

Original Article

Abundance and characteristics of microplastics in commercially sold fishes from Cebu Island, Philippines

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Abstract: This study documents microplastics (MPs) in the top three commercially sold fishes viz. *Auxis rochei*, *Rastrelliger kanagurta* and *Chanos chanos* in major public markets of Cebu Island, Philippines. MPs were found in the gastrointestinal tracts (FGIT) and quantified and characterized according to size, type, and color. In general, nine (97.3%) of 81 FGIT samples contained 635 total pieces of MPs with size ranging 0.01 to 0.50 and 1.00 to 2.00 mm. Transparent microfibers (91%) were the most predominant MPs, with blue (48%) as the most common MP color observed, followed by red (39%), black (8%) and white (5%). *Chanos chanos* proved to be the most susceptible fish to MP ingestion with a mean average of 11.6 pieces per individual fish, followed by *A. rochei* with 6.6 pieces, and *R. kanagurta* with 5.3 pieces. The results indicated that MPs were ubiquitous and high in commercially sold fishes in major public wet markets of Cebu Island, Philippines. The ingestion of fishes is of primary concern as a route of human exposure to MPs because they filter a large volume of seawater and are typically eaten whole without gut removal. Further study is needed on the potential consequences of MPs to aquatic populations to assess comprehensive exposure integrating multiple sources and routes.

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Introduction

Since the 1930s, plastics are essential raw materials in the global plastic industry due to various applications (Lusher et al., 2013; Setala et al., 2014). This resulted in widespread occurrence of the plastics in the marine environments and has drawn global attention, because its pollution may give a considerable impact on marine organisms (Bendell, 2018). However, the information available on the abundance of plastics in aquatic organisms in the Philippine waters, mainly fishes, remain to be scarce.

As plastics are usually broken down by UV light, various environmental conditions often result in the formation of secondary microplastics (MPs hereafter, both singular and plural, Nematdoost Haghi and Banaee, 2017). Due to their small size, various fragmented forms, and a wide range of potential sources, MPs are proven difficult to pinpoint and separate from bodies of water (Farel et al., 2018). As

most plastic wastes end up accumulating in the oceans and degraded slowly by sunlight, microbes or mechanical abrasion, it is noteworthy to study the abundance of MPs in fishes as well as their characteristics (e.g. type, size and composition). The tendency of fishes to accumulate MPs and their unhealthy physiological effects (Nematdoost Haghi and Banaee, 2017; Banaee et al., 2019) reveal importance of such MPs studies in fishes.

Understanding how MPs affect marine organisms has not been only the interest of marine biologists but also the policy makers and environmental managers. Many studies have documented that MPs are ingested by various marine organisms such as bivalves, zooplankton, copepods, fish, etc. (Browne et al., 2008; Lusher et al., 2013; Chua et al., 2013; Rochman et al., 2014; Watts et al., 2014) and transported up through high trophic levels in the food chain (Cedervall et al., 2012; Setala et al., 2014). Therefore, the widespread

