Investigation of Microplastic Accumulation in the Gastrointestinal Tract in Birds of Prey

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INVESTIGATION OF MICROPLASTIC ACCUMULATION IN THE GASTROINTESTINAL TRACT IN BIRDS OF PREY

by

Julia Carlin

A thesis submitted in partial fulfillment of the requirements
for the Honors in the Major Program in Biology
in the College of Sciences
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Thesis Chair: Dr. Linda Walters, Ph.D.

ABSTRACT

Plastic pollution is unavoidable in the natural environment. Consequences of plastic ingestion include exposure to environmental pollutants and toxin accumulation, causing endocrine disruption, inflammatory and physiological stress in organisms. Microplastics have been shown to transfer across food webs, however, limited studies have examined microplastic accumulation across terrestrial food webs. Furthermore, few studies have examined plastic pollution in apex predatory animals. A study was conducted to quantify the abundance of plastic pollution in the gastrointestinal tract in birds of prey. Two species were investigated, one which forages in terrestrial habitats and one which forages in aquatic environments including *Buteo lineatus (*red-shouldered hawk) and *Pandion haliaetus* (osprey), respectively. The gastrointestinal tract was necropsied, chemically digested, and examined for microplastic prevalence. Overall, microplastics are significantly more abundant per gram of gastrointestinal (GI) tract tissue in species that forage on small rodents and terrestrial reptiles (*B. lineatus*) as compared to species that forage on fish and aquatic invertebrates (*P. haliaetus*). *Buteo lineatus* averaged 0.81 (±0.15) fibers and 0.14 (±0.04) fragments per gram of GI tract tissue while *P. halieatus* averaged 0.31 (±0.09) fibers and 0.04 (±0.02) fragments per gram of GI tract tissue. There was a significant interaction between type and color in both *B. lineatus* and *P. haliaetus* GI tract tissues. Micro-Fourier-transform infrared spectroscopy (μ-FTIR) was run on haphazardly selected samples and found that rayon was the most common polymer identified in both species. The significant difference found between species could be indicative that terrestrial raptors may experience greater bioaccumulation than aquatic species foraging at comparable trophic levels. However, the significant interaction between type and color in both species indicates a potential common source of pollution that affects both environments. Further investigation on the source of polymers is necessary in order to develop conservation and management strategies aimed at decreasing the output of synthetic fibers into the environment. Due to the abundance of polymers found in these species, understanding the potential biological and physiological effects of plastics is essential to informing superior management strategies that can better protect and preserve wildlife from increasing anthropogenic pressures.

ACKNOWLEDGMENTS

I would first like to thank my committee chair, Dr. Linda Walters (UCF Biology), for all her guidance, mentorship, and belief in me which has made all the difference in my undergraduate career and research success. Thank you to my committee member, Dr. Melinda Donnelly, for all your invaluable advice, support, and assistance throughout this journey. Thank you to Casey Craig, Jacob Hromyak, Adam Searles, Megan Robbins, and all members of the Coastal and Estuarine Ecology Lab at UCF for constant support and encouragement. Thank you to Samantha Little (Audubon Center for Birds of Prey) for believing in me during the beginning of this research idea and helping make all of my necropsies and sampling possible. Lastly, thank you to UCF for providing the opportunities for me to grow as an intellectual, researcher, and human being.

DEDICATION

For my mom, dad, and sister, who encouraged me to work towards my dreams, taught me to look at the world with passion, enthusiasm, and curiosity, and inspired me to create the change I wish to see.

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INTRODUCTION

Plastics are ubiquitous. Reaching regions far from human activity, plastic pollution is unavoidable in the natural environment [1]. Plastic has become virtually indispensable to daily use and manufacturing. Due to this, there is a positive correlation between plastic pollution and human population densities [2]. The convenience plastic products pose has caused an increase of the annual production from 1.5 million tons in the 1950s to 335 million tons in 2016 [2, 3]. Plastic material is resistant to corrosion, durable, light, has a low production cost, and is chemically inert [3, 4]. The qualities of plastic that make it in high demand also are the underlying reasons as to why plastic is detrimental to the environment.

The most commonly used plastics include polyethylene (cosmetic products), polyvinyl chloride (PVC), polypropylene (boating/fishing gear), and polystyrene (shipping material) [5]. Plastic materials are not biodegradable or take decades to degrade due to the strong chemical structure of polymers [4, 6]. If plastics do biodegrade, they often release toxic by-products into the environment [7]. Moreover, plastics will fragment over time via UV radiation, photodegradation, mechanical transformation such as wave action, and microbial degradation. The fragmentation of plastics produces microscopic particles of plastic material known as microplastics [3, 8].

Microplastics are any plastics smaller than 5mm in diameter. There are two general classifications of microplastics, primary and secondary [3]. Primary microplastics are any plastic that is intentionally manufactured to be < 5mm in size. Direct usage of primary microplastics are often seen in cosmetic products such as incorporation of microbeads in facial exfoliants and toothpastes [9]. Along with microbeads, nurdles are another source of primary microplastics. Nurdles are resin pellets which serve as the raw material and precursors for common “user plastics”. Nurdles are an abundant environmental pollutant because they are manufactured and transported worldwide, and often unintentionally released into oceans and rivers [10].

Secondary microplastics result from the fragmentation and the breakdown of larger plastics [5]. Two important types of secondary microplastics are fibers and fragments. These two types of plastic are the most abundant types of microplastic found in animals collected from the environment [3]. Fibers are thread-like, uniformly shaped polymers that often originate from synthetic textile materials [11]. Synthetic fibers make up 60% of all fiber production [12]. Washing synthetic clothing is one of the most significant sources of fiber pollution, potentially producing thousands of fibers each wash which can escape into the environment [2]. Fibers are one of the most abundant plastic pollutants in the environment, seen to reach concentrations of thousands of fibers per cubic meter [13]. When plastic materials are exposed to UV radiation, mechanical weathering, or photodegradation, the composition of polymers will weaken, producing smaller, irregularly shaped fragments [11].

