Indigo-dyed cellulose fibers and microplastics in surface-feeding seabird chick regurgitates from the Gulf of Alaska

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ABSTRACT

We provide new evidence of anthropogenic materials ingestion in seabirds from a remote oceanic area, using regurgitates obtained from black-legged kittiwake (*Rissa tridactyla*) chicks from Middleton Island (Gulf of Alaska, USA). By means of GPS tracking of breeding adults, we identified foraging grounds where anthropogenic materials were most likely ingested (and then brought to chicks). They were mainly located within the continental shelf of the Gulf of Alaska and near the Alaskan coastline. Anthropogenic cellulose fibers showed a high prevalence (85% occurrence), whereas synthetic polymers were less frequent (20%). Most fibers (60%) were blue and we confirmed the presence of indigo-dyed cellulosic fibers, characteristic of denim fabrics. In terms of mass, contamination levels were 0.077 µg/g wet weight and 0.009 µg/g wet weight for anthropogenic microfiber and microplastics, respectively. These results represent the only recent data of contamination by anthropogenic fibers in seabirds from the Gulf of Alaska.

KEYWORDS: anthropogenic materials, cellulosic fibers, indigo dye, microplastics, Pacific Ocean, seabirds

1. INTRODUCTION

In recent years, anthropogenic cellulosic microfibers (Sanchez-Vidal et al., 2018; Remy et al., 2015; Le Guen et al., 2020; Athey et al., 2020) have been considered for their occurrence in biota in addition to microplastics (Cole et al., 2011; Hale et al., 2020). When anthropogenic cellulosic microfibers are included in the total count of anthropogenic items, they may outnumber microplastic counts by a factor of 10 (Stanton et al., 2019). Anthropogenically modified cellulosic microfibers include natural cellulose, such as cotton, flax, hemp, sisal, kenaf or ramie (Ciechanska et al., 2009), as well as semi-synthetic cellulose such as viscose, rayon or the so-called Lyocell fibers (Ganster and Fink, 2009). The latter are mainly obtained from wood pulp (Sixta, 2008) applying chemical reactions or organic solvent addition as in the case of Lyocell process (Ganster and Fink, 2009). Nowaday, they are widely used for clothing, interior textiles and hygiene products beside natural cellulose fabrics (Bredereck and Hermanutz, 2005). Among natural cellulose textiles, denim fabrics are one of the most used (Paul, 2015); they are made of cotton, but they contain colorants (mainly indigo dye) and other chemical additives to improve the mechanical performance and the durability of the final product (Paul, 2015). Athey et al. (2020) reported that a wash of one pair of used jeans can release > 50,000 microfibers. Most of them are retained by wastewater treatment plants (WWTPs), but some persist in the effluents and reach the aquatic environment. The effluents of the WWTPs analyzed by Athey et al. (2020) contained 22 ± 18 microfibers/l with indigo denim fibers being near half of anthropogenically modified cellulose microfibers. Anthropogenic cellulosic microfibers are typically small, with characteristic dimensions of up to a few mm in length and often < 15 μm in diameter (Suaria et al., 2020), matching the criteria proposed by Uddin et al. (2020) for microplastics. Rivers and wastewater discharge are considered the main sources of anthropogenic microfibers, both cellulosic and synthetic, to the oceans, together with coastal tourism and commercial fishing (Desforges et al., 2014; Egger et al., 2020). Moreover, they are easily transported by the atmosphere (Dris et al., 2017), and, therefore, atmospheric long-range transport, together with aquatic transport via oceanic currents, are the main pathways for the

contamination of remote areas (Mishra et al., 2021). In water, due to their small dimensions and to the low density, they can float on the sea-surface (Zobkov et al., 2019) and they can be easily ingested by plankton (Collignon et al., 2012), fish (Cannon et al., 2016; Morgana et al., 2018; Brandon et al., 2020) and seabirds (De Pascalis et al., 2022; Clark et al., 2023). In their review, Wang et al. (2021) reported that 78% of seabird species had microplastics in their digestive tracts, and Clark et al. (2023) identified the Mediterranean and Black Seas, and the Northeast Pacific, Northwest Pacific, South Atlantic and Southwest Indian Oceans as high exposure risk to plastic ingestion for seabirds. Quantifying microplastics contamination in seabirds is essential not only for biomonitoring purposes (O'Hanlon et al., 2017), but also for assessing potential adverse effects (Qiao et al., 2019). Monomers and additives pose an additional threat when released from ingested microplastics by contributing to hormonal imbalance and/or cytotoxicity (Andrady, 2017). Contaminants such as metals or persistent organic pollutants (POPs) can be present on microplastics and other anthropogenic materials, adsorbed by chemical affinity to the surface or within the polymer structure, and transferred to the organism through the "Trojan horse" effect (Diepens and Koelmans, 2018).

The black-legged kittiwake (*Rissa tridactyla*, Linnaeus, 1758) is a widespread pelagic gull that breeds in arctic and subarctic zones across the Northern Hemisphere (Coulson, 2011; CAFF, 2020) and has often been the target of biomonitoring studies for microplastic ingestion in several areas of its distribution range, such as Portugal (Basto et al., 2019), Ireland (Acampora et al., 2017), Denmark (Hartwig et al., 2007), Canadian Arctic (Poon et al. 2017), and the Gulf of Alaska (Robards et al., 1995). Black-legged kittiwakes are small cliff-nesting gulls that aggregate in large breeding colonies (Hatch et al., 1993). Usually, foraging areas are located 5-40 km from colonies, but birds do sometimes forage at greater distances (Suryan et al., 2000; Osborne et al., 2020). Kittiwakes are surface-feeders with a mainly piscivorous diet, but invertebrate prey like krill (Euphasiidae family) are also consumed (Hatch, 2013). Common fish prey in Alaska include

capelin (*Mallotus villosus*), Pacific sand lance (*Ammodytes hexapterus*), Pacific herring (*Clupea pallasii*) and sablefish (*Anopoploma fimbria*) (Hatch, 2013).

Considering its ubiquity from about 35° N to the high-Arctic (CAFF, 2020), the easy access to breeding sites, and tendency to regurgitate when handled, we chose this species for assessing microplastic and anthropogenic material contamination in the Gulf of Alaska. More specifically, we expect to: 1) update the current status of contamination by anthropogenic materials in the Gulf of Alaska after the pioneristic work of Robards et al (1995); 2) evaluate the relative abundance of anthropogenic cellulose vs. microplastics; 3) test the usefulness of collecting chick regurgitates as an easy and non-invasive tool for monitoring pollution by anthropogenic materials, including cellulosic microfibers.

