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Published in:
Environmental Pollution

DOI:
10.1016/j.envpol.2019.113635

Publication date:
2020

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Abstract

Though the number studies on microplastic ingestion by fish is growing, data on fish species characteristic of the South African coastline are scarce. This study quantified and characterised (physically and chemically) microplastics ingested by four species of juvenile fish (viz. *Oreochromis mossambicus* [Peters, 1852], *Terapon jarbua* [Forsskål, 1775], *Ambassis dussumieri* [Cuvier, 1828] and *Mugil* sp.), within four mangroves along the east coast of South Africa. Microplastics were isolated from whole fish using a proteinase K digestion method, and then quantified and characterised in terms of shape, chemical nature (plastic type), colour and length. Fibres (68%) and fragments (21%) were the dominant shapes found. Of the 174 fish sampled, 52% contained microplastic particles, with 0.79 ± 1.00 particles per fish. The average number of particles per fish did not differ significantly across species within sites and across sites but was higher than in juvenile fish of other species sampled in oceanic habitats. The main plastic types collected using 10 µm filters and identified with Fourier Transform Infrared Spectroscopy (FTIR), were rayon (70.4%), polyester (10.4%), nylon (5.2%) and polyvinylchloride (3.0%). Particle length ranged from 0.1 - 4.8 mm, averaging 0.89 ± 0.77 mm, but irrespective of length, particles were mostly blue in colour. This study provides evidence that juvenile fish inhabiting mangroves are consuming significant quantities of microplastics. Importantly, it should be noted that rayon, though the most abundant plastic type

found, is a semi-synthetic, fibre made from regenerated cellulose that is commonly reported in studies of this nature. The habitats studied serve as nurseries for numerous fish species; however, more detailed studies are needed to assess whether microplastic ingestion could compromise the health of these fish or whether these effects are dependent on species, feeding habit and/or plastic type.

Capsule: South African juvenile fish associated with mangroves had 0.79 ± 1.00 microplastic particles per fish. Rayon (70.4%), polyester (10.4%), nylon (5.2%) and polyvinylchloride (3.0%) were the main plastic types ingested.

Keywords: Estuary; ingestion; marine; plastic; rayon; debris

Introduction

Reports of microplastic (≤ 5 mm in length) ingestion by aquatic organisms date back to the beginning of mainstream plastic production (Carpenter et al., 1972) and over the last decade have been recorded in a wide range of fauna Wright et al. (2013), including fish (Jovanović, 2017). Microplastic ingestion in fish in particular, has been reported for a wide variety of water bodies, including inland lakes (e.g. Biginagwa et al., 2016), ocean channels (Lusher et al., 2013), deep ocean (e.g. Anastasopoulou et al., 2013) and more recently estuarine habitats (Vendel et al., 2017). However, these reports represent a geographic bias in that they emanate largely from the developed world; data for African marine environments and species that typify the continent's coastal habitats are particularly scarce (but see Naidoo et al., 2016).

The interest in microplastic ingestion by fish is based on the negative health consequences, which have been reported from laboratory studies for a range of organisms and particle sizes. Whilst particles sizes used in experiments can differ greatly, from a lower limit of 3 µm upwards, variable combinations of the following have been observed: decreased growth (Naidoo and Glassom, 2019), decreased feeding and/or weight loss (Besseling et al., 2013; de Sá et al., 2015), transfer to organs (Browne et al., 2008), inflammation (Wright et al., 2013), liver toxicity and pathology (Rochman et al., 2013b), endocrine disruption (Rochman et al., 2014) and decreased reproductive output (Sussarellu et al., 2016). Plastic particles can also act as vectors that transport persistent organic pollutants (Rios et al., 2007). This has been suggested to contribute to the negative health effects mentioned above (but see Bakir et al., 2016). Some authors do dispute their potential as the main vectors for these pollutants (e.g. Beckingham and Ghosh, 2017). However, in light of the possible negative health effects, microplastic ingestion by fish has the potential to affect fisheries by affecting fish growth (Markic and Nicol, 2014; Naidoo and Glassom, 2019). This could be especially problematic in developing countries that are heavily reliant on fish stocks (Lamberth and Turpie, 2003).