While secondary microplastics are more common in the natural environment, both primary and secondary microplastics have increased in prevalence over time, bringing potentially deleterious effects to wildlife [8, 11]. Ingestion of microplastics from wildlife has become commonplace. Microplastic presence have been reported in oysters and crabs [14]. A study by Waite et al. [14], investigated microplastic presence in oysters and crabs in the Indian River Lagoon, Florida, and found an average of 16.5 microplastic pieces per adult oyster. There has also been an abundance of studies conducted on microplastic presence in fish [3,11, 16, 17]. Indicating the widespread nature of microplastics, pelagic and benthic fish exhibit similar compositions of microplastics in their gut content [3].

Seabirds also ingest plastics, commonly mistaking them for food [9]. Other marine organisms, along with seabirds, have been documented ingesting nurdles in the environment [10]. Organisms can also incorporate microplastics into their tissues through natural trophic interactions. In both laboratory settings and field studies, microplastics have been confirmed to transfer through food webs [15]. However, the extent to bioaccumulation and its potential deleterious effect in higher trophic organisms remains mostly unknown [15]. Beyond ingestion of plastics, microplastics can adhere to organisms, or become incorporated via uptake by organisms' gills which has shown to produce strong inflammatory responses [15]. The consumption of plastics from marine organisms pose a potentially detrimental effect on the animal's overall survival and reproduction [7, 10, 15, 16, 19, 26].

Through plastic ingestion, organisms are exposed to toxic materials. Adverse effects on organisms from ingested plastics is of increasing concern [9,13]. Due to microplastics high surface-area-to-volume ratio, they have the capacity to easily uptake and transfer toxic materials across their surfaces [13]. Furthermore, microplastics serve as a reservoir for environmental pollutants [10]. Due to the differences in chemical composition of microplastics, each plastic material has a different affinity to environmental pollutants [10]. Plastics incorporate toxins onto their surface through either absorption or manufacturing [10]. Plastics can absorb toxins because of the hydrophobic surface of plastics, which attracts hydrophobic environmental pollutants from aquatic environments [13] Alternatively, various plastics, such as plastic resins, are directly manufactured with chemical additives that cab be deleterious to wildlife if ingested [10]. For example, PCBs have been identified as one toxic substance contained in plastic pellets in marine ecosystems [10]. Upon ingestion of plastic particles, the transfer and buildup of such toxic chemicals into an organism's tissue can occur. This transfer and accumulation of pollutants in tissues due to ingestion of microplastics was demonstrated in laboratory experiments on fish [13]. One field study showed a positive correlation between pollutant PCB concentrations and mass of ingested plastics in the fat tissue of the great shearwater, *Puffinus gravis* [10].

While extensive research is currently being conducted in marine ecosystems, there is limited research on microplastic abundance and diversity in terrestrial wildlife. Furthermore, there is a lack of research investigating microplastic presence in top predatory animals. Studies on marine bird species documented that at least 44% ingest plastics [8]. While there is research documenting plastic ingestion in shorebirds [1, 20, 21], to my knowledge, there is no published research on microplastic accumulation in birds of prey. Birds of prey offer interesting insights for potential conservation efforts dealing with plastic pollution. The raptors' foraging habitats also have the potential to serve as indicators as to where plastic pollution is of greatest concern. Comparing osprey, who's primary diet comes from fish, to red shouldered hawks, who's primary food source is small mammals and amphibians, can show differential plastic abundances in either the marine, freshwater, or terrestrial ecosystem. Beyond that, this research can shed light on the ability of microplastics to transfer along food webs. Studies have shown that higher trophic level organisms have a greater chance of deleterious effects due to accumulation of toxins in microplastics along the food web [3]. As top predators, birds of prey can expand our current understanding on potential bioaccumulation of toxins via microplastic accumulation. Through bringing awareness to plastic pollution in both marine and terrestrial ecosystems, investigating how it affects top predators in the food web, further conservation efforts can be made to decrease microplastic presence to other wildlife in similar habitats.

This study addresses this important knowledge gap by investigating microplastic abundance in seven raptor species. The following questions were addressed: 1) Are microplastics present in birds of prey?, 2) What was the most abundant type (fiber vs fragment) and color of microplastic found in birds of prey?, 3) Was there a correlation between general foraging location (terrestrial vs aquatic ecosystems) and microplastic abundance?, 4) Which species of raptor exhibited the highest microplastic particle per gastrointestinal tract tissue sample ratio?, and 5) What was the most common polymer found in each species?

Shorebird ingestion of microplastics have been largely documented [1]. The diversity of predatory birds' diets suggests that microplastics will therefore also be present in birds of prey. Majority of literature on microplastic prevalence in the environment focuses on aquatic ecosystems [2, 7, 8]. Therefore, I hypothesize that *Pandion haliaetus* (osprey), which forages primarily on fish from both fresh and saltwater ecosystems, would have the greatest mean abundance of microplastics per gram of gastrointestinal (GI) tissue as compared to all other species. A common source of marine plastic pollution comes from the fragmentation of boat ropes, and therefore polypropylene, nylon and PET fibers are expected to be most commonly found in *P. haliaetus* [5]. *Buteo lineatus* (red-shouldered hawk), *Strix varia* (barred owl), *Megascops asio* (eastern-screech owl), *Bueto jamaciensis* (red-tailed hawk), and *Accipiter cooperii* (cooper's hawk) diet is composed predominantly of small rodents and terrestrial reptiles and therefore a greater mean abundance of "user plastics" (ie: trash, recyclable materials) can be expected to be found in the gastrointestinal tract of such species.