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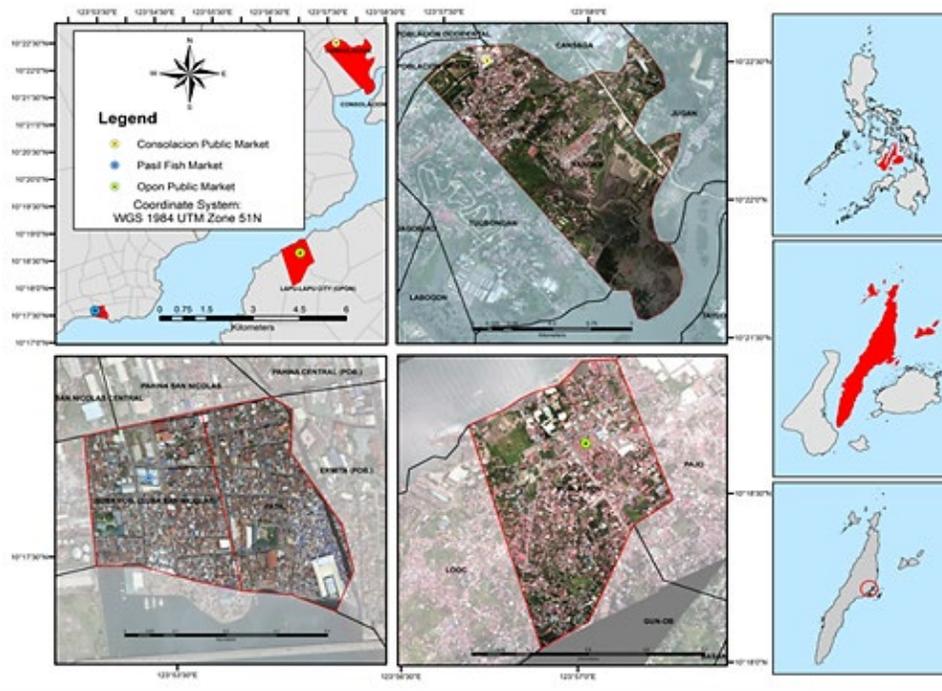


Figure 1. Geographic location of the study areas (major public markets) in Cebu Island, Philippines.

use of MPs is expected to lead its accumulation in the environment. Thus, greater exposure risk of wild organisms and human populations occurs over time.

Marine fisheries in the Philippines contribute to fish production as the primary source of protein for most of the population (Macusi et al., 2011). However, the possible effects of MPs on human compels need for research on MP contamination in major public wet markets in Cebu Island, the Philippines. Considering the importance of marine resources to the livelihood of Cebuano people, as well as the lack of information regarding the levels of MPs in the fishes, this study aims to: (1) investigate the abundance of MPs in the top three commercially sold fishes in a wet public market in Cebu Island, Philippines and (2) characterize MPs based on type, size, color and composition. This study will shed light on the risks of consuming seafood, particularly on fishes sold in major public markets of Cebu Island, Philippines. This study was carried out in three major public wet markets of Cebu, Philippines during 2017-2018.

Materials and Methods

Description of the study area and context: The study was conducted in three public markets in Cebu

Island, Philippines, namely (1) Consolacion Public Market, Consolacion, (2) Pasil Fish Market, Barangay Pasil, and (3) Lapu-lapu Public Market, Barangay Opon, Lapu-lapu City. These sites were selected to covers whole distribution of the Cebu Island, Philippines (Fig. 1). Pasil Fish Market is located in Barangay Pasil downtown Cebu City. It is the largest seafood market in the Visayas. It is located near the Pasil Fish Port where fisher folks arrive with their fish mainly caught in the nearby open Cebu Strait. Lapu-lapu Public Market in the Island of Mactan, is a large public market in the district where most of the population purchase their commodities, including fish which are mainly caught in their jurisdiction, the Olango group of Islands. Consolacion Public Market is the only public market in the Municipality of Consolacion where dry and wet goods are sold. One of the primary commodities that are usually bought by the consumer in the area is fish which are mainly caught in the seas of Carmen.

Ethical considerations: The researchers were granted clearance from the Ethics Review Committee of Velez College. A transmittal letter was given to the Head of the Marine Biology Section, Dr. Danilo T. Dy, in the University of San Carlos (USC) – Talamban Campus

Cebu, Philippines as permission to utilize their Marine Research Station in Mactan Island as a research locale. Then, a certification for exemption from the Institutional Animal Care and Use Committee (IACUC) was obtained.

Sample collection: MPs were assessed in fish samples, following the protocol of Rochman et al. (2015). For the identification of the top three commercially sold fishes, the researchers utilized an open-ended interview with 30 local fish vendors which were selected randomly from the public wet markets. In gathering fish samples, a selective method was utilized based on the interview result. Samples were processed in Marine Research Station, Maribago, Mactan Island facilitated by USC-Department of Biology. All fish samples were securely wrapped with aluminium foil and zip lock bags and stored in the freezer to avoid possible contamination. Then the sampling site and fishing data were also recorded.

