Microplastics in the stomach contents and gastrointestinal tracts of North American insectivorous bats (#111483)

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Microplastics in the stomach contents and gastrointestinal tracts of North American insectivorous bats

Ashleigh B Cable [Corresp., 1], **Emma V Willcox** [1], **Leah N Crowley** [1], **Christy Leppanen** [2]

[1] School of Natural Resources, University of Tennessee - Knoxville, Knoxville, Tennessee, United States

[2] The Center for Tobacco Products, United States Food and Drug Administration, Silver Spring, Maryland, United States

Corresponding Author: Ashleigh B Cable
Email address: acable5@vols.utk.edu

Background. Microplastics are ubiquitous environmental contaminants of increasing concern to aquatic and terrestrial species. Bats are a group of aerial insectivores that consume emergent aquatic and terrestrial insects. Microplastics have been detected in numerous insectivorous bird species and in bats in the Amazon. There are currently no published studies investigating dietary pathways of microplastic exposure in North American (NA) insectivorous bats. **Methods.** We extracted, quantified, and characterized microplastics from the stomach contents of *Myotis lucifugus* (little brown bats) and gastrointestinal tracts (GITs) of *Eptesicus fuscus* (big brown bats). We compared microplastic concentrations in GITs to procedural blanks. We used linear regression to investigate the relationship of GIT concentrations with bat body mass. **Results.** We show that insectivorous bats ingest microplastics and that higher microplastic GIT concentrations are related to lower body mass, potentially indicative of poorer body condition and reduced fat storage. Fat reserves are an important energy resource for bats to survive while migrating, reproducing, hibernating, and surviving diseases such as the non-native fungal disease white-nose syndrome. This study provides a baseline for understanding microplastic exposure of NA bats and the possible health implications of contact with environmentally relevant concentrations.

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Ashleigh B. Cable[1], Emma V. Willcox[1], Leah N. Crowley[1], Christy Leppanen[2]

[1] School of Natural Resources, University of Tennessee, Knoxville, Tennessee, Unites States of America

[2] The Center for Tobacco Products (CTP), United States Food and Drug Administration (FDA), Silver Spring, Maryland, United States of America

Corresponding Author:
Ashleigh B. Cable[1]
2431 Joe Johnson Drive, Knoxville, Tennessee, 37996
Email address: ashleigh.cable@gmail.com

Abstract

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Introduction

Microplastics (MPs), or tiny plastic particles sized 1–5 mm, are human-sourced contaminants that are widespread and abundant in the environment (Thacharodi et al., 2024). Nano- (<1 mm) and meso-(5–25 mm) sized plastic particles are often grouped together with MPs in research studies (Correia et al., 2023). Considerable research on MPs has focused on uptake by aquatic organisms, but recent studies also show that terrestrial organisms are exposed (Ayala et al., 2023; Carlin et al., 2020; Fackelmann et al., 2023; Hoang & Mitten, 2022; Masiá et al., 2019; Sherlock et al., 2022; Wayman et al., 2024; Weitzel et al., 2021; Winkler et al., 2020). Potential health concerns related to MP exposure are numerous across taxa and include physiological reactions to the constituents from which they are made, such as bisphenol A (BPA), a known endocrine-disrupting compound (Flint et al., 2012). Additionally, the physical properties of MPs are implicated in the disease plasticosis, indicated by particles that embed in or change the structure of tissues (Charlton-Howard et al., 2023). Microplastics have been associated with many sublethal effects in organisms including reduced body condition (Welden & Cowie, 2016), altered gut microbiomes (Fackelmann et al., 2023), altered fatty acid composition (McCann Smith et al., 2024), organ damage (Rivers-Auty et al., 2023), depressed immune systems, oxidative stress, and inhibited growth (Osman et al., 2023).

Aerial insectivores are among the terrestrial organisms that are exposed to MPs. In the last five years, numerous studies have documented MP exposure in birds (Carlin et al., 2020; Fackelmann et al., 2023; Liu et al., 2023; Masiá et al., 2019; Schutten et al., 2024; Teboul et al., 2021; Tokunaga et al., 2023; Wayman et al., 2024). One published study documents exposure in multiple bat species in the Brazilian Amazon (Correia et al., 2023). To date, no studies in the literature have investigated MP exposure in NA insectivorous bats. Many of these bat species are of conservation concern due to a myriad of threats, including habitat loss or degradation, urbanization, agricultural intensification, wind turbine fatalities, and the disease white-nose syndrome (WNS). The objectives of this study were to 1) test a method for extracting microplastics from bat stomach contents, 2) characterize and quantify MP concentrations in bat gastrointestinal tracts, and 3) investigate the possible relationship of MP concentrations with bat sex, age, and body condition.

