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Microplastics in different tissues of fish and prawn from the Musa Estuary, Persian Gulf

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Highlights

- Microplastics (MPs) have been determined in tissues of fish and crustaceans from the Musa estuary and Persian Gulf
- 828 MPs of mainly a fibrous nature were detected in all tissues and species examined
- Mean abundance ranged from 7.8 in tiger prawn to 21.8 in bartail flathead
- The means by which MPs enter non-digestive tissues is unclear
- The occurrence of MPs in seafood for human consumption is cause for concern

Abstract

Commercially-important species of fish and a crustacean from four sites in the Musa estuary and a site in the Persian Gulf have been analysed for the presence and location of microplastics (MPs). A total of 828 MPs were detected in the guts (gastrointestinal tracts), skin, muscle, gills and liver of demersal and pelagic fish (*Platycephalus indicus*, *Saurida tumbil*, *Sillago sihama, Cynoglossus abbreviatus*) from all five sites and in the exoskeleton and muscle of the tiger prawn, *Penaeus semisulcatus*, from three sites. On an individual basis, MPs were most abundant in *P. indicus* (mean = 21.8) and least frequently encountered in *P. semisulcatus* (mean = 7.8), but when normalized on a mass basis, MPs ranged from 0.16 g^{-1} for *C. abbreviatus* to 1.5 g^{-1} for *P. semisulcatus*. Microscopic analyses (polarized light, fluorescence, SEM/EDS) revealed that MPs were mainly fibrous fragments (with a few angular fragments) of various colour and size (< 100 μm to > 1000 μm) and with strong C and O signatures. Additional particles detected that were distinctly different in colour, morphology, brittleness and elemental composition (part-metallic, and containing Cu) were suspected of being fragments of antifouling paint. The means of entry of

MPs into tissues not involved in digestion are unclear but could be related to translocation or adherence. Regardless of the mode of accumulation, the presence of MPs in heavily fished species of fish and crustacean raises concerns about the potential transfer of synthetic materials into humans.

Keywords: Microplastics; fish; prawns; accumulation; microscopy; Persian Gulf

1. Introduction

While the effects of chemical pollutants on marine ecosystems have been studied for many decades, the pervasiveness and impacts of litter on marine life have been recognized more recently (Auta et al., 2017; do Sul and Costa, 2014). Plastics, as identifiable primary objects or secondary fragmented pieces, comprise the largest pool of litter on both a mass and number basis and enter the oceans via rivers, sewage discharge, land run-off, and spillages and discharges from ships at sea (Moore, 2008; Andrady, 2011; Barnes et al., 2009; Gregory and Andrady, 2003). Given the expected future demand and discharges of plastic, coupled with the resistance of synthetic polymers to environmental degradation, it is clear that the marine plastic inventory will continue to increase beyond at least the next decade (Jambeck et al., 2015).

Of particular concern with respect to both the direct impacts on marine life and transfer through the foodchain are readily ingestible microplastics (MPs), or synthetic particles ranging from a few micrometers to five millimeters in any dimension (Alomar et al., 2016; Turner, 2017; Abbasi et al., 2017). Primary MPs include abrasive micro-beads in face scrubber cosmetics and toothpaste, synthetic fibres and pre-production resin pellets, while secondary MPs are generated in situ by the mechanical and oxidative breakdown of larger plastics (Hidago-Ruz et al., 2012). As well as the inherent composition of the polymer and the presence of any additives, the chemistry of MPs may be modified by the adsorption of toxic substances from ambient sea water to the hydrophobic plastic surface (e.g. organic pollutants; Teuten et al. 2007; Ziccardi et al. 2016) or to more hydrophilic, hydrogenous or biogenic phases coating the surface (e.g. heavy metals; Ashton et al., 2010; Holmes et al., 2014).

A wide range of marine organisms, including bivalves, zooplankton, fish, invertebrates, birds and cetaceans, incidentally take up MPs from sediment or the water column because they mistake them for food (Cole et al. 2013; Lusher et al. 2015; Ferreira et al. 2016). Ingesting MPs of no nutritional value may induce physical and chemical toxicity, block or damage the digestive tract, or decrease individual fitness, ultimately resulting in death (Wright et al., 2013; Luís et al. 2015; De Sá and Guilhermino 2015). Moreover, MPs of tens of micrometers in dimension have the propensity to translocate from the gut to the circulatory system in many organisms where they may reside for relatively long periods of time (Browne et al., 2008; van Cauwenberghe et al., 2015; Collard et al., 2017). While the effects of translocated MPs on chronic animal health are unknown, their presence is of particular concern because consumption of contaminated food, including fish and shellfish, may act as a vehicle for the ingestion and translocation of MPs in humans (Li et al., 2015; Rist et al., 2018).

The Musa is one of the biggest estuaries in the northern Persian Gulf and is the most important fishery resource for people in the cities of Mashahr (population 150,000), Sarbandar (75,000) and Hendijan (50,000). While the coast of the estuary is flanked by agricultural land, there are also various industrial plants and extensive docks that support the petrochemical and shipping industries and municipal and industrial sewage from the catchment is poorly treated (Hosseini et al., 2013; Rastegari Mehr et al., 2016). Given these conflicting uses of the estuary, the aim of the present study was to determine whether MPs are accumulating in different organs of five abundant and commercially valuable species of fish and crustacean that are heavily consumed by local people. Specifically, we target the skin, gastrointestinal tract, liver, muscle and gills of two demersal fish, the bartail flathead (*Platycephalus indicus*) and greater lizardfish (*Saurida tumbil*), one pelagic fish, the northern whiting (*Sillago sihama*), and one mesopelagic species fish, the tongue sole (*Cynoglossus abbreviatus*), and the skin and muscle of the tiger prawn, *Penaeus semisulcatus*.