2. MATERIAL AND METHODS

2.1 Regurgitate sampling

Spontaneous regurgitates were collected from 20 black-legged kittiwake chicks aged 5-20 days on July 17, 2021, in the breeding colony on Middleton Island (59°26'15.3" N, 146°19'39.4" W), Alaska (USA). As is the case in many waterbirds, kittiwake chicks recently fed by attending parents tend, when handled, to regurgitate their entire stomach content as an antipredator defense. Regurgitate samples were collected at the nest by gently inserting the gape of a chick that was regurgitating directly into the opening of a 45 ml falcon vial, which was immediately closed. We collected one regurgitate sample per individual. Every precaution for avoiding sample contamination was adopted (see 2.6). Moreover, at regular intervals during the sampling procedure, three field blanks were collected by the same personnel and using the same materials and procedures to detect possible contamination during sampling arising from the operator, sampling environment, or collection materials. Samples were preserved by adding ethanol at 10% v/v relative to the sample volume (2 mL for blanks). All samples and blanks were maintained at -20°C before laboratory analyses.

Regurgitates were collected under license from the U.S. Fish and Wildlife Service and Alaska Department of Fish and Game, as detailed in the next paragraph.

2.2 GPS tracking

To estimate the areas used to collect food for the chicks by kittiwakes breeding on Middleton Island, we deployed GPS dataloggers (8 g, Axy-Trek, TechnoSmart, Rome, Italy) on 18 randomly selected chick-rearing adults (15 males and 3 females) from nests located near those where we sampled chicks (it was not possible to track the adults attending the sampled chicks). Tracking occurred between July 12 and July 22, 2021 (i.e. from 5 days before until 5 day after the day of regurgitate sampling). Dataloggers were deployed on tail feathers using Tesa tape within a few minutes of capture at the nest following established procedures (Osborne et al., 2020). The combined weight of tag and tape was approximately 2.2% of adult body mass, which is well below the recommended thresholds of 3-5% that should avoid disrupting natural flight behaviour (Barron et al., 2010). Dataloggers were set to record one location every 3 min and most of them were retrieved within 2-4 days after tagging. Locations within a 3 km radius around the colony and incomplete trips were excluded using the 'tripSplit' function ('track2KBA' package) (Beal et al., 2021). We used the 'kernelUD' function from the 'adehabitatHR' package (Calenge, 2006) to calculate 25%, 50%, and 75% utilization distribution (UD) kernels over all locations (href = 14.1 km, grid cell size of 1 x 1 km) to illustrate the core foraging area of chick-rearing kittiwakes. Overall, we obtained 72 foraging trips (mean 4 trips per individual, min-max 1-9 trips) within the sampled time period.

Capture, handling and tagging procedures were approved by the McGill Animal Care Committee (protocol MCGL-7814), under state permit #21-089 issued by the Alaska Department of Fish and Game and federal permit #MB33779D-1 issued by the US Fish and Wildlife Services.

2.3 Anthropogenic material extraction

Regurgitate samples and field blanks were analyzed in parallel. Samples were defrosted at room temperature (22-23°C), transferred into a 500 ml glass beaker cleaned with Mill-Q filtered water and weighed. Organic matter digestion was achieved following the protocol for marine vertebrate digestive tracts, regurgitates and scat (Lusher et al., 2018). KOH solution (10% w/v) was added to each sample at a ratio of 1:3 (KOH solution:sample volume); samples were shaken and incubated at 40°C for 72 h in a heater (Karami et al, 2017). Due to the high presence of lipids in regurgitate samples, ethanol (≥99.8% for gas-chromatography, Sigma-Aldrich, Steinheim, Germany) was added to the solution as described by Dawson et al. (2020); ethanol was added according to the state of saponification, at a ratio of 1:10 (ethanol:sample volume) if the solution was clear, and 1:4 or 1:2 if the solution was dark with a visible layer of lipid. After ethanol addition, samples were incubated in the heater for 1 h at 60°C. Two-step filtration was applied to digested suspensions to retain coarse and fine materials, reducing the possibility of filter clogging: a first filtration through a metal sieve with a pore size of 65 µm, and a second one using a cellulose membrane filter (pore size 20 µm; Ø = 47 mm, StonyLab, China) (Wiggin and Holland, 2019). The metal sieve and cellulose filters were visually inspected using a stereomicroscope equipped with a digital camera (Leica EZ4, Leica Microsystems, Buccinasco, Milan, Italy) to isolate suspected anthropogenic materials. Their identification followed an assessment of shape, structure, and color according to the indication of Lusher et al. (2018) and Uddin et al. (2020). Suspected anthropogenic materials were transferred, using metal tweezers and needles, to steel filters (Paul GmbH & Co., pore size 25 µm - 70 mm Ø) within glass Petri dishes. Once the visual inspection of a sample was completed and all suspected anthropogenic materials were transferred to the same steel filter, it was photographed under a stereomicroscope (Leica EZ4, Leica Microsystems, Buccinasco, Milan, Italy) and each item within each filter was labeled on the filter image by a unique code. Each item was measured (length and width) with the free imaging software ImageJ and classified according to shape and color.

2.4 µ-FTIR analysis

To identify the chemical composition, each isolated item was analyzed by micro-Fourier Transform Infrared Spectroscopy (μ-FTIR). Analyses were carried out in transmission mode with a Spotlight 200i FTIR Microscopy System (Perkin Elmer) equipped with a mercury cadmium telluride (MCT) single detector (100 × 100 μm, spectral resolution 0.5 cm^{-1} and sensitivity 40,000/1 RMS). Spectra were acquired with 32 co-added scans. in 4,000 -550 cm^{-1} range and with a resolution of 4 cm^{-1}. A point mode approach described in a previous paper (Reinold et al. 2021) was applied for the collection of the spectra of the identified particles. Every ten measurements a background spectrum was collected to check instrument performance and cleanliness. In the case of suspected cross contamination, the instrument was cleaned, and analysis was repeated. Patented COMPARE™ spectral comparison algorithm was used for performing the spectral comparison with spectra available in a commercial library. At least four spectra were recorded for each suspected anthropogenic material item and IR spectra were compared with those of the library, recording the match of each μ-FTIR spectrum with the one selected from the library. Each item was photographed under the microscope of the instrument and the best transmittance spectrum in relation to the library identification procedure was recorded. A positive identification with the reference library was assigned for matches ≥ 70%. In the case of semi-synthetic materials (e.g. Rayon) and natural cellulose fibers of anthropogenic origin (cotton), the possibility of unequivocally discriminate these materials by IR spectra is challenging, due to dye masking, weathering and adsorption processes (Comnea-Stancu et al., 2017; Cai et al., 2019; Saito et al., 2021). Following Comnea-Stancu et al. (2017), we considered both dyed cellulose fibers and Rayon fibers as part of a unique category, i.e. “anthropogenically-modified cellulose-based fibers” or simply “anthropogenic cellulose fibers”.