MATERIALS AND METHODS

Data Collection

Avian samples were collected from Audubon Center for Birds of Prey in Maitland, Florida. All individuals were collected after either dying naturally in the wild, or within 24 hours in Audubon’s care. In both cases, no food was given to the raptor by Audubon. The location where each raptor was picked up by Audubon either deceased or prior to death is shown in Figure 1. It is important to note that this map represents the last known location of each bird and is not necessarily indicative of foraging ranges. Species included; *Buteo lineatus (*red-shouldered hawk) (n = 28), *Pandion haliaetus* (osprey) (n = 16), *Megascops asio*, *Strix varia* (barred owl) (n = 8), (Eastern screech owl) (n = 4), *Coragyps atratus* (black vulture) (n = 2), *Cathartes aura* (turkey vulture) (n = 2), *Bueto jamaciensis* (red-tailed hawk) (n = 2), and *Accipiter cooperii* (cooper's hawk) (n = 1). Numbers represent availability between January and May 2018. A necropsy was performed on each bird, extracting the gastrointestinal tract, from the esophagus to the large intestine (Figure 2). Samples were then frozen in a -40°C freezer until chemically digested.

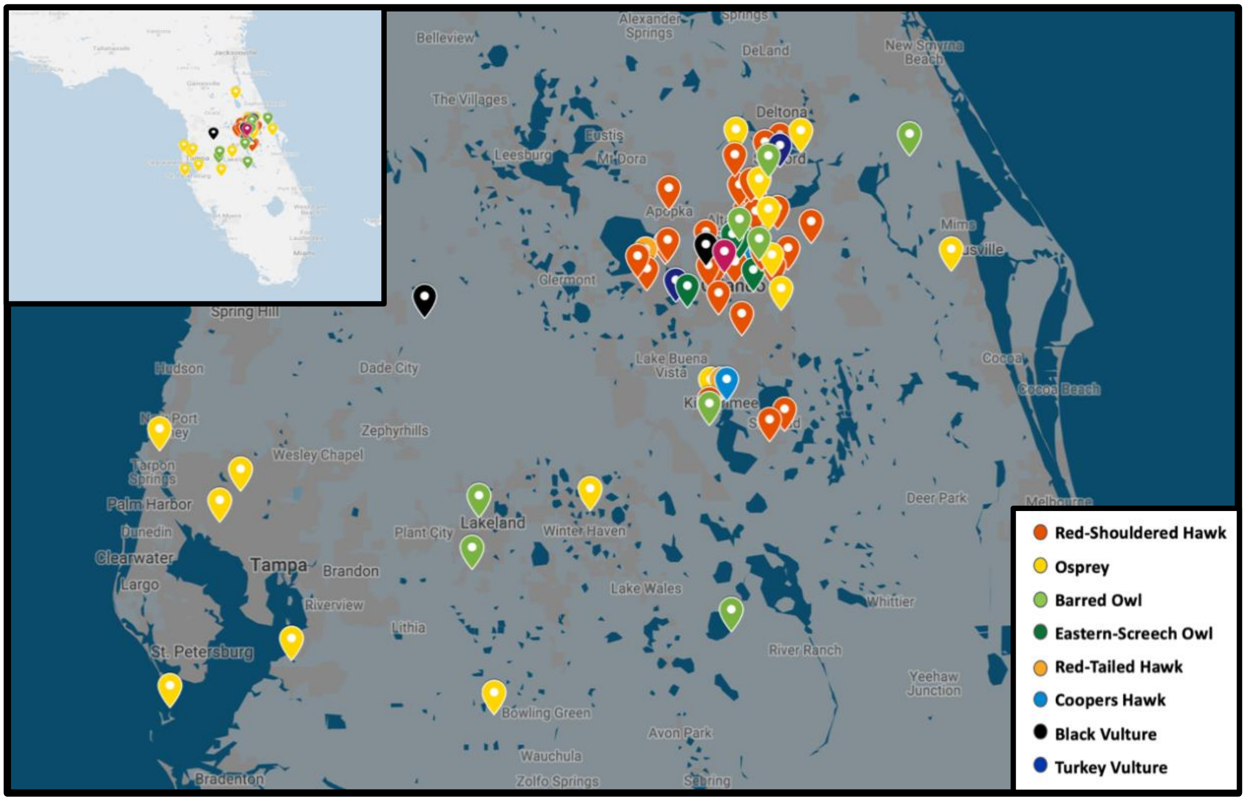

Figure 1: Sample distribution where birds were picked up deceased or prior to death by Audubon Center for Birds of Prey.

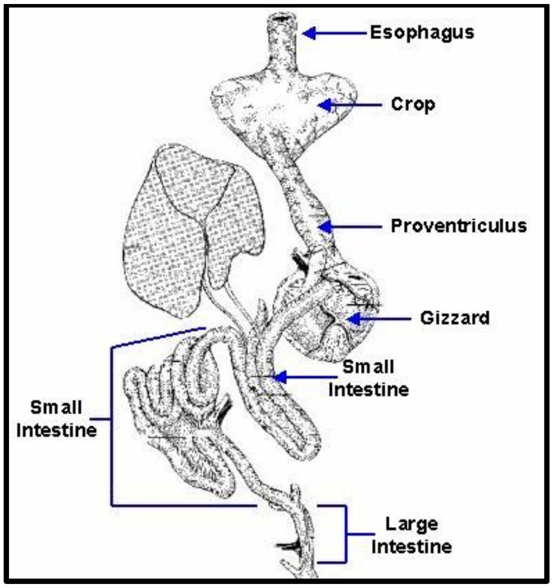

Figure 2: Necropsied section of raptor gastrointestinal tract. (© Fernbank Science Center)

Digestion/Microscopy

Each sample was chemically digested in an Erlenmeyer flask using 10% potassium hydroxide (KOH) at a ratio three times the biological mass (3:1) [16]. Samples are then placed in the shaking incubator for 48 hours at 65°C and 65 rpm. After incubation, samples are stored at room temperature for 24 hours. Previous tests confirmed that plastic particles are resistant to 10% potassium hydroxide and incubation and therefore polymers should not deteriorate in any manner [17]. Each solution was filtered using a glass vacuum filtration apparatus (Figure 2) and glass fiber filter papers (90mm, 2.5μm). Remaining solution was filtered once more using Whatman Qualitative filter papers No. 5 (90mm, 0.7μm). Filter papers were placed in petri dishes and secured with tape to limit contamination. The filter papers were then examined using a dissecting microscope at 30x magnification.

