Microplastics in commercially sold fishes from General Santos City Fish Port Complex, Philippines

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Abstract. The excessive global production and inadequate recycling of plastics have led to the accumulation of plastic waste, which degrades into microplastics and poses a threat to marine organisms and human health. The need to study microplastic contamination in wet markets in General Santos City Fish Port Complex (GSCFPC), is crucial for assessing exposure risks and implementing measures to mitigate pollution and protect public health. This study aimed to quantify and characterize the ingested microplastics in the gastrointestinal tract of *Decapterus macarellus*, *Euthynnus affinis*, and *Selar crumenophthalmus*, and compare the data among the different fish species. There were seven (7.07%) of 99 individuals contained ten microplastic particles with a mean size of 0.789±0.379 mm. The most abundant microplastic recovered were microfragments (90%) with a dominant color of blue (100%). Based on the polymer composition, ethylene-propylene copolymer was the most prevalent (50%), followed by poly (vinyl stearate) (30%), polyethylene (10%), and polypropylene (10%). The results revealed that all fish species examined exhibited similar susceptibility to microplastic contamination, with *D. macarellus* ingesting 0.15 particles per individual, followed by *S. crumenophthalmus* with 0.12 particles per individual and *E. affinis* with 0.03 particles per individual. Additionally, there was a significant difference ($p < 0.05$) in microplastic size, indicating that *D. macarellus* was likely to ingest larger debris compared to *S. crumenophthalmus*. The study revealed evidence of microplastic contamination in commercial fish species at the GSCFPC. Highlighting the potential risks to human health and the environment of Southern Mindanao, further assessment of smaller fish species and trophic transfer is necessary for a comprehensive understanding of the contamination patterns.
Key Words: commercial fish, gastrointestinal tract, microplastic.

Introduction. Plastics are synthetic materials created by combining molecular monomers through polymerization, which results in their durable, flexible, and non-water-soluble characteristics (Desidery & Lanotte 2022) and utilized for a wide range of uses across various industries worldwide (Plastics Europe 2019). The excessive dependence on plastics has resulted in a significant rise in global production, escalating from 2 million tons in 1950 to 380 million tons in 2015 (Geyer et al 2017). Due to the inadequate global recycling rate of plastics (less than 10%), a substantial amount of plastic waste, ranging from 60 to 99 million tons, was mismanaged in 2015 (Lebreton & Andrady 2019). The accumulated plastic wastes have been causing pollution in aquatic environments over the years. These plastic wastes will eventually degrade into tiny particles measuring less than 5mm through a combination of chemical, mechanical, and biological processes, posing a serious threat to the ecosystem (Ivleva et al 2016; Granby et al 2018; Akarsu et al 2020; Amelia et al 2021). The plastic particles with a size range of < 5 mm in both length and width dimensions are categorized as microplastics (MPs) (GESAMP 2019).

MP pollution is an emerging and alarming issue that has garnered global attention due to its detrimental effects on marine organisms and their habitat ecosystems (Gall & Thompson 2015; Rochman et al 2015; Provencher et al 2019; Zhang et al 2020). When MPs are ingested by marine organisms, they pose a deleterious effect not only on the marine organisms but also on humankind (Rochman et al 2015; Avio et al 2017; Abbasi et al 2018; Zeytin et al 2020; Amelia et al 2021; Keziya et al 2022; Wei et al 2022; Agbekpornu & Kevudo 2023; Rojoni et al 2024). The minute size of MPs has led to documented cases of ingestion in various marine species, including bivalves, zooplankton, seabirds, sea turtles, and small pelagic fishes (Possatto et al 2011; Lusher et al 2013; Watts et al 2014; Gutow et al 2015; Neves et al 2015; Abbasi et al 2018; Ghosal et al 2018; Kolandhasamy et al 2018; Pellini et al 2018). This ingestion poses a vulnerability in the marine food web, as there is a potential for the transfer of MPs to higher trophic levels (Cedervall et al 2012; Setälä et al 2014; Gall & Thompson 2015; Di Mauro et al 2017; Sun et al 2018; Zhang et al 2019). The impacts of MP ingestion extend beyond the bioaccumulation of chemical substances in marine organisms and also affect human health, including enhanced inflammatory response, toxicity, and disruption of the gut microbiome (Hermsen et al 2017; Wright & Kelly 2017; Smith et al 2018; Rojoni et al 2024). Research on MP contamination in wet markets, where fresh seafood is sold, is essential to assess human exposure and consumption risks (Abiñon et al 2020). By studying the extent of MP contamination in these settings, we can assess the associated risks and implement appropriate measures to mitigate as well as for marine environmental monitoring programs in the future (Koelmans et al 2017; Hermsen et al 2018; Rochman 2020). Investigating MP levels and sources in major public wet markets will provide valuable insights into how consumers may be exposed to MPs and the overall impact on public health (Abiñon et al 2020). This research will inform policies and interventions aimed at reducing MP pollution, ensuring food safety, and safeguarding the well-being of individuals who rely on these markets for their daily sustenance (Bucol et al 2020; Rochman 2020; Cabansag et al 2021).

