

Presence of microplastic among commercially important Pelagic fish in Zamboanga Peninsula and Agusan del Norte, Philippines

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Abstract. Microplastic pollution has been an ongoing problem over the years. The ingestion of microplastics by a diverse range of marine organisms such as fish, mammals, and invertebrates had posed serious physical consequences, including increased mortality. This study documents the microplastic ingestion of pelagic fish from Mindanao waters. Three hundred seventy-seven fish individuals representing 15 pelagic species were collected from the fish landing sites of Dipolog, Pagadian, and Buenavista. Our results showed that 88% of the total fish examined had microplastics, of which 55% were fibers and 45% were fragments. Its sizes range between 0.066 mm to 3.3 mm, while 9 different colors were noted, with black being the most dominant indicating that fishing activities were prevalent in these areas. The abundance of ingested microplastic items was not significantly correlated with fish size or weight, suggesting that ingestion may be due to accidental swallowing. FT-IR analysis identified five plastic polymers, namely, rayon, polyamide nylon, polypropylene, polyethylene, and polyester. The findings of the study highlighted the possible danger of microplastic contamination in fish which can pose a threat to human health and the marine ecosystem.

Key Words: fiber, fragment, FT-IR, marine pollution, pelagic fish ecology, environmental monitoring.

Introduction. Plastics are among the chief sources of ocean pollution worldwide. Dangers posed by these organic materials may include entanglement in and ingestion of plastics to most marine organisms that could either be damaging or fatal to the organisms (Laist 1997; Denuncio et al 2011; Lazar & Gracan 2011; van Franeker et al 2011; Abreo & Macusi 2016; Abiñon et al 2020; Karuppasamy et al 2020; Lopes et al 2020; Sathish et al 2020; Suwartiningsih et al 2020; Paler et al 2021; Kalaiselvan et al 2022; Yona et al 2022; Harikrishnan et al 2023; Prusty et al 2023; Agao-Agao et al 2024; Nurhasanah et al 2024; Riaz et al 2024). It has been reported that countries like China, Indonesia, the Philippines, Thailand, and Vietnam discard more plastics, with the Philippines providing 0.28-0.75 million metric tons of these pollutants to the sea per year (Jambeck et al 2015). Generally, plastics may occur as primary and secondary microplastics. Primary microplastics are particles that are most common in personal care commodities, microfibers from apparel, and also from the washing of synthetic cloths or fabrics. Primary microplastics are directly introduced into the marine ecosystem via rivers, sewage discharges, and land run-off (Andrady 2011). Secondary microplastics, on the other hand, are derived from the breaking down of waste plastic objects (i.e. plastic bags, fishing nets) by the weathering process (Gregory & Andrady 2003). Once in the oceans, microplastics can either float or sink and become bioavailable to a wide range of marine organisms, including invertebrates, fish, marine mammals, and birds (Cole et al 2013; Murphy et al 2017; Nelms et al 2019; Adika et al 2020; Amin et al 2020).

In Southeast Asia, pelagic fish are of particular interest since they are the most commonly eaten variety of fish in the region. Small and semi-pelagic species are also economically important globally and account for the majority of biomass, particularly in upwelling areas (FAO 2016). A large number of fish species used for human consumption have been observed to ingest microplastics in the Pacific, Atlantic, and Indian oceans, as well as the Mediterranean Sea although low concentrations of microplastics have been found per individual (Lusher 2015).

In the Philippines, evidence of microplastics in the gastrointestinal tracts (GITs) of pelagic and demersal fishes from various locations in the country was reported (Espiritu et al 2019; Paler et al 2019; Abiñon et al 2020; Bucol et al 2020; Palermo et al 2020; Cabansag et al 2021; Agao-Agao et al 2024). However, no studies to date have reported the presence of microplastics in the GITs of pelagic fish from Mindanao waters, specifically the Zamboanga Peninsula and Agusan del Norte. Zamboanga Peninsula and Agusan del Norte, were of particular interest as these areas are known to be highly productive during the Northeast Monsoon (Locally known as Amihan). Zamboanga Peninsula is located in the western part of Mindanao where the axis of monsoon winds runs parallel to its coast, producing an upwelling mechanism driven by strong winds and cooler temperatures during the Northeast Monsoon (Villanoy et al 2011). This mechanism brings cold, nutrient-rich water to the surface, enabling plankton bloom, and thereby providing food for planktivorous fish such as sardines and other pelagic fish. On the other hand, Butuan Bay's primary production is driven by the nutrients coming from the Agusan River and possible double estuarine circulation from the Bohol Sea (Cabrera et al 2011). Both mechanisms result in increased fishery production in these 2 areas. Hence, this study was conducted to investigate the presence, characteristics, and implications of microplastic ingestion by commercially important pelagic fish in Mindanao. The findings are expected to highlight the potential dangers of microplastic pollution in contaminating fish, posing risks to human health and the environment.

