Contaminants and Human Health Risks Associated with Exposure to Microplastic Ingestion of Green Mussels (*Perna viridis*) Collected from The Kedonganan Fish Market, Bali

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Abstract

Microplastics (MPs) are pollutant agents that have been absorbed and detected in aquatic ecosystems at high concentrations. This study aimed to investigate the presence of MPs pollution in green mussel (*Perna viridis*) products sold at the Kedonganan fish market, Badung, Bali. A total of 150 mussels with an average weight of 3,2 ± 0,71 g/mussels from three traders each composed and followed by the pre-treatment stage using 5 M NaCl solution, extraction with wet oxidation peroxidation (WPO) + Fe(II) catalyst and filtered. The highest percentage for the form of MPs was successively obtained by the Line form in Trader A at 85,42% and the lowest in Trader C at 50,00%. The highest form of fragments was obtained in Trader C at 42,86%. Film and filament forms were only obtained in Trader A. The highest MPs color was black and the lowest was gray. The highest average MPs particle size was found in the form of a filament of 1944,37 ± 88,41 μm which was found in Trader A. Estimates of MPs intake per year/capita in Indonesia showed that exposure to MPs through consumption of green mussels in this study amounted to 498,330 MPs/year/capita items. Overall, the green mussel from Trader A had the highest percentage and size of MPs, with the shape of fragment MPs being dominated by Trader C and the color of the MPs being dominated by black. MPs exposure to green mussel consumption in Indonesia is very high, but no health impact category has yet been found for this estimate.

Keywords: environmental health, health risks, microplastics, shellfish, traditional markets

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INTRODUCTION

Microplastics (MPs) pollution of the environment has drawn a lot of attention, particularly due to its detrimental effects on the viability of the earth's flora and fauna (Bhatt *et al*., 2021; Yuan *et al*., 2022). The marine environment continues to be the primary repository for plastic waste contamination even though MPs are known to circulate in all ecosystem matrices (Fok *et al*., 2017). According to Sendra *et al*. (2021) MPs have a variety of physicochemical characteristics, including composition, particle size, form, and the polymers that make up the plastic itself. One of the issues with determining the risks these contaminants pose to the target species is their diversity (Kögel *et al*., 2020). Although the target species are found in both marine and terrestrial environments, living things that are in extremely polluted locations are the priority targets for these toxins (Richard *et al*., 2021).

According to classification, MPs are referred to as coming from a "primary" source if they are formed in smaller (5 mm) or microscopic sizes and as a "secondary" source if they are created through the fragmentation of microplastic waste (Hartmann *et al*., 2019; Lehtiniemi *et al*., 2018). As previously mentioned, MPs can be made from a variety of monomers, including high and low density polyethylene (HD/LD-PE), polyethylene terephthalate (PET), polypropylene (PP), polystyrene (PS), and polyvinyl chloride (PVC) (Lambert *et al*., 2017). MPs also have a wide range of chemical characteristics. It's interesting to note that MPs can also be broken down to create "nano plastic" (1–100 nm), a type of plastic whose toxicological characteristics are still unknown (EFSA, 2016; Kögel *et al*., 2020).

More than 3,3 billion individuals use seafood as an animal protein source, making seafood consumption one of the requirements with a 20 kg per capita consumption rate. When compared to the previous year, when it was 54,56 kg/capita, Indonesia's seafood consumption in 2021 increased by 1,48% to 55,37 kg/capita (MMAF, 2022). The World Health Organization (WHO) has advocated for regular seafood eating since it may have positive impacts on human health. (Dawson *et al*., 2022).

However, according to the state of the ecosystem right now, a lot of seafood is contaminated with environmental toxins, which can lead to disease, particularly in humans (Smith *et al*., 2018; Wasilah *et al*., 2021; Wiradana *et al*., 2020). Heavy metals (Rosiana *et al*., 2022), antibiotic residues (Okocha *et al*., 2018), pesticide residues (Olsvik *et al*., 2019), and up to MPs (Bhuyan, 2022) are a few examples of pollutants that can lower the quality of seafood. MPs are typically extracted from fishery commodity tissues, such as the gastrointestinal tract, spleen, and liver, which are typically discarded rather than ingested by people, in contrast to other types of environmental pollutants. The ability of shellfish to operate as a "filter-feeder" in the aquatic environment and transmit MPs to human consumers is still a source of worry, however (Walkinshaw *et al*., 2020; Wiradana *et al*., 2019). However, aquatic biota, particularly shellfish, is being employed as a potential method for biomonitoring and determining the standard of an ecosystem (Bertrand *et al*., 2018; Wiradana *et al*., 2020). This aquatic organism, however, is also one of the fisheries products that people can eat.

