The potential of aerial insectivores for monitoring microplastics in terrestrial environments

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HIGHLIGHTS

• Microparticles (MP) found in tree swallows (≥83%) at WWTP & reference sites
• At both sites, fecal sacs of chicks (90%) contained MPs and all were fibers.
• Reference chicks had more MPs (feces) that were larger, more diverse in GI tracts.
• Reference birds had more aquatic diet versus WWTP chicks had more terrestrial diet.
• MP numbers were not correlated between sample types or with chick condition and size.

GRAPHICAL ABSTRACT

Abstract

Limited research has been conducted on microplastics in terrestrial ecosystems and biota, despite being some of the most ubiquitous environmental pollutants. We investigated the presence of microplastics (over 125 μm) in tree swallow (Tachycineta bicolor) chicks (10 d. o.), an aerial insectivore whose diet involves terrestrial and/or freshwater sources. Swallows nested immediately downstream (300 m) of the discharge pipe of a large, urban wastewater treatment plant (WWTP) or at a rural conservation area (40 km apart). Anthropogenic microparticles (including microplastics) were identified in nearly all WWTP chicks (90%; $N = 20$) and reference chicks (83%; $N = 20$). All microparticles were fibers (100%) in the gastro-intestinal (GI) tracts of WWTP nestlings, whereas unexpectedly, they were more diverse in the GI tracts of reference chicks, with ~15% characterized as pre-production plastic pellets. The fecal sacs of most nestlings (90%) contained microparticles, and all were characterized as fibers suggesting their excretion by tree swallows. Compared to WWTP chicks, the reference chicks had more microparticles in their fecal sacs and larger particles (length, width) in their GI tracts, likely reflecting the more aquatic-based diet of the reference chicks fed insects caught adjacent to the nearby dam, compared to the more terrestrial-based diet of the WWTP chicks. The numbers of microparticles were not correlated between GI tracts and fecal sacs, nor with the chicks’ condition or size (weight, organs, feathers). We recommend sampling macroinvertebrate prey to permit stronger conclusions regarding WWTPs as possible sources of microplastics for swallows, and to determine if such macroinvertebrates may be a non-lethal method to characterize microparticle diversity ingested by birds as presently identified in chicks’ GI tracts. We conclude that sampling fecal sacs only, while not indicative of the diversity of microplastics ingested by terrestrial passerines (e.g., tree swallows), is useful for determining their exposure to microparticles.

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1. Introduction

Plastic pollution has become one of the leading drivers of global contamination, permeating marine, freshwater and terrestrial ecosystems (Lusher, 2015; Lusher et al., 2015; Li et al., 2018; Choi et al., 2021). It is reported even in remote marine and freshwater environments, including deep-sea sediments and Arctic sea ice (Van Cauwenberge et al., 2013; Fischer et al., 2015; Zhang et al., 2016; Geilfus et al., 2019). Environmental sources of plastic waste are diverse and include maritime and terrestrial sources (GESAMP, 2015). Most plastic waste is thought to come from terrestrial sources, which include landfills, atmospheric cycling and deposition, agricultural, urban and industrial runoff, and littering (Cozar et al., 2014; Shahul Hamid et al., 2018; de Souza Machado et al., 2018). Wastewater treatment plants (WWTP) are another important source of microplastics to freshwater and terrestrial ecosystems (Estabanati and Fahrenfeld, 2016; Murphy et al., 2016; Raju et al., 2018; Crossman et al., 2020).

Once in the environment, plastics degrade into smaller fragments via abiotic or biotic processes (Auta et al., 2018; Shahul Hamid et al., 2018; GESAMP, 2015). These small, fragmented pieces of anthropogenic debris are known as microplastics, defined as plastic particles smaller than 5 mm in size, and can take two forms: primary or secondary microplastics (Cole et al., 2011). Primary microplastics are manufactured to be <5 mm in size (e.g., cosmetic microbeads, pre-production pellets). Secondary microplastics are the result of larger pieces of plastic breaking down into particles <5 mm in size, and include microfibers, tire wear particles and fragmented plastic packaging (Rochman et al., 2019). Microplastics are often categorized by morphology and colour to help identify their source and to determine if they are primary or secondary (Helm, 2017). Microplastics are composed of many different material types; the most common include polypropylene (PP), polyethylene (PE), and polystyrene (PS) (Erni-Cassola et al., 2019). In the environment, microplastics are a mixture of different sizes, morphologies and chemistries that may have different interactions with the biological and physical environment.

The presence of microplastics is best documented in marine ecosystems (e.g., Van Cauwenberge et al., 2013; Tubau et al., 2015; Obbard et al., 2014), with research rapidly increasing in freshwater ecosystems where microplastic pollution is reported globally (Eerkens-Medrano et al., 2015). Furthermore, microplastic contamination is reported within marine and freshwater biota at all trophic levels, from plankton to fish, birds and whales (Auta et al., 2017; Horton et al., 2017; Brookeson et al., 2019).

Currently, there is limited research concerning microplastics in terrestrial ecosystems, including terrestrial-freshwater interfaces (e.g., Provencher et al., 2018), despite the reporting of evidence that terrestrial environments are significant sources of microplastics to freshwater systems and biota (Jambek et al., 2015; de Souza Machado et al., 2018; D’Souza et al., 2020). A handful of studies report microplastics in mostly agricultural soils (Nizzetto et al., 2016a, 2016b; de Souza Machado et al., 2018), with the application of WWTP biosolids that are rich in clothing fibers and cosmetic microbeads, considered a suspected pathway (Mahon et al., 2016; de Souza Machado et al., 2018). Microplastic fibers were reported in agriculture fields 15 years after the application of WWTP biosolids as fertilizer (Horton et al., 2017; Nizzetto et al., 2016b; Zubris and Richards, 2005). Nizzetto et al. (2016a) predicted that agricultural soils alone could contain more microplastics than oceanic basins. Collectively, these studies suggest that, like aquatic organisms, there is strong potential for terrestrial species to be exposed to and ingest microplastics.

