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Incidence of microplastic fiber ingestion by Common Terns (*Sterna hirundo*) and Roseate Terns (*S. dougallii*) breeding in the Northwestern Atlantic

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ABSTRACT

Ingestion of microplastics has been documented across marine species, but exposure remains sparsely described in many seabird species. We assess microplastic (between 0.2 and 5.0 mm) ingestion in two Northwestern Atantic - breeding species for which exposure to microplastics is entirely or largely undescribed: Common Terns (*Sterna hirundo*) and Roseate Terns (*S. dougallii*). Common Tern microplastic load did not vary between life stages ($p = 0.590$); microplastic load did differ in Common Tern adults breeding at two of three colonies explored ($p = 0.002$), with no other regional differences observed. Roseate Terns ingested significantly more microplastics than Common Terns ($p = 0.007$). Our results show that microplastic ingestion by terns varies regionally and inter-specifically, but not by life stage, trends potentially explained by dietary differences. We provide the first quantification of microplastic fiber ingestion by terns in the Northwestern Atlantic and identify trophic dynamics related to microplastic ingestion, representing an important step toward understanding the risk of the pollutant to terns across regions, as well as toward the use of terns as potential bioindicators of microplastics.

1. Introduction

Microplastic pollution is widespread in marine ecosystems, and ingestion of this pollutant suite has been documented in most organisms investigated (Jambeck et al., 2015; GESAMP, 2015; Provencher et al., 2017; Secretariat of the Convention on Biological Diversity et al., 2012), even as far back as the 1970s (Connors et al., 2017). The ingestion of microplastics, which are defined as plastic particles between 1.0 μm and 5.0 mm in size (Frias and Nash, 2019), has been shown to cause various detrimental impacts to marine organisms including tissue damage, impaired physical development or growth, and the possible leaching of toxic chemicals into body tissues (Anbumani & Kakkar, 2018; Carbery et al., 2018; Gall and Thompson, 2015; Rochman et al., 2013; Tanaka et al., 2020). Thus, with estimates of 2 million tons of microplastic fibers alone entering global marine systems annually, it is crucial to gain an understanding of the prevalence of the pollutant across marine organisms (Mishra et al., 2019). While the need to understand microplastic pollution has been recognized for decades (Qin et al., 2020), methods for the isolation and identification of microplastics have only recently become sufficiently reliable, leaving many systems sparsely described or entirely undescribed. Establishing microplastic levels present across systems is critical to subsequently address more complex questions relating to microplastic trophic transport, ingestion risk, and management options (Granek et al., 2020).

Seabirds are one of the most threatened groups of birds due to a range of anthropogenic threats (Dias et al., 2019), and there is growing concern for their conservation as we continue to gain understanding of microplastic pollution in marine environments (Wilcox et al., 2015). Additionally, seabirds have been explored as useful bioindicators of marine macroplastics (Acampora et al., 2016; Avery-Gomm et al., 2018), and therefore likely have potential to be used similarly for monitoring microplastics both in the environment and in their prey items (Avery-Gomm et al., 2018), many of which are ecologically and economically important. Although macroplastic ingestion has been studied in a number of seabirds (Provencher et al., 2014), many species and systems remain under-described or entirely undescribed when it

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comes to microplastic ingestion.

Terns are small, migratory seabirds in the larid family that represent one group for which microplastic exposure is under-described despite conservation concerns (Arnold et al., 2020; Gochfeld and Burger, 2020). We are aware of few studies globally that address plastic ingestion by terns, and fewer that specifically investigate microplastic ingestion by terns in the Northwestern Atlantic (Braune and Gaskin, 1982; Hays and Cormons, 1974; Moser and Lee, 1992; Rapp et al., 2017), where a significant number of populations breed each year (Arnold et al., 2020; Gochfeld and Burger, 2020). Previous studies in the region have found small numbers of plastic particles (of unreported size) in the stomachs of terns (Braune and Gaskin, 1982; Moser and Lee, 1992) and others have found microplastic particles (as small as 1.0 mm) in tern pellets (regurgitated indigestible materials; Hays and Cormons, 1974). Other research has found no incidence of plastic ingestion by select species of tern in the region (Rapp et al., 2017). Because the tern diet consists of fishes from taxa and age classes known to ingest smaller plastics (Hipfner et al., 2018; Lenz et al., 2016; Peters, 2018), low incidences of macroplastics found in the few previous studies conducted in terns are unsurprising, and we expect that terns would instead be more likely impacted by plastics in the currently under-explored micro size range, including one of the most ubiquitous microplastic morphologies, microfibers (Athey and Erdle, 2021). Therefore, examining the incidence of ingestion of these smaller plastics by terns is important for understanding the risk of microplastic pollution to tern species. It is also an important starting point for assessing the trophic dynamics of ingestion and the value of terns as indicators for this pollutant suite, which includes numerous polymers, blends of materials, as well as differing morphologies and sizes (Rochman et al., 2019).

Our study aims to fill these gaps in knowledge by investigating microplastic ingestion in two species of tern breeding in the Northwestern Atlantic: the Common Tern (*Sterna hirundo*), which is state threatened in the study region, and the Roseate Tern *(S. dougallii*), which is federally endangered (Arnold et al., 2020; Gochfeld and Burger, 2020). Specifically, we aim to 1) describe and quantify microplastic ingestion levels, 2) identify any differences in microplastic ingestion between adults and pre-fledge chicks, 3) identify any geographical patterns in microplastic ingestion by New Hampshire (NH)-, Massachusetts (MA)-, and New Jersey (NH)-breeding Common Terns, and 4) identify any interspecific variation in microplastic ingestion. In addressing these questions, our study aims to establish current rates of microplastic ingestion experienced by terns while also gaining a preliminary understanding of the mechanisms of plastic ingestion across life stages, colonies, and species, a first step toward assessing the potential use of terns as indicators for the pollutant.

Previous research indicates that microplastics are present in most marine systems (Mishra et al., 2019; Provencher et al., 2017), therefore, both of the species and life stages investigated in this study are expected to experience some degree of microplastic ingestion. We expect that small, fish-specialized seabirds like terns are most likely to ingest plastics via secondary ingestion (i.e. through their prey items as opposed to via primary ingestion directly from the environment), therefore, because adults of both tern species investigated feed their chicks a similar diversity of fishes to that which they consume themselves (Nisbet, 1973), we expect the two life stages (aim 2) to exhibit similar microplastic loads. Adult and young terns may, however, ingest fishes of different sizes due to their different gape limitations. Therefore, we expect adults to ingest a higher proportion of microplastics on the large end of the size distibution as compared to chicks, as microplastic size tends to correlate with body size across species (Jâms et al., 2020). When addressing microplastic ingestion across the three colonies (aim 3), we expect to see some differences in the abundance of microplastics ingested due to likely underlying regional differences in microplastic pollution in the environment and the prey base (Auta et al., 2017; Cohen et al., 2019; Welden and Lusher, 2017). Because adult terns across NJ, MA, and NH are expected to ingest fishes of similar size, and again assuming body size correlates with the size of plastics ingested in fishes (Jâms et al., 2020), we do not expect to see any differences in the size of microplastics ingested across the three colonies. Lastly (aim 4), because Common and Roseate terns vary in the major prey species they rely on (Arnold et al., 2020; Gochfeld and Burger, 2020), and assuming that those major prey species might vary in their microplastic ingestion, the two are expected to vary in their microplastic load. Because the two species ingest prey items of similar size, we do not expect them to differ in the sizes of microplastics they consume (Jâms et al., 2020).

