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Microplastics in the diet of nestling double-crested cormorants (*Phalacrocorax auratus*), an obligate piscivore in a freshwater ecosystem

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1 **Microplastics in the diet of nestling double-crested cormorants (*Phalacrocorax***
2 ***auratus*), an obligate piscivore in a freshwater ecosystem**

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19 **Microplastics in the diet of nestling double-crested cormorants (*Phalacrocorax***
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22 **Abstract**

23 Anthropogenic debris, namely plastic, is a concern across aquatic ecosystems worldwide,
24 with freshwater systems being understudied relative to marine. In this study, we
25 quantified and characterized debris in the diet of double-crested cormorant chicks
26 (*Phalacrocorax auratus*) from three sites in two of the Laurentian Great Lakes to 1)
27 determine whether or not the diet of double-crested cormorants in the Laurentian Great
28 Lakes includes anthropogenic debris, 2) characterize the size, shape and type of debris
29 incorporated, and 3) examine relationships between the amount of debris ingested and
30 their proximity to industrial/urban centres. Overall, >86% of cormorants in our study
31 had anthropogenic debris (mostly fibers) in their digestive tracts with no correlation
32 between site and the amount of debris ingested. The ingested debris includes
33 microplastics, natural fibres from textiles, and other anthropogenic materials (e.g., glass).
34 To the best of our knowledge, this is one of the first studies to examine anthropogenic
35 debris in a diving bird in the Laurentian Great Lakes, and one of few studies
36 investigating this in freshwater birds.

37 **Keywords:** Marine Litter, Microplastics, Freshwater, Birds

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39 **Introduction**

40 Anthropogenic debris, in particular plastic, has become a contaminant of concern
41 in aquatic ecosystems around the globe (Thompson et al. 2009). Found ubiquitously
42 across marine habitats, including the open ocean (Carpenter and Smith 1972), coral
43 reefs (Donohue et al. 2001) and the deep sea (Goldberg 1997; Galgani et al. 2000),
44 anthropogenic debris is diverse and includes macroplastics (plastic particles > 5mm),
45 microplastics (plastic particles < 5mm), glass, and fibrous materials from textiles.
46 Freshwater ecosystems are also contaminated by anthropogenic debris, including in the
47 sediments and surface waters of the Laurentian Great Lakes (Eriksen et al. 2013;
48 Corcoran et al. 2015; Ballent et al. 2016). Contamination from anthropogenic debris can
49 negatively affect wildlife and ecosystem health, depending upon the level of exposure
50 and the type and size of the debris (Rochman et al. 2016). These impacts can result
51 from entanglement (Derraik 2002), smothering or ingestion (Gregory 1978; Oehlmann et
52 al. 2009), and can be physical or chemical in nature (Rochman et al. 2013; Tanaka et al.
53 2013). Despite the growing body of research on the sources, contamination, fate, and
54 effects of anthropogenic debris in marine ecosystems and food webs, there is
55 comparatively less information about anthropogenic debris in freshwater ecosystems
56 (Wagner et al. 2014; Dris et al. 2015), specifically with regard to exposure and effects
57 of anthropogenic debris (Thompson et al. 2009) and the sources and sinks of debris in
58 freshwater environments (Anbumani & Kakkar 2018).

It has long been asserted in both the ecological and ecotoxicological literature that birds are suitable candidates for monitoring changes in ecosystems, particularly with respect to changes resulting from anthropogenic pollution (Furness 1993; Montevecchi 1993). Marine bird species in particular have been used to monitor anthropogenic debris in marine environments (Furness 1985; Moser & Lee 1992; Cadee 2002; Avery-Gomm et al. 2012; Herzke et al. 2016). The corresponding literature on freshwater birds is scarce (Eerkes-Medrano et al. 2015) and we seek to address this paucity of knowledge by investigating the occurrence of anthropogenic debris in a freshwater avian species.

