Environment International 134 (2020) 105303

Contents lists available at ScienceDirect

Environment International

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

Full length article

Microplastic study reveals the presence of natural and synthetic fibres in the diet of King Penguins (*Aptenodytes patagonicus*) foraging from South Georgia

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ARTICLE INFO

Handling Editor: Dr. Da Chen

Keywords:
Antarctic predator
Microplastics
Fibres
Mesopelagic fish
Breeding stage
Foraging strategies

ABSTRACT

Marine ecosystems are experiencing substantial disturbances due to climate change and overfishing, and plastic pollution is an additional growing threat. Microfibres are among the most pervasive pollutants in the marine environment, including in the Southern Ocean. However, evidence for microfibre contamination in the diet of top predators in the Southern Ocean is rare. King Penguins (*Aptenodytes patagonicus*) feed on mesopelagic fish, which undergo diel vertical migrations towards the surface at night. Microfibres are concentrated in surface waters and sediments but can also be concentrated in fish, therefore acting as contamination vectors for diving predators feeding at depth. In this study, we investigate microfibre contamination of King Penguin faecal samples collected in February and March 2017 at South Georgia across three groups: incubating, chick-rearing and non-breeding birds. After a KOH digestion to dissolve the organic matter and a density separation step using a NaCl solution, the samples were filtered to collect microfibres. A total of 77% of the penguin faecal samples (36 of 47) contained microfibres. Fibres were measured and characterized using Fourier-Transform Infrared spectroscopy to determine their polymeric identity. Most fibres (88%) were made of natural cellulosic materials (e.g. cotton, linen), with only 12% synthetic (e.g. polyester, nylon) or semi-synthetic (e.g. rayon). An average of 21.9 ± 5.8 microfibres g^{-1} of faeces (lab dried mass) was found, with concentrations more than twice as high in incubating penguins than in penguins rearing chicks. Incubating birds forage further north at the Antarctic Polar Front and travel longer distances from South Georgia than chick-rearing birds. This suggests that long-distance travelling penguins are probably more exposed to the risk of ingesting microfibres when feeding north of the Antarctic Polar Front, which might act as a semi-permeable barrier for microfibres. Microfibres could therefore provide a signature for foraging location in King Penguins.

1. Introduction

At a time when marine ecosystems are experiencing substantial disturbances (Richardson and Polocsanska, 2008) such as climate change (IPCC, 2007; Brierley and Kingsford, 2009; Doney et al., 2012;IPCC, 2018), overfishing (Jennings and Kaiser, 1998) and species invasions (Elton, 1958; Katsanevakis et al., 2014), plastic pollution has been recognized as another major threat for the ocean. Global plastic production has increased substantially over the last 60 years, from 0.5 million tonnes (MT) in 1960 to 348 MT in 2017 (Plastics Europe, 2018), and almost 300 MT of plastic debris is estimated to be floating at the sea surface globally, with more deposited on the seafloor and along shorelines (Boerger et al., 2010; Browne et al., 2011; Eriksen et al., 2014).

Most plastic debris in the ocean is thought to derive from land-based sources: beaches; rivers; wastewater discharges, and transport of land litter by wind. Items of large plastic debris have long been the focus of public concern, mainly due to the various documented negative impacts on wildlife and their obvious visibility (Gall and Thompson, 2015; Zettler et al., 2017). However, microplastics (plastic particles < 5 mm, Arthur et al., 2009) are now recognized as key components of plastic contamination in marine environments. Most microplastics form from the breakdown of larger plastic items (Gregory and Andrady, 2003; Barnes et al., 2009; Wright et al., 2013), although some primary

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https://doi.org/10.1016/j.envint.2019.105303
Received 2 July 2019; Received in revised form 25 October 2019; Accepted 1 November 2019

microplastics deriving from textiles, cosmetics, industrial and medical applications can be introduced directly into the ocean as micron-sized particles (Gregory, 1996; Fendall and Sewell, 2009). Microplastics are now ubiquitous, occurring in environments from the equator to the poles and from the coast to abyssal sediments (Zarfl and Matthies, 2010; Lusher et al., 2015; Van Cauwenberghe et al., 2015).

By far the most abundant microplastics in oceanic surface waters are microfibres (Barrows et al., 2017), which are threadlike particles derived from clothes, carpets and similar products. For instance, more than 1900 microfibres can be released from a single polyester fleece jacket per wash (Browne et al., 2011) and a 5 kg wash load containing polyester textiles releases over 6,000,000 microfibres (De Falco et al., 2018). Although ~90% of microplastics are thought to be retained by wastewater treatment plants (Ziajahromi et al., 2016), it is now widely recognized that washing clothes releases microfibres in wastewater because their small size allows them to pass easily through treatment systems. Microfibres are generally assumed to be made from synthetic materials such as polyester or polyamide ('nylon'), but fibres of natural materials, e.g. wool and cotton, are also found in the ocean (Barrows et al., 2018). In 2017, more than 100 MT of fibres were produced worldwide (Textile Exchange, 2018), of which natural fibres accounted for about 30%, with the remainder being predominantly synthetic fibres (Carr, 2017; Textile Exchange, 2018). Because of the amount of textile fibres produced annually, and the fact that there is presently no global regulation of the discharge of fibre-contaminated wastewater, there is an urgent need to monitor and assess the presence and impacts of microfibres, both natural and synthetic, on marine ecosystems.