2. Methods

2.1. Study location and sample collection

Fecal samples were collected at three sites: White and Seavey Island, NH (42.9685, −70.6255), Monomoy National Wildlife Refuge, MA (41.5419, −70.0069), and Barnegat Bay Islands, NJ (39.6365, −74.2099; Fig. 1). Adult Common Tern fecal samples were collected at all three sites; samples from Common Tern chicks were collected in NH and NJ only. Samples from Roseate Tern chicks were only collected at the NH site, as the species does not nest in large numbers at the other sites.

Samples were collected in the summer (May–August) of 2019 during established colony monitoring and management activities performed annually throughout the breeding season. Samples were collected from Common Tern adults when they defecated as a defense response to human presence in their colony, and from pre-fledged (<15 days old) Common and Roseate tern chicks when they defecated in response to handling for regular banding and monitoring procedures. Fecal samples were not collected from adult Roseate Terns, as the species does not regularly defecate as a defense response. For each sample collected, the entire fecal deposit was scraped into a glass scintillation vial using a wooden popsicle stick and was stored at −20 °C until laboratory processing.

To reduce microplastic contamination during field sampling, fecal samples were only collected from non-synthetic substrates including skin, cotton, rock, vegetation, and metal. Samples were stored in sterile glass scintillation vials that were resealed immediately after sample deposition to reduce airborne contamination (as per Brander et al., 2020). Field control blanks were collected alongside fecal sample collection on White and Seavey Island in NH for analysis using the same

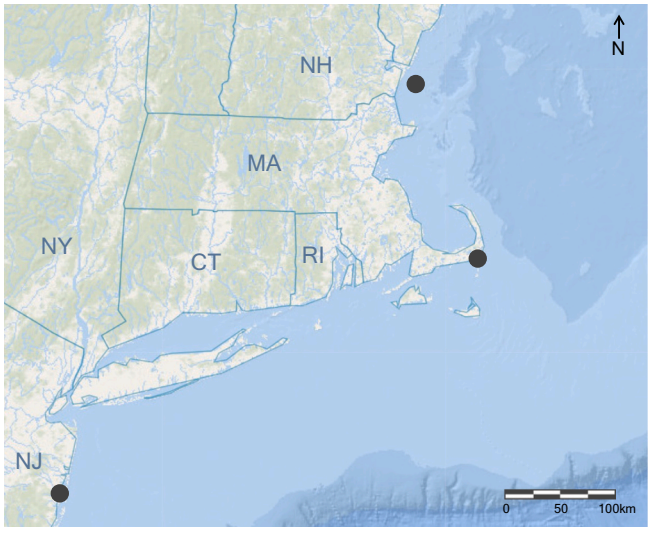

Fig. 1. Map of the Northeastern U.S. coast showing the location of the tern colonies from which we collected fecal samples in summer 2019. Colonies are located at White and Seavey Island, NH, Monomoy National Wildlife Refuge, MA, and Barnegat Bay, NJ.

procedures applied to the fecal samples. The field controls consisted of a fecal sample substitute (canned pumpkin puree) which was dropped on the various collection substrates and then sampled as we would feces. Blanks of the fecal substitute alone were also collected and analyzed so as not to confound environmental microplastics with those present in the substitute itself.

2.2. Microplastic isolation and identification

Fecal samples were dried for ~24 h at 65 °C to allow for measurement of their dry weight. After drying and weighing, the samples were transferred into reaction beakers, during which step the wooden sticks used to collect the samples were boiled for 20 min in 40 mL of DI water to release any fecal matter caught in the wood. After the boil step, the samples were dried for a second time (for ~24 h at 65 °C) before undergoing chemical digestion to remove biogenic materials. The samples were digested using a wet peroxide oxidation reaction (solution containing equal parts of 0.05 M Fe(II) solution and 30% H_2O_2). The resulting solution was diluted with 360 mL of DI water and vacuum filtered through a 2.0 μm paper filter (as per Hidalgo-Ruz et al., 2012). Each filter was visually inspected for potential microplastics under a dissecting scope. Particles suspected to be microplastic based on lack of cellular structure, presence of uniform color and morphology, and low degree of brittleness (as per Hidalgo-Ruz et al., 2012) were enumerated. In half of the fecal samples analyzed, the suspected microplastic particles were also removed from the filter, characterized by shape and color, and stored between glass cover slides for further analysis.

To control for contamination during microplastic isolation in the lab, cotton clothing was worn during processing, and all work surfaces were cleaned before use with a lint roller. Any DI water used during sample processing was filtered twice through an 11.0 μm filter, and the Fe(II) solution used in the chemical digestion was filtered twice through a 25.0 μm filter before use to reduce water- and solution-borne microplastic contamination. All glassware and equipment used during sample processing was rinsed three times with filtered DI water before use, and filters (11.0 μm) and foil was placed over the top of beakers during drying and digestion to allow for evaporation while maintaining control of air-borne contamination (Brander et al., 2020). To further control for air-borne contamination, we conducted the chemical digestion and sample filtration under a fume hood and conducted all microscope work under a plastic sheet. Procedural control blanks, which underwent the same methods as the fecal samples and field controls (i.e., drying, chemical digestion, filtration, and microscope inspection), were processed alongside a subset of the fecal samples (Brander et al., 2020).

For each fecal sample from which suspected microplastics were collected, a subset of 25–30% of the total isolated fibers was randomly selected for material type identification and further characterization. Fragments only represented 14.6% of the particles isolated from the fecal samples and were therefore excluded from such analyses due to small samples sizes; thus, all further analyses described refer to fibers alone. Each fiber underwent identification in the Brander Lab at Oregon State University using a Thermo Nicolet is 30 Fourier-transformed infrared spectrometer (FTIR) and an iN5 microscope fitted with an Attenuated Total Reflectance (ATR) accessory and germanium tip. A gold-plated slide containing one fiber in a drop of 70% filtered ethanol, to avoid fiber loss during transport, was placed on the μFTIR for each reading. Ethanol was allowed to evaporate once the slide was placed under the μFTIR microscope. The reflectance of the fiber was measured using a fixed aperture (128 to 512 scans) on the cleanest and largest section of the fiber. A geranium tip probe was inserted into the machine and lowered to make contact into ~1-2 μm of the material surface of the sample for additional spectral analysis. The spectral outputs from both methods (overall reflectance and attenuated reflectance) were compared to FTIR spectral libraries (Cowger et al., 2021) with a minimum threshold of 70% used to determine the material identity of each particle (per recommendations from Cowger et al., 2020). Using Open Specy (Cowger et al., 2021), spectra were baseline corrected and smoothed, and matched to microplastic-specific spectral libraries. Based on the FTIR results, each fiber was characterized into one of four categories: synthetic, semi-synthetic, dyed natural (natural fibers, typically cellulose, with a manufactured dye applied), and natural (per approaches described in Baechler et al., 2020 and Harris et al., 2021). Particles characterized as synthetic, semi-synthetic, and dyed natural were considered anthropogenically derived, and were examined in ImageJ for physical characterization including color and length.