Birds may be an ideal model to begin to understand microplastic contamination in terrestrial ecosystems as for other environmental contaminants. For example, European starlings (Sturnus vulgaris) accumulated triclocarban and triclosan when foraging in agricultural soils amended with WWTP biosolids (Sherburne et al., 2016). Other terrestrial birds, such as tree swallows (Tachycineta bicolor), have additional potential to demonstrate the transfer of contaminants (e.g., microplastics) from aquatic to terrestrial ecosystems (Bartrons et al., 2013; Koch et al., 2020). The foraging niche of tree swallows integrates terrestrial and freshwater environments since they feed predominantly on aerial insects that include aquatic-emerging insects (e.g., Diptera, Ephemeroptera, Odonata) (Dods et al., 2005). Plastics are widely found in freshwater invertebrates (Windsor et al., 2019; Simmerman and Coleman Wasik, 2020; Garcia et al., 2021) and were reportedly transferred through larval development to adult mosquitoes (Al-Jalibachi et al., 2019) that are commonly eaten by tree swallows. The trophic transfer of microplastics occurs through the freshwater food web of Eurasian dippers (Cinclus cinclus), birds that consume riverine macroinvertebrates and are restricted to fast-flowing montane rivers (D’Souza et al., 2020). Tree swallows are also a good candidate species to potentially identify specific point-sources (e.g., WWTPs) of plastics as with other contaminants (Fernie and Letcher, 2018; Custer et al., 2020), since they are colonial nesters that forage within a 400 m radius of their nests. With the increasing focus on microplastics in wildlife toxicology, there is a need to explore and validate non-lethal methods for assessing and monitoring microplastics in terrestrial birds as with aquatic species (Provencher et al., 2018; D’Souza et al., 2020; Bourdages et al., 2021).

2. Methods and materials

All monitoring, protocols and procedures involving the tree swallows were conducted with appropriate scientific permits (SC-OR-2019-00053; CH-019-008) and approved under the Guidelines of the Canadian Council of Animal Care by Environment and Climate Change Canada (permit #KF10-2019). The study was conducted as part of a broader study on the exposure, accumulation and potential effects of per- and poly-fluoroalkyl substances (K. Fernie, pers. comm.).

2.1. Study sites

The two tree swallow breeding colonies are located in southern Ontario, Canada, and monitoring of the birds at both sites was initiated in 2004 as part of an ongoing environmental contaminants research program (KJF). The reference site, the Mountsberg Conservation Area (43°27′N, 80°02′W), has a natural conservation area and wildlife sanctuary.
established in 1964), has 49 nest boxes situated adjacent to a large constructed reservoir with upstream low-intensity agricultural input (e.g., small hobby/horse farms, hay production) and no direct urban inputs or known applications of biosolids. Located ~40 km south east of the reference site, the second study site has 49 nest boxes located 300 m downstream of the effluent discharge pipe of the Woodward Wastewater Treatment Plant (WWTP) (43°25′N, 79°77′W). This WWTP uses secondary treatment processes and is the major WWTP for the city of Hamilton, Ontario (2019 population: 763,000).

2.2. Tree swallow chicks: field and laboratory collections

The breeding activity of tree swallows is highly synchronous and was monitored from May through July 2019. Following previously published protocols (Gilchrist et al., 2014; Fernie and Letcher, 2018), we collected 10-day old tree swallow nestlings, randomly selecting two siblings from each brood of 4 to 6 chicks, and randomly selecting the broods, at the reference site (N = 20 chicks) and the WWTP site (N = 20 chicks). Immediately prior to sampling the chicks each day, two field blanks were collected at each site by waving an empty sample glass jar in the air five times then immediately sealing it. Voluntarily-excreted fecal sacs from chicks (one fecal sac/chick) were collected directly into a pre-labelled glass jar, placed on ice in the field, then transferred to -20 °C until processing. Each chick was identified individually, and we recorded their body weight, wing chord length, length of the ninth primary feather (right wing), and estimated body condition (weight/wing chord length). The chicks were euthanized by decapitation, disected in the laboratory. Using the same methodology (above), two laboratory blanks were collected immediately before and after necropsies each day by waving an empty sample glass jar in the air five times then immediately sealing it. Briefly, blood was collected at decapitation, centrifuged, and plasma or red blood cell aliquots of intestine were subjected to digestion, described by feature and morphology, prepared for analysis by Raman spectroscopy, photographed and measured using ImageJ. Particle length was defined as the longest measurement of the particle.

2.3. Stable isotope analysis

Following Sun et al. (2020), red blood cells (RBCs) of individual chicks were analyzed for stable carbon (13C and 12C) and nitrogen (15N and 14N) at the Ján Veizer Stable Isotope Laboratory (Ottawa, Canada). Briefly, RBC samples were lipid-extracted and freeze-dried, then analyzed for C and N isotopes using an isotope ratio mass spectrometer (IRMS; Delta Advantage, Thermo) coupled to an elemental analyzer (EA; vario EL cube, Elementar) via a Confo III interface (Thermo). The samples were flash combusted (~1800 °C; Dumas combustion) and the resulting gas products carried by helium through the EA to be cleaned, separated, and sent to IRMS via interface. The SI ratios for C and N are expressed as δ values (‰) relative to their respective international standards, Vienna Pee Dee Belemnite and atmospheric N2, and normalized to calibrated international standards. The analytical precision inferred from internal standards showed ±SD ≤ 0.2‰ for δ13C and δ15N. The RPD of duplicates (n = 7) measured every 10 samples was 0.4% and 0.9% for δ13C and δ15N, respectively. The results for δ13C and δ15N in the concurrently analyzed in-house reference material (double-crested cormorant egg/liver) were within the acceptable range (±3 SD).