Several types of shellfish that are used as bioindicators include the bivalves *Mytilus galloprovincialis* which are economically valuable but are capable of accumulating MPs contamination in the Mediterranean Sea (Grbin *et al*., 2019). Some seafood including mollusks sold in Australia has also been contaminated with MPs in both wild caught and instant seafood (Rist *et al*., 2018). MPs contamination levels were also found in green mussels (*Perna viridis*) sold in markets in Thailand. The results show that the average MPs abundance in green mussels is 7,32 ± 8,33 particles/shells and 1,53 ± 2,04 particles/g (wet weight) with the highest MPs in the form of fragments (75,4%) (Imasha and Babel, 2021). MPs contamination was also reported in mussel shells (*Pilsbryoconcha exilis*) collected from the Perancak River, Jembrana, Bali (Yunanto *et al*., 2021). MPs contamination was also found in blood clams (*Tegilarca granosa*) and tofu clams (*Meretrix meretrix*) in the waters of Banyuurip Village, Gresik, East Java (Yona *et al*., 2021). However, there are no recent reports that reveal MPs contamination in shellfish products such as green mussels sold at traditional fish markets in Bali.

Kedonganan Fish Market is a traditional market that sells fishery products from fishermen which located in Badung, Bali. Fishery products sold at Kedonganan Market are dominated by snapper, grouper, crustaceans, crabs, and shellfish products including green mussels. The results of previous studies revealed that fish resources around Keodnganan Beach and Kedonganan PPI had as many as 16 types of fish caught which were dominated by Tuna by 54% (Pratiwi *et al*., 2020). Catchings such as lemuru fish, on the other hand, are in the excellent category at the Kedonganan market, with a composite score of 66,7 (Suariningsih *et al*., 2021). Most of the catch sold in this market comes from Java Island and around the waters of Jimbaran Beach. Interestingly, fishery products that are commercialized in this market are still not monitored for food safety, including from the MPs contamination aspect. The purpose of this study was to monitor MPs contamination in green mussels (*Perna viridis*) sold at the Kedonganan Fish Market based on the type, size, and color of the MPs found. The results of this study can be used as an early warning to fishermen, traders, and even consumers to always care about environmental sustainability in order to support the quality of fishery products, especially those sold at the Kedonganan Fish Market.

MATERIALS AND METHODS

Study Area

This study used a purposive sampling method on three traders selling green mussels at the Kedonganan Fish Market. A total of 50 green mussels in three traders (n = 150 green mussels) with an average weight of green mussels 3,2 ± 0,71 g/head (Wiradana *et al*., 2020). The green mussel samples that have been collected were then put into a sterile plastic clip measuring 1 kg and equipped with a sample label and put into a cooling box, then transported to the laboratory for further evaluation.

Pre-treatment

The green mussel samples that have been collected were then put into a sterile beaker glass to be washed using sterile aqua bikes to remove residual contaminants and polluting agents. Clean green mussels are then sorted, and the meat part was taken for material extraction purposes. The green mussel meat for each trader was then put into a sterile beaker glass for further evaluation.

Extraction and Identification of MPs

Green mussel meat that has been collected from each trader was then extracted using a 10% KOH solution for 24 hours. Green mussel meat was then filtered using Whatman filter paper with Cat No: 714–104, with a pore size of 0,45 µm with a diameter of 47 mm. The filtration process was also equipped with a vacuum pump. Each screening process includes a control blank in the form of an aquadest. A total of 1–2 samples were filtered, depending on the time required for the screening process everyday. The MPs attached to the filter paper were then observed with a stereomicroscope at 20× magnification equipped with an Optilab Advance microscope camera and Image Raster 3.0 software.

The MPs were determined in terms of shape, color, and size categories. The structure of the MPs was identified visually following the procedure from Prata and the six size categories used to follow the procedure from Simon-Sánchez *et al*., (2019) i.e.: < 50 µm, 50–100 µm, 100–200 µm, 200–300 µm, 300–500 µm, and > 500 µm.