Material and Method

Description of the sampling areas. Within the Zamboanga Peninsula, fish collections were done in two (2) sites, namely, at Dipolog City and Pagadian City. Dipolog City, which is the capital of Zamboanga del Norte has a total land area of 7,301 km², making it the largest province of the Zamboanga Peninsula region. It has a 400 km coastline that runs from the northern to the southern part of the province. On the other hand, Pagadian City, which is the capital of Zamboanga del Sur occupies the southern section of Zamboanga Peninsula and consists of four (4) bays such as Sibuguey, Dumanguilas, Maligay, and Pagadian. Within Agusan del Norte, fish collections were done at Buenavista, a fishing coastal municipality. It is bounded on the north by Butuan Bay, south by Las Nieves, west by Nasipit, and east by Butuan.

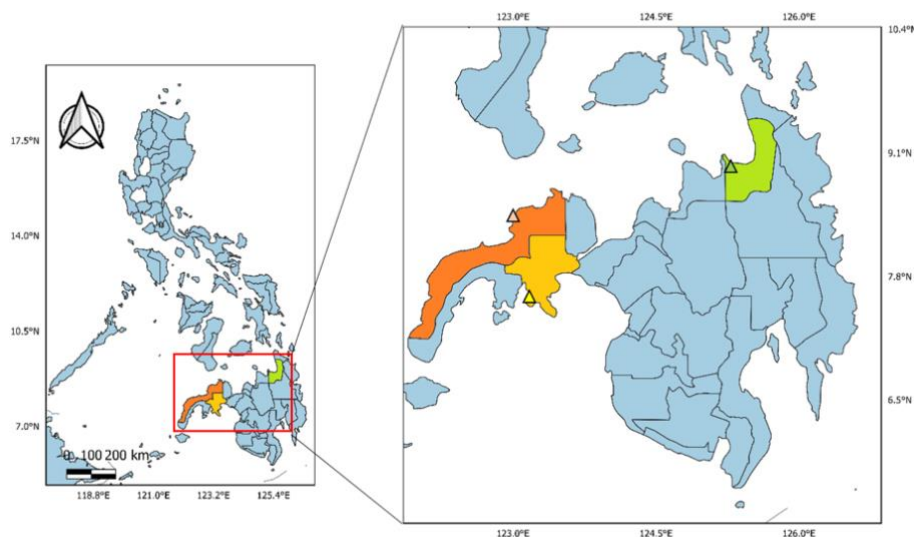


Figure 1. Map of the location sites showing the fish ports of Dipolog, Zamboanga del Norte (orange), Pagadian, Zamboanga del Sur (yellow), and Buenavista, Agusan del Norte (green)

Biota sampling. Fish samples were collected from the fish landing sites in the cities of Dipolog and Pagadian, Zamboanga Peninsula, and in Buenavista, Agusan del Norte during the northeast monsoon period that occurred in the country between January and February 2021. The collected fish samples were placed inside Ziplock bags, properly labeled with the date and location of collection, and then stored in a cooler during transport. Upon arrival in the laboratory, the samples were immediately stored in the freezer until dissection to prevent possible contamination. A total of 377 fish samples belonging to 15 species of pelagic fish were collected.

Contamination control. To prevent contamination, a cotton laboratory coat and sterile latex gloves were worn throughout the whole process. The glassware and dissecting instruments were washed with dishwashing liquid, rinsed with distilled water, and then ethanol. The work surface was pre-cleaned with 70% ethanol before dissection to ensure a sterile environment. All fish were washed before dissection and all dissections were carried out inside the improvised biological safety cabinet to prevent potential contamination with airborne microplastics. Furthermore, two procedural blanks (i.e. petri dish with Whatman No. 4, without gut tissues) were placed together with the samples and then checked for possible airborne microplastic contamination.