In the Philippines, marine fisheries have played a significant role in food production (Macusi et al 2011; BFAR 2023). Surprisingly, the country ranks third globally as a major contributor to marine plastic waste, estimated to range from 0.28 to 0.75 million tons annually (Jambeck et al 2015; Bucol et al 2020). However, despite the evident threats posed by MPs, there remains a significant gap in understanding their presence in fish resources, particularly in South Western Mindanao (Abreo 2018; Espiritu et al 2019; Bucol et al 2020; Palermo et al 2020; Bonifacio et al 2022). Previous research on MPs has mainly concentrated on sediment analysis and the examination of bivalves. The investigation of MP ingestion in commercially sold fishes in the Philippines has become a growing area of interest (Bucol et al 2020; Cabansag et al 2021).

Given the significance of fish resources to the livelihoods in Southern Mindanao, it is essential to document the MPs ingested by commercially sold fishes from the General Santos City Fish Port Complex (GSCFPC). The GSCFPC is considered to be the second largest fish port complex and was considered the country's premier fish producer and is likewise hailed as the tuna capital of the Philippines (Tan 2016). Hence, fish dealers and consumers across the region obtain their fish supplies in the said complex which are subsequently distributed to local markets. Thus, this study was designed to investigate the MPs among commercially and significantly available fish species. Specifically, to quantify the ingested MPs in the gastrointestinal tract of *Decapterus macarellus* (Cuvier, 1833), *Euthynnus affinis* (Cantor, 1849), and *Selar crumenophthalmus* (Bloch, 1793), characterize the extracted MPs based on their type, size, color, and composition, and compare the collected data of MPs among fish species.

Material and Method

Description of study area. The fish samples were directly gathered from landing site Market 2 of the GSCFPC. The fish landed were sourced from the Fisheries Management Area 3 (FMA-3), a special delineation of area for fishery resource management purposes that covers regions 9, 12, and the Bangsamoro Autonomous Region in Muslim Mindanao (BARMM) (BFAR & USAID 2020). FMA-3 through the implementation of Fisheries Administrative Order (FAO) No. 263 encompasses various fishing grounds, including Moro Gulf, Celebes Sea, Iliana Bay, Dumanquillas Bay, and Saranggani Bay (Figure 1). The collection of samples between species was done on December 2022 and January 2023. In addition, all experimental procedures were conducted in a controlled laboratory setting at the Science Department of Mindanao State University – General Santos.