Estimates of Human Exposure to MPs Through Seafood Consumption

Two approaches were used to estimate human exposure to MPs through the consumption of green mussels. First, it is based on the recommendations set by the European Food Safety Authority (EFSA) regarding the consumption of fish: children from 1 year old (40 g/week); children aged 2–6 years (50 g/week); >children aged 6 years (200 g/week), and adults or the general population (300 g/week). Second, based on data set by the European Market Observatory for Fisheries and Aquaculture Products (EUMOFA) and the National Fisheries Service (NOAA) but adjusted for the level of fish consumption in Indonesia, which is 55,37 kg/year/capita. Estimates of MPs intake in humans were based on the average total number of MPs in mussel meat collected in this study (i.e. the number of MPs found in mussel meat/150 specimens) (Barboza *et al*., 2020): (1) Human MPs intake per week (MPs items/week): average MPs items in shellfish meat (MPs items/g) × recommended seafood intake per week (g) (EFSA); (2) Annual human MPs intake (MPs items/year): average MPs items in shellfish meat (MPs items/g) × recommended seafood intake per week (g) × number of weeks per year (52 weeks); (3) Human MPs intake per week per capita (MPs items/week/capita): average MPs items in shellfish meat (MPs items/g) × seafood consumption per week per capita (g); (4) Annual human MPs intake per capita (MPs items/year/capita): average MPs items in shellfish meat (MPs items/g) × seafood consumption per year per capita in selected regions (g).

RESULTS AND DISCUSSION

Percentage of MPs

The results of the identification of MPs in green mussels collected at the Kedonganan showed that there were 4 forms of MPs. The form of MPs includes Line, Fragment, Film, and Filament (Figure 1).

Based on percentage, MPs in Line form have the highest percentage when compared to other forms in this study. MPs with the highest Line form were found in shellfish traders B, namely 85,42%, followed by shellfish traders A with 79,17% and shellfish traders C with 50,00%. MPs with the highest Fragment form were found in shellfish traders C (42,86%), followed by shellfish traders B (14,58%) and shellfish traders A (10,42%). Interestingly, MPs in the form of film and filament had the lowest percentage in this study and were only found in shellfish traders A, at 6,25% and 4,17% respectively (Figure 2). Several recent reports report the number of forms of MPs that vary from location to country. Contamination of MPs has been found in fish and shellfish from estuaries in the Sundarbans. This study revealed that the abundance of MPs varied in all samples, ranging from 5,37 ± 1,07 to 54,30 ± 16,53 MPs item/g wet weight in muscle samples. The results of the FT-IR analysis also confirmed that the dominant types of PP, PE, and PA polymers were found in the muscle tissue of fish and shellfish (Sultan *et al*., 2023). MPs contamination was also found in wild oysters and clam culture *Crassostrea gigas* and *Ruditapes philippinarum* clam in Jiaozhou Bay, China. In contrast to the findings of this study, the results of this study indicated that the majority of MPs were in the form of fiber (92,97%) (Zhang *et al*., 2022).

Size of MPs

Based on their size, MPs in the form of filaments have the highest average size of 1944,37 μm which is only found in shellfish traders A. Furthermore, Line-shaped MPs are found in all shellfish traders with the highest size obtained in shellfish traders A at 1518,45 μm, followed by shellfish trader B at 1145,85 μm and C shell traders at 962,39 μm. MPs in the form of the film were only found in shellfish traders A with an MPs particle size of 1312,63 μm. MPs in the form of fragments had the lowest size in this study, namely shellfish trader A (551,12 μm), shellfish trader B (552,72 μm), and shellfish trader C (291,82 μm) (Figure 3).

Green mussels collected from the Tambak Lorok beach area, Java Sea have an average MPs size of 211,163 μm (Khoironi *et al*., 2018), lower when compared to the findings in this study. Likewise, *Mytilus spp*. which was successfully collected from the waters of the Portuguese Sea has a particle size ranging from 36–4439 μm which is dominated by fibers (50%), films (22%), and spherules (18%) (Marques *et al*., 2021). Plastic debris can consist of a complex mixture of particles that can be categorized based on visual characteristics including size, color, and shape, and are relatively diverse pollutants, covering a wide range of sizes and shapes from larger waste to the nanoscale (Kiran *et al*., 2022). Interestingly, the various types of polymers that make up MPs that have floating to sinking properties also affect the differences in the size and type of MPs that accumulate in an area.(Hartmann *et al*., 2019).

