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The occurrence of microplastic in specific organs in commercially caught fishes from coast and estuary area of east China

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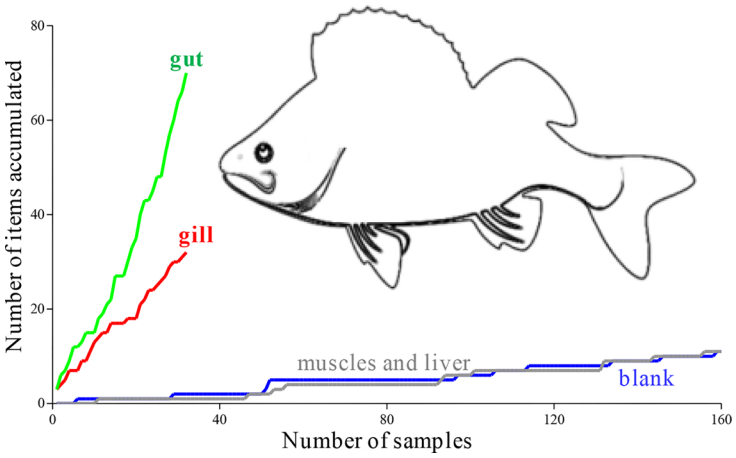
GRAPHICAL ABSTRACT

Microplastics > 20μm are often present in gills and guts but are rarely when occurred in fish muscles and liver from *Lateolabrax maculatus*.



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ABSTRACT

It is important to understand where microplastics go in the body of organisms. They can readily affect target organs and transport microplastic-associated chemicals to humans via consumption. The plastics (> 20 μm) in guts and gills of 13 species of fishes from coast estuary areas of China were examined for the presence of microplastics. Muscle and liver were analyzed from a commercial species, the Asian seabass (*Lateolabrax maculatus*), of which 73% of the suspected items were verified by micro-Fourier Transform Infrared Spectroscopy. We targeted the organ specific distribution of microplastics. Microplastics were detected in gut and gills in 22%-100% and 22%-89% of total individuals, respectively. Microplastics in gut varied from 0.3 to 5.3 items/ind. and varied from 0.3 to 2.6 items/ind in gill, respectively. The size of microplastics in gills were smaller than those found in the guts. No microplastics were detected in the liver or muscle tissue of *L. maculatus*, and several non-plastic items detected in muscles can be attributed to background contamination. Further research is required using a larger number of specimens and better quality control and quality assurance are required to assess the presence of small microplastics or nanoplastics in fishes internal organs and muscle.

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1. Introduction

The presence of microplastics (< 5 mm) in oceans and marine organisms is of great concern to biodiversity and is a potential hazard to human consumers of seafood [1–5]. Microplastics have been detected throughout the world in a variety of marine and freshwater biota, including bivalves, birds and fish [6–9]. Many laboratory studies have also shown that a variety of organisms from a range of trophic levels uptake microplastics and any associated chemical contaminants bound to these microplastics [10–14].

Fishes were the most common organism used in microplastics studies [15,16]. Being model species in environmental science and ecology, fish are widely used as biomonitors to assess the health of aquatic ecosystems [17]. More than 150 fish species from all kinds of habitats have been reported to contain microplastics [18]. Zebra fish, medaka and other species have also been used in microplastic exposure experiments. The endpoints such as mortality, growth inhibition and metabolism disorder have been selected [19–21].

Ingestion is widely considered to be the primary uptake pathway of microplastics in fish [22–24]. Once ingested, they are expected to be resided in the intestinal tract (gut) and ultimately be eliminated [25,26]. After ingestion, physical damage occurred, including internal and/or external abrasions, ulcers and blockages of the digestive tract [4]. While some contaminants that are ingested may accumulate in tissues and internal exposure, the exposure is more transient in nature for microplastics. In some laboratory studies, gills were also considered as an important pathway to collect microplastics in fish, although this has rarely been reported in the field [16,27].

The occurrence of microplastics in muscle and liver has been reported in several case studies [28–30]. They suggested that microplastics up to 5 mm accumulated in the muscle of fish and the plastic film located inside the liver. If such findings could be further proved with better quality control and quality assurance, we need to reconsider the risks associated with microplastics in fishes because human consume the fish meat directly and microplastics will accumulated in fish, affecting the target organs. In laboratory conditions, the uptake and accumulation of polystyrene in zebrafish liver and gill can result inflammation and lipid accumulation [19].

In addition to muscle and liver, the fish gill is a sophisticated, delicate organ that has multiple physiological functions in addition to gas exchange. They included osmoregulation, acid-base regulation and excretion of nitrogenous, disruption of any of these functions could potentially be fatal [31]. Whether microplastics can interact with fish gill in natural conditions is still unknown.

