Marine Pollution Bulletin 203 (2024) 116401

Contents lists available at ScienceDirect

Marine Pollution Bulletin

journal homepage: www.elsevier.com/locate/marpolbul

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

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ARTICLE INFO

Keywords:
Anthropogenic materials
Plastic pollution
Microplastics
Mesoplastics
Pacific Ocean
Kittiwakes

ABSTRACT

We provide 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. 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 (in the micro- and mesoplastics dimensional range) 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^{-1} wet weight and 0.009 μg g^{-1} wet weight for anthropogenic microfibers and synthetic polymers, respectively. These results represent the only recent report of contamination by anthropogenic fibers in seabirds from the Gulf of Alaska.

1. Introduction

Plastic pollution is recognized as a pervasive global environmental emergency, which is becoming an increasingly concerning environmental issue even in remote areas, such the Arctic (Bergmann et al., 2022). In addition to synthetic polymers, anthropogenic cellulosic microfibers have also been considered as pollutants in recent years because of their environmental abundance (Sanchez-Vidal et al., 2018; Remy et al., 2015; Le Guen et al., 2020; Athey et al., 2020). Indeed, they may outnumber microplastic counts by a factor of 10 in different environmental matrixes (e.g. 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 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). Nowadays, 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 on average 22 microfibers L^{-1} with indigo denim fibers constituting nearly half of anthropogenically modified cellulose microfibers.

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https://doi.org/10.1016/j.marpolbul.2024.116401
Received 24 January 2024; Received in revised form 15 April 2024; Accepted 18 April 2024
Available online 6 May 2024

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) for oceans waters, 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). Hence, atmospheric long-range transport, together with aquatic transport via oceanic currents, are the main pathways for 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 global plastic exposure 'hot-spots' 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). For instance, phthalates, the most common plasticizers, are considered endocrine disrupting compounds (Kamrin, 2009). Moreover, 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 recent review of Baak et al. (2020a) on plastic ingestion by seabirds in the circumpolar Arctic emphasized the lack of recent information for most species. Moreover, most of the literature on plastic contamination in Arctic seabirds focused on the Atlantic Ocean (Amélineau et al., 2016; Poon et al., 2017; Baak et al., 2020b), while fewer information is available for other regions, such as the Pacific Arctic (Day, 1980; Robards et al., 1995; Padula et al., 2020). 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 (about 400 g) 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). In the Pacific region, common fish prey 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. Moreover, Baak et al. (2020a) recommended prioritizing this species for microplastic pollution biomonitoring across the Arctic marine food webs. With this study, we aimed at: 1) update the current status of contamination by anthropogenic materials in the Gulf of Alaska after the pioneristic works of Day (1980) and 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 × 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, processing blanks exactly as regurgitate samples and during processing of regurgitate samples (three blanks in total). 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 and Hernandez-Milian, 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 and Hernandez-Milian (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 4000–550 cm^{-1} range and with a resolution of 4 cm^{-1}.

A positive identification with the spectra of the reference library was assigned for matches ≥70 %. Further details of spectrum acquisition and identification are reported in Supplementary Information (SI- *Integration of μ-FTIR analysis*). 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 4000 cm^{-1}. Further details of spectrum acquisition are reported in Supplementary Information (SI- *Integration of μ-Raman spectroscopy*). 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 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 overestimate 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 <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. During laboratory analysis, all materials and procedures were strictly provided to prevent contamination of samples by microplastic and external materials in general (laboratory contamination). Procedures adopted to prevent sample contamination in laboratory are reported in Supplementary Information (SI- *Integration of quality control and quality assurance*).

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 fiber 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 Fig. 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 Montague Island, 80 km northwest 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