Interestingly, MPs belong to a size that is ideal for accumulating in small aquatic species such as zooplankton (Desforges *et al*., 2015), shellfish, and crustaceans in marine food webs. Studies have proven that smaller MPs are ingested by a wide range of species from zooplankton to marine mammals (Nelms *et al*., 2019). It should also be noted that the shape, size, and density of MPs can affect the position of the particles in the water column, which can determine how MPs particles move to water bodies, which in turn causes differences in the amount of each water, sediment, and even marine biota (Kowalski *et al*., 2016) including clams. A study showed that the size of MPs in the form of fragments was higher in sediment samples, followed by seawater, and the lowest in *M. edulis* shells from Southwest Coast, UK (Scott *et al*., 2019). However, further study is still needed to measure the MPs in this shellfish product under natural conditions as well as with seawater and sediment. However, based on the literature that the MPs size is higher than 100 μm has the potential to translocate MPs in clam meat, where they can be ingested by humans and then translocated to human organ tissue (Naidu *et al*., 2022).

MPs Color on Green Mussel Flesh

Color is one of the important parameters that can be used to identify MPs and potential contamination in sample preparation. The present

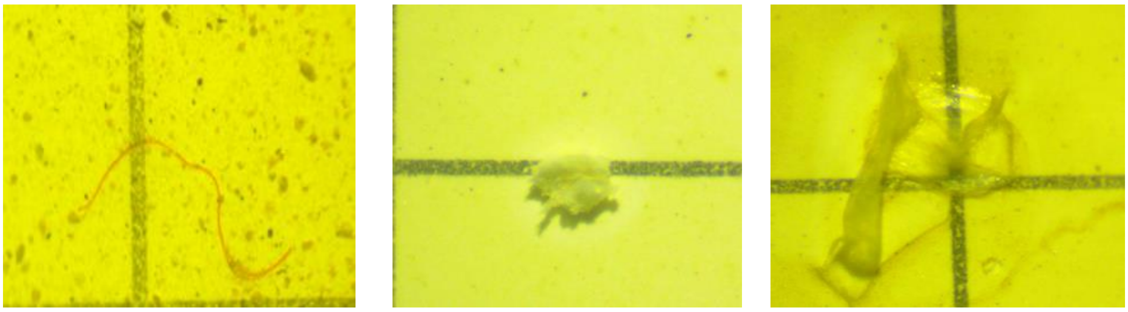

Figure 1. The form of MPs found in green mussels. a) Line, b) Fragment, and c) Filament.

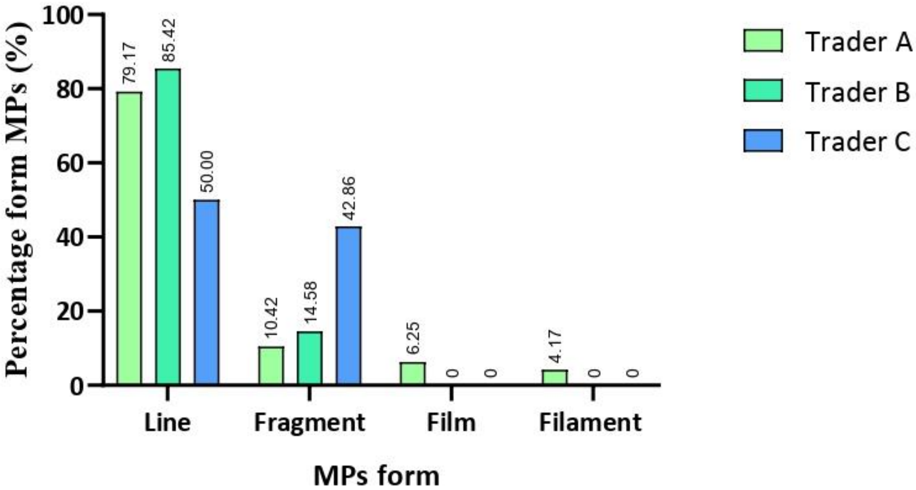

Figure 2. Percentage of MPs forms found in green mussels.

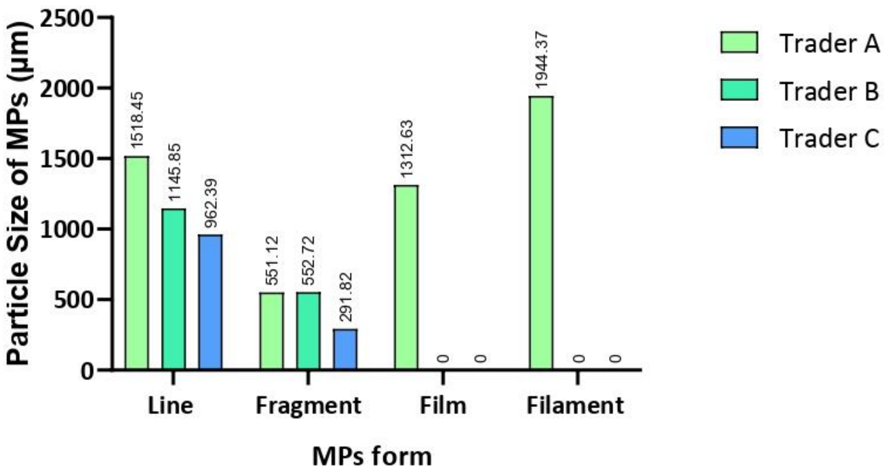

Figure 3. MPs particle size found in green mussels.

