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Ingestion of Microplastics by Commercial Fish in Skudai River, Malaysia

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Abstract

Microplastics are plastic particles less than 5 mm and have been classified as contaminants of emerging concern. In recent years, the ubiquity of microplastics has caused a serious threat to aquatic animals worldwide. Over the past decade, the ingestion of microplastics has been extensively reported in various marine animals. However, studies on ingested microplastics in the aquatic animal in freshwater ecosystems are still scarce. Therefore, the presence of microplastics in the gastrointestinal (GI) tract of freshwater fish in Skudai River was investigated. Sixty fish were caught belonging to 6 species and 3 feeding habits. The analysis shows all species ingested microplastics. By individual, only 40% of the fish ingested microplastics. Microplastics with size between 1 to 5 mm were the most dominant particles found in the GI tract. There was a significant difference in number of microplastics among different species. A positive correlation was observed between the number of microplastics and Fulton's condition, body weight and weight of the GI tract. This investigation represents the first study on the interaction between microplastics and aquatic animals in fishing and urban area of this country, where fish are consumed by local people.

***Keywords**:* Plastic pollution; Microplastics ingestion; Wild freshwater fish; river; Fishing area

1. Introduction

The occurrence of small plastic particles less than 5 mm also defined as microplastics has become a global issue. Microplastics are categorized into two sources. Microbeads and small scrubbers used in personal care products and also plastic pellet are classified as a primary source. Meanwhile, the secondary source of microplastics originated from the fragmentation of larger plastic polymers (e.g. film, fibers, and sharp-edged fragment) (Wagner *et al.*, 2014). The abundance of microplastics is not only polluted the environment but also affected aquatic biota through ingestion due to their smaller size. Microplastics are ingested either accidentally or directly when they consumed lower-trophic level organisms that have initially ingested microplastics (Nelms *et al.*, 2018). On the other hand, the particles could also be swallowed together with the preys (Peters and Bratton, 2016). The retained microplastics could block the digestive tract and may lead to false satiation (Rummel *et al.*, 2016).

In other circumstances, this smaller particle can be absorbed in the tract and translocated into different tissues (Abbasi *et al*., 2018). Microplastics are also reported as a potential vector to carry other chemical pollutants and able to transfer to organisms in the food web (Anbumani and Kakkar, 2018). Worth noting that most of the studies on microplastics pollution were mainly recorded in marine ecosystems (Sanchez *et al*., 2014). However, the investigation on microplastics in freshwater environments and associated biota is still limited (Slootmaekers *et al*., 2019; McNeish *et al*., 2018). Only a few studies have reported on microplastics ingestion by freshwater biota in the natural population, for example, wild gudgeons, Gobio gobio from French Rivers (Sanchez *et al*., 2014) and sunfish, Lepomis spp. in Brazos River Basin, USA (Peters and Bratton, 2016).

Skudai River is reported as among the polluted river with rubbish in Peninsular Malaysia. In fact, Hangzo and Cook (2014) reported that 11 tons of rubbish were collected from Skudai River every month. Regrettably, their findings showed that this rubbish was originated from the residents living along the waterway. As of today, the common type of trash accumulated in this urban river are mostly plastic-based products such as cups, bottles, variety of plastic wrappers, fishing nets and polystyrene plates. Nevertheless, the sampling location in Skudai River is well-known for fishing activity. All of the captured fish in this river are edible species and are sold in the local market. However, as of today, there is no information on the occurrence of microplastics in urban river of Malaysia, specifically in this river. Thus, a preliminary study is pertinent as to understand the interaction of plastic particles and fish community in such area.

The aim of this work is to determine (a) the presence and occurrence of microplastics in different species and feeding habit of freshwater fish in Skudai River, (b) the relationships between number of microplastics and body condition, body weight, and weight of GI tract, (c) types of microplastics found and their possible sources. To the best of our knowledge, this study is the first evidence on the ingestion of microplastics by freshwater fish in urban river of Malaysia.