Figure 3: Vacuum filtration apparatus. (© Fishersci)

Micro-Fourier-Transform Infrared Spectroscopy

To determine the identification of plastic found in samples, micro-fourier-transform infrared spectroscopy (FTIR) was used. Post microscopy, the samples were separated categorically by species and spectroscopy was run on a subsample of each, chosen through a random number generator. Using this, plastics can be differentiated from non-plastics and the most abundant type of polymer can be identified in each species.

Limiting Contamination

To limit aerial contamination while processing samples, filtration was completed in a fume hood. Before and after each sample, all equipment was washed 3X with filtered, deionized water. To quantify potential contamination during microscopy, 5 blank filter papers were set up surrounding the microscope, 6-inches apart on all sides, for a one-hour duration. This was replicated 5 times for a total of 25 blanks to obtain an average aerial microplastic particle contamination per hour. Procedural controls tested microplastic retention. 10 polypropylene fibers were added to each of eight samples containing a solution of KOH. Samples were placed in a shaking incubator, vacuum filtered, and examined via microscopy. Number of remaining polypropylene fibers were counted to obtain a retention factor and percent loss during the digestion and quantification procedure.

Statistical Analyses

The collected data was analyzed using IBM SS Statistics 24 software with a univariate general linear model. A one-way analysis of variance (ANOVA) compared *P. haliaetus* and *B. lineatus* to determine any significant difference in the mean abundance of microplastic per gram of GI tract tissue. Additionally, separate two-way ANOVAs for *P. haliaetus* and *B. lineatus* were run to determine any significant difference between the following factors; color, type (fiber vs fragment), and an interaction between type and color.

RESULTS

Limiting Contamination Trials

Five blanks were set up surrounding the microscope for the duration of one hour. This was replicated 5 times for a total of 25 blanks. The average number of microplastic contamination per hour was 0.68 (±0.02). Polypropylene fibers were added to a KOH solution, digested and quantified. 71 out of 80 polypropylene fibers were recovered, giving an 88.7% recovery. Therefore, approximately 11.3% of fibers originally in the gastrointestinal tract of the samples were lost in throughout the methods.

Species Comparisons

Analyses were only conducted on *P. haliaetus* and *B. lineatus* because the other species did not have an adequate sample size to produce enough statistical power to detect significant differences between them. A full enumeration of results for each species is found in Table 1. *Buteo lineatus* and *M. asio* showed the greatest average microplastic abundance per gram of GI tract tissue for both fibers and fragments (Table 1). One microbead was found both in one *B. lineatus* and one *B. jamaicensis* sample (Table 1). Four macroplastics were found in one *C. atratus* (Figure 4). *Bueto lineatus* had significantly more microplastics (both fiber and fragment) per gram of GI tract tissue sample than *P. haliaetus* (one-way ANOVA; $p = 0.013$, $F = 6.46$, df =1, 87; Table 2, Figure 5).

Table 1: Average total microplastics (fibers and fragments) comparison in each species and average number of microplastics (fiber and fragment) per gram of GI tract tissue sample.

Species	**Mean weight of GI tract tissue (g) (±S.E.)**	**Mean Total Number of Fibers per sample (±S.E.)**	**Mean Total Number of Fragments per sample (±S.E.)**	**Mean Fiber per Gram of GI Tract Tissue (±S.E.)**	**Mean Fragment per Gram of GI Tract Tissue (±S.E.)**	**Total Number of Microbeads**	**Total Number of Macro-plastics**
Buteo lineatus **Red-shouldered hawk (n = 28)**	26.26 (±1.22)	21.14 (±3.83)	3.57 (±0.80)	0.81 (±0.15)	0.14 (±0.04)	1	0
Pandion haliaetus **Osprey (n = 16)**	56.47 (±3.01)	17.50 (±5.35)	2.44 (±0.86)	0.31 (±0.09)	0.04 (±0.02)	0	0
Strix varia **Barred owl (n = 8)**	38.34 (±4.27)	4.53 (±1.60)	2.29 (±0.81)	0.09 (±0.03)	0.04 (±0.02)	0	0
Megascops asio **Eastern screech owl (n = 4)**	56.47 (±3.01)	9.50 (±3.97)	1.00 (±1.00)	0.65 (±0.11)	0.07 (±0.20)	0	0
Coragyps atratus **Black vulture (n = 2)**	84.24 (±9.05)	8.00 (±0)	2.00 (±2.00)	0.10 (±0.01)	0.02 (±0.02)	0	4
Cathartes aura **Turkey vulture (n = 2)**	72.55 (±15.25)	17.50 (±10.50)	0.50 (±0.50)	0.24 (±0.24)	0.01 (±0.01)	0	0
Bueto jamaicensis **Red-tailed hawk (n = 2)**	27.14 (±1.07)	0.5 (±0.35)	0	0.01 (±0.01)	0	1	