2. Materials and methods

2.1. Sampling and sample preparation

Fish and prawn samples were caught from along the coastal waters of the Persian Gulf during June 2015 by a trawl net from five locations (see Figure 1), one of which served as a control site (S5; the fishery port of Hendijan located outside the estuary and 70 km from any petrochemical facilities). At each station, up to five samples of each species were collected, with a total catch of 56 specimens among all species. Samples were transported in a cooler to the laboratory where they were stored at -20 °C pending processing and analysis.

Given the ubiquity of MPs in the indoor environment (Gasperi et al., 2018), suitable measures were undertaken to prevent plastic and fibre contamination in the laboratory. Thus, all chemical reagents were filtered (8-μm, Whatman No. 540) before use and white cotton laboratory coats, single-use latex gloves and face masks were used throughout sample manipulation and processing. Working surfaces were thoroughly cleaned with ethanol and all glassware, tools and fish and prawn skin surfaces were washed successively with a commercial dishwashing liquid, HPLC-grade distilled water and ethanol before being dried in an oven at 105 ^{o}C (glassware and tools) or at room temperature in a metal cabinet (skin surfaces). Analysis of two procedural blanks (without tissues) and distilled water contained in two wide dishes that had been left exposed during the duration of sample processing revealed no contamination from MPs under the working conditions in the laboratory.

2.2. Extraction of MPs

As required, specimens were thawed and the fork length from the mouth to the central point of the caudal fin and body weight were recorded. Each fish was gutted and dissected in a metal tray using a scalpel, forceps and scissors and the muscle, skin, gills, liver and gut (gastrointestinal tract) retrieved. The pooled livers, guts and gills from each species and at each site were transferred directly to covered petri dishes while pooled muscles and skin, after separation, were homogenized using an Electric Meat Grinder (KENWOOD MG510, UK) before about 15 g of each was retained and stored in a petri dish. For the (smaller) prawns, tissue retained for analysis was restricted to the muscle and skin (exoskeleton) that was pooled from individuals and homogenized as above.

Tissues of fish and prawn were subject to digestion to remove organic matter and leave behind silica/aluminosilicates and any plastic (Karami et al., 2017). Thus, tissues were emptied into a series of 500 mL glass beakers to which approximately 30 mL of 35% H_2O_2 (Arman Sina) and 30 mL of 4% KOH (Merck) were added. The contents were digested for 72 h at 60 ºC in an oven to dissolve the soft organic components of the tissues, before a 10:40 ml mixture of 68% $HClO_4$ and 65% HNO_3 (both Merck) was added to completely digest more resistant material like the gills and skin-exoskeleton. After a few minutes of acid extraction, digests were diluted with warm distilled water to preserve the integrity of MPs. Plastics were separated from all tissues with the exception of the gut by shaking digests at 350 rpm for 5 min and subsequently centrifuging triplicate aliquots for 5 min at 4000 rpm. Supernatants were directly filtered under vacuum through S & S grade 589/3 filters which were subsequently stored and dried (at room temperature) in individual petri dishes pending analysis.

For MPs embedded in the gastrointestinal tract of fish, remaining digests were agitated at 350 rpm for 5 min in a solution of concentrated sodium iodide (NaI, Merck; density = 1.6-1.8 g cm^{-3}) to separate plastics from additional material that had been ingested with subsequent filtration and storage undertaken as above.

2.3. Observation and validation of MPs

A visual assessment of material retained on the filters, and including any arising from the procedural control, was made according to colour, size and morphology (elongated fibre versus angular fragment) and at up to 200 x magnification using a Carl-Zeiss binocular microscope. The presence of plastic was verified by the colours returned by polarized light microscopy using an

Olympus BX41TF microscope and by fluorescence microscopy using an Olympus CX31 microscope. Images from all microscopic techniques were captured using an Olympus Pen EPL 1 digital camera.

Based on the optical microscopy results, the topography and elemental composition of selected MPs were determined through high vacuum SEM/EDS. We used a Tescan VEGA 3 electron microscope (with a resolution of 2 nm at 20 kV) and an Oxford Instruments X-Max 50 silicon drift detector with AZtec and INCA software after samples that had been carefully brushed from the filters were mounted on double-sided adhesive carbon tabs on aluminium SEM stubs.

3. Results

3.1. Size and weight of fish and prawns

Table 1 summarises the catch from each sampling site (note that the number of species caught at each site varied and that some species were absent from sites 1, 2 and 3). Also shown are the mean, minimum and maximum lengths and weights of each of the five species, serving to illustrate differences in size among species and between sites and, for a given species, differences in age and, therefore, propensity to have accumulated MPs.

3.2. MPs in fish and prawns

Table 2 shows the number of MPs in the tissues of the five species at each site, with data pooled for the number of individuals indicated in Table 1. Note that MPs were detected visually (Figure 2), with the synthetic nature of samples confirmed by fluorescence and polarized light microscopies for characteristic response to visible and ultraviolet light (Woodall et al., 2015; Wang et al., 2016; Figure 3) and, for selected samples, by SEM/EDS for surface morphology and elemental composition (mainly carbon). By comparison, no particles of this nature were observed on the two filters arising from the procedural controls.