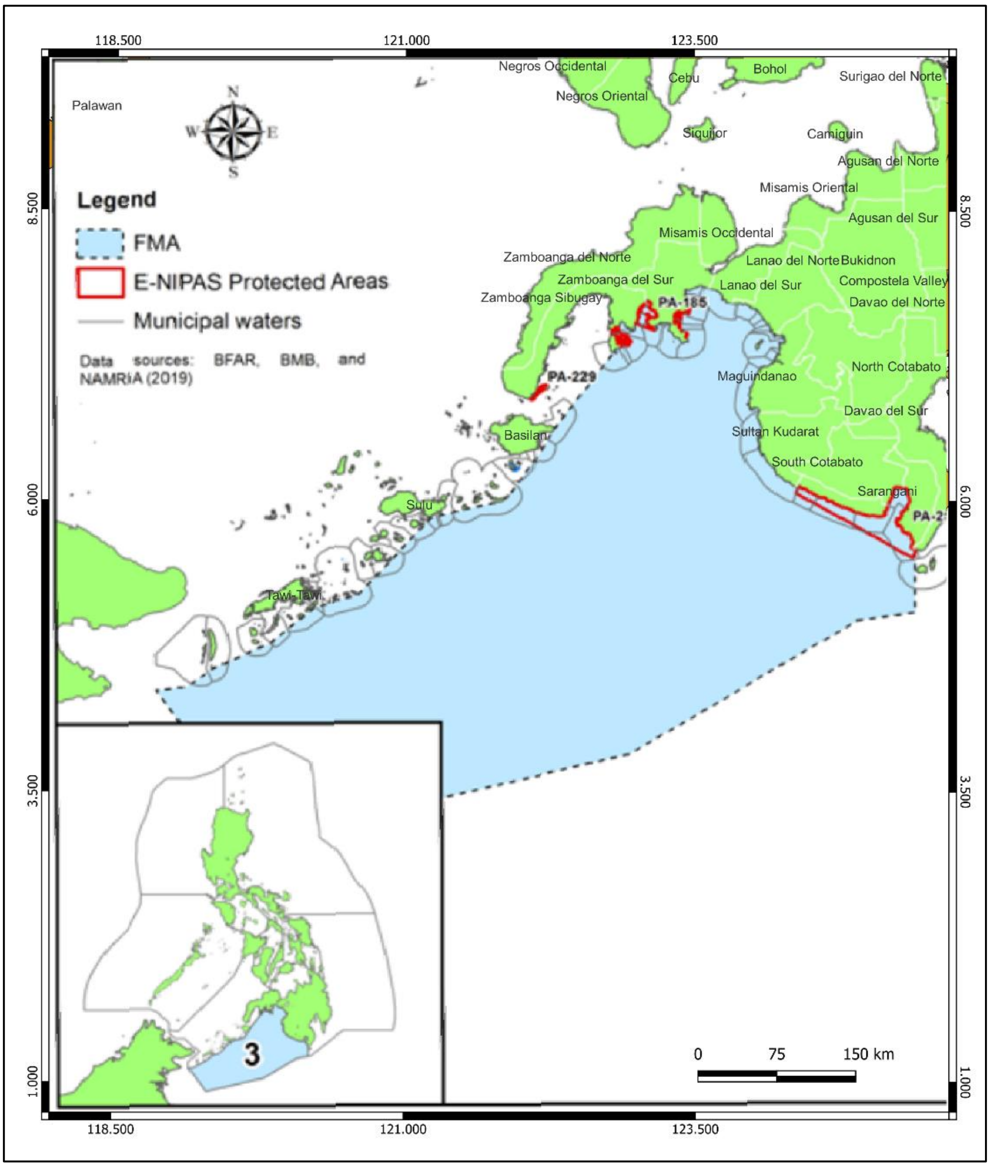

Figure 1. A) The map of the Philippines illustrating the boundaries and location of Fisheries Management Area 3. B) FMA-3 showing the fishing grounds of the collected samples landed in GSCFPC. Retrieved from USAID Fish Right Program (2020).

Sample collection. A total of 99 samples were randomly collected from the GSCFPC. The sample size was determined at a 95% confidence interval with a 10% margin of error. The selected fish has sample size of 33 each species for the *D. macarellus*, *E. affinis*, and *S. crumenophthalmus* (Figure 2).

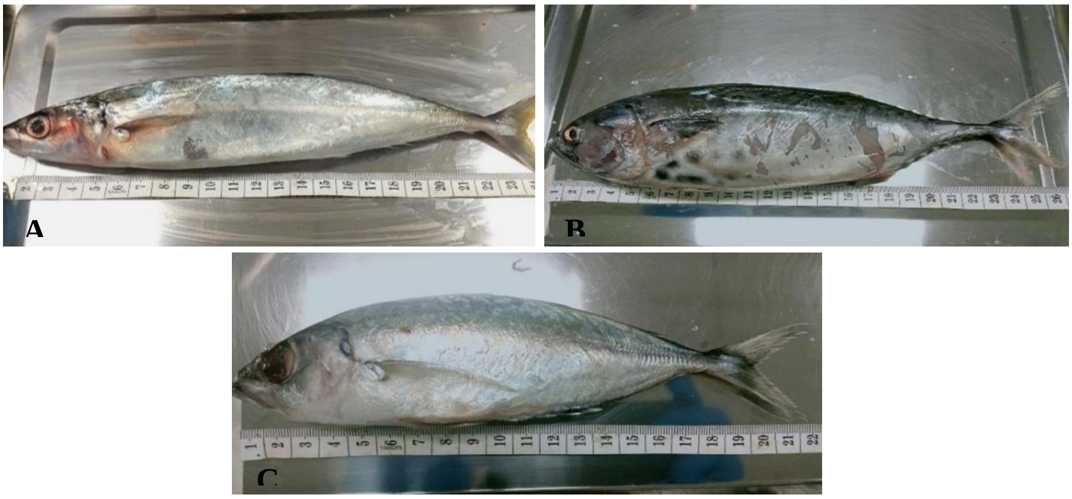

Figure 2. The selected commercially available fish species in GSCFPC: (A) *Decapterus macarellus*, (B) *Euthynnus affinis*, and (C) *Selar crumenophthalmus*.

The nomenclature of these species was following online Fishbase by Froese & Pauly (2024). The collection of fish samples was done by following the protocol of Abiñon et al (2020). Prior dissection process, morphometric data such as the snout-to-vent length, total length, and body weight were recorded using measuring tape and a top-loading balance.