Ingestion of microplastics by low trophic level organisms (e.g. zooplankton) may be a potential pathway for transfer into the marine food chain (Setälä et al., 2014; Nelms et al., 2018). In addition to physical effects on single organisms, the potential ecological implications are even worse for larger organisms as microplastics are known to accumulate persistent organic pollutants (POPs) and can release these toxic substances upon ingestion (Rios et al., 2007; Teuten et al., 2009) and even induce pathologies (Rochman et al., 2013). Similar microfibre composition in both invertebrates and shorebird faeces along the Eastern Atlantic Ocean suggests that birds mainly ingest microfibres through their prey, confirming microfibre transfer through the food web (Lourenço et al., 2017). Recent studies report microplastics (both fibres and fragments) in mesopelagic fish from the North Pacific (Boerger et al., 2010; Davison and Asch, 2011) and North Atlantic Oceans (Lusher et al., 2016; Wieczorek et al., 2018), with contamination rates ranging between 9% and 75% of individuals. In addition, 73% of fish from seven mesopelagic fish species collected at depths of 300–600 m in the Northwest Atlantic Ocean contained plastics (98% microfibres) in their digestive tract, similar to fibres sampled in surface waters (Wieczorek et al., 2018). Many mesopelagic fish species undergo diel vertical migrations (DVM), i.e. they reside at depth during the day to avoid visual predators, migrate towards the surface at dusk to feed on zooplankton during the night, and descend back to depth at dawn (Clark and Levy, 1988; Brierley, 2014). This migration serves as an active mechanism for transporting microplastics from the surface deeper into the ocean (Wright et al., 2013). In turn, mesopelagic fish could act as a potential source of microplastics to larger predatory organisms, including seabirds and marine mammals that feed at the surface during the night or at depth during the day.

There is increasing evidence that predators feeding at depth are also affected by plastic contamination. Microplastics have been found in the digestive tract of a deep-diving cetacean, the True's Beaked Whale (*Mesoplodon mirus*), which can feed at depths exceeding 2000 m (Aguilar de Soto et al., 2017) on cephalopods and mesopelagic fish (Lusher et al., 2015). A study of 51 scats of South American Fur Seals (*Arctocephalus australis*) found no microplastic fragments, but 67% of individuals contained large numbers of microfibres (Perez-Venegas et al., 2018). Microplastic fragments have been found in the scats of sub-Antarctic Fur Seals (*Arctocephalus tropicalis*) presumably as a result of ingestion by their prey, including myctophid fish (Eriksson and Burton, 2003). Only a few studies report microplastics in the faeces of birds. Reynolds and Ryan (2018) analysed 283 faecal samples from 7 different species of ducks for microplastics. Authors detected the presence of microfibres in 5% of the samples and they also found differences across species, suggesting that microfibre ingestion can be influenced by foraging behaviour (Reynolds and Ryan, 2018). In a study focusing on Northern Fulmars (*Fulmarus glacialis*), microplastics have been found in 47% of the faecal samples (Provencher et al., 2018).

Although plastic ingestion by seabirds has been the focus of numerous studies, data for deep-diving seabirds remain scarce compared to birds that feed close to the surface (Ryan, 1987; Brandão et al., 2011; Codina-García et al., 2013; Provencher et al., 2014). Evidence to date has suggested that penguins are not so strongly impacted by plastic debris ingestion, probably because penguins target live prey and do not pay attention to inert items (including floating plastic), unlike other seabirds that scavenge such as albatrosses and petrels (Ropert-Coudert et al., 2019). However, entanglement (mainly from abandoned or lost fishing gear) has been reported for 7 of the 18 penguin species, with African (*Spheniscus demersus*) and Little (*Eudyptula minor*) penguins being the most affected (Ryan, 2018). In addition, there is a risk of indirect microplastic contamination via transfer from their prey such as pelagic or mesopelagic fish (Nelms et al., 2018), as suggested in a recent study showing that microplastic fibres and fragments were found in the scats of Gentoo Penguins (*Pygoscelis papua*) from two different colonies in the Scotia Sea (Bessa et al., 2019a).

The King Penguin (*Aptenodytes patagonicus*) breeds at sub-Antarctic islands throughout the Southern Ocean, where it is one of the most important avian consumers (Woehler, 1995). King Penguins are capable of diving to a depth of 400 m (Charrassin et al., 2002) and feed mainly on mesopelagic fish (especially on myctophids, which account for ≥90% of their diet by mass) (Adams and Klages, 1987; Cherel et al., 2002). King Penguins target the Antarctic Polar Front to forage, which is known to be a productive zone in many sectors of the Southern Ocean (Bost et al., 1997; Charrassin and Bost, 2001; Sokolov et al., 2006), and is especially important for King Penguins breeding at South Georgia (Scheffer et al., 2010).

In this study, we examined fresh faecal samples collected from King Penguins breeding at South Georgia for microplastics. Our objectives were to determine if there were microplastics in the faecal samples and to examine variability in microplastic abundance and composition across three groups: incubating; chick-rearing and non-breeding birds.