Here, we quantify anthropogenic debris ingested by double-crested cormorant chicks (*Phalacrocorax auratus*) raised in the freshwater Laurentian Great Lakes. Double-crested cormorants (hereafter referred to as 'cormorants') are diving piscivorous birds that feed primarily on pelagic and benthic fish (Neuman et al. 1997). Their feeding behavior makes cormorants an interesting and unique study species to examine anthropogenic debris because they do not feed on the surface in contrast to the preferred feeding strategy of well-researched species like fulmars and gulls (Camphuysen et al. 1997; Farmer & Leonard 2011). Since plastic tends to float on the surface of the water (Gasperi et al. 2014) or deposit in the surficial sediment (Corcoran et al. 2015), it is anticipated that diving birds are ingesting anthropogenic debris at a lower rate than surface-feeders (Poon et al. 2017). Consequently, any contamination of

anthropogenic debris in cormorants may be a result of trophic transfer from their prey. Anthropogenic debris in the diet of cormorants was quantified previously by Acampora et al. (2017), but only in cormorants inhabiting marine environments. We sampled cormorants from three freshwater study sites in the Great Lakes, two sites in close proximity to an urban and industrial centre in Lake Ontario and another more remote location in Lake Erie. We hypothesized that contamination of the cormorants would occur across all sites, but with higher concentrations found in cormorants at sites in closer proximity to urban/industrial centres based on previous research (e.g. Galgani et al. 2015).

Methods

Field Collection

As part of an unrelated study (Wallace et al. 2018), 30 cormorant chicks were sampled from three colonies across the Laurentian Great Lakes in 2014 (Fig. 1), with 10 chicks sampled from each colony. Two colonies were located in Lake Ontario near the city of Hamilton, Ontario (Centre Island [43.3046° N, 79.8028° W] and Pier 27 [43.2833° N, 79.7937° W]), and one colony was located in eastern Lake Erie (Mohawk Island [42.8345° N, 79.5226° W]). All of the chicks were sampled before they fledged (~6-7 weeks post-hatch) which ensures that their diet was restricted to what was provided by the parent birds only. During sampling, large chicks that appeared to be greater than one kilogram in mass were opportunistically selected, captured by hand, and placed in a dark, covered

box until processed. Individuals were sacrificed by decapitation using a guillotine, following standardized operating protocols and animal care approval (Environment and Climate Change Canada ACC SOP-07). The age of the sampled chicks was estimated by using the daily average mass of known age chicks (Dunn 1975b) to generate a growth curve using a polynomial regression, and back calculating the estimated age of the chicks in this study.

Dissection

Immediately following euthanization, the liver and lung tissues were collected for an independent study (Wallace et al. 2018), and the remains, with stomach and gastrointestinal (GI) tract intact, were frozen at the Canada Centre for Inland Waters in Burlington, Ontario, Canada. In 2016, the chicks were removed from the freezer, dissected and their digestive tracts were transferred to the University of Toronto to examine the incidence of anthropogenic debris. Once thawed, the proventriculus of each individual was bisected and the gizzard was emptied. Following the methodologies of van Franeker & Meijboom (2002) and Holland et al. (2016), the digestive tract was opened and carefully rinsed through a 0.5mm sieve. The contents on the sieve were then transferred to a clean glass jar for chemical digestion.

Digestion

From the sieve, all content was transferred to a clean glass jar (rinsed three times with Reverse Osmosis (RO) water to rinse out any debris) with 4-Normal potassium

hydroxide solution. The jar was left sealed for 14-16 days to digest all organic materials, allowing the separation of organic and inorganic material. Following the digestion period, the contents of the jar were sieved and rinsed again with RO water to remove as much waste material as possible. The remaining matter was placed in a large glass petri dish for microscopy.

Quantifying Anthropogenic Debris

To separate all anthropogenic debris from the remaining material, each sample was first observed under an OMANO microscope at 40× magnification, and particles that were perceived as being anthropogenic were removed and placed in smaller glass petri dishes. Following the completion of debris removal, all particles were then transferred to double-sided tape in separate petri dishes to allow for photography and chemical analysis to determine polymer type. Once particle extraction was complete, each particle was photographed and measured. Size metrics of length and width were recorded for each particle. Particles were also grouped by the following categories: fibers, fragments, pellets, films, fibre bundles and spheres.

Quality Analysis and Quality Control

For quality assurance and control purposes, all tools were rinsed with RO water three times, and between each sample to avoid cross contamination and procedural contamination. For each 10 cormorant samples, one lab blank was run during the dissection and initial sorting of anthropogenic debris and a second blank was run during the transfer of all sorted particles to double-sided tape. The contents of these dissection and transfer blanks were then summed for

each set of 10 samples. Using a combination of particle morphology, color (e.g., black fibers) and chemical identification (e.g., yellow polyethylene fragment), the average content of the blanks was subtracted from each sample to account for procedural contamination (See SI for more information).