Among the catch, 828 pieces of MP were detected, being encountered across all tissues from each species. In only isolated cases (e.g. the liver of *P. indicus* from sites 1, 2 and 5 and the gut of *P. indicus* at site 5) were MPs absent, with numbers exceeding 25 in the skin of *S. sihama* at site 2, the gills of *P. indicus* at site 4 and the skin of *P. indicus* at site 5. On this basis, there were no clear differences in the total number of MPs accumulated by each species or between sites (and including the control site), but numbers tended to be higher in the skin, muscle and gills than the gut and liver of *S. sihama* and *P. indicus* and were always greater in the skin than in muscle from *P. semisulcatus*. When considered on an individual basis, or after total numbers for each species had been normalized for the number of samples analysed, MPs are most abundant in *P. indicus* (mean = 21.8) and least frequently encountered in *P. semisulcatus* (mean = 7.8); when normalized on a mass basis, however, the mean abundance of MPs ranged from 0.16 g^{-1} for *C. abbreviatus* to 1.5 g^{-1} for *P. semisulcatus*. By comparison, a recent study by Akhbarizadeh et al. (2018) in the northeast of the Persian Gulf reports an average abundance of MPs in muscle of the fish, *P. indicus*, *Sphyraena jello* and *Epinephelus voioides*, and the shrimp, *Alepes djedaba*, of 1.85 ± 0.46, 0.57 ± 0.17, 0.78 $\pm$ 0.22 and 0.80 ± 0.12 g^{-1}, respectively.

Nearly all MPs encountered were filamentous fragments (consisting of single fibres) of different size and colour and as illustrated in Figure 2. In only five cases were non-fibrous plastics found among the different species of fish: specifically, two white fragments in the muscle of *C. abbreviatus* from site 5, one yellow fragment in the gills of *S. sihama* from site 4 and one blue fragment in the gastrointestinal tract of both *S. sihama* at site 4 and *S. tumbil* at site 1.

The size distributions of MPs are shown in Table 3 for individual tissues and in Figure 4 for whole organisms. Thus, there is a wide range of lengths of (mainly) filamentous material across all species, with the most abundant sizes between either 100 and 250 μm (*S. sihama, P. indicus, P. semisulcatus*) or 250 to 500 μm (*C. abbreviatus*, *S. tumbil*). With respect to the different tissue types, the digestive organs appear to contain a high proportion of relatively large MPs, while particles above 250 μm are absent from the liver.

The colour distribution of the MPs that had visibly accumulated is shown in Figure 5. Thus, overall, 71% of MPs consisted of black or grey filamentous fragments, with blue and green fragments comprising about 12% of the MP pool. White-transparent and red-pink fragments contributed about 7 and 8%, respectively, with yellow-orange material lowest in overall abundance at about 1.3%. There were no clear differences in colours accumulated by different species or in different organs. However, there were notable differences in the distribution of certain colours between the different sites; for instance, only one white-transparent fragment and no yellow-orange fragments were recorded at site 1 while six yellow-orange and 20 white-transparent fragments were observed at sites 4 and 5, respectively.

In addition to the MPs described above and quantified in Tables 2 and 3, a number of fragmented particles of between a few tens of nm to a few hundred μm in diameter were observed in the guts and gills of (mainly) pelagic fish that were distinctly different. Thus, EDS revealed the presence of metals, and mainly Cu, in addition to C and O, while manipulation during analysis and SEM imagery showed that the material was highly brittle (Figure 6). It is possible that these particles were of metal construction, at least in part. However, given the detection of both organic material and Cu, we suspect that these particles are small flakes of paint impregnated with Cu. Most contemporary antifouling paint formulations employ Cu as a biocide and are generated abundantly at boat maintenance and repair facilities and are also shed from boat hulls and other painted maritime structures while in use (Turner, 2010).

4. Discussion

This study is one of an emerging number demonstrating the accumulation of MPs by marine organisms. Of the MPs detected, and consistent with previous environmental studies, they are mainly fibrous (Lusher et al., 2013; Rochman et al., 2015; Pazos et al., 2017), with sizes ranging from < 100 μm to > 1000 μm. MPs are generally larger in the gills and gastrointestinal tract than in other organs because larger material can readily enter the digestive environment with relatively little obstruction; the abundance of MPs in the digestive environment is also rather variable, reflecting variations in the amount and type of consumed food both between individuals of the same species and among different species.

Despite some planktivorous fish seeming to select MPs that are visually similar to their diet (i.e. blue fragments) (Ory et al., 2017), without information on the colour distribution of MPs in the water column or sediments of the Musa estuary and Persian Gulf there is no evidence in the present study for the preferential ingestion or accumulation of MPs according to appearance. We also do not have specific information on the type of plastics found in the organisms sampled, although MPs retrieved from littoral sediments of the Persian Gulf indicate a predominance of polyethylene, nylon and polyethylene terephthalate (Naji et al., 2017).

On an individual basis, MP abundance ranges from about 8 for the prawn, *P. semisulcatus*, to over 20 for the demersal fish, *P. indicus*, that forages in the sediment and where most of the denser MPs reside. These values are higher than those reported for fish in previous studies; for example, up to 7.2 items per individual were observed in coastal and freshwater fish from China (Jabeen et al., 2017), up to about 4 per individual were detected in the semi-pelagic Mediterranean fish, *Boops boops* (L.). (Nadal et al., 2016), and an average of 1.6 items per fish were recorded in various demersal fish in Spanish coastal waters (Bellas et al., 2016). However, it is important to appreciate that these studies focused on the retrieval of MPs from the digestive tract only. When our data are restricted to the gut, the average number of MPs per individual ranges from about 1.5 in *S. sihama* to 3 in *C. abbreviatus* (see Table 2).

The discrepancies referred to above arise from the general assumption that accumulation of plastics by fish and other organisms proceeds mainly through ingestion and is, therefore, dependent on factors like feeding strategy and gut structure as well as the extent of local plastic pollution (Romeo et al., 2015; Jabeen et al., 2017). Thus, MPs may be accumulated directly and incidentally or deliberately while feeding from the water column or sifting through contaminated sediment, or indirectly through the consumption of contaminated prey (Cannon et al., 2016; Jovanović, 2017). The detection of MPs in the present study in organs not directly involved with ingestion-digestion suggests that other factors may be significant for the accumulation and, potentially, translocation of MPs in fish.