Several sections of Yangtze River and Yangtze Estuary of China were suggested as a hotspot for microplastic pollution and fishes from these waters have been detected with high level of microplastics in gut in recent studies (e.g. microplastics reached up to 340 items/kg in sediment and 13,617 × 10^3 items/km^2 in water) [32–35]. It provides us with an ideal background to study fish with high levels of microplastic exposure in field conditions. In the present study, we carried out a study to:1) estimate the background contamination of microplastics in fishes by measuring them in gill and gut of fishes from the case area -2) investigate microplastics in muscle and liver from a very common commercial fish species, Asian seabasses (*Lateolabrax maculatus*), using thorough quality control and error analysis procedures. We described the distribution of microplastics in different fish organs and the possibility whether they can accumulate in fish tissues.

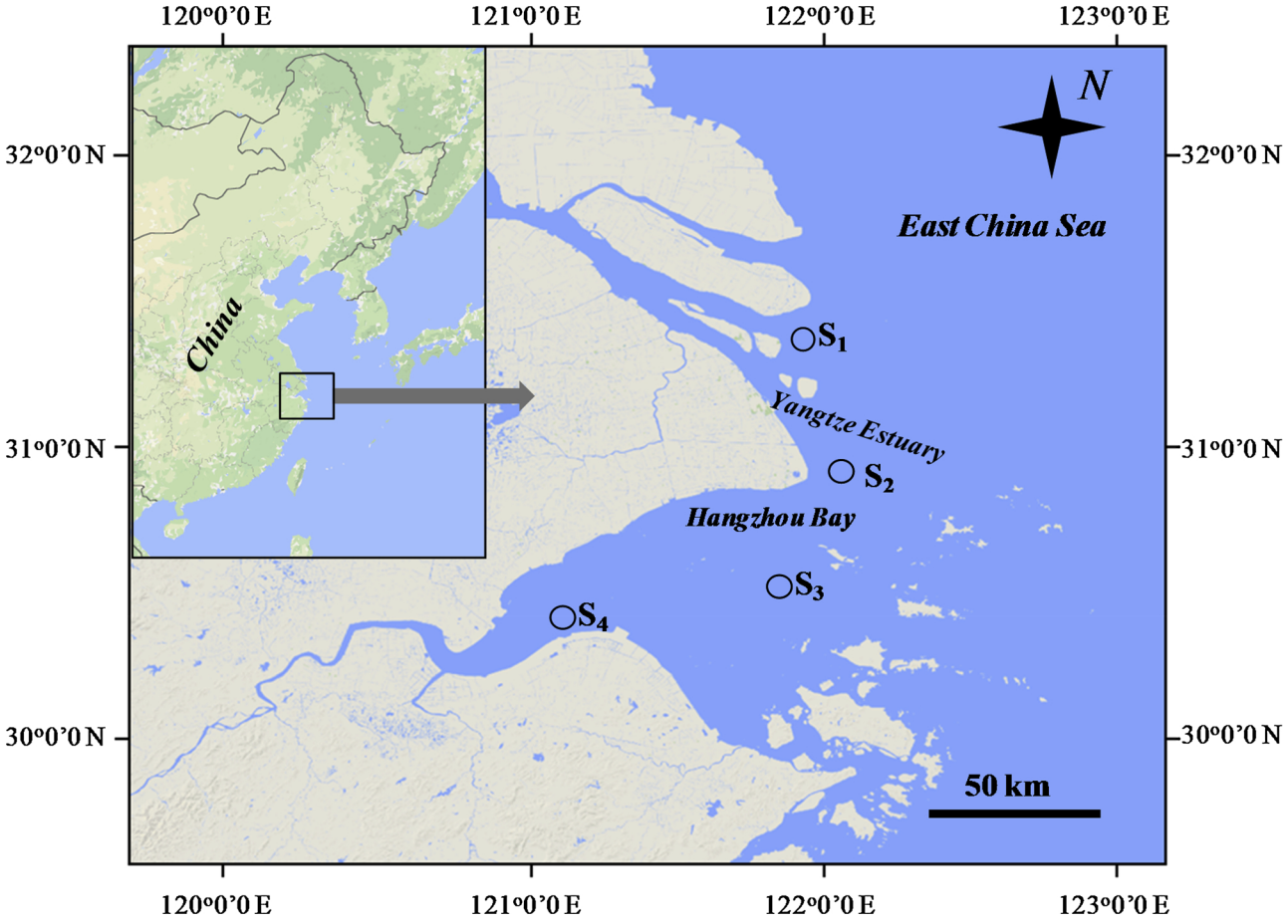

Fig. 1. The sampling sites of fishes.

2. Materials and methods

2.1. Sample collection

From October to November, 2017, 14 species (Supplementary Materials Table 1) of fish (217 individuals) were collected from 4 areas at Hangzhou Bay and Yangtze Estuary where intense plastic associated industry, family workshops and trading markets were located [36–38] (Fig. 1). They potentially introduced the microplastics into environment. All fish were directly captured using local fishing boats within 8 h and the location of sites were recorded. Freshly caught fish were stored at −20 °C. Within each species, seven to nine individuals of a similar size were selected and examined for microplastics in guts and gills.