MPs extraction and quality control. To reduce any possible contamination, all experimental procedures including dissection were performed in a closed air-conditioned laboratory. Moreover, the materials used throughout the extraction and processing of samples were made of glass or steel. Materials made of polymer were extremely evaded in any procedure for possible cross-contamination. Laboratory materials were washed thoroughly, rinsed with distilled water, and air-dried in an inverted position to prevent any fibers from entering the glassware (Li et al 2015; Lusher et al 2017). After obtaining morphometric data, fish samples were initially thawed and rinsed with distilled water and were dissected on a sanitized benchtop. The gastrointestinal (GI) tract of the fish samples was then extracted and immediately transferred to a 25 mL glass tube with a cap. To minimize potential of contamination in the process, a distinct tube was prepared as a negative control, which indicates that the sample tube does not contain GI tract but was analyzed in the same method as other samples (Jabeen et al 2017). The purpose of the negative control is to verify the potential presence of MP contamination originating from external sources during the experimental process.

Prior to the onset of the experiment, a simulation was conducted to establish a MP extraction protocol based on the existing studies (Avio et al 2015). The MPs extraction protocol used in this study was adapted from Foekema et al (2013) and modified by Dehaut et al (2016) and Lopes et al (2022). According to Dehaut et al (2016), the effective digestion of biological tissues and minimal degradation of polymers was achieved by employing a 10% (w/v) KOH (potassium hydroxide) solution and incubating at 60°C for a duration of 24 hours. The addition of KOH solution to the extracted GI tract follows a ratio of 2:1 in terms of volume. Optimizing the said protocol, Lopes et al (2022) suggested that the addition of 10% Polysorbate-20 (Tween-20) resulted in an improved filtration flow rate in the extraction process, increased the recovery rates of polymers, provided a protective effect from polymer degradation, and did not interfere in Fourier Transform Infrared (FTIR) spectrum of the polymers found in the marine environment. Following the incubation process, the contents were filtered under a vacuum filtering assembly using a 0.45 μm polyvinylidene difluoride filter membrane. The remaining organic particles in test tubes were rinsed with distilled water for complete filtration. Afterward, the filter membrane containing the filtrates was observed for potential MP presence. The summary of the procedural steps for extracting microplastics is shown in Figure 3.

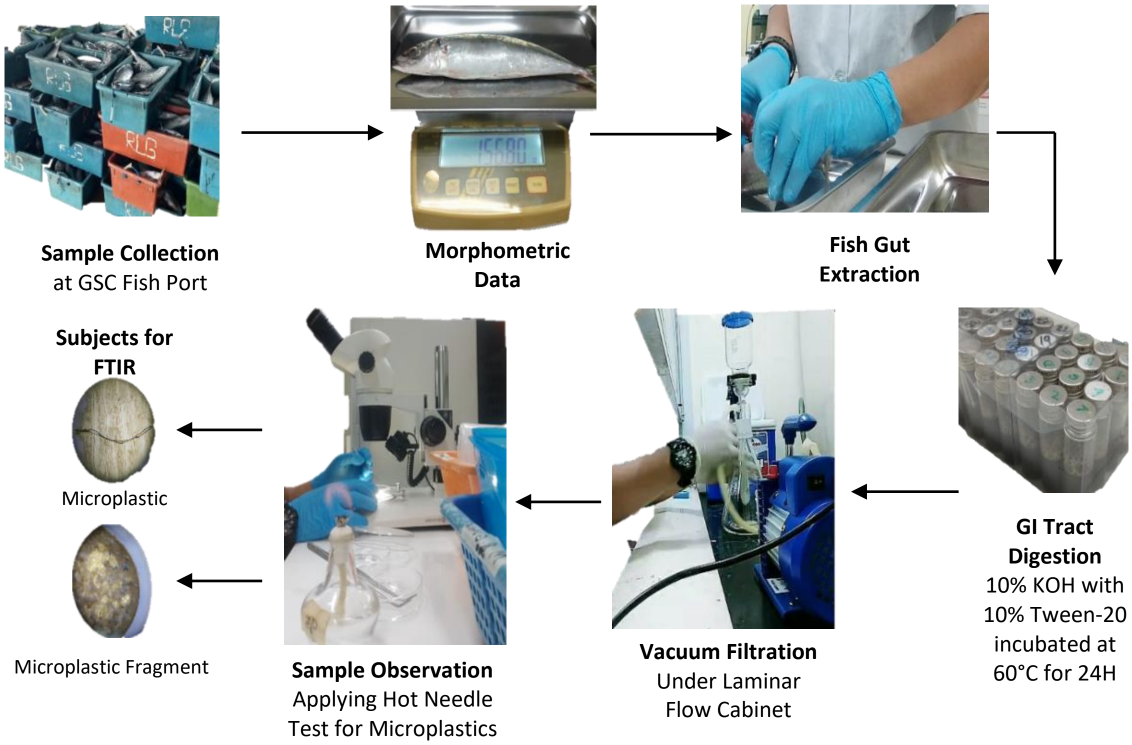

Figure 1. The summary of the procedural steps for extracting microplastic particles from the gastrointestinal tract of fish species.