Fish identification and processing for microplastic determination. Before dissection, all fish samples were photo-documented, and total body length was recorded. Species were identified using the FishBase data (Froese & Pauly 2019). Following the methods described by Güven et al (2017) with slight modification, all individual fish samples were processed for the presence of microplastic. The fish were placed in a metal tray and a longitudinal incision in the abdominal area was made to remove the gastrointestinal tract of the specimen (esophagus to anus). The stomach and intestines were cut longitudinally using a scalpel and then placed in a sterile petri dish to avoid contamination. The GI tract contents were treated with 35% hydrogen peroxide (H_2O_2) for chemical digestion. The samples were digested at $50^\circ C$ for a minimum of 24 hours until all the contents were digested. After digestion, the samples were filtered using Whatman No. 4 (pore size: 20-25 μm) and visually inspected under a dissecting microscope (EL224 Exacta Optech) for the presence of plastic particles. Plastic fragments that were found were mounted on a glass slide and photographed and

visually analyzed under 40x and 100x magnification using a compound microscope (Model 10 Boeco). Plastic particle colors were recorded and sizes (μm) were measured using the stage micrometer.

Polymer identification using Fourier Transform-Infrared Spectroscopy (FTIR). To determine the type of plastic polymer, microplastic obtained from the GI tract of the fish samples was further analyzed by using Attenuated Total Reflectance (ATR) Fourier Transform-Infrared Spectroscopy with a Perkin Elmer Spectrum Two FT-IR Spectrometer. Spectra were collected in absorbance mode in the range of $4,000\text{-}450\text{ cm}^{-1}$. The initial finding revealed that only microplastics obtained from the 4 fish species (*Selaroides leptolepis* (Cuvier, 1833), *Selar crumenophthalmus* (Bloch, 1793), *Hemiramphus far* (Forsskål, 1775), and *Auxis thazard* (Lacepède, 1800)) can be read or detected using the FT-IR, while the rest of the microplastics coming from the 9 fish species were undetectable. In this case, the determination of the type of plastic polymer was limited to the microplastics collected from the 4 fish species. Hence, a total of 4 vials (one vial representing each fish species), which contain between 2-5 microplastics (mainly fragments), were used for the analysis. Fibers were too small to be pressed by the diamond. The FTIR analysis was performed by a professional technical representative at the Material Science and Polymer Chemistry Laboratory of Caraga State University. No spectral library or database was used in this study. All polymer characterization was based on Hummel (2002).

Statistical analyses. One-way analysis of variance (ANOVA) was used in testing the differences in microplastic numbers among the three sampling stations and Pearson's correlation analysis was performed to determine if there is a relationship between fish length and microplastic numbers, fish weight and microplastic numbers, fish length and microplastic length, and fish weight and microplastic length. A significance level of 0.05 was used for ANOVA and 0.01 for Pearson correlation. All statistical analyses were done using Statistical Package for the Social Sciences (SPSS) software version 25.

Results

Presence of microplastic in fish. A total of 377 fish individuals were inspected for the presence of microplastic (Table 1). Out of these 377 fish, 333 individuals (or 88% of the total fish examined) had microplastics, with a total of 92 microplastic particles removed and recorded from their gastrointestinal tracts. Among these fish species examined, the top three species that showed the highest occurrence of microplastic in their digestive tract were *S. leptolepis* (26.09%), followed in decreasing order by *S. crumenophthalmus* (21.74%) and *H. far* (11.96%). Conversely, plastic particles were not extracted from the GITs of *Thunnus albacares* (Bonnaterre, 1788), *Pterocaesio pisang* (Bleeker, 1853), and *Euthynnus affinis* (Cantor, 1849).

Table 1

Fish collected, sample size, average fish length and weight, and ingested microplastic particles