Commercial species: Three representative commercial fish species viz. *Auxis rochei* (Scrombridae), *Rastrelliger kanagurta* (Scrombridae) and *Chanos chanos* (Chanidae) were collected from major public wet markets in Metro Cebu, Philippines. Only those fish species with age ranging between 1 and 2 years were included for MP analysis. *Auxis rochei* is highly commercialized and found in marine brackish waters. It is endemic in pelagic-neritic zones and distributed in the Atlantic, Indian, and Pacific oceans. This species is carnivorous feeding on small fishes, crustacean, and squid. *Rastrelliger kanagurta* is found in the pelagic-neritic area and distributed from East Africa to Indonesia. It is an omnivorous fish, feeding on phytoplankton, zooplankton, shrimps, and small fish. *Chanos chanos* is a benthopelagic organism found in marine, fresh, and brackish waters. It is distributed mostly in the Indo-Pacific waters. This species is omnivorous feeding on cyanobacteria, soft algae, small benthic invertebrates, and pelagic fish eggs.

Microplastic identification and analysis: Samplings were performed once every week for three weeks, gathering a total of 81 fishes. The samples have

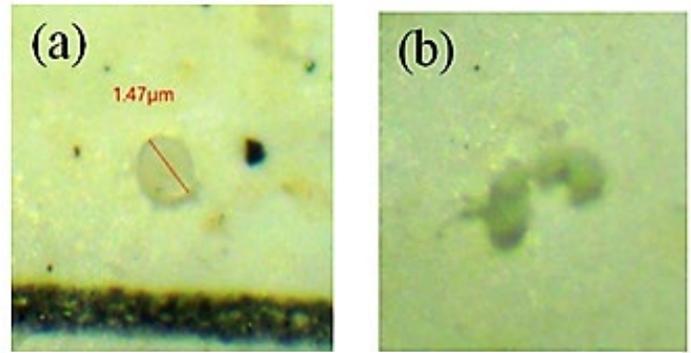


Figure 2. (a) Before and (b) after result of the hot needle test on MPs identification under the stereomicroscope.

roughly the same in length with size ranging from 33-36 cm. Each fish was placed in an aluminium foil which was then brought and processed in Marine Research Station on the same day. The MPs in the fish gut was extracted and inspected based on Budimir et al. (2018). Dissection was done under the laminar flow cabinet with controlled airflow to prevent contamination with the use of synthetic-free gloves (De Witte et al., 2014). All beakers and apparatuses used in the study were also rinsed several times with distilled water to avoid potential contamination with MPs. The gastrointestinal (GI) tract of the fishes was isolated by cutting lengthwise in the belly from head to anus. The GI tract was separated from the animal and weighed in a large tared jar covered with aluminium foil to prevent any contamination. Reagents were prepared by separately dissolving technical grade powdered NaOH and SDS (sodium dodecyl sulphate) (5g/L) in a distilled water. Digestion method was done by adding a 10 mL NaOH and 5 mL SDS 0.5% to the jar per 1 g of fish tissue. It was incubated in the oven at 50°C for 24 h, then incubated for another 24 h after the jars are gently shaken. After the incubation process, the contents were vacuum filter using a 0.45 μm filter membrane and the jars were rinsed with 95% ethanol and Milli-Q water three times and filtered separately. Then 10 mL of technical 2M HCl was added and rinsed with distilled water after 5 min. If there are still organic materials left, 30% hydrogen peroxide was added in the same way as HCl. All MPs were placed in a filter paper and inspected under the microscope. MPs were placed in a petri dish covered with filter papers; identified and confirmed