Materials & Methods

Bat stomach content collection

We received bat stomach contents collected during previous field studies as part of WNS surveillance. At the time of collection, WNS was recently introduced to NA, and large quantities of dead bats were found in caves. The samples used in this study were collected from three locations in the Northeast USA: Bennington County cave, Vermont (n=42), Hampden County mine, Massachusetts (n=25), and Warren County mine, New York, USA (n=25). We believe that they were all collected by hand from sites by researchers and know that they were all from a single species, *Myotis lucifugus* (little brown bat). The samples were not collected for the purpose of studying microplastics and there was no protocol in place to limit contamination at

time of stomach content collection; therefore, we only used these samples to test a method of MP extraction and quantification and cannot determine the source of MPs in these samples.

Bat gastrointestinal tract (GIT) collection

We collected bat carcasses through public health monitoring programs in Tennessee, USA. We received dead bats that had been collected throughout the state and submitted to facilities in Knox and Davidson Counties. The bats were often submitted following human or pet exposure and were missing brain tissue from rabies tests that were performed. We chose *Eptesicus fuscus* (big brown bat) as our study species because we had the highest number of carcasses of that species. Bats were frozen at -20 degrees Celsius, then thawed for three hours on the day of necropsy and necropsied in a dedicated lab at the University of Tennessee College of Veterinary Medicine to extract the full, intact GIT from esophagus to anus. During necropsy, we placed bats on metal pans, necropsied them with small metal scissors and scalpels, and stored all extracted organs in aluminum foil. We refroze each sample in a labeled whirl Pak bags until further analysis. Unlike the collection method of the stomach content samples discussed previously, this GIT collection protocol ensured reduced risk of MP contamination, thus allowing us to test the dietary pathway of exposure.

Extraction of microplastics

We adapted methods previously used to extract MPs from GITs of birds of prey (Carlin et al., 2020). We rinsed each GIT sample with pure deionized water (DI water) twice to remove external microplastics. This step was not conducted with the stomach contents samples as that would have washed away all the material. We weighed each GIT sample and digested tissues in a 10% potassium hydroxide (KOH) solution and placed them on a shaker at 180 Revolutions per Minute (RPM) speed for 24 hours. Samples remained in the KOH solution for 3–12 days

depending on tissue digestion success. We found that applying heat during digestion was not required, possibly due to the small sample mass. We used a glass vacuum filter system and glass membrane filters (Whatman, grade GF/F borosilicate glass microfiber filters, 47 mm diameter, 0.42 mm thickness) to separate out undigested from digested material. We originally used filters with a 2.7 um pore size for stomach content samples but determined that a smaller pore size could be used, so we adopted 0.7 um pore size for GIT samples. We stored samples on the glass filters in closed glass petri dishes in a dark filing cabinet until we counted particles in subsequent steps.

Limiting and quantifying contamination

To avoid introducing contamination to samples, all benches were wiped down at the beginning of each session with paper towels and DI water. We wore 100% white cotton lab coats, covered all samples with aluminum foil while processing, and rinsed all glassware with pure DI water. We incorporated wo procedural blanks every digestion session in the lab to quantify background contamination following the same procedures that we used to digest tissue samples (rinsing glassware, adding 10% KOH, filtering through vacuum filter, storing filters in petri dishes, and counting and characterizing particles). The procedural blanks only contained the average amount of KOH solution that was added to the previous ten GIT samples and did not contain any tissues. We attempted to limit the number of people in the lab, but it was a shared space, and we could not always control lab use.

Counting and characterizing microplastics

We examined each glass filter under a microscope (Motic, Model SMZ-171) and scanned left-to-right and up-to-down. We scanned on multiple magnification settings ranging 7.5–50X to count particles of every size. We placed a dot, made with a fine tipped marker, beside every

piece of plastic to avoid counting particles more than once. We characterized each particle type based on a Standard Operating Procedure prepared by the Rochman Lab (https://rochmanlab.files.wordpress.com/2021/01/microplastic-identification-and-characterization-sop-1.pdf). We used a hot needle method to determine if some particles were plastics. First, we would heat the needle with a flame and place it on the edge of a particle. If it melted or bent with heat, we classified it as a MP. If it did not melt or bend, we applied pressure to the particle with the needle. If the particle broke under pressure, we did not classify the particles as a MP. Chitinous insect parts that remained post-digestion were not classified as MPs. They did not melt or bend with heat, and they would often shatter under needle pressure. The hot needle method has been used for nearly a decade (De Witte et al., 2014) and there are now many suggestions to improve the method (Beckingham et al., 2023). If we were not sure about a particle, we were conservative and did not classify it as a MP. We also recorded the color of each particle based on the above referenced document, as color is often characterized in MP research (Carlin et al., 2020; Masiá et al., 2019). We used a Moticam X3 camera and software to take pictures and measure the longest side of a subset of particles per sample.

