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First record of synthetic micro-fibre ingestion by Mute Swans *Cygnus olor* and Whooper Swans *C. cygnus*

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Abstract

Despite an acute focus on the ingestion of large and small synthetic debris by seabirds, scant consideration has been given to their occurrence in other avian species inhabiting coastal and inland wetland areas. Here, we assess ingestion of synthetic micro-fibres (*i.e.* microplastics and other non-natural fibres, 0.5–5 mm in size) by Mute Swans *Cygnus olor* inhabiting a large freshwater reservoir ($n = 12$ faecal samples), and from Whooper Swans *C. cygnus* wintering on a remote offshore Atlantic island ($n = 11$ faecal samples). Samples were chemically digested to eliminate labile organic matter including natural fibres. In total, 79 synthetic micro-fibres were recovered at frequencies of 4.2 ± 0.8 and 2.6 ± 0.7 (mean \pm s.e.) per sample, ranging from 0–10 and 0–7 micro-fibres per sample, for Mute Swan and Whooper Swan faecal samples, respectively. The number of synthetic micro-fibres recovered did not differ significantly between species or sites. Similarly, there was no difference in the number of synthetic micro-fibres detected per gram of faecal sample. Overall, our preliminary data further bolster emerging records for the ingestion of synthetic debris by non-marine waterbirds inhabiting freshwater and coastal areas.

Key words: Anthropocene, microplastic, plastic pollution, synthetic fibre, waterfowl, wetland birds.

In recent years, the proliferation of large and small synthetic debris items within natural environments has become one of the most topical environmental issues of the 21st century (Cunningham *et al.* 2020). Although the ingestion of large items of synthetic debris (> 5 mm in length) is considered a major threat to wildlife (Derraik 2002; Gall & Thompson 2015), primarily through inflammation, physical damage and blockage of the gastrointestinal tract (Pierce *et al.* 2004), ingestion of small synthetic debris (< 5 mm) by wildlife is also of growing concern (Cole *et al.* 2011). For example, ingestion of small synthetic debris can result in bioaccumulation and biomagnification of chemical contaminants within food webs (Lourenço *et al.* 2017; Tanaka *et al.* 2020). Yet, despite concerns for potential wide-ranging environmental impacts, the overwhelming majority of studies have focused on the occurrence and effects of synthetic debris in marine rather than freshwater or terrestrial ecosystems (Reynolds & Ryan 2018; O'Hanlon *et al.* 2017; Wong *et al.* 2020).

A plethora of studies have examined ingestion of synthetic debris by seabird species, but ingestion of synthetic debris by other avian species remains poorly understood (Lourenço *et al.* 2017; Reynolds & Ryan 2018; Rossi *et al.* 2019; Coughlan *et al.* 2021). In particular, the extent to which small synthetic debris is ingested by waterbirds inhabiting a mosaic of terrestrial, freshwater and marine habitats in coastal areas is largely unknown (English *et al.* 2015; Coughlan *et al.* 2020). In addition, the vast majority of studies have focused data acquisition through necropsy of

seabird carcasses or the examination of regurgitation pellets, *i.e.* boluses comprised of items that cannot be digested such as shell fragments and stones (Provencher *et al.* 2017, 2018). However, as birds can also ingest synthetic debris that are small enough to pass through the entire gastrointestinal tract (*e.g.* Reynolds & Ryan 2018), a lack of studies attempting to report ingestion of synthetic debris through assessment of bird faecal samples is considered a missed opportunity for environmental monitoring (Provencher *et al.* 2018), especially as many bird species will not be readily available for necropsies in sufficient number to provide a meaningful sample size.

Like many Anatidae, both resident and migratory swan species tend to inhabit a mixture of inland and coastal habitats (Rees *et al.* 1997). Although herbivorous, swans frequently ingest grit to aid the mechanical breakdown of food items within the gizzard, which has previously resulted in the accidental ingestion of anthropogenic debris (O'Halloran *et al.* 1988; Spray & Milne 1988; Hong *et al.* 2013). Most notably, the ingestion of spent gunshot and lost or discarded anglers' weights has been linked to elevated blood lead levels and subsequent swan death (O'Halloran *et al.* 1988; O'Connell *et al.* 2009), which can considerably reduce and limit swan numbers over time (Wood *et al.* 2019). Pertinently, faecal samples have previously been used to detect trace element poisoning in swans wintering in a marine lagoon (Wang *et al.* 2017). Therefore, in the present study, we assess faecal samples obtained from two swan species inhabiting two contrasting habitats for the presence of synthetic micro-fibres

(*i.e.* microplastics and other non-natural fibres such as blended textiles). Faecal samples were obtained from Mute Swans *Cygnus olor* inhabiting a large freshwater reservoir situated *c.* 24 km from the coast, and also from Whooper Swans *C. cygnus* wintering on an offshore island in the northeastern Atlantic Ocean. In particular, we add to the current paucity of studies that have documented the presence of synthetic micro-fibres in bird faecal samples. In addition, the ingestion of synthetic micro-fibres by Mute Swans and Whooper Swans is documented for the first time.