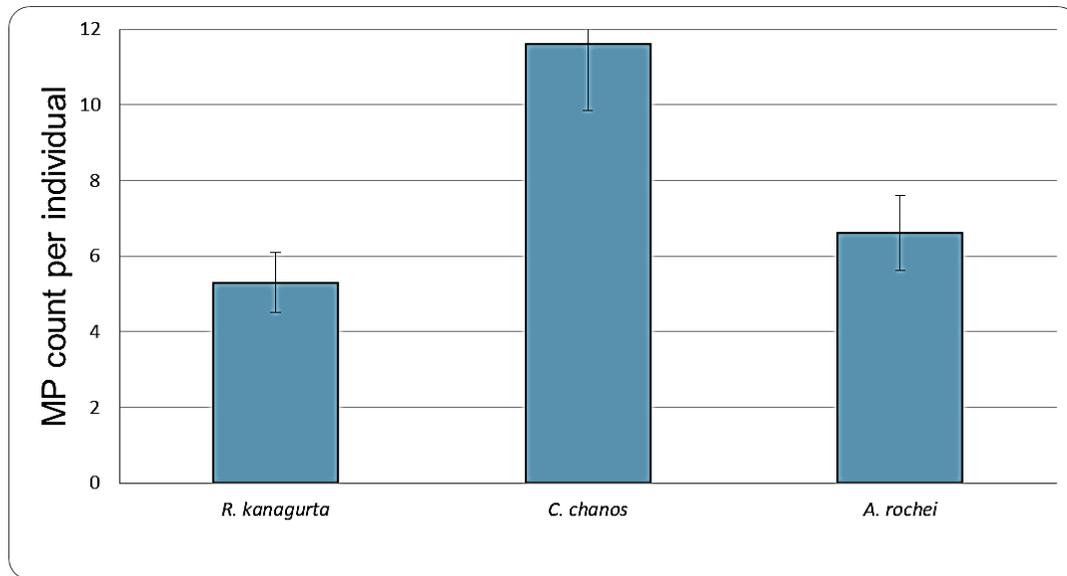


Figure 3. Average amount of ingested MPs per individual fish.

using the stereomicroscope and a hot needle technique, respectively. Hot needle procedure was done by heating the tip of a thin needle and poking a suspected MPs under the stereomicroscope (De Witte et al., 2014). Plastic tends to shrink or warp when exposed to heat (Fig. 2). Amscope™ (digital microscope camera) used for documentation. Precautionary methods were made such as closing the petri dish always and opening it only under the laminar flow cabinet to prevent from any contamination during the whole procedure. The researchers also wore proper laboratory gown and sterile, non-synthetic gloves. All authors made sure that all MPs present in the GI tracts of fishes sampled have homogenous color, and undeformed feature before undergoing further analysis (Lusher et al., 2014). For microfibers, there should be consistency in terms of thickness throughout lengths without debris found on both ends of the fibers sampled in all fishes. Throughout the MP extraction process, all containers were appropriately covered with aluminium foil. To reduce contamination, some of the members who did the experiment wore protective gears such as mask, gloves and goggles.

Data analysis: The normality and homoscedasticity of the gathered data were tested using Shapiro-Wilk and Levene's tests, respectively. All data (mean average and %) were statistically analyzed using

Kruskal–Wallis test to investigate differences on the amount of the ingested MPs between fish species with subsequent post hoc Dunn's test analysis at 5% level of significance was performed using Statistical Package for the Social Sciences (SPSS) software (IBM Co. Ltd, USA).

Results

MPs were detected in all the fishes (Fig. 3) and out of 81 fishes (Fig. 4), 79 (97%) had the ingested MPs in their digestive tract. Measured densities of MPs showed a statistically significant difference in all fishes (Kruskal-Wallis test, $\chi^2(2) = 13.75$, $df=3$, $P=0.001$, <0.05). *Chanos chanos* (with mean rank of 52.89) had higher MP density, followed by *A. rochei* (mean rank = 40.89) and *R. kanagurta* (mean rank = 29.22). A significant difference in MPs density was found between *R. kanagurta* and *C. chanos* (Dunn's test, $t = -23.67$, $SD = 6.38$, $P = 0.001$) while there was no significant difference in MPs density between *R. kanagurta* and *A. rochei* (Dunn's test, $t = -11.67$, $SD = 6.38$, $P = 0.203$) and *A. rochei* and *C. chanos* (Dunn's test, $t = 12.00$, $SD = 6.38$, $P = 0.180$) (Fig. 3).