Microplastics and age, sex, and body condition

We calculated MP concentration by taking the number of particles divided by the mass of the GIT tissue plus the added KOH solution. Microplastic concentration for procedural blanks (i.e., controls) were the number of particles divided by the mass of the KOH solution only. We log transformed MP concentrations from GITs to normalize the data. We used an ANOVA to test if MP concentrations in bat samples were significantly different than control samples. We used ANOVA to also determine if MP concentrations varied by age and sex. We graphed body mass and MP concentrations by collection month to investigate seasonal patterns of MP concentrations

and bat mass. We fit a linear regression model in R version 4.3.2 to determine if MP GIT concentration had a relationship with bat mass, a proxy for estimating fat stores (McGuire et al., 2018). Bat mass was the dependent variable and MP concentration was the independent variable.

Results

Method testing with bat stomach contents

Methods used previously for extracting MPs from GITs of birds of prey (Carlin et al. 2020) and adapted for this study extracted 306 MPs from 85 of the 92 *M. lucifugus* stomach contents. Fibers made up over half of the observed plastics (56%), followed by fragments (35%), films (4%), foams (3%), and fiber bundles (2%). Blue particles made up over half of the observed plastics (56%), followed by clear (16%), and red particles (12%). The remaining 16% included black, brown, gray, green, pink, purple, white, and yellow particles.

Microplastics concentrations and characterizations

We extracted 574 plastic particles from 26 *E. fuscus* GITs collected from 15 Tennessee counties (Fig.1) and 28 plastic particles from 10 control samples. The average MP concentration in GITs was 7.5 *n*/g ± 10.9 SD (Table 1). The majority of microplastics were fibers (*n*=574; 94%). We detected plastics that were small enough to be classified as “nanoplastics” and large enough to be considered “mesoplastics”; however, we refer to all plastics as “microplastics” for the purposes of this paper. The fibers we measured ranged from 0.3–11.1 mm, fragments from 0.1–0.5 mm, and films from 0.9–2.8 mm in length. We also identified 9 fiber bundles, 16 fragments, 7 films, and 2 spheres (Fig. 2). We identified 26 fibers and 2 foams in our 10 control samples. Control samples had an average MP concentration of 1.2 *n*/g ± 0.5 SD (Table 1). All plastics in control samples were clear fibers (64%), blue fibers (18%), purple fibers (11%), or blue foams (7%). The most abundant MPs in GIT samples were clear fibers (52%), blue fibers

(35%), red fibers (8%), purple fibers (2%), blue fragments (1%), clear fiber bundles (1%), and white films (1%). We also identified fibers of other colors and other plastics occurring in smaller numbers.

Microplastics and age, sex, and body condition

Microplastic concentrations were significantly higher in GIT samples than control samples (Table 1; p=0.01). Concentrations were higher in males (*n*=13) than females (*n*=13), but this was not significant in the ANOVA analysis (p=0.08). Higher concentrations in adults (*n*=18) compared with juveniles (*n*=7) was also not significant (Table 2; p=0.96). The linear model determined that MP concentrations in the GIT had a significant negative association with bat body condition, with lower body mass associated with higher GIT MP concentrations (Fig. 3; p=0.007, adjusted r^2=0.23).

Eptesicus fuscus were collected in April (*n*=1), May (*n*=8), June (*n*=4), July (*n*=5), August (*n*=3), September (*n*=1), and November (*n*=4). We did not compare body mass and MP concentrations across months statistically due to limited sample size. Mean bat body mass appeared similar in all months (12.4–14.1g range) except November (17.5g ± 3.7 SD), when bats weighed the most (Fig. 4). Microplastic concentrations were lowest in April (0.7 *n*/g), but the sample size was a single bat. May had the highest MP concentrations (11.7 *n*/g ± 18.3 SD; Fig. 4).