Microplastic identification and data analyses. Each filter membrane was placed in a Petri dish under a laminar flow cabinet before examination. Identification of MP particles was carried out under a Euromex stereomicroscope and was photographed. All suspected MP particles were documented. To verify the suspected MP particles, a hot needle test which involved heating the tip of a thin needle and probing a suspected MP under the stereomicroscope was performed (De Witte et al 2014). It is known that plastic tends to contract or deform when exposed to heat. Additionally (Figure 4), sizes of the MPs were digitally measured using ImageJ software, described by Rochman et al (2015). All confirmed MP particles were then clustered according to each species and were kept in a sealed Petri dish for further analysis. Subsequently, the samples were sent to the Center for Sustainable Polymers at Mindanao State University – Iligan Institute of Technology for FTIR composition analysis.

All statistical analyses were carried out using both MS Excel 2019 and the IBM SPSS v.22 software. The collected data for MPs ingestion were analyzed using the Kruskal-Wallis H Test at a significance level of = 0.05 with post hoc test i.e., Mann-Whitney U Test, to investigate the differences in the amount of ingested MPs and their size between species (Abiñon et al 2020).

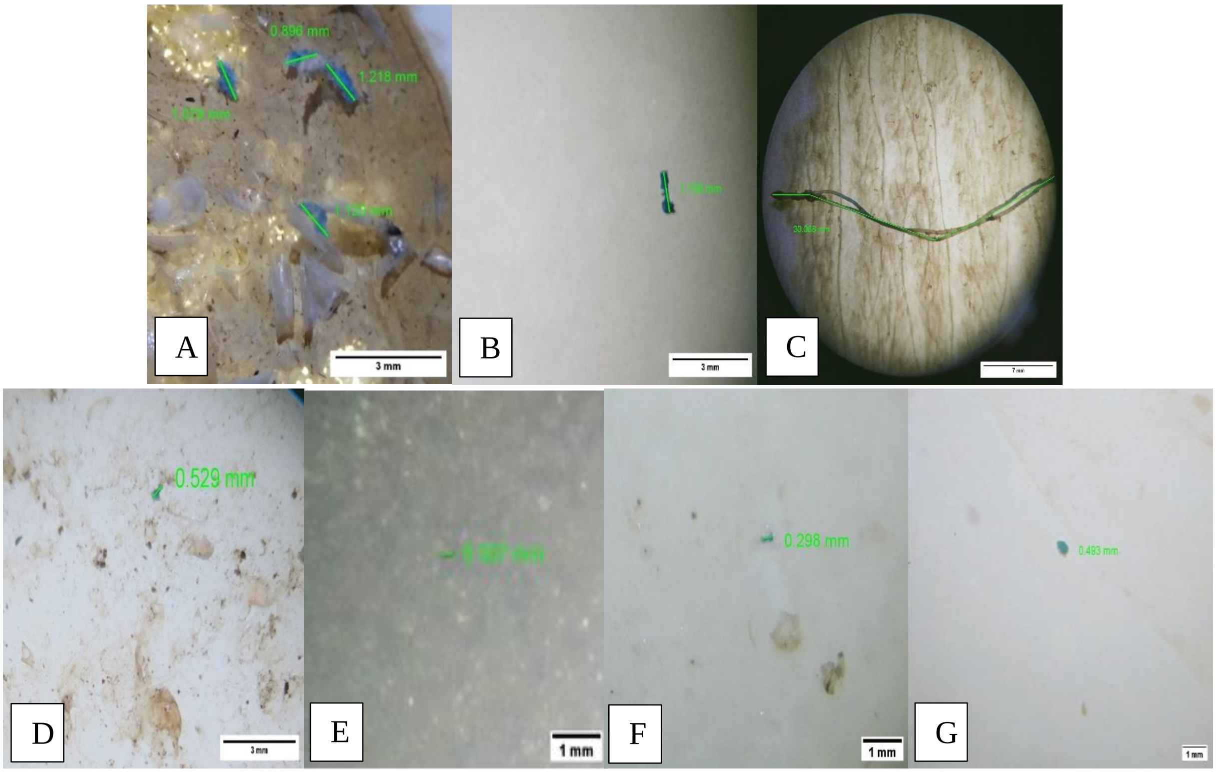

Figure 4. Photo documentation of putative microplastics measured using Image J software in all fish species. A & B) *D. macarellus* samples, C) *E. affinis* sample, and D, E, F & G) *S. crumenophthalmus* samples.

Results and Discussion

Characteristics of the collected fish species. Based on the analysis of morphometric data of the collected species (Table 1), it is observed that *E. affinis* exhibits the largest measurements across various parameters with a snout-vent length range of 14.4 to 16.6 cm, total length of 23.7 to 27 cm, and body weight of 140.92 to 269.82 g. Followed by *D. macarellus* and *S. crumenophthalmus* with snout-vent length range of 9.5 to 13.7 cm and 8.5 to 12 cm, total length of 9.8 to 28.5 cm and 19.5 to 24.5 cm, and body weight of 69.81 to 245.68 g and 86.87 to 177.88 g, respectively.