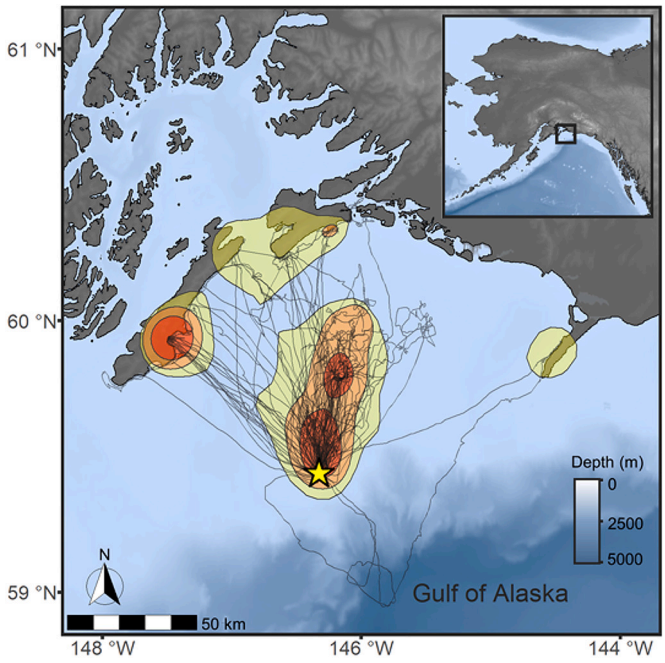

Fig. 1. Foraging areas of chick-rearing adult black-legged kittiwakes breeding at the Middleton Island colony (yellow star) derived from GPS tracking. Dark lines represent 72 foraging trips from 18 GPS-tracked individuals. Yellow, light orange and dark orange polygons represent 75 %, 50 % and 25 % utilization 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). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

by red (15.6 %), white (13.3 %), black (6.6 %), and green (4.5 %). The distribution of fiber size and color is shown in Fig. 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 and Hernandez-Milian (2018), Mishra et al. (2021) 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 (Fig. 3).

Among anthropogenic fibers, four of them were composed by petroleum-based synthetic polymers: 2 red fibers of polyester (PET), 1 red fiber of polyacrylonitrile (PAN), and one white fiber of polyethylene (PE). Two of these fibers were smaller than 5.0 mm in length (1 red PET and 1 white PE) and were therefore classified as microplastics, whereas the other two (1 red PET and 1 red PAN) belonged to the mesoplastic dimensional range, although they were slightly above the 5 mm threshold (5.33 and 5.50 mm, respectively). Spectra of the different polymers are shown in Fig. 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

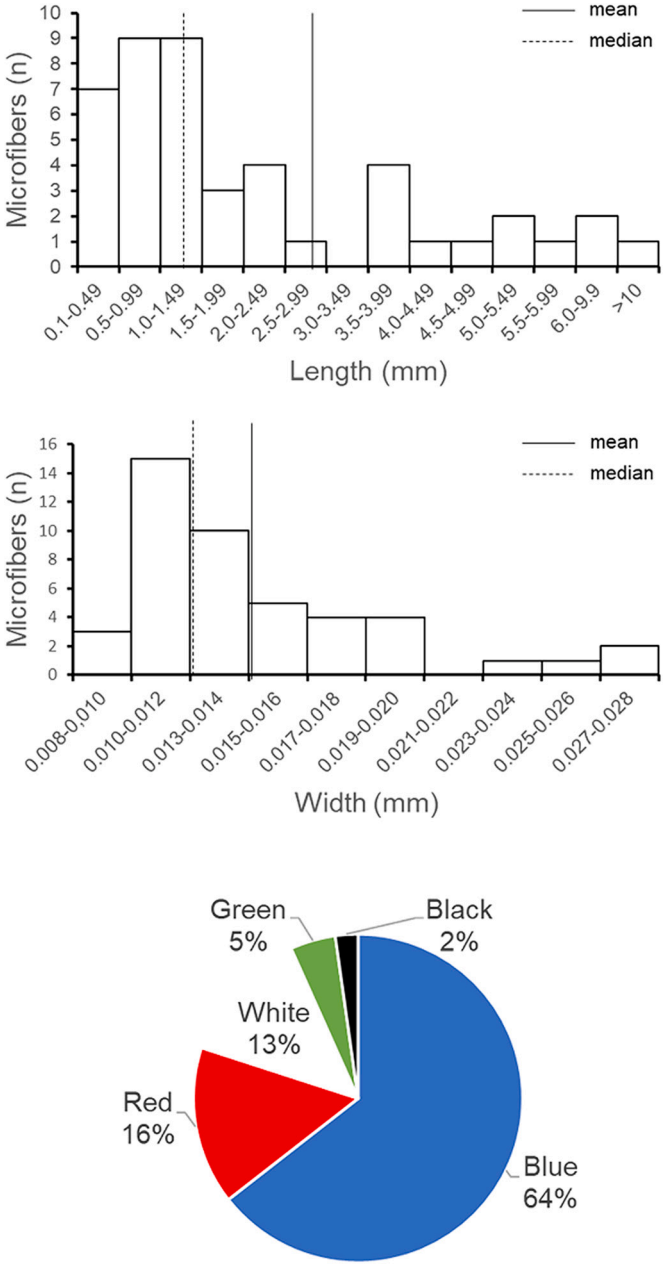

Fig. 2. Size (upper panel: length; middle panel: width) and color (lower panel) distributions of the anthropogenic fibers detected in black-legged kittiwake regurgitate samples (n = 45 fibers).