2.2. Organ specific analysis and hydrogen peroxide treatment

Once fish were defrosted, their weight and length were recorded, then the guts and the whole gill raker and filaments were removed and weighed (Supplementary Materials Table 1). In addition, liver and subsamples of muscle were removed from the mid lateral section of each *L. maculatus* and weighted (Fig. 2; Supplementary Materials Table 2).

The gut, gill, liver and each part of muscle sample were put into 300 ml glass bottles and digested separately. Extraction of microplastic was carried out according to the method described by Jabeen et al [18]. Approximately 200 ml of H_2O_2 (30%, V/V) was used to digest biological tissues. Bottles were covered with glass cap and placed in oscillation incubators at 65 °C with 80 rpm for 72 h. In order to improve the efficiency of digestion and reduce contamination, a high temperature of 65 °C was selected. It has a little influence on the morphology of plastics and make them discolored. Nevertheless, it has little influence on the identification of polymer composition. All of the liquid in the bottles was filtered and the filter (Millipore Nylon NY2004700) was covered and stored in dry Petri dishes for further observation. The pore size of filter was 20 μm.

2.3. Quality control of experiments

To ensure a working environment free of plastic contamination, all apparatus were rinsed three times with filtered water to reduce the chances of contamination. Necroscopies were carried out by full steel scalpels and all the liquid were filtered with a 1-μm filter (Whatman glass microfiber filters GF/B) by using a vacuum with a pump prior to use. Cotton laboratory coats were worn during the whole procedure of experiment. In order to reduce the influence from air-borne contamination, necroscopies were carried out less than 5 min per fish and fishes were covered with aluminum foil before used. In our laboratory, we keep an air cleaner (Allerair 6000 V) run routinely to reduce air-borne plastic contamination.

The fishes were caught by nylon (polyamide) nets which is a potential contamination in polymer identification. There were 1 fragment and 4 fibers (Supplementary Materials Figure S1) were verified as polyamide in samples, accounting for 2.9% of all verified items. These identified polyamide microplastics were quite different in shape and size, and we cannot confirm that these items necessarily came from the net, which meant a possible overestimation of polyamide in our results. Fishes surface were rinsed by filtered water, while we didn't rinsed fish gill before anatomy. As adherence might be a primary pathway for microplastic uptake in fish gill, a washing process for gill might clear some particles attaching on the surface of gill and underestimate the level of microplastics in gill.

Batches of blank controls were run without gut, gill, liver or muscle tissue and were performed simultaneously to evaluate background contamination (Fig. 2). In every blank control, 10 ml filtered water were used as a substitutes for fish tissue because they have the similar volume. 200 ml of H_2O_2 (30%, V/V) were then added and the blanks were treated in parallel to the sample treatment including digestion, filtration and observation. For gut and gill analysis, a total number of 81 blank controls were performed. For liver and muscle analysis of *L. maculatus,* a total number of 160 blank controls were performed. All of the suspected items from blank controls were also identified via micro-Fourier Transform Infrared Spectroscopy (μ-FT-IR).

For gut and gill groups, 2 out of 7 fibers recovered from blank controls were finally confirmed as polymer resulting in an average contamination of 0.025 items/ind. (0.011 items/g). In liver and muscle groups, 3 out of 11 fibers recovered from blank controls were confirmed as polymer resulting in an average contamination of 0.095 items/ind. (0.008 items/g). All of the items in blank controls were significantly less abundant than in the gut and gill groups ($p < 0.05$).

2.4. Observation, identification and validation of microplastic

Suspected plastic particles on the filters were observed and photographed by using a microscope (Micro Imaging GmbH, Goottingen, Germany) at different (25-80x) magnifications. Visual assessments were used to quantify and sort the suspected microplastics based on their properties. They were classified into fibers, fragments, pellets, sheets and films.

Overall, from the 452 suspected microplastic particles collected, 175 particles were validated using μ-FT-IR for a quick snap of polymer composition in fishes. For suspected microplastics found in *L. maculatus*, all were analyzed to precisely characterize polymer composition (Supplementary Materials Table 3). In total, 83 out of 113 particles (73%) were verified (the remainder were missing or damaged during the process). Polymer composition was measured under the attenuated total reflection mode of an μ-FT-IR (Bruker, LUMOS). Data were collected at a resolution of 4 cm^{-1} with a 32-s scan time. All spectra were compared with databases from Bruker to verify the polymer type [39]. The spectrum matches were at least 70% for identified particles. The number of microplastics reported was recalculated by excluding the verified non-plastic items.

2.5. Data analysis

Paired *t*-test and individual *t*-test were used to compare the difference between two paired and individual groups. Mann-Whitney U test was used to compare the difference between two individual groups with heterogeneous variances. The 0.05 and 0.01 significance levels were chosen. The data analysis in the current study was processed using SPSS 22.0.