2. Materials and Methods

2.1 Fish collection and analysis

Fish were captured using cast net in September and October 2017. The specimens were euthanized via pithing, kept in zip lock bags and stored at -20 °C (Peters and Bratton, 2016). Each specimen was cleaned with ultrapure water (PURELAB® Flex, Elga). Weight of body (g) and total body length (cm) were recorded before the removal of GI tract (from the top of oesophagus to anus) were performed. Non-related tissues and the gall bladder were excised carefully from the tract. The GI tract was weighed individually prior to tissue digestion.

2.2 Isolation of microplastics from gastrointestinal (GI) tracts

The digestion of GI tract was carried out following Karami *et al*. (2017) with a slight modification. An amount of 1:10 (v/w) of 10% potassium hydroxide (KOH) was added in 250 mL glass beaker containing GI tract. Beakers were covered with aluminum foil as to prevent air-borne fiber contamination. The reaction was left standing for 2 days at room temperature. The digest was filtered through 22 μm filter membrane (No. 541, 47 mm, Whatman) with a pump vacuum. The filter was then transferred into 10 mL of saturated sodium chloride (NaCl) solution and shaken for 2 min by using orbital shaker (Model No. 721, Hotech Instruments Corp.). The solution was filtered again, oven dried at 30 °C for 2 min and ready to be observed under light microscopy.

2.3 Microplastics inspection and identification

Microplastics were observed under microscope (HSZ-600) with 40x–45x magnification. The inspection of microplastics was carried out by following procedure in Peng *et al*. (2017). Particles that possess organic structure and small sand grain were carefully identified and discarded from the analysis. Meanwhile, particles that did not tear apart and crushed by tweezer were measured for size. The inspected microplastics were also assessed based on color and shape. The color description by Peng *et al.* (2017) was followed, such as blue (including green), red (including pink), yellow (including orange), black (including grey), transparent and white. Meanwhile, five categories of shapes are fragment of which derived from large plastics fragmentation, film (thin polymer), fiber/line (thin, fibrous or thread-like polymer), foam and bead (Free *et al.*, 2014). In this study, particles with size more than 5 mm were discarded from the analysis.

2.4 Statistical analysis

The number of microplastics ingested by fish is tested for normality using Shapiro-Wilk test. In this study, the distribution of data was not normal, thus, the mean number of microplastics among species was analyzed by a non-parametric test (Kruskal-Wallis). Then, two multiple comparison tests were performed as to observe the differences. Dunn test was used to determine which species is significantly different. Meanwhile, Nemenyi test was carried out since the data has outliers. The number of ingested microplastics between Fulton's condition, weight of GI tract, and body weight (BW) were analyzed using a linear regression. The Fulton's condition factor (K) was calculated based on formula reviewed by Froese (2006):

$$K = \frac{W}{L^b} \times 100$$

where, W is the weight of fish in gram, L is the total length of fish in cm, b is the weight-length relationship with value = 3, while the factor 100 is applied to bring K close to unity. The statistical analysis was done using RStudio 3.3.3 at 0.05 significant level.

3. Results and Discussion

Overall, 60 fish were captured from Skudai River, belonging to 6 species and 3 feeding habits (Table 1). The total length of fish ranged from 13.4 to 44 cm and the body weight ranged between 75 and 511 g. The weight of gastrointestinal (GI) tract was also recorded between 2 and 37 g. A total of 24 (40%) fish had ingested microplastics in the GI tract (Figure 1). Of these, 8 fish at least ingested one microplastics. One individual *Clarias gariepinus* contains hook and line in the GI tract. Meanwhile, two mesoplastics (more than 5 mm) were found in the GI tract of *Pangasius hypophthalmus*. All these particles were excluded prior to filtration step.