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 results revealed the presence of anthropogenic materials, consisting of cellulose and plastic polymer fibers mainly in the microplastics dimensional range, in chick stomach regurgitates of a surface-feeding seabird species breeding in the Gulf of Alaska, the black-legged kittiwake. Previous studies concerning plastic contamination in seabirds from this area were performed by visual sorting of relatively large plastic fragments (in the micro- and mesoplastics range) in stomach contents of

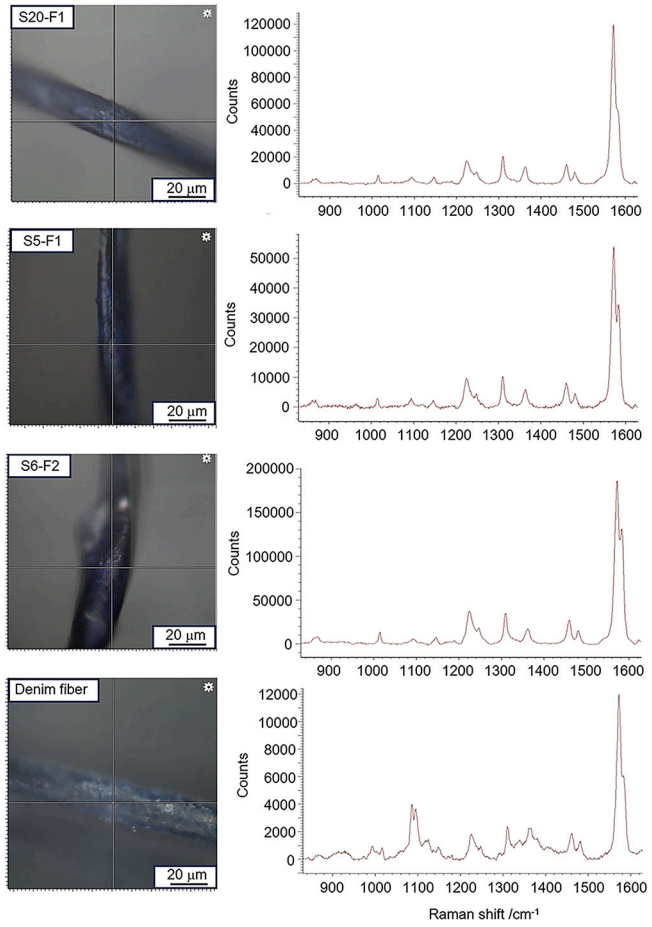

Fig. 3. Microscope images and Raman spectra of the three blue fiber S20-F1, S5-F1 and S6-F2 (Table S1) compared with a reference spectrum of Demin fabric fiber (Image and spectrum on the bottom). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

adult birds (Day, 1980; Robards et al., 1995; Bond et al., 2010; Padula et al., 2020). Moreover, the study by Padula et al. (2020) was performed only in the Aleutian Archipelago rather than in the central part of the Gulf of Alaska, and it mainly focused on phthalate contamination of bird tissues. Only the studies by Day (1980) and Robards et al. (1995) were performed in the same area of our study, but they employed a different, highly invasive, sampling methodology (shooting of adult birds). The latter study reported a plastic occurrence of 7.8 % (0.3 items per bird) in black-legged kittiwake stomach contents, mainly in the form of light-colored fragments, and found that surface-feeding petrels, such as fork-tailed storm-petrel (*Oceanodroma furcata*) and northern fulmar (*Fulmarus glacialis*), were more contaminated than other seabird species, such as the black-legged kittiwake (Robards et al., 1995). Baak et al. (2020b) confirmed the contamination difference between fulmars and kittiwakes, likely because of longer stomach retention of plastics in petrels compared to gulls (Ryan, 2015). 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, and 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 catch their prey (mainly fish) at greater depths through pursuit-diving. Poon et al. (2017) concluded that pursuit-diving avian predators were the least affected by microplastics contamination. Taken together, these studies suggest that surface-feeding, mostly zooplanktivorous, seabirds may more easily mistake microplastics for their natural prey or passively ingest them because microplastics are particularly abundant were zooplankton occurs, since both microplastics and zooplankton are transported by the same currents (Collignon et al., 2012; Suaria et al., 2020; De Pascalis et al., 2022).