2.3. Data analysis

We measured the relative abundance of microplastics (the number of microplastics per gram of feces; hereafter referred to as microplastic load) for each fecal sample collected across our species, state, and life stage groups. To do so, the relative proportion of each particle category (synthetic, semi-synthetic, dyed natural, and natural) in each fecal sample was estimated based on the FTIR results from the subset of fibers isolated from each sample. The microplastic load for each particle category was then calculated for each fecal sample by multiplying the total microplastic load observed in that sample by the proportion of particles of each category from that sample group. Means comparisons were used to compare microplastic load between species, between life stages, and across states. Because our data were not normally distributed, even after transformation efforts (Shapiro-Wilk test $p < 0.001$ regardless of log or square root transformation), we used non-parametric tests including the Kruskal-Wallis one-way ANOVA (KW) and the Mann-Whitney Wilcoxon rank sum test (MWW). Like the microplastic load data, the fiber length data were also not normally distributed (Shapiro-Wilk test $p < 0.001$ for all transformations attempted), thus we used the same non-parametric means tests to compare particle lengths between groups. All statistical tests were performed in R (R Core Team 2021 Version 4.1.1).

3. Results

3.1. Particle characteristics and identification

From the 120 fecal samples and 25 controls analyzed, we isolated 571 suspected microplastic fibers. 87.5% of the fecal samples contained microplastic loads that were higher than the average load found in blanks. From a randomly selected subset of 237 suspected microfibers chosen for FTIR analysis, 170 produced usable spectra (spectral results detailed in Table A2 and Fig. A1). These fibers accounted for 29.8% of the total microfiber samples collected, and of them, 123 were collected from fecal samples and 47 from controls. Of the 123 suspected microplastic fibers with usable spectra isolated from fecal samples, 17.9% were synthetic, 0.8% were semi-synthetic, 45.5% were dyed natural, and 35.8% were natural (i.e. 64.2% were anthropogenically-derived). Many of the anthropogenically-derived fibers isolated from fecal samples were blue (42.5%), with other well-represented colors including transparent (11.3%), gray (10.4%), pink (10.3%), black (6.6%), and multicolored (i.e. characterized by >1 color; 4.7%). The fibers isolated from our controls showed similar trends, with a makeup of 19.1% synthetic, 2.1% semi-synthetic, 59.6% dyed natural, and 19.1% natural (80.8% anthropogenically-derived). Many of the anthropogenically-derived particles isolated from the controls were also blue (47.3%), with other well-represented colors including gray (11.8%), pink (11.8%), transparent (7.5%), black (4.3%), and multicolor (4.3%).

3.2. Microplastic prevalence and size

Microplastic fiber load was measured and reported as the number of synthetic fibers per gram of feces, which was calculated by multiplying the total fiber count observed per gram of feces by the proportion of FTIR-analyzed samples identified as synthetic for the sample group. To

add context to subsequent values, we note that fecal samples had an average mass of 0.03 g. Though the results reported here refer to estimates for synthetic fiber load only, we note that the results for the other anthropogenically derived fibers (semi-synthetics and dyed naturals) follow the same trends as those reported for synthetic fibers (Table A1 and Figs. 2-5). Unlike our reporting for microplastic fiber load, our size results are detailed for all anthropogenically derived fibers (synthetic, semi-synthetic, and dyed natural), as the sample size of synthetic fibers alone was too small to make robust comparisons between groups.

Our controls, which account for both field and laboratory contamination, contained significantly fewer microplastics than fecal samples (MWW W = 436.0, $p < 0.001$ two-tailed). Common Tern adults and chicks (on the colony in NH) had similar microplastic loads (MWW W = 382.0, $p = 0.590$ two-tailed; Table A1 and Fig. 2), although adults ingested significantly larger fibers than chicks (MWW, W = 82.0, $p = 0.015$ two-tailed; Fig. 3), with adults ingesting fibers with a mean length of 2.3 ± 1.2 mm and chicks those with a mean length of 1.1 ± 0.7 mm. Results from the assessment of microplastic fiber load in Common Tern adults across states (Table A1 and Fig. 4) showed that birds breeding in NH had a significantly lower microplastic burden than those breeding in MA (KW $p = 0.010$, $\chi^2 = 9.1$, df = 2; MWW W = 377.0, $p = 0.002$ two-tailed), but that their microplastic fiber load did not differ from that of birds breeding in NJ (KW $p = 010$, $\chi^2 = 9.1$, df = 2; MWW W = 187.0, $p = 0.109$ two-tailed). The microplastic fiber load found in NJ birds also did not differ from that of birds in MA (KW p = 0.010, $\chi^2 = 9.1$, df = 2; MWW W = 237.0, $p = 0.194$ two-tailed). The size of fibers ingested by Common Terns in the three states did not differ from one another (KW $\chi^2 = 2.5$, $p = 0.281$, df = 2). In NH, Roseate Tern chicks had a significantly higher microplastic load than Common Tern chicks (MWW W = 155.0, $p = 0.007$ two-tailed; Fig. 5), with Roseate Terns ingesting 2.8 times the load of Common Terns (Table A1). The length of anthropogenic fibers ingested did not differ between the two species (MWW W = 50.5, $p = 0.531$ two-tailed).

4. Discussion

Our study provides the first recorded observation of microplastic fiber ingestion by Common Terns in the Northwestern Atlantic, and by Roseate Terns globally, both of which are ecologically important species that play key predatory roles in marine ecosystems. Specifically, we found that terns breeding on colonies in the Northwestern Atlantic dispel means of approximately 50 to 250 microplastic fibers and 115 to 550 other anthropogenically derived microfibers (semi-synthetic and dyed natural) per gram of feces. Though the numbers reported are elevated slightly past their actual value due to contamination, we believe this estimate represents a minimum microfiber load ingested by these terns, as additional microplastics fibers may lodge in gut tissue or be expelled through regurgitation (Lavers et al., 2019). For example, a preliminary analysis of NH-breeding Common Tern pellets from 2019 showed microplastic loads of similar magnitudes to those reported here for fecal samples (McDowell et al., 2021). Furthermore, our methods likely missed smaller microplastic fibers (<200 μm in length) due to biases in the visual identification and physical removal of microfibers from the filters. For these reasons, the actual microplastic load experienced by the terns is almost certainly higher than reported here, though the exact magnitude of the potential difference remains unknown.