2.4. Preparation, quantification and characterization of suspected microplastics

Digestions and extraction of the microplastics from the GI tracts and fecal sacs were completed as described in Munno et al. (2018). Briefly, the samples were digested with 20% KOH solution in an oven (60 °C) for 24 h, then sieved (125 µm stainless steel mesh sieve) and rinsed with RO water, to capture only microplastics above 125 µm. Each sample was examined under microscope (OLYMPUS SZ61, magnification range 6.7×–45×), and suspected anthropogenic microplastics extracted, described by colour and morphology, prepared for analysis by Raman spectroscopy, photographed and measured using ImageJ. Particle length was defined as the longest measurement of the particle.

2.5. Chemical identification

Raman spectroscopy was used to chemically identified a subsample of suspected anthropogenic microplastics (Xplora Horiba Raman XploRA PLUS confocal microscope, Piscataway, NJ, USA; operating with LabSpec6 software v. 6.5.1.24). We subsampled from each sample by colour-morphology (e.g., blue fiber, black fragment). At least 10% of all microplastics of each colour category was randomly selected from each sample and chemically identified (including lab and field blanks). We chemically identified a total of 166 microplastics (26.7% of all microplastics) from the chicks’ GI tracts (WWTP: N = 57 particles; reference: N = 33 particles) and fecal sacs (WWTP: N = 36 particles; reference: N = 40 particles). The Raman spectrometer was equipped with a charge coupled device detector (-60 °C, 1024 × 256 pixels), and spectra were acquired using a 100× LWD objective (NA = 0.8) resulting in laser powers of 11.2 mW and 20.2 mW at 100% filter for the 532 nm and 785 nm lasers, respectively. Spectral resolution ranged from 1.3 cm⁻¹ (785 nm excitation laser, 600 grooves/mm) to 3.3 cm⁻¹ (532 nm excitation laser, 1200 grooves/mm). Each spectrum was corrected manually and matched to reference spectra using Bio-Rad KnowItAll and ID Expert software from the KnowItAll Raman Spectral Library and the Spectral Library of Plastic Particles (SLoPP and SLoPP-E) (Munno et al., 2020). Database matching software may apply corrections to the spectra automatically (baseline, vertical clipping, intensity distortion, horizontal offset, vertical offset, Raman intensity distortion). Matches between each spectrum and reference spectra were assigned visually based on a combination of hit quality index score, peak intensity and peak alignment. If particles were larger than 1 mm they were analyzed using Fourier transform infrared (FTIR) spectroscopy with an FPA-based Alpha II FTIR setup with OPUS/3D technology (Bruker Ltd., Milton, ON, CA). Particles were then categorized into material types based upon chemical identification results. Material types include plastic (e.g. PP, PE, PVC, natural, anthropogenic (unknown), anthropogenic (synthetic) and anthropogenic (cellulosic)) (Table 1). This allowed for the identification and categorization of the microplastics and other anthropogenic materials. In total, 73.5% of the particles examined were confirmed to be anthropogenic particles. We did not correct our anthropogenic particle counts according to our results from Raman spectroscopy.

2.6. Quality assurance/quality control

All tools were rinsed with RO water three times before and between the processing of each sample to avoid cross-contamination and procedural contamination. To account for contamination of microplastics in the field and lab, the blank samples were digested, picked, and counted using the same procedures and methods as were used for all samples. The anthropogenic particles found in the blanks were tallied and averaged for each sample type (GI tract or fecal) and site (WWTP or reference) based on morphology and colour. They were then subtracted from each sample of the corresponding sample type and site, i.e., samples were blank-corrected.

2.7. Statistical analysis

All of the chicks (N = 40) were included in the statistical analysis, including those with no microplastics found in their GI tracts and fecal
sacs (N = 2 WWTP chicks, 5 reference chicks). Two statistical outlier samples (i.e., a WWTP GI tract with 111 particles, a reference fecal sac with 56 particles) were identified (i.e., beyond two standard deviations) and removed from further statistical analyses. (Statistical outcomes remained the same when the two statistical outliers were included.) Data were tested for normality and homogeneity of variance but could not be successfully transformed. Nested ANOVAs on ranked data, with ‘brood’ nested within ‘site’ to account for variation between siblings in each brood, were used to identify significant differences in the diet of the chicks (δ13C, δ15N), and the number and size (length, width) of microparticles in their GI tracts or fecal sacs, between sites, within each site (i.e., GI tracts vs. fecal sacs), and overall (both sites combined). We were surprised to find that the tree swallow chicks at both sites (≥ 75%) appear to have ingested a similar number of anthropogenic particles into their GI tracts. The WWTP in this study serves a large, urbanized area with nearby industrial sectors that include many plastics manufacturing companies, leading to our prediction that a higher number of microparticles would be ingested by the tree swallow chicks raised immediately downstream of the WWTP discharge pipe. While the similar numbers of microparticles in the chicks’ GI tracts did not support this prediction, they are consistent with those of other studies. WWTPs were not a source of increased microplastic burdens in downstream macroinvertebrates (Windsor et al., 2019), and while urbanization affected microplastic contamination in water and aquatic biota (macroinvertebrates, trout), effects on contamination were not necessarily consistent with the expected effect of urban point source contamination (Simmerman and Coleman Wasik, 2020). Moreover, the WWTP in the present study used secondary treatment processes at the time, and secondary treatment processes can remove up to 99% of microparticles in the inflow (Schell et al., 2021 and references therein; see also Iyare et al., 2020).

