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Plastic ingestion by estuarine mullet *Mugil cephalus* (Mugilidae) in an urban harbour, KwaZulu-Natal, South Africa

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Abstract

Coastal urban environments have high plastic pollution levels, and hence interactions between plastic debris and marine life are frequent. We report on plastic ingestion by mullet *Mugil cephalus* in Durban Harbour, KwaZulu-Natal, South Africa. Of 70 mullet (13.0–19.5 cm total length), 73% had plastic particles in their guts, with a mean of 3.8 particles per fish (SD 4.7). Plastic ingestion showed no relation to digestive tract content or fish length. White and clear plastic fibres were ingested most commonly. This urban population of *M. cephalus* had a higher incidence of plastic ingestion than has been reported in studies on fish from other coastal areas or the oceanic environment.

Introduction

Plastic has become essential to many daily activities due to its low mass, high durability and low production cost (Koelmans et al. 2014); as a result, global plastic production has increased steadily and currently exceeds 299 million tonnes per year (PlasticsEurope 2015). Single use of plastics by modern society has added to the contamination of the global marine environment (Browne et al. 2011). The presence of plastic in the environment facilitates the interaction with plastic by organisms, and the potential for its ingestion. For example, fish were found to ingest plastics in coastal areas (e.g. Dantas et al. 2012), the open ocean (e.g. Boerger et al. 2010) and even at depths of 850 m in the Mediterranean Sea (e.g. Anastasopoulou et al. 2013). Increasing plastic production and discards may make these encounters more frequent (Thompson et al. 2009). Ingestion rates in some habitats may be high. For example, Davison and Asch (2011) estimated that planktivorous fish in the North Pacific Central Gyre could consume between 12 000 and 24 000 tonnes of plastic per year. Fish that ingest plastics show signs of a false sense of satiation (Ramos et al. 2012), slowed digestion rates (Jackson et al. 2000) and liver toxicity (Rochman et al. 2013), and there is a possibility that they could have increased buoyancy, which may affect vertical migration (Boerger et al. 2010). Estuarine biota, especially those near industrial hubs, may encounter higher water column and sediment plastic loads than those in the open ocean (Bakir et al. 2014). Given that estuaries are important nursery areas for juvenile fish (Cyrus and Forbes 1996; Forbes and Demetriades 2008), plastic debris may pose a severe threat to this life-history stage. Durban Harbour on South Africa's east coast has been reported to contain 144 species of juvenile fish and the threat of plastic pollution in this environment may potentially affect fish stocks (Harris and Cyrus 1999; Markic and Nicol 2014). The most commonly caught species in Durban Harbour is the flathead mullet *Mugil cephalus* (Linnaeus, 1758) (Pradervand et al. 2003). Juvenile and subadult mullet usually use estuaries as nursery areas (Lamberth and Turpie 2003). Under laboratory conditions, a single individual of this species (measuring just 25 mm standard length) was found to ingest as many as 45 particles (Hoss and Settle 1990). However, the incidence of *in situ* plastic ingestion by *M. cephalus* needs to be quantified. *Mugil cephalus* was used therefore as a sentinel species to investigate the ingestion of plastics in the heavily industrialised Durban Harbour. It is considered to be a good indicator species, being cosmopolitan in coastal ecosystems globally, with the exception of the polar regions, and found in fresh water, brackish water and marine environments (Whitfield et al. 2012). Mullet are consumed by carnivorous fish and birds entering coastal systems, providing a potential pathway for the transfer of plastics to high trophic levels (Farrell and Nelson 2013). We hypothesised that *M. cephalus* from Durban Harbour would ingest plastic particles, and that the amount of plastic ingested would be influenced by the total length and digestive tract contents of the fish. If larger fish ingested more plastic particles than smaller fish, this would imply that by ingesting more food, larger fish may encounter more plastics. If smaller fish ingested similar amounts of plastic to larger fish, this would imply that more plastic is retained in the digestive tract of small than large fish when other waste material is egested.