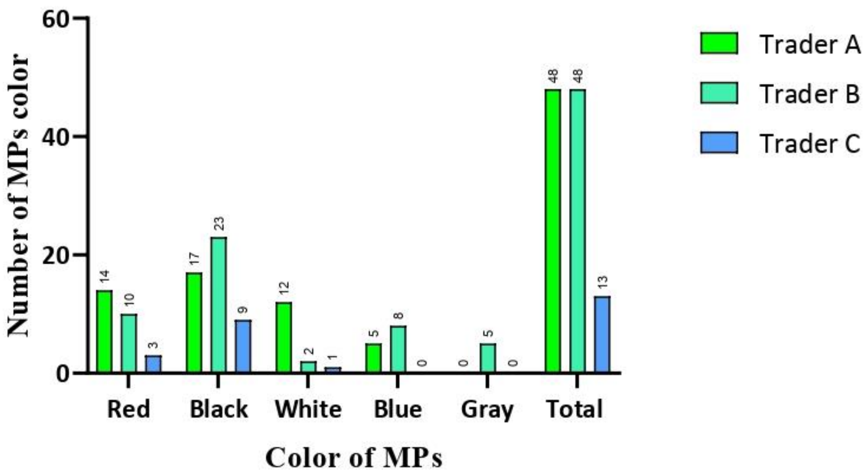

Figure 4. Color number of MPs found in green mussels.

Table 1. Estimates of human intake of MPs from seafood consumption based on MPs found in mussels compared to recommendations from the relevant EFSA consumption of seafood per week by children of various ages and adults or the general population

	Children			**Adults or the general population**
	(1 year)	**(2–6 years)**	**(> 6 years)**	**(> 18 years)**
mussel meat/week*	40 g	50 g	200 g	300 g
MPs items/week	360	450	1,800	2,700
mussel meat/year	2,080	2,600	10,400	15,600
MPs items/year	18,720	23,400	93,600	140,400

*amount of seafood consumption based on recommendations from the EFSA.

study, 5 colors of MPs were found including red, black, white, blue, and gray. The total number of colored MPs that were identified for each green mussel trader at the Kedonganan Fish Market was Trader A (48 pcs), Trader B (48 pcs), and Trader C (13 pcs) respectively. The highest MPs color found in this study was black to be precise at Trader B (23 pcs), followed by Trader A (17 pcs) and Trader C (9 pcs). Then followed by the highest red color at Trader A (14 pcs) and the lowest at Trader C (3 pcs). MPs with the highest white color were found in Trader A (12 pcs), and blue color in Trader B (8 pcs) (Figure 4).

Various colors of MPs/NPs have been documented in several studies including red, orange, yellow, brown, tan, off-white, white, gray, blue, and green (Murray and Örmeci, 2020; Rochman *et al*., 2019). MPs in blue and red were the colors most frequently reported (Zhang *et al*., 2020). Apart from shellfish, colored MPs have also been detected in wild fish indicating that colored MPs have the potential to be mistaken by fish as their natural prey. It also shows that if various marine or freshwater fish have a color preference for ingesting MPs, future study is urgently needed to understand the harmful effects of MPs on fish and shellfish based on their color preference (Okamoto *et al*., 2022). Experimental studies show that goldfish (*Carassius auratus*) ingest more white MPs than other colors (Xiong *et al*., 2019). In contrast to the results of this study which showed that the highest finding of black MPs was 47% in trader B when compared to other MPs colors, the MPs color found in shells collected from four locations in Hong Kong was dominated by red (67%) and the lowest black (6%) (Joyce and Falkenberg, 2023). It is very important to measure the color of MPs to provide information to researchers about the color preferences consumed by aquatic biota. For example, black MPs may be captured by fish because this color is associated with some common prey that has the same color (Ory *et al*., 2018), and white or blue MPs are more likely to be ingested by plant-eating fish (Boerger *et al*., 2010).