Table 1

Means of the morphometric data of the collected species

Species name	*Snout-vent length (cm)*	*Total length (cm)*	*Body weight (g)*
D. macarellus	12.04±1.01	25.05±1.87	164.15±36.75
E. affinis	16.00±0.52	25.78±0.77	215.82±28.66
S. crumenophthalmus	9.42±0.62	21.47±1.11	124.72±18.29

Occurrence of microplastics in fish. MP particles were detected in all three fish species examined. Among the 99 samples analyzed, only seven individuals (7.07% of the total) were found to have ingested MP particles in their gastrointestinal tract. Categorizing by species, out of the 33 samples each, two samples (6.06%) were detected from *D. macarellus*, one sample (3.03%) was from *E. affinis*, and four samples (12.12%) were from *S. crumenophthalmus*. It is noteworthy that only one individual had multiple MP particles found in the digestive system of *E. affinis* species. Overall, a total of ten plastic particles were recovered across all fish species examined. However, considering the size limit of the MPs (< 5 mm), only nine particles were identified. One plastic particle exceeded the five mm threshold and was excluded from the particle size analysis and other statistical analysis, although it was still considered in the characterization analysis (Table 2).

Table 2
Summary of the MPs count, mean size, and abundance per individual recovered from sampled species

Species name	*No. of samples*	*MPs count*	*MPs abundance/individual*
D. macarellus	33	5	0.15
E. affinis	33	1	0.03
S. crumenophthalmus	33	4	0.12
Total	99	10	0.10

Out of the 99 fish samples analyzed, only seven individuals were detected to have ingested MP particles, suggesting a lower level of MPs contamination compared to Abiñon et al (2020) conducted in Cebu, Philippines, that 79 out of 81 commercially fish species were found to have MPs in their GI tracts. On the other hand, the results of this study align with the findings of Paler et al (2021) conducted in Central Philippines, where only five *Siganus* spp. were positively identified with MPs in their GI tract. The relatively low abundance of MPs in this study may be attributed to the sample size, which is recommended to be at least 50 individuals per research unit according to Hermsen et al (2018). Despite the small number of samples of MPs, this study is essential to report as it could provide valuable information. Moreover, the chosen commercial fish species exhibit a pelagic feeding behavior i.e. *E. affinis* inhabits offshore areas while *D. macarellus* and *S. crumenophthalmus* inhabit coastal areas (Carpenter & Niem 2001; Froese & Pauly 2024). Furthermore, Froese & Pauly (2024) described the feeding behavior of these commercial fish species. *E. affinis* as opportunistic predators feed on a wide variety of prey items like small fish (anchovies, herrings, sardines, and other schooling fish species), cephalopods and crustaceans when available. *D. macarellus* and *S. crumenophthalmus* depend their food on planktonic organisms (copepods, crustaceans, and small fish) including larvae and eggs of various marine organisms. Hence, MPs that prevalently settle in beach sediments to surface waters and sediment regions (Graca et al 2017; Koelmans et al 2017), thus *D. macarellus* and *S. crumenophthalmus* ingest more MPs compared to *E. affinis*. However, there are some unintentional ingestions of MPs may still occur due to their opportunistic behavior, in the case of *E. affinis*. In situations where plastic particles adsorb chemical cues similar to those associated with the fish's preferred food, there is a higher likelihood that the fish may consume the plastic (Savoca et al 2017).

Characteristics of microplastics collected. Although it is recognized that fish consumption of MPs does not yield accurate data regarding the extent of plastic pollution, it can still provide valuable information about the prevalent types of plastic and their sources (Roch et al 2015). All MP particles collected were classified based on their type, size, color, and composition (Table 3). The fish samples contained two types of MPs, with microfragments comprising the majority (90%) and microfibers being the least prevalent (10%) specifically retrieved in *E. affinis*. The abundance of microfragments suggests that the region may have experienced pollution from larger debris such as synthetic rubbers, plastic bottles, and/or plastic packaging, which have undergone degradation over time due to various environmental factors. The consumption of fragmented MPs, particularly those with irregular and sharp edges, can lead to blockages in the digestive system of fish which can result in issues such as abrasion, damage to the stomach wall, and overall physical deterioration (Wright et al 2013; Jabeen et al 2017). Additionally, the presence of a 30-mm blue polyethylene fiber, evidently derived from a rope, provides evidence of fishing practices contributing to the contamination of MPs in the area (Ribic et al 2010).

