01	DCCO	3207	80	Dark	Fiber	4.41
DCCO	3202	33	Dark	Fiber	0.29	DCCO	3207	81	Light	Fiber	1.75
DCCO	3202	34	Light	Fragment	0.47	DCCO	3207	82	Dark	Fragment	0.13
DCCO	3202	35	Light	Fiber	3.72	DCCO	3207	83	Mid	Fiber	0.13
DCCO	3202	36	Light	Fiber	4.76	DCCO	3205	84	Mid	Fiber	2
DCCO	3202	37	Dark	Fragment	0.11	DCCO	3205	85	Mid	Fragment	0.06
DCCO	3202	38	Light	Fragment	1.08	DCCO	3205	86	Mid	Fiber	1.02
DCCO	3210	39	Mid	Fiber	0.8	DCCO	3205	87	Dark	Fragment	0.28
DCCO	3210	40	Dark	Fragment	0.2	DCCO	3208	88	Mid	Fiber	0.54
DCCO	3210	41	Dark	Fragment	0.11	DCCO	3208	89	Dark	Fiber	2.35
DCCO	3210	42	Dark	Fragment	0.16	DCCO	3208	90	Mid	Fiber	1.85
DCCO	3210	43	Light	Fragment	0.31	DCCO	3208	91	Mid	Fiber	2.23
DCCO	3210	44	Dark	Fragment	0.08	DCCO	3208	92	Light	Fiber	7.81
DCCO	3210	45	Light	Fiber	2.17	DCCO	3208	93	Dark	Fiber	4.72
DCCO	3210	46	Light	Fragment	0.42	DCCO	3208	94	Dark	Fragment	0.48
DCCO	3209	47	Mid	Fragment	0.1	DCCO	3204	95	Dark	Fragment	0.14
DCCO	3209	48	Dark	Fragment	0.11	DCCO	3204	96	Light	Fragment	0.57
						DCCO	3206	N/A	N/A	N/A	0

Appendix Table 1d. Royal Tern (ROTE) microplastic data

Species	Specimen	Plastic Item	Color	Type	Size (mm)
ROTE	3135	1	Dark	Fiber	0.93
ROTE	3135	2	Mid	Fiber	2.58
ROTE	3135	3	Dark	Fiber	0.72
ROTE	3135	4	Dark	Fiber	0.18
ROTE	3135	5	Mid	Fiber	2.11
ROTE	3135	6	Mid	Fiber	0.78
ROTE	3135	7	Dark	Fiber	0.53
ROTE	3135	8	Mid	Fiber	1.45
ROTE	3135	9	Mid	Fiber	0.37
ROTE	3135	10	Dark	Fragment	0.31
ROTE	3135	11	Dark	Fiber	0.85
ROTE	3135	12	Mid	Fragment	0.34
ROTE	3135	13	Mid	Fragment	0.12
ROTE	3135	14	Mid	Fiber	1.73
ROTE	3135	15	Mid	Fiber	0.57
ROTE	3135	16	Dark	Fiber	1.26
ROTE	3142	17	Mid	Fiber	1.34
ROTE	3142	18	Mid	Fiber	0.16
ROTE	3142	19	Dark	Fragment	0.19
ROTE	3142	20	Light	Fiber	2.18
ROTE	3142	21	Dark	Fiber	0.74
ROTE	1108	22	Mid	Fragment	0.15
ROTE	1108	23	Mid	Fragment	0.1
ROTE	1108	24	Light	Fragment	0.12
ROTE	1108	25	Mid	Fiber	0.52
ROTE	1108	26	Mid	Fragment	0.08
ROTE	1108	27	Mid	Fragment	0.07
ROTE	1108	28	Dark	Fragment	0.08
ROTE	1108	29	Dark	Fragment	0.09
ROTE	1108	30	Mid	Fragment	0.07
ROTE	1108	31	Dark	Fiber	0.43
ROTE	1108	32	Dark	Fiber	0.96
ROTE	1108	33	Mid	Fragment	0.1
ROTE	3141	34	Light	Fiber	5.08
ROTE	3141	35	Dark	Fiber	2.09
ROTE	3141	36	Mid	Fragment	0.1
ROTE	3141	37	Dark	Fragment	0.1
ROTE	3141	38	Dark	Fragment	0.05
ROTE	3141	39	Dark	Fragment	0.12
ROTE	3140	40	Mid	Fiber	1.04
ROTE	3140	41	Mid	Fiber	1.11
ROTE	3140	42	Mid	Fiber	1.15
ROTE	3140	43	Mid	Fiber	2.23
ROTE	3140	44	Mid	Fiber	0.97
ROTE	3140	45	Mid	Fiber	2.44
ROTE	3140	46	Dark	Fragment	0.23
ROTE	3140	47	Mid	Fiber	2.33
ROTE	3140	48	Mid	Fiber	2.36
ROTE	3140	49	Mid	Fiber	1.56
ROTE	3140	50	Dark	Fragment	0.14
ROTE	3140	51	Light	Fragment	0.43
ROTE	3137	52	Mid	Fiber	0.4
ROTE	3137	53	Light	Fiber	2.19
ROTE	3137	54	Light	Fragment	0.45
ROTE	3137	55	Light	Fiber	2.37
ROTE	3137	56	Light	Fiber	0.5
ROTE	3137	57	Light	Fiber	0.73
ROTE	3137	58	Light	Fiber	2.25
ROTE	3137	59	Light	Fiber	1.62
ROTE	3137	60	Dark	Fragment	0.13
ROTE	3137	61	Dark	Fragment	0.24
ROTE	3137	62	Dark	Fragment	0.19
ROTE	3137	63	Mid	Fragment	0.16
ROTE	3137	64	Mid	Fragment	0.11
ROTE	3137	65	Mid	Fragment	0.47
ROTE	3137	66	Mid	Fiber	0.57
ROTE	3137	67	Dark	Fragment	0.22
ROTE	3137	68	Mid	Fragment	0.28
ROTE	3138	69	Light	Fiber	2.71
ROTE	3138	70	Mid	Fragment	0.13
ROTE	3138	71	Mid	Fiber	0.78
ROTE	3138	72	Mid	Fiber	1.61
ROTE	3138	73	Mid	Fiber	0.83
ROTE	3138	74	Dark	Fragment	0.11
ROTE	3138	75	Mid	Fiber	0.23
ROTE	3136	76	Mid	Fiber	1.68
ROTE	3136	77	Mid	Fiber	1.69
ROTE	3136	78	Mid	Fiber	1.18
ROTE	3136	79	Mid	Fiber	1.81
ROTE	3136	80	Light	Fiber	1.61
ROTE	3136	81	Mid	Fiber	3.45
ROTE	3139	82	Mid	Fiber	1.83
ROTE	3139	83	Mid	Fiber	0.47
ROTE	3139	84	Mid	Fiber	1.44
ROTE	3139	85	Mid	Fiber	0.36
ROTE	3139	86	Dark	Fragment	0.2
ROTE	3139	87	Light	Fiber	0.61
ROTE	3139	88	Mid	Fiber	1
ROTE	3139	89	Mid	Fiber	2.93