Methods

Sample collection

In June 2018, a total of 12 faecal samples were collected from resident adult Mute Swans inhabiting Chew Valley Lake, Somerset, UK (51°20.082'N, 2°37.082'W; Fig. 1), where *c.* 350 Mute Swans occur during winter months (*i.e.* 0.7% of the British population, which is estimated at *c.* 50,500 birds; Woodward *et al.* 2019, 2020). Similarly, 11 faecal samples were collected from migratory adult Whooper Swans wintering on Foula Island, in the Shetland archipelago, UK (60°08.022'N, 2°3.608'W; Fig. 1) – seven in October 2018, four in February 2019 – with 146 Whooper Swans counted wintering on Shetland during the January 2020 international swan census (Brides *et al.* 2021). In all cases, fresh faecal samples were collected from monospecific roosting sites. The samples were all collected by hand at distances of ≥ 1 m apart, to enhance the probability that they were produced by different individual birds.

Individual faecal samples were collected by inverting a clear plastic food-grade bag (freezer bag) over the operator's hand, taking care to pick-up only faecal matter. The internal surfaces of the sampling bags did not touch the operator's clothing, and were exposed to the air for < 60 s. Samples were collected from grass locations and possible contamination through contact with soil is considered to be low. Moreover, no soil was observed during laboratory processing. All samples were refrigerated within 90–120 min of collection (at *c.* 5°C for ≤ 5 months). Although it is unknown if Whooper Swans sampled in October were subsequently re-sampled in February, each faecal sample was treated as an individual datum point in the analysis.

Digestion, separation and microscopy

Samples were placed in 400 mL glass beakers and weighed on an analytical balance (0.01 g; Mettler Toledo AB104). To eliminate labile organic matter and natural fibres, samples were digested in solutions of iron(II) sulfate heptahydrate ($\text{FeH}_{14}\text{O}_{11}\text{S}$) and 30% hydrogen peroxide (H_2O_2) at 60°C (0.75 g of $\text{FeH}_{14}\text{O}_{11}\text{S}$ per 50 mL H_2O_2), until total digestion had occurred (Masura *et al.* 2015). Following Coughlan *et al.* (2020), an application ratio of 20 mL per 10 g of faecal sample was employed, while a density based separation technique was subsequently used to isolate synthetic micro-fibres from denser undigested mineral components through flotation, using a saturated solution of NaCl (*i.e.* 275 g L^{-1}). The resulting supernatant was decanted carefully and vacuum-filtered onto filter