3. Results

3.1. Validation of microplastics using μ-FT-IR

Visual observation and μ-FT-IR provided important information about the natural or synthetic origin of suspected items with different and similar morphotypes (Fig. 3). Of the 175 selected items from gut,

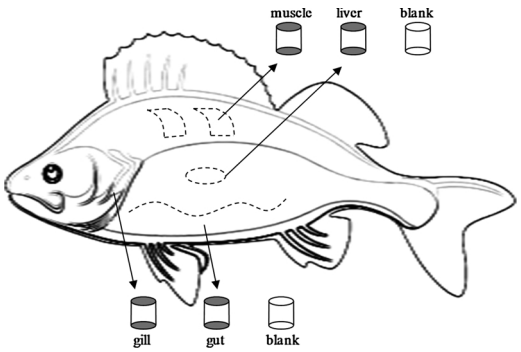

Fig. 2. Anatomy of fish organ specific analysis and the batch of blank controls.

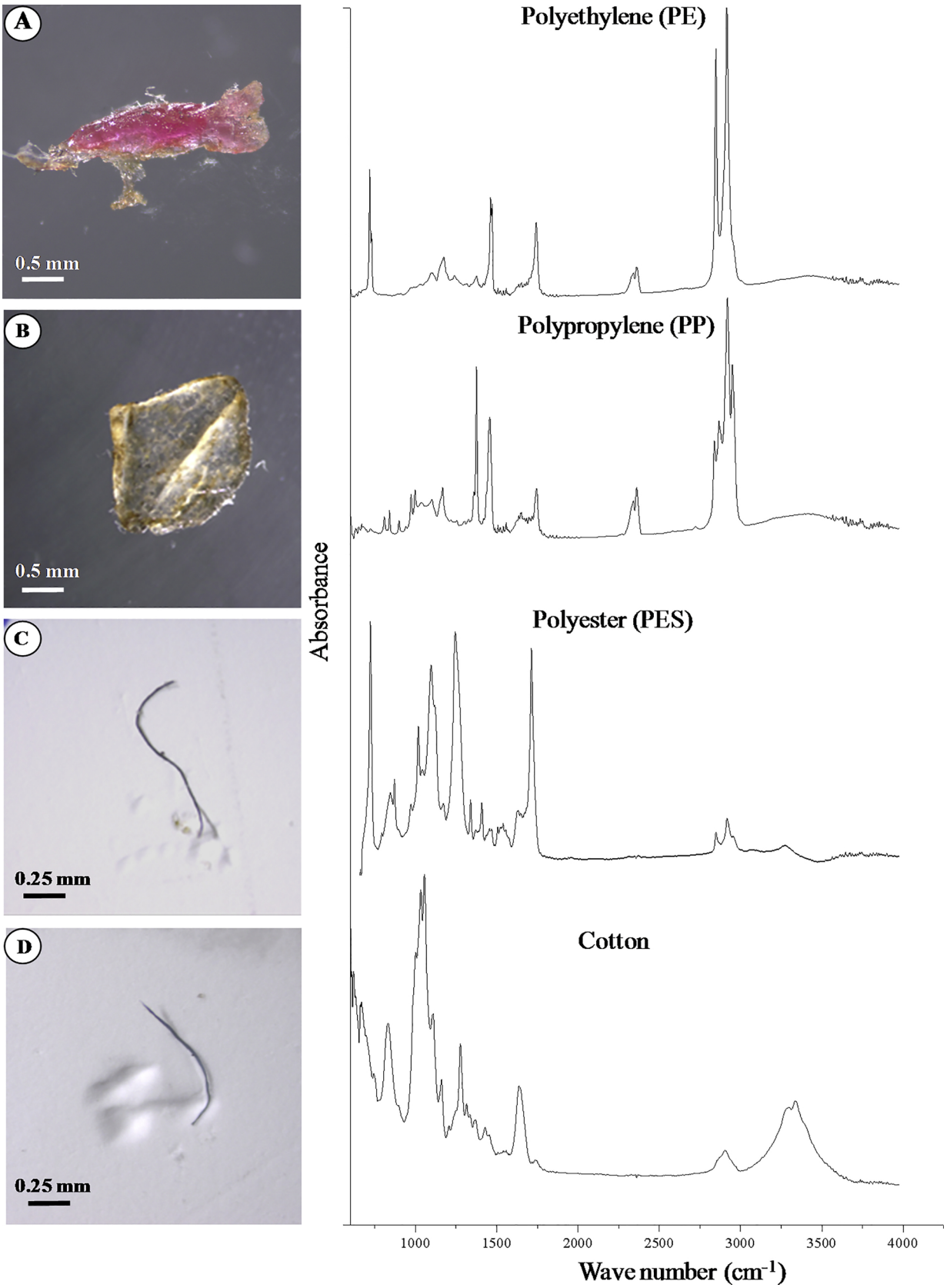

Fig. 3. Photographs and spectra of examples of the most common plastics from gut (A and B) and gill (C) and the non-plastic from muscle (D) identified by using μ-FT-IR.

gill, muscle and blank control, 132 items were confirmed as plastics using μ-FT-IR. (Supplementary Materials Table 4).10 polymer types were identified (Supplementary Materials Table 4) and the dominant polymer was polyester, followed by polypropylene and polyethylene (Fig. 3A-C). A total of 43 natural based material items such as cotton were also confirmed by μ-FT-IR (Fig. 3D). In addition, no suspected items were found in liver samples.