Whilst a number of studies have quantified and even characterised microplastic particles ingested by fish based on physical characteristics (e.g. Boerger et al., 2010; Davison and Asch, 2011; Kripa et al., 2014; Ferreira et al., 2016; Mizraji et al., 2017) reports that detail the chemical composition of particles ingested by fish are scarcer. The impact of these particles on organisms could be influenced by both the physical and the chemical nature of the plastic they are composed of; polyvinylchloride and polystyrene for example, can have carcinogenic properties, while other types may be less harmful (Rochman et al., 2013a). Fish feeding strategies may also influence the typology and abundance of microplastics

ingested by different species (Mizraji et al., 2017). Additionally, particle abundance and type can differ across habitats owing to density differences (Moore et al., 2005) and feeding strategies can differ across species (Ferreira et al., 2019) which suggests that different species could be exposed to different suites of plastics. It is therefore important to both quantify and characterise the different types of plastics ingested by fish in a wider range of natural and managed systems, and species. In this regard, only few studies have investigated plastic ingestion by fish in estuarine and specifically mangrove habitats within estuaries, compared with other marine environments (Vendel et al., 2017). Such information is important, since these systems are a crucial pathway through which microplastics reach the ocean (Possatto et al., 2015). These systems offer important ecosystem services by providing refugia to fish and thus food provision to humans (van Niekerk and Turpie, 2012). For example, the economic value associated with South African estuarine fisheries was estimated to be 1.2 billion rand per annum (van Niekerk and Turpie, 2012). These mangroves are also nursery sites for many species of juvenile fish, as they offer food and cover from predators (Laegdsgaard and Johnson, 2001). There is a high probability that juvenile estuarine fish will interact with microplastics as these habitats are usually in close proximity to contamination sources and these particles are likely to be retained in this environment (Naidoo et al., 2015, 2016). Mangrove pneumatophores have also been suggested to increase the retention of plastics in estuarine systems (Martin et al., 2019). At the time of this study, there were no published reports on the abundance, type, length, colour and chemical composition of microplastics ingested by juvenile fish species associated with South African mangrove systems. This motivated the present study which quantified and characterised (physically and chemically) microplastics ingested by four species of juvenile fish (viz. Tilapia - *Oreochromis mossambicus* [Peters, 1852], Thornfish - *Terapon jarbua* [Forsskål, 1775], Glassfish - *Ambassis dussumieri* [Cuvier, 1828] and Mullet - *Mugil* sp.) in four mangroves in KwaZulu-Natal (KZN), South Africa. Many of the predominantly open estuaries on the east coast of South Africa support dense mangrove strands (Ward and Steinke, 1982) and we compared three of them from an urban setting and one located in a semi-rural Ramsar site. The shape, chemical composition, colour and length of microplastics were recorded and compared among sites and species. Knowledge on systems and fish species can inform future pollution management strategies, particularly for mangroves which are subject to numerous anthropogenic pressures globally (Friess et al., 2019). Our focus on juvenile fish is based on the fact that many fish populations are rapidly depleting owing to overfishing, climate change, habitat destruction and other anthropogenic impacts; a healthy juvenile population is essential for sustaining fish stocks (Wallace et al., 1984; Harris and Cyrus, 1999).

Methods

Fish collection

In total, 174 juvenile fish, between the fry and fingerling stages of development, of four species (*O. mossambicus* , *T. jarbua* , *A. dussumieri* and *Mugil* sp.) were collected (if present) in the St. Lucia (28° 23'S, 32° 25'E), Umgeni (29° 48'S, 30° 02'E), Durban Harbour (29° 52'S, 31° 04'E) and Isipingo (30° 00'S, 30° 57'E) estuaries, in KZN, South Africa. Detailed information on these systems can be found in the National Biodiversity Assessment of these estuaries (see van Niekerk et al., 2019). St. Lucia has the largest mangrove area (288 ha), followed by Durban Harbour (13.4 ha), Umgeni (27 ha) and the Isipingo (3.8 ha) (van Niekerk et al., 2019). While the St. Lucia and Isipingo estuarine systems are freshwater

dominated, the Durban Harbour and the Umgeni are more marine dominated (van Niekerk et al., 2019). Fish collection was done by actively pursuing fish, using dip nets on a single day at each estuary. Collections were done within January 2019, which corresponded to the wet season. Owing to species salinity preferences, *O. mossambicus* was present in the St. Lucia and Isipingo estuaries only, where salinity ranges from fresh to brackish conditions (Whitfield et al., 1981). *T. jarbua* and *A. dussumieri*, which are typical in marine conditions (Whitfield et al., 1981), occurred in the Umgeni and Durban Harbour estuaries only. Fish were placed in 99% anhydrous alcohol for euthanisation, a method approved by the Ethics Committee at the University of KwaZulu-Natal [AREC/011/016D]). Fish were transported to the laboratory in individual hermetically sealed plastic bags. Thereafter, the surface of each fish was dried with paper towel and its mass recorded to three decimal places. Fish were then placed individually in a new set of hermetically sealed polyethylene bags filled with commercial salt and stored at 4°C until further processing. Metal forceps (rinsed with deionized water before use) were used to handle the fish samples at all stages.