We were surprised to find that the tree swallow at the reference site had ingested larger microparticles and excreted more microplastics than the WWTP chicks raised immediately downstream of the WWTP discharge pipe. The presence of low-intensity agriculture (e.g., horse farms, hay production), the lack of WWTP biosolid applications, and the lack of urbanization upstream and surrounding the reference site, led to our prediction that there would be fewer suspected microplastics ingested by the tree swallow chicks raised at this site. Yet our findings did not support this prediction. Several factors may possibly explain the increased number of microparticles in the fecal sacs of the reference chicks compared to the WWTP chicks. There were important dietary differences between chicks at the sites, with reference chicks having significantly lower δ13C (nested ANOVA (ranked data): site: F1,37 = 623.91 p < 0.001) and higher δ15N (nested ANOVA: F1,37 = 216.12 p < 0.001) than WWTP chicks. Microplastics were found in the feces (45%) and regurgitates (50%) of Eurasian dippers, considered transitory in the dippers (D’Souza et al., 2020). In the present study, comparatively more tree swallow chicks downstream of the WWTP (90%) and at the reference site (90%) had anthropogenic particles in their fecal sacs (Table 2), indicating that tree swallow chicks are able to excrete microparticles that they consume. Surprisingly, when accounting for the variation among siblings, there was a significantly greater number of microparticles in the fecal sacs of the reference chicks (8.7 ± 12.7 microparticles/chick) than the WWTP chicks (5.9 ± 6.0 microparticles/chick) (nested ANOVA on ranked data: p = 0.06; site(brood): F18,39 = 2.12 p = 0.05; Fig. 1B). A similar pattern was evident in the size of the microparticles found in the tree swallow chicks: compared to the WWTP chicks, the reference chicks had larger microparticles in their GI tracts (nested ANOVA on ranked data: length: site: F1,527 = 4.31 p = 0.04, site(brood): F18,527 = 5.86 p < 0.001; width: site: p = 0.57, site(brood): F18,527 = 1.78 p = 0.02), but microparticles were similar in size in the fecal sacs of chicks at both sites (length: width: p-values > 0.10). The differences between study sites in the size of microparticles in the chicks’ GI tracts, likely reflect the differences between sites in microparticle morphologies in the GI tracts discussed below. Furthermore, when considered collectively, the significant differences between sites in the numbers (fecal sacs) and size (GI tracts) of microparticles in the tree swallow chicks, demonstrates the importance of sampling multiple siblings across broods in order to address the large variability among siblings and other individuals in ingested and excreted microparticles.

Our results suggest the ubiquity of microplastics in terrestrial birds, since most of the tree swallow chicks at both sites (≥ 75%) appear to have ingested a similar number of anthropogenic particles into their GI tracts. The WWTP in this study serves a large, urbanized area with nearby industrial sectors that include many plastics manufacturing companies, leading to our prediction that a higher number of microparticles would be ingested by the tree swallow chicks raised immediately downstream of the WWTP effluent discharge pipe. While the similar numbers of microparticles in the chicks’ GI tracts did not support this prediction, they are consistent with those of other studies. WWTPs were not a source of increased microplastic burdens in downstream macroinvertebrates (Windsor et al., 2019), and while urbanization affected microplastic contamination in water and aquatic biota (macroinvertebrates, trout), effects on contamination were not necessarily consistent with the expected effect of urban point source contamination (Simmerman and Coleman Wasik, 2020). Moreover, the WWTP in the present study used secondary treatment processes at the time, and secondary treatment processes can remove up to 99% of microparticles (Schell et al., 2021 and references therein; see also Iyare et al., 2020).

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### Table 1

Category and description of the possible chemical identified by Raman spectroscopy.

<table>
<thead>
<tr>
<th>Identification category</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plastic (by polymer type)</td>
<td>Used when a specific polymer type was identified (e.g., PP, PE, PVC).</td>
</tr>
<tr>
<td>Natural</td>
<td>Hair, wool, minerals, rock</td>
</tr>
<tr>
<td>Cellulosic (considered natural because it cannot be identified as anthropogenic)</td>
<td>Cotton, holly, rayon</td>
</tr>
<tr>
<td>Anthropogenic (unknown)</td>
<td>Dye, pigment or additive found in both synthetic and natural materials.</td>
</tr>
<tr>
<td>Anthropogenic (synthetic)</td>
<td>Dye, pigment or additive used solely in plastics manufacturing.</td>
</tr>
<tr>
<td>Anthropogenic (cellulosic)</td>
<td>Presence of a colour (i.e., not clear or white) identified via visual microscopy or within the Raman spectrum, with a cellulosic base material.</td>
</tr>
</tbody>
</table>

### Table 2

The numbers of anthropogenic particles identified in the gastro-intestinal (GI) tract and fecal sacs of 10-day old tree swallow chicks raised at a rural conservation area (reference) or 300 m downstream of the effluent discharge pipe of a large urban wastewater treatment plant (WWTP). Means ± standard deviations (SD), and ranges (minimum (min.); maximum (max.)), of particle numbers found in the birds are reported.