Microplastic fiber load did not differ between Common Tern adults and chicks. This result provides support for our assumption that secondary ingestion is the leading microplastic ingestion mechanism in terns. If terns instead ingested microplastics via primary ingestion (i.e., directly out of the water column or terrestrial environment), we would have expected to see varying microplastic loads between life stages; specifically, we would expect pre-fledge chicks to ingest fewer microplastics than adults, as chicks only interact with a small range of the terrestrial environment while adults interact with a larger range of both the terrestrial and aquatic environments. While we are aware of few papers that investigate age-driven differences in microplastic ingestion specifically, the results from studies comparing plastic (mainly macroplastic) ingestion in chick and adult birds are quite variable, with adults of some species ingesting more plastic than chicks (Avery-Gomm et al., 2013; Spear et al., 1995), while some species have higher ingestion in chicks (Acampora et al., 2014; Van Franeker and Meijboom, 2002), and others, like ours, show little difference in plastic load between the life stages (Acampora et al., 2016; Spear et al., 1995). While the two life stages in our study ingested similar microplastic loads, the anthropogenically-derived fibers ingested by chicks were significantly smaller in length than those ingested by adults. If we assume secondary ingestion of fibers and if microplastic size varies with body size (Jâms et al., 2020), the observation that chicks ingest smaller fibers may potentially be a result of their diet containing fewer fish on the large end of the prey size spectrum as compared with adults, as chicks are limited on the upper size threshold by gape size. However, this is speculative, and more data are needed for confirmation.

Of the three sites explored in this study, the birds in MA exhibited the highest microplastic loads, followed by those in NJ and NH, with no differences observed in the sizes of fibers ingested. Only the sites with

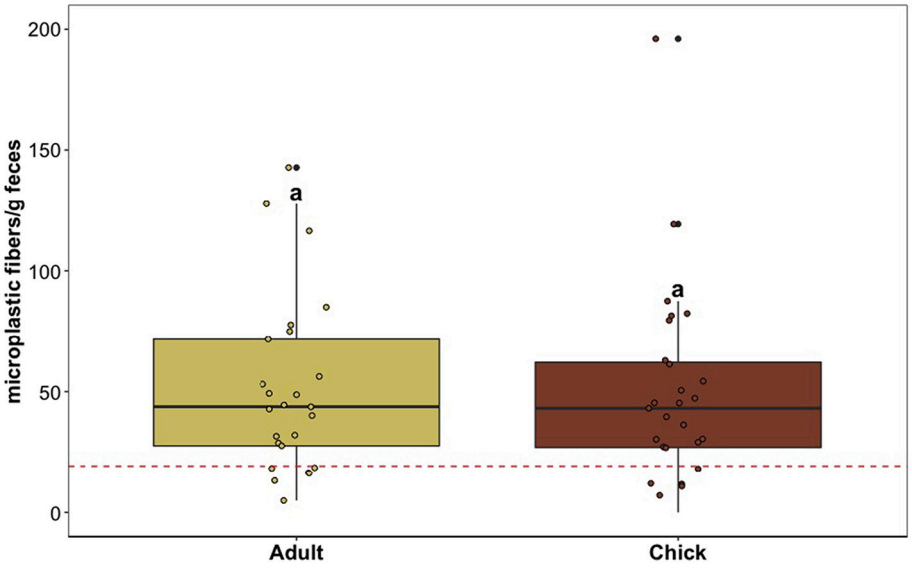

Fig. 2. Boxplot showing the estimated number of synthetic microfibers per gram of feces collected from adult (3+ years old; $n = 26$) and chick (<15 days old; $n = 27$) life stages of Common Terns (*Sterna hirundo*) from White and Seavey Island, NH in 2019. Mann-Whitney Wilcoxon tests revealed no significant difference between the two life stages ($p = 0.590$) as indicated by "a." Horizontal dashed line indicates estimated number of microfibers per gram of feces introduced through field and lab contamination.

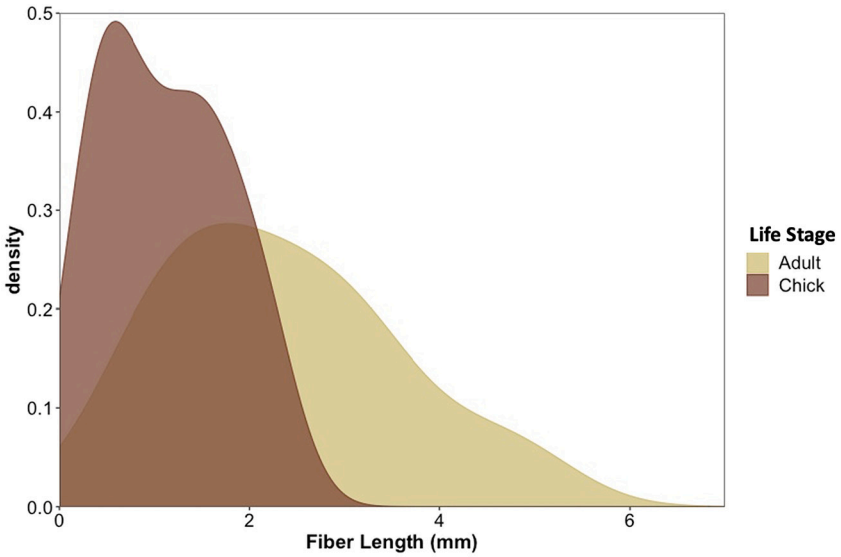

Fig. 3. Distribution of lengths of anthropogenically derived microfibers collected from Common Tern (*Sterna hirundo*) adults (3+ years old) and chicks (<15 days old) on White and Seavey Island, NH in 2019. Mann-Whitney Wilcoxon tests revealed a significant difference between the two life stages ($p = 0.013$).