Accipiter cooperii **Coopers hawk (n = 1)**	21.99	9.00	0	0.41	0	0	0

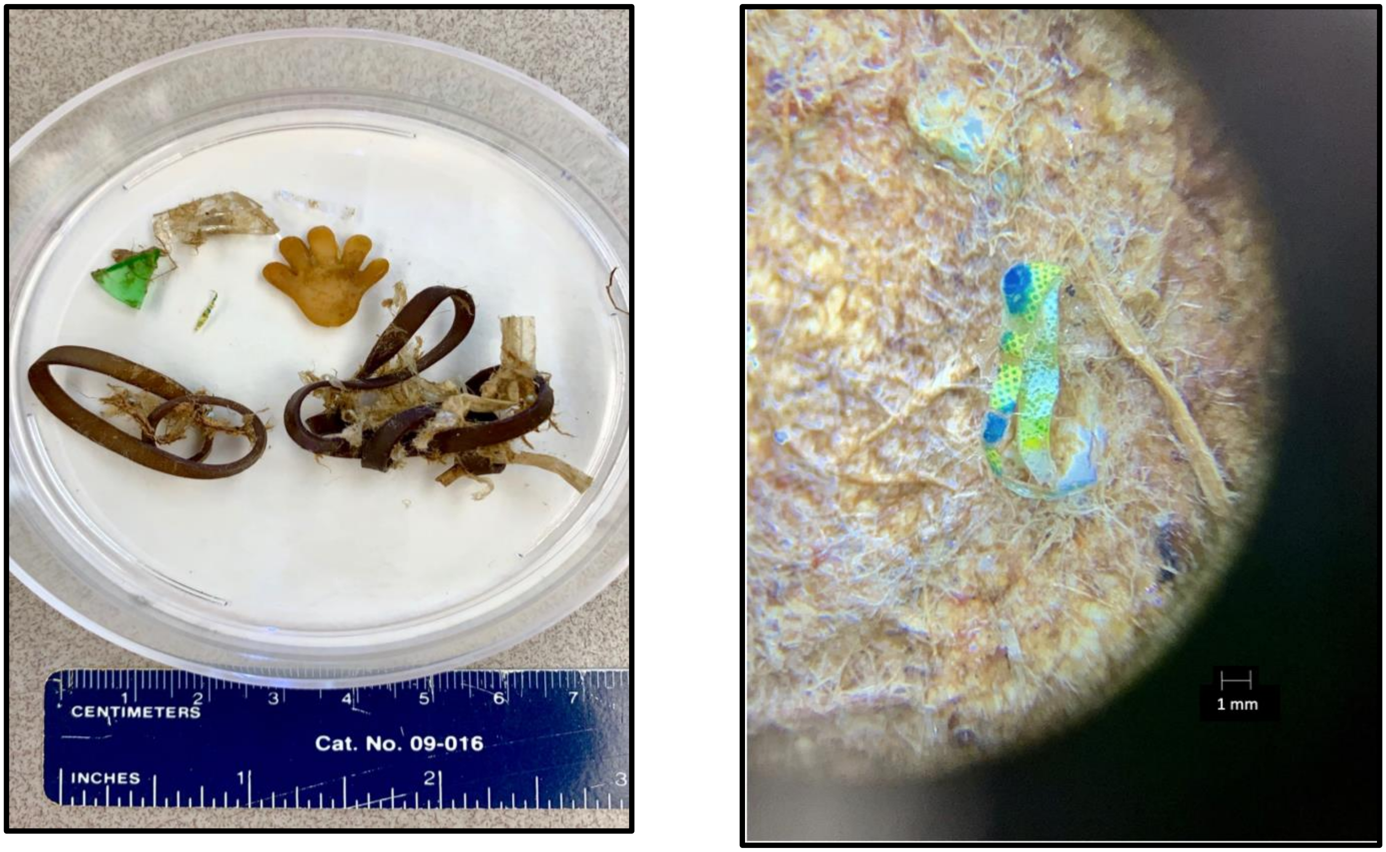

Figure 4: Macroplastics found in a single *Coragyps atratus* (black vulture)

Buteo lineatus

For *B. lineatus* there was a significant interaction between type and color (two-way ANOVA, $p < 0.001$ $F = 7.65$, df = 6, 391; Table 3, Figure 6). Furthermore, it was found that the blue color microplastic was significantly more abundant than all other colors (Table 3, Figure 6) except for clear microplastics. Blue fibers were more abundant than all other fibers and fragments, except for clear fibers (Figure 6). Fibers were significantly more abundant than fragments (Table 3, Figure 6).

Pandion haliaetus

From the microplastic particles found in *P. haliaetus* GI tract tissue, there was a significant interaction between type and color (two-way ANOVA, $p < 0.001$ F=4.68, df = 6, 223; Table 4 Figure 6). Furthermore, there were significantly more blue colored microplastics than all other colors, except for clear microplastics (two-way ANOVA; $p < 0.001$, F = 7.23, df = 6, 223; Table 4, Figure 6). Fibers were significantly more abundant than fragments ($p < 0.001$, F = 28.91, df = 1; Table 4; Figures 6). Compared to all other fibers and fragments, blue fibers were more abundant, except for clear fibers (Figure 6)

Table 2: One-way ANOVA results analyzing mean microplastic abundance per gram of GI tract tissue sample between *B. lineatus* and *P. haliaetus*.