Species	Fish samples	Ave length (cm)	Ave weight (g)	Microplastic (MP)		
				Fibers	Fragments	Total MP
<i>Thunnus albacares</i> (Bonnaterre, 1788)	2	47.50	1430	0	0	0
<i>Hemiramphus far</i> (Forsskål, 1775)	31	28.08	65.48	5	6	11
<i>Sphyraena jello</i> Cuvier, 1829	30	32.28	168	3	0	3
<i>Pterocaesio pisang</i> (Bleeker, 1853)	30	14.78	37.60	0	0	0
<i>Katsuwonus pelamis</i> (Linnaeus, 1758)	22	27.61	395.47	2	2	4
<i>Decapterus kurroides</i> Bleeker, 1855	20	13.63	21.68	1	0	1
<i>Amblygaster leiogaster</i> (Valenciennes, 1847)	6	17.17	47.13	1	0	1
<i>Sardinella lemuru</i> Bleeker, 1853	71	12.41	16.74	9	0	9
<i>Selar crumenophthalmus</i> (Bloch, 1793)	29	15.79	44.52	14	6	20
<i>Decapterus macrosoma</i> Bleeker, 1851	7	13.71	23.90	5	0	5
<i>Auxis thazard</i> (Lacepède, 1800)	20	22.05	128.59	3	1	4
<i>Euthynnus affinis</i> (Cantor, 1849)	12	26.50	271.65	0	0	0
<i>Cheilopogon unicolor</i> (Valenciennes, 1847)	31	22.19	93.39	2	7	9
<i>Hemiramphus lutkei</i> Valenciennes, 1847	16	24.62	43.56	0	1	1
<i>Selaroides leptolepis</i> (Cuvier, 1833)	50	18.89	78.12	6	18	24
TOTAL	377	47.50	1,430	51	41	92
			55%	45%		

Microplastic type and color. In the present study, there were 2 types of microplastic extracted from the GIT of pelagic fish, namely, microfibrers and micro fragments (Figure 2). Among these 2 types of microplastic particles, fibers (55%) were commonly found in all fish analyzed compared to fragments (45%). The majority of the fish species inspected contained more fibers extracted from their GITs (Figure 3), except for the species *K. pelamis*, *C. unicolor*, *H. lutkei*, and *S. leptolepis* where more fragments were observed. Only *H. far* showed an equal proportion of fibers and fragments found in their digestive tract.

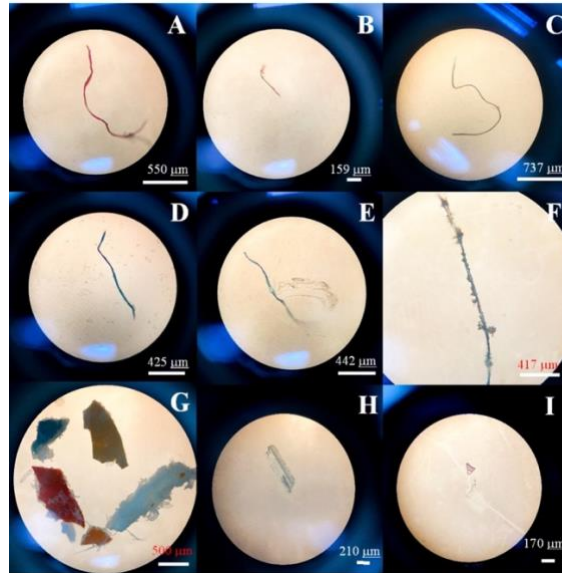


Figure 2. Microplastics were found in the gut contents of some commercial pelagic fish under 40x and 100x magnification. (a,b=red fiber; c=black fiber; d-f=blue fiber; g1= blue fragment; g2=orange fragment; g3=yellow fragment; g4=white fragment; h=green fragment; i=red fragment).

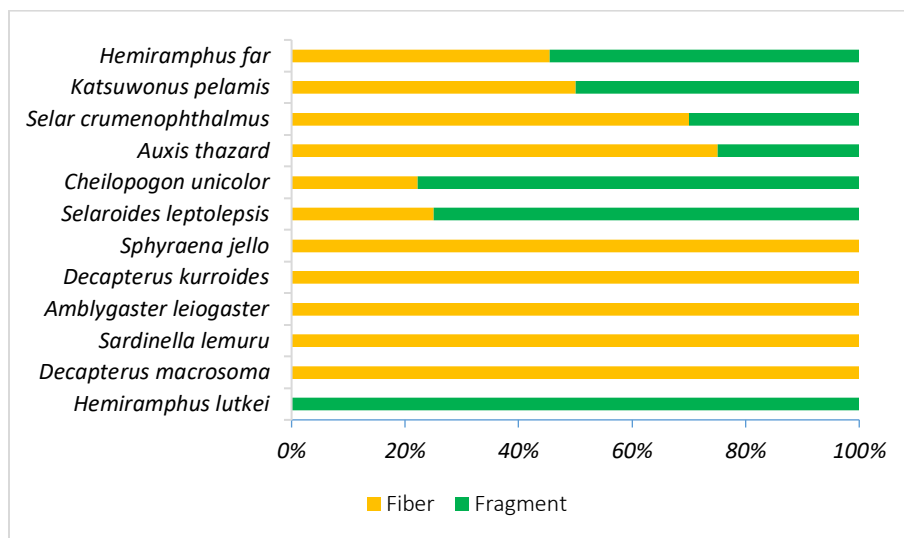


Figure 3. Microfiber and micro fragments were ingested by 12 pelagic fish from the three sampling areas.

