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Ubiquitous microplastics in the upper gastrointestinal tracts of Florida coastal seabirds

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Abstract Plastic pollution is increasingly recognized as a global problem. In particular, plastic pieces <5 mm in size ('microplastics') are of interest due to their prevalence and association with harmful, persistent organic pollutants (POPs). Very little is known about the prevalence of microplastics in coastal birds. Yet, these water-associated birds are at a high risk of ingesting microplastics that accumulate near the water's surface. This study describes the microplastics found in the proventriculus and ventriculus of four species of coastal birds regarding quantity, size, type (fiber or fragment), and color (light, mid, or dark). A total of 643 microplastic particles were identified, with 43 of the 44 study specimens containing microplastics (97.7% frequency). The 'fiber' type and the 'mid' color 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 medium-sized seabirds, but more work is needed to determine microplastic patterns between taxa and foraging environments.

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

Introduction

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

Most plastic is buoyant in aquatic environments and subject to abrasion or photodegradation, and these two processes act to reduce the plastic into increasingly smaller pieces (Barnes et al. 2009). The smaller plastic pieces, called "microplastics", have become of interest in recent years and are defined in the scientific literature as plastic particles <5 mm in size. Currently, microplastics are categorized as either primary or secondary, depending on the source of the plastics.

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Primary microplastics are manufactured to be small, such as industrial pellets and the abrasive microbeads used in soaps and cosmetics. Secondary microplastics are particles that have degraded from larger plastic items (e.g., fibers directly shed from clothing during laundering, photodegraded monofilament fishing line, and disposable shopping bags).

Because of their small size, marine microplastics can readily be taken up by a range of organisms, from zooplankton to apex predators (Cole et al. 2013, Provencher et al. 2014, Zhao et al. 2016). While the full extent of the damage from microplastics is poorly known, they can act as vectors for persistent organic pollutants (POPs) due to their shared hydrophobicity (Mato et al. 2001, Moore 2008), and POPs are known to biomagnify at higher trophic levels (Teuten et al. 2007).

Coastal birds are at particular risk of ingesting microplastics because many birds feed on prey at the ocean's surface, where buoyant plastic pieces commonly accumulate (Moser and Lee 1992). Plastics can physically damage the gastrointestinal tract of seabirds and can even cause starvation in large quantities (Pierce et al. 2004). Coastal birds assimilate POPs associated with plastic sources into their tissues (Ryan et al. 1988, Tanaka et al. 2013). Once ingested, microplastics and POPs can reduce body condition, alter reproduction rates, and increase mortality in seabirds (Lavers et al. 2014, Spear et al. 1995). For example, the pesticide Dichlorodiphenyltrichloroethane (DDT) resulted in a severe population decline of Brown Pelican (*Pelecanus occidentalis*) before it was banned in 1972 but DDT remains a microplastic associated POP (Bakir et al. 2014). Rani et al. (2015) detected over 200 chemicals associated with microplastics, all with wide ranging biological effects.

The purpose of this study was to conduct a preliminary assessment of microplastics in the upper gastrointestinal tracts of four medium-sized coastal bird species common to southeast Florida, as no published studies exist examining microplastic content in these species. Our four study species were: Brown Pelican, Royal Tern (*Thalasseus maximus*), Laughing Gull (*Leucophaeus atricilla*), and Double-crested Cormorant (*Phalacrocorax auratus*). Although a varying portion of these species' populations seasonally migrates, most of these birds commonly inhabit the region year-round along the coastline (eBird 2021). These species are generalists, mostly foraging for prey on or near the ocean surface, targeting fishes and invertebrates. The Brown Pelican employs a scoop-foraging feeding style whereas the other three more directly target individual prey items (Aygen and Emslie 2006, Johnson et al. 2002, Pierotti and Annett 1990, Shields 2014). All four species are classified as "least concern" by the IUCN (IUCN 2021).