Sample preparation

All glassware and handling equipment were washed and rinsed with deionized water before use. As suggested by Woodall et al. (2015), samples were filtered under vacuum in a clean room dedicated to microplastic research. The air ducts in this room are blocked off from the main air conditioning and white cotton lab coats are worn within. Glassware used for filtration were covered in aluminum foil before use and after the filtration of each sample. Petri dishes and metal forceps were pre-checked for plastic contamination under a dissecting microscope. Fish were removed from salt, rinsed and rehydrated in 18.2 MΩ Milli-Q water. Each fish was then examined under a dissecting microscope for surface contamination, possibly originating from salt during storage or prior handling. Any surface contamination found was removed and fish were transferred to 30 mL pre-cleaned glass bottles, which were then sealed with end caps to serve as digestion vials. All steps were carried out in a laminar flow hood to prevent contamination.

Fish digestion and microplastic isolation

The direct inspection of fish guts for microplastics is challenging, particularly in juvenile fish, as the gut content and oil globules cloud observations. Therefore, many studies use nitric acid to digest fish tissue, aiding observation (Lusher et al., 2017). However, plastics such as nylon (polyamide), can be degraded by the acid, making this method more appropriate for large scale monitoring on a limited budget (Naidoo et al., 2017). Since we wanted to account for all plastics, digestions were carried out on whole fish using Proteinase K (3.0 - 15.0 unit/mg solid lyophilized powder, from *Engyodontium album*). The protease, filter papers and chemicals were all obtained from Sigma-Aldrich. The digestion method was adapted from Cole et al. (2014) and has been shown by Karlsson et al. (2017) to have a high recovery (97%) of plastics, with minimal alteration of their physical and chemical nature for identification.

One mg/mL of proteinase K in Tris EDTA Buffer (pH 8.0, prepared with Milli-Q water) was used for sample digestions. One mL of the solution was pipetted into in each digestion vial and shaken for 1 min on a Hati roto mixer to facilitate fish tissue digestion. All pipette tips and the tip of the pipette used for all digestion steps were pre-rinsed with deionised water before use. Three 1L replicates of Milli-Q water was filtered to serve

as controls and these revealed no contamination of fibres. Digestion vials were incubated in an oven at 39°C overnight. Upon recovery, samples were shaken once more and visually observed. Vials containing undigested fish tissue were placed back into the oven for further digestion. Fish bones, scales, otoliths and eye balls did not digest.

Once all digestible tissue was no longer visible, samples were filtered, under vacuum, through a 10 µm Whatman cyclopore polycarbonate filter (47 mm diameter). After the sample was poured into the sample holder, the sides of the digestion vial and the sample holder were rinsed three times, with 2 mL and 3 mL of Milli-Q water, respectively. The filter was then removed and placed within a closed Petri dish, which was then covered in foil and placed in an oven at 39°C overnight. The sample holder and filter holder were rinsed with Milli-Q water between samples.

Quantification and characterization of microplastics

After sample filtration, the filters were observed under a dissecting microscope (Microtec, HM-3), whilst still enclosed within Petri dishes. Particles that resembled microplastics were isolated and characterised, based on guidelines by Hidalgo-Ruz et al. (2012), placed on Whatman qualitative filters (No. 4) and enclosed in Petri dishes for imaging and plastic type diagnosis. Since 10 µm filters were used, this was the lower cut-off size for the diameter of fibres that would be retained. Exposure of filters to air was limited to < 1 min during particle transfer. To assess levels of potential airborne contamination during exposure of the filters to air two filters were left exposed on the lab bench within the room in which all sample viewing was conducted. Analysis of these exposed filters revealed the presence of a single clear fibre after 2 h. The transfer of particles from isolation filter to the one used for photography took <1 min, which validated the method used. It should be noted that visual observations of microplastic particles are subject to inaccuracies based on particle size and method(s) of observation (Song et al., 2015). For example, those authors reported that for sediment samples clear and white fragments can be disregarded as microplastics using microscopy for observation, yet spectral characteristics revealed otherwise. This under- representation of particles is probably widespread in the literature, since it is not always feasible to subject all particles to spectral analysis.

Imaging was done using a light microscope (Leica M205 C) at 5× magnification, with Petri dishes closed, and particle length was measuring using ImageJ. Of the 137 particles viewed, 91% were measured in terms of their longest length (mm). Thereafter samples were individually transferred to a Bruker Vertex 70 Fourier Transform Infrared Spectrometer (FTIR), equipped with a Hyperion 1000 Microscope attachment and a liquid nitrogen cooled mercury cadmium telluride detector (Bruker, Ettlingen, Germany). The instrument measured the absorbance of the sample using OPUS 7.5 software (Bruker, Ettlingen, Germany). Samples were prepared on a diamond compression cell and 30 background scans were run before analysing each sample. Sample spectra were based on 32 scans from 600 - 4000 λ. cm^{-1}, at a resolution of 4 cm^{-1} (Comnea-Stancu et al., 2017). Sample spectra were compared to reference spectra from a basic polymer library, a Bruker optics attenuated total reflection (ATR) polymer library and a synthetic fibres ATR library with 8, 234 and 337 entries, respectively. Sample evaluation was performed using a quality index adapted from Woodall et al. (2014). Polymer identification was based on the Euclidean distance between spectra, using the quick identity test option in the spectral software. Samples were compared to reference spectra and samples with a score of zero represented a perfect match, while

144 samples with a score of ≤ 0.600 were accepted as the reference polymer. Although the possibility of a cross-match between other organic matter
145 and rayon does exist, as in the case of all other studies of this nature, care was taken to not include fibres that resembled natural materials by
146 selecting fibres with no cellular structures, equal diameter and of a singular colour throughout (Reynolds and Ryan, 2018).