Results of laboratory experiments have reported the occurrence of MPs in the circulatory system or non-digestive organs of marine invertebrates (Browne et al., 2008; von Moos et al., 2012) and in the liver of zebrafish (Lu et al., 2016). However, particles employed in these studies were on the order of tens of micrometers in diameter or less, thereby facilitating passage across the gill or gut epithelium through cell internalization and subsequent translocation. Collard et al. (2017) suggest that detection of larger MPs (and of dimensions comparable to those observed here) in the livers of European anchovies (*Engraulis encrasicolus*) may result from two processes: the agglomeration of smaller particles and/or passage through the gut barrier by some form of intracellular or paracellular endocytosis. The former mechanism is unlikely in the present study because SEM images revealed distinct and relatively smooth fibrous fragments, and without knowledge of the locations of MPs in (homogenized) tissue the latter mechanism cannot be fully explained.

Alternatively, it has recently been suggested that adherence affords an additional means by which fibrous MPs may associate with organs independent of the digestive system, in a manner by which seaweeds accumulate plastics (Gutow et al., 2016). Thus, under laboratory conditions, about 50% of microfibres exceeding 100 μm in marine mussels could be accounted for through adherence, with surface area and “stickiness” two important controls in this respect (Kolandhasamy et al., 2018). Regardless of the mechanisms by which MPs enter or associate with non-digestive tissues, their occurrence has a number of implications for evaluating the inventory, location and toxicity of MPs in marine animals, as well as for human health through seafood consumption. Specifically, if the gut is considered as the sole receptacle, where MPs may either be in transit or entrapped, the total number of MPs accumulated by an individual may be considerably underestimated. With respect to toxicity, accumulation outside the digestive tract may induce histological changes and oxidative stress (Lu et al., 2016) or release contaminants associated with or adsorbed to MPs (Ashton et al., 2010). The potential for MPs to be transferred to humans should not be underestimated given that the soft tissue of the species considered are important to the regional fishing industry. According to the Institute of Standards and Industrial Research of Iran in 2010, daily average fish muscle consumption is about 7 g/person/day, meaning that about 5 MPs could be consumed on a daily basis. While there is currently no regulatory framework concerning the presence of MPs in sea food (European Food Safety Authority 2016), this does not exclude the possibility that MPs are able to interact with human cells and tissues and facilitate the delivery of harmful contaminants to the bloodstream (Santillo et al., 2017).

5. Conclusions

This study has demonstrated the presence of MPs of mainly a fibrous nature and of length < 100 μm to > 1000 μm in various commercially important species of fish and a crustacean collected from the Musa estuary and the Persian Gulf. Average quantities of MPs ranged from 0.16 g^{-1} for the mesopelagic fish, *C. abbreviatus,* to 1.5 g^{-1} for the prawn, *P. semisulcatus,* with particles encountered in various tissues from both digestive and non-digestive organs across all species. The occurrence of MPs outside the digestive system suggests that material can be translocated following ingestion or that additional, non-ingestive mechanisms (e.g. adherence) are significant.

The presence of MPs in non-digestive organs has the potential to induce toxic effects on individuals and affords an exposure route to humans who consume contaminated fish.

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Figure captions:

Figure 1: Locations of the five sampling sites along the coast of the Musa estuary.

Figure 2: Examples of MPs encountered in fish and prawn tissues and as captured by binocular microscope. Note that fibres in panels (b) and (g) are extremely thin and, therefore, have a relatively high propensity to penetrate tissue, and that fibres in panels (c), (d), (f), (h), (i) and (j) exhibit partial entrapment in half-digested tissues.

Figure 3: An image and the composition of a fibre obtained by SEM/EDS (W% = weight percent and A% = atomic percent) (a); fibre images obtained using upper-light fluorescence microscopy (b,c); fibre images obtained by polarized downward projecting light microscopy (e,g) and corresponding images obtained without polarized light (d,f).

Figure 4: The net distribution of MPs among different size categories (in μm) in the five species.

Figure 5: Overall colour distribution of the MPs observed in the samples.

Figure 6: SEM/EDS image and composition of a particle of a relatively brittle and non-fibrous natu

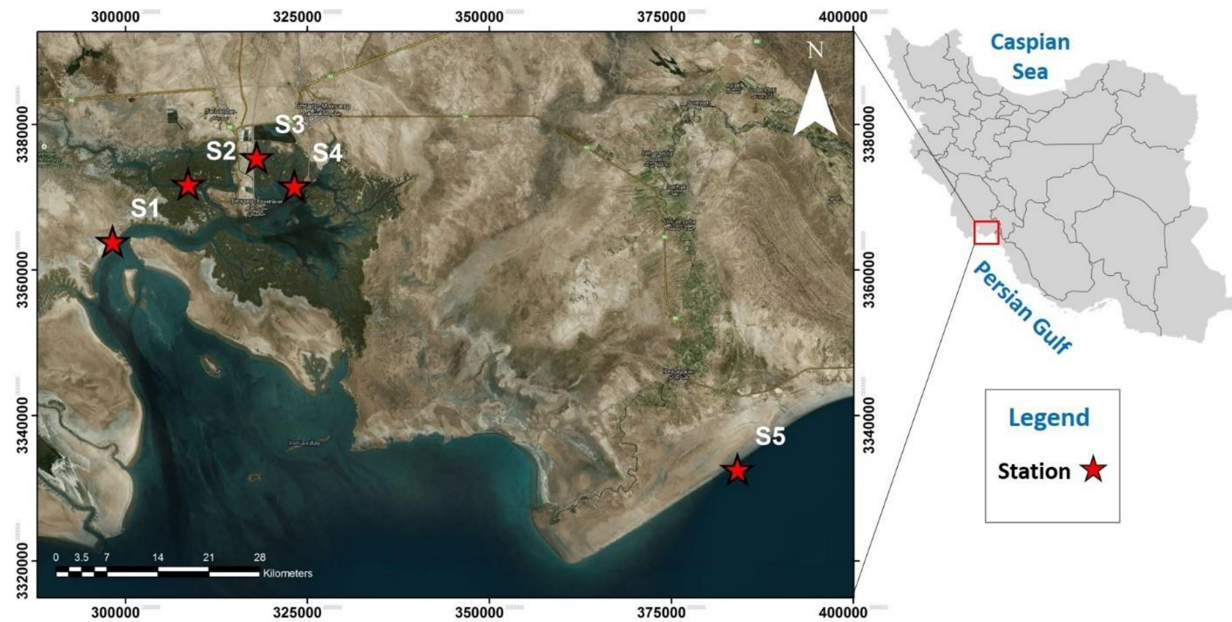
Table 1: Number of species caught from each site (*n*) together with the mean (and minimum and maximum) lengths (cm) and weights (g).