2. Material and methods

2.1. Faecal sample collection

A total of 47 faecal samples were collected from adult King Penguins breeding at the Hound Bay colony, South Georgia (54°39′S, 36°27′W) from the 19th of February until the 11th of March 2017 as part of the 2016–2017 Antarctic Circumnavigation Expedition (ACE). Samples were collected from the ground using a clean metal spatula immediately after observing a bird defecate, and care was taken to not pick up any underlying soil or silt. After each use, the metal spatula was rinsed with pre-filtered ethanol solution to remove external contamination. Immediately after collection, the samples were placed in sterile 2 mL Eppendorf tubes. The tubes were filled with pre-filtered (pore size < 1 μm) 80% ethanol solution and closed immediately after in order to minimize sample exposure to the air. Samples were kept frozen (−20 °C) until the microplastics extraction phase. One third of the samples were collected from non-breeding adults (n = 16), another third from incubating birds (n = 16), and the remaining samples from chick-rearing adults (that were brooding small chicks 1 to 2 weeks of age) (n = 15). All faecal samples were collected by the same two fieldworkers, both wearing the same field equipment provided by the British Antarctic Survey, which included an orange suit. They were all

brand new clothes (same fabric and same colour for both fieldworkers).

2.2. Microplastic extraction

Extraction of microplastics was performed according to the protocols described in Avio et al. (2015) and Bessa et al. (2019a,b). Samples were defrosted and the ethanol was removed from the Eppendorf tubes using a sterile syringe. The needle was held next to the tube wall and below the liquid surface, in order to minimize the chances of capturing any fibres floating in the tubes. The remaining content of the tube (i.e. the faecal sample) was transferred into a clean metal cup. Wet and lab dried masses of each sample were measured, with sample drying being achieved overnight in a laboratory oven at 50 °C. The dry content of the metal cup was placed in a clean mortar to be triturated. The powder obtained was then placed in an Erlenmeyer glass and completely covered (ratio > 5:1) with 40 mL of a pre-filtered 10% potassium hydroxide (KOH) solution (prepared by diluting 10 g of KOH in 100 mL of milli-Q water) for pre-digestion of the organic matter. The samples were kept at 50 °C overnight to accelerate the reaction, and then transferred into clean graduated glass cylinders. 100 mL of filtered hypersaline solution (prepared by adding NaCl in milli-Q water until density reached 1.2 g mL^{-1}) was then added to the samples for density gradient separation. After stirring, the samples were left to settle for 10 min and the supernatant was collected. This process was repeated twice, and the edges of the cylinder were rinsed every time with milli-Q water to avoid loss of particles. Samples were then vacuum-filtered onto clean glass microfibre filters (1.2 µm nominal pore size), labelled and stored in 47 mm petri dishes securely closed using parafilm (© Nescofilm). All samples were then examined using a stereomicroscope (45x magnification). All fibres found in the samples were counted and classified according to their colour.

2.3. Contamination control

Procedural blanks (n = 17) were run after every third sample to assess the level of external contamination associated with the laboratory extraction protocol (preparation of the solutions and quality of equipment used). Milli-Q water was filtered using the same equipment and filtration apparatus as the samples. All lab-ware and equipment used was carefully rinsed with milli-Q water prior to use and precautions were taken to minimize aerial contamination. In addition, 17 procedural air blanks were run during sample handling and processing to determine the levels of aerial contamination during laboratory procedures. Clean glass microfibre filters were left exposed next to the samples for the entire duration of the microfibre extraction procedure. The filtering equipment was kept covered as much as possible and exposure of the samples was kept to the minimum. White cotton lab coats were used at all times during laboratory procedures.

2.4. Microfibre characterisation

Both fibres extracted from penguin faecal samples and procedural blanks were analysed using Fourier Transform Infrared (FT-IR) spectroscopy to determine their polymeric composition. µFT-IR analyses were conducted at ISMAR-CNR using a LUMOS standalone FT-IR microscope (Bruker Optik GmbH) equipped with a motorized XY sample stage and an automated Attenuated Total Reflection (ATR) probe (Ge crystal). All fibres were carefully hand-picked using forceps and placed on a glass slide for analysis. Prior to each scan, fibre length and diameter were measured to the nearest micron from the digital images collected by the instrument. Following background scans, ATR spectra were recorded by averaging 64 scans per item with a spectral resolution of 4 cm^{-1} (range 4000–650 cm^{-1}). CO_2 interference (adsorption at 2300–2400 cm^{-1}) was removed for clarity. After acquisition, infrared spectra were processed and analysed using OPUS 7.5 software (Bruker). Polymer identification was performed by comparison with commercially available libraries and a custom library compiled within the framework of the JPI-OCEANS project BASEMAN by the Alfred Wegener Institute in Helgoland, Germany (Primpke et al., 2018). Only matches > 75–80% with reference spectra were accepted as verified polymers.

2.5. Data analysis

All statistical analyses were performed using the R software (R Development Core Team, 2015). The alpha level for all significance tests was set at 0.05 and results are generally presented as mean ± standard error (SE).