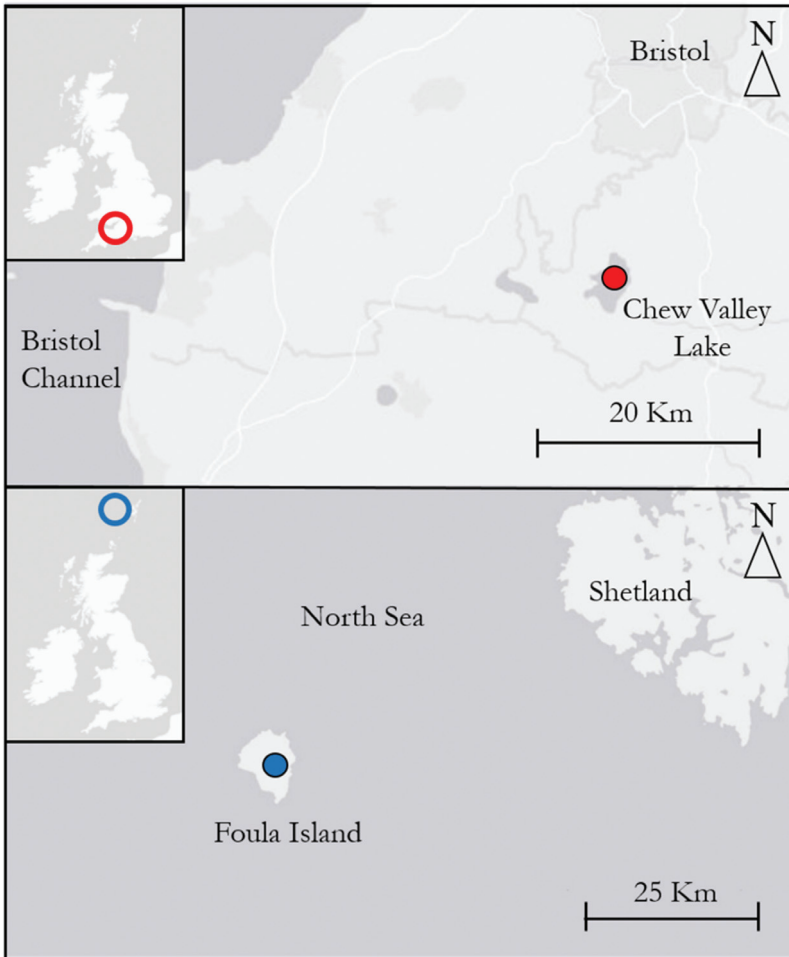


Figure 1. Faecal samples were collected from resident adult Mute Swans inhabiting Chew Valley Lake, Somerset, UK, and from migratory adult Whooper Swans wintering on Foula Island, Shetland archipelago, UK ($n = 12$ and $n = 11$, respectively).

pads (Whatman grade 41, 47 mm diameter, 20 m pore). All filter pads were placed in clean glass Petri dishes and dried at room temperature. Each sample was then examined, all synthetic micro-fibres were identified visually (as by Zhao *et al.* 2016), and also measured with a line-gauge ruler under a stereomicroscope (Olympus SZX16).

Recovered synthetic micro-fibres were classified by colour tone in relation to seven primary colour groups which, for simplification, were subsequently regrouped as three colour tones (either as light/clear, mid, or dark tone) (Zhao *et al.* 2016; Provencher *et al.* 2017; Bessa *et al.* 2019). This study focused on the detection of

synthetic micro-fibres ≥ 0.5 mm in size, because the analysis of smaller synthetic debris is considered problematic given uncertainties around airborne contamination by ultra-small micro-fibres (Torre *et al.* 2016).

To minimise potential contamination, glassware rather than plastic apparatus was used throughout. Glassware was acid washed prior to use, and covered with fresh aluminium foil to minimise the risk of contamination during the entire extraction procedure. Further, wherever possible, a stereomicroscope was used periodically to check the apparatus for the presence of synthetic micro-fibres, prior to sample processing. In addition, prior to processing the first sample of each batch, all glassware was double-rinsed with distilled water and a procedural control sample was processed using this distilled water. This damp filter pad was then put into a Petri dish and used as a laboratory contamination control, by placing it directly alongside the benchtop area in use (where it remained exposed to the laboratory air during the processing of samples) and was also analysed for micro-fibres at the end of the process ($n = 3$). Finally, analysts wore 100% white cotton lab coats, non-fibrous cotton based under-clothing and nitrile gloves on analysing the samples, to reduce the potential for human contamination.

Statistical analyses

All data were assessed for normality of residual distributions (Shapiro-Wilk test) and homoscedasticity of variances (Levene's test). For normally distributed synthetic micro-fibre count data, as residuals conformed to homoscedasticity assumptions,

a two-sample *t*-test was employed to test for differences in synthetic micro-fibre counts between the assessed species and their respective sites. Due to a non-normal distribution ($P < 0.05$) and heteroscedastic residuals ($P < 0.05$), numbers of synthetic micro-fibres recovered per gram of faecal sample were assessed using a Welch's *t*-test, to test for differences between species and sites. Similarly, differences in the mass of collected faecal samples between species/sites was also considered using a Welch's ANOVA. All data were analysed in SPSS v23.0, 2015 (IBM Corp., Armonk, New York, USA).

Results

Potential laboratory contamination was negligible, with only one synthetic micro-fibre being detected by control filter pads; *i.e.* a rate of 0.04 per processed faecal sample. Overall, synthetic micro-fibres were recovered from 91.7% and 81.8% of Mute Swan and Whooper Swan samples, respectively. All synthetic micro-fibres had a maximum length within the size range of 0.5–5 mm. No other debris shape-types were detected. In total, 79 synthetic micro-fibres were recovered at frequencies of 4.2 ± 0.8 and 2.6 ± 0.7 (mean \pm s.e.) per sample, ranging from 0–10 and 0–7 micro-fibres per sample, for Mute and Whooper Swan faecal samples, respectively. When standardised in relation to the quantity of faecal mass per sample, synthetic micro-fibres were detected at mean (\pm s.e.) frequencies of 0.08 ± 0.02 and 0.22 ± 0.07 fibres g^{-1} per sample, ranging from 0–0.3 and 0–0.75 fibres g^{-1} , for Mute and Whooper Swan faecal samples, respectively.