Material and methods

A total of 70 late-juvenile and subadult mullet were collected using a castnet on 11 November 2014 at the Bayhead mangroves of Durban Harbour, KwaZulu-Natal, South Africa (29°53′20.44″ S, 31°00′35.35″ E). Mullet were euthanised by a concussion method, which was approved by the Ethics Committee of the University of KwaZulu-Natal (Reference: 113/14/Animal), then placed on ice and transported back to the laboratory. The total length (TL; cm) of each fish was recorded, the digestive tract removed and the total mass (g) noted. Digestive tracts were dissected and their contents, consisting mainly of benthic microalgae and sediment, were placed into individually marked Petri dishes. All Petri dishes had been rinsed, dried with compressed air and checked under a microscope for any plastic contaminants. Each dish was covered with a second dish and placed in an oven at 60 °C for 24 h. Those containing digestive tract contents were examined under a dissecting microscope for microplastics, following the methods of Boerger et al. (2010). Forceps used for examining digestive tract contents were checked first for plastic contamination under a dissecting microscope. In addition, a cotton laboratory coat was worn to ensure that synthetic fibres from clothing did not contaminate samples. To determine whether contamination was a confounding factor under laboratory conditions (see Davison and Asch 2011; Foekema et al. 2013), three replicates of empty Petri dishes were situated as follows: (i) open on the bench top; (ii) open in the desiccator; and (iii) covered with another dish, in the desiccator. On examining these dishes after 24 h, fibre contaminants were present in one of the open bench-top dishes and in two of the open dishes left in the oven, but none were found in the covered dishes. Therefore, it was assumed that this investigation was at minimal risk of airborne contamination. Plastics found in samples were characterised according to colour and type following Lusher et al. (2013). Small particles were removed carefully and placed into glass cavity slides to be photographed, identified and measured under a Nikon ECLIPSE Ti Series inverted microscope, fitted with a DS-US camera powered by NIS-Elements BR software. After plastics had been removed from samples, the dry masses of digestive tract contents were recorded.

Statistical methods

A general linear model (GLM) was used to assess the relationship between the number of plastic particles found in mullet and their total length and the dry mass of their digestive tract contents, using R 3.2.1 (R Development Core Team 2015). In all, four rows with missing data were deleted and a Hosmer–Lemeshow goodness-of-fit test was used to assess the fit of the model using the Resource Selection package for R. There was no evidence that the model did not fit well (c2 = −7.716, df = 8, $p = 1.000$).