2.5.1. Concentrations and dimensions of microfibres in the samples

After testing for normality using Shapiro-Wilk tests, Kruskal-Wallis tests were performed to compare the concentrations of microfibres encountered in samples from the different groups (chick-rearing, incubating and non-breeding birds, as well as in the procedural blank samples). In the event of a significant p-value, these tests were followed by post-hoc Mann-Whitney U-tests to identify which group was different than the others. Numbers of microfibres per sample were calculated as the number of microfibres counted per sample, minus their respective procedural blank and procedural air blank fibres. Concentrations of microfibres per sample were calculated as the net number of microfibres found per gram (lab dried weight) of the faecal sample. In some instances, there were fewer fibres in the sample than in the blank, in which case the counts were set to zero. Similarly, Kruskal-Wallis tests followed by Mann-Whitney U-tests were performed to compare the length and diameter of microfibres between the three different groups (chick-rearing, incubating and non-breeding birds) and the procedural blanks. The Bonferroni correction was applied to correct the level of significance when multiple comparisons were performed simultaneously.

2.5.2. Colours of microfibres

Multidimensional Scaling (MDS) ordination was performed based on a Bray-Curtis dissimilarity matrix to investigate whether the colours of microfibres in the faecal samples and the procedural blanks were similar. We compared the colours in the samples with those in the procedural blanks to understand if the microfibres were coming from different populations (i.e. if all fibres from the blanks were of a certain colour that was not found in the samples). A betadisper test was run to test homogeneity of dispersion among groups (three groups and procedural blanks), which is a condition for adonis (betadisper and adonis functions from package vegan in R; Oksanen et al., 2019). Adonis tests whether colour composition among groups is similar or not. See Supplementary Material 1 for more information on MDS and Adonis.

2.5.3. Polymer composition

MDS ordination was performed based on a Bray-Curtis dissimilarity matrix to investigate whether the polymer compositions of microfibres contained in the three different groups (chick-rearing, incubating and non-breeding birds) and in the procedural blanks were similar. Betadisper and adonis tests were also run for this analysis.

3. Results

3.1. Microfibre' quantification among groups

The only man-made items found in the faecal samples were microfibres, which were present in 77% of the samples (36 out of 47). A total of 264 fibres were counted in all samples (63 in chick rearing, 108 in incubating, 93 in non-breeding). Only three fibres were found in procedural air blanks (0.188 ± 0.090 microfibres per sample, n = 17) indicating very low aerial contamination levels during sample handling. A total of 59 microfibres were found in the procedural blanks (n = 17),

Table 1
Concentrations of microfibres in King Penguin faecal samples for the three different groups. All results are given as number of microfibres.g^{-1} (lab dried weight) after correcting for experimental contamination levels. 'mf' = microfibres.

Group	Total number of microfibres	Concentration of microfibers (mean ± SE)
All groups (n = 47)	264	21.9 ± 5.8 mf·g^{-1}
Chick-rearing (n = 15)	63	7.0 ± 3.2 mf·g^{-1}
Incubating (n = 16)	108	26.0 ± 8.7 mf·g^{-1}
Non-breeding (n = 16)	93	31.7 ± 14.2 mf·g^{-1}

indicating a higher contamination risk of 3.1 ± 0.3 microfibres per sample, but still significantly lower than the mean number of microfibres extracted from penguin samples (W = 43; p = 0.006). After accounting for procedural contamination, 111 fibres were counted in the samples (15 in chick-rearing birds, 55 in incubating birds and 41 in non-breeding birds) and an average density of 21.9 ± 5.8 microfibres·g^{-1} (lab dried weight) was obtained across all groups (Table 1).

The Kruskal-Wallis test applied to the microfibre concentrations showed significant differences between groups (Kruskal-Wallis: χ^2 = 5.8254, p-value = 0.043). There were significantly higher concentrations of fibres in lab dried faeces from incubating birds than from birds brooding chicks (Mann-Whitney *U* test: W = 55; p = 0.031; Table 1, Fig. 1). However, no significant difference was observed between chick-rearing and non-breeding individuals (W = 93; p = 0.833) or between incubating and non-breeding birds (W = 153; p = 1).

3.2. Microfibre dimensions

Mean fibre length in penguin samples was 1684 ± 92 µm (range: 186–9280 µm) and mean fibre diameter was 18.5 ± 0.53 µm (range: 5–100 µm, Table 2). There were no statistical differences among groups, including procedural blanks, for microfibre length (Kruskal-Wallis: χ^2 = 3.2959, p-value = 0.348) and for microfibre diameter (Kuskal-Wallis: χ^2 = 7.2681, p-value = 0.064) (Table 2).

3.3. Variations in microfibres' colours encountered

Most fibres found were either black (50%), grey (19%) or blue (18%) in colour. Additional details on the colour composition in the faecal samples and in the procedural blanks are given in Supplementary Material 2.

Table 2
Mean ± SE length and diameter (µm) of microfibres in penguin faecal samples for the three groups and the procedural blanks.

Group	Length (µm)	Diameter (µm)
All groups	1684 ± 92	18.5 ± 0.53
Chick-rearing	1607 ± 151	18.1 ± 1.51
Incubating	1746 ± 173	17.6 ± 0.51
Non-breeding	1667 ± 138	19.7 ± 0.85
Procedural blanks	1573 ± 197	18.0 ± 0.68

The sample sizes were unbalanced between groups and the betadisper condition was not met for tests of microfibre colour variability (F = 7.77; p < 0.001), meaning that the dispersions among groups (chick-rearing, incubating, non-breeding and procedural blanks) were heterogeneous. The adonis function was then rerun on 56 samples (14 samples for each group, balanced design) and the results for adonis (based on Bray-Curtis dissimilarity matrices and 999 permutations) showed that there was no significant effect of 'group' on the colour composition of microfibres (F = 1.41, R^2-group = 0.075, p = 0.19) and that around 92% of the variance remained unexplained. Accordingly, all ellipses are overlapping in the MDS ordination plot (Supplementary Material 3).