2.5 μ-Raman spectroscopy of cellulosic fibers

After μFTIR analysis, several blue cellulose fibers were analyzed by μ-Raman-spectroscopy (inVia Renishaw™ instrument combined with a Leica stereomicroscope with 4 magnifications 5×, 20×,

50× and 100× and a motorized x–y stage). Magnification was set depending on the fiber size. Non-polarized μ-Raman spectra were obtained in a nearly backscattered geometry, using two laser sources at two fixed wavelengths (532 and 785 nm). The CCD detector had a spectral resolution FWHM of 0.5 cm^{-1}, in the spectral range between 50 and 4,000 cm^{-1}. To enhance the Raman scattering and allow a better vision of a single fiber, a polished aluminum foil was placed on a slide and used as a support for the analysis, as reported in Ferrero et al. (2022). Aluminum enhances the Raman signal by amplifying the electron cloud density around metallic structures as described in Ferrero et al. (2022). Laser power was limited to avoid heating effects and microfiber combustion or thermal degradation. In this respect, a one second test, with five accumulations, fixing the laser intensity at 50%, was carried out at the border of each microparticle; if too intense, 60 accumulations of 1 s with a laser intensity of 5–10% was used. Calibration was done using an integrated internal standard of silicon wafer before each experimental session. Finally, the baseline was subtracted from each spectrum to remove background noise. Spectra were matched to those of standard materials cataloged in the Bio-Rad KnowItAll Spectral Database and with spectra recorded from reference standards provided by AITC (Italian Association of Textile and Color Chemistry, www.aictc.org).

2.6 Quality control and quality assurance

Since microplastics and residues of anthropogenic materials are ubiquitous, it is crucial to perform quality control checks to prevent sample contamination and thus an overestimation of the presence of microplastics in samples (Provencher et al., 2019). During fieldwork, care was taken to prevent contamination from clothes and the environment; the vial was opened for as little time as possible (mostly less than 10 s) and regurgitates were introduced directly into the vials, avoiding contact with any other surfaces. Field blank samples were collected to monitor environmental contamination during sampling operations or potentially arising from materials and reagents used in sampling. Control samples underwent all the steps of the process from field collection to every

process in the laboratory. Thus, they were both field and procedural blank samples. In the laboratory, all the materials used were strictly non-plastic and they were all cleaned with Milli-Q filtered water. All solutions were filtered using a 0.45 µm pore size cellulose membrane filter. To prevent contamination from the laboratory environment, laboratory work was conducted under a dedicated hood. All the laboratory surfaces were regularly cleaned with ethanol and every beaker or solution was covered with aluminum foil and opened only for the minimum time required. To prevent the release of synthetic fibers from clothes, white cotton lab coats were worn. After sorting and labeling anthropogenic particles on the acquired filter images, only labeled particles were further considered. Moreover, chemical identification was performed in a clean room with a filtered air system.

Despite all precautions, six fibers were isolated from the three blank samples (min 1, max 3 per sample, mean 2 ± 1 SD) having black, white, purple and blue colors (maximum one fiber per color per sample). Among them, one was identified as nylon (spectra correlation = 89%; black color), three were cellulose fibers (spectra correlation >84%; 2 white and 1 purple), 2 were not identified (black and blue color). Following Suaria et al. (2020), results in samples were blank-corrected by subtracting the largest number of fibers found in blanks, taking into account chemical composition and color. Hence, for each regurgitate sample, one fiber each for white, purple, black and blue colors were excluded from the final results. One white and/or one purple fibre was excluded from the sample results when the polymers in samples were either cellulose or not identified, while one black and/or one blue fiber was excluded in sample results irrespective of their polymeric composition, because such fibers were not chemically identified in blanks. By this procedure, 1 to 3 fibers were excluded from the results of each sample (28 fibers across all samples).

As benchmarks of efficiency of the extraction and purification methodology, we relied on mass recovery tests performed in a previous study conducted in our laboratory by Winkler et al. (2022), that reported mean (± SD) recovery rates of low- (polystyrene, PS) and high-density

(polyethylene terephthalate, PET) polymers to be 97.1 ± 2.4% and 41.0 ± 16.8%, respectively. Despite low recovery of PET particles, no correction for recovery rate percentages was applied since underestimation was preferred to overestimation of the microplastic content.

3. RESULTS

Core foraging areas (25% kernel UD) of chick-rearing black-legged kittiwakes breeding at the colony from which regurgitate samples were collected are shown in Figure 1. Regurgitate content most likely came from pelagic foraging areas located within 50 km north of the colony site on Middleton Island and coastal areas near the coastline of Montague Island, 80 km north-west of the colony site.

Overall, in 17 out of 20 regurgitate samples (85% occurrence) we found 45 microfibers (range: 0-5 fibers per sample; mean: 2.3 ± 1.6 SD) and 6 fragments, which are particles of irregular shape (min-max 0-1 items per sample, mean 0.30 ± 0.47 SD, 33% occurrence; Table S1). Among microfibers, the most abundant color was blue (60%), followed by red (15.6%), white (13.3 %), black (6.6%), and green (4.5%). The distribution of fiber size and color is shown in Figure 2. Mean and median length of fibers were 2.8 mm and 1.3 mm, and mean and median width were 0.015 mm and 0.013 mm, respectively (Table S1). Fragments were identified as cellulose or were not chemically identified (Table S1). For this reason, and because of their irregular shape, they were not considered unequivocally as anthropogenic materials.