<table>
<thead>
<tr>
<th>Site</th>
<th>N</th>
<th>Number of particles</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Quantity</td>
<td>Mean ± SD</td>
</tr>
<tr>
<td>Fecal sac</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WWTP</td>
<td>20</td>
<td>118</td>
<td>5.9 ± 6</td>
</tr>
<tr>
<td>Reference</td>
<td>20</td>
<td>174</td>
<td>8.7 ± 12.7</td>
</tr>
<tr>
<td>GI tract</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WWTP</td>
<td>20</td>
<td>226</td>
<td>11.3 ± 24.7</td>
</tr>
<tr>
<td>Reference</td>
<td>20</td>
<td>102</td>
<td>5.1 ± 7</td>
</tr>
</tbody>
</table>
p < 0.001; site(brood): $F_{18,37} = 11.60 \ p < 0.001$) and $\delta^{15}$N values (nested ANOVA (ranked data): site: $F_{1,37} = 540.87 \ p < 0.001$; site (brood): $F_{18,37} = 9.60 \ p < 0.001$), indicating a stronger freshwater, lower trophic dietary signature than the more terrestrial, higher trophic dietary signature of the WWTP chicks. Microparticle numbers (fecal sacs, GI tracts) and concentrations (GI tracts) were not significantly correlated with foraging location (δ$^{13}$C) or trophic position (δ$^{15}$N) (Spearman’s correlations controlling for non-independence within broods: all $p$-values $\geq 0.14$), demonstrating the complexities of microplastic exposure and ingestion by birds. In riverine fish, ingested microplastic abundance was associated with foraging location, but not with trophic position unlike macroinvertebrates (Garcia et al., 2021). We hypothesize that the more terrestrial-based diet of the WWTP chicks potentially reduced their ingestion of microparticles from the nearby river receiving the WWTP effluent. In addition, studies have shown that microplastic fragments and fibers can be transported via atmospheric transport to remote and pristine habitats (Rillig, 2012; Allen et al., 2019). Given that the reference site in this study is approximately 18 to 27 km from the nearest two urban-manufacturing centers (i.e., Milton and Guelph, ON, respectively), atmospheric deposition may be an additional pathway for microplastic exposure of the reference tree swallow chicks. Moreover, the dam adjacent to the nest boxes at the reference site could be another potential source of anthropogenic particles for the chicks, as dams were reported to concentrate microplastics within sediments and surface waters (Di and Wang, 2018; Watkins et al., 2019) and adult swallows were observed feeding over the water adjacent to the dam (K. Fernie, pers. obs.). Future research should investigate the potential for birds to be exposed to and ingest anthropogenic particles through their diet (e.g., aerial insects, macroinvertebrates), atmospheric deposition and the accumulation of such materials at dams and reservoirs.

We also investigated the profiles and proportions of morphologies in a subsample of the microparticles found in the birds. (Analytical logistics precluded generation of anthropogenic particle morphologies for individual birds.) In the tree swallow chicks downstream of the WWTP, 100% of the microparticles observed in GI tracts and fecal sacs
were fibers (Fig. 2A). Similarly, in the reference chicks, 100% of the microparticles in the fecal sacs were also fibers. These results suggest that tree swallow chicks regularly ingest and excrete fibers, and that these types of microparticles may be ubiquitous in the environment. Fibers were also the dominant type of microplastic in guano of Arctic seabirds (Provencher et al., 2018) and fecal samples and regurgitates of Eurasian dippers (D’Souza et al., 2020). Our findings of 100% fibers in fecal sacs from tree swallow chicks at both sites, further suggests that the birds can excrete fibers relatively easily, but that other morphologies of microplastics may not be (easily) excreted and thus accumulate, potentially contributing to plastics-associated chemical contaminant burdens, physiological changes and/or physical complications (Azzarello and Van Vleet, 1987; Ryan, 1988; but see D’Souza et al., 2020). While the GI tracts of the reference chicks were dominated by fibers, they also contained two other particle morphologies: fibers (82%), pellets (16%), and fragments (2%), in contrast to the GI tracts of the WWTP chicks that contained fibers only (Fig. 2A). The observed site differences in microparticle morphology profiles of the GI tracts suggest that while WWTP effluent-receiving waterways may be dominated by microfibers, other waterways, even those with limited human-impacted landscapes, may have more variety of anthropogenic particle types that birds may be exposed to, ingest and accumulate. Recent research has estimated the retention and release of microparticles from WWTPs, and characterized some of the temporal-environmental complexities and additional sources of microplastics (e.g., run-off) involved in determining microparticle profiles and concentrations in receiving waters and biota (Simmerman and Coleman Wasik, 2020; Schell et al., 2021). We recommend future research investigate possible environmental sources of microparticles and their transfer through terrestrial food webs of birds, expanding on the limited research to date with freshwater avian food webs (Windsor et al., 2019; D’Souza et al., 2020).