3.4. FTIR characterisation: Synthetic vs natural fibres

The polymer composition of 295 fibres was identified using µFTIR: 236 from penguin samples (89.4% of all fibres collected) and 59 from procedural blanks (100% of the fibres counted). The three fibres from the procedural air blanks and 28 fibres extracted from the faecal samples were too small to be handled with laboratory forceps and were not identified.

Of the fibres identified from penguin samples, 84.7% were cellulosic (n = 200 fibres), 3.0% were wool (n = 7 fibres) and only 12.3% (n = 29 fibres) were synthetic. Overall, 87.7% of the fibres analysed were natural fibres of vegetal or animal origin. Cellulose was the most abundant polymer found in the faecal samples among all groups (accounting for 46.7% in the chick-rearing group, 53.7% in the incubating group and 55.6% in the non-breeding group) followed by cotton (accounting for 35% in the chick-rearing group, 30.5% in the incubating

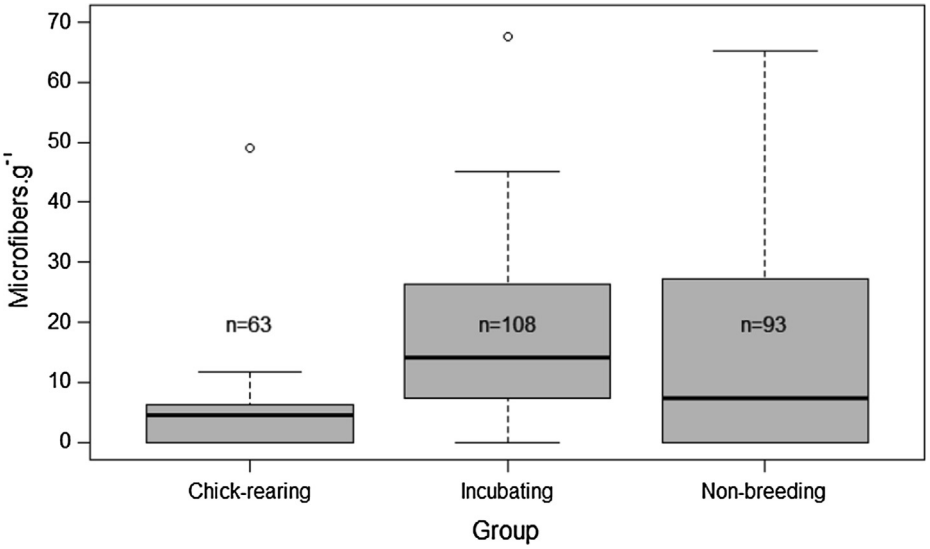

Fig. 1. Concentrations of microfibres found in King Penguin faecal samples for the three groups: chick-rearing (63 microfibres), incubating (108 microfibres) and non-breeding (93 microfibres) birds. Concentrations are given in microfibres·g^{-1} (lab dried weight) and are corrected for experimental contamination levels.

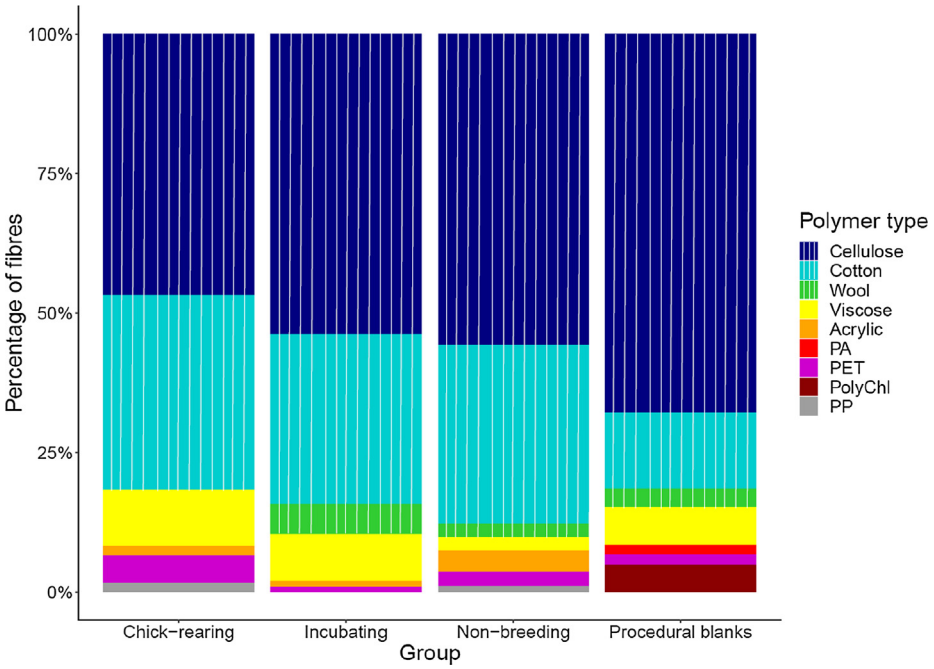

Fig. 2. Proportions of microfibres found in King Penguin faecal samples across the different groups: chick-rearing (63 microfibres), incubating (108 microfibres), non-breeding (93 microfibres) birds and procedural blanks (59 microfibres). White hatched categories refer to natural polymer types. PA = Polyamide (nylon), PET = Polyethylene terephthalate (polyester), PolyChl = Polychloroprene, and PP = Polypropylene.