Table 3
Summary of the MPs characteristics recovered from different fish species as categorized based on their type, size, color, and composition

Sample	*Type*	*MPs size (mm)*	*Color*	*Composition*
DM 1	Fragment	0.896, 1.072, 1.120, 1.218	Blue	Ethylene/propylene copolymer (ethylene content 60%)
DM 18	Fragment	1.159	Blue	Polypropylene (PP)
EA 11	Fiber	30.068	Blue	Polyethylene (PE)
SC 17	Fragment	0.307	Blue	Ethylene/propylene copolymer (ethylene content 60%)
SC 21	Fragment	0.529	Blue	Poly (vinyl stearate)
SC 23	Fragment	0.298	Blue	Poly (vinyl stearate)
SC 30	Fragment	0.493	Blue	Poly (vinyl stearate)

Note: DM = *D. macarellus*; EA = *E. affinis*; SC = *S. crumenophthalmus*.

All of the recovered MP particles were observed to be blue in color. The color of MP particles discovered matched the findings of Carr et al (2016), Ory et al (2017), Abiñon et al (2020), and Zhang et al (2020). It was observed that blue particles were preferentially also consumed by other commercial fish species like *Chanos chanos*, locally known as milkfish or bangus, *Rastrelliger kanagurta*, locally known as Indian mackerel or alumahan, and *Auxis rochei*, locally known as bullet tuna or bodboron (Abiñon et al 2020). The consistency in color suggests that the color selectivity of MPs could potentially influence their attraction to natural food sources for aquatic organisms (Andrady 2011). Moreover, Ory et al (2017) suggest that copepod preys resemble blue MPs. These results align with the colors of MPs documented in numerous previous studies where the presence of blue-colored MPs was found to be more prevalent (Rochman et al 2015; Zhang et al 2019; Abiñon et al 2020).

In terms of composition, the most abundant MP type was the ethylene-propylene copolymer (50%), followed by poly (vinyl stearate) (30%), polyethylene (10%), and polypropylene (10%). The presence of ethylene-propylene copolymer (EPR) aligns with the findings of Graca et al (2017), which propose a correlation between the ingestion of this specific polymer by marine fish and their distribution in nearshore and benthic areas. It is found that EPR is widely present in beach sediments and marine bottom areas, increasing the likelihood of ingestion by fish species that feed in these areas. This association can be attributed to the low-density property of EPR, which is commonly utilized in plastic packaging, automotive components, and construction materials due to its resistance to heat, flexibility, and weatherability. On the other hand, poly (vinyl stearate) is utilized in various industries such as plastics, coatings, adhesives, and cosmetics as a lubricant or anti-blocking agent. Similarly, polypropylene is known for its versatility in packaging, automotive parts, and electrical appliances while polyethylene is widely used in packaging, bottles, and containers. However, the polyethylene identified in this study originated from a rope likely used in fishing practices. This suggests that fishing practices, maritime transport, and tourist activities are potential sources of MP contamination in the region.

On average, the MP particles that were detected had a total size of 0.789±0.379 mm, ranging from the smallest size of 0.298 mm to the largest size of 1.218 mm. When looking at specific species, *D. macarellus* had an average size of 1.094±0.12 mm, while *S. crumenophthalmus* had an average size of 0.407±0.12 mm. Among fish species, the most common size range for microplastic particles was between 0.789 and 1.168 mm, while the least common size range was between 1.168 and 1.547 mm. These findings coincide with the studies of Graca et al (2017), Abiñon et al (2020) and Ghosh et al (2021), where most MPs identified are within the size range of 0.10-2.0 mm (Figure 5).