Microplastic debris characteristics: Four types of MPs, including microfibers, microfragments, micropellets and microfilms were recovered in all fishes (Fig. 5f). A total of 635 pieces of MPs were detected and categorized according to size, type, and

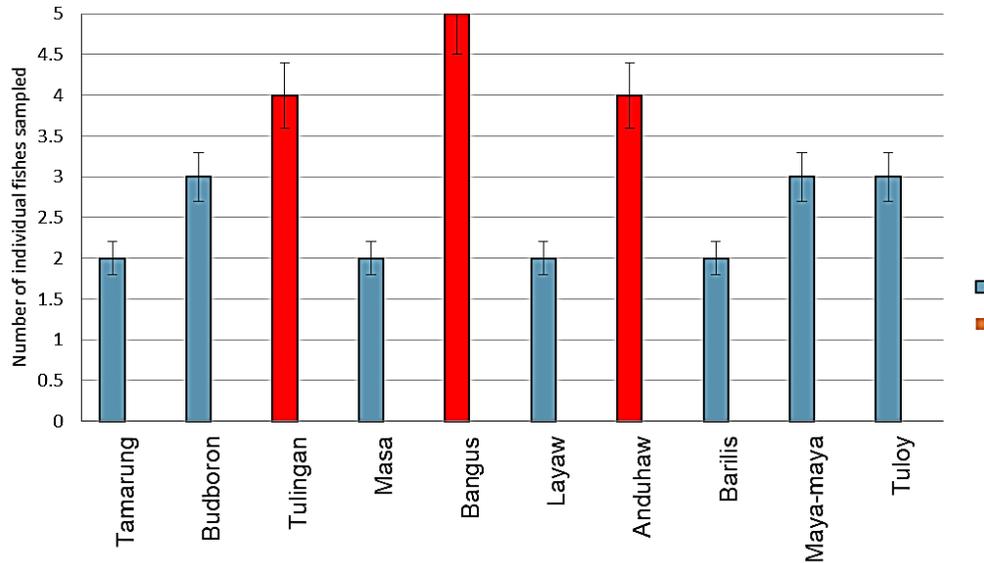


Figure 4. Identification of top 3 commercially sold fishes in three large public markets in Cebu Island, Philippines.