group and 32.1% in the non-breeding group) (Fig. 2). Synthetic fibres accounted for 18.3% of chick-rearing birds, 10.5% of incubating birds and 9.8% of non-breeding birds. Of the 29 synthetic fibres extracted from penguin samples, 13 were purely synthetic (i.e. acrylic, polyester and polypropylene) and 16 were semi-synthetic (i.e. viscose/rayon). The most common synthetic fibre type was polyester (6 fibres). Similar proportions were found in the procedural blanks: 85% of fibres were of natural origin (82% cellulosic and 3% wool), although a lower proportion of cotton was found (13.6%). Acrylic (5 fibres) and polypropylene (2 fibres) were only found in penguin samples, whereas polyamide (nylon, 1 fibre) and polychloroprene (3 fibres) were only found in the procedural blanks (Fig. 2). The betadisper condition for adonis was met ($F = 0.35$; $p = 0.79$), meaning that the dispersion among groups (chick-rearing, incubating, non-breeding) was homogeneous and the adonis test revealed that there was no significant difference in microfibre composition between penguin samples and procedural blanks ($F = 0.81$, R^2-group $= 0.041$, $p = 0.61$; see Supplementary Material 4 for the MDS plot). Details concerning the FTIR analysis of microfibres found in penguin faecal samples across the three groups and the procedural blanks are given in Supplementary Material 5.

All main variables are presented in Supplementary Material 6, following the guidelines suggested in Provencher et al. (2017).

4. Discussion

This study provides the first evidence of microfibre ingestion by King Penguins. Microfibres were found in most samples (~77%), with an average concentration of 21.9 ± 5.8 microfibres.g^{-1} of lab dried faeces. However, most fibres (~88%) were made of natural cellulosic materials (cotton, linen), with only a few purely synthetic fibres (polyester, polypropylene and acrylic).

4.1. Quantities of microfibres

The quantity of microfibres in faecal samples from incubating birds was twice as high as chick-rearing birds. Two possible hypotheses might explain this difference. Firstly, adults might offload fibres to their chicks in regurgitated meals, lowering the level of contamination in the faeces of the chick-rearing group. This phenomenon occurs in petrels that accumulate plastic in their gizzards (Ryan, 1988; Rodríguez et al., 2012). Secondly, microfibre dispersal processes might be restricted across frontal systems. The Antarctic Polar Front is associated with dynamic mesoscale features such as eddies, which might assist the transfer of biotic and abiotic materials across the frontal system (see Waller et al., 2017) but the transport is still mainly oriented eastward, potentially limiting the cross-front transport and making the Antarctic Polar Front a semi-permeable barrier for microfibres. As a result, it is possible that there is a dilution in microfibre concentrations south of the Antarctic Polar Front. Incubating King Penguins perform longer foraging trips than chick-rearing birds, and target the Antarctic Polar Front, a productive area of particular importance for this species (Scheffer et al., 2010). Individuals feeding at lower latitudes, close to the Antarctic Polar Front, might be more exposed to the risk of ingesting microfibres, in which case microfibres in faecal samples could provide a potential signature of foraging at the Antarctic Polar Front.

4.2. Types of microfibres

The fact that there were no significant differences in the colour and the composition of fibres found across the three groups (chick-rearing, incubating and non-breeding birds) suggests that the origins of the fibres are similar for each group. Most microfibres in penguin faecal samples were black, blue and grey, similar to the colours reported in other studies (Gago et al., 2018). That high proportions (> 80%) were natural fibres is also in keeping with the emerging trend from other studies. Remy et al. (2015) showed that most fibres ingested by invertebrates in the Mediterranean Sea also were cellulosic, and 80% of the microfibres collected from surface sediments in southern European deep seas were made of cellulose (Sanchez-Vidal et al., 2018). Stanton et al. (2019) found that textile fibres collected from the river Trent (UK) are dominated by natural, not microplastic, fibres. This pattern might change in future as we produce more clothes from synthetic materials compared to clothes from natural sources. The relatively high proportion of microfibres from natural origins in the Southern Ocean also might be a consequence of slow degradation rates of both natural and synthetic fibres due to low temperatures in the region.

4.3. Origins of the microfibre contamination

Until recently, it was thought that the Southern Ocean experienced negligible microplastic pollution because it is distant from human populations and oceanographically isolated by the Antarctic Polar Front, which may act as a barrier to dispersal (Clarke et al., 2005; Fraser et al., 2011; Fraser et al., 2016). However, Fraser et al. (2018) demonstrated that eddies and surface waves in the Southern Ocean can strongly enhance connectivity for particles drifting at the surface of the ocean, which can even cross fronts, therefore suggesting that the Southern Ocean might not be isolated biologically.