In terms of the composition of anthropogenic particles found in the chicks, 74% of the 166 microparticles sub-sampled for Raman spectroscopy (27% of the total suspected anthropogenic particles) were confirmed as anthropogenic in origin, consisting of, in total (GI tracts + fecal sacs): anthropogenic cellulose (33%), acrylic (22%), cellulosic (21%), anthropogenic unknown (5%), PET/polyester (4%), PP (3%), PE (2%), unknown (2%), natural (organic) (2%), polyurethane (1%), natural (inorganic) (1%), polyethyleneimine (1%), anthropogenic synthetic (1%), and copolymer (1%). Of the plastic microparticles, the three most frequent types were acrylic, PET/polyester, and PP. Similarly, processed cellulose and PET/polyester were commonly found in the few terrestrial biota studies (e.g., Carlin et al., 2020) available for comparison, suggesting that these may be ubiquitous in terrestrial food webs. When comparing the diversity of materials found in the nestlings between sites, it is far from what we expected (Fig. 2B, Table 3). Initially, we believed chicks situated near a large urban center that is highly industrialized would contain a greater variety of plastic types compared to chicks situated in a rural conservation area (Canada, E, 2020; see also Windsor et al., 2019; Simmerman and Coleman Wasik, 2020; Schell et al., 2021). Instead, the plastic polymers found in the GI tracts of the reference chicks appeared more diverse than in the GI tracts of the WWTP chicks (Fig. 2B), and contained plastics commonly used for manufacturing day-to-day plastic items (PP and PE) (Table 3) supporting our hypothesis of atmospheric transportation as a source (discussed earlier). Within each site, the three most common types of anthropogenic particle types remained relatively consistent in the GI tracts and fecal sacs, namely anthropogenic cellulosic, cellulosic, and acrylic (Table 3), but the profiles differed in the GI tracts of the WWTP chicks (anthropogenic cellulosic > acrylic > cellulosic) versus the reference chicks (cellulosic > anthropogenic cellulosic = polypropylene), and in the fecal sacs of the WWTP chicks (cellulosic > anthropogenic cellulosic > acrylic) versus the reference chicks (acrylic = anthropogenic cellulosic > cellulosic) (Table 3).

3.2. Comparing the microparticles in the fecal sac to the GI tract: a possible non-lethal tool?

One of our major objectives was to determine if non-lethal sampling (i.e., fecal sacs) of passerine chicks could be used instead of lethal sampling (e.g., GI tract collections) for assessing and/or monitoring suspected microparticles in terrestrial birds. In the current study, there were a similar number of anthropogenic particles found in the GI tracts and fecal sacs of the tree swallow chicks across both sites (nested ANOVA on ranked data: p ≥ 0.43), and no significant correlations in the number of microparticles between the GI tracts and fecal sacs overall (Spearman’s r = −0.13 p = 0.43) or within each site (Spearman’s r-values = −0.12, −0.31, p-values > 0.19) (Fig. 3). However, we observed a greater diversity of microplastic morphologies and polymer types in the GI tracts than fecal sacs of the tree swallow chicks (Fig. 2A, B, C. Sherlock, Kj. Fernie, K. Munno et al. Science of the Total Environment 807 (2022) 150453

<table>
<thead>
<tr>
<th>Table 3</th>
</tr>
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<tbody>
<tr>
<td>The types of polymers (total numbers (#) and percentages (%) of particles) identified in a subsample of anthropogenic particles found in the GI tracts and fecal sacs of tree swallow chicks (overall) at the reference site or 300 m from the effluent discharge pipe of a large, urban wastewater treatment plant (WWTP) using secondary treatment. The red asterisks indicate the three most common polymers for each site and sample type.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Polymer type</th>
<th>Overall</th>
<th>GI tract</th>
<th>Fecal sac</th>
<th>WWTP</th>
<th>Reference</th>
<th>WWTP</th>
<th>Fecal sac</th>
</tr>
</thead>
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<tr>
<td>Acrylic</td>
<td>17</td>
<td>18.9*</td>
<td>20</td>
<td>26.3*</td>
<td>13</td>
<td>22.8*</td>
<td>4</td>
</tr>
<tr>
<td>Anthropogenic (cellulosic)</td>
<td>30</td>
<td>33.3*</td>
<td>25</td>
<td>32.9*</td>
<td>25</td>
<td>43.9*</td>
<td>5</td>
</tr>
<tr>
<td>Anthropogenic (unknown)</td>
<td>6</td>
<td>6.7</td>
<td>7</td>
<td>3.9</td>
<td>4</td>
<td>7</td>
<td>2</td>
</tr>
<tr>
<td>Polyurethane</td>
<td>2</td>
<td>2.2</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>3.5</td>
<td>0</td>
</tr>
<tr>
<td>Cellulosic</td>
<td>14</td>
<td>15.6*</td>
<td>21</td>
<td>27.6*</td>
<td>7</td>
<td>12.3*</td>
<td>7</td>
</tr>
<tr>
<td>PET/polyester</td>
<td>3</td>
<td>3.3</td>
<td>3</td>
<td>3.9</td>
<td>1</td>
<td>1.8</td>
<td>2</td>
</tr>
<tr>
<td>Natural</td>
<td>4</td>
<td>4.4</td>
<td>1</td>
<td>1.3</td>
<td>2</td>
<td>3.5</td>
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<tr>
<td>Copolymer</td>
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<td>0</td>
<td>0</td>
<td>1</td>
<td>1.8</td>
<td>0</td>
</tr>
<tr>
<td>Polystyrene</td>
<td>5</td>
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<td>0</td>
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<td>5</td>
</tr>
<tr>
<td>Polyethylene</td>
<td>3</td>
<td>3.3</td>
<td>1</td>
<td>1.3</td>
<td>0</td>
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<td>3</td>
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<tr>
<td>Anthropogenic (synthetic)</td>
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<td>1</td>
<td>1.3</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td>Unknown</td>
<td>3</td>
<td>3.3</td>
<td>1</td>
<td>1.3</td>
<td>2</td>
<td>3.5</td>
<td>1</td>
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<tr>
<td>Polyethyleneimine</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
</tbody>
</table>

| Overall | 90 | 100 | 76 | 100 | 57 | 100 | 33 | 100 |
| GI tract | 90 | 100 | 76 | 100 | 57 | 100 | 33 | 100 |
| Fecal sac | 90 | 100 | 76 | 100 | 57 | 100 | 33 | 100 |
| WWTP | 90 | 100 | 76 | 100 | 57 | 100 | 33 | 100 |
| Reference | 90 | 100 | 76 | 100 | 57 | 100 | 33 | 100 |