147 Statistical analyses

148 Plastics found in fish were enumerated and thereafter characterised by shape, chemical nature (plastic type), colour and particle length (mm).
149 Literature does point out the distinction between rayon and other microplastics since rayon is synthesized from naturally occurring polymers and
150 hence considered semi-synthetic or regenerated fibre (Comnea-Stancu et al., 2017). However, rayon particles are frequently included in the
151 category of microplastics for studies of this nature (e.g. Bessa et al., 2018; Halstead et al., 2018; Su et al., 2019b) given the fact that they are the
152 product of an non-natural manufacturing process and are not a natural dietary component of aquatic organisms.

153 Given the uneven sample sizes for the different species and sites, nested ANOVA was used to test for any difference in mean particle abundance
154 and mean particle length across species, within sites and across sites (data for all species within a site pooled). All differences were considered
155 significant at the 0.05 level. The relationships among the total number of particles, particle length and fish mass were investigated using Spearman
156 rank correlations. Particle length was averaged per fish if more than one particle was found.

157 Results

158 Of the 174 fish analysed, 91 (52%) contained microplastics (Table 1). A total of 137 plastic particles were found, with an average number of 0.787
159 ± 1.00 particles per fish average (Table 1). The number of particles per fish did not differ significantly across sites ($F = 2.58$, $df = 3$, $p = 0.06$) or
160 among species, within sites ($F = 1.41$, $df = 2$, $p = 0.25$). Nevertheless, data exhibited a few trends which are worth mentioning: (1) The maximum
161 number of particles per fish was six, found in mullet collected from Durban Harbour, while tilapia from both St. Lucia and Isipingo and glassfish
162 from Durban Harbour, contained a maximum of two particles per fish; (2) mullet from Umgeni exhibited the highest average number of particles;
163 (3) the lowest average number and frequency of occurrence of ingested particles was observed in tilapia from the St. Lucia Estuary (Table 1).

164 The total number of particles per fish was not significantly correlated with fish mass ($R = 0.106$, $n = 174$ $p = 0.162$). Fish mass and particle length
165 were also not significantly correlated ($R = 0.086$, $n = 86$, $p = 0.433$). The average length of ingested particles was 0.894 ± 0.769 mm and ranged
166 from 0.1 - 4.8 mm and did not differ significantly across sites ($F = 0.53$, $df = 3$, $p = 0.66$) or among species within sites ($F = 1.46$, $df = 2$, $p = 0.24$).