Of the 29 items detected in muscle and blank control samples, 24 items (83%) were non-plastics and no plastic was found in muscle samples (Fig. 4). By contrast, 19 non-plastics were confirmed out of 127 items (15%) from gut and gill samples.

3.2. The occurrence of microplastics in 13 species of coastal fishes

Microplastics were prevalent in the guts (22%-100%) and gills (22%–89%) of fish collected from all 4 sampling sites (Table 1). The average abundance of microplastics in gut varied from 0.3 to 5.3 items/ind. (i.e., 0.1 to 8.8 items/g) and microplastics in gill varied from 0.3 to 2.6 items/ind. (i.e., 0.1 to 5.2 items/g) (Table 1). The average abundance of microplastics in gut was the highest in the great blue spotted mudskipper *Boleophthalmus pectinirostris*at S_4 (5.3 ± 2.4 items/ind. or 8.8 ± 7.4 items/g) (Table 1). The average abundance of microplastics in gill was the highest by individual in the spiny-head Croaker *Collichthys lucidus* (2.6 ± 1.6 items/ind.) and by weight in the anchovy *Coilia ectenes* (5.4 ± 3.9 items/g) at S_3 (Table 1).

Of all the microplastics recovered, fibers were the most prominent form ($p < 0.01$), followed by fragments in gut and gill (Supplementary Materials Figure S2). In particular, the average proportion of fiber reached 90% in gill samples. The microplastics > 1 mm were the most common size in gut, accounting for 60% of the total items, while microplastics < 1 mm were more common in gill, accounting for 55% of the total items. ($p < 0.05$ Supplementary Materials Figure S3).

3.3. The microplastic pollution in different organs from L. Maculatus

In the present study, microplastics are commonplace in the gut and gill of *L. maculatus*, while no microplastic were extracted from muscle or liver samples. In all sampling sites, the mean of microplastic abundance among blank, liver and muscle have no significant difference ($p > 0.05$) but were significantly lower than in gut (1.1 to 2.9 items/ind., i.e., 0.3 to 0.9 items/g) or in gill (0.6 to 1 items/ind., i.e., 0.2 to 0.5 items/g) ($p < 0.05$, Fig. 5). Similar to other species, microplastic consisted primarily of fibers in *L. maculatus* ($p < 0.05$).

4. Discussion

4.1. Plastic from samples versus non-plastic from blank

In order to avoid the error from visual inspections, the validation of microplastic with spectroscopic analyses were highly recommended. It have gradually become a vital step in this research [22,40,41]. The success rate of visual identification depended on methods applied and varied between studies. In some reports, the "microplastics" isolated from organisms were natural based instead of synthetic plastic. Studies using μ-FTIR or μ-Raman validation suggested plastics typically contributed 40%-90% of suspected items in marine, coastal and freshwater fishes (with a sample size from 93 to 1203 individuals) [18,42–44]. They were comparable to what we currently found in gut and gill

However, in contrast to their high presence in gut and gills, plastics was absent in muscle and less than 30% in all blank samples. Furthermore, 100% of the items from both blank and muscle samples were fiber. The presences and patterns of non-plastic fibers in muscle samples questioned about whether they came from background contaminants (e.g., cotton laboratory coat). For routine monitoring, randomly selecting and analyzing suspected microplastics in a small sample size might insufficient for estimating microplastic contamination in large populations; moreover, to accurately determine microplastic contamination and risks to biota, particularly in individual fish or organs, 100% item validation is highly recommended (Supplementary Materials Table 3).

Even though great efforts were applied to minimize contamination from experimental procedures, complete insulation of contamination is impossible, especially for air borne contamination. Guides of microplastic methodology always highlighted the importance of procedural controls [22]. In our studies, we proposed that a prompt experimental process can effectively avoid airborne contamination. When more samples are analyzed, the cumulative number of items found in organs and blanks, both plastics and non-plastics, inevitably increased (Fig. 6A). However, the rate of increase was different between groups (Fig. 6B). As the number of experimental groups increased, items recovered from blanks and muscle increased at a similar slow rate. Such a similarity indicates that items in muscles may be from low level contamination during laboratory procedures. This highlights the importance of setting up batches of control blanks when assessing extremely low levels of microplastic accumulation. It can help to ensure microplastics came from those samples and not from inevitable laboratory contamination. In contrast with muscle and blanks samples, the rate of item accumulation was much higher in gill and gut samples and very different from the control blanks (Fig. 6B), increasing our confidence that microplastics measured in these samples are not from laboratory procedures.