Discussion

Emerging research on MPs has proven that they are ubiquitous environmental contaminants. We show in this study that insectivorous bats are no exception to the terrestrial wildlife species that ingest MPs. To our knowledge, this is the first study to document dietary exposure routes of MPs to bats in North America; however, other studies have similar findings in

25 bat species captured in the Brazilian Amazon (Correia et al. 2023) and it is likely a global issue. The MP concentrations that we detected in bat GITs were comparable to GITs of migratory birds and nestling birds in Wisconsin and Illinois, USA (Hoang & Mitten, 2022). The presence of MPs in GITs suggests that bats are exposed from their insectivorous prey, by contaminated drinking water, or by incidental or intentional ingestion of particles suspended in the air. All are possible explanations as ontogenic transfer of MPs from aquatic-to-terrestrial systems through metamorphosis has been documented in arthropods (Al-Jaibachi et al., 2019; Grgić et al., 2023; Yıldız et al., 2022). Considering that bats are known to consume a variety of arthropods (Deeley et al., 2023; Maslo et al., 2022), exposure via prey items is possible. Moreover, free-ranging MPs in freshwater systems and the air are abundant and widespread, thus available for uptake (O'Brien et al., 2023; Thacharodi et al., 2024). Bats visit water sources for foraging and drinking purposes (Kalcounis-Rueppell et al., 2007; Rydell et al., 2022), thus, drinking water could be a route of exposure. Microplastic pollution in the air might also have substantial influence on bat exposure, as MPs are abundant in the atmosphere (O'Brien et al., 2023) and bats use the aerosphere to forage. Future research could explore MP concentrations in arthropods that are known bat prey sources, surface water and air from foraging and drinking areas, and concentrations in bat organs and bat guano to understand accumulation patterns. Studies that are able to collect fresh bat carcasses that have not yet been frozen could assess histopathology of gastrointestinal tracts to determine if bats experience plasticosis (Charlton-Howard et al., 2023). Additionally, studies on the potential role that plasticosis may have in nutrient absorption and fat storage in bats would be highly valuable. If plastics damage cells in GITs, it might make it more difficult for bats to absorb nutrients. For example, celiac disease in

humans can compromise the structural integrity of the intestinal lining and inhibit nutrient and vitamin absorption (García-Manzanares & Lucendo, 2011).

While MPs are abundant in the environment, there may be seasonal patterns of bat exposure. For example, bats may not be exposed during hibernation when they limit foraging activities. We were unable to test any seasonal patterns of MP GIT exposure with the limited data; however, we did anecdotally notice that lower MP concentrations may occur during the time immediately post-hibernation and the largest concentrations may occur post-migration and during the early pregnancy period. It is also possible that weather events could alter exposure, as flooding can increase MPs up to 14 times (Gündoğdu et al., 2018). This warrants additional research into seasonal patterns of exposure and influence of severe weather events, as bats may be more vulnerable to contaminants and other stressors during periods of high energy expenditure and prey consumption.

It is also possible that not all sympatric insectivorous bat species are exposed to MP equally. It is likely that species traits and geographic factors such as foraging behaviors (i.e., foraging mostly over water sources versus terrestrial areas) and proximity to contaminated sites and urban areas would also influence MP exposure. *Eptesicus fuscus* is an urban-adapted bat species, and we received carcasses from programs where the initial source was a direct human-wildlife interaction. Therefore, the bats in this dataset may be more exposed to anthropogenic disturbance and urban development, and, thus, may have higher MP concentrations than bats foraging in less-developed areas.

Our most interesting finding was that higher GIT MP concentrations were associated with bats having lower mass, a proxy for fat reserves (McGuire et al., 2018). However, we caution that we had a limited sample, and there are likely other factors that influence bat mass.

Regardless, our findings indicate that this topic should be explored further. Fat storage is a crucial resource for species that migrate and hibernate. Fat storage is even related to disease survival probability, as bats that hibernate and are infected with the non-native pathogen that causes white-nose syndrome are more likely to survive the winter if they start off with higher fat reserves (Cheng et al., 2019; Perry & Jordan, 2020). Our finding warrants future research exploring this possible relationship between higher MP GIT concentrations and bat mass, on how MPs might block the GIT, inhibit metabolic processes, the possibility that MP consumption fills the bat with non-nutritional volume and mass that cannot add to body mass and fat stores, or other sublethal impacts related to fat storage and breakdown processes. MPs are persistent in the environment, lasting hundreds of years, and are continuously released in ecosystems. Research on remediation and restoration of aquatic systems and how this might benefit bats and other wildlife would be valuable to future conservation efforts to help recovery of species impacted by emerging environmental contaminants.

Conclusions

This study provided evidence that insectivorous bats are exposed to microplastics. Moreover, concentrations in the GIT may influence bat health condition. For example, MP GIT concentrations had a significant influence on bat mass. Future research could investigate the role of MPs in arthropods, drinking water, and particles suspended in the air column in bat ingestion and uptake. Understanding how pollution and environmental contaminants affect bats is critical for monitoring bat populations globally

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401

Table 1(on next page)

Summary table of microplastics (MPs) found in the gastrointestinal tracts (GITs) of big brown bats (*Eptesicus fuscus*).