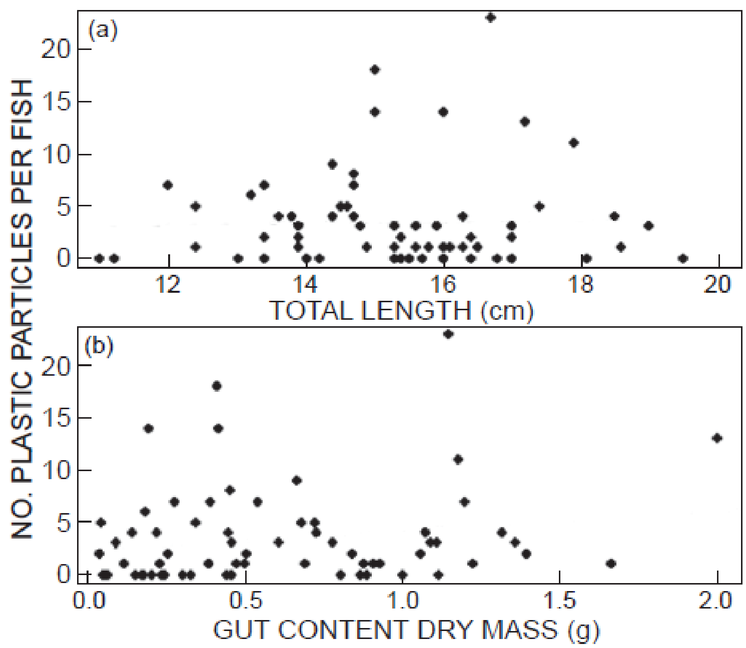


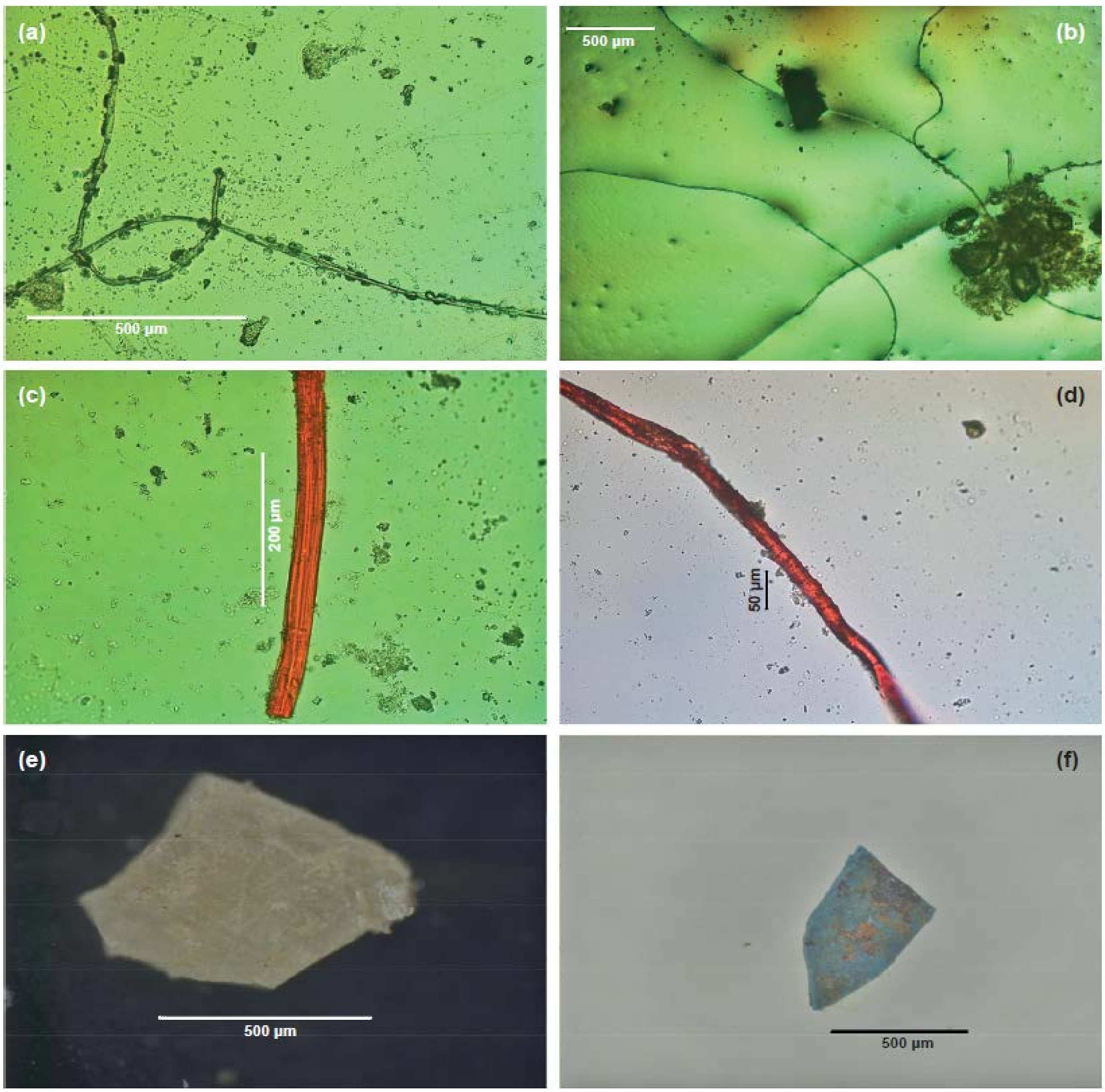
Figure 1: Relationship between number of plastics ingested by *Mugil cephalus* and (a) fish total length and (b) fish gut content dry mass