Even if chemically identified as cellulose, microfibers were considered of anthropogenic origin due to their unnatural shape and uniform color (blue, red, white, green, black), following Lusher et al. (2018), Mishra et al. (2019) and Uddin et al. (2020). In the case of blue cellulose material (3 fibers), we applied μ-Raman spectroscopy to confirm their anthropogenic origin. All of them were identified as indigo-dyed cellulose fibers as their spectra matched that of an indigo-dyed denim fiber (Figure 3). In fact, indigo dye is typically used in denim fabrics.

Among microfibers found in regurgitate samples, four of them were identified as microplastics by μ-FTIR analysis, having three different synthetic polymers: polyester (PES, 2 red fiber; 50% of microplastics), polyacrylonitrile (PAN, 1 red fiber; 25% of microplastics) and polyethylene (PE, 1 white fiber; 25% of microplastics). Spectra of the different polymers are shown in Figure 4 together with those from the library (spectra correlation were >90% for the three polymers). Hence, microplastics were found in 4 samples (20%) with a mean 0.2 items per sample (range: 0-1 items per sample).

Considering the wet weight (w.w.) of each regurgitate sample (range 4.6 - 37.9 g, mean 16.0 g, Table S2), a mean of 0.17 ± 0.021 (SE) g^{-1} w.w. of anthropogenic items were encountered, of which 0.017 ± 0.0064 (SE) items g^{-1} w.w. were μFTIR-confirmed microplastics. Moreover, considering the length and width of each fiber, we derived the relative volume and, by approximating the density of each fiber to 1 g cm^{-3}, we derived the mass of anthropogenic fibers/microplastics for each sample (μg g^{-1} w.w.). Mean contamination levels per unit mass were 0.077 ± 0.012 (SE) μg g^{-1} w.w. for anthropogenic fibers and 0.009 ± 0.0045 (SE) μg g^{-1} w.w. for μFTIR-confirmed microplastics.

4. DISCUSSION

Our study is the first to assess microplastics in seabirds from the Gulf of Alaska since the previous monitoring (1988-1990) by Robards et al. (1995). At that time, plastic occurrence in black-legged kittiwakes was 7.8% (0.3 items per bird), mainly in the form of light-colored fragments. Robards et al. (1995) suggested that surface-feeding seabirds that feed on zooplankton, such as parakeet auklet (*Aethia psittacula*), fork-tailed storm-petrel (*Oceanodroma furcata*) and northern fulmar, were more contaminated than piscivorous species such as the black-legged kittiwake. Baak et al. (2020) confirmed the contamination difference between fulmars and kittiwakes nevertheless they were both surface feeders. Similarly, Amélineau et al. (2016) found in eastern Greenland (70° N) a high microplastic contamination (9 ± 11 SD items per chick meal from gular pouches) in little auks (*Alle*

alle), an Arctic zooplankton-feeding seabirds. These authors confirmed greater microplastic contamination in zooplanktivorous birds, which may mistake microplastics for their natural prey or passively ingest them because microplastics are particularly abundant were zooplankton occurs, since they are both transported by the same currents (Collignon et al., 2012; Saura et al., 2020; De Pascalis et al., 2022). In the Canadian Arctic (74° N), Poon et al. (2017) found high levels of microplastics in northern fulmars (3.4 ± 3.1 SD item/bird; 89 % occurrence), a lower contamination in black-legged kittiwakes (0.18 ± 0.60 SD item/bird; 9 % occurrence), and no contamination in two seabird species (*Uria lomvia, Cepphus grylle*) which are pursuit-diving seabirds catching their prey (mainly fish) at greater depths. Poon et al. (2017) concluded that species adopting pursuit-diving behavior to catch their prey were the least affected by microplastics contamination.

Regarding μ-FTIR confirmed microplastics, our findings are similar to those reported by Poon et al. (2017) and Baak et al. (2020) for the same species in the Arctic region of the Atlantic Ocean. The finding of a similar contamination in such distant areas suggest the presence of a widespread contamination across the whole Arctic region as reviewed by Mishra et al. (2021). Unfortunately. comparing our microplastic findings (20% occurrence, 0.2 items per sample, all microfibers in the dimensional range 0.3-5 mm) with those of Robards et al. (1995) in the same area and for the same species (7.8% occurrence, 0.3 items per sample, mainly fragments in the dimensional range 0.5-28 mm) to assess temporal changes is challenging because of the difference in the methodology and analyzed sample (regurgitate and stomach content in this study vs. Robards et al., 1995 respectively). Nevertheless, our data suggest that plastic contamination may have shifted from relatively larger fragments to very small plastic microfibers in a 30 year period, with most of the contamination nowadays consisting of anthropogenic cellulose microfibers (85% occurrence, 2.0 items per sample, dimensional range 0.2-32 mm). Cellulose microfibers were recently suggested as a new contamination issue by several authors as they represented the prevalent form of contamination in different environmental matrices (Sanchez-Vidal et al., 2018; Remy et al., 2015; Le Guen et al., 2020; Suaria et al., 2020; Ferrero et al., 2022). Athey et al. (2020)

reported that a washing of new blue jeans released 210 microfibers g^{-1}, with amounts decreasing at subsequent washes (130 microfibers g^{-1}), and that the effluents of two WWTPs released annually to surface waters 1.1×10^9 indigo denim microfibers. Accordingly, most of the microfibers we found in black-legged kittiwake chick regurgitates were blue cellulosic ones and at least some of them were indigo-dyed, thus presumably derived by denim fabrics. The WWTPs considered in the work of Athey et al. (2020) serve near the same number of people as Alaska's inhabitants (around 730,000 people). If we considered that the same potential release would reach the Gulf of Alaska, which has a dimension of over 1,500,000 km^2, we may estimate a yearly load of 730 microfibers km^{-2}, not far from the findings reported by Egger et al. (2020) for seawater from the same area.

Considering color and polymers of the fibers found in our study, those found by Bourdages et al. (2021) in northern fulmars from the Canadian Arctic were almost identical (blue 58% vs 60%, white 21% vs. 13.3%, red 17% vs. 15.6% and black 4% vs 6.6%, polyester 25% vs. 50% and polyethylene 4% vs. 25%; Bourdages et al. (2021) vs. this study, respectively). Color and polymer composition as well seems to indicate a similar widespread contamination in the whole Arctic region.