4.2. Concentrations of microplastics in different species

In our studies, habitats and feeding habits were two important factors involved in microplastic ingestion. All demersal species including *Boleophthalmus pectinirostris*, *Tridentiger barbatus and Acanthogobius ommaturus* had the highest microplastic ingestion (Supplementary Materials Table 5). However, whether ecological habitats play a key role in microplastic ingestion is still unclear and largely differed from investigation areas. Some studies reveled high microplastics abundance in benthic and demersal species which were supposed to ingested plastics from seabed [18,45]. In contrast, mass buoyant plastics at sea surface are also believed to induce high microplastics ingestion in pelagic species [46–48]. We proposed that the vertical distribution of microplastics concentration from sea surface to bottom are critical to their ecological risks. For feeding habits, two kinds of herbivorous fish (*Coilia ectenes* and *Coilia mystus*) showed lowest microplastics ingestion in all sampling sites. A narrow diet source and low trophic level for those herbivorous fish may reduce their possibility for plastic ingestion [49].

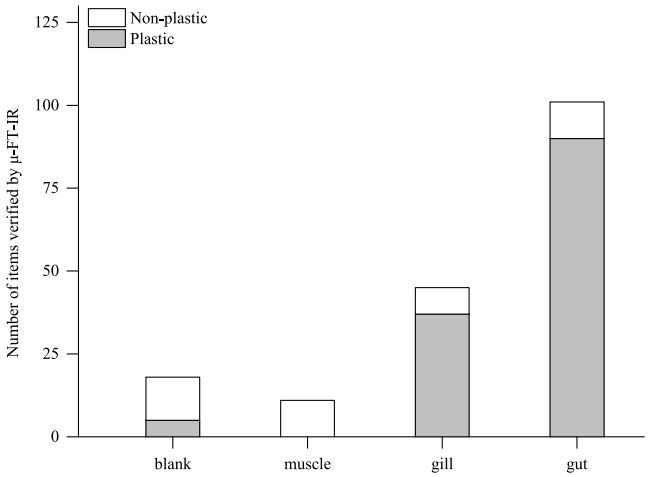

Fig. 4. The composition of plastics and non-plastics in different organs and blank controls in all fishes (n = 175).

Table 1
The abundance (± standard deviation) and frequencies of microplastics in gut and gill from 13 species of coastal fishes.

genus species	number	abundance of microplastics (Mean ± SD)				frequencies	
		gut		gill		gut	gill
		items/ind.	items/g	items/ind.	items/g	%	
S_1							
Coilia ectenes	9	0.7 ± 1.1	1.3 ± 2.2	0.3 ± 0.5	0.6 ± 0.9	33	33
Cynoglossus robustus	9	0.7 ± 0.9	1.9 ± 2.5	0.9 ± 0.8	2.6 ± 2.3	44	67
Hemibarbus maculatus Bleeker	9	0.9 ± 1.2	0.4 ± 0.5	0.9 ± 0.6	1.1 ± 0.8	44	78
Coilia mystus	9	0.3 ± 0.7	0.5 ± 1.0	0.3 ± 0.5	0.7 ± 1.0	22	33
S_2							
C. ectenes	9	0.3 ± 0.5	0.3 ± 0.5	0.4 ± 0.5	0.7 ± 0.8	33	44
Harpodon nehereus	9	2.2 ± 1.2	1.9 ± 1.2	0.8 ± 1.4	1.4 ± 2.6	89	33
Collichthys lucidus	9	0.6 ± 1.0	0.8 ± 1.8	0.7 ± 1.1	1.3 ± 2.1	33	33
Acanthogobius ommaturus	8	3.9 ± 2.1	2.0 ± 2.1	0.9 ± 0.8	0.5 ± 0.6	100	67
Tridentiger barbatus	8	4.5 ± 2.0	6.2 ± 4.0	1.4 ± 1.7	2.8 ± 3.6	100	56
S_3							
C. ectenes	9	0.8 ± 0.7	2.6 ± 2.4	1.4 ± 1.1	5.4 ± 3.9	67	78
Liza haematocheila	9	0.7 ± 1.1	0.2 ± 0.3	1.9 ± 2.3	0.5 ± 1.0	33	33
Co. lucidus	8	0.5 ± 0.9	0.8 ± 1.5	2.6 ± 1.6	5.2 ± 3.8	25	89
Scomber japoicus	9	0.8 ± 0.8	0.1 ± 0.1	2.4 ± 2.0	0.2 ± 0.2	56	78
A. ommaturus	9	3.6 ± 1.3	4.0 ± 2.5	1.2 ± 1.2	1.5 ± 1.5	100	78
Thamnaconus septentrionalis	9	0.7 ± 1.0	1.3 ± 2.1	0.6 ± 1.3	1.4 ± 3.7	33	22
Pampus cinereus	9	1.1 ± 1.3	0.7 ± 0.9	0.9 ± 0.6	1.5 ± 1.6	56	78
H. nehereus	9	2.8 ± 1.6	6.3 ± 4.1	1.0 ± 1.1	2.5 ± 2.8	78	56
S_4							
C. ectenes	9	1.0 ± 1.5	2.1 ± 3.1	1.2 ± 1.3	3.7 ± 3.4	45	67
L. haematocheila	8	1.9 ± 1.6	0.1 ± 0.1	0.9 ± 0.8	0.1 ± 0.1	75	63
Co. lucidus	9	2.6 ± 2.1	8.1 ± 10.4	1.6 ± 1.6	3.4 ± 3.8	78	56
Boleophthalmus pectinirostris	9	5.3 ± 2.4	8.8 ± 7.4	1.2 ± 1.2	2.8 ± 2.9	100	56