Estimated Intake of MPs by Humans Consuming Green Mussels

Based on the mean total MPa found in green mussel meat (9 MPs items/g meat, n = 150) and on the recommended weekly intake of seafood for different age groups by EFSA (2011) this study successively ranged from 360 MPs items/week (children aged 1 year), 450 MPs items/week (children aged 2–6 years), 1,800 MPs items/week (children aged > 6 years), and 2,700 MPs items /week (> 18 years to adulthood) (Table 1). In addition, based on the average total MPs found in mussel meat and seafood consumption per capita in Indonesia, the estimated human MPs consumption through the consumption of green mussels is 10 MPs/week/capita items and 498,330 MPs/capita items. year/capita (Table 2).

Table 2. Estimated human MPs intake from seafood consumption based on MPs found in green mussels from Kedonganan Market as well as per capita fish consumption in Indonesia in 2021

Indonesia	
Per capita consumption	55.37
green mussel/week/capita	1,065
MPs items/week/capita	10
green mussel/year/capita	55,370
MPs items/year/capita	498,330

Seafood is a source of animal protein that can support human health. However, consuming seafood containing MPs can pose a risk to human health, especially if consumed over a long period in areas with high levels of fish consumption or areas that have been reported to be contaminated by high amounts of plastic-polluting agents. The MPs consumption estimates made in this study are adjusted to the recommendations from the EFSA regarding the consumption of mussels (EFSA, 2016) (Table 1) showed that adults or the general population who consume 300 g of shellfish per week are estimated to consume an average of 2,700 MPs/week or 140,400 MPs/year. These values are certainly higher when compared to previous studies which estimated MPs consumption in marine fish (*Dicentrachus labrax*, *Trachurus trachurus*, *Scomber colias*) from the North East Atlantic Ocean by the general population of 824 MPs/year (Barboza *et al*., 2020). This is also higher when compared to the estimated consumption of seafood from the Persian Gulf, which is 17 MPs/week or around 877 MPs/year (Akhbarizadeh *et al*., 2018). However, these findings are low when compared with estimates of the annual intake of MPs from seafood consumption by adults from Atlantic Ocean waters (Mahu *et al*., 2023). The difference in estimates of seafood intake versus MPs is due to the high contaminant pressure over a wide range of habitats and feeding preferences (Mahu *et al*., 2023).

Based on fish consumption per capita (Table 2), this study estimate showed that the consumption of MPs by humans through the consumption of seafood, in this case, green mussels, depends on several factors such as geographical location, age, lifestyle, and local culture, and differs in each region. country. Apart from fishery products, humans also consume other types of food which are also known to have the potential to be contaminated by MPs, for example, rice (Tian *et al*., 2023), vegetables (Hao *et al*., 2023), fruits (Pinto-Poblete *et al*., 2023), and meat products (Kedzierski *et al*., 2020). Contamination of food by MPs during the harvesting, preparation, and consumption processes may have become a common situation today that needs further investigation (Catarino *et al*., 2018). Exposure to MPs in humans can occur through several routes such as ingestion, absorption by the skin, to inhalation so human absorption of MPs is likely to be much greater than estimates based on the consumption of fish products alone (Cox *et al*., 2019; Prata, 2018). Considering that this is a preliminary study, further study on the effects of exposure to MPs on other fishery commodities, the effects of exposure, and their toxicity are still urgently needed.

CONCLUSION

This study provides the first information regarding evidence of MPs contamination in mussels sold at the Kedonganan Fish Market, Bali. All samples collected from mussel traders analyzed had MPs that varied based on shape, particle size, and color which indicated MPs contamination in the aquatic environment. In addition, the presence of MPs in green mussel meat which is commonly consumed highlights the need for further assessment of the polymer type of these MPs and the toxicity of MPs in experimental animals. Based on the average MPs found in green mussels and adjusted for recommendations from the EFSA regarding intake of seafood consumption, 18,720–140,400 items MPs/year. Estimates of MPs intake per year/capita in Indonesia show that exposure to MPs through consumption of green mussels in this study amounted to 498,330 MPs/year/capita items. The results of these findings can contribute to setting limits on seafood intake related to MPs contamination and increasing public and relevant authorities' awareness of the risks MPs pose to environmental and human health. In addition, the findings from this study are supported by assembled literature, further study is needed regarding MPs contamination in other marine fishery products, types of MPs polymers, and the dangers of MPs to physiological disorders in experimental animals.

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