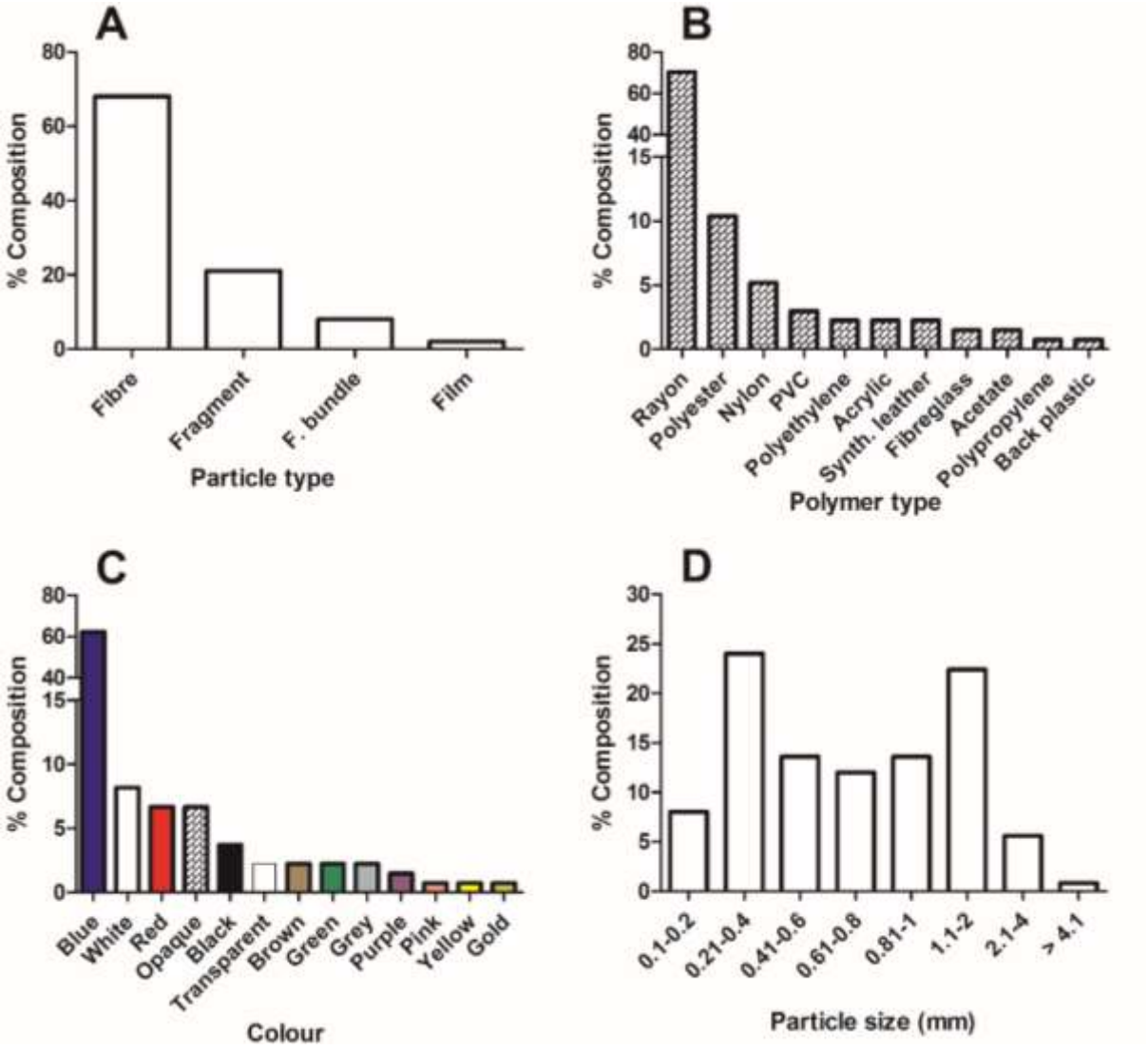
167 The ingested particles fell into four shape categories and eleven plastic types (Figure 1). Despite this varied typology, the distribution of particles
168 across these types was skewed. Fibres (68%) and fragments (21%) were the dominant shapes found, while rayon (70.4%), polyester (10.4%),
169 nylon (5.2%) and polyvinylchloride (PVC, 3.0%) were the most abundant plastic types. Fibres composed of rayon dominated in all species and
170 sites. Although the spectral sensitivities may vary in the four fish species sampled, the colour of particles are important since this may affect

171 particle selection by visually dependent feeders, especially if the microplastic resembles their prey items (e.g. Ory et al., 2017). The most common
172 colours of microplastics found were blue, white, red, opaque and black (Figure 2).

Table 1. Characteristics of microplastics ingested by four fish species from each sampling site.

Species	***O. mossambicus***	***O. mossambicus***	***T. jarbua***	***Mugil* sp.**	***Mugil* sp.**	***A. dussumieri***	
Common name	**Tilapia**	**Tilapia**	**Thornfish**	**Mullet**	**Mullet**	**Glassfish**	**Total**
Site	**St. Lucia**	**Isipingo**	**Umgeni**	**Umgeni**	**Harbour**	**Harbour**	
Fish mass (g)*	0.13 ± 0.07	0.12 ± 0.18	0.07 ± 0.01	0.09 ± 0.05	0.41 ± 0.20	0.12 ± 0.09	0.16 ± 0.17
Fish with particles (number, %)	11 (38%)	13 (45%)	14 (48%)	17 (59%)	16 (55%)	20 (69%)	91 (52%)
No. of particles/fish*	0.41 ± 0.57	0.59 ± 0.73	0.66 ± 0.81	1.14 ± 1.25	1.00 ± 1.46	0.93 ± 0.75	0.79 ± 1.00
Min/Max no. of particles/fish	1/2	1/2	1/3	1/4	1/6	1/2	1/6
Particle length (mm) *	0.64 ± 0.46	0.92 ± 1.18	0.94 ± 0.81	0.88 ± 0.55	1.09 ± 0.77	0.76 ± 0.77	0.89 ± 0.77

All values are based on 29 individuals.
*Values represent mean ± SD



178

179 Figure 1. The distribution (%) of microplastic particles ingested by juvenile fish across different (A) shape, (B) plastic type, (C) colour and (D)
180 size categories. The particles were extracted from four fish species sampled from four estuaries with mangroves in KwaZulu-Natal, South Africa.
181 Data for sites and species are pooled.