Materials and Methods

All coastal 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. 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. All collections were of previously deceased specimens, so IACUC approval was not needed.

Specimens were collected opportunistically from the centers, so sample size depended on the number available. All the processing occurred in a clean fume hood, with the proper clothing worn (hat/hairnet, cotton clothing, and nitrile 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). For each specimen, the proventriculus and gizzard (hereafter, “upper GI tract”) were removed, rinsed externally with filtered DI water, and opened with a longitudinal incision. Any macroscopic pieces of plastic or other debris (e.g., fish hooks) were removed before placing the organs in a potassium hydroxide solution (10% KOH) at a 3:1 potassium hydroxide to organ volume ratio. All samples during the dissolution process were kept in glass mason jars sealed with aluminum foil and kept at room temperature for two to three weeks to allow the organic matter to dissolve completely. Once completely dissolved, the remaining liquid was filtered via a Buchner funnel paired with a vacuum flask through a 1 μm filter (Whatman brand, Grade GF/B Glass Microfiber filters). After passing the dissolved solution through the filter, filters were kept in covered Petri dishes and dried at room temperature for a minimum of 48 hours before the microscopic visual examination.

Several samples had high levels of residual lipids that did not dissolve entirely with 10% KOH alone, even when additional solution was added. For these samples, *ca*. 15-20% of the industrial-strength degreaser Solvalene (Superior Industries, Inc.; Chattanooga, TN) per total specimen volume was added to the 10% KOH solution in *ca*. 10 ml increments until the lipid layer was dissolved. Over 24 hours on a warm stir plate was required to emulsify the lipids into particles small enough to pass through the filter. Solvalene was verified by the manufacturer not to dissolve or affect plastics (pers. comm. H. Wilson, Superior Industries, Inc., December 1, 2020).

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 all microplastic items (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 procedure outlined by Zhao et al. (2016) and following the guide for identifying microplastics from Barrows et al. (2017).

First, anthropogenic particles were identified via the following criteria: no cellular or organic structures were visible, fibers were equally thick, not tapered at the end and had a three-dimensional bend, fibers were not segmented, nor did they appear as twisted flat ribbons, colored items were clear and homogeneously colored, potential microscopic anthropogenic litter that was transparent or whitish was examined with extra care and under higher magnification, particles were not lustrous, and fibers were bendable or soft. The second step was to identify the characteristics of each piece. Individual plastic pieces were classified into two groups: fragments and fibers. Colors were classified as light, mid, or dark, and each particle’s longest dimension was measured in mm.

RStudio (version 1.3.1073; RStudio Team 2020) was used for statistical analysis. Data were assessed for normality and homoscedasticity assumptions (data fit a normal distribution via Shapiro-Wilk test and ensuring homogeneity of variances). The hypotheses’ evaluation was run accordingly in parametric (ANOVA) or non-parametric (Kruskal-Wallis) analyses depending on whether the data fit a normal or non-normal distribution. Standard descriptive statistics were used to describe the standard morphometric data for the bird specimens (weight, wing chord length, and tarsus length) and the size (mm) of all the microplastics found, in total and for all species. A one-way ANOVA evaluated 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. Two negative binomial tests were also used to evaluate differences in the rates of microplastic ingestion between (1) species and (2) feeding methods. Significance was assessed at $\alpha = 0.05$.

Results

A total of 44 coastal birds were collected and processed. The Laughing Gull had the highest number of specimens (n=13), followed by Brown Pelicans (n=12), Double-crested Cormorants (n=10), and finally Royal Tern (n=9). Most of the specimens