In terms of color distribution, a variety of hues were observed for microplastic (Figure 4), with black as the dominant shade in fibers (Figure 4A) and orange in fragments (Figure 4B). This is followed by green and blue, while other colors, such as red, yellow, and white were less common. The dominant black coloration in microfibers observed in our study aligns with previous works, which also reported the prevalence of black fibers in the digestive tracts of fish (Lusher et al 2013; Bellas et al 2016; Murphy et al 2017; Bottarri et al 2019; Suwartiningsih et al 2020). Ory et al. (2017; 2018) suggested that the microplastic color can resemble the prey of the fish, which is the case for planktivorous fish, hence leading to ingestion by these marine organisms. Conversely, Boerger et al. (2010) reported that pelagic fish in the North Pacific Central Gyre do not have their microplastic ingestion influenced by the colors of the plastic. Additionally, odors emitted by plastics may encourage marine fish to consume plastic particles (Savoca et al. 2019). In this study, the predominant

black color in microfibers might have come from black fishing nets, which are commonly used in these areas, indicating that fishing practices could play a role in the microplastic contamination of the waters.

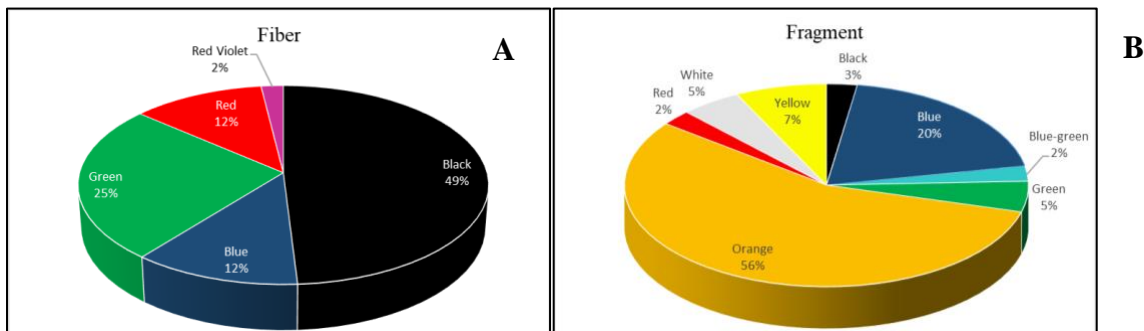


Figure 4. Pie chart showing the different colors of (A) microfiber and (B) micro-fragment and their relative frequency.

Microplastic length/size. The length of the extracted particles from pelagic fish ranged between 0.06-3.33 mm (60-3330 μm) for fibers (Figure 5) and between 0.13-3.17 mm (130-3170 μm) for fragments with a size of <0.5 mm being the most dominant in both microplastics.

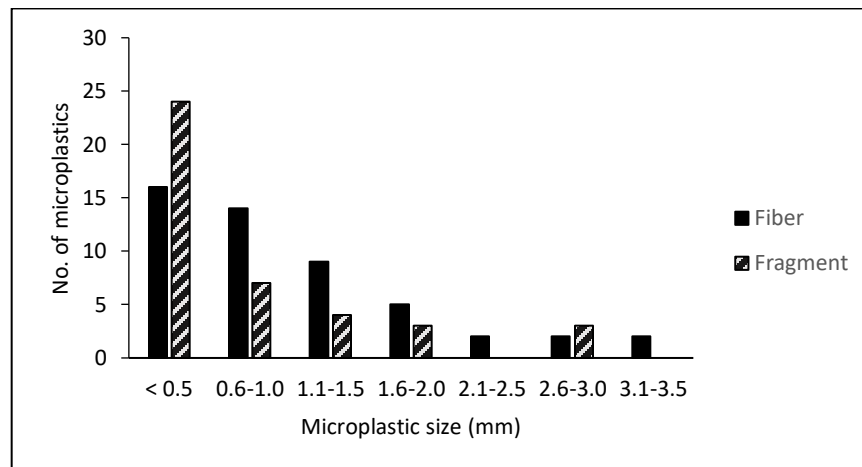


Figure 5. Number of microplastics according to their size interval.