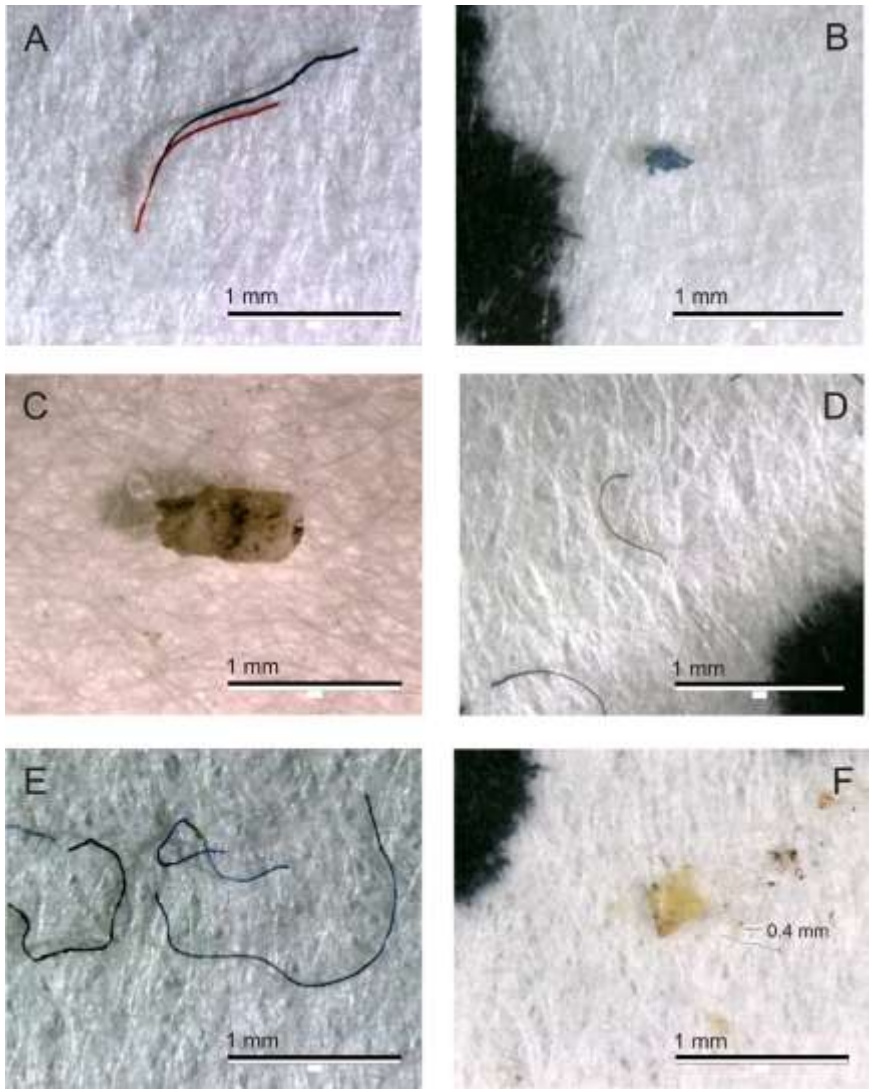


Figure 2. Microplastic particles found in juvenile fish of four fish species sampled from four mangroves in KwaZulu-Natal, South Africa. A: particles of rayon (red and blue), B: blue fragment of PVC, C: opaque fragment of PVC from Durban Harbour mullet, D: polyester fibre, E: rayon fibres and F: polyethylene fragment from a thornfish in Umgeni.

Discussion

Comparison between sites and species

The eastern seaboard of KZN, the location for all four study sites, is one of the most densely populated coastal regions in South Africa. Rapid urbanisation and a wide range of industrial and tourist activities along the coastline and upstream of the rivers that flow into many of its estuaries, introduce pollutants into many water bodies in the region (O'Donoghue and Marshall, 2003). Most recently, reports of microplastic ingestion in fish sampled in the region point towards the prevalence of plastic pollution in coastal systems in KZN (Naidoo et al., 2016). The findings of the present study support this assertion but also show the susceptibility of juvenile fish associated with KZN mangroves to microplastic ingestion. Furthermore, ingestion appears to be independent of species (at least for the four investigated here) and site; for instance, the quantity of microplastics ingested by fish in the most protected site (St. Lucia) was comparable with fish from Umgeni and Durban Harbour which are open to numerous anthropogenic activities. Whilst microplastic data were not assessed for this study, literature indicates that microplastics are present in the sediment and water fractions at the three urban sites investigated here (Naidoo et al., 2015). At the time of this study, environmental microplastic data were not available for St. Lucia. However, we believe that even mangrove systems that are sheltered from urbanisation can become contaminated by microplastic particles as a consequence of plastic inputs from runoff during rainfall events, domestic and industrial effluent that enter rivers that flow into estuaries and washing of clothes in rivers systems by local communities (Le, 2017). The plastic retention capability of mangrove forests is reported to be high (Martin et al., 2019) and thus particles entering this system are possibly trapped in, and could possibly be transferred to fish either directly by prey mis-identification and indirectly via their invertebrate prey (Farrell and Nelson, 2013). Coupled with this, in systems such as St. Lucia which has been closed for > 15 years (Tweddle et al., 2016), plastics could accumulate as a consequence of limited water exchange with the ocean (Claessens et al., 2011).