		S. sihama	*P. indicus*	*C. abbreviatus*	*S. tumbil*	*p. semisulcatus*
S1	*n*	4	3	4		
	length	20.1 (17.2-20.1)	17.3(16.5-18.5)	17.7 (14.2-20.5)		
	weight	67.8 (49.3-95.1)	23.8 (18.3-32.7)	33.1 (14.2-56.6)		
S2	*n*	4	1	4		
	length	16.6 (13.0-20.0)	16.0	17.7 (14.2-20.5)		
	weight	39.4 (14.2-62.4)	16.8	33.1 (14.2-56.6)		
S3	*n*					5
	length					7.8 (5.5-10.0)
	weight					5.4 (2.3-10.6)
S4	*n*	4	4	3	4	3
	length	16.6 (15.5-18.0)	19.5 (18.5-21.5)	23.7 (23.0-24.0)	15.7 (13.0-18.0)	7.3 (6.0-8.5)
	weight	45.6 (36.4-53.3)	46.7 (35.7-63.6)	115.9 (109.3-123.0)	36.1 (18.6-50.4)	5.2 (2.5-8.0)
S5	*n*	5	4	4		4
	length	20.5 (18.5-24.5)	20.5 (20.0-22.0)	23.8 (22.5-26.0)		7.6 (4.5-10.5)
	weight	72.2 (51.8-119.9)	41.7 (39.1-46.5)	88.4 (75.2-115.4)		4.9 (1.4-8.7)
total	*n*	17	12	15	4	12
	length	18.6 (13.0-24.5)	19.0 (16.0-22.0)	24.6 (14.2-21.7)	15.7 (13.0-18.0)	7.6 (4.5-10.5)
	weight	57.2 (14.2-119.9)	36.8 (16.8-63.6)	75.8 (14.2-123.0)	36.1 (18.6-50.4)	5.2 (1.4-10.6)

Table 2: Number of MPs detected in the five species pooled from each site (with the number of species given in Table 1). Also shown is the total number of MPs in each species, the mean number when normalized for the number of individuals analysed and the average mass of individuals, and the mean number per individual when only the gut was considered.

		S. sihama	*P. indicus*	*C. abbreviatus*	*S. tumbil*	*P. semisulcatus*
S1	skin	7	27			
	muscle	14	7			
	gut	1	11			
	gills	15	22			
	liver	6	0			
S2	skin	29	14	8		
	muscle	20	21	10		
	gut	9	4	11		
	gills	12	12	12		
	liver	4	0	5		
S3	skin					23
	muscle					12
	gut					
	gills					
	liver					
S4	skin	14	14	8	6	21
	muscle	19	14	12	12	14
	gut	12	12	18	11	
	gills	20	27	13	8	
	liver	11	13	24	17	
S5	skin	11	27	13		14
	muscle	11	13	12		10
	gut	4	0	15		
	gills	8	23	8		
	liver	12	0	11		
total		239	261	180	54	94
mean/individual		14.1	21.8	12.0	13.5	7.8
mean/g		0.25	0.59	0.16	0.37	1.51
mean/gut		1.5	2.3	2.9	2.8	