The average number of microplastics by all specimens was 1.07 ± 1.76 (mean ± SD) items per fish. Meanwhile, an average of 1.08 ± 1.77 items per individual was found among fish that ingested microplastics. The results show 64 ingested microplastics were found in the GI tract, comprised of film (43.28%), fragment (28.36%), fiber (20.9%) and foam (2.99%) (Figure 2a). The most dominant color ingested in GI tracts was blue (42.19%) followed by white (26.56%), red (21.88%), black (7.81%) and yellow (1.56%) (Figure 2b). Meanwhile, large microplastics (1 to 5 mm) was the prevalent size found in fish (Figure 2c), with P. hypophthalmus as the highest species ingesting this particle size. The predominant size and shape also varied among species (Table 2).

Based on feeding habit, the abundance of ingested microplastics was found highest in herbivore (1.50 ± 1.73 items per fish) than of omnivore (0.82 ± 1.78 items per fish). However, no significant difference between ingested microplastics and feeding habit was observed ($p = 0.09$). When the results were compared across fish species, *P. hypophthalmus* shows the highest mean of ingested microplastics, where the maximum microplastics found in the GI tract of this species was 9 particles (Table 2). There was significant difference between number of microplastics and fish species (Kruskal-Wallis test, $p = 0.002$). Meanwhile, both Dunn and Nemenyi tests showed the significant difference was observed between *C. gariepinus* and *P. hypophthalmus* as well as *A. testudineus* and *P. hypothalamus* ($p < 0.05$).

All fish species in our study ingested microplastics indicating the interaction of microplastics and fish community occurs in this river. Interestingly, *Oxyeleotris marmorata* ingested two types of microplastics (fiber and fragment) although only one individual

Table 1. Feeding habit and physical measurement of fish. Body weight (BW) and GI tract are in gram.

Fish species	Common name	N	Feeding habit	BW	GI tract
Oreochromis mossambicus	Tilapia	18	Herbivore	135-511	5-11
Cyclocheilichthys apogon	Beardless barb	2	Herbivore	123-131	2-3
Clarias gariepinus	African catfish	21	Omnivore	90-281	4-26
Anabas testudineus	Climbing perch	13	Omnivore	75-120	3-5
Pangasius hypophthalmus	Pangasius catfish	5	Omnivore	109-218	28-37
Oxyeleotris marmorata	Marble goby	1	Carnivore	213	13

Table 2. Basic descriptive statistics of the ingested microplastic of each fish species.

Fish species	Mean±SD	% of ingestion	Dominant		
			Color	Size	Shape
O. mossambicus	1.61±1.79	55.56	White	1.0-5.0	Film
C. apogon	0.50±0.71	50	Blue	0.1-0.5	Film
C. gariepinus	0.33±0.8	19.05	Blue	0.1-0.5	Equal
A. testudineus	0.38±0.87	23.08	Blue	1.0-5.0	Fiber
P. hypophthalmus	4.00±3.16	100	Blue	1.0-5.0	Film
O. marmorata	2.00	100	Blue and black	<0.1 and 1-5	Fiber and fragment

managed to be captured during sampling period. Certainly, the sample size is too low for a proper actual representation of plastic particle pollution level of the site. However, this can be a basic indication to further investigate more individual in future work (Slootmaekers *et al.*, 2019). Correlation coefficients (cc) based on combined fish samples demonstrated that the body condition has a moderate strong correlated to the number of ingested microplastics (cc = 0.4082, $p < 0.05$). Similarly, the ingested microplastics and body weight has also indicated a moderately strong correlation (cc = 0.5082, $p < 0.05$). Meanwhile, a strong correlation between microplastics and GI tract weight was observed (cc = 0.7273, $p < 0.05$). In general, a bigger fish more ideally requires a large volume of food to reach energy demands than of smaller ones. Hence, the chance to ingest more microplastics particles by them is also higher (Horton *et al.*, 2018).