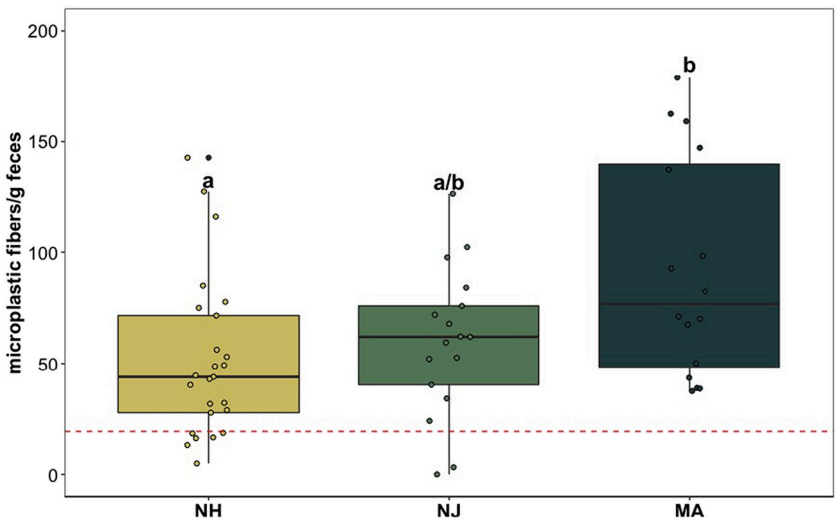

Fig. 4. Boxplot showing the estimated number of synthetic microfibers per gram of feces collected from Common Tern (*Sterna hirundo*) adults breeding in NH, NJ, and MA in 2019. A Kruskal-Wallis test indicated significant differences across the groups ($p = 0.010$). Locations with different letters represent significant differences according to post-hoc Mann-Whitney Wilcoxon tests. Horizontal dashed line indicates estimated number of microfibers per gram of feces introduced through field and lab contamination.

the lowest (NH) and highest (MA) microplastic loads differed significantly from one another. There are many possible explanations for why birds at different colonies might experience variation in microplastic load, including system-specific oceanographic dynamics (Auta et al., 2017; Cohen et al., 2019; Welden and Lusher, 2017) and anthropogenic uses (Quesadas-Rojas et al., 2021) leading to geographically differing microplastic levels in the environment and in prey items ingested by the seabirds. The results might also be explained by dietary differences, as terns across colonies can vary greatly in diet. For example, Common Terns at the colony in MA feed primarily on sand lances (*Ammodytes* spp.; Staudinger et al., 2020), unlike other colonies in the region that tend to have more variable diets (Arnold et al., 2020). While there are no data detailing microplastic ingestion for tern prey items in the Northwestern Atlantic nor those within the appropriate age/size class (juvenile fishes ~1-6 cm in length; Black, 2006), studies conducted elsewhere found higher prevalence of microplastic fiber ingestion in bottom-dwelling sand lance (Pacific sand lance, *A. personatus*) than in pelagic herring (Pacific herring, *Clupea pallasii*), the Atlantic relative (Atlantic herring, *C. harengus)* of which is a common prey item for terns in NJ and NH (Bertram et al., 2016; Hipfner et al., 2018). Therefore, it is possible that similar trends could occur within the Northwestern Atlantic prey base, leading to elevated microplastic load in tern populations that rely heavily on sand lances. Regardless of the mechanism behind the geographical differences in microfiber load observed, the fact that members of the same species display those differences in their feces is promising for the use of Common Terns as indicators of microplastic pollution (in their prey items and possibly by proxy in their environments).

Common and Roseate terns differed significantly in their microplastic fiber load when sampled at a single colony in NH, which we anticipated based on dietary differences between the two species. Differences in plastic ingestion between closely related species living and breeding in mixed-species colonies is not uncommon (Caldwell et al., 2020). However, our results, which showed that Roseate Terns ingested more microfibers, were of particular interest because Roseate Terns in NH primarily consume sand lances (*Ammodytes* spp.) while Common Terns forage more often on herrings (*C. harengus* and *Alosa* spp.) and hakes (*Merliccius* sp. and *Urophycis* sp.). The increased ingestion of microfibers by Roseate Terns may therefore, as speculatedc for MA Common Terns, be the result of higher levels of microplastic ingestion in

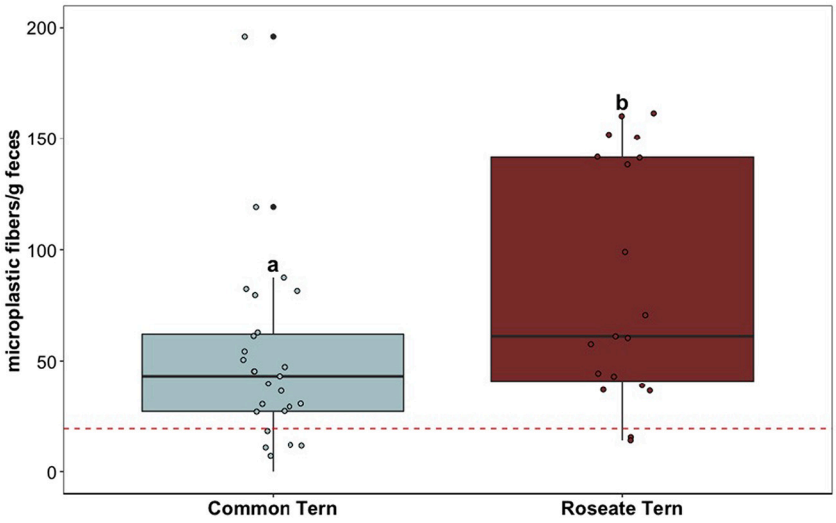

Fig. 5. Boxplot showing the estimated number of synthetic microfibers per gram of feces collected from Common Tern (*Sterna hirundo*) and Roseate Tern (*S. dougallii*) chicks (<15 days old) on White and Seavey Island, NH in 2019. Different letters represent significant differences according to a Mann-Whitney Wilcoxon test ($p = 0.007$). Horizontal dashed line indicates estimated number of microfibers per gram of feces introduced through field and lab contamination.

the sand lances they rely on (Arnold et al., 2020; Gochfeld and Burger, 2020). This again assumes secondary ingestion as the driving mechanism of microplastic exposure in terns, as is supported by the results of this study. The speculation that sand lance ingestion, rather than that of other prey items, is driving our results is just one hypothesis, and sampling of prey items will be necessary to test the theory. As we expected, Common and Roseate terns of the same life stage did not differ in the size of plastics ingested, a trend which may again be attributed to microplastic size correlating with body size (Jâms et al., 2020), as the two seabird species tend to ingest fishes of a similar size. While we do not know the relative threat that the ingestion of the microplastic levels observed here pose to our study species, there is concern over the fact that federally endangered Roseate Terns were found to ingest elevated levels of the pollutant as compared with their co-occurring, congeneric counterparts.

Because only a few studies have assessed microplastic loads in seabird feces, we cannot make a conclusive statement regarding the severity of microplastic ingestion in the species and colonies described by our study as compared with others. That being said, our results are approximately 2500 to 12,500 times higher than a recent study which found that guano collected from Northern Fulmar (*Fulmarus glacialis*) in the Arctic contained a mean of 0.02 plastic particles per gram of fecal matter (Hamilton et al., 2021). Our results are approximately 4 to 20 times higher than those found in another study on Northern Fulmar guano, which found an average of 12.79 microplastics/g feces (Provencher et al., 2018). It should be noted that in both studies mentioned, the samples referred to "guano" were collected by removing fecal precursor samples from dissected sections of the end of the seabird GI tract, therefore, their results are not necessarily directly comparable to ours. Both of these studies were, however, conducted in geographical areas for which models estimated lower concentrations of microplastics than were estimated for our study areas in the Northwestern Atlantic (Van Sebille et al., 2015), presenting a possible non-methodology-driven explanation for the differences in microplastic load observed.