Regarding µ-FTIR confirmed plastic fibers, the four fibers composed by petroleum-based synthetic polymers formally belonged to two different dimensional categories, micro- and mesoplastics, according to the 5 mm threshold (Barnes et al., 2009). Yet, in this work they were considered together in the category of synthetic polymers, because the two longer fibers were just above the 5 mm threshold and because other classifications increased, for fibers, the dimensional limits of 5 mm. Indeed, according to the International Organization for Standardization (ISO 4484-2:2023), microplastics include fibers with length $\leq$ 15 mm and the same definition was adopted in a recent update of the EU Drinking Water Directive (Directive 2020/2184).

Considering fibers composed by petroleum-based synthetic polymers, our findings are similar to those reported by Poon et al. (2017) and Baak et al. (2020b) 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 plastic contamination across the whole Arctic region (see reviews in Baak et al., 2020a, Kühn and van Franeker, 2020, Mishra et al., 2021, Collard and Ask, 2021 and Bergmann et al., 2022). Comparing our findings regarding plastics (20 % occurrence, 0.2 items per sample, all fibers in the dimensional range 0.3–5.9 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 not feasible because of the difference in the methodology of collection and analyses of samples (regurgitate in this study vs. stomach content in Robards et al., 1995). Regurgitates reflect plastic contamination of chick meal (proventriculus content), while hard fragments, originating from multiple meals, may accumulate within the gizzard that can be analyzed only by dissection of dead animals, by gastric lavage or pellet analysis (for species producing pellets). Nevertheless, our data suggest that chick meal contamination consists of very small fibers only, most of which were anthropogenic cellulosic fibers (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. Furthermore, microfibers were the most abundant anthropogenic particles (mostly blue-colored) in WWTPs effluents as well as in Arctic sediments and fish (Athey et al., 2020). 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. However, we should point out that Egger et al. (2020) focused on plastic fragments (rather than microfibers) sampled with a neuston net trawl (500 µm mesh size) whereas Athey et al. (2020) and Barrows et al. (2018) focused on all anthropogenic items (largely consisting of microfibers) originating from the analysis of water samples. More specifically, Barrows et al. (2018) reported that 91 % of the anthropogenic materials found in seawater

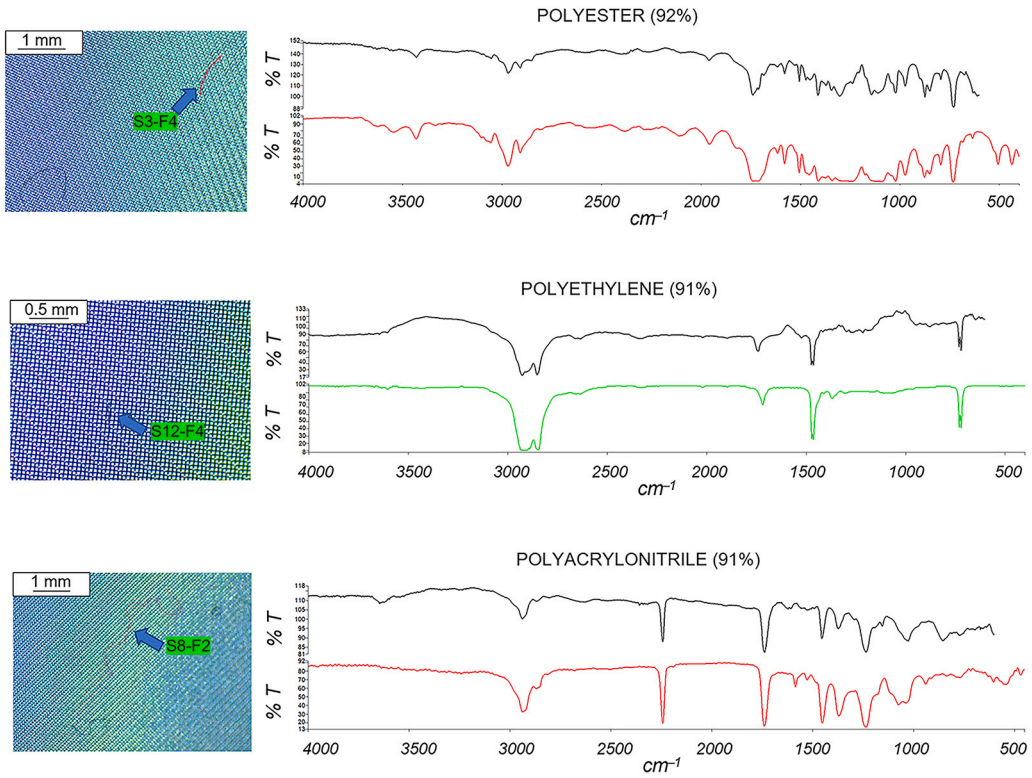

Fig. 4. Microscope images of three microplastics found in black-legged kittiwake regurgitate samples (right side) with their respective μ-FTIR spectra (%T = percentage of transmittance; cm^{-1} = wavenumber per cm). Each unknown spectrum (black line above) is compared with the best match from library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

were microfibers, with a high proportion of blue-colored fibers and a dimensional interval ranging mainly between 0.1 and 1.5 mm, similarly to values reported in our study (median length 1.3 mm).