One of the most important issues of studying contamination in top predators is the evaluation of possible bioaccumulation and biomagnification phenomena. Microplastics in seawater have been analyzed extensively in most of the world's oceans, including mid-North Pacific (Pan et al., 2022), Northeast Greenland (Morgana et al., 2018), Northwest and South Atlantic and Antarctic (Suaria et al., 2020) and the North Pacific and Gulf of Alaska (Egger et al., 2020). The latter study grouped microplastic concentrations from the Gulf of Alaska with those originating from the open ocean outside the North Pacific subtropical gyre because of the similarity in concentrations. The median microplastic concentration in that geographically combined group of samples was 17,238 items/km^2, which corresponds to 0.043 item/m^3 (considering a trawl height of 40 cm, Egger et al., 2020). Taking this median concentration as a proxy for the contamination of the feeding area of

black-legged kittiwakes from Middleton Island (involving a large sector of the Gulf of Alaska, as demonstrated by our GPS tracking data), we attempted to calculate a bioconcentration factor as the mean number of microplastics in regurgitates on a fresh weight basis (microplastics per kg of regurgitate) divided by the mean number of microplastics in the same mass of water (microplastics per kg of water). If we consider only the μFTIR-confirmed microplastics (17 items/kg wet weight), we obtain a value of 400,000. Conversely, if we consider the total number of anthropogenic fibers (74 items/kg wet weight), we obtain a value of 1,700,000. These calculations are merely tentative; in fact, if we consider, for example, the data of Barrows et al., (2018) regarding contamination by anthropogenic materials in seawater from the Arctic region (Gulf of Alaska included), much lower bioaccumulation factors were calculated. Beyond the inconsistency of literature data in microplastics and anthropogenic material contamination in seawater, mainly due to the considerable heterogeneity in analytical methodologies and in the amplitude of the anthropogenic material categories considered by different authors, the calculation presented here aim to stress the perspective of a very high bioconcentration potential of microplastics and anthropogenic items in seabirds in relation to their foraging environment, as already stated for meso-plastic materials (van Franeker et al., 2015). Microplastics in kittiwake regurgitates are probably ingested primarily through diet (fish and invertebrates; Hatch, 2013) rather than being directly ingestion from water. Considering the small dimension of microfibers and the mainly piscivorous diet of black-legged kittiwakes, a direct ingestion of these materials (by mistaking them with prey) seems unlikely. Moreover, when foraging, kittiwakes may also ingest water, but the expected number of microplastics in the small volume of water ingested during foraging can be considered negligible as well. Thus, the most probable origin of the anthropogenic materials found in regurgitates is their presence in prey, which means the contamination transfer along the food chain. The transfer of microplastics and anthropogenic items along the marine food chain is well documented (Mishra et al., 2021), but it remains unclear the entity of the bioconcentration potential and which are the characteristics which enhance this phenomenon. The review of Walkinshaw et al. (2020) analysed

the concentrations of microplastics in fish and marine fauna globally. They reported concentrations even above 1 microplastic item per g of fresh weight for mussels and oysters, 0.01-1 microplastic items per g of fresh weight in chub mackerel (*Scomber japonicus*), between 0.01-0.1 microplastic items per g of fresh weight in anchovies (Engraulidae family) and Atlantic herring (*Clupea harengus*), and fewer than 0.001 microplastic items per g of fresh weight in skipjack (*Katsuwonus pelamis*) and yellowfin tunas (*Thunnus albacares*). The authors of that review concluded that microplastics do not biomagnify along the food chain, but instead organisms at lower trophic levels are more contaminated on a mass basis than top predators. Filter feeders, such as mussels on the seafloor or zooplankton at the surface, are considered to have the greatest exposure to microplastic contamination (Fang et al., 2018) and present higher microplastics concentration than fish (Morgana et al., 2018; Liboiron et al., 2019). It remains unclear whether a size- and/or a color-selection occur along the food chain and, if they happen, at which trophic level they occur.

To our knowledge, our study is one of the first to perform μ-RAMAN spectroscopy on blue cellulose microfibers in seabirds, confirming that such fibers were cellulosic and dyed with indigo, a characteristic of denim fabrics. Anthropogenic cellulose microfibers are emerging as a new contamination element in environmental pollution studies. Considering reported concentrations of anthropogenic items in the Gulf of Alaska's seawater, we tentatively derived very high bioaccumulation factors. Studies in remote areas are essential for the global monitoring of this environmental issue, which is both alarming and rapidly evolving. Due to the broad distribution of black-legged kittiwakes in the boreal region (Coulson, 2011, from about 35° N to the high Arctic), the relatively easy access to breeding sites, and the tendency to regurgitate when handled, chick regurgitates should be regarded as an effective and non-invasive monitoring tool for assessing contamination from anthropogenic material in Arctic food webs.