Because there is still no concrete understanding of how microplastics within the size range we observed might impact the health of seabirds, we also cannot make any conclusion about the physical risk of the pollutant to our study individuals. That being said, one experimental study did find that the ingestion of plastic particles as small as 5.00 mm in size was responsible for the transfer of toxic plastic additives into seabird body tissues (Tanaka et al., 2020), therefore it is plausible to assume that similar leaching of chemicals might occur in terns that ingest plastic fibers of sizes within that same order of magnitude, which represents the high end of the size range of microplastics found in our study. However, it is unclear whether the volumes of chemicals that might realistically leach from microplastics into body tissues do so in appreciable amounts compared to other sources of exposure (Bank, 2022). Though information on the physical impacts of microplastic ingestion in seabirds is lacking, the baseline understanding that this study provides regarding microplastic ingestion by terns is an important starting point for understanding the relative threat of plastic pollution to terns and other small seabirds in the Northwestern Atlantic.

5. Conclusions

The results of our study provide valuable quantifications and characterizations of microplastic ingestion levels for Common and Roseate terns breeding in the Northwestern Atlantic, while also illuminating dynamics and mechanisms of microplastic ingestion between life stages, geographical areas, and species. Our study also investigates these same trends in non-synthetic anthropogenic microfibers (many of which are cellulose with a manufactured dye and chemicals applied). Inclusion of these fibers has recently been identified as an important step toward understanding the entire suite of anthropogenic microfibers (which includes microplastics) polluting marine organisms, as they make up a significant proportion of the pollutant suite and have been underestimated in the past (Athey and Erdle, 2021).

Our findings show potential for the use of terns (specifically, Common Terns) as bioindicators for anthropogenic microfibers (including microplastics), and they provide increased understanding of the trophic transfer of this persistent pollutant suite. Specifically, our results suggest that terns primarily ingest anthropogenic microfibers through secondary consumption, and that variation in diet may result in different levels of exposure to the pollutant. Future work should focus on robust sampling of microfibers ingested by tern prey fishes, of those present in the surface waters surrounding the colonies and foraging grounds, and of those that might be lodged in body tissue or expelled via alternate means (regurgitated pellets).

CRediT authorship contribution statement

Aliya Caldwell: Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Writing – original draft, Visualization, Project administration, Funding acquisition. **Susanne Brander:**

Validation, Resources, Writing – review & editing. **John Wiedenmann:** Formal analysis, Writing – review & editing, Supervision, Funding acquisition. **Gemma Clucas:** Investigation, Writing – review & editing. **Elizabeth Craig:** Conceptualization, Methodology, 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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Appendix A. Appendix

Table A1
Summary (sample size, frequency of occurrence, and mean ± standard deviation) of synthetic (plastic), semi-synthetic, and dyed natural fibers estimated in Common Tern (*Sterna hirundo*) and Roseate Tern (*S. dougallii*) adults and chicks breeding in NH, MA, and NJ in 2019. Controls encompass both field and laboratory contamination and numbers reported for biological (fecal) samples were not corrected to account for contamination.

	Sample size	Mean # synthetic fibers/g feces	Mean # semi-synthetic fibers/g feces	Mean # dyed natural fibers/g feces
Common Tern				
Chick				
NH	27	49.5 ± 40.8	3.2 ± 2.6	134.1 ± 110.5
Adult				
NH	26	89.4 ± 197.5	5.8 ± 12.7	242.3 ± 535.2
NJ	20	108.3 ± 133.8	7.00 ± 8.6	293.5 ± 362.6
MA	19	173.5 ± 265.8	11.2 ± 17.2	470.1 ± 720.3
Roseate Tern				
Chick				
NH	21	141.3 ± 186.7	9.1 ± 12.1	382.7 ± 506.0
Controls	25	19.3 ± 23.5	1.2 ± 1.5	52.3 ± 63.7

Table A2
Results, including color, length (mm), and material type (from two spectral libraries: Omnic [Primpke et al., 2018] and OpenSpecy [Cowger et al., 2021]) for each particle isolated from tern feces ($n = 123$). Particle length was only collected and reported for anthropogenically derived fibers.

Color	Length (mm)	Material type (Omnic)	Omnic % Match	Material type (OpenSpecy)	Open Specy % match
Transparent	2.2	Polyethylene terephthalate	86.56	Fibre Polyester	96
Transparent	1.6	Polyethylene terephthalate	88.67	Polyethylene Terepthalate	96
Multicolor	4.5	Polyethylene terephthalate	88.63	Polyethylene Terepthalate	98
Gray	na	Cellulose Acetate Filter ATR 64	94.18	Cellulose	97
Transparent	na	Cellulose Acetate Filter ATR 64	91.74	Fibre Viscose	95
Pink	0.4	Cellulose Acetate Filter ATR 64	84.45	Cellulose	98
Blue	1.6	Poly(Vinyl Acetate:Ethyelene)	70.07	Acrylic	82
Transparent	na	Cellulose Acetate Filter ATR 64	88.92	Cellulose	95
Blue	2	Cellulose Acetate Filter ATR 64	91.93	Cellulose	97
Blue	2	Cellulose Acetate Filter ATR 64	85.93	Cellulose	95
Transparent	na	Wool Raw Cashmere Mongolia #267	88.51	Wool Raw Cashmere Mongolia	91
Blue	0.6	Fibre Cotton Combers #47	91.95	Cellulose	98
Pink	1.2	Cellulose Acetate Filter ATR 64	91.58	Cellulose	98
Black	1.4	Poly(acrylonitrile)	74.25	Acrylic	87
Blue	1.3	Cellulose Acetate Filter ATR 64	84.78	Celllulose	98
Blue	0.4	Cellulose #31	80.04	Cellulose	97
Multicoclor	4.6	Cellulose Acetate Filter ATR 64	87.2	Cellulose	98
Transparent	na	Cellulose Acetate Filter ATR 64	91.14	Cellulose	99

(*continued on next page*)

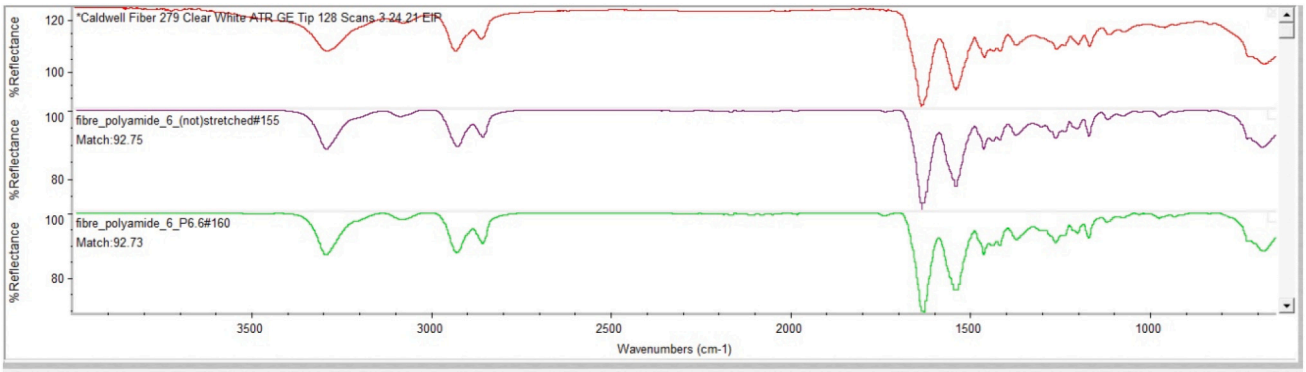
Table A2 (*continued*)