Previous studies suggest that there may be a difference in microplastic ingestion by intertidal fish of different feeding guilds (Mizraji et al., 2017). Literature suggests that whilst some species like mullet are generalistic feeding fish that are indiscriminant feeders, other species such as glassfish are selective feeders that feed largely on zooplankton (Dyer et al., 2015). Differences in plastic ingestion by species with different feeding strategies was also suggested to occur by Wright et al. (2013), but we found no significant evidence of this in the four species compared here. Similarly, Vendel et al. (2017) found that a fish's feeding guild did not determine the quantity of microplastics ingested when comparing 29 species of estuarine fish. Halstead et al. (2018), also found no difference in the abundance of microplastics ingested by three species of benthic feeding fish in an urban estuary. It must be noted though that all the studies quoted above focused on adult and sub-adult fish as opposed to juvenile fish. Whilst all fish exhibit the tendency to feed near the sediment, certain species can adopt feeding strategies that could increase/decrease the number of microplastics ingested. Juvenile mullet, thornfish and tilapia most often feed near the sediment, on microphytobenthos or benthic invertebrates (www.fishbase.org), in shallow water ecosystems, and this could explain the lack of significant

differences in microplastic ingestion levels between species within sites. Rayon particles, that are frequently reported in fish, are abundant in sediment and would this would explain the large portion of rayon particles in these species (Gago et al., 2018). Microplastics can also move through food chains (Setälä et al., 2014), and therefore glassfish that feed in the water column were also exposed to these particles.

Our study indicated that fish in this study area consumed similar levels of microplastics to other benthic feeding estuarine fish from urban settings. For example, we found that 52% of the 174 fish we investigated ingested microplastics, while Halstead et al. (2018), found that 43% of 93 fish from Sydney Harbour ingested microplastics. Estuarine and coastal fish seem to consume higher amounts of plastic particles than fish in oceanic habitats (see review by Jovanović, 2017), however it must also be noted that methodological differences amoung studies make comparisons difficult. As alluded to above, this is possibly because plastics can concentrate in estuaries, making their interaction with fish more frequent in these systems. For example Ramos et al. (2012) found that 13.4% of 425 fish had ingested blue nylon threads, in a tropical estuary where fishing is the main microplastic source, while Steer et al. (2017) found that only 2.9% of the 347 fish larvae they examined had ingested microplastics in the English channel, where fishing is common, but particles could possibly be wider spread. Some freshwater fish higher up rivers also seem to exhibit high levels of plastic ingestion. Karlsson et al. (2017), for example, found that 68% of 62 brown trout had consumed plastics. This suggests that fish feeding closer to terrestrial and freshwater plastic sources may be interacting more frequently with particles, prompting higher levels of ingestion.

Main plastic types and sources

Most of the plastics found in fish were fibrous in shape. This is the case with many studies that have investigated microplastic ingestion in marine biota (as reviewed by Gago et al. (2018); and is especially so for estuarine fish (Ramos et al., 2012; Ferreira et al., 2016; Vendel et al., 2017; Halstead et al., 2018). The most common microfibres reported in the environment and fish are acrylic, polypropylene, polyethylene, polyester and rayon (Gago et al., 2018; Halstead et al., 2018), which were all also encountered in this study. Rayon, also known as viscose in some locations, was by far the most abundant plastic ingested here. It is a semi-synthetic cellulosic fibre, manufactured from wood pulp and the derivatization of cellulose with carbon disulfide (Comnea-Stancu et al., 2017; Gago et al., 2018). Rayon is used in the manufacture of clothing and therefore fibres can be sourced from abrasion while washing (Comnea-Stancu et al., 2017).

Washing machine effluent can also contain polyester and acrylic fibres (Napper and Thompson, 2016), which were also found within fish in the current study. It has been estimated that > 1 900 fibres could be formed from machine washing a single garment (Browne et al., 2011). The type of material may also be important in creating fibres as it is estimated that 700 000 fibres could be released from a 6 kg load of acrylic material (Napper and Thompson, 2016). There have been reports of these fibres passing through sewage treatment plants into natural waterways (Browne et al., 2011), which is probably why they are so prevalent. Halstead et al. (2018), for example, estimated that a single mullet in an urban estuary has the potential to consume 11 000 microfibres annually. Many of these fibres can ultimately end up in the deep sea (Woodall et al., 2014).