Micro-fibres recovered from Mute Swan samples had dark (48%; e.g. navy blue, black, dark red) or mid-colour tones (52%; e.g. blue, green, red). Similarly, only dark (72.4%) and mid-colour tones (27.6%) were detected for synthetic micro-fibres recovered from Whooper Swan samples. Light colour tones (e.g. clear, white-blue, yellow) were not detected in either species.

The total number of synthetic micro-fibres recovered did not differ significantly

between the species or sites assessed (two-sample t-tests: both $t_{21} = 1.42$, n.s.; Fig. 2). Similarly, the number of synthetic micro-fibres recovered per gram of faecal sample did not differ significantly between the species or sites (Welch's t-test: both $t_{11,94} = 1.78$, n.s.; Fig. 2). However, the mass of the faecal samples collected was significantly greater for Mute Swans (57.0 ± 7.8 g) than for Whooper Swans (15.9 ± 2.6 g) (Welch's t-test: $t_{13,45} = 4.99$, $P < 0.001$).

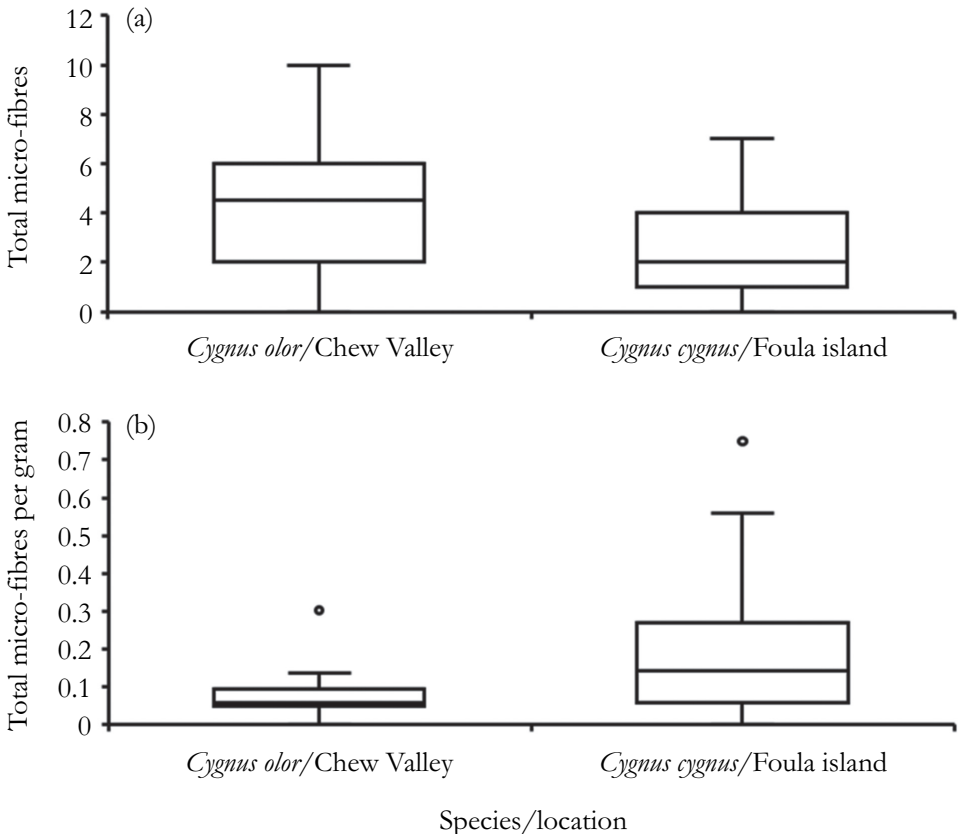


Figure 2. Median counts with interquartile ranges (IQR), and maximum and minimum IQR values, are shown for (a) the total number of recovered synthetic micro-fibres, and (b) the total number of synthetic micro-fibres detected per gram of faecal sample, in relation to both species and sampling sites. Outlier values are also shown.

Discussion

Here, ingestion of synthetic micro-fibres by Mute and Whooper Swans is documented for the first time. Although previous studies have catalogued the ingestion of synthetic debris by Anatidae inhabiting wetlands and coastal locations of Canada, continental Europe and South Africa (*e.g.* Holland *et al.* 2016; Gil-Delgado *et al.* 2017; Reynolds & Ryan 2018), the present study further bolsters the inference that the ingestion of synthetic micro-fibres by waterbirds is a widespread occurrence across multiple habitat types. In addition, we would suggest that the ingestion of synthetic micro-fibres by both resident and migratory swan species is a frequent occurrence, even in remote coastal locations. Further, our data lend support to the concept of using non-invasive faecal sampling for increased and more efficient detection of contaminant ingestion by bird species (Wang *et al.* 2017; Provencher *et al.* 2018).