When comparing the diversity of materials found in the nestlings between sites, it is far from what we expected (Fig. 2B, Table 3). Initially, we believed chicks situated near a large urban center that is highly industrialized would contain a greater variety of plastic types compared to chicks situated in a rural conservation area (Canada, E, 2020; see also Windsor et al., 2019; Simmerman and Coleman Wasik, 2020; Schell et al., 2021). Instead, the plastic polymers found in the GI tracts of the reference chicks appeared more diverse than in the GI tracts of the WWTP chicks (Fig. 2B), and contained plastics commonly used for manufacturing day-to-day plastic items (PP and PE) (Table 3) supporting our hypothesis of atmospheric transportation as a source (discussed earlier). Within each site, the three most common types of anthropogenic particle types remained relatively consistent in the GI tracts and fecal sacs, namely anthropogenic cellulosic, cellulosic, and acrylic (Table 3), but the profiles differed in the GI tracts of the WWTP chicks (anthropogenic cellulosic > acrylic > cellulosic) versus the reference chicks (cellulosic > anthropogenic cellulosic = polypropylene), and in the fecal sacs of the WWTP chicks (cellulosic > anthropogenic cellulosic > acrylic) versus the reference chicks (acrylic = anthropogenic cellulosic > cellulosic) (Table 3).
Table 3). This may reflect the ability of the chicks to ingest a large diversity of anthropogenic debris, but because of the morphology of some microparticles, be less likely to excrete specific types of microparticles (i.e., hard fragments and pellets) as easily, and therefore those microparticles may not be present in the fecal sacs. The size of these microparticles may be a contributing factor, since fibers may be substantially smaller than fragments and pellets. Overall, the microparticles were significantly wider (nested ANOVA on ranked data; tissue: $F_{1,1037} = 3.52 \ p = 0.06$; tissue (brood): $F_{38,1037} = 1.55 \ p = 0.02$) and longer (nested ANOVA on ranked data; tissue: $F_{1,1037} = 3.44 \ p = 0.05$; tissue (brood): $F_{38,1037} = 3.44 \ p < 0.001$) in the chicks’ GI tracts (width: $0.14 \pm 0.6$ mm; length: $2.3 \pm 1.8$ mm) than fecal sacs (width: $0.03 \pm 0.04$; length: $2.1 \pm 1.8$ mm), with a seemingly greater proportion of smaller microparticles in the fecal sacs than the GI tracts (Fig. 4A, B). It

**Fig. 3.** There were no significant correlations between the number of suspected microparticles found in the fecal sacs and GI tracts of the tree swallow chicks overall ($N = 40$; Spearman’s $r = -0.13 \ p = 0.43$), at the reference (REF) site ($N = 20$; Spearman’s $r = -0.31 \ p = 0.19$), or immediately downstream (300 m) of the effluent discharge pipe of the wastewater treatment plant (WWTP) ($N = 20$; Spearman’s $r = -0.12 \ p = 0.63$). Statistical methods involved Spearman’s rank order correlations controlling for non-independence within broods.

**Fig. 4.** A The frequency of all particle sizes (after blank correction) across all collected anthropogenic particles ($N = 619$) in the fecal sacs (red) and gastro-intestinal tracts (blue) of the tree swallow chicks (both sites combined). The enlarged inset (B) shows the trend for particles to be 0–4 mm in length. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
is possible that the larger microparticles found in the GI tracts than the fecal sacs could reflect longer retention times in the chicks that may be biologically important (discussed below).

Interestingly, when examining the GI tracts of the tree swallow chicks, pieces of shells of various shades of yellow and white were discovered that were not evident in the fecal sacs. In addition, only yellow and white coloured pellets and fragments were found in the GI tracts of the reference chicks, where 100% of these fragments were white, 73% of pellets were yellow and 27% of pellets were white. When examined together, the pre-production pellets and fragments resembled the shells that the tree swallows had ingested. Adult birds, including tree swallows, often ingest shells to support eggshell formation and nestling development since shells are a main source of calcium and at times a form of grit (St. Louis and Breebaart, 1991). Our findings suggest that the tree swallows were likely actively selecting this morphology of microparticles and shows that anthropogenic particles were directly fed to the 10-day old chicks who had not yet fledged from the nest as was evident with Eurasian dippers (D’Souza et al., 2020). More evidence is required to substantiate the possibility that birds may be actively selecting plastic fragments when seeking calcium-rich materials (e.g., shell fragments, small bones) during egg formation and chick development.

The differences among tissues in the number, morphologies and sizes of microparticles, and the lack of correlations in microparticle numbers between tissues, suggests that fecal sacs should not be used solely for quantifying and characterizing anthropogenic particles in passerine birds. We recommend that fecal sacs be used as a non-lethal indicator to identify that an organism is exposed to and ingesting anthropogenic particles. With the increasing need to develop and validate non-lethal, minimally invasive methods, we recommend that future research investigate the concentrations, numbers and types of anthropogenic particles found in dietary prey (e.g., aerial insects, macroinvertebrates) or voluntary regurgitates of multiple bird species (e.g., D’Souza et al., 2020) as a potential substitute for the use of GI tracts. The results of our study suggested that the lethal collection of the GI tract was required to fully understand the numbers and types of anthropogenic particles ingested; thus, it would be highly valuable to determine if non-lethal, minimally invasive alternative methods (e.g., macroinvertebrate prey) would provide comparable information on particle characteristics to more accurately understand the exposure and ingestion of microparticles by birds since sampling only the fecal sacs would underestimate the chicks’ ingestion of anthropogenic particles.