Fig 1.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350000
375000
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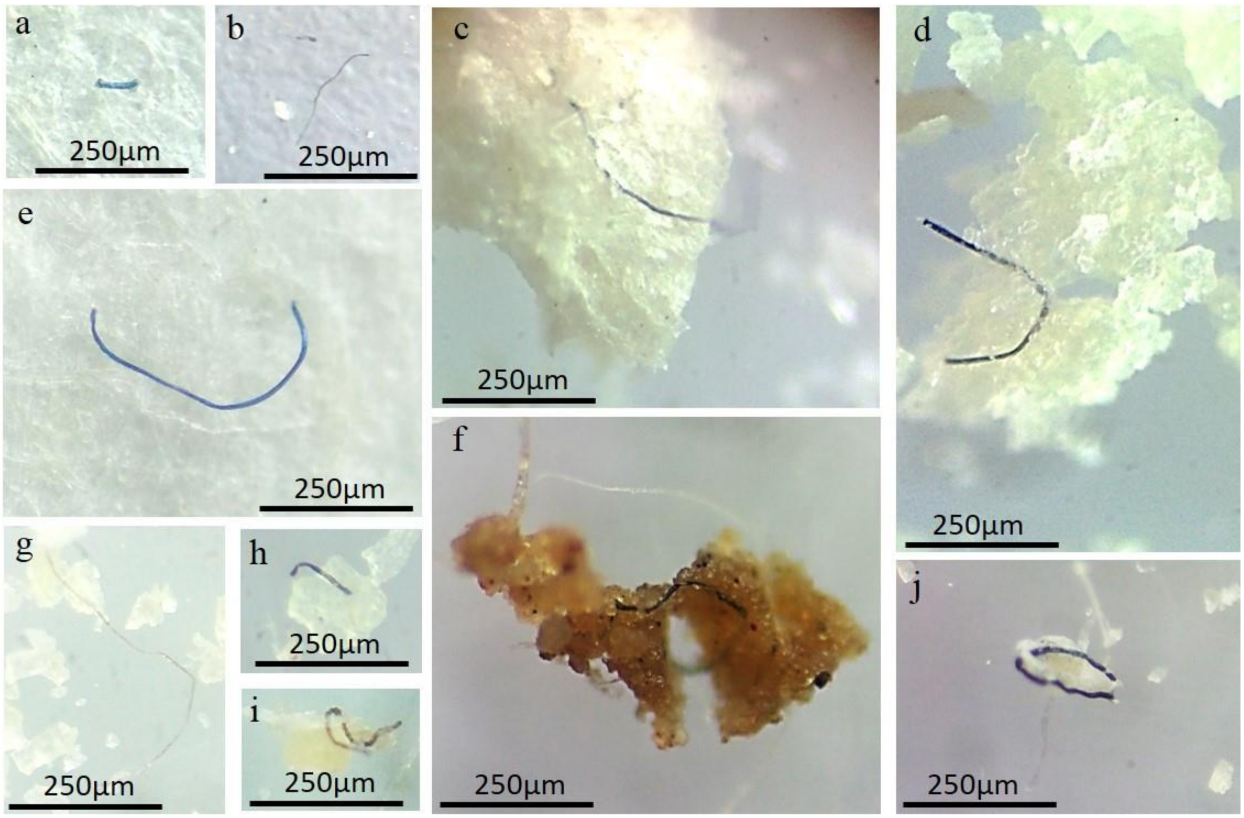
3380000
3360000
3340000
3320000
N
S1
S2
S3
S4
S5
0
3.5
7
14
21
28
Kilometers
Caspian
Sea
Persian Gulf
Legend
Station

Fig 2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250µm
b
250µm
c
250µm
d
250µm
e
250µm
f
250µm
g
250µm
h
250µm
i
250µm
j
250µm

Fig 3

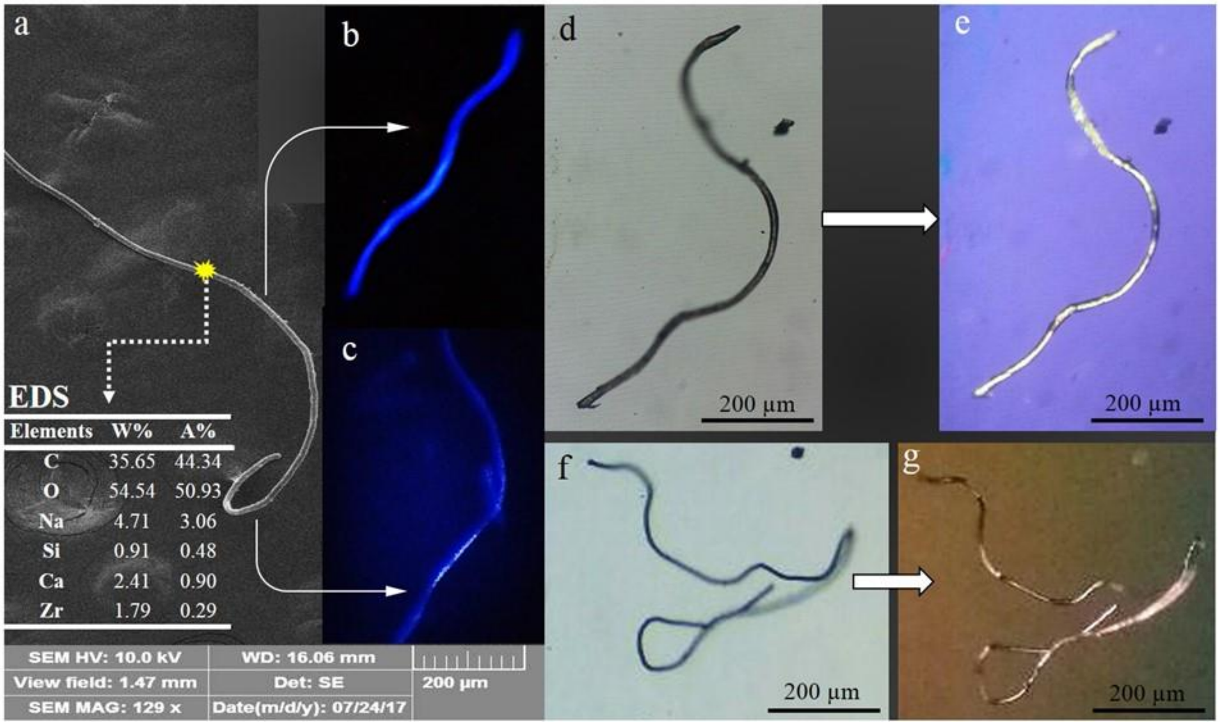

a
b
c
d
e
f
g
EDS
Elements W% A%
C 35.65 44.34
O 54.54 50.93
Na 4.71 3.06
Si 0.91 0.48
Ca 2.41 0.90
Zr 1.79 0.29
SEM HV: 10.0 kV
WD: 16.06 mm
View field: 1.47 mm
Det: SE
200 µm
SEM MAG: 129 x
Date(m/d/y): 07/24/17
200 µm
200 µm
200 µm
200 µm