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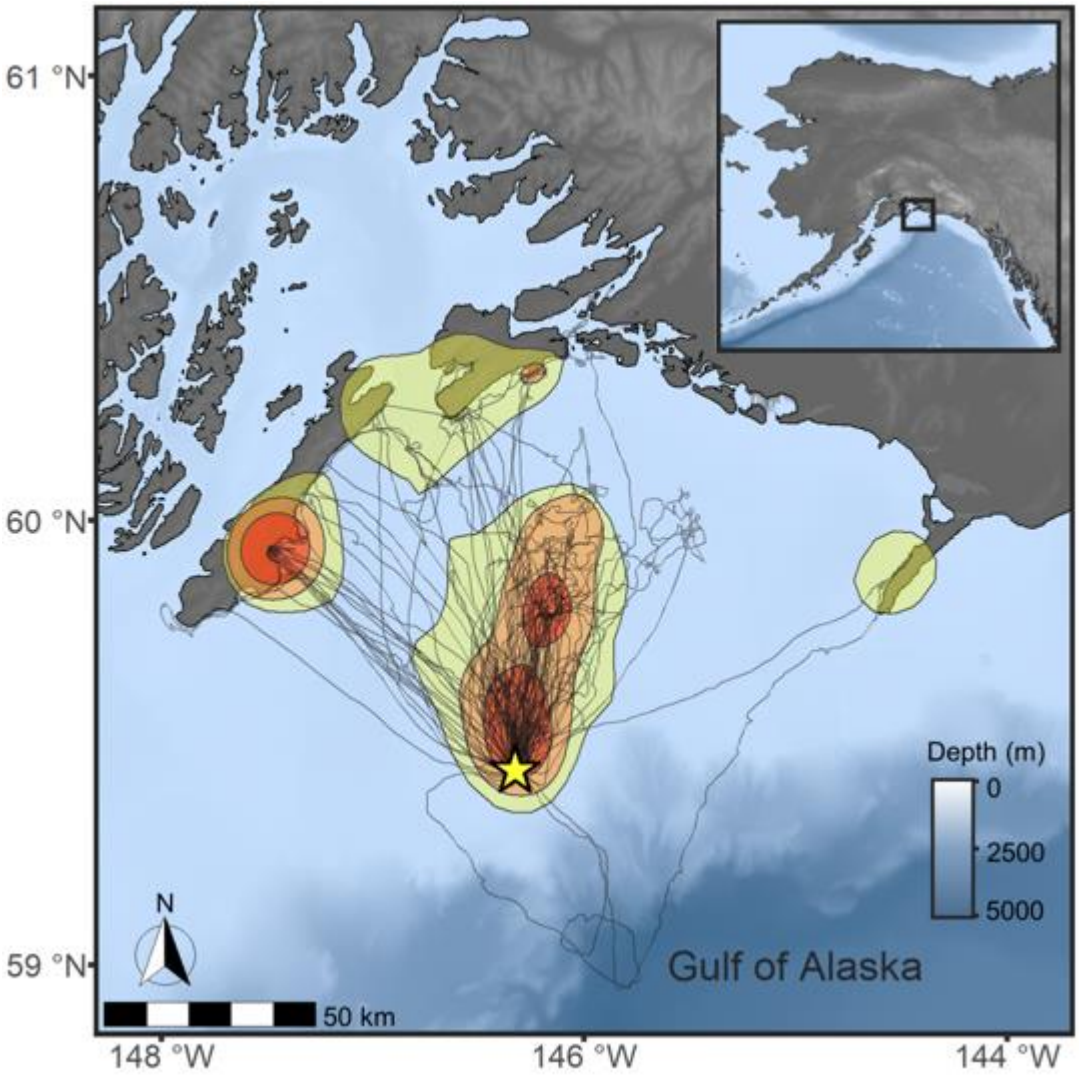
FIGURE CAPTIONS

Figure 1. Foraging areas of chick-rearing adult black-legged kittiwakes breeding at the Middleton Island colony (yellow star) derived from GPS tracking. Dark lines represent 72 foraging trips from 18 GPS-tracked individuals. Yellow, light orange and dark orange polygons represent 75%, 50% and 25% utilization distribution kernels, respectively, and they represent increasingly concentrated GPS locations, i.e. the most likely foraging areas of tracked individuals. Inset: location of the study area within Alaska (USA).

Figure 2. Size (upper panel: length; middle panel: width) and color (lower panel) distributions of the anthropogenic fibers detected in black-legged kittiwake regurgitate samples (n = 45 fibers).

Figure 3. Microscope images and Raman spectra of the three blue fiber S20-F1, S5-F1 and S6-F2 (Table S1) compared with a reference spectrum of Demin fabric fiber (Image and spectrum on the bottom).

Figure 4. Microscope images of three microplastics found in black-legged kittiwake regurgitate samples (right side) with their respective μ-FTIR spectra (%T = percentage of transmittance; cm^{-1} = wavenumber per cm). Each unknown spectrum (black line above) is compared with the best match from library reference spectra (coloured lines below). Spectral identification was (from the top): polyester (PES, 92% match), polyethylene (PE, 91% match) and polyacrylonitrile (PAN, 91% match).



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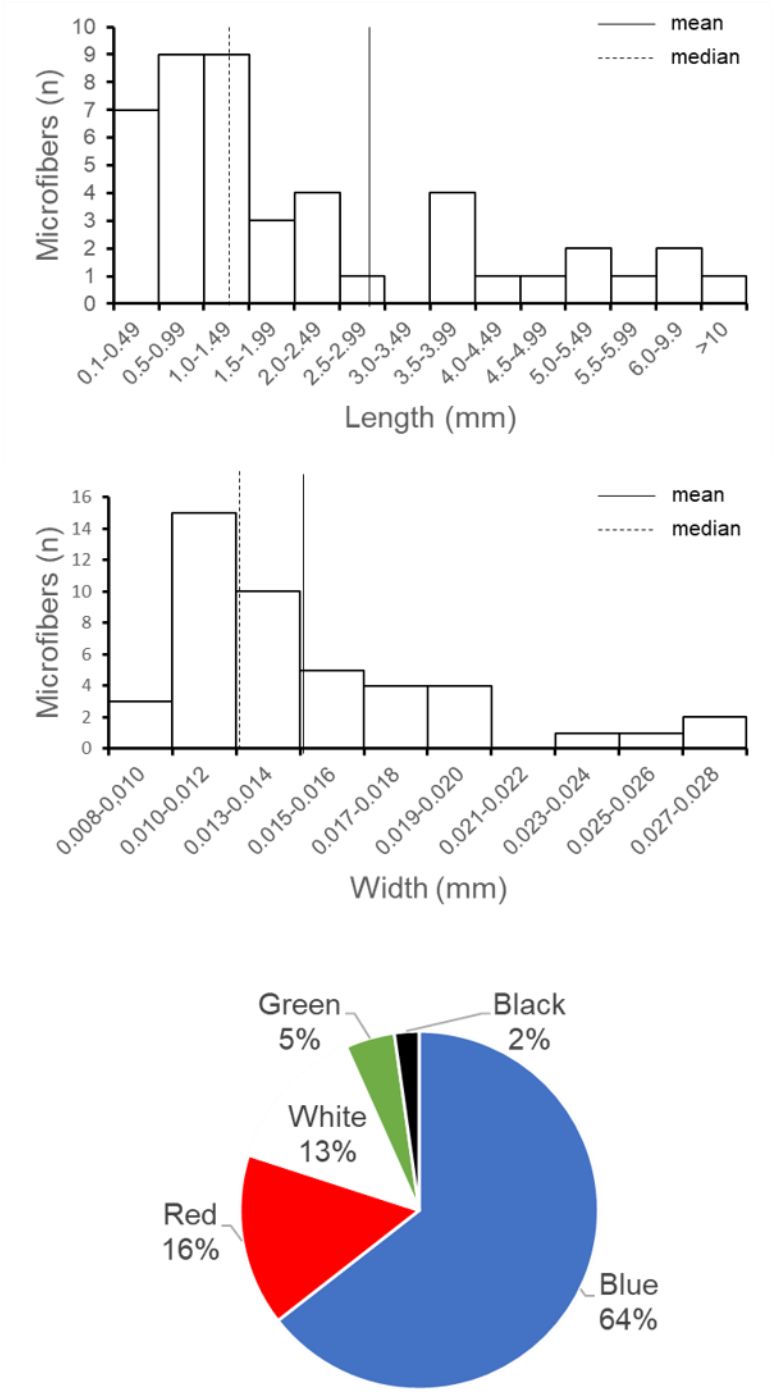