Statistical analysis of microplastics. The difference in the number of fish samples among fish species per sampling site used in the current study may prevented the formulation of a significant correlation between fish length/size and amount of microplastic ingested. The abundance of microplastic found between the upwelling sites of Zamboanga peninsula and nutrients from the Agusan River in Butuan revealed no significant difference based on the result gathered from one-way analysis of variance ($p=0.759>0.05$) despite having more microplastic found in Pagadian ($n=39$) than in Buenavista ($n=37$) and Diplolog ($n=16$). Pearson correlation revealed no relationship between either the fish length ($r=-0.235$, $p=0.418$) and fish weight ($r=-0.21$, $p=0.470$) and the amount of ingested microplastic particles. Moreover, there was no correlation between the fish length ($r=-0.362$, $p=0.203$) and fish weight ($r=-0.77$, $p=0.794$) and the length of ingested microplastic.

Identification of microplastic polymer using FTIR. For microplastic polymer classification in this study, micro-fragments (a total of 31) extracted from the 4 fish species, namely *S. leptolepis*, *S. crumenophthalmus*, *H. far*, and *A. thazard* were used for the analysis. The results revealed rayon, polyamide nylon, polyester, polypropylene, and polyethylene as polymer compositions of microplastics (Table 2). All 4 fish species shared peaks for the polymers rayon and polyamide nylon, while 3 fish species shared peaks for polyesters. However, polypropylene was unique only for *S. leptolepis*, while polyethylene was for *S. crumenophthalmus*.

Table 2

Characteristic bands of selected microplastic particles representing the polymer type collected from 4 species of pelagic fish

<i>Species</i>	<i>Characteristic band (cm⁻¹)</i>	<i>Polymer Type</i>
<i>Selaroides leptolepis</i> (Cuvier, 1833)	3,272; 2,921; 1,150; 1,000	Rayon
	1,627	Polyamide nylon
	1,371	Polypropylene
<i>Selar crumenophthalmus</i> (Bloch, 1793)	3,277; 1,115; 1,066	Rayon
	2,851	Polyethylene
	1,727	Polyester
	1,576; 1,465; 1,256	Polyamide nylon
<i>Hemiramphus far</i> (Forsskål, 1775)	3,265; 1,036	Rayon
	2,850; 1,466	Polyester
	1,540	Polyamide nylon
<i>Auxis thazard</i> (Lacepède, 1800)	1,120; 1,068	Rayon
	2,924	Polyamide nylon
	1,259	Polyester

Discussion. The present findings provide evidence of microplastic ingestion by commercially important pelagic fish from selected areas in the Zamboanga Peninsula and Agusan del Norte. This highlights the vulnerability of these fish to the growing microplastic pollution affecting both global oceans and Philippine waters. Studies on the consumption of plastic particles by marine fish (Lusher et al 2013, 2015; Neves et al 2015; Rummel et al 2016; Nelms et al 2019; Bottari et al 2019; Abiñon et al 2020; Adika et al 2020; Bakir et al 2020; Maaghloud et al 2020; Neto et al 2020; Sathish et al 2020; Zakeri et al 2020; Koongolla et al 2022; Piskula & Astel 2023; Nurhasanah et al 2024; Riaz et al 2024) are widely growing, an indication of the seriousness of the impact posed by microplastic pollution.

The majority of the microplastics extracted from the pelagic fish in our study were microfibers. This agrees with the earlier findings of Lusher et al (2013), Neves et al (2015), Güven et al (2017), Bessa et al (2018), Bottari et al (2019), Hastuti et al (2019), Abiñon et al (2020), and Bakir et al (2020) who reported fibrous microplastics as more abundant in the marine environment compared to fragments. Possible sources of microfibers may due to synthetic fibers discharged from washing clothes (Browne et al 2011), or cellulose acetate fiber as a result of the breaking down of cigarette filters (Wright et al 2015), and degradation of fishing items namely, rope and net (Cole 2016). Probably, the abundance of microfibers in the regions of Zamboanga Peninsula and Agusan del Norte may be attributed to the long-term degradation of fishing nets.