Samples were collected from 15 counties in Tennessee, United States of America in years 2019 and 2020 , and procedural blanks (i.e., control samples). Concentrations are reported as number of MPs divided by the mass of the tissue sample (g) plus KOH solution (g) for the GITs and number of MPs divided by the KOH solution (g) for procedural blanks.

1

Sample type	MP count (mean ± SD)	Total count (range)	MP concentration (mean ± SD)	Total MP concentration (range)
GITs (*n*=26)	22.1 ± 25.6	574 (1–112)	7.5 ±10.9	194.3 (0.3–54.6)
Controls (*n*=10)	2.8 ± 1.7	28 (1–6)	1.2 ± 0.5	11.6 (0.4–2.0)

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3
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Table 2 (on next page)

Mean and standard deviation values for microplastic (MP) concentrations (number of MPs divided by sample and KOH mass (g)) in big brown bat (*Eptesicus fuscus*) gastrointestinal tracts.

Samples were collected from 15 counties in Tennessee, United States in years 2019 and 2020. The number in parentheses indicates sample size.

1

Sex	Juvenile	Adult	Unknown	All
Male	3.5 ± 0.8 (3)	13.1 ± 16.0 (10)		10.9 ± 14.5 (13)
Female	6.7 ± 5.7 (4)	2.9 ± 2.6 (8)	2.6 (1)	4.0 ± 3.9 (13)
All	5.3 ± 4.4 (7)	8.6 ± 12.8 (18)	2.6 (1)	

2

3

4

Figure 1

Map of 15 Tennessee (TN) counties and the average number of microplastics extracted from gastrointestinal tracts (GITs) from bats collected in 2019 and 2020.