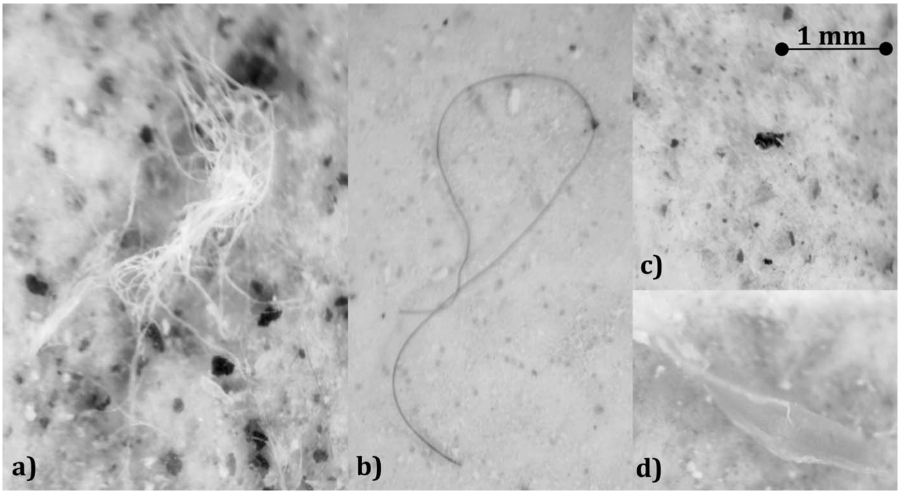

Figure 1. Examples of found microplastics. Examples of found microplastics from coastal bird upper gastrointestinal tracts: a) light fiber (Brown Pelican, specimen 3167), b) mid fiber (Brown Pelican, specimen 3172), c) mid fragment (Laughing Gull, specimen 3169), d) mid fragment (Double-crested Cormorant, specimen 3202), with a scale bar in the upper right-hand corner. Scale bar is the same across all photos; differences in color (shade) and texture are due to varying consistencies of dissolved upper GI tract liquids.

were from the Florida Keys Wild Bird Center (n=43), with one Royal Tern coming from the South Florida Wildlife Center (n=1).

A total of 643 microplastic particles were found across all specimens in varying shapes, sizes, and colors (Figure 1). Of the 44 study specimens, 43 contained microplastics (97.7% frequency). The mean length for the particles found was 1.145 (±1.23 mm), and the median particle length was 0.74 mm, with 95% of the particles found being smaller than 3 mm (Figure 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%. 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%).

Microplastic size data were not normal even after transformations, so the non-parametric Kruskal-Wallis test was used for comparisons. There was no difference between size of microplastics and species (p-value = 0.2578, Kruskal-Wallis chi-squared = 4.0343, df = 3) (Figure 3).

Negative binomial tests were run for each separate predictor (independent) variable. For the first test, the type of microplastic (fiber vs. fragment) was the first categorical predictor, and species was the second. The results of the first test showed that species affected the total number of microplastics ingested (p values for each species < 0.05) and that 'type' affected the total number of microplastics ingested because there were differences between the two levels of the independent variable fragment and fiber ($p = 1.9e^{-6}$, $z = -4.76$). Fiber was the type more

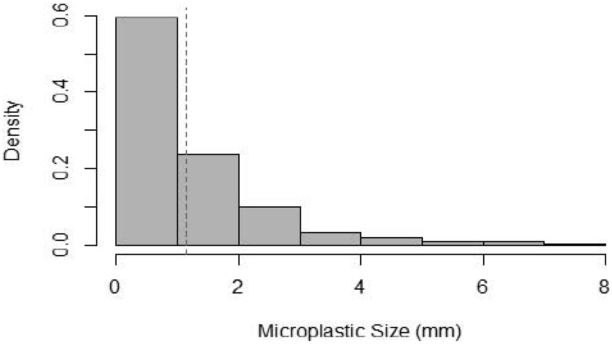

Figure 2. Microplastic size composition overall. Histogram representing the size of all microplastics (n=643 total microplastic fibers and fragments) found from upper gastrointestinal tracts of 44 four coastal bird study species from southeast Florida. The dashed line represents the mean per bird of the population.