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). These results indicate widespread contamination across the North American Arctic.

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 petroleum-based synthetic fibers (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 could be obtained. 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 suggested for mesoplastics (van Franeker and Law, 2015).

The occurrence of anthropogenic materials in kittiwake chick regurgitates highlights the need for a careful evaluation of their adverse effects at individual and population levels. For instance, the conservation status of black-legged kittiwakes was recently rated as vulnerable according to the massive population declines in the past decades and the expected continuing decline in the near future (BirdLife International, 2018). Main threats include climate change, fisheries, hunting, tourism and marine oil and plastic pollution (CAFF, 2020). It is thus essential to assess the potential adverse effects of plastic contamination on this species across its distribution range. Even if some evidence, based on visual sorting, may be suggestive of a very low incidence of plastic marine debris in chick meals of other species from the Aleutian Islands (Bond et al., 2010), we showed a much higher prevalence. Given that meta-analyses of microplastic pollution on marine species coherently revealed a wide spectrum of adverse effects (e.g. Bucci et al., 2020), we strongly suggest to not underestimate the possible adverse effects deriving from anthropogenic materials in chick meals.

Anthropogenic fibers in kittiwake regurgitates can be assumed

through fish and invertebrate prey (Hatch, 2013), whereas a direct ingestion of these materials (by mistaking them with prey) seems unlikely considering the mainly piscivorous diet of the species. In addition, kittiwakes may also ingest fibers directly from sea water during drinking and accidentally during foraging, although the amount of fibers assumed through these routes is difficult to establish and likely to be small. Given the above, we regard prey as the most probable origin of the anthropogenic materials found in regurgitates, which implies transfer of contaminants along the food chain. The transfer of microplastics and anthropogenic items along marine food webs is well documented (Mishra et al., 2021), but it remains unclear the extent of the bioconcentration potential and which are the fiber characteristics enhancing this phenomenon. The review of Walkinshaw et al. (2020) analyzed 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 and 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 web, 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 occurs along the food chain and, if it happens, at which trophic level it occurs.

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. A recent study by Caldwell et al. (2022) reported that blue microfibers were the prevailing anthropogenic material in tern (*Sterna* spp.) faeces, but did not analyze them by μ-RAMAN spectroscopy to confirm indigo-dye presence. Anthropogenic cellulose microfibers are emerging as a novel environmental pollutant. 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, kittiwake chick regurgitates should be regarded as an effective and non-invasive monitoring tool for assessing contamination from anthropogenic materials in Arctic food webs.

CRediT authorship contribution statement

Paolo Tremolada: Writing – review & editing, Writing – original draft, Visualization, Supervision, Methodology, Formal analysis, Data curation, Conceptualization. **Francesco Saliu:** Writing – review & editing, Supervision, Methodology, Investigation. **Anna S. Winkler:** Writing – review & editing, Supervision, Methodology, Investigation. **Cecilia P. Carniti:** Investigation. **Melisa Castelli:** Investigation. **Marina Lasagni:** Supervision, Methodology. **Sergio Andò:** Investigation. **Don-Jean Leandri-Breton:** Writing – review & editing, Visualization, Supervision, Investigation. **Marie Claire Gatt:** Writing – review & editing, Investigation. **Joan Ferrer Obiol:** Writing – review & editing, Investigation. **Marco Parolini:** Supervision, Resources. **Chinatsu Nakajima:** Writing – review & editing, Investigation. **Shannon Whelan:** Resources, Project administration, Data curation. **Akiko Shoji:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition. **Scott A. Hatch:** Writing – review & editing, Supervision, Resources, Project administration, Investigation, Funding acquisition. **Kyle A. Elliott:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition. **Jacopo G. Cecere:** Writing – review & editing, Supervision, Investigation, Conceptualization. **Diego Rubolini:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Investigation, Funding acquisition, Data curation, Conceptualization.

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.

Data availability

Data will be made available on request.

Acknowledgements

We thank two anonymous reviewers for constructive criticism on a previous draft of the manuscript. The study was partly funded by the Italian Ministry for University and Research (PRIN 2017 funding scheme, grant number 20178T2PSW to D.R.)

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marpolbul.2024.116401.

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