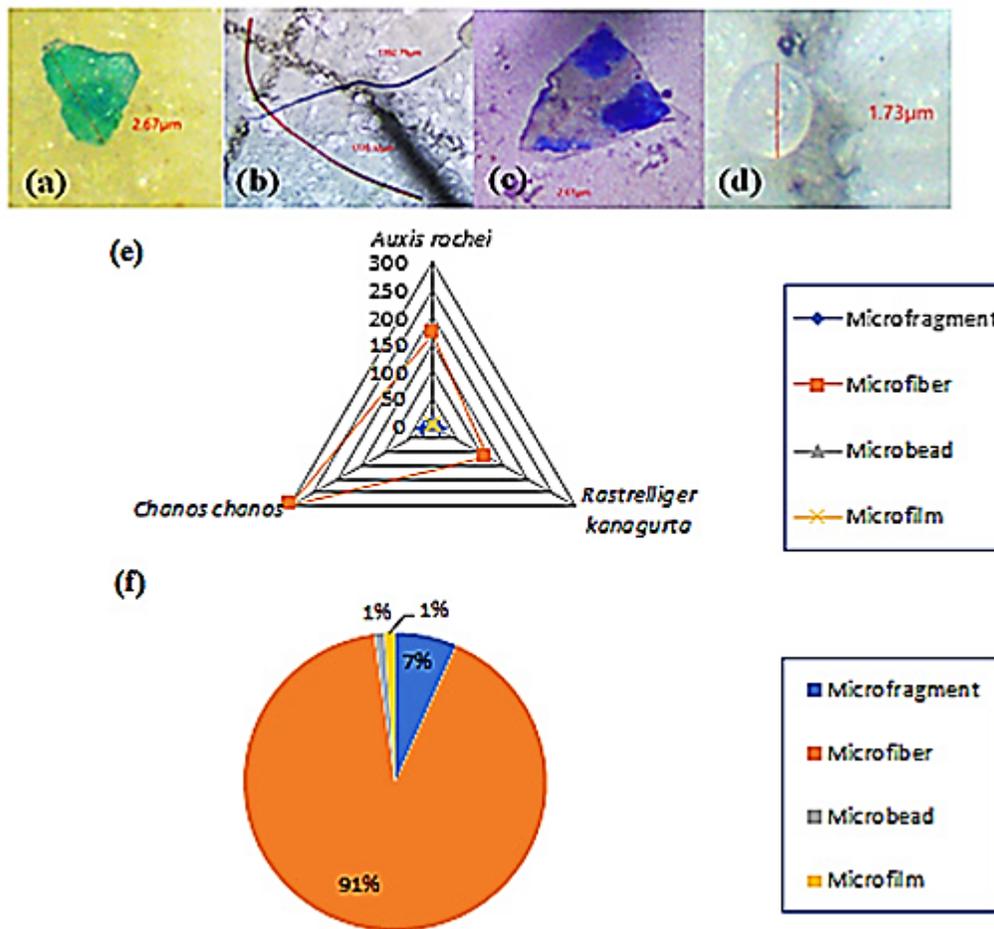


Figure 5. MPs types found in fish samples: (a) Microfragment, (b) microfiber, (c) microfilm, and (d) micropellet along with the (e) distribution of MPs types between fish samples and (f) its total proportion in all samples.

color. Microfibers were the most common type of MPs found in all fishes sampled, with a proportion of

80 to 91%. Microfragments were the second most abundant MPs type, making an average of 7% of each