Potential impacts of plastics and associated pollutants on fish health

Although feeding performance was not tested here, fish can display decreased feeding performance when exposed to plastics < 90 µm (Miranda et al., 2019), however this is not true for all species (Jacob et al., 2019). Consumption of particles of the types found within the fish species studied here have been shown to have negative consequences. For example, glassfish fed a mixture of the plastics types found in this study, such as polyethylene, polystyrene and polyvinyl chloride, were also shown to have slower growth compared with controls during chronic exposure (Naidoo and Glassom, 2019). In other fish species, endocrine disruption was observed after chronic ingestion of both virgin and marine sourced polyethylene particles (Rochman et al., 2014). It must be noted however, that these were laboratory-based experiments in which fish were given a plastic feed daily, unlike the snap-shot we investigated here. The size of particles found in experiments mentioned above (~ 0.5 mm), and those found here (> 0.1 mm), were much larger than those shown to translocate into circulatory systems of fish (< 20 µm), by Su et al. (2019a). However, translocation of associated pollutants is possible (Bakir et al., 2014), which may have possible negative health impacts. For example, blue rayon fibres, are able to adsorb polycyclic aromatic hydrocarbons (PAHs) from estuaries, and have thus been used for monitoring environmental concentrations (Sakamoto and Hayatsu, 1990; Kummrow et al., 2006). Given that the bulk of the particles found here are rayon, this warrants further investigation into pollutants associated with these particles within mangroves which are widely reported to exhibit high levels of organic and inorganic pollutants.

Particle length and colour

The average length of ingested particles found here was 0.894 ± 0.769 mm. This size is common for fibres and are within a size range that can easily be ingested by marine biota (Gago et al., 2018), including fish and their prey items (Ory et al., 2017). Fish mass did not correlate with particle length, which suggests that larger fish may not have consumed longer particles; i.e. these fish species appear to be indiscriminate in their selection of particles. As in other studies, example Vendel et al. (2017), the number of particles consumed also did not correlate with fish mass, negating the possibility that larger fish consume more particles than smaller fish.

Blue was the most common colour of fibres found in the mangrove associated fish examined here. Blue, black, white and red were also the most common colours of plastic found in other estuarine fish (Ramos et al., 2012; Ferreira et al., 2016). Blue fibres seem to be the most common colour of fibres in general, as confirmed by South African nearshore water (Nel and Froneman, 2015), global seawater and sediment microfibre assessments (Gago et al., 2018). Other common microplastic colours are transparent and black (Gago et al., 2018), which were also dominant here. Microplastic colour is important since it may influence uptake by visual predators. For example, Ory et al. (2017) found that small pelagic fish (*Decapterus muroadsi* [Temminck and Schlegel, 1844]), ingested mainly blue particles, possibly because this resembled the colour of the copepods they prey on. White and opaque colours, which were also found here, could also resemble copepods and other prey that that fish consume (Carpenter et al., 1972; Wright et al., 2013).

Concluding remarks and recommendations

The levels of microplastic ingestion by the juvenile fish studied here is important in the context of ingestion levels reported in other studies. Ingestion levels do not appear to be species or site dependent but high particle abundance and frequency of occurrence across all species may be reflective of the high levels of microplastics within mangrove systems. Rayon seems to be most prevalent in fish studied here which is in keeping with trends in the past and current literature. The main caveat of this study is that we could not obtain the same suite of species at all sites due to site and species peculiarities. Future studies should also consider comparing estuarine filter-feeding fish to benthic feeding fish. Fish do, however, change their feeding preferences during development (Ferreira et al., 2019) which should also be factored in when assessing the risks of microplastic ingestion in a species. In the context of assessing risk, the processing methods used in this study can serve as an effective approach for quantifying and characterising microplastic ingestion in fish. However, this should be coupled with techniques that identify the pollutants that adsorb onto microplastic particles for a more holistic assessment of how microplastics affect fish health and functioning. It must be acknowledged that the possibility of particles behind the gills contributing to abundance counts cannot be discounted entirely. Each fish was checked rigorously for surface contamination to minimize this possibility but we recommend that future studies consider removing the gills or head of specimens and analyzing these separately for microplastics.

Acknowledgements

The authors would like to acknowledge the Marine Biology and Ecology Research Centre (MBERC) team at the University of Plymouth, including Madeleine Steer for useful tips on proteinase digestion, Andy Atfield for organising lab equipment and Andrew Tonkin for assistance with the use of the µFTIR. Funding for this project was made possible by the National Research Foundation (NRF) grant number (SFP170605237015) and the Association of Common Wealth Universities, Blue Charter Fellowship, awarded to T. Naidoo. Mangrove research groups at the University of KwaZulu-Natal and the University of the Western Cape are funded by the NRF Marine and Coastal Research Grant (MCR180611344089).

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