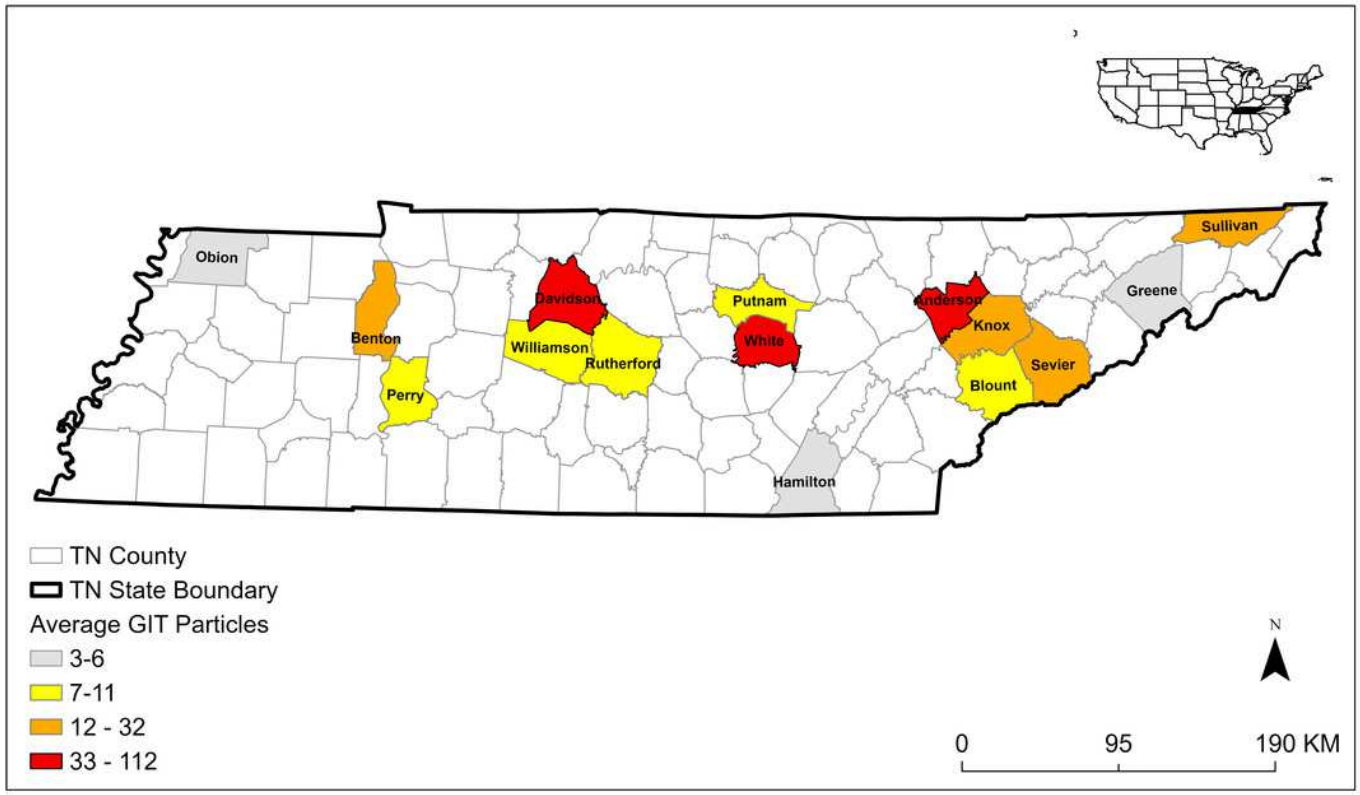


Figure 2

Microscopic images of plastic particles extracted from gastrointestinal tracts of big brown bats (*Eptesicus fuscus*) collected in 2019 and 2020 in Tennessee, USA.

Figure 2A: sphere, Figure 2B: fragment, Figure 2C: fiber bundle.

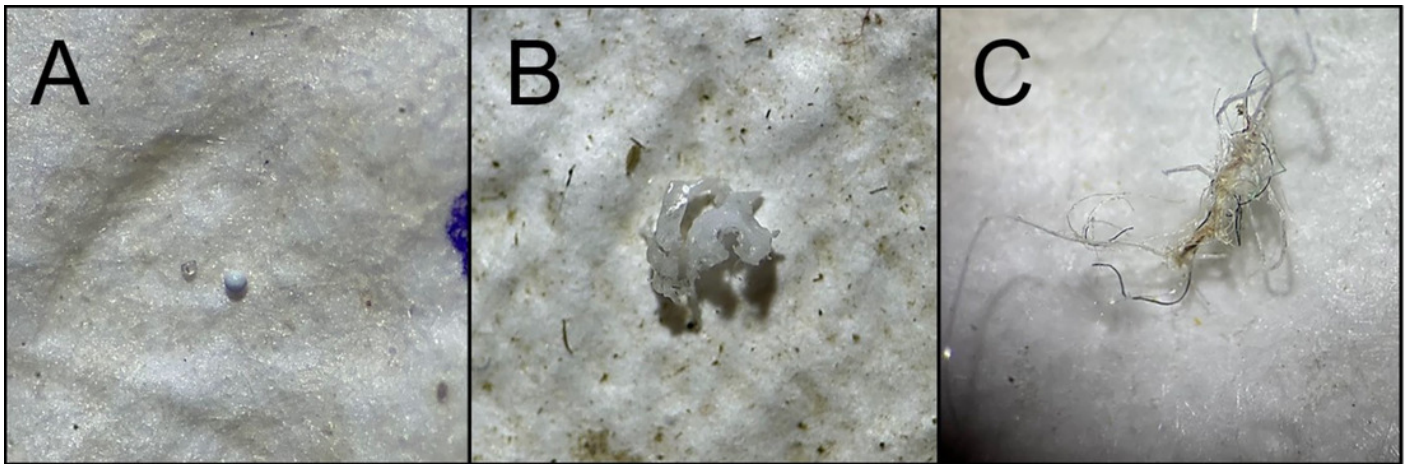


Figure 3

Plot showing the relationship between microplastic concentrations in the gastrointestinal tracts of big brown bats (*Eptesicus fuscus*).

Samples were collected from 15 counties in Tennessee, USA in years 2019 and 2020. The line shows the fit linear model (Body mass=15.77-1.37(MP concentration); $F_{1,24}$=8.72, p=0.007, adjusted r 2 =0.23) and the gray shading is the 95% confidence interval. There is a significant relationship between microplastic concentrations and bat mass.