	Degrees of Freedom	**Mean Square**	**F value**	**p value**
Species	1	2.080	6.458	p = 0.013
Total	87			

Table 3: Two-way ANOVA results analyzing mean microplastic abundance per gram of GI tract tissue for *B. lineatus.*

	Degrees of Freedom	**Mean Square**	**F value**	**p value**
Color	6	199.04	16.99	p < 0.001
Type	1	640.31	54.65	p < 0.001

(Fiber or Fragment)				
Type x Color	6	89.66	7.65	p < 0.001
Total	391			

Table 4: Two-way ANOVA results analyzing mean microplastic abundance per gram of GI tract tissue for *P. haliaetus.*

	Degrees of Freedom	**Mean Square**	**F value**	**p value**
Color	6	73.80	7.23	p < 0.001
Type (Fiber or Fragment)	1	294.86	28.91	p < 0.001
Type x Color	6	47.70	4.68	p < 0.001
Total	223			

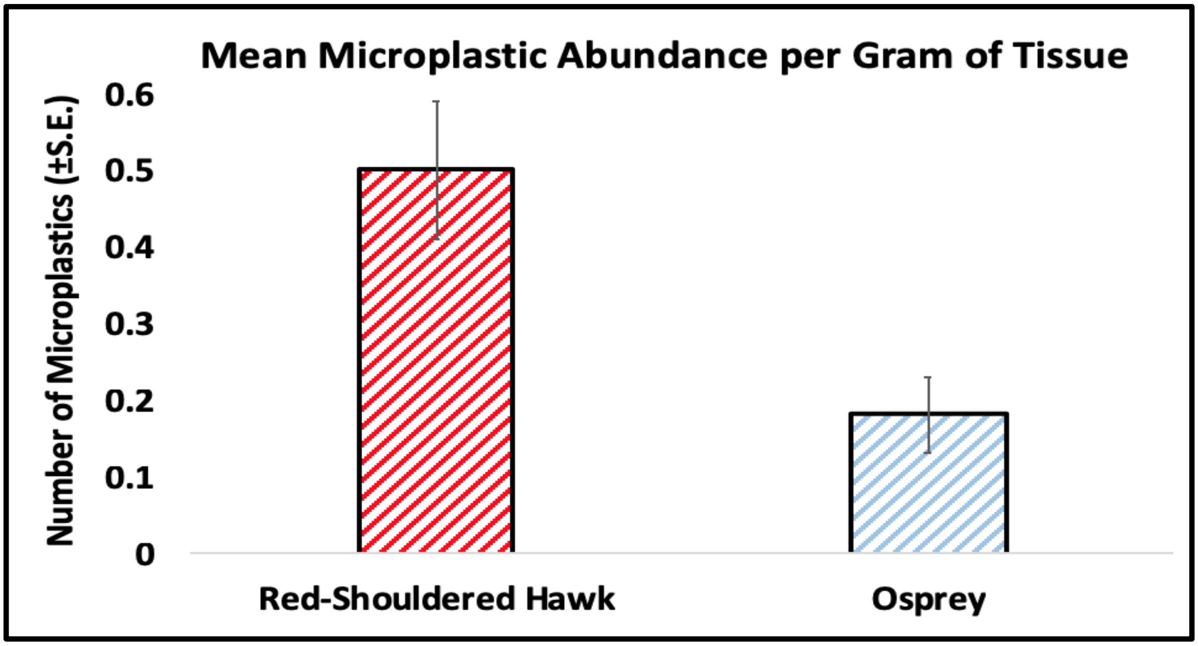

Figure 5: Comparison of *B. lineatus* and *P. haliaetus* for mean microplastic abundance per gram of GI tract tissue.

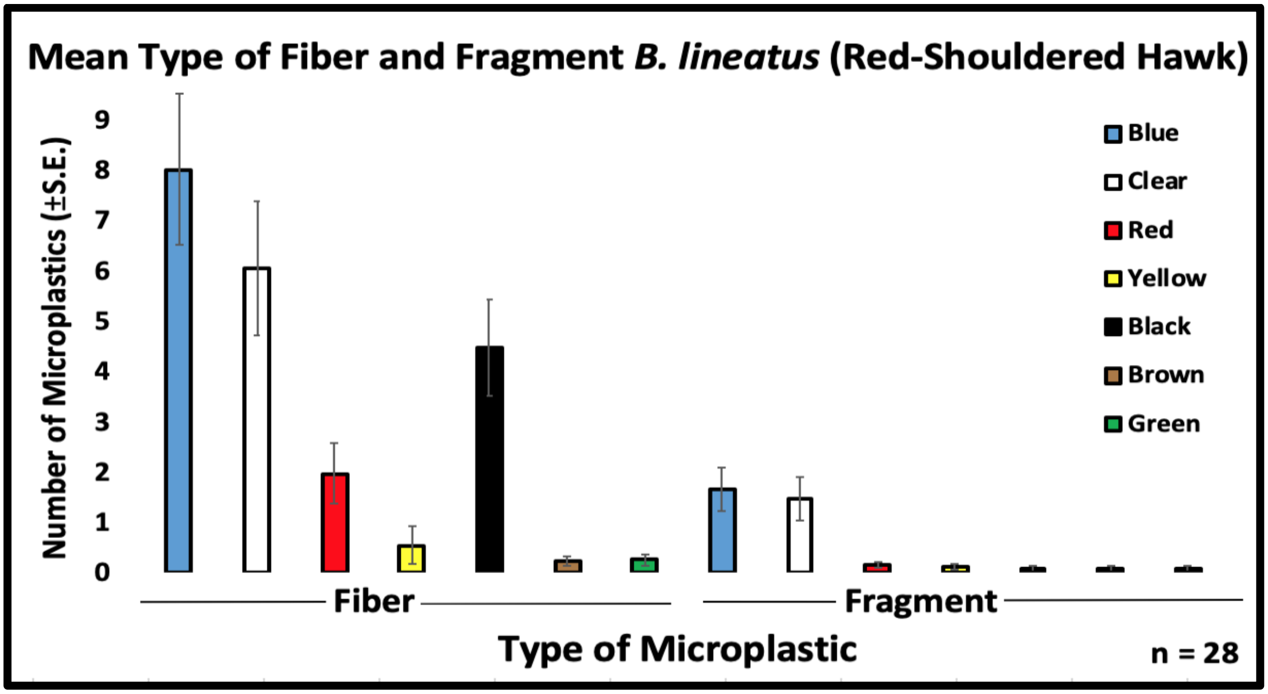

Figure 6: Mean number of microplastics found in *B. lineatus* per gram of GI tract tissue, categorized by type of microplastic (fiber and fragment) and by color.