Previously, the ingestion of synthetic debris by Anatidae has been linked to the availability of historical or current sources of anthropogenic detritus at study sites (*e.g.* Gil-Delgado *et al.* 2017; Reynolds & Ryan 2018). Although the exact availability and origin of synthetic micro-fibres at our study sites is currently unknown, Chew Valley Lake is a popular amenity site for public visitation, recreational angling and dinghy sailing events (Environmental Agency 2016), so it is perhaps unsurprising that resident wildlife will have contact with synthetic debris. Furthermore, as both urban and agriculture sources have been identified as drivers of increased

nutrient loading in Chew Valley Lake (Environmental Agency 2016), which is supplied by an expansive 5,911 ha catchment area, human habitation and agricultural waste are also likely to contribute to the presence of both large and small synthetic debris. Nonetheless, although spatial and temporal variation of their distribution and abundance can occur, synthetic micro-fibres are considered a widespread contaminant of freshwater environments worldwide (*e.g.* Crew *et al.* 2020; Stanton *et al.* 2020). Similarly, despite the remote offshore location of Foula Island and a resident population of ≤ 33 people, an extensive amount of synthetic debris is frequently washed ashore (*e.g.* domestic waste and fishing gear), which can be blown further inland during storm conditions (S. Gear pers. comm.). Overall, on balance, we argue that the ingestion of synthetic micro-fibres by Mute and Whooper Swans is likely a result of environmental contamination by synthetic micro-fibres, which may have been produced from the breakdown of larger synthetic debris items (Law & Thompson 2014). Additionally, it is possible that micro-fibres have been dispersed in high quantities locally *via* release from synthetic clothing (Napper & Thompson 2016; De Falco *et al.* 2019).

Although synthetic micro-fibres and micro-fragments can result from the degradation of larger particles within the digestive tracts of birds (Provencher *et al.* 2018), since synthetic debris items > 5 mm in length were not detected by the present study, it appears that the synthetic micro-fibres found were most likely ingested accidentally as small fibres (< 5 mm).

However, larger debris items could potentially be retained for an extended period within the gastrointestinal tracts (Holland *et al.* 2016; Ryan 2016). Therefore, although faecal sampling represents a non-invasive method of sampling ingestion of contaminants by bird species, faecal samples will likely underestimate the quantity of synthetic debris taken by birds. For example, while Provencher *et al.* (2018) documented a correlation between the quantity of synthetic debris in faecal precursor samples (*i.e.* faecal samples taken from the cloaca during necropsy) and the amount of synthetic debris recovered from the upper gastrointestinal tract (*i.e.* esophagus, crop, proventriculus and gizzard) of Northern Fulmars *Fulmarus glacialis*, the quantity of synthetic debris in the upper gastrointestinal tract was generally greater than that of the faecal precursor samples. Although further research is required, it appears that synthetic micro-fibres can lodge in the intestinal wall for extended periods of time, possibly months, while also being capable of rapidly passing through the digestive system (Provencher *et al.* 2018). Nevertheless, knowledge of debris items that rapidly pass through species is environmentally important, especially as a growing number of studies indicate that ingestion of small synthetic debris can lead to the release of chemical contaminants (Tanaka *et al.* 2020). Further, the fate of synthetic micro-fibres following their excretion by swans will also need to be considered. In particular, due to poor digestive performance, swan faeces often contain undigested food items that attract swans and other smaller Anatidae to forage

on swan faeces (*i.e.* coprophagy: Black & Rees 1984; Vogrin 1997; Shimada 2012). As such, coprophagy may lead to increased ingestion of synthetic micro-particles resulting in increased bioaccumulation of chemical contaminants. This could be particularly problematic for smaller waterbirds if they also ingest synthetic micro-particles from the wider environment at the same rate as swans, which could then be further increased through engaging in coprophagy.

Whilst the present study provides a preliminary insight into the ingestion of synthetic micro-fibres by swan species, further in-depth assessments are required to ascertain the prevalence of ingestion, and the overall impact of synthetic micro-fibres on Anatidae individuals and populations. In particular, the extent of absorption and subsequent impacts of chemical contaminants requires investigation, especially in relation to bird health and developmental life stages (*i.e.* embryotic and neonate stages). Nevertheless, our findings lend support to the premise that synthetic micro-fibres could be a problematic pollutant for non-marine waterbird species.

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