Results

Plastic particles were found in 51 fish, comprising 72.8% of the 70 mullet sampled. A total of 260 plastic particles at a mean of 3.8 particles per fish (SD 4.7) were consumed and a maximum of 23 particles were found within a single fish of 16.7 cm TL. Plastic particles ranged from 0.2 to 15 mm; however, only a subset of particles were measured. Mullet length ranged from 11.0 to 19.5 cm, with the majority within the 13.0–17.9 cm size range. The GLM suggested that neither fish length ($t = 0.803$, df = 62, $p = 0.425$), nor the dry mass of digestive tract contents ($t = 0.501$, df = 62, $p = 0.618$), nor the interaction between them ($t = -0.683$, df = 62, $p = 0.497$), exhibited a significant relationship with the number of plastic particles ingested per fish (Figure 1). Fibres were the most common plastics found in the digestive tracts under study, comprising 51.2% of the total. Fragments, polystyrene, films, monofilament line and twine contributed 34.6%, 7.3%, 5.0%, 1.5% and 0.4%, respectively, to the overall composition of plastics found. Fragments were the most colourful with white (41.8%), clear (22.0%), opaque (13.2%) and black (5.5%) the most abundant, whereas other types of plastics were mainly clear and white, except twine that was red only. Of the total, 4.8% of fragments could not be identified conclusively as plastic due to their small size and fouling from other gut contents (Figure 2). These fragments were, however, included in all analyses due to their strong resemblance to plastic fragments.

Discussion

In all, 73% of *M. cephalus* examined contained plastic particles, validating the hypothesis that this species in Durban Harbour is prone to plastic ingestion. This proportion



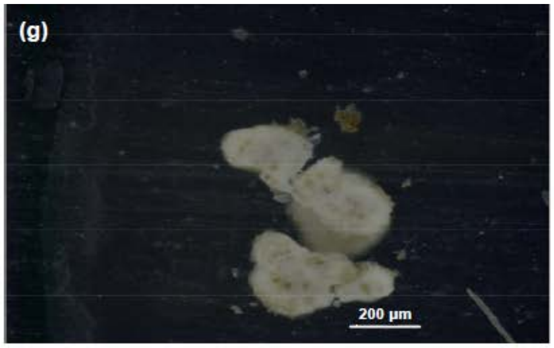


Figure 2: (a–d) Examples of fibres, (e) opaque fragment with fracture lines, (f) blue fragment with fouling on its surface, and (g) polystyrene particles found in the digestive tracts of *Mugil cephalus* specimens collected in Durban Harbour

is higher than that found in other *in situ* studies of plastic ingestion by fish (Supplementary Table S1, available online), with the next highest being ingestion rates of 51–52% by red gurnard *Aspitrigla cuculus* and blue whiting *Micromesistius poutassou* collected in the English Channel (Lusher et al. 2013). Compared to fish examined by Boerger et al. (2010), the maximum number of plastic particles ingested by a single *M. cephalus* from Durban Harbour was four times lower, yet the average number of particles ingested by *M. cephalus* was considerably higher. Among marine environments, including the harbour in this investigation, the lowest incidence of plastic ingestion was found in fish from deep waters (Anastasopoulou et al. 2013). The high incidence of plastic ingestion by *M. cephalus* in this study may be due to their mode of indiscriminate benthic feeding (Whitfield et al. 2012), because plastics accumulate in intertidal sediments of Durban Harbour (Naidoo et al. 2015). In a study by Hoss and Settle (1990), experimentally fed early juvenile *M. cephalus* ingested five times more plastic than did five other fish species, with up to 75% of fish consuming polystyrene spheres 210–350 µm in diameter. These experimentally fed plastics were at the lower end of the size range found in our samples. *Mugil cephalus* may change their diet as they grow, with increasing amounts of detritus and sediment in the guts of larger fish (De Silva and Wijeyaratne 1977), which may affect the type and quantity of plastic ingested. Boerger et al. (2010) recorded a higher number of plastic particles on average in larger fish. However, we did not find differences in plastic ingestion relative to fish size (Figure 1), implying either that: (i) smaller mullet may be able to access areas that have higher sediment plastic concentrations; (ii) smaller mullet may retain more plastic particles per gram of digestive tract because their digestive tracts are less developed and take longer to egest the particles; or (iii) the total size range of fish in our samples was not wide enough to show differences. Nevertheless, the possibility of plastic being retained in the digestive tract and any associated negative effects on fish warrants further experimental investigation. Smaller juvenile mullet (2.1–2.5 cm) than those investigated here were found to consume plastic particles (Hoss and Settle 1990) and could be included in further investigations. Most plastic types ingested by *M. cephalus* were consistent with other studies on ingestion of microplastics by fish (e.g. Boerger et al. 2010; Ramos et al. 2012; Foekema et al. 2013; Lusher et al. 2013) (Supplementary Table S1, available online). However, habitat use may affect the type of plastic most likely to be ingested. For example, fibres formed the lowest proportion of the plastic ingested by pelagic-feeding fish in the Mediterranean Sea