Microplastics (including fibres) have been found in intertidal sediments from South Georgia (Barnes et al., 2009), as well as in marine sediments in the Atlantic sector of the Southern Ocean (Van Cauwenberghe et al., 2013), Terra Nova Bay (Munari et al., 2017), in the Antarctic Peninsula region (Waller et al., 2017; Reed et al., 2018; Absher et al., 2019; Lacerda et al., 2019), in the Ross Sea (Cincinelli et al., 2017) and in the Pacific sector of the Southern Ocean (Isobe et al., 2019). In addition, Waller et al. (2017) estimated that over a decade between 0.5 and 25.5 billion are released into the Southern Ocean from local sources (i.e. ships and research stations). Given that microplastics are present in the Southern Ocean, the potential exists for them to be in the diet of fish and higher predators.

King Penguins mainly feed on mesopelagic fish ($\geq$90% of their diet by mass; Adams and Klages, 1987; Cherel et al., 2002) and are likely to indirectly ingest microplastics via contaminated prey, even if they feed at depth during the day. Several studies have shown that fish act as a source of microplastic contamination for marine predators. For instance, mesopelagic fish are thought to be the source of plastic fragments in fur seals scats at Macquarie Island (Eriksson and Burton, 2003). Microfibres also have been found in the stomach contents of Pacific Sand Lance (*Ammodytes personatus*) and Pacific Herring (*Clupea pallasii*) consumed by Rhinoceros Auklets (*Cerorhinca monocerata*) (Hipfner et al., 2018).

Our results suggest that trophic transfer (i.e. from fish to penguins) likely represents an indirect pathway for microfibre contamination in King Penguins. However, the possibility that the microplastics found in penguin faeces are a result of direct, accidental consumption cannot be excluded. Other potential sources of microfibre contamination in faeces include external contamination from the soil during sample collection, and contamination from our field clothing. Blanks from the field could have been taken in order to measure any background air contamination, but this was not done since the faecal samples were not collected with the objective of a plastic-contamination study in mind. Samples were collected for a diet study, and contamination blanks were not required for that. All samples were however collected by the same two fieldworkers, both wearing the same brand new field clothing provided by the British Antarctic Survey. Although orange garments were included in that field equipment, no orange fibres were found in any of the faecal samples, which would seem to exclude that source of contamination.

In addition, both operators used exactly the same sampling technique, immediately closing the clean vials after collecting the samples, limiting exposure time to the air. As a result, these potential biases are likely to be consistent for all samples, and thus do not result in significant differences among groups.

4.4. The potential impacts of microfibres on King Penguins

Since plastic production and plastic waste are increasing, it is expected that the number of species impacted will continue to increase in the future.

Chemicals may leach from plastics into seabird stomach oil at a faster rate than into seawater (Tanaka et al., 2015). As a result, microplastics may introduce harmful substances into food webs provided they are retained long enough in organisms, with unknown ecological effects that might be amplified due to bioaccumulation and biomagnification (Teuten et al., 2009). The long residence time of plastic in marine ecosystems could harm marine life for many decades even in a scenario involving the immediate cessation of production and discarding of plastics. Microfibres contain chemicals and plastic additives such as dyes or flame retardants that are commonly used for textiles (Machado et al., 2018) that might enhance bioavailability of toxic compounds to organisms ingesting microfibres (Henry et al., 2019). We might expect that because most fibres found in the diet of King Penguins are mostly from a natural origin, these fibres have little impact on penguins. However, so-called “natural” fibres also often contain just as much chemical dyes and other additives as synthetic microfibres, that could be deleterious to penguins.

However, microplastics do not necessarily leach chemicals into seabirds. Indeed, Koelmans et al. (2016) found that the flux of hazardous hydrophobic organic chemicals (HOCs) from ingested microplastic was much lower than the flux of HOCs bioaccumulated from prey, rejecting the hypothesis that microplastic ingestion is always associated with an increase exposure to HOCs. In a study comparing Persistent Organic Pollutants (POP) concentrations in the liver and muscle tissues of fulmars with those in the plastic that they ingested (present in the stomach contents), Herzke et al. (2016) found that plastic is relatively passive in terms of POP contamination in tissues, and that the POP concentrations in body tissues reflect those of simultaneously ingested prey. In addition, if leaching occurs, this does not only concern the chemicals present in the plastic itself (e.g. flame retardants or heavy metals) but also hydrophobic waterborne pollutants that can adhere on the hydrophobic surface of plastics (Cole et al., 2011). This is especially true for microplastics which have a large surface to volume ratio (Betts, 2008; Ashton et al., 2010). However, marine predators are also subject to these pollutants with their prey as pollutants are known to bioaccumulate in food webs (Gobas et al., 1993; Kelly et al., 2007).

Recent studies highlight that ingested plastic can cause gut inflammations and if particles are very small, they may be able to penetrate the digestive tract barrier to reach the blood or other organs and affect their functioning. Indeed, Lu et al. (2016) found that 5 μm diameter microplastics can accumulate in the gills and the liver of zebrafish, and can cause lipid accumulation in fish liver and oxidative stress. In another study, Mattsson et al. (2017) demonstrated that nanoplastics were responsible for reducing survival rates in zooplankton and that they could pass from the circulatory system to the brain tissue and alter fish behaviour. These findings provide new insights into the toxic effects of microplastics on fish, but it remains unknown whether this toxicity could also occur in fish predators such as King Penguins. This concept is called “translocation” and is proposed as a priority for research on microplastics (Paul-Pont et al., 2018).