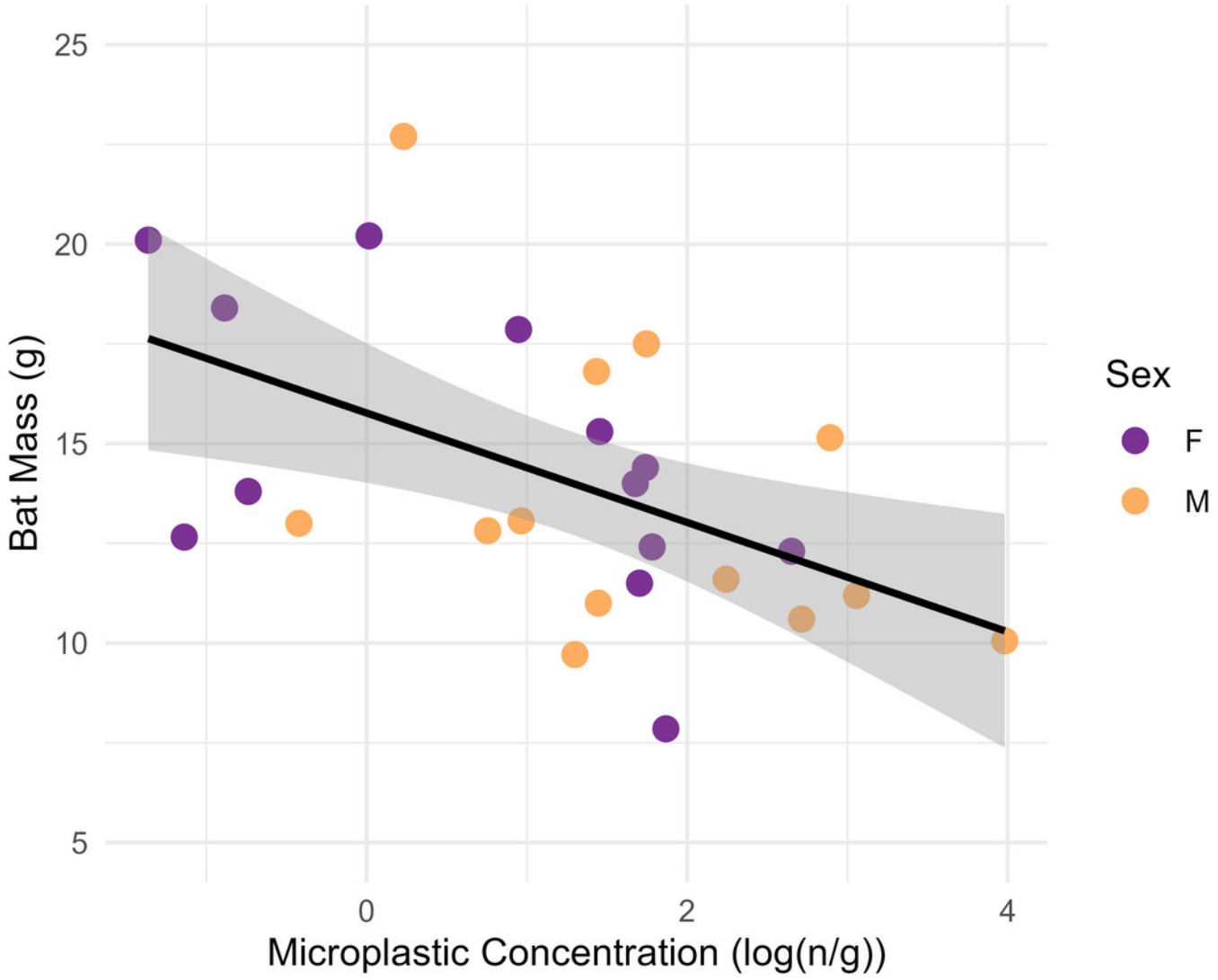
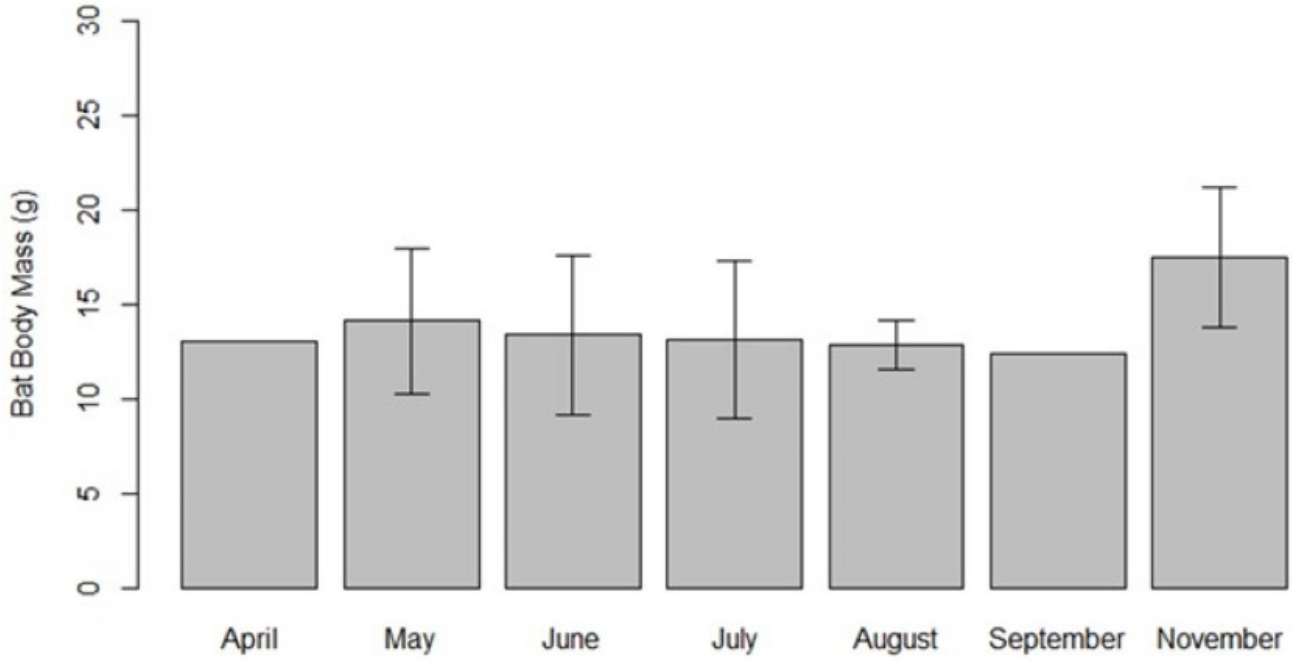
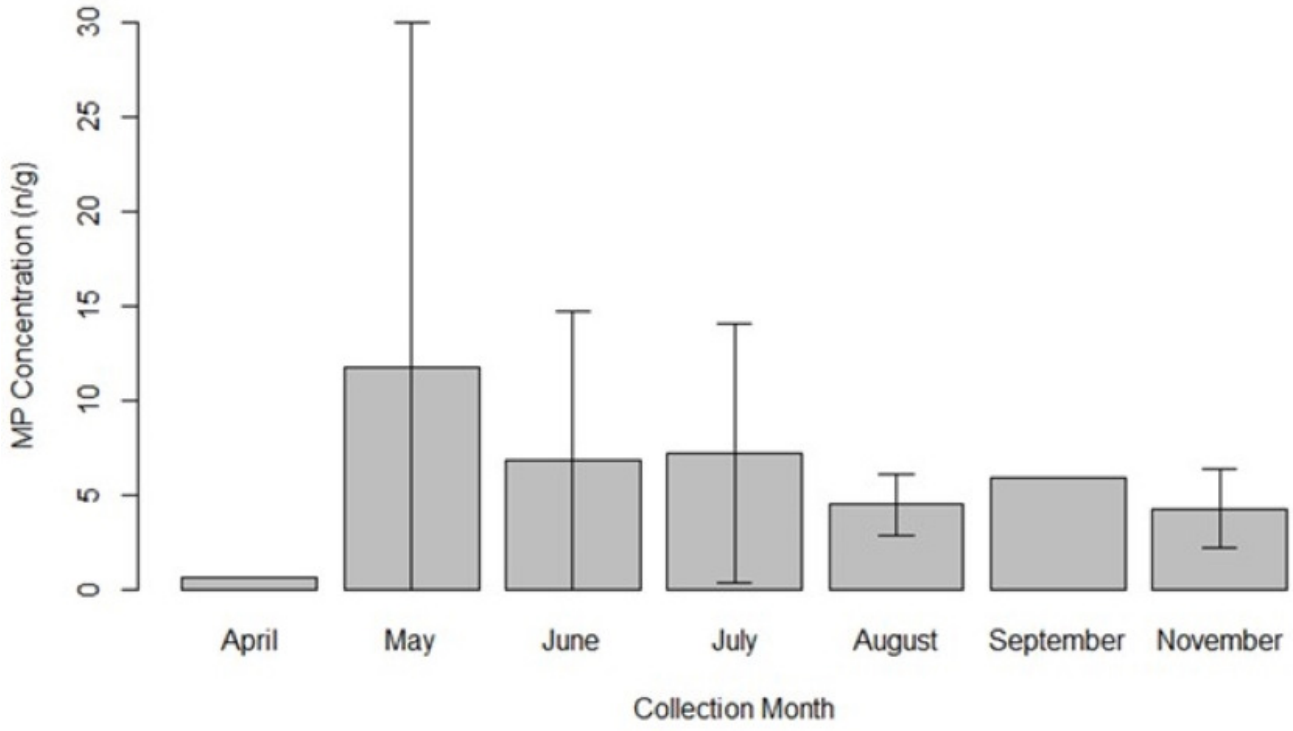


Figure 4

Bar graph showing the average bat body mass (grams) and microplastic concentrations (number of particles per gram of tissue) in gastrointestinal tracts of 26 big brown bats (*Eptesicus fuscus*).

Samples were collected from 15 counties in Tennessee, USA in years 2019 and 2020. The error bars indicate the standard deviation when there was more than one sample.


Bat Body Mass (g)
0
5
10
15
20
25
30
April
May
June
July
August
September
November


MP Concentration (n/g)
0
5
10
15
20
25
30
April
May
June
July
August
September
November
Collection Month