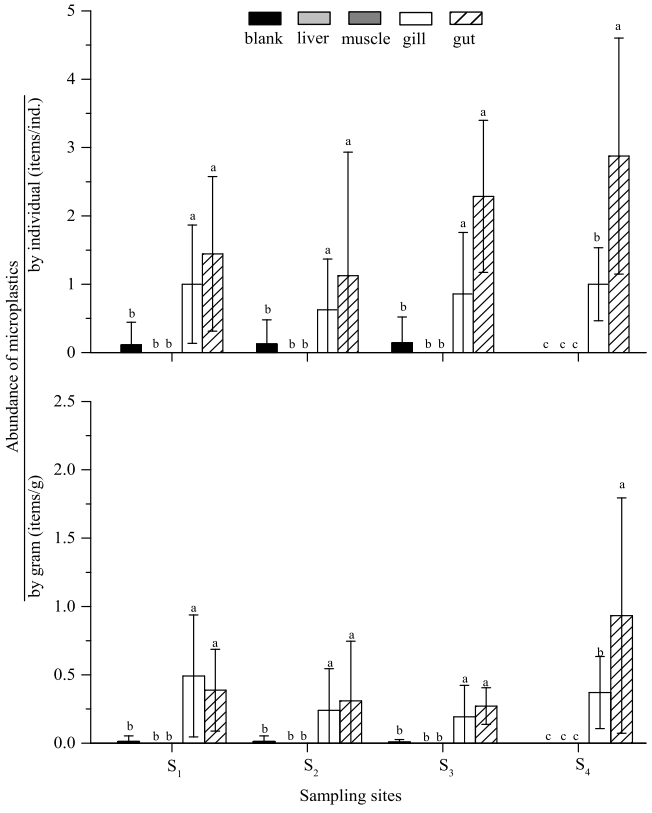

Fig. 5. Comparison of abundance of microplastics between different groups and blank control. Each value represents the mean ± standard deviation. A paired *t*-test was used to compare the difference between every group one by one; the letters above the bars indicate significant differences ($p < 0.05$).

However, there was no clear trend of the microplastics logged in gill between species or sampling sites. Although microplastics in gut were more closely related to the total frequency of microplastics in fishes ($p < 0.01$), microplastics in gill didn't related with the detection frequency (Supplementary Materials Figure S4). Particles including microplastics in water can be passive captured by gill throughout filtering [50,51]. It is an non-selective and more transient process in comparison with microplastic ingestion [52]. Hence, the microplastics residue level in fish gill could be less correlated with individual or feeding type.

4.3. The different shape and size patterns of microplastics in gill and gut

Fibers were more commonly found in gut ($p < 0.01$), while the size of microplastics in guts was significantly higher than in gills ($p < 0.05$) (Supplementary Materials Figure S5). Our research indicated that microplastics could be captured via fish gills in natural conditions and numbers were comparable to ingestion. While the intestinal tract in most cases may be the dominant pathway for microplastic uptake, branchial uptake of waterborne microplastics maybe an important pathway for short-term acute toxicity in fish [21,51]. Gills should be considered as another vulnerable organs may affected by microplastics. Moreover, the intestinal tract and gill are exposed to different types of microplastics because the types of microplastics in gut and gill were different.

In particular, small fibrous microplastics were more likely to be lodged in gills than occur in guts. The finding was different from laboratory-based evidence that reported microplastics did not adhere strongly or accumulate at high amounts in zebrafish gills [21]. This calls into question, that the risk of microplastics can be ascertained without the consideration of size and types. As small size plastics are more readily ingested, they can entered internal organs and increase the bioavailability of associated chemicals [4]. Many of lower trophic organisms exert limited selectivity between particles and capture anything of appropriate size [53]. Exposure experiments are excepted with more environmentally relevant [54]. Further, the time of plastics lodged at gills are important because microplastics may temporarily