### 3.3. Possible ecological implications of microplastic ingestion

Previous studies have reported that ingestion of microplastic debris may lower overall fitness in seabirds (Ryan, 1988). A range of potential adverse consequences may also occur in relation to the chemicals that plastics are produced with, and those chemicals they can absorb from the environment (Teuten et al., 2009). Roman et al. (2019) reported that chemicals from microplastics ingested by Japanese quail (Coturnix japonica) caused minor delays in sexual maturity and growth of chicks but not adult body weight. We investigated whether the total number of anthropogenic particles found in the tree swallow chicks (GI tracts + fecal sacs combined across both study sites) showed any correlations with their condition and size, and found no significant associations with their estimated body condition, body weight, organ weights (i.e., GI tract, liver, spleen, lungs), or primary feathers (i.e., length) (Spearman’s rank correlations controlling for non-independence within broods: p-values > 0.12); nor were there any significant correlations with these morphological measures and the number of anthropogenic particles in the GI tracts versus fecal sacs when examined across both sites or by individual study site (p-values > 0.10). Similarly, there was no relationship between the plastic load and body condition of 17 individual birds (representing 12 terrestrial species) from Shanghai, China (Zhao et al., 2016). We recommend future research be undertaken with multiple avian species at varying ages to characterize possible, more subtle physiological and biochemical effects of microparticles on birds.

Plastics were recently found to transfer through the freshwater food web of free-ranging Eurasian dippers (D’Souza et al., 2020). Although we did not directly assess trophic transfer in this study, our findings of microparticles in the tree swallows suggest that this mid-trophic species likely contributes to trophic transfer of microparticles as insectivorous predators and prey. As aerial insectivores, the preferred prey of tree swallows are caddisflies, dragonflies, damselflies and mayflies that develop in freshwater systems before emerging as adults. Microplastics and particles are found in these and other macroinvertebrates (e.g., Ehlers et al., 2019; Windsor et al., 2019; Simmerman and Coleman Wasik, 2020; Garcia et al., 2021). Since tree swallows are prey for other predators, and trophic transfer of microplastics is known to occur between avian species (Hammer et al., 2016), it is conceivable that tree swallows may be a source of microplastics when consumed by higher trophic predators. Future studies should investigate the presence and types of microplastics in the prey of birds at all trophic levels, including insects consumed by aerial insectivore birds, to better characterize trophic transfer of microplastics especially in terrestrial food webs.

Our study and the findings reported by others (e.g., Provencher et al., 2018; D’Souza et al., 2020; Bourdages et al., 2021) indicate that birds ingest microfibers but do not retain them like other microplastics. Consequently, tree swallows may be a vector of microfibers from the aquatic environment to the terrestrial environment through their fecal material. We recommend that future research characterize the concentrations of fibers and other anthropogenic particles ingested and excreted by birds to facilitate comparisons among studies, as well as the passage time of fibers and other particles through the birds (see D’Souza et al., 2020). Tree swallow colonies are often small flocks when migrating, ranging from hundreds to hundreds of thousands in one flock (Cornell Lab of Ornithology, 2019). Here, the average number of microparticles found in each fecal sac of the chicks was 67.5 microparticles. If each swallow deposits this number of anthropogenic particles, they may be conduits of potentially large numbers of microplastics that contaminate terrestrial sites and ecosystems.

### 4. Conclusions

Overall, the results of our study show that anthropogenic particles are found in both the GI tracts and fecal sacs of tree swallows, a terrestrial passerine that feeds on aquatic-emerging aerial insects. It appears that tree swallows are an appropriate species for investigating and monitoring the exposure, ingestion and possible toxicity of microplastics to terrestrial birds. The dietary differences between sites of the tree swallows, the high variability of microplastic ingestion among individuals and smaller samples sizes, limited our ability to strongly conclude that WWTPs are not a source of anthropogenic particles to these birds. Nevertheless, our results strengthen the findings of other research suggesting microplastics are ubiquitous in terrestrial birds and ecosystems. We recommend that fecal sacs may be used as a non-lethal indicator to identify that a bird is ingesting anthropogenic particles, and that future research determine if other non-lethal sampling methods (e.g., macroinvertebrate or other prey samples, voluntary regurgitates) are a viable alternative to using GI tracts to better characterize the anthropogenic particles ingested by birds. Moreover, we recommend that future research determine the sources and transfer of microplastics in freshwater and terrestrial food webs of birds (e.g., tree swallows), through characterizing microparticles in surface waters, prey species (e.g., aerial insects, aquatic macroinvertebrates), and concurrently in bird samples (e.g., fecal samples, regurgitates). Future research should investigate atmospheric deposition as a potential source of exposure to anthropogenic particles for birds; determine concentrations and passage times of fibers and other particles through birds, and the possibility of more subtle physiological and biochemical
changes in birds from ingesting microplastics and related environmental chemicals.

CRediT authorship contribution statement

**Cassandra Sherlock**: Methodology, Validation, Formal analysis, Investigation, Writing – original draft, Writing – review & editing. **Kim J. Fernie**: Conceptualization, Methodology, Validation, Investigation, Resources, Data curation, Writing – review & editing, Supervision, Project administration, Funding acquisition. **Keenan Munno**: Methodology, Validation, Formal analysis, Writing – review & editing. **Chelsea Rochman**: Conceptualization, Methodology, Validation, Investigation, Resources, Writing – review & editing, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References