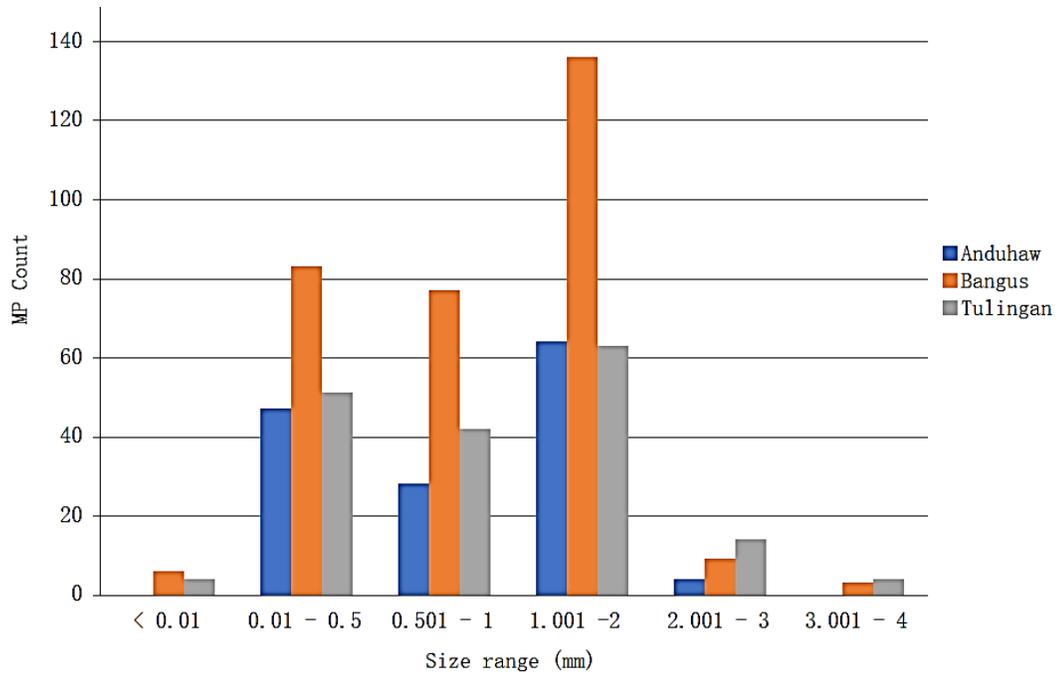


Figure 6. Size distribution of MPs in three study sites

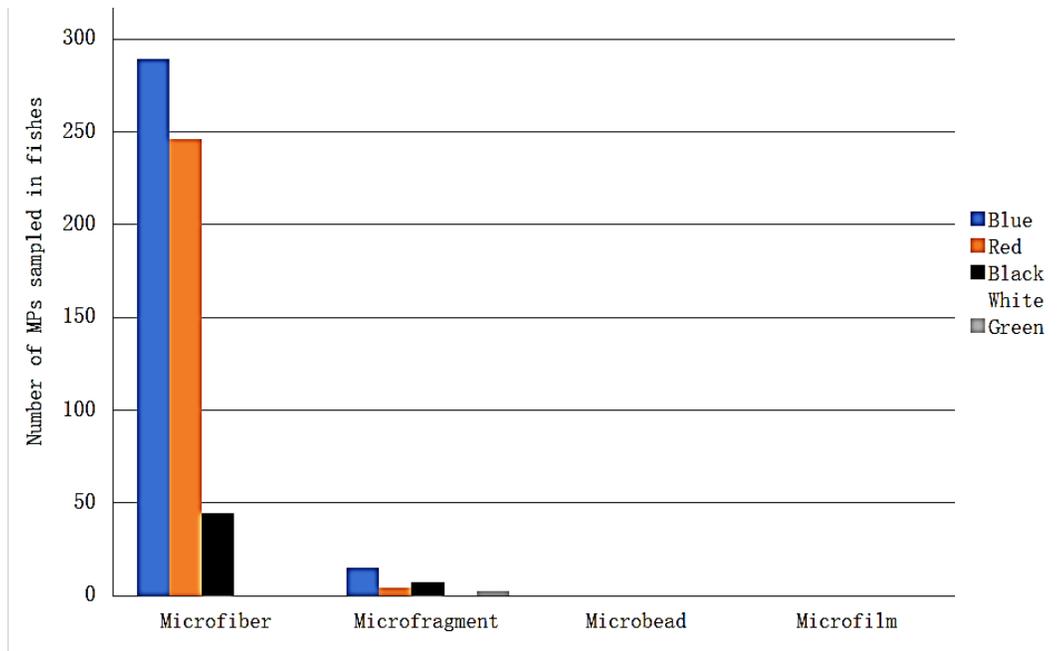


Figure 7. MP distribution according to color and type.

sample. The other two types, micropellets and microfilms were only found in a few fish samples at 1% for each MPs type (Fig. 5). Both microfragments and microfibers were present in *A. rochei* while in *R. kanagurta*, micropellet, microfragment, microfiber and microfilm were the most common MP types. In *C. chanos*, microfragment, microfiber and microfilm

were present. Only microfoam was not present in all fishes (Fig. 7).

Most detected MPs in fishes have sizes ranging 0.015-3.800 mm, with 1.001-2.000 mm as the most common size class (Fig. 7). Moreover, the classification of MP by size showed that the most abundant MPs were in the range of 0.01-0.5 to 1.001-

2.000 mm (Fig. 6). The dominant color group of MPs was the colored one particularly blue (48%) color is the most prevalent in all fishes (Fig. 7) followed by red (39%), black (8%) and white (5%).