(Anastasopoulou et al. 2013), but the highest proportion in benthic- or demersal-feeding fish, such as *M. cephalus* collected in Durban Harbour and gudgeons *Gobio gobio* from an urban French river (Sanchez et al. 2014). Although there are no comparable studies of plastic ingestion by benthic fish from deep seas, Woodall et al. (2014) reported that the deep sea has become 'a major sink' for fibres, with concentrations of up to 15 fibres per 50 ml in the Mediterranean deep-sea sediment, whereas up to 10 fibres per 50 ml were found in Durban Harbour (Naidoo et al. 2015). Potentially, therefore, rates of ingestion may be similar to those found in urban environments. Fibres are probably derived from clothing and other synthetic textiles, because fibres found in marine environments around the world closely resemble those found in washing machine effluent and have the potential to pass through sewage treatment works (Browne et al. 2011). Other sources of fibres found in the harbour may include weathered fishing and boat-mooring equipment (Guastella and Smit 1994; Murray and Cowie 2011). Most of the plastics ingested by *M. cephalus* were clear or white. However, clear and white plastic particles together constituted the majority of all plastic particle colours in nearby sediments (Naidoo et al. 2015) and in offshore waters (Ryan 1988), implying that ingestion of particles of these colours is not selective. This concurs with studies of fish in the North Pacific Central Gyre (Boerger et al. 2010) and the English Channel (Lusher et al. 2013), and of estuarine fish of the Goiana Estuary, in north-east Brazil (Dantas et al. 2012; Ramos et al. 2012). However, possible selective feeding on clear and white plastic particles by longnosed lancetfish *Alepisaurus ferox* was observed by Choy and Drazen (2013). The high incidence of plastic ingestion by *M. cephalus* found in this study may have negative impacts as reported for other fish species (Jackson et al. 2000; Ramos et al. 2012; Rochman et al. 2013), which could ultimately lead to population effects.

Conclusion

This is the first *in situ* investigation of ingestion of microplastics by fish in South African waters. *Mugil cephalus* from Durban Harbour had a high incidence of plastic ingestion, with fibres being the primary type reported. Fibres may originate from domestic and industrial sources which pass through water treatment works and enter the sediment where the fish forage while feeding on benthic algae. Future work to investigate a range of species, coupled with laboratory experiments, is required to gain more information on the extent of plastic ingestion in urban settings and the biological effects thereof. *Acknowledgements* — We thank Roy Jackson for technical assistance, Dr Brent Newman from the Council for Scientific and Industrial Research for the provision of samples, and Niche-Fambo Fru Azinwi for help with the Nikon microscope. Constructive comments from two anonymous reviewers helped to improve the quality of the final manuscript. Funding from the National Research Foundation made this research possible.

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