Chemical Identification

The particles were subsampled for chemical ID by category (i.e., fibers, fragments, pellets, films, fibre bundles and spheres) and site. Within each site, if the number, x , of particles in a category was $x \leq 10$, all particles were analyzed. If the number of particles, x was $10 < x \leq 50$ in a category, 15 were randomly selected and analyzed. If the number of particles, x was $50 < x \leq 100$ in a category, then 20 particles were randomly selected and analyzed. All particles from the lab blanks were analyzed. This approach resulted in a total of 120 particles being analyzed out of 273 (i.e., 44%). The digestion process, where chemicals were used, does not affect the appearance or size of the particles.

Two separate methods were used to identify the polymer classification of the particles. Particles that were large enough to manipulate by hand, approximately bigger than 1mm, were analyzed using Fourier transform infrared (FTIR) spectroscopy, with an FPA-based Alpha II FTIR setup with OPUS/3D technology (Bruker Corporation). Smaller particles were analyzed using Raman spectroscopy with an Xplora Plus (Horiba Scientific) with LabSpec 6 software. Both methods rely on generating a spectrum for each particle, and comparing it to a reference library of known spectra using specific

spectral peaks that correspond to identifiable functional groups. This allowed for the identification and categorization of the particles as various anthropogenic materials.

Statistical Analysis

All birds ($n = 10$ for each sampling site), were included in the statistical analysis, including those with no debris found in their gut content. To test our data for homogeneity of variance, we performed a Bartlett's test on our two variables of interest: number of particles and length of particles (number of particles: $K\text{-squared} = 5.4964$, $p > 0.05$, length of particles: $K\text{-squared} = 2.3422$, $p > 0.05$). Since both tests were not significant we did not transform our data, and we performed two individual single-factor ANOVA tests on the number of particles per bird per site and the length of the particles per bird per site. All analyses were performed in R (version 3.5.0).

Results & Discussion

Contamination in cormorants from the Laurentian Great Lakes

In total, 26 out of 30 (86.7%) cormorant chicks contained anthropogenic debris in their GI tract. An average of 5.8 particles were found per bird ($SD = 4.0$) across all sites. A maximum of 15 particles were found in the digestive tract of a single bird (Table 1). The average length of particles varied across individuals (Fig. 2), with a small number of individuals showing high variability in particle length, and others showing low variability. In individuals with low variability, the lengths of the particles tended to be relatively short (i.e., $< 3\text{mm}$). In general, fibres were the most common type of debris, with

fragments being second most common (Fig. 3). These results clearly demonstrate incorporation of anthropogenic debris into the diets of pre-fledgling cormorants in the Laurentian Great Lakes. The fact that >86% of all individuals sampled ingested anthropogenic debris suggests that cormorants across the Laurentian Great Lakes, and likely other diving piscivorous birds across other contaminated freshwater ecosystems in general, are incorporating anthropogenic debris into their diet.

Chemical analysis showed that in general, all ingested particles can be categorized in three broad groups: microplastics, anthropogenic natural fibres from textiles (e.g., cotton), and other anthropogenic materials (e.g., paint, synthetic leather, glass). None of the particles that we originally counted as anthropogenic were determined to be of natural origin by chemical analysis (e.g., plants, shells). As such, we are confident that particles that were not chemically identified were anthropogenic. The plastic polymers found in the cormorants' GI tracts were diverse, including some of the more commonly used types of plastic (i.e., polyethylene and polypropylene). Although the type of materials varied across the different sites, natural fibres from textiles and other anthropogenic materials made up the majority of particles across all sites (Table 2, Fig. 3).

Incorporation of anthropogenic debris in birds, particularly those who have yet to begin foraging for themselves, is concerning due to the well-documented effects that ingestion of debris has shown previously. Ingestion can cause death and decreased

fitness in birds (e.g. Ryan 1988) and other organisms (Rochman et al. 2016). For example, physiological effects such as hepatic stress can occur in fish as a result of debris ingestion (Rochman et al. 2013), and anthropogenic debris has been shown to affect the survival of pre-fledgling birds (Sievert & Sileo 1993).