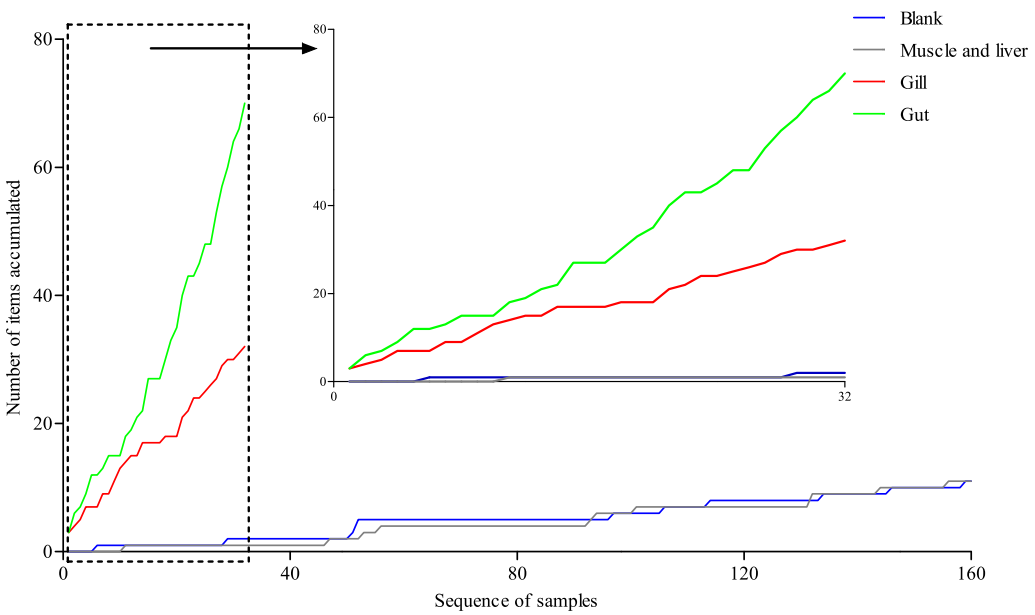

Fig. 6. (A) Cumulative number of items (plastics and non-plastics) found in different organs and the control blank over 160 samples. (B) Inset figure showing cumulative number of items (plastics and non-plastics) from 32 samples.

attach on the surface of gill and washed by water with limited ecology risks.

4.4. Where do microplastics go?

Our case study provides a possible answer to the distribution of microplastics in fish organs, that is microplastic > 20 μm cannot be observed in liver and muscle while their prevalence in gut and gill were confirmed.

The translocation of exogenous particles inside the body have been widely researched in the field of pathology. For fishes, gill and gut epithelium are considered as two possible translocation pathways for exogenous particles and have been proved in the cases of nanoparticles and dissolved substances [28,50]. High level of microplastic pollution in research areas have been reported in water and sediments from previous studies [34,55]. In theory, microplastics might entered internally in fish if they were taken up and presented in gill or gut.

However, the nature of particles including size, shape and composition will largely affect such processes. In our cases, the translocation of typical microplastics (on the scale of millimeter) from the intestinal tract to the circulatory system was hard because they were too large. Mammalian epidemiological studies suggest that the polystyrene microspheres at a size of 15.8 μm were mechanically filtered by capillary beds and not adsorbed into internal organs [56]. A case laboratory study based on gold fish also found microplastics couldn't enter into liver [57].

Much smaller microplastics (e.g. nanoparticles) may be more likely to be accumulated into internal organs. In this study plastics < 20 μm could not be detected due to methodological limitations, which is similar to the range of microplastic sizes focused on in other studies on accumulation in organs. Here, they found microplastics in liver and muscle tissues ranging from micrometers to millimeters in size [28–30]. If more findings like this are reported, we need reconsider and redefine the risks microplastics pose to aquatic biota as these may be greater than what is currently expected. In addition, the behavior and risk of nanoplastics can be very different in fish and should be also assessed [58].

While we isolated suspected particles from fish muscles, we cannot definitively prove they were the result of bioaccumulation in fish. Furthermore, without the use of blank controls or validation, there is a risk of overestimating microplastic presence in biological tissues. In addition, we demonstrated that microplastic contamination in fish in laboratory-based studies need to be validated in field populations. As we could see the size and shape patterns of microplastics were quite different between organs. And there could be different mechanisms involved in the uptake of microplastics. Test and verification of such mechanisms in laboratory conditions will better establish our understanding of the ecological risk of microplastics to fish in the field.

The amount and type of microplastics in fishes depend on species and amount of microplastic contamination at sampling sites [59]. The lack of routine and large scale work can incur the misunderstanding of risk assessment. It should be noted that virtual risks of microplastics in the environment have been argued in some places [27,60,61]. One of the most poignant viewpoint was that the risks can be overestimated without being validated using evidence from field-based studies. The human health risks via consumption of microplastics and their associated chemicals in fish is important. On the other hand, where microplastics or nanoplastics go in the body and the vulnerable target organs can help understanding the ecotoxicology of microplastics. As intestinal tract analysis have been widely used [16], a routine analysis based on muscles and internal organs should also be considered.

5. Conclusion

In the present study, the occurrence of microplastics were found in the gut and gill from fishes. The shape and size patterns of microplastics are different between gut and gill. Fibrous microplastics with small size more likely lodged in gill. But no evidence of microplastics larger than 20 μm in liver or muscle could be observed in *L. maculatus*. Several suspected non-plastic items detected in muscle samples were similar to those present in background contamination. Blank control and validation are necessary to avoid overestimating the level of microplastics in fish organs. We did not observe microplastics > 20 μm in muscle or liver tissues, even though they were present in the intestinal tract and gills of *L. maculatus*. However, smaller microplastics and even nanoplastics should be investigated in the future. Researches with larger sampling size and through quality control are compulsory for the assessment of ecotoxicology microplastics contamination in the internal organs of a range of species of fish.

Conflict of interest

The authors declare no competing financial interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jhazmat.2018.11.024.

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