frequently ingested (Figure 4). For the second test, the color of the microplastics (light, mid, or dark) was the first categorical predictor, and species was the second. In both tests, the number of microplastics was the discrete response variable. The results of the second test showed that species affected the total number of microplastics ingested (all p values < 0.05); the 'mid' color was the only color to

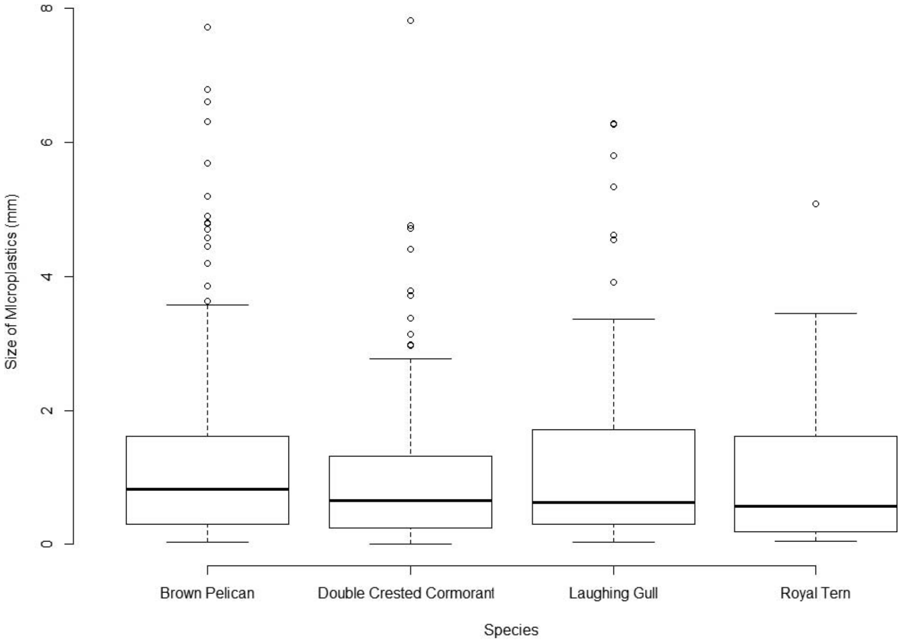

Figure 3. Microplastic size comparison by species. Box-and-whisker plot comparing the size of microplastics (mm) ingested between each of the four coastal bird study species from southeast Florida.

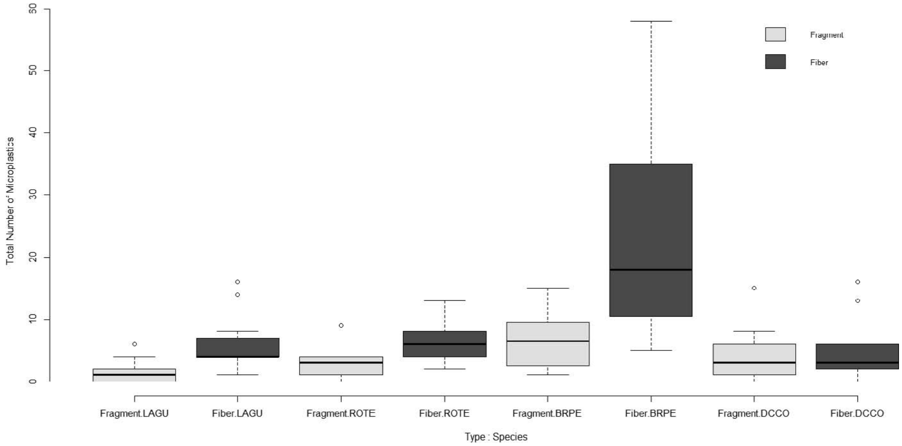

Figure 4. Fiber vs fragment breakdown. Box-and-whisker plot comparing microplastic type (fiber vs fragment) within and across the four coastal bird study species from southeast Florida. 'Fiber' was the type to be significantly ingest more frequently than fragment. LAGU = Laughing Gull, ROTE = Royal Tern, BRPE = Brown Pelican, DCCO = Double-crested Cormorant.

affect the total number of microplastics and was the only color to be significantly different between species ($p = 1.1e^{-6}$, $z = 4.87$) (Figure 5).