Color	Length (mm)	Material type (Omnic)	Omnic % Match	Material type (OpenSpecy)	Open Specy % match
Pink	0.8	Cellulose Acetate Filter ATR 64	89.29	Cellulose	99
Transparent	na	Cellulose #31	84.52	Cellulose	98
Blue	1.5	Fibre Polyester	76.1	Polyvinylchloride	84
Orange	2	Cellulose Acetate Filter ATR 64	81.56	Cellulose	95
Transparent	1.6	Polyethylene terephthalate	94.08	PET	96
Transparent	na	Cellulose Acetate Filter ATR 64	97.43	Cellulose	97
Transparent	na	Cellulose Acetate Filter ATR 64	91.03	Cellulose	97
Black	3.2	Phenoxy Resin	61.47	Polyvinylchloride	83
Transparent	na	Cellulose Acetate Filter ATR 64	94.93	Cellulose	98
Blue	3.6	Wool Raw Cashmere Mongolia #267	84.35	Fur Dog	96
Transparent	na	Cellulose Acetate Filter ATR 64	91.36	Cellulose	98
Transparent	na	Cellulose #31	83.67	Cellulose	97
Transparent	na	Cellulose #31	86.56	Cellulose	98
Transparent	na	Cellulose Acetate Filter ATR 64	92.7	Cellulose	98
Transparent	na	Cellulose Acetate Filter ATR 64	93.84	Cellulose	98
Blue	0.8	Methyl Alcohol, 99.9% Spectrophotometric grade	71.37	Cellulose	97
Pink	5.4	Fur Dog #62	89.38	Fur Wild Boar	85
Blue	0.6	Cellulose Acetate Filter ATR 64	79.44	Cellulose	98
Blue	1.1	Fibre Linen #122	56.39	NA	
Transparent	na	Cellulose Acetate Filter ATR 64	89.02	Cellulose	98
Transparent	na	Cellulose Acetate Filter ATR 64	72.39	Cellulose	95
White	na	Cellulose Acetate Filter ATR 64	87.02	Cellulose	96
White	na	Cellulose acetate filter ATR 64	92	Cellulose	97
White	na	Cellulose Acetate Filter ATR 64	93.02	Cellulose	97
White	na	Cellulose Acetate Filter ATR 64	94.42	Cellulose	93
White	na	Fibre Cotton Comberse #47	88.29	Cellulose	97
Transparent	na	Cellulose #31	84.25	Cellulose	98
White	na	Cellulose Acetate Filter ATR 64	86.74	Cellulose	98
Blue	0.5	Indigo Synthetic	76.13	Fibre Linen	89
Blue	2.3	Cellulose Acetate Filter ATR 64	91.1	Cellulose	99
Blue	na	Cellulose#31	83.88	Cellulose	98
Pink	na	Cellulose Acetate Filter ATR 64	84.12	Cardboard/Cellulose	97
White	na	Cellulose Acetate Filter ATR 64	95.13	Cellulose	98
Blue	1.7	Cellulose Acetate Filter ATR 64	92.32	Cardboard/Cellulose	97
Black	0.6	Cellulose#31	87.57	Cardboard/Cellulose	97
White	na	Cellulose Acetate Filter ATR 64	92.67	Algae *Fucus Serratus*	70
White	na	Cellulose acetate filter ATR 64	93.27	Cellulose	98
White	na	Cellulose #31	83.87	Cellulose	96
White	na	Cellulose acetate filter ATR 64	91.14	Cardboard/Cellulose	94
Gray	1.7	Cellulose Acetate Filter ATR 64	86.43	Cellulose	97
White	0.9	Nylon 6#147	92.08	Polyamide	95
Yellow	1.4	Cellulose #31	87.92	Cellulose	98
White	na	Cellulose Acetate Filter ATR 64	93.97	Cellulose	95
White	9.1	Polyethylene terephthalate #136	86.12	Polyethylene Terepthalate	98
Red	1.6	Wool Raw Cashmere Afghanistan #266	73.16	Fibre Tussah Silk	83
White	na	Cellulose Acetate Filter ATR 64	92.63	Cellulose	98
White	na	Cellulose acetate filter ATR 64	92.54	Cellulose	98
Black	3.3	Cellulose Acetate Filter ATR 64	89.34	Cellulose	98
Multicolor	2.1	Fibre Linen #122	83.12	Cellulose	85
Transparent	na	Fur Dog #62	82.92	Fur Red Deer	85
Transparent	na	Fibre Cotton Combers #47	75.13	Cellulose	94
Transparent	na	Cellulose Acetate Filter ATR 64	93.16	Cellulose	98
Blue	1	Fibre Cotton Combers #47	69.81	Cellulose	93
Transparent	na	Fibre Linen #122	43.93	NA	
Blue	1.5	Cellulose Acetate Filter ATR 64	84.26	Cellulose	90
Blue	1.7	Cellulose Acetate Filter ATR 64	92.37	Cellulose	97
Multicolor	2.2	Cellulose #31	90.74	Cardboard/Cellulose	95
Multicolor	0.9	Cellulose Acetate Filter ATR 64	88.61	Cellulose	96
Blue	0.4	Fibre Linen #122	76.97	Cellulose	94
Transparent	na	Cellulose Acetate Filter ATR 64	91.73	Cellulose	98
Gray	1.4	Cellulose Acetate Filter ATR 64	83.48	Cellulose	64
Black	3.9	Polyethylene terephthalate	87.58	Polyethylene terepthalate	98
Blue	1.2	Cellulose #31	85	Cellulose	96
Gray	0.6	Cellulose #31	82.8	Cellulose	98
Pink	1.8	Cellulose #31	91.08	Cardboard/Cellulose	98
Blue	0.4	Cellulose Acetate Filter ATR 64	88.73	Cellulose	98
Transparent	na	Cellulose Acetate Filter ATR 64	93.05	Cellulose	98
Transparent	0.8	Nylon	90.11	Polyamide 6	97
Blue	1.6	Cellulose Acetate Filter ATR 64	96.81	Cellulose	97
Blue	0.6	Cellulose Acetate Filter ATR 64	92.96	Cellulose	97
Blue	1.1	Cellulose Acetate Filter ATR 64	83.8	Cellulose	96
Blue	na	Cellulose Acetate Filter ATR 64	93.68	Cellulose	98
Transparent	na	Cellulose Acetate Filter ATR 64	91.35	Cellulose	97