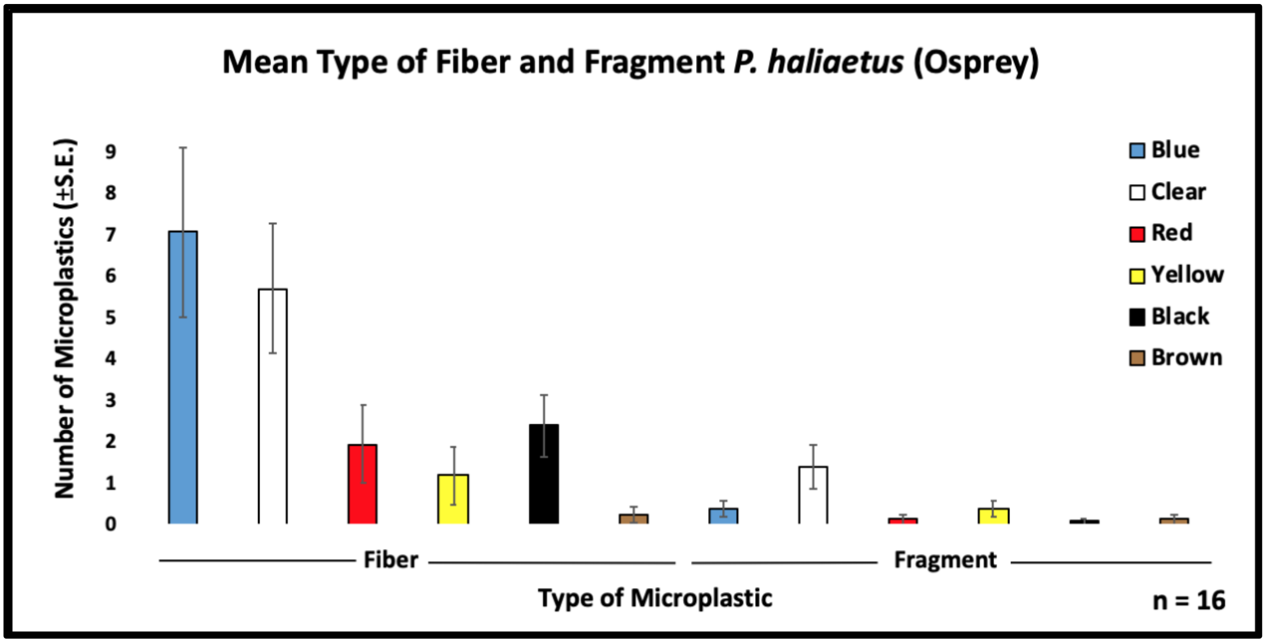

Figure 7: Mean number of microplastic found in *P. haliaetus* per gram of GI tract tissue, categorized by type of microplastic (fiber and fragment) and by color.

Polymers Found: Micro-Fourier Transform Infrared Spectroscopy

Five randomly selected samples of *B. lineatus* were analyzed with μ-FTIR. Four polymers of rayon and one polymer of polyethylene terephthalate (PET) were identified. From five randomly selected *P. haliaetus* samples, 3 rayon polymers and 1 polyamide polymer were identified. Identification and quantification of synthetic polymers found in *B. lineatus* and *P. haliaetus* are shown in Figure 8. It is important to note that polymers identified are not necessarily indicative of the total polymer composition found in the entire gastrointestinal tract of each bird, but rather representative of haphazardly selected samples which were large enough to run through μ-FTIR spectroscopy.

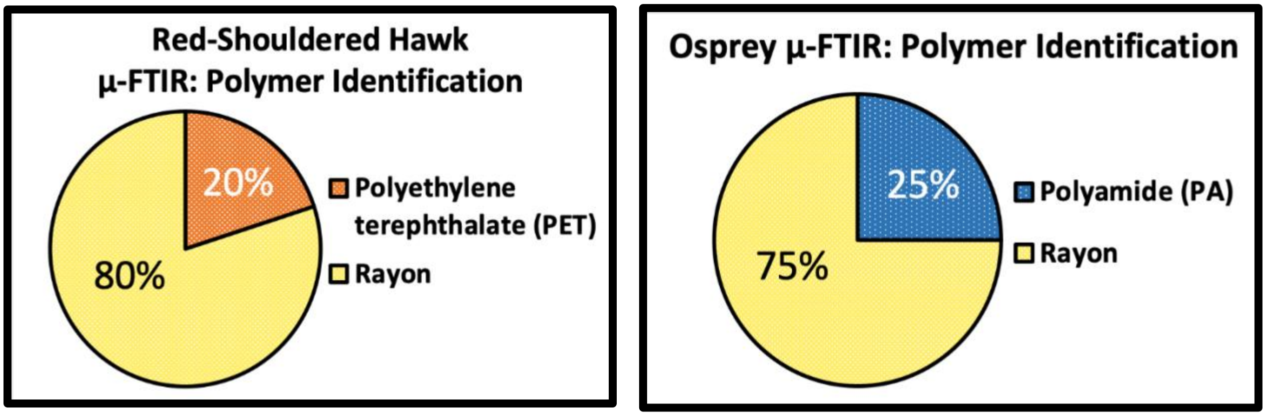

Figure 8: Identification and quantification of synthetic polymers found in *B. lineatus* (red-shouldered hawk) and *P. haliaetus* (osprey) from haphazardly selected samples.