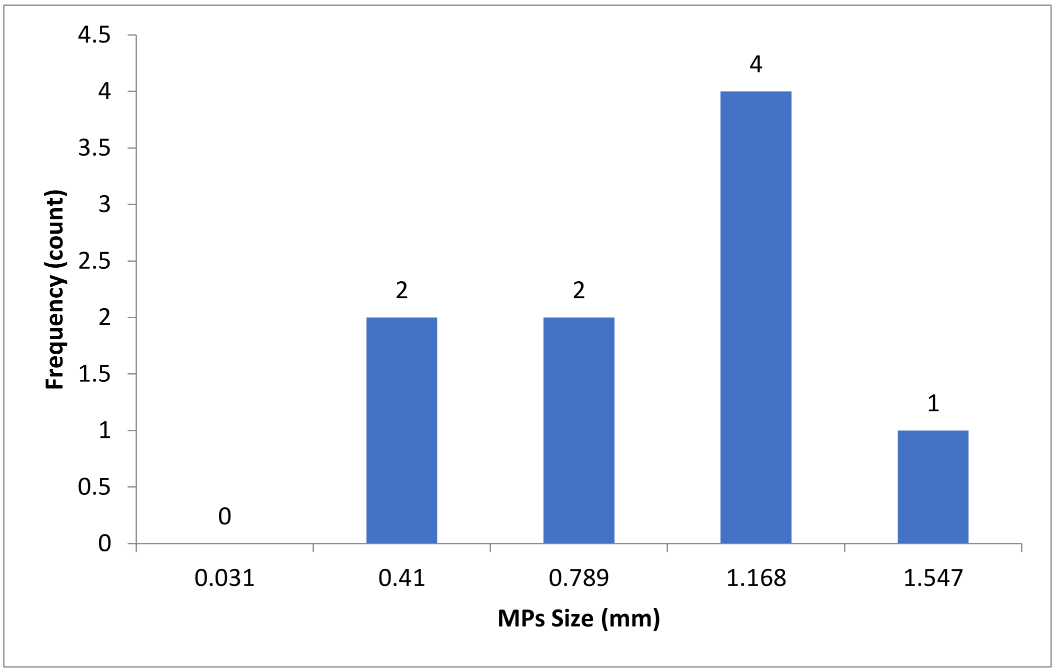

Figure 5. Histogram of the frequency of microplastic size (mm).

Correlational analysis of MPs. The limited size of the dataset prevented the establishment of a correlation between the frequency of MP occurrence and the morphometric data. However, a non-parametric statistical analyses was used (Table 4). Kruskal-Wallis H Test at a significance level of $p = 0.05$ with post hoc Mann-Whitney U Test revealed no statistical difference in MPs ingestion count between *D. macarellus* and *S. crumenophthalmus.* Kruskal-Wallis H Test in MPs ingestion count between *D. macarellus* and *S. crumenophthalmus* revealed a p-value at 0.157 while Mann-Whitney U Test also revealed a p-value at 1.00, therefore we accept the null hypothesis where the distribution MPs count is the same across species at $p > 0.05$.

Table 4

Summary of statistical analyses between *D. macarellus* and *S. crumenophthalmus*

Test samples	*Kruskal-Wallis H test*			*Mann-Whitney U test*	
	Test statistic	*Asymptotic significance*	*Decision*	*Asymptotic significance*	*Decision*
Microplastic count	2.000[a,b]	0.157	Retain the null hypothesis	1.00[1]	Retain the null hypothesis
Microplastic size	6.000[a,b]	0.014	Reject the null hypothesis	0.16[1]	Reject the null hypothesis

Note: [a] The test statistic is adjusted for ties; [b] Multiple comparisons are not performed because there are less than three test fields; [1] Exact significance is displayed for this test.

On the other hand, the microplastic particle size shows a statistically significant difference in both *D. macarellus* and *S. crumenophthalmus*. Kruskal-Wallis H Test in MPs size between *D. macarellus* and *S. crumenophthalmus* revealed a p-value at 0.014 while Mann-Whitney U Test revealed a p-value at 0.016, therefore we reject the null hypothesis where the distribution MPs size is the same across species at $p > 0.05$.

The findings indicate that fish species examined were similarly susceptible to contamination by MP particles. Specifically, *D. macarellus* showed the highest abundance at 0.15 particles per individual, followed by *S. crumenophthalmus* at 0.12 particles per individual, and *E. affinis* at 0.03 particles per individual. On the other hand, the significant difference between MP size indicates that *D. macarellus* were likely to ingest larger debris compared to *S. crumenophthalmus.* The difference in MP size ingestion between *D. macarellus* and *S. crumenophthalmus* may be attributed to their contrasting body sizes, with *D. macarellus* having a larger mouth size and morphology, which allows it to consume larger particles compared to *S. crumenophthalmus*. Feeding behavior and potential size selectivity may also play a role in the disparity in MP size ingestion. However, further research is necessary to comprehensively comprehend and quantify the impact of these factors on MP ingestion in these fish species.

Conclusions. MP ingestion by commercially sold fish species is a significant concern as it provides a potential pathway for the transfer of MPs to human systems. The results in this study provide evidence of MPs contamination in sold fishes in General Santos City Fish Port Complex, the second largest fish port in the Philippines. Although the ingestion of MPs was observed in only a small fraction of the fish samples, it is essential not to underestimate the significance of the risks posed to human health and the environment. These findings are consistent with previous research, suggesting that the abundance of MPs may be influenced by the feeding behavior of the fish species and the characteristics of the plastic particles. This study contributes to our knowledge of MP pollution in the region under investigation and emphasizes the necessity for further research and measures to mitigate the contamination of plastics.

Consumption of fish without removing the GI tract poses a greater risk of human exposure to MPs, which is likely to occur with smaller fish species. It is highly advised to conduct a comprehensive evaluation of commonly purchased fish species, particularly smaller ones, to understand the potential pathway of MP contamination to humans. Moreover, obtaining a larger sample size and conducting a thorough assessment of MPs in other tissues of fish species would yield more reliable data on MP contamination in fish available in markets. Lastly, further research is needed to investigate the transfer of MPs through the food chain in marine ecosystems, tracking how MPs can move to higher trophic levels.

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Conflict of interest. The authors declare that there is no conflict of interest.

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