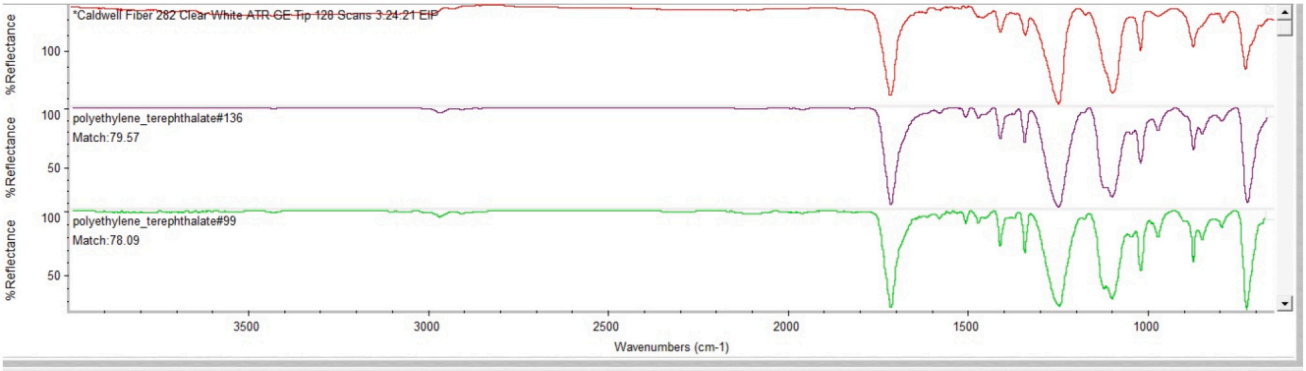
(*continued on next page*)

Table A2 (*continued*)

Color	Length (mm)	Material type (Omnic)	Omnic % Match	Material type (OpenSpecy)	Open Specy % match
Transparent	na	Cellulose #31	88.03	Cellulose	98
Blue	1	Fibre Polyester	82.16	NA	
Purple	4.9	Cellulose #31	85.27	Cellulose	98
Gray	0.8	Wool Raw Cashmere Mongolia #267	83.32	Fur Dog	94
Green	2.3	Polyethylene terephthalate	93.72	PET	99
Transparent	na	Wool Raw Cashmere Mongolia #267	89.98	Wool Raw Cashmere Mongolia	89
Blue	na	Fibre Linen #122	85.13	Cardboard/Cellulose	97
Gray	na	Cellulose Acetate Filter ATR 64	90.55	Cellulose	98
Purple	na	Cellulose Acetate Filter ATR 64	92.71	Cellulose	98
Transparent	na	Cellulose Acetate Filter ATR 64	94.69	Cellulose	98
Purple	1.6	Polyethylene terephthalate	84.57	Polyesterterpthalate	99
Pink	0.7	Cellulose Acetate Filter ATR 64	91.94	Cardboard/Cellulose	95
Transparent	3.3	Epoxide Resin	87.27	Polyester	98
Transparent	7.7	Copolyamide	74.02	Copolyamide	90
Transparent	2.4	Polyethylene Terephtalate	86.54	Polyester	87
Multicolor	1.9	Cellulose #31	74.95	Cellulose	97
Red	0.7	Cellulose Acetate Filter ATR 64	86.43	Cellulose	98
Blue	1	Cellulose Acetate Filter ATR 64	95.37	Cellulose	97
Transparent	1.4	Polytetrfluoroethylene	97.41	Teflon/PFTE	98
Multicolor	na	Cellulose Acetate Filter ATR 64	93.74	Cellulose	99
Blue	0.5	Cellulose #31	86.33	Cellulose	98
Pink	1.1	Fur Dog #62	74.26	Fur Red Deer	83
Blue	0.4	Cellulose #31	86.62	Cellulose	97
Transparent	2	Fibre Polyamide	92.75	Fibre Polyamide	96
Transparent	1.4	Polyethylene Terephtalate	87.19	Polyesterterpthalate	98
Transparent	2	Polyethylene Terephtalate	79.57	Polyethylene terepthalate	98
Pink	0.7	Cellulose #31	91.43	Cellulose	98
Purple	0.6	Hydroxyethyl Cellulose #100	62.18	Cardboard/Cellulose	96
Blue	4.7	Methyl Alcohol Spectrophotometric Grade	70.24	Cellulose	98
Blue	2.5	Cellulose Acetate Filter ATR 64	64.06	Cellulose	97
Transparent	na	Cellulose #31	81.58	Cellulose	93
Transparent	na	Cellulose Acetate Filter ATR 64	82.01	Cellulose	93



	Index	Match	Compound Name	Library Name
1	141	92.75	fibre polyamide 6 (not)stretched#155	Sebastian database 2
2	136	92.73	fibre polyamide 6 P6.6#160	Sebastian database 2
3	140	92.63	fibre polyamide 6#156	Sebastian database 2
4	149	91.94	nylon 6#147	Sebastian database 2
5	139	86.71	polyamide 6#157	Sebastian database 2
6	159	86.44	polyamide#137	Sebastian database 2
7	137	86.26	polyamide 6#159	Sebastian database 2
8	138	85.96	polyamide 6#158	Sebastian database 2
9	252	85.02	copolyamide#280	Sebastian database 2
10	251	83.23	polyamide#281	Sebastian database 2



	Index	Match	Compound Name	Library Name
1	160	79.57	polyethylene terephthalate#136	Sebastian database 2
2	75	78.09	polyethylene terephthalate#99	Sebastian database 2
3	185	76.53	polytehylene terephthalate#174	Sebastian database 2
4	76	75.70	polyethylene terephthalate#98	Sebastian database 2
5	291	75.49	polyethylene terephthalate#323	Sebastian database 2
6	188	74.69	polyester#171	Sebastian database 2
7	187	73.88	polyethylene terephthalate#172	Sebastian database 2
8	231	72.89	polyethylene terephtalate#227	Sebastian database 2
9	191	72.05	fibre polyester#217	Sebastian database 2
10	134	71.91	polybuthylene terephthalate#163	Sebastian database 2

Fig. A1. Example of Fourier-transformed infrared spectroscopy - attenuated total reflectance (FTIR-ATR) results for two microplastic fibers (a and b) isolated from tern feces. Spectral images from the fibers are stacked atop images of the top two match results from the Omnic Library (Primpke et al., 2018). Tables containing the top 10 match results from the Omnic library are stacked below the spectra. Fiber **a** (length = 2.0 mm) is Polyamide (92.75% confidence of top match) and fiber **b** (length = 2.0 mm) is Polyester (79.57% confidence of top match).

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