Although adverse biological effects of the ingestion of microfibres on primary consumers are coming to light (Watts et al., 2015; Jemec et al., 2016; Woods et al., 2018), whether these translate into impacts on higher trophic-level predators is, as yet, unclear. More specifically, little is known concerning the impact of microfibres on seabirds. A study looking at food transit rates in African Penguins by assessing the time necessary to excrete food with a marker highlighted that to excrete 95% of the marker, penguins needed 21 h on average (Laugksch and Duffy, 1986). It is not unreasonable to believe that most microfibres ingested by King Penguins are excreted rapidly (short residence time in organisms), in which case microfibres might not have a major physical impact on the birds. More data are needed to better understand the potential effects of microfibres for this particular species.

Of more concern than the toxicity of microfibres is the eventuality in which large quantities of microfibres are ingested by the penguins’ prey. Impacts at the base of the food chain such as blockage or damage of digestive tracts, false food satiation due to the fact that a proportion of the stomach volume is filled with nutritionally worthless plastic, or transfer of toxic compounds, could directly impact the population

dynamics of these prey organisms and therefore affect food availability for penguins. In addition, potential bioaccumulation and biomagnification processes could amplify the negative effects of chemicals observed for prey species (Cole et al., 2013; Besseling et al., 2013; Teuten et al., 2009), that could in turn have negative consequences on higher predators such as fish (Lusher et al., 2013; Romeo et al., 2015) and seabirds (Furness, 1983; Ryan, 2019). For this reason, it is urgent that strong measures are taken to address the problem of microfibre releases into the environment and that plans are implemented to monitor microfibre contamination of marine ecosystems over the long term.

4.5. Perspectives and recommendations

It remains unclear whether the microfibres found in penguins originate from trophic transfer (via consumption of contaminated mesopelagic fish) or from direct consumption (e.g. while drinking seawater). It would be highly relevant to investigate whether or not prey (e.g. mesopelagic fish) caught in the foraging area of the penguins contain high levels of microfibres and to assess the associated impacts on these organisms, which are likely to be transferred to higher predators. An alternative to our method would be to assess microfibre contamination levels in faecal samples collected from captive King Penguins fed with wild-caught mesopelagic fish (also see Nelms et al., 2018) and to track where these fish were caught. This approach could also address the most pressing question, which would be to determine the residence times of fibres in penguins in order to identify the likelihood of transfer of pollutants. However, if microfibre contamination comes from penguins' prey, residence time is likely to depend on prey type, body condition, foraging trip duration, breeding stage, as well as fibre polymer type and size. Therefore, more data are needed to assess transit time in this particular penguin species. More generally, comparing the levels of contamination of several penguin species with different foraging strategies such as African Penguins as epipelagic predators, King Penguins which are able to reach the mesopelagic zone, or Gentoo Penguins which feed closer to the seabed and might be more exposed to microplastics in sediments would identify which foraging strategy is the most impacted by microfibre contamination. This in turn could help to identify species at relatively high and low risk of microfibre contamination in future. Overall, the levels of fibres contamination need to be explored in prey items as well as in the environment where the animals feed and in other penguin species to better understand fluxes and impacts in the entire Southern Ocean food web. Finally, it is important that microplastic studies use a standard approach, following similar sampling and processing techniques as well as standard assessment methods (Provencher et al., 2017; Provencher et al., 2019).

5. Conclusions

Our findings suggest that trophic transfer represents an indirect pathway for microfibre contamination through sub-Antarctic food webs. Given the abundance of fibres in pelagic fish (Boerger et al., 2010; Davison and Asch, 2011; Lusher et al., 2016; Wieczorek et al., 2018) and other seabird prey such as invertebrates (Lourenço et al., 2017), it is likely that secondary ingestion of fibres occurs in many if not most seabirds. This is consistent with the dominance of fibres in the faeces of Northern Fulmars, where they are much more abundant than in stomach contents (Provencher et al., 2018). The higher fibre loads in the faeces of incubating King Penguins compared to chick-rearing birds may result from inter-generational transfer to chicks or greater exposure to microfibres because incubating penguins feed at the Antarctic Polar Front. If the latter hypothesis is correct, microfibres could provide a signature for foraging location in King Penguins. This work emphasizes the need to assess the levels of microfibres' contamination in prey species and in the environment where the penguins feed as well as in other predatory species to better understand fluxes and impacts in the entire Southern Ocean food web.

Declaration of Competing Interest

The authors declared that there is no conflict of interest.

Acknowledgments

We thank the staff at the British Antarctic Survey base at King Edward Point (South Georgia), Quark Expeditions and the crew and staff of the Ocean Endeavour, the crew of the FPV Pharos South Georgia for their help with the fieldwork logistics, Steeve Mathieu and Patrice Cabanne for their assistance during the extraction phase of microplastics, as well as two anonymous reviewers for their help to improve this manuscript. The Antarctic Circumnavigation Expedition (ACE) was a research cruise of the Swiss Polar Institute, supported by funding from the ACE Foundation. Funding for this research was provided by the ACE Foundation (projects 5 and 19), the Natural Environment Research Council's Collaborative Antarctic Science Scheme (CASS-129) and a Trans-Antarctic Association Grant to RBS.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2019.105303.

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