Discussions

The results indicated that *A. rochei*, *R. kanagurta* and *C. chanos* were vulnerable to MP contamination, of which *C. chanos* accumulated most MPs (± 11.6 pieces per individual fish) following *A. rochei* (± 6.6) and *R. kanagurta* (± 5.3). This result was in congruence with the study of Ory et al. (2017). MP abundance in fishes can be explained series of environmental factors (i.e., wave action and water currents) as well as human-mediated activities (i.e. disposal of waste materials and wastage produced from industrial and municipal facilities). To cite, the total population in Metro Cebu (798,634), Lapu-lapu City (550,467) and Mandaue City (331,320) when combined (1,680,421) was higher than those of any other cities in the Philippines Higher population in these cities could contribute to MP contamination in marine ecosystems. The higher degree of development in Metro Cebu indeed, is caused the higher MP pollution in marine environment.

Dominance of the microfibers in this study indicated high change of ingestion events. *Chanos chanos* had ingested the most amounts of MPs as a benthic-pelagic species. It lives near the bottom as well as in midwaters or near the surface and feeds on free swimming organisms. Philips and Bonner (2015) showed high plastic ingestion from lower to higher trophic level. Both *A. rochei* and *R. kanagurta* are pelagic-neritic species found in coastal waters and mid-waters. Microfibers come from industrial and municipal waterways which indirectly and directly contaminate freshwater and marine ecosystems (Lusher et al., 2014). Also, sewage treatment facilities produce voluminous amounts of microfibers through the production of textiles and become part of the waterways, which can be hardly determined when studied on-site (Bessa et al., 2014). Other industries probably generate a considerable number of microfibers from the fishing nets and ropes which

contribute to high microfiber accumulation in the studied fishes. Our result is pivotal for management purposes in studied markets in Metro Cebu since successful management of MP pollution can be attained based on the source of MP origin.

In recent studies, ingestion of MPs among fishes could be attributed to their accidental identification of MPs as their prey (Boerger et al., 2010) since these MPs have similar color, shapes and texture with that of food (Foekema et al., 2013; Lusher et al., 2013, 2015; Ory et al., 2017).

The highest value of microfibers is found in high volume and number (>85%) on coastal shorelines and even on the surface of oceans globally. Uncertain with its main source, most research suggests that these microfibers are debris produced from synthetically-made fibers (with dimensions <5 mm) which can easily pass through microfiber materials (Lusher et al., 2015; Bessa et al., 2018). Other domestic materials such as clothing and plastic materials from laundry are also considered as a major source of microfibers (Bagchia et al., 2016; Carr et al., 2016).

Moreover, the color of the dominant MPs found was consistent with previous studies (Bagchia et al., 2016; Carr et al., 2016; Ory et al., 2017). Their color selectivity may potentially contribute to the likelihood of floating MPs to their natural food for aquatic organisms (Andrady, 2011). Blue particles were preferentially ingested by *C. chanos* and *A. rochei* (Ismail et al., 2019), and *R. kanagurta* (Martin et al., 2017). The results were in concordance with the MP colors observed in many previous studies (Ogata et al., 2009; Rochman, 2015; Tekman et al., 2017) where blue color is more abundant.

MP ingestion among fish species is a major concern to human systems since these MPs can be engulfed in bulk and can be left in their gut for a long period. Left unnoticed, these fishes with high MPs in their guts are commonly bought by market-goers daily. Moreover, wide-scale assessment on the most commonly-purchased fishes is also recommended as this will identify the possible route of MP contamination to humans. Suggested guidelines to minimize MP exposure include correct assessment of MPs during

the preparation, handling, and storage of seafood products. Market-surveys, done comprehensively and seasonally, in seafood products should be conducted to assure human health safety.

Conclusions: The results indicate presence of MPs in commercially sold fishes in major public wet markets of Cebu Island, Philippines. Vulnerable to MP contamination, commercially sold fishes contaminated with MPs have direct exposure to humans and may pose harm when eaten without the removal of MPs in their gut. In this case, there is a need to conduct assessments and identification of MPs in other marine species (e.g., oysters, mussels and planktons) which are not only known as a source of food and livelihood among fisherfolks but also considered as excellent bioindicators in marine ecosystems.

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