Fig 4

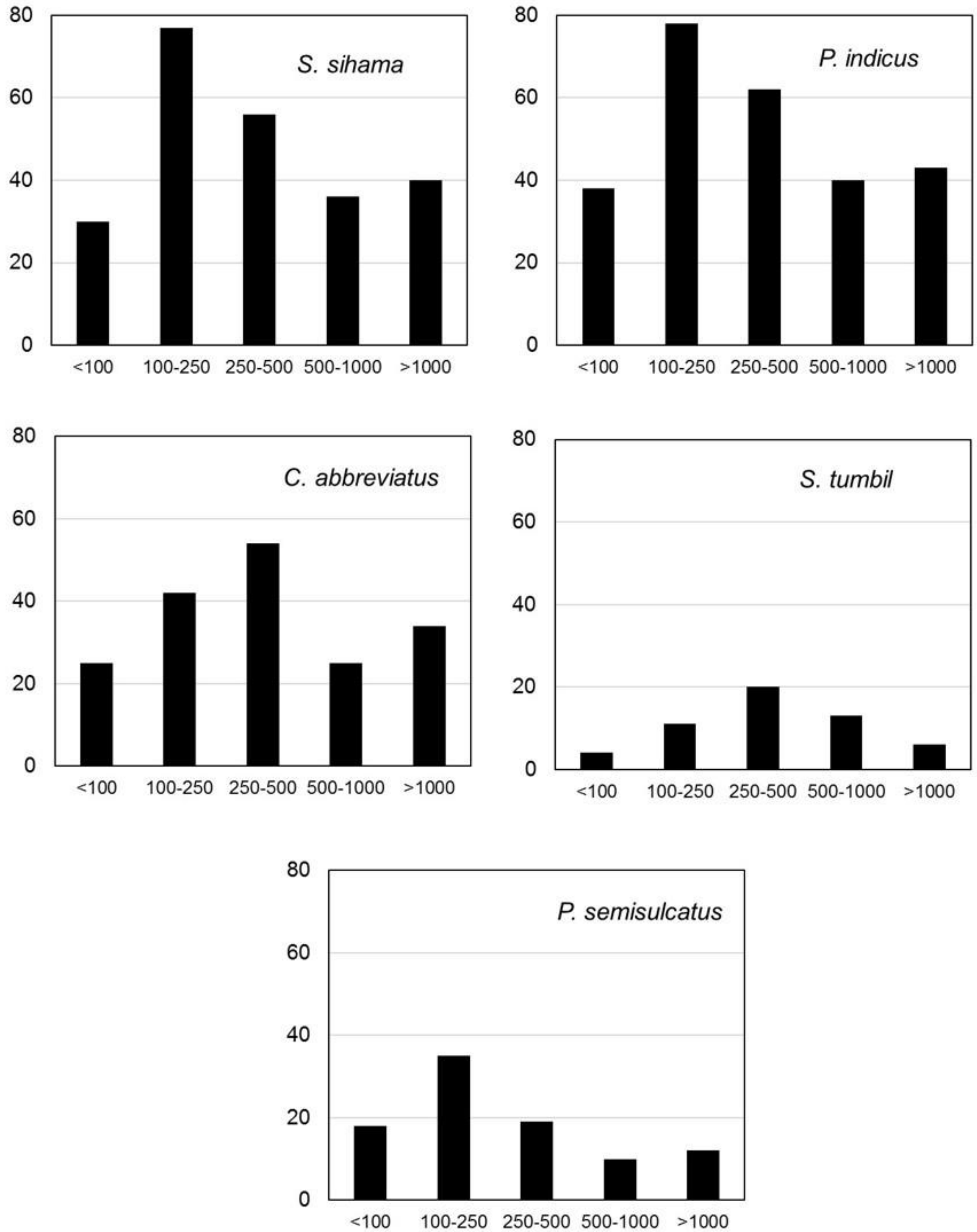

S. sihama
P. indicus
C. abbreviatus
S. tumbil
P. semisulcatus
0
20
40
60
80
<100
100-250
250-500
500-1000
>1000

Fig 5

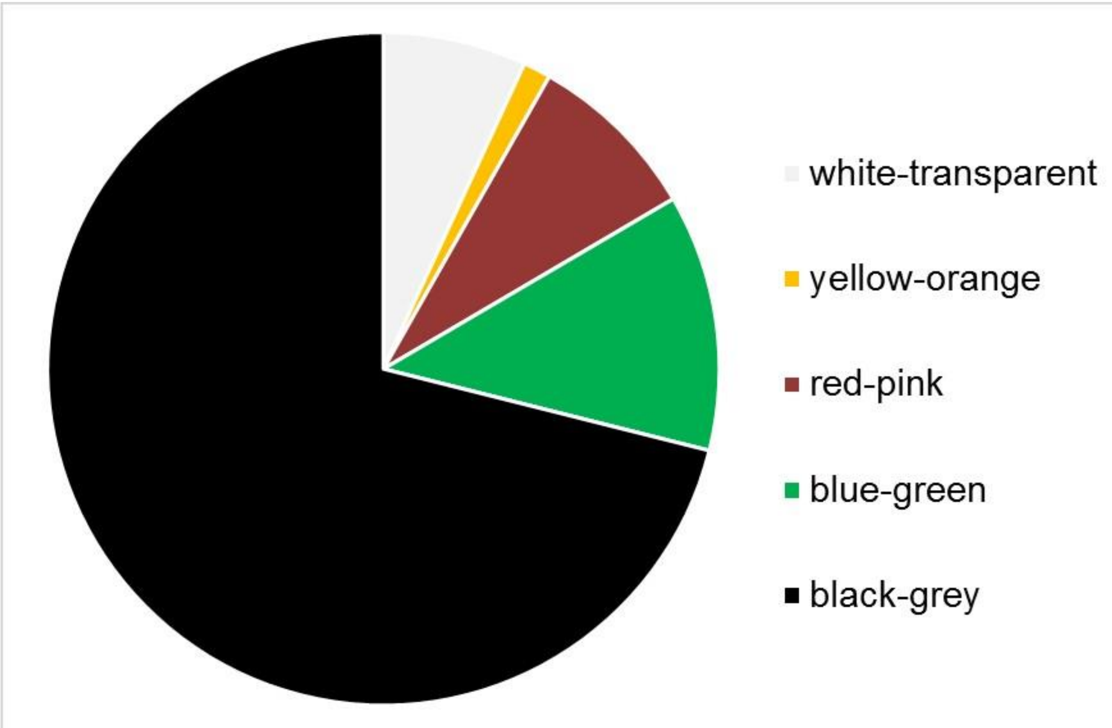
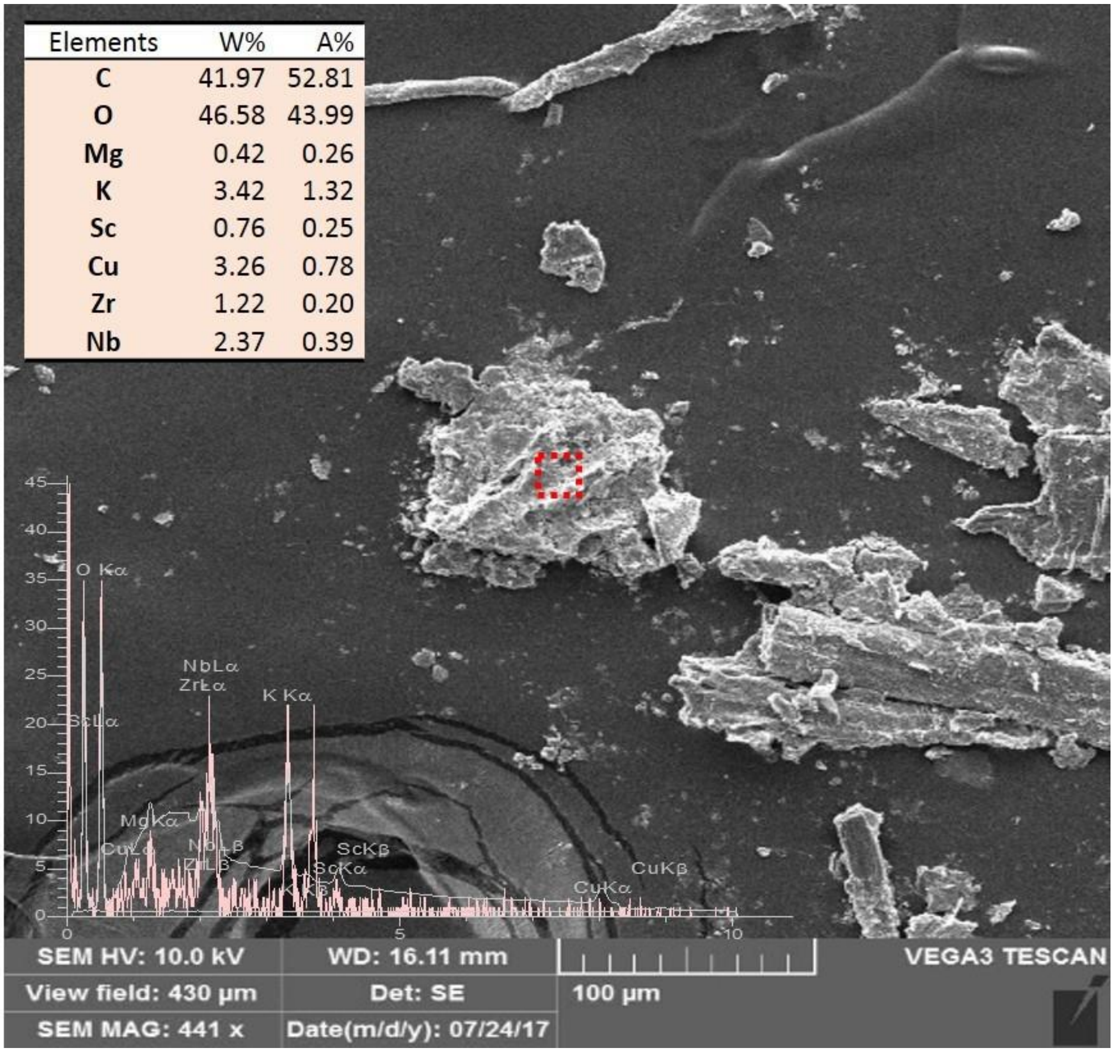

white-transparent
yellow-orange
red-pink
blue-green
black-grey

Fig 6



Elements W% A%
C 41.97 52.81
O 46.58 43.99
Mg 0.42 0.26
K 3.42 1.32
Sc 0.76 0.25
Cu 3.26 0.78
Zr 1.22 0.20
Nb 2.37 0.39
O Kα
NbLα
ZrLα
K Kα
ScLα
MgKα
CuLα
ScKβ
ScKα
CuKα
CuKβ
SEM HV: 10.0 kV
WD: 16.11 mm
VEGA3 TESCAN
View field: 430 μm
Det: SE
100 μm
SEM MAG: 441 x
Date(m/d/y): 07/24/17

re (W% = weight percent and A% = atomic percent).