Anthropogenic debris can impair physiological function in birds through mechanical, behavioural and chemical means. Ingested plastic specifically can injure the digestive system (Bennet 1960; Ryan 1987) by causing blockages or ulcerations (Azzarello & Van Vleet 1987). Such debris may also reduce the effectiveness and efficiency of foraging and digestion (Day et al. 1985) through mechanisms such as false satiation, where the individual consumes a level of debris that results in a false sense of being nutritionally satiated (Ryan 1988). Furthermore, it is now widely known that anthropogenic debris can be a source of toxic chemicals to wildlife (Baltz & Morejohn, 1976; van Franeker 1985; Rochman et al. 2014; Tanaka et al. 2015; Jang et al. 2017). Thus, ingestion of debris in cormorant chicks might have negative effects on the physiology, growth, development, and potentially on the behaviour, of these birds.

Additional research is required to characterize the potential physiological and physical effects of anthropogenic debris on cormorants and other birds inhabiting freshwater ecosystems.

Variation among sites

There were no significant differences among sites in terms of the number or length of particles per bird (ANOVA; $p > 0.05$ in both cases) (Fig. 2 and 4). This finding is contrary to our hypothesis, and suggests a relatively uniform distribution of debris across all three sites with no notable variance in the sites closer to urban/industrial centres. The chemical composition of particles found in the birds was not uniform across the three sites, with the cormorants at Centre Island having the highest number and variety of plastic-based debris (Table 2), and the cormorants at Mohawk Island having very little plastic-based debris. The fact that the cormorants at Mohawk Island had low levels of specifically plastic-based debris is unsurprising because the individuals at the two other sites (which showed higher levels of plastic-based debris) are located in Hamilton Harbour, near a number of manufacturing facilities, the sewage outflows of three wastewater treatment plants, and a large urban centre.

We found little evidence of a relationship between the amount of debris found in the gut and the proximity to urban and industrial centres. Numerous studies have shown that in the Laurentian Great Lakes, as well as other freshwater systems, debris concentrations correlate negatively with distance from the city centre (Zbyszewski et al. 2011; Eriksen et al. 2013; Wang et al. 2017). As a major shipping port, a national manufacturing centre, and with effluent from three wastewater treatment plants entering its waters, Hamilton Harbour is a prime candidate for anthropogenic debris pollution. However, although far from a city centre, Mohawk Island is 4.9 km

239 downstream from the mouth of the Grand River, which receives the effluent from 30
240 municipal wastewater treatment plants, and the mouth of the Grand is a source of
241 plastics to Lake Erie (Cable et al. 2017). Therefore, the fact that there is no relationship
242 between proximity to urban centres and the amount of debris found in our samples
243 indicates that nesting location of these cormorants does not necessarily play an
244 important role. Debris pollution, particularly fibres, may be ubiquitous across the
245 Laurentian Great Lakes. It may also indicate that the debris is potentially taken up by the
246 birds via their food source, and thus foraging locations may be more relevant than
247 nesting sites. Nearshore environments typically are the most common areas for
248 anthropogenic debris in the Laurentian Great Lakes (Ballent et al. 2016). With a foraging
249 range extending up to 20 km (Hobson et al. 1989), but usually not exceeding 3 km
250 (Custer et al. 1992), cormorants feed in nearshore environments but not on the shore
251 itself. In Lake Erie, 65% of foraging flocks of cormorants were within 2.5 km from shore,
252 and most frequently foraged in depths of 8 to 10 m (Stapanian et al. 2002). Cormorants
253 from Mohawk Island had a higher proportion of goby in their diet, suggesting they feed
254 more heavily from the benthic zone compared to cormorants from Hamilton Harbour
255 (King et al. 2017). Further, based upon stable isotopes (δN , δC) in their red blood cells,
256 cormorants from Hamilton Harbour forage in Lake Ontario in addition to the harbour
257 itself (King et al. 2017).