A negative binomial test was run to assess differences in the rate of occurrence of microplastics (number of particles/bird) between species. There were significant differences in the rates of occurrence for microplastic ingestion between species ($p = 1.358e^{-8}$, $R^2 = 0.457$). Brown Pelicans contained significantly more microplastics

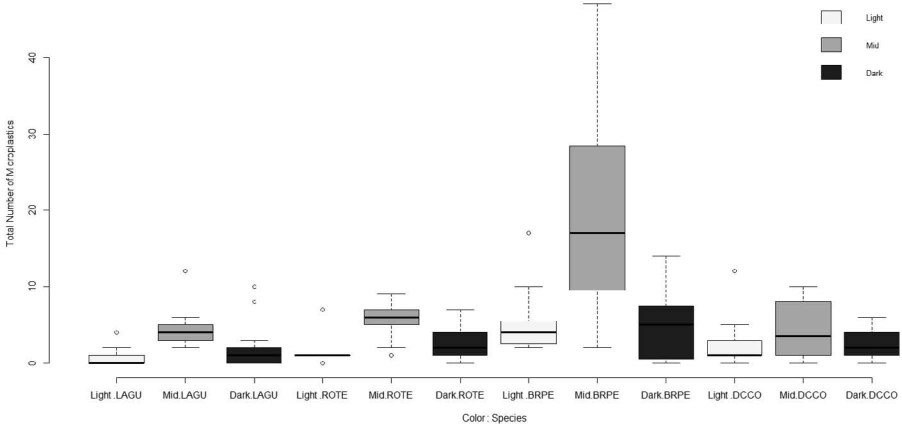

Figure 5. Color composition. Box-and-whisker plot comparing the three color classification groups (light, mid, and dark) of microplastics ingested within and across the four coastal bird study species from southeast Florida. The 'mid' color was ingested significantly more than 'light' or 'dark' colors. Species abbreviations: LAGU = Laughing Gull, ROTE = Royal Tern, BRPE = Brown Pelican, DCCO = Double-crested Cormorant.

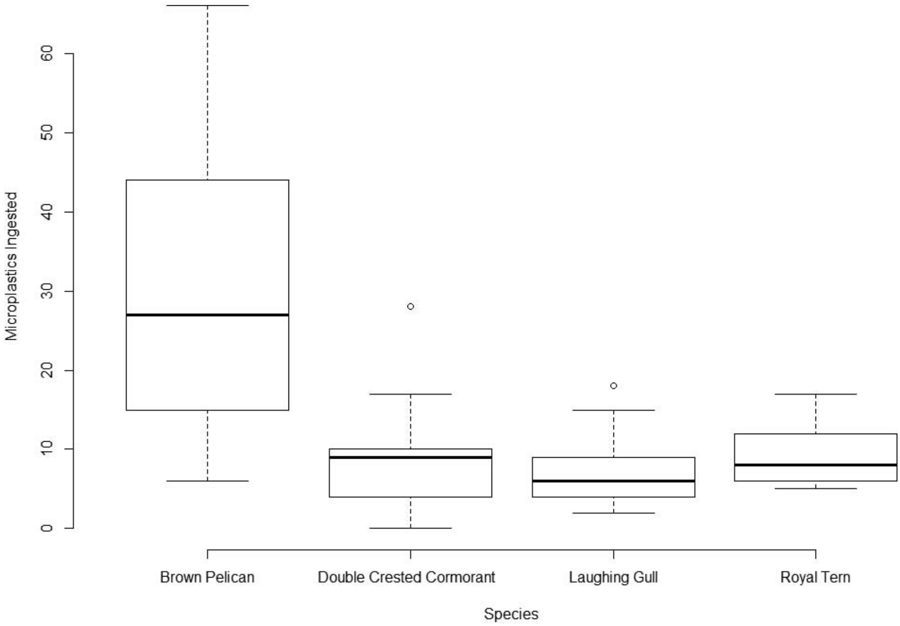

Figure 6. Total microplastics ingested (species). Box-and-whisker plot comparing the total number of microplastics ingested in each of the four coastal bird study species from southeast Florida; Brown Pelican was the only species that ingested significantly more microplastic particles.