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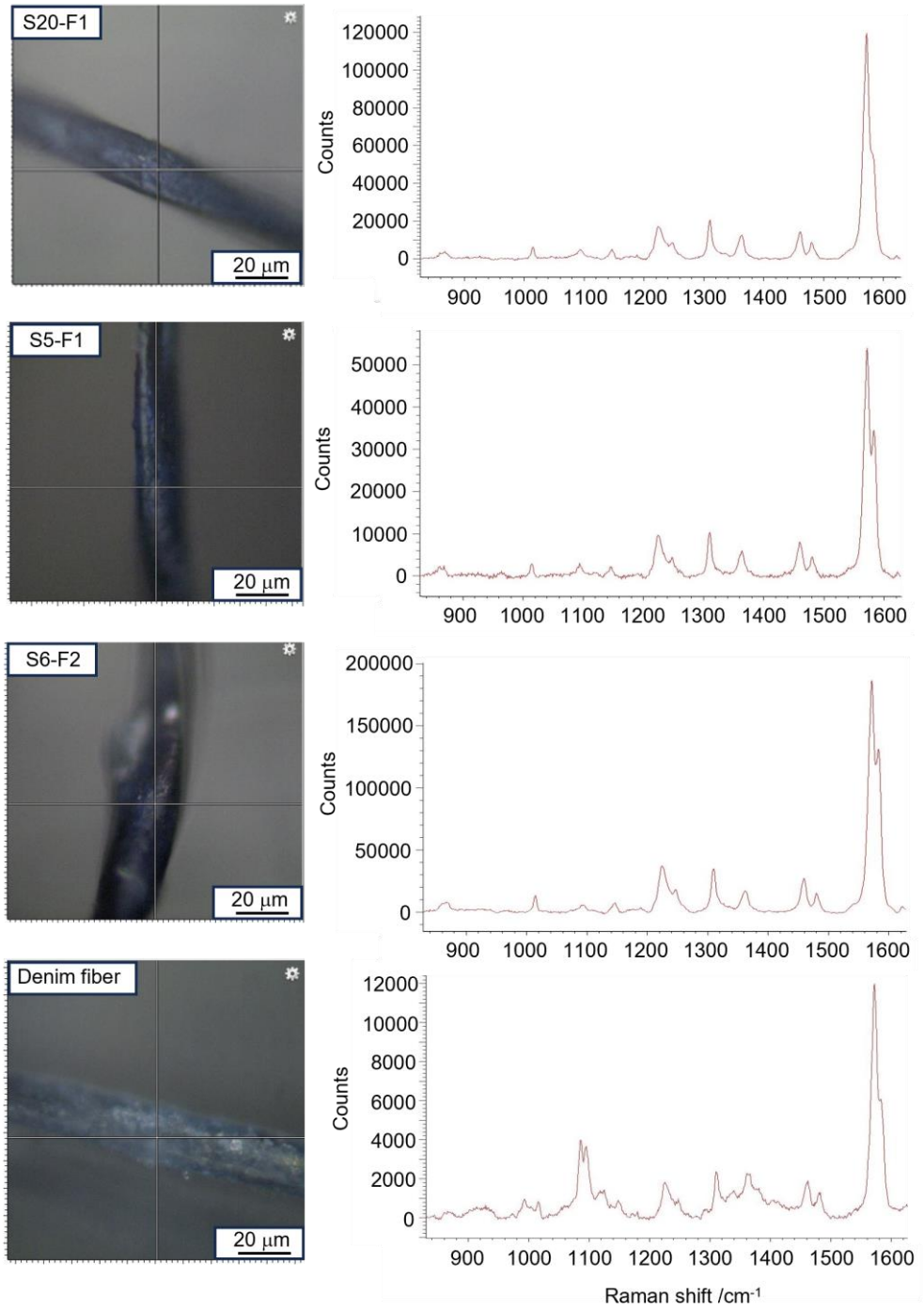

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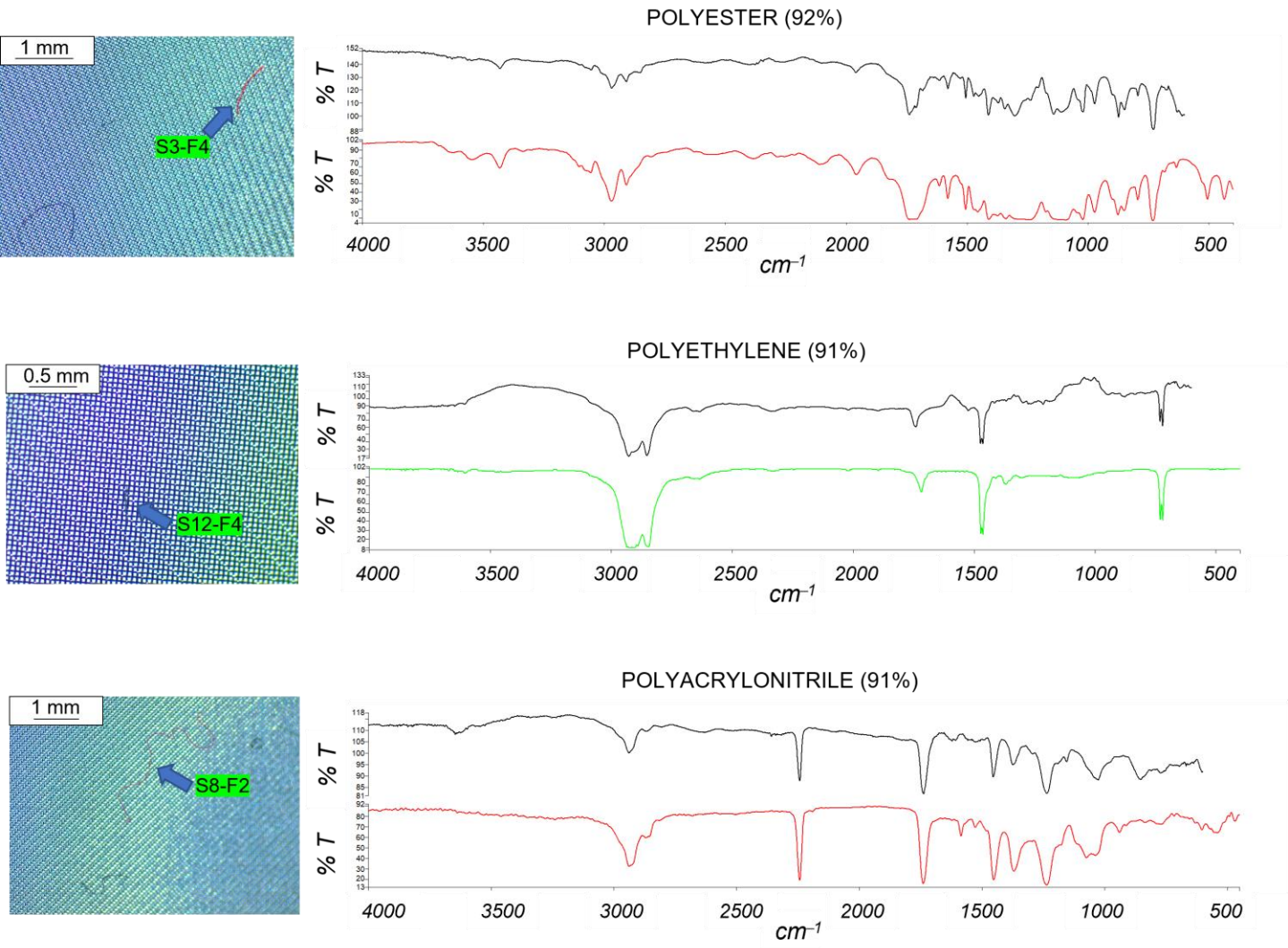