The dominant black coloration in microfibers observed in our study aligns with previous works, which also reported the prevalence of black fibers in the digestive tracts of fish (Lusher et al 2013; Bellas et al 2016; Murphy et al 2017; Bottarri et al 2019; Suwartiningsih et al 2020). Ory et al (2017 2018) suggested that the microplastic color can resemble as prey of the fish, which is the case for planktivorous fish, hence leading to

ingestion by these marine organisms. Conversely, Boerger et al (2010) reported that microplastic ingestion by pelagic fish in the North Pacific Central Gyre is not influenced by plastic colors. Moreover, odors produced by plastics may induce marine fish to consume plastic particles (Savoca et al 2019). In the present study, the dominant black coloration in microfibers may have originated from black fishing nets which were widely used in these regions suggesting that fishing practices may contribute to microplastic contamination of the waters.

The microplastic sizes recorded in the GITs of pelagic fish in the current study were smaller (0.06-3.33 mm) compared to the large microplastic sizes like 0.5-11.70 mm in North Atlantic (Lusher et al 2016), 0.10-4.90 mm in Hongkong (Cheung et al 2018), 1-5 mm in the coast of Portugal (Bessa et al 2018). Small microplastic particle sizes may not block the GITs of pelagic fish (Neves et al 2015) because these can be eliminated together with their feces. For large-sized particles, these microplastics will likely remain in the digestive tract triggering the animals to have a feeling of being full which may lead to malnutrition and eventually a decrease in the fish population (Boerger et al 2010).

Our findings revealed no significant correlation between fish length/weight and ingested microplastic items. This is in line with previous works which demonstrated no correlation (Foekema et al 2013; Guven et al 2017; Hastuti et al 2019; Palermo et al 2020) between either the fish length ($r=-0.235$, $p=0.418$) and fish weight ($r=-0.21$, $p=0.470$) and the amount of ingested microplastic particles. In contrast, some authors reported that the number of microplastic was positively correlated with the weight of the digestive tract and fish length (Suwartiningsih et al 2020; Cheung et al 2018; Peters et al 2016) and fish weight (Alshawafi et al 2018) while Boerger et al (2010) reported that larger fishes have a higher number of plastics found in their guts compared to smaller fishes. It is evident in the present work and those previously mentioned studies that biological factors such as fish length and weight are not enough to explain in determining the amount of microplastic ingested by fish, but ingestion may be due to accidental swallowing or ingestion of microplastic particles that are incorporated in their food source or prey (Foekema et al 2013).

The main polymers identified were rayon, polyamide nylon, polyester, polypropylene, and polyethylene. Rayon is found in clothes/fabrics, furnishings, and sanitary products that enter into the sea/coastal areas using wastewater treatment (Browne et al 2011; Lusher et al 2013). Polyamide nylon (PA) is widely used in textile/clothing fibers and fishing items (Challa 1993; Mondal et al 2019) and is the most frequently used material in fishing nets and gears in the Philippines (Green et al 2004). Polyester (PET) is utilized to manufacture fishery and textile/fabric fibers, films, and wrapping items. Polyethylene (PE) can be found in bottles, sachets, toys, and housewares, while polypropylene (PP) comes from automotive manufacturing and packaging forms. The results may indicate that fishing activities, maritime equipment, and plastic trash are potential sources of microplastic contamination in the areas of Zamboanga Peninsula and Agusan del Norte.

Conclusion. The ingestion of microplastics by commercially important pelagic fishes poses a vital concern from the ecological and economic point of view as it endangers the health of the marine environment, food security, and financial stability. In this study, the findings suggest that some fishes caught in Zamboanga Peninsula and Agusan del Norte were contaminated with microplastics. The susceptibility of marine fishes to the ever-growing presence of plastic wastes in the seas must be addressed to safeguard marine life and its ecosystem. With this information, the study proposes joint actions between industries, fisherfolks, local government units, and the academe to develop practices that may reduce microplastic pollution and effective plastic waste management within the marine system. It is also recommended that future research relating to microplastics in sediments and demersal fish in other regions with strong upwellings be investigated. Lastly, the findings may provide an opportunity to promote public awareness campaigns and form policymaking that addresses microplastic pollution.

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

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