DISCUSSION

A plethora of studies discuss anthropogenic litter in aquatic species such as oysters, crabs, and shorebirds [1, 14, 22]. In fact, it is estimated that 90% of shorebirds ingest plastic [22]. However, microplastics in terrestrial birds are less well studied, which pose the question if these birds are faced with similar threats [22]. This disparity augmented the importance of this study. The emphasis placed on plastic ingestion in aquatic species influenced the prediction that *P. haliaetus* would contain the greatest abundance of microplastics in the gastrointestinal tract. However, the results of this study did not support the related hypothesis. Microplastics were found not only in *P. haliaetus,* but consistently found in each species examined in this study. Furthermore, *B. lineatus* had a significantly greater mean microplastic abundance per GI tract tissue than *P. haliaetus* (Table 2). This significant difference may be elucidated through foraging behaviors of raptors.

Rodents constitute 65% of *B. lineatus'* diet with the remainder consisting of terrestrial reptiles and amphibians [23]. *P. haliaetus'* diet is composed predominantly of fish originating from diverse habitats including estuaries, rivers, and oceans [24]. The results from this study show that *B. lineatus* had a greater mean microplastic abundance per gram of GI tract tissue than *P. haliaetus.* This suggests that terrestrial birds of prey may experience greater bioaccumulation of microplastics than aquatic species foraging at comparable trophic levels. This study was conducted in a highly urbanized environment where rodents have a higher likelihood of relying on sources of anthropogenic waste for sustenance, therefore increasing their chance of exposure to microplastics. As a result, birds of prey feeding within such terrestrial food webs may experience higher levels of bioaccumulation of anthropogenic materials, such as microplastics. Fish and their food sources may be exposed to lower concentrations of microplastics than rodents because rodents experience a direct source of anthropogenic litter from foraging in trash-cans and landfills. Furthermore, terrestrial birds of prey are not only exposed to microplastics through secondary sources but are often exposed directly from foraging in landfills [26]. This is compared to fish, which are often exposed to indirect sources of microplastics in the aquatic environment originating from wastewater treatment plants, domestic and industrial drainage, and runoff [27]. Therefore, birds of prey foraging within such aquatic food webs may experience lower levels of bioaccumulation of anthropogenic litter than their respective terrestrial predatory birds.

Although differences in foraging behaviors exist, both *B. lineatus* and *P. haliaetus* were similar qualitatively in the composition of microplastics found in their gastrointestinal tracts. In both species, there was a significant interaction between type and color which was due to the predominance of blue fibers over other plastic types-colors ($p < 0.001$, Table 2, Figures 6, 7). Very little literature exists on the qualitative nature of microplastics in the environment. However, the occurrence of blue fibers found in birds of prey is consistent with a study conducted by Waite et al. [14] where blue fibers were most common in crabs and oysters (87%, 74% of fibers, respectively). Furthermore, Waite et al. [14] found that at three different sites throughout the Indian River Lagoon, blue fibers consistently dominated in prevalence (87%). Because organisms in aquatic and terrestrial habitats harbored predominantly blue fibers, microplastics may affect organisms similarly across habitats. This suggests that blue fibers and clear fibers may be produced more abundantly, discarded more frequently, or more commonly disposed of during manufacturing or industrial drainage, as microplastic pollution often originates in these types of manufacturing and industrial processes [26]. Predators may also have a preference towards dark-colored prey, and therefore blue fibers simulate normal trophic interactions. This preference is also reflected in fish, where predators primarily consume microplastics that are congruent in color and shape as their typical food particles [16]. This partiality may explain the disparity between blue fibers and all other types/colors of microplastics found.

The source of polymers is an important factor in developing conservation and management strategies aimed at decreasing the output of synthetic fibers into the environment. According to Carbery et al. [15], over 30 fish species have been reported to have rayon and polyamide fibers in their digestive systems. This prevalence of rayon fibers is consistent for aquatic and terrestrial species. Rayon was the most abundant polymer found in *B. lineatus* and *P. haliaetus* (n = 4, n = 3). However, PET and polyamide were found exclusively in *B. lineatus* and *P. haliaetus*, respectively. Rayon and PET are common synthetic fibers used in the textile industry. Through runoff and industrial drainage, these fibers may be released into the environment. Polyamide is a common polymer in the manufacturing of boat ropes. Due to the continual fragmentation via UV radiation, photodegradation, mechanical transformation such as wave action, and microbial degradation, boat ropes can fragment over time, releasing plastic fibers directly into the marine environment [3,8]. Through bioaccumulation, *P. haliaetus* may experience higher concentrations of potential exposure to polyamide through foraging in marine environments where fragmentation of boat ropes is common. It is important to note; the identified polymers are not necessarily representative of the most abundant polymers found in each species, it is rather simply a representation of common polymers found in *B. lineatus* and *P. haliaetus.* Macroplastics were found in one sample of *C. atratus*. A study by Plaza and Lambertucci [27] reported *C. atratus* foraging in and near landfills. Foraging in landfills may be a source of macroplastic ingestion. While there is limited research regarding *C. atratus* ingestion of plastics, it has been shown that seabirds commonly mistake microplastics for food [9]. Similarly, species such as *C. atratus,* which are typically larger than shorebirds and therefore consume larger prey, could easily mistake plastics for food resulting in ingestion of macroplastics. Foraging in terrestrial, overpopulated urban areas can also attribute to the commonness of wildlife mistaking plastic for food.

This study is the first to provide thorough statistical analysis comparing plastic ingestion across terrestrial and aquatic birds of prey. These results highlight the need for further research on plastics in the terrestrial realm and for more comprehensive comparisons between the aquatic and terrestrial environments. Overall, microplastics are significantly more abundant per gram of GI tract tissue in species that forage on small rodents and terrestrial reptiles (*B. lineatus*) as compared to species that forage on fish and aquatic invertebrates (*P. haliaetus*). Blue and clear fibers are significantly more abundant than other color and types of microplastics. Due to the abundance of polymers found in these species, understanding the potential biological and physiological effects of plastics is essential to providing management strategies that can better protect and preserve wildlife from increasing anthropogenic pressures.

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