than the other examined species, followed by Double-crested Cormorants and Royal Terns, with Laughing Gulls containing the least amount (Figure 6).

Finally, a negative binomial test assessed differences in the rate of occurrence of microplastics between different feeding methods (scoop versus targeted). There was a significant difference in the rate of microplastic ingestion between the two feeding types ($p = 1.194e^{-9}$, $R^2 = 0.4397$), with the scoop foraging method showing a higher incidence of microplastic ingestion (Figure 7).

Discussion

Although Carlin et al. (2020) examined microplastics in Florida accipitrid hawks, this is the first study to quantify microplastics in these four specific Florida coastal bird species. Ultimately, 97.7% of the specimens (43 of 44) contained a total of 643 particles, with 14.6 particles per bird.

‘Fiber’ was the most abundant type and found significantly more frequently than ‘fragment’, which is consistent 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), 89.2% thread/fiber and 10.8% other in Zhu et al. (2019), and 86% microfibers and 13% microfragments in Carlin et al. (2020). Fibers are used frequently in furniture, clothing, and hygiene products; global fiber production reached 111 million metric tons in 2020, with polyester fibers

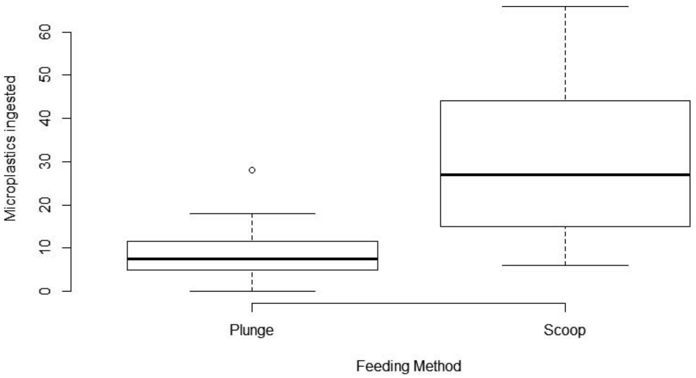

Figure 7. Total microplastics ingested (feeding method). Box-and-whisker plot comparing the total number of microplastics ingested between the two different feeding methods employed by the study organisms (scoop for Brown Pelican and targeting for Laughing Gull, Double-crested Cormorant, and Royal Tern). The scoop feeding method leads to a significantly higher incidence of microplastic ingestion.

accounting for 52.2% of the total (Anon. 2020). Although less common, microplastic fragments are still dangerous to animal life, causing 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. Prior bird studies concur with this result; Zhao et al. (2016) reported 81.6% of the ‘mid’ color type, Zhu et al. (2019) reported 91.1% of the blue/mid color type, and Carlin et al. (2020) found that most fibers were clear or royal blue. Color prevalence 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).

Species type did significantly affect the total number of microplastics, but no statistically significant relationship was found between species and particle size; all four study species contained similarly sized particles, which is consistent with other bird microplastic studies. This study found 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. The small size of particles found in this study, compared to 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) suggested three possible routes: (1) incidental ingestion while foraging, (2) fragmentation of macroplastic particles within the upper GI tract, or (3) secondary ingestion for carnivorous birds. The three ingestion routes are not mutually exclusive, as they all likely contribute to the results of this study.