The percentage of fish community ingested microplastics in Skudai River (40%) is equivalent with a variety of fish species across different freshwater ecosystems worldwide such as in Brazos River Basin, USA (Peters and Bratton, 2016) and River Thames, UK (Horton *et al.*, 2018) at 44.95% and 33%, respectively (Table 3). Meanwhile, when the data was segregated based on different fish species, by considering more than 10 individual samples, the percentage of ingested microplastics in *O. mossambicus* (55.56%) was higher than the percentages reported by various freshwater fish in several geographical locations (Table 3) except fish species in Poyang Lake, Ponghai (Yuan *et al.*, 2019) and Qinghai Lake, China (Xiong *et al.*, 2018). In their studies, microplastics were found in 90.91% of the eleven *Carassius auratus* and 100% of the whole ten individuals of *Gymnocypris przewalskii* contained microplastics in the GI tracts. Similarly, fish species in Wascana Creek, Canada (Campbell *et al.*, 2017) and Pajeú river, Brazil (Silva-Cavalcanti *et al.*, 2017) also shown high percentages of ingested microplastics compared with those in our recent work.

Significant differences in microplastics ingestion were found between *P. hypophthalmus* and *C. gariepinus* and *A. testudineus*. Noting that all these species are known as omnivorous fish based on their feeding behavior. However, the significant differences between them may be explained by their living and feeding preference area in the aquatic environments. For example, *A. testudineus* is classified as a pelagic fish where they preferentially live and feed away from the bottom of river. As for this species, fiber was the prevalent shape found in their GI tracts. This was consistent with previous findings on the ingestion of microplastics by various pelagic freshwater fish such as *Lepomis spp*. (Peters and Bratton, 2016), *Rutilus rutilus* (Horton *et al*., 2018), and *Carassius auratus* (Yuan *et al*., 2019). Fiber was also reported as the most prevalent shape ingested by marine (Bellas *et al*., 2016; Neves *et al*., 2015; Lusher *et al*., 2013) and estuarine fish (Bessa *et al*., 2018b; Vendel *et al*., 2017; Pazos *et al*., 2017). In fact, fibers are much lighter compared with other microplastics morphology (e.g. fragment and film) and typically floated in the water column for a longer time (Campbell *et al*., 2017). Although *P. hypophthalmus* and *C. gariepinus* are known as benthopelagic fish, yet the dominant morphology of ingested microplastics were different between both species (Table 2). Film was the most dominant shape found in *P. hypophthalmus*. Conversely, the shapes of microplastics in African catfish were equivalent to each other. Worth knowing, both species are generally consuming all types of feed, able to adapt their feeding behavior based on food availability and are regarded as a final predator (Rodríguez *et al*., 2018). Therefore, this indicates that the ingestion of microplastics between both species were not selectively based.

The results showed that most of the ingested microplastics were mostly larger and colourful. Thus, the present study highlighted several possible factors based on the environmental issues. The sampling site is located near to sewage treatment plant (STP). Until recently, STP is indicated as the major point sources of microplastics pollution in the freshwater environment (Kang *et al*., 2018; Wagner *et al*., 2014). In addition, the population density in the vicinity of the river is 1767.85 person/km^2 and about 325 km^2 of watershed area. Hence, this high population density size may also contribute to the abundance of microplastics in the water way. Recently, the Department of Environment & Department of Irrigation and Drainage, Malaysia have reported that Skudai River was among the polluted river with highest amount of rubbish in Peninsular Malaysia (Aruna, 2014). Regrettably, rubbish can still be observed in this river and mostly deposited in the riverbank. The existence of rubbish pile nearby the sampling site might contribute further to the high abundance of film and fragment in the river. Meanwhile,

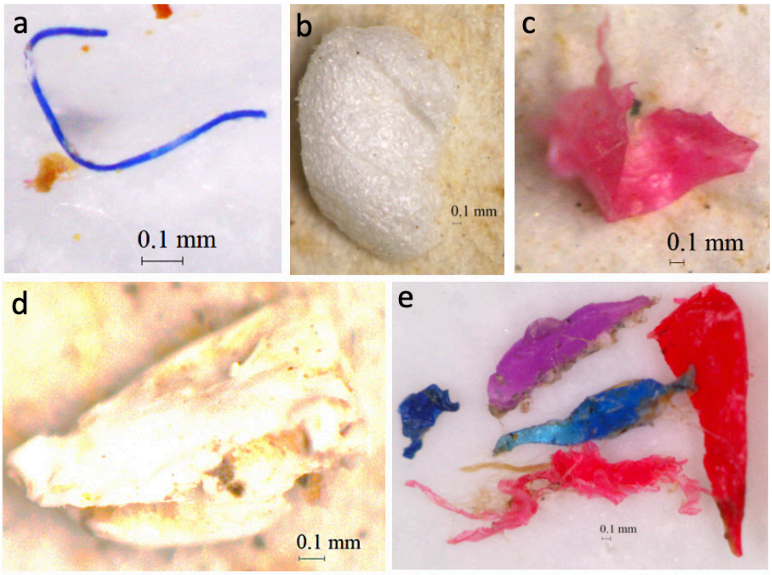
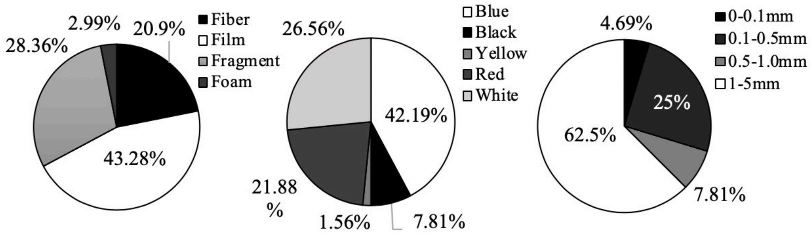


Figure 1. Microplastics obtained from the GI tract of different fish species at Skudai River, for example a) fiber (from A. testudineus), b) foam (from C. gariepinus), c) film and d) fragment (from O. mossambicus) and e) a mixture of colorful film and fragment from all species.



a) Type of microplastics   b) Color of microplastics   c) Size of microplastics

Figure 2. Percentage of microplastics based on (a) type/shape, (b) color, and (c) size.

Table 3. Ingestion of microplastics (MP) by different species of freshwater fish worldwide. The literature review was carried out on 07 March 2019 by searching related articles with Topic: Microplastics* Ingestion* Freshwater Fish* in Web of Science, from 1970-2019. Only wild-caught freshwater fish ingested microplastics in GI tract were selected for comparison and N indicated as number of fish sample.

Location	Fish Species (N)	Fish ingested MP (%)	References
Poyang Lake, China	*Carassius auratus* (11)	90.91	Yuan *et al.*, 2019
Flemish rivers, Belgium	*Gobio gobio* (78)	9	Slootmaekers *et al.*, 2019
Marne and Seine Rivers, Paris	*Squalius cephalus* (60)	25	Collard *et al.*, 2018
Qinghai Lake, China	*Gymnocypris przewalskii* (10)	100	Xiong *et al.*, 2018
River Thames, UK	*Rutilus rutilus* (64)	33	Horton *et al.*, 2018
Taihu Lake, China	6 species (20-40)	NA	Jabeen *et al.*, 2017
Wascana Creek, Canada	5 species (181)	73.50	Campbell *et al.*, 2017
Pajeú river, Brazil	*Hoplosternum littorale* (48)	83	Silva-Cavalcanti *et al.*, 2017
Brazos River Basin, USA	Lepomis spp. (436)	44.95	Peters and Bratton, 2016
Watersheds of the Gulf of Mexico	44 species (419)	8.20	Phillips and Bonner, 2015
French Rivers	*Gobio gobio* (186)	12	Sanchez *et al.*, 2014
Skudai River	6 species (60)	40	Present study

some of the plastic waste were also discarded from cars across the bridge. According to Peters and Bratton (2017), roadway adjacent to the sampling site was considered as the potential non-point source of pollution through the direct disposal of trash illegally. This debris ultimately entered river via rain and wind events (Fischer *et al.* 2016).

In this present work, blue was the most common color identified in all shapes of microplastics. Although most of the previous studies on freshwater fish less mentioned about the predominant color (Table 1), our result reported similar findings with those found in various marine fish (Herrera *et al.*, 2019; Bessa *et al.*, 2018b; Pazos *et al.*, 2017; Ferreira *et al.*, 2018; Peters *et al.*, 2017). Similarly, the large microplastics particles found in this study is consistent with other studies in both aquatic biome (Slootmaekers *et al.*, 2018; Collard *et al.*, 2018). These microplastics characters are significant since most of the fish mistaken them for food (Ory *et al.*, 2017). For instance, Herrera *et al.* (2019) supported the argument by Ory *et al.* (2017) on the resemblance of blue microplastics as natural prey copepods of which are also in blue color. According to Herrera *et al.* (2019), blue was the most dominant color found in the Atlantic chub mackerel (Scomber colias) and the percentage of blue copepod (Labidocera sp.) was also high in their sampling site of Canary Islands' surface water.

Most of the captured fish in Skudai River are commonly consumed by local people. Indeed, non-edible part such as GI tract will be removed and thought harmless to consumers. However, similar to aquatic organisms, microplastics are also exposed to a variety of persistent pollutant in the water (Pazos *et al.*, 2017). Previous works have demonstrated that microplastics can sorb and could act as a vector for several organic pollutants (Hartmann *et al.*, 2017) and metals (Brennecke *et al.*, 2016). In addition, once ingested, microplastics able to translocate to other part of body such as circulatory system and muscle (Akhbarizadeh *et al.*, 2018). The great concern is when the microplastics desorbed in these tissues and subsequently may potentially risk to human health (Rochman *et al.*, 2015). In fact, Bakir *et al.* (2014) reported that the desorption in GI tract could be 30 times greater than in seawater itself.

The selection of internal organ and isolation method to identify ingested microplastics are currently not uniform (Jabeen *et al.*, 2017). For example, the digestion method by chemical reaction such as potassium hydroxide (KOH), hydrogen peroxide (H_2O_2) and sodium hypochlorite (NaClO) were commonly used in both marine (e.g. Collicutt *et al.*, 2019; Jabeen *et al.*, 2017) and freshwater fish (e.g. Yuan *et al.*, 2019; Xiong *et al.*, 2018). Among these, KOH was identified as the most suitable solution to digest GI tract of fish (Bessa *et al.*, 2018a; Kühn *et al.*, 2017). In this study, no foam was produced during the process. However, a brown slimy layer was observed on surface solution in most of the sample. Indeed, it has affected the visibility of sieved particles on filter paper during microscopic inspection. The result shows that the additional step using separation density (saturated NaCl) in this study has improved the detection and analysis step. On the other hand, direct observation of organ contents under the microscope in identifying microplastics was also carried out by several studies (Horton *et al.*, 2018; Neves *et al.*, 2015). This might underestimate the number of microplastics presence (Jabeen *et al.*, 2017). Thus, the utilization of chemical solution is necessary as to produce reliable results of the ingested microplastics in fish. This is pertinent since the smaller microplastics can be deliberately miscount under direct observation (Jabeen *et al.*, 2017). In addition, different types of organ samples to investigate the microplastics ingestion are of major concern since most of the previous works only considered stomach contents rather than the GI tract (Miranda *et al.*, 2016; Rummel *et al.*, 2016). Jabeen *et al.* (2017) strongly suggested to investigate the whole GI tract (from top of esophagus to anus) which is able to provide an all-inclusive report on the number of ingested microplastics in fish.

4. Conclusion

This study reported on the first evidence on the occurrence of microplastics ingestion in the most important ornamental and economic fish in Malaysia urban river and fishing resource. All species ingested microplastics indicates the widespread occurrence of plastic particles among the captured fish. Thus, more fish species should be considered to ascertain the potential impact of microplastics in freshwater ecosystems. Nevertheless, some of the captured fish in this study could potentially serve as indicator species in monitoring anthropogenic particles.

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