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SUPPLEMENTARY INFORMATION

Indigo-dyed cellulose fibers and microplastics in surface-feeding seabird chick regurgitates from the Gulf of Alaska

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Table S1. Microfibers and fragments found in regurgitate sample (S1-20), identification code, length, width, color, shape, polymer and correlation percentage of polymer identification (%). NI = polymer could not be identified based on reference spectra.

Sample	Particle	Length mm	Width mm	Color	Shape	Polymer	%
S1	F1	2.85	0.009	Blue	Fiber	NI	-
S2	F1	3.701	0.017	Red	Fiber	NI	-
S3	F1	1.548	0.024	Blue	Fiber	NI	-
	F4	0.996	0.018	Red	Fiber	Polyester	92%
	F5	2.032	0.014	Blue	Fiber	Rayon	76%
	F7	1.43	0.015	Black	Fiber	Rayon	70%
	F8	32.579	0.019	Green	Fiber	NI	-
S4	F1	1.873	0.014	Blue	Fiber	Rayon	71%
	F2	0.257	0.015	White	Fiber	NI	-
	F5	5.333	0.018	Red	Fiber	Polyester	90%
	F6	0.657	0.025	Red	Fiber	NI	-
	F7	0.848	0.012	Blue	Fiber	NI	-
S5	F1	0.707	0.013	Blue	Fiber	Cellulose (Denim)	80
	F3	0.586	0.009	Blue	Fiber	NI	-
	P1	0.129	0.071	Blue	Fragment	NI	-
S6	F1	1.761	0.010	Blue	Fiber	Cellulose	72
	F2	1.222	0.011	Blue	Fiber	Cellulose (Denim)	77
	F3	0,775	0.015	Blue	Fiber	Cellulose	79
S7	F2	0.423	0.013	Black	Fiber	NI	-
	F3	2.008	0.011	Blue	Fiber	Cellulose	80
	F4	0.651	0.014	Black	Fiber	Cellulose	78
S8	F1	0.858	0.011	Blue	Fiber	NI	-
	F2	5.497	0.012	Red	Fiber	Polyacrylonitrile	91
	F3	1.487	0.014	Blue	Fiber	NI	-
	F4	1.092	0.012	Blue	Fiber	NI	-
	F6	3.723	0.020	White	Fiber	NI	-

	P1	0.075	0.061	White	Fragment	Rayon	88
S9	F2	1.266	0.020	Blue	Fiber	Rayon	71
	F3	1.152	0.012	Blue	Fiber	Rayon	70
	P1	0.118	0.046	Black	Fragment	NI	-
S10	F1	0.999	0.010	Blue	Fiber	NI	-
	F3	0.934	0.012	Blue	Fiber	Rayon	80
	F4	1.191	0.013	Blue	Fiber	Rayon	70
	F5	5.674	0.009	Blue	Fiber	Rayon	70
S11	F1	2.157	0.011	Green	Fiber	NI	-
	F2	0.197	0.018	Blue	Fiber	NI	-
S12	F2	7.558	0.014	Blue	Fiber	NI	-
	F4	0.266	0.012	White	Fiber	Polyethylene	91
S13	F1	2.281	0.010	Blue	Fiber	Rayon	79
	F2	1.155	0.012	Blue	Fiber	NI	-
S14	-	-	-	-	-	-	-
S15	F1	4.907	0.011	Blue	Fiber	NI	-
S16	-	-		-	-	-	-
S17	F1	7.173	0.027	White	Fiber	NI	-
	F2	0.433	0.019	White	Fiber	Cellulose	72
	P1	0.131	0.054	Blue	Fragment	NI	-
S18	F1	4.012	0.013	White	Fiber	NI	-
	F3	0.377	0.027	Red	Fiber	NI	-
	P1	0.069	0.053	White	Fragment	Cellulose	90
S19	-	-		-	-	-	-
S20	F1	3.575	0.013	Blue	Fiber	Cellulose (Denim)	71
	F3	3.524	0.016	Red	Fiber	NI	-
	P1	0.075	0.046	Red	Fragment	NI	-
	F5	0.215	0.016	Blue	Fiber	Rayon	70

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16 Table S2 – Wet weight (w. w.) of regurgitate samples

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Sample ID	Wet weight (g)
S1	10.6
S2	9.1
S3	37.9
S4	10.3
S5	26.6
S6	16.1
S7	11.1
S8	16.2
S9	13.0
S10	26.7
S11	4.6
S12	6.1
S13	15.5
S14	9.0
S15	15.7
S16	9.8
S17	22.8
S18	26.0
S19	20.5
S20	12.1
MEAN	**16.0**
St. dev.	**8.4**

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Declaration of interests

☒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.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: