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# Abundance and characteristics of microplastics in commercial marine fish from Malaysia

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**Abstract**

Plastic debris is widespread and ubiquitous in the marine environment and ingestion of plastic debris by marine organisms is well-documented. Viscera and gills of 110 individual marine fish from 11 commercial fish species collected from the marine fish market were examined for presence of plastic debris. Isolated particles were characterized by Raman spectroscopy, and elemental analysis was assessed using energy-dispersive X-ray spectroscopy (EDX). Nine (of 11) species contained plastic debris. Out of 56 isolated particles, 76.8% were plastic polymers, 5.4% were pigments, and 17.8% were unidentified. Extracted plastic particle sizes ranged from 200 to 34900 μm (mean = 2600 μm ±7.0 SD). Hazardous material was undetected using inorganic elemental analysis of extracted plastic debris and pigment particles. The highest number of ingested microplastics were measured in *Eleutheronema tridactylum* and *Clarias gariepinus*, suggesting their potential as indicator species to monitor and study trends of ingested marine litter.

*Keywords:* Microplastics; Commercial fish; Ingestion; Raman spectroscopy.

## 1. Introduction

Despite global recognition of plastic as a pervasive contaminant, plastic marine pollution is still a growing environmental threat that affects marine biota and ecosystems (Walker, 2018). Global plastic production is increasing and surpassed 335 million metric tons (MT) in 2016 (PlasticsEurope, 2017). Between 4.8–12.7 million MT are estimated to enter oceans annually (Jambeck et al., 2015). Due to its ubiquity and longevity in the environment, marine plastic debris has adverse effects on marine wildlife and ecosystems, tourism industry, fisheries, damage maritime equipment, and navigation safety (Karbalaei et al., 2018). The Convention on Biological Diversity (CBD, 2016) reported that 75% of all marine debris is plastic and almost 800 marine species were affected by marine debris via entanglement or ingestion (Walker et al., 1997; Worm et al., 2017). Numbers of impacted species will likely increase with continued mismanagement and leakage of plastic into marine ecosystems (Walker, 2018).

Macroplastics (>10 mm) may remain in the environment for significant periods and break down under biological, chemical, and mechanical forces into smaller particles known as mesoplastics (1–10 mm) (Karami et al., 2017a), microplastics (0.001–1 mm) (Karami et al., 2016), or even nanoplastics (<0.001 mm) (Karami et al., 2018). Microplastics may also be released in to the marine environment from primary sources from microbeads used in industrial and cosmetic products (Fendall and Sewell, 2009; Pettipas et al., 2016) and through various environmental weathering processes (e.g., UV exposure, biodegradation, and physical process) (Gregory, 1996; Andrady, 2011).

The relatively small size of micro- and mesoplastics causes them to be more readily ingested by a broad range of marine species from planktonic invertebrates to large marine mammals (Desforges

et al., 2015; Li et al., 2016; Zhao et al., 2016; Jabeen et al., 2017; Cho et al., 2019). Recently, microplastics were detected in organisms cultured for human consumption such as mussel (Van Cauwenberghe and Janssen, 2014) and fish (Rochman et al., 2015) as well as in abiotic products such as salt (Karami et al., 2017b). A recent study by Karami et al. (2017a) has shown the presence of microplastics in edible flesh of four commonly consumed fish and estimated an annual ingestion of 246 anthropogenic particles by the consumers. Presence of plastic debris in human food is a concern due to ability of plastic debris to sorb persistent organic pollutants which could expose organisms and consumers to synergistic impacts from contaminants (Ogata et al., 2009).

Fish and other seafood (molluscs and crustaceans) are important sources of protein for people in Malaysia compared to other sources of protein such as chicken, beef and mutton. Fish are also an excellent source of essential fatty acids, amino acids, minerals (iodine and selenium) and vitamins (vitamins A, D, and B12) (Nurnadia et al., 2011; Karbalaei et al., 2017). Data from 2000 showed that Malaysian per capita consumption of fish was 58 kg per person (Nurnadia et al., 2011). Consumption of fish is the major route of exposure to microplastics in humans with top predator fish containing considerably elevated concentrations of microplastics due to bioaccumulation and biomagnification (Carbery et al., 2018). In Malaysia, a total of 0.199 trillion microplastics are estimated to enter the marine environment from personal care and cosmetic products (Praveena et al., 2018). Therefore, consumption of seafood could be an important source of human exposure to microplastics in Malaysia and elsewhere where consumption of seafood forms an important part of the diet.

This study investigated microplastic contamination in viscera and gills of 11 commonly consumed marine fish species from Malaysian markets to determine abundance of plastic debris in these fish species. Atomic composition of select anthropogenic particles were analyzed using field emission

scanning electron microscopy (FESEM) equipped with an energy-dispersive X-ray spectroscopy (EDX) to measure potential presence of hazardous compounds on these particles. Selected fish in the present study include Torpedo scad (Megalaspis cordyla), Orange-spotted grouper (Epinephelus coioides), Indian mackerel (Rastrelliger kanagurta), Kawakawa (Euthynnus affinis), Longtail tuna (Thunnus tonggol), Threefinger threadfin (Eleutheronema tridactylum), African catfish (Clarias gariepinus), Cachama (Colossoma macropomum), Delagoa threadfin bream (Nemipterus bipunctatus), Grass carp (Ctenopharyngodon Idella), and Oxeye scad (Selar boops) are common consumed fish in Malaysia and mostly used in other studies (Ahmad et al., 2016; Anual et al., 2018).

## 2. Methods

### *2.1 Materials and chemicals*

Ethanol 95%, potassium hydroxide (KOH), and sodium iodide (NaI) were obtained from R&M Chemicals (UK). Solutions of KOH (10% w/v) and NaI (4.4 M, 1.5 g/mL) were prepared by dissolving powder/pellet in ultrapure deionized water (Milli-Q Gradient system, Millipore, France). GF/D microfiber filter membrane (pore size: 2.7 µm) and filter membrane No. 540 (pore size: 8 µm) were purchased from Whatman. Filter membranes (149 µm) were supplied by Spectrum Laboratories (USA).

### *2.2 Sample collection*

A total of 110 individual fish from 11 commercial fish species [fish per species (n)=10] sold for human consumption were collected from the local fish market in Seri Kembangan, Malaysia (Table 1). Fish species were provided by the fish market and each fish was photographed for further identification. These species include commonly used fish consumption in Malaysia

(Ahmad et al., 2016). To increase representativeness of samples, fish species were randomly selected from one store. Fish were dissected before sale to customers using scissors and forceps to remove the gills and viscera including digestive tract from the top of the oesophagus to the anus, kidney, liver, heart and spleen. Viscera and gills of fish were collected and placed in pre-cleaned Ziploc bags (rinsed twice with deionized water and once with ethanol) and were transferred to the laboratory of aquatic toxicology at Universiti Putra Malaysia and maintained at -20°C. In order to control external contamination entering from the space of fish markets, an empty and opened pre-cleaned Ziploc bags were used as a blank (n=5 each species) during the dissection of each species of fish. All blank samples were analyzed same as viscera and gills of fish.

**<insert Table 1 here>**

*2.3 Extraction*

Plastic isolation from viscera and gills of the sampled fish was performed according to the method of Karami et al. (2017c). Excised organs and gills of fish were placed together in a 250 mL DURAN glass bottle (Schott, Germany) sealed with premium cap and pouring ring (Schott, Germany). Then 200 mL of KOH (10% w/v) was added to each bottle and was subsequently incubated at 40 °C for 72 h. Digestates were filtered over 149 µm filter membrane using a vacuum pump (Gast vacuum pump, DOA-P504-BN, USA) connected to a filter funnel manifold (Pall Corporation, USA). To separate potential plastic particles from other digestion resistant materials (e.g., exoskeleton of invertebrates), the 149 µm filter membrane was soaked in 10-15 mL NaI solution (4.4 M, 1.5 g/mL) and sonicated at 50 Hz for 5 min., agitated on an orbital shaker at 200 rpm for 5 min., and eventually centrifuged at 500 × g for 2 min. Finally, the supernatant of the mixture containing plastic particles was filtered through another filter membrane (pore size: 8 µm).

To ensure total isolation of plastic debris, this stage was performed twice. The filter papers were dried at 40°C and stored in Petri dishes for visual identification of particles.

*2.4 Visual identification*

Visual inspection of 8 µm filter membranes was conducted using Motic SMZ-140 Stereomicroscope (×110 magnification). All particles resembling plastic debris were sampled based on morphological characteristics including shape and size. Sampled particles were photographed (AxioCam, ERc 5S, Germany) equipped with a Stereomicroscope.

*2.5 Raman spectroscopy and FESEM-EDX analysis*

Extracted particles were assessed over a range of 150 to 3000 $cm^{-1}$ using a micro-Raman spectrometer (Horiba LabRam HR Evolution) equipped with a Single Mode Open Beam Laser Diode (Innovative Photonic Solutions) operating at a wavelength of 785 nm coupled with a charge-coupled device detector (Horiba Synapse). Baseline correction (Labspec 6, Horiba Scientific) was applied on all particles prior to library search to increase quality of spectra. Analyzed spectra were compared to the following spectral libraries: Raman polymers and monomers from Bio-Rad Sadtler and Raman Forensic from Horiba using the KnowItAll software from Bio-Rad. In addition, the Correlation Algorithm (KnowItAll, Bio-Rad) was utilized to compare every query spectrum to the data bases spectra. Furthermore, the elemental composition of some of the isolated anthropogenic particle was investigated by FESEM (Hitachi Ultra-high resolution SU8010) operating at 5 keV and equipped with an Oxford-Horiba Inca XMax50 energy-dispersive X-ray (EDX; Oxford Instruments Analytical, High Wycombe, England). Detection limits (DLs) were 1000 pg/µg for most metals.

*2.6 Contamination prevention*

To avoid airborne plastic contamination of samples, experiments were conducted in a horizontal laminar flow cabinet (AHC-4A1-ESCO). Ethanol 70% was used to clean surfaces. Nitrile gloves and cotton lab coat were worn during experiments. All liquids (ethanol, deionized water, NaI, and KOH) were filtered through 2.7 µm filter membrane. Glassware and instruments, including forceps, were washed using dishwashing liquid, rinsed with deionized water, and finally with ethanol. Procedural blanks were run simultaneously during collection of fish organs at the market (blank Ziploc bag), digestion step (blank KOH solution), and density separation (blank NaI solution) to account for potential contamination.

*2.7 Statistical analyses*

Statistical analysis was conducted using SPSS (IBM SPSS Statistics V. 24). Descriptive statistics, histograms, box plots and tests of normality were conducted on all data to identify if parametric or non-parametric tests were appropriate. A non-parametric Kruskall-Wallis test was used ($p$<0.05) for pairwise comparison to compare total number of plastic polymers or pigment particles among different species.

## 3. Results

A total of 56 particles >149 µm were extracted from excised organs and gills of 11 commercial fish species. A total of 43 particles (76.8%) were confirmed as plastic polymer, three particles (5.4%) were identified as pigment, and 10 particles (17.8%) were unidentified (Fig. 1a). Collected blanks were completely free of MP contamination. Microplastics were categorized into three shapes (fragment, fiber and film) and eight maximal length classes (149-500- 500-700, 700-1000, 1000-5000, 5000-10000, 10000-20000, 20000-30000, 30000-34900 µm) (Fig. 2) with an average

size of 2612.83 μm ± 6974.53SD. The smallest and biggest size of microplastics were detected in Threefinger threadfin (215 μm) and Cachama (3490 μm), respectively. The most frequent type of plastic polymer was polyethylene (88.4%), followed by polypropylene (9.3%) and polyethylene terephthalate (2.3%) (Fig.1b). Only one type of pigment has been identified in this study (i.e., phthalocyanine). Fragments were the most dominant plastic type (67.4%), followed by Fibres (16.3%) and films (16.3%) (Fig. 1c). Frequency of occurrence (%FO) of microplastics varied among species, with the %FO of ingested microplastics being higher in African catfish (60%) and Indian mackerel (50%) (Fig. 3).

**Fig. 1**

**Fig. 2**

**Fig. 3**

Fig. 4 shows images of some extracted anthropogenic particles. Analysis of inorganic composition of some of the isolated pigment particles, micro-, meso-, and macroplastics has revealed that all examined particles contained carbon (C), oxygen (O), calcium (Ca). Only a few particles contained sodium (Na), magnesium (Mg), chlorine (Cl), potassium (K), and phosphorus (P) (Fig. 5).

**Fig. 4**

**Fig. 5**

Results indicate there were significant differences in total number of isolated plastic polymers or pigment particles among different fish species (Kruskall-Wallis non parametric one way-ANOVA, $p<0.05$). Microplastic concentrations in Oxeye scad, Kawakawa, and Delagoa threadfin bream

were significantly different with African catfish and Threefinger threadfin. Significant differences were observed between Torpedo scad and Threefinger threadfin.

## 4. Discussion

There is a growing body of literature documenting plastic ingestion by aquatic organisms under laboratory and natural conditions (Neves et al., 2015; Nobre et al., 2015; Rummel et al., 2016; Catarino et al., 2018; Akhbarizadeh et al., 2018; Barboza et al., 2018; Hanachi et al., 2019; Hastuti et al., 2019). Marine organisms can passively ingest or mistake plastic for prey (Wright et al., 2013). A study conducted by Jambeck et al. (2015) estimated global plastic waste inputs from land into the ocean, based on solid waste, population density and economic status data from 2010, according to their modelled estimate, six Southeast Asian countries namely, Indonesia, Philippines, Vietnam, Thailand, Malaysia and Burma are among the top 20 countries mismanaging high quantities of plastic waste (i.e., ~ 1.4 to 3.7 million MT of marine plastic debris annually) (Jambeck et al., 2015). Although these countries have been reported as contributing large quantities of plastic marine pollution, developed countries have exacerbated the problem by exporting plastic waste to these countries, which often lack waste management infrastructure (Lui et al., 2018).

Several studies have investigated presence of plastic debris in the gastrointestinal tracts of aquatic organisms used for human consumption. For example, Neves et al. (2015) assessed presence of microplastics in stomach contents of 263 commercial fish caught from Portugal where 19.8% of individual fish contained microplastics ranging in size from 200 and 4800 μm. In another study, Rochman et al. (2015) measured plastic debris in fish and shellfish collected from fish markets in Indonesia and the US. They found 28% and 25% of individual fish contained anthropogenic

particles (size >500 μm) with mean plastic debris numbers of 1.4 ±3.7 SD and 0.5 ±1.4 SD per fish in samples from Indonesia and the US, respectively. All anthropogenic particles collected from fish in Indonesia were plastic, while anthropogenic particles collected from fish in the US were fibres. Miranda and Carvalho-Souza (2016) also found microplastics (ranging in size from 1000 to 5000 μm) in digestive tracts of two important edible fish species, King mackerel (*Scomberomorus cavalla*) and the Brazilian sharpnose shark (*Rhizoprionodon lalandii*) from Brazil with ingestion rates of 62.5% and 33%, respectively.

Given that viscera of fish and bones are used as ingredients for feeding farmed organisms (e.g., fishmeal for poultry and fish), microplastics may likely be transferred directly to tissues of farmed organisms and indirectly to humans (Hantoro et al., 2019). Our recent study highlighted that marine-derived fish meal is a source of microplastics which if transferred to cultured fish poses risks for aquaculture derived fish (Hanachi et al., 2019). For example, microplastics were isolated from farmed blue mussel (*Mytilus edulis L.*) (Mathalon and Hill, 2014) and farmed Manila clams (*Venerupis philippinarum*) (Davidson and Dudas, 2016), likely due to use of plastics in fish farm infrastructure including plastic lines which could result in availability of plastics to the organisms or food that produced from fish and other animals (e.g. fishmeal).

With respect to the morphology, plastic debris can be categorized into fibres, fragments, films, foams, and beads (Lusher et al., 2017). The isolated micro-, meso-, and macroplastics were mostly in the shape of fragment (67.4%) which is in agreement with the widespread distribution of this type of plastic debris in the marine environment (Eriksen et al., 2014). Approximately, 5.4% of the extracted particles were identified as phthalocyanine which is mainly employed as a synthetic pigment in the plastic industry (Charvat, 2005). Polyethylene was the most prevalent isolated plastic polymer (88.4%) followed by polypropylene (9.3%) and polyethylene terephthalate (2.3%).

Presumably this could be due to high demand and production of polyethylene which contribute to disposal of this plastic polymer into aquatic environments (PlasticsEurope, 2017). This in turn can potentially result in ingestion by aquatic organisms (Hidalgo-Ruz et al., 2012). A recent study by Khalik et al. (2018) was the first report on the presence of microplastic in Malaysian marine waters from two regions, namely Kuala Nerus and Kuantan port. Microplastics were identified based on physical (colour, shape, density) and chemical characteristics (ATR-FTIR analysis), and the results showed that fragment type and high density (> 1.02 g cm−3) of microplastic were the most prevalent characteristics found in Malaysian marine waters. As such, polyester, polystyrene, polyamide, polyvinyl chloride, polypropylene, and polyethylene were identified.

Microplastic levels detected in this study were compared with those studies in fish species reported worldwide on markets, fishing areas, coastal regions and sea (Table 2). Full genus name of species extracted from Table 2 were compiled in Table S1 (see Supplementary material). Research has shown that a high variety of commercially important fish species are often contaminated with microplastics in their gastrointestinal tracts, gills, liver and muscle. For example, in anchovy, large microplastics have been detected in 80 per cent of livers ranged from 124 μm to 438 μm (Collard et al., 2017). Also, Karami et al. (2017a) reported 59.0% plastic polymers in excised organs (viscera and gills) and eviscerated flesh of four commonly dried fish species (Greenback mullet, Belanger's croaker, Indian mackerel, and Spotty-face anchovy) in Malaysian markets, which in two species (Greenback mullet and Belanger's croaker), the eviscerated flesh contained higher microplastics concentrations than the excised organs. Recently, presence of microplastics was detected in the muscle of commercially important species, bartail flathead (*Platycephalus indicus*), greater lizardfish (*Saurida tumbil*), northern whiting (*Sillago sihama*), tongue sole (*Cynoglossus abbreviatus*) (Abbasi et al., 2018), Shrimp scad (*Alepes djedaba*), *E. coioides*, and Pickhandle

barracuda (*Sphyraena jello*) (Akhbarizadeh et al., 2018). These findings have led to increasing concerns about potential impacts of microplastics on human health.

Fibers and fragments were the dominant shape of plastic debris. Polyethylene and polypropylene were the most commonly reported plastic polymers found in fish from the fishing areas, markets, coast and sea (Table 2). Variations in microplastics shape, polymers and size likely reflect different sources and waste management strategies in different countries and sampling locations. In this study, African catfish and Threefinger threadfin showed high ingestion rates (%90 and %100, respectively), which were within ranges found in other studies. In a study conducted by Hastuti et al. (2019), the majority of analysed fish samples including Cichlidae, Scatophagidae, Siganidae, Mugilidae, Mullet, Chanidae, Clupeidae, Fringescale sardine, and Balistidae showed high microplastic ingestion rate in a range of %70-100. High ingestion rate was also observed in Acoupa weakfish (%194.3) (Ferreira et al., 2018), Bartail flathead (%115.6) (Akhbarizadeh et al., 2018), Catfish (%366.6) (Silva-Cavalcanti et al., 2017), and Japanese anchovy (%228.1) (Tanaka and Takada, 2016). Different ingestion rates in species may be due to fish consumption behavior, prevalence of ocean plastics and population density in different regions (Liboiron et al., 2016).

Occurrences of plastic debris in gastrointestinal tracts of organisms could be due to trophic transfer of smaller species being prey for higher trophic organisms (Farrell and Nelson, 2013). For example, remains of shrimp exoskeletons were found in excised organs of some fish species in the current study. Presence of microplastics in shrimp has been reported by Devriese et al. (2015). Fish sampled in this study (e.g., African catfish *C. gariepinus* and Indian Mackerel *Rastrelliger kanagurta*) feed on zooplankton (Spataru et al., 1987; Sivadas and Bhaskaran, 2009), which have been reported to ingest microplastics (Cole et al., 2013; Desforges et al., 2015).

Fish feeding in pelagic zone are likely to ingest more anthropogenic particles than demersal feeders, likey due to the low density of the highly prevalent plastic polymers in the environment (e.g., polyethylene and polypropylene) (Hidalgo-Ruz et al., 2012). However, fouling or biofouling may increase the density of these polymers (Morét-Ferguson et al., 2010; Karami, 2017) causing them to sink and become available for demersal feeders. For example, in this study excised organs or gills of almost all demersal feeders contained PE particles (density: 0.91–0.96 g/cm$^3$).

Surface morphology of microplastics can change significantly through degradation in the environment (e.g., erosion, photooxidation and temperature) causing surface abrasion. Inorganic and organic contaminants can sorb into pores or cracks of microplastics (Kowalski et al., 2016). Toxic effects of plastic debris carrying pollutants have been measured under laboratory conditions. For example, Karami et al. (2016) and Rochman et al. (2013) demonstrated that both virgin microplastics and microplastics-loaded with contaminants can elicit hepatic toxicity. Due to the hydrophobic nature of plastics and widespread distribution of persistent organic pollutants in aquatic ecosystems, plastic debris are commonly found with a mixture of contaminants (Ogata et al., 2009). Inorganic composition of select extracted plastic and pigment particles were analyzed using FESEM-EDX to identify potential sorbed contaminants. However, no contaminants were detected.

## Conclusion

Excised organs and gills of nine of 11 commercial marine fish species contained plastic debris suggesting a potential route of microplastic exposure to humans. This study showed that most microplastics sampled were fragments. The most frequent size category of microplastics were between 149 and 500 μm. Fish viscera and gills are often discarded prior to human consumption,

so further investigation of plastic contamination of edible fish tissues is recommended to assess potential plastic pollution in human food. Additional routes of human exposure to microplastics (e.g., skin contact and inhalation) is required to assess comprehensive exposure integrating multiple sources and routes.

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