Different feeding methods exhibited by the four medium-sized study species could partially explain the higher microplastic content found in Brown Pelicans. Caldwell et al. (2020) investigated this idea and found that plastic ingestion is greater in species that exhibit a more generalist feeding style as opposed to specific. Brown Pelicans use a scoop foraging style where they plunge dive underwater, mouth agape, and scoop their prey along with a large volume of water into their gular pouch. They then tilt their neck down to expel all water and then consume their prey (Shields 2014). This feeding method exposes Brown Pelicans to larger amounts of water (and associated floating plastic particles) than the other three study species as the latter feed by targeting individual prey items (Aygen and Emslie 2006, Johnson et al. 2002, Pierotti and Annett 1990).

Another possible reason for Brown Pelicans containing significantly more microplastics is that they are also the physically largest of the four study species and prey upon larger fishes. Rather than mistaking the small microplastic items for prey, the higher levels of microplastics in Brown Pelicans could be explained via bioaccumulation of plastics in prey (Nordberg et al. 2009), as suggested explicitly for microplastics in accipitrid hawks from Florida by Carlin et al. (2020). The Brown Pelican's diet consists mainly of the coastal marine fishes menhaden (*Brevoortia* spp.), mullet (*Mugil* spp.), and clupeid herrings and pilchards (Blus et al. 1979, Fogarty et al. 1981). For example, Atlantic Menhaden (*Brevoortia tyrannus*) gastrointestinal (GI) tracts were reported to contain microplastics (Avio et al. 2015), and Parker et al. (2020) found in Striped Mullet (*Mugil cephalus*) that microplastics can pass from the GI tract to the liver, where they are retained in the fish – the first study to show microplastic translocation and retention in marine vertebrates. As these latter two prey fishes for Brown Pelicans have been shown to contain microplastic particles, the significantly higher microplastics found here in Brown Pelicans could be attributed to secondary ingestion.

Future studies could further investigate this idea by subjecting prey fishes identified from coastal bird stomachs to the same microplastic identification techniques. 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). Tertiary ingestion may also contribute to the high particle numbers as both mullet and menhaden feed on detritus as well as zooplankton, which have also been found to ingest microplastics (Cole et al. 2013). Based on the estimate from the larger but similar American White Pelican (Werner 2004), Brown Pelicans consume more total prey by weight than the other three study species (*ca*. 1 kg/day versus *ca*. 0.6 kg/day for Double-Crested Cormorants per Glahn and Door 2002), so biomagnification processes may be responsible for their significantly higher quantity of microplastics.

The study had some limitations. For example, the individual birds in this study may not represent a proper random sample of the study species, as the bird carcasses were collected opportunistically from wildlife centers. Although none of these birds died specifically due to ingested microplastics, the associated co-morbidity effects may have made them weaker or more likely to be injured, thereby putting them at a higher probability of becoming a center patient. Additionally, microplastic particles were only identified by visual characters due to budget and time limitations. However, such particles could be further identified via Fouriertransform infrared spectroscopy (FTIR) to determine the exact type of plastic. Future work should attempt to characterize the kinds of plastic better (e.g., Nicastro et al. 2018 and Carlin et al. 2020) to suggest possible sources for waste reduction, an approach identified by Thompson et al. (2005).

Nevertheless, the demonstrated presence of microplastics in these four coastal birds highlights the need for a complete survey at other trophic levels to assess potential sources and ecological impacts within the coastal ecosystem. Exposed plastic is projected to persist for hundreds of years (Barnes et al., 2009) and will continue to fragment into progressively smaller pieces, increasing the probability of direct and indirect ingestion. Similar microplastic prevalence results from marine-associated birds in other geographic regions (e.g., South Korea in Nam et al. 2021 and Arctic Canada in Bourdages et al. 2021) further emphasize the global nature of the problem. Future studies should further explore how different foraging strategies affect plastic ingestion or how plastic particles could bioaccumulate up trophic levels across ecosystems.

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