258 We hypothesize that the incorporation of debris into the diet of cormorant chicks
259 may be related to trophic transfer from the prey (e.g., fish) of the parent cormorants.
260 The size of the particles in our samples are generally smaller than the pre-production
261 pellets and fragments that are typically consumed by surface feeding birds (Robards et
262 al. 1995). In addition, the main category of debris (e.g., fibers) found in the cormorant
263 chicks may suggest that the source is from prey fish. Substantial evidence showing the
264 widespread occurrence of fiber ingestion in fish has been published (e.g. Biginagwa et
265 al. 2016; Rummel et al. 2016), and unpublished data (K. Munno, University of Toronto,
266 Toronto, Ontario, personal communication, 2018) shows that fish in Lake Ontario have a
267 high incidence of fiber ingestion. Moreover, round goby (*Neogobius melanostomus*) in
268 Lake Michigan tributaries had the highest concentration of microplastics in their guts
269 compared to 10 other fish species (McNeish et al. 2018), and thus the high prevalence of
270 goby as prey items may account for a proportion of the microplastic loading in
271 cormorant diets.

272 The feeding mechanism for these young birds also supports our hypothesis that
273 trophic transfer is involved in the uptake of fibers and anthropogenic debris by the
274 cormorant chicks. In birds that feed their chicks by regurgitation, regurgitation of food
275 involves bringing up food from the crop (Klinghammer & Hess 1964), a temporary
276 storage area in the GI tract. This suggests that prey items fed to young were not mixed
277 with the stomach contents of the parents. This is noteworthy because cormorants are

known to incorporate anthropogenic debris into their nests (Podolsky & Kress 1989). It is possible that the stomach could contain debris obtained while building nests or some other non-feeding activity. It is also possible that the chicks themselves could have directly ingested some of these particles should the nesting site have contained such debris. However, we think it is unlikely that this occurred, as the vast majority of our particles were small fibres, likely too small for cormorant chicks to see, or pick up and swallow. The absence of macroplastic (plastic debris >5 mm) in the digestive tracts of the chicks, which is more common at the nesting sites (Podolsky & Kress 1989), suggests there is no evidence of chicks ingesting plastic material directly. As such, we hypothesize that the parent cormorants consume fish that have ingested anthropogenic debris, and then regurgitate those fish to their nestling chicks.

Moreover, it has been shown that older nestling cormorants tend to be fed whole fish rather than the semi-liquid food provided to younger chicks (Dunn 1975a), which indicates that our slightly older nestling individuals likely ingested whole fish and therefore obtained any debris already contained in that fish. We estimated the ages of the chicks based on body mass, as we did not know the date of hatch. The estimated average age of chicks differed among colonies with the chicks on Centre Island ($\bar{x} = 22.3$ days) being younger than those from Mohawk Island ($\bar{x} = 25$) and Pier 27 ($\bar{x} = 25.8$). It does not appear that the range in age, or size, is related to plastic burden. Anecdotally, during the dissection stage of our study, we found multiple mostly intact fish in the GI

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298 tracts of several individual birds. Unfortunately, all of the fishes' GI tracts were digested
299 from the bird's stomach acids so we were unable to quantify the contents in the fish, but
300 this demonstrates that the individual birds were ingesting whole, potentially
301 contaminated fish.

302 Trophic transfer of debris has been demonstrated in laboratory experiments,
303 including transfer from mussels to crabs (Farrell & Nelson 2013), and recently in a
304 freshwater food chain including algae, water fleas, and two levels of fish (Chae et al.
305 2018). Still, very few studies have demonstrated trophic transfer in nature. Furtado et al.
306 (2016) and Hammer et al. (2016) both showed trophic transfer of debris in birds in field
307 studies, but both demonstrated that the ingested plastic was from consuming other
308 waterbird species (i.e., White-faced storm-petrels (*Pelagodroma marina*) and Northern
309 Fulmars (*Fulmarus glacialis*) respectively). Trophic transfer of anthropogenic debris has
310 been discussed widely with regards to many different systems, and likely takes place in
311 both pelagic and benthic food webs (Setälä et al. 2013). Further work is needed to more
312 definitively demonstrate trophic transfer in general, and in this specific food web. This is
313 crucial to quantitatively address the cumulative impact of anthropogenic debris in
314 ecosystems holistically, incorporating the various potential interactions between trophic
315 levels, including bioaccumulation and/or biomagnification.

316

Overall, the results of our study show that anthropogenic debris is found in the GI tracts of pre-fledgling cormorants in the Laurentian Great Lakes. This study contributes to our limited understanding of the presence of anthropogenic debris in freshwater, in the diets of freshwater birds, and in particular diving freshwater birds. Through our study, we suggest that the incorporation of anthropogenic debris is potentially due to the trophic transfer of debris from prey fish to the parent cormorants, and then regurgitated to the nestling chicks. More research is needed to determine whether trophic transfer is responsible for the uptake of anthropogenic debris into cormorant chicks, and future studies should aim to determine if this type of potential trophic transfer can result in bioaccumulation and/or biomagnification of anthropogenic debris in these top-level predators, as has been shown to occur with contaminants such as mercury (Suedel et al. 1994; Hammerschmidt & Fitzgerald 2006). Future research is also required to determine the physiological and physical implications of uptake of anthropogenic debris in birds. Documenting the incidence of ingestion of anthropogenic debris in cormorants residing in freshwater improves our understanding of the fate of anthropogenic debris in freshwater ecosystems and the risk it may pose to wildlife.

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30

Table 1. Site-by-Site Average Length and Average Number of Particles. The average length of particles (mm) and the average number of particles per individual cormorant chick across all three sites.

Site	Avg. Length of Particles/Bird (mm)	Max. Length of Particles/Bird (mm)	Min. Length of Particles/Bird (mm)	Avg. # of Particles/Bird	Max. # of Particles/Bird	Min. # of Particles/Bird
Pier 27 (L. Ontario)	4.24	22.1	0.11	5.1	7	0
Centre Island (L. Ontario)	3.55	117	0.09	5.9	15	0
Mohawk Island (L. Erie)	2.61	19.4	0.3	6.3	12	0

Table 2. Chemical Categories and Corresponding Number of Particles. The categories are divided based on the chemical makeup of particles analyzed through FTIR and Raman spectroscopy and demonstrate the polymeric diversity available to the ecological community.

Debris Category	Pier 27		Centre Island		Mohawk Island	
Polyester	1	2.8%	0	0.0%	0	0.0%
Acrylic	3	8.3%	1	2.5%	2	6.5%
Poly(Acrylic Acid): Ethylene	0	0.0%	1	2.5%	0	0.0%
Polyamide	1	2.8%	0	0.0%	0	0.0%
Polyethylene	6	16.7%	5	12.5%	0	0.0%
Vinyl Acrylic	0	0.0%	1	2.5%	0	0.0%
Polypropylene	3	8.3%	1	2.5%	0	0.0%
Poly(Divinyl Benzene): Styrene	0	0.0%	1	2.5%	0	0.0%
PVC	0	0.0%	1	2.5%	0	0.0%
Polyacrylonitrile	1	2.8%	0	0.0%	1	3.2%
Rayon	3	8.3%	4	10%	1	3.2%
Natural Fibre From Textiles	7	19.4%	12	30%	9	29.0%
Other Anthropogenic Materials	10	27.8%	9	22.5%	17	54.8%
No Signal	1	2.8%	4	10%	1	3.2%
Total	36	100%	40	100%	31	100%

Figure 1. Study Area. The Laurentian Great Lakes have shores in both Canada and the United States. The red star denotes Hamilton, Ontario, Canada. Mohawk Island is a cormorant nesting site on Canadian land, in the eastern portion of Lake Erie. Both Centre Island and Pier 27 are nesting sites in the harbour of Hamilton ON, an industrial centre on the western end of Lake Ontario. Basemap layers provided by Esri.

Figure 2. Average length of particles. This boxplot displays the interquartile range and the bar inside the box denotes the median. All particle lengths were plotted per bird, and separated according to sites. Some birds show high variance in particle size (usually due to one or two anomalous particles) while other show very little variation. ANOVA testing returned nonsignificant results ($p = 0.057$, $F\text{-value} = 124.1$, $df = 2,27$)

34

Particle Types

Figure 3. Types of Particles Across Sites. All particles from all sites are displayed here. Fibres are the most common category of particle found, with fragments being the next most common and all other categories appearing sporadically, some only once across all sites.

Figure 4. Particles per Sample: The number of particles found per sample is displayed per bird and separated according to sampling site. The four individuals that had zero debris particles are included. ANOVA testing showed no significant differences between sites ($p = 0.546$, $F\text{-value} = 0.75$, $df = 2,27$)

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Draft