

## Exploring Microplastic Contamination in Wetland Mollusks: Species-specific Findings from a Major Wetland Habitat

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Received: June 26, 2024; Revised: September 7, 2024; Accepted: September 26, 2024

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### Abstract

Microplastic (MP) pollution in aquatic ecosystems is an emerging global environmental concern, yet its impact on wetland mollusks remains inadequately understood. This study aimed to assess and quantify MPs in three economically significant edible mollusk species *Bellamya angularis*, *Pila ampullacea*, and *Cristaria plicata* sourced from Panlabuhan and Tugno lakes, situated within the expansive Agusan Marsh, a major wetland ecosystem in the Philippines. Soft tissue samples were subjected to KOH digestion followed by Fourier Transform Infrared Spectroscopy analysis to characterize MPs. Eight polymer types were identified within the mollusk tissues, with polyethylene terephthalate (PET) being the most prevalent. PET fibers were identified as the primary MP source across the study sites. Morphological analysis revealed that fibers predominated over filaments and fragments, with black and blue-colored MPs being the most common. Quantitative analysis showed that MP levels varied between species, with *B. angularis* having higher concentrations per gram of tissue compared to *P. ampullacea* and *C. plicata*. Tugno Lake had higher MP levels than Panlabuhan Lake. The feeding habits of mollusk species likely influence MP ingestion, with predation and omnivory playing a potential role. This research discussed the broader implications of MP contamination in mollusks, with a focus on the potential human health risks associated with consuming MP-contaminated mollusks.

**Keywords:** Gastropods; Bivalves; Agusan Marsh; Microplastics

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## 1. Introduction

Microplastics (MPs), defined as plastic particles smaller than 5 mm, are ubiquitous in aquatic environments due to their chemically stable properties, which allow them to persist and accumulate in both the water column and sediments for long periods (Wang *et al.*, 2021). MPs are known to absorb additional pollutants and can be ingested by various organisms, including zooplankton and benthic species, leading to toxic effects and their spread through the food chain (Jin-Feng *et al.*, 2018). Their small size, widespread use, and improper disposal have made MPs one of the most pervasive forms of pollution globally (Baroja *et al.*, 2021), raising concerns about their bioavailability, retention time, and impact on aquatic organisms (Sendra *et al.*, 2021).

Mollusks, particularly bivalves and univalves, have received significant attention in MP research due to their susceptibility to ingesting MPs. Bivalves, as filter feeders, are among the most affected organisms, while univalves are exposed to MPs through their feeding behaviors and diet (Akindele *et al.*, 2019). Both groups of mollusks play essential roles in ecosystem functions such as nutrient cycling, carbon sequestration, and water filtration, making them critical bioindicators for assessing environmental pollution (Baroja *et al.*, 2021; Oehlmann and Schulte-Oehlmann, 2003). Their widespread distribution, stationary behavior, and ability to filter large volumes of water make them valuable indicators of environmental contamination, particularly in freshwater ecosystems (Sendra *et al.*, 2021).

Wetland ecosystems, characterized by slow-moving or stagnant waters, are particularly vulnerable to MP contamination due to their function as natural catch basins for pollutants from surrounding human activities, including agriculture, urbanization, and industrial operations (Hamidian and Dalvand, 2023). These ecosystems support diverse aquatic species and serve as important sources of food and livelihood for local communities. However, the accumulation of MPs and their potential to bioaccumulate in edible species pose serious risks to both ecosystem health and human populations that rely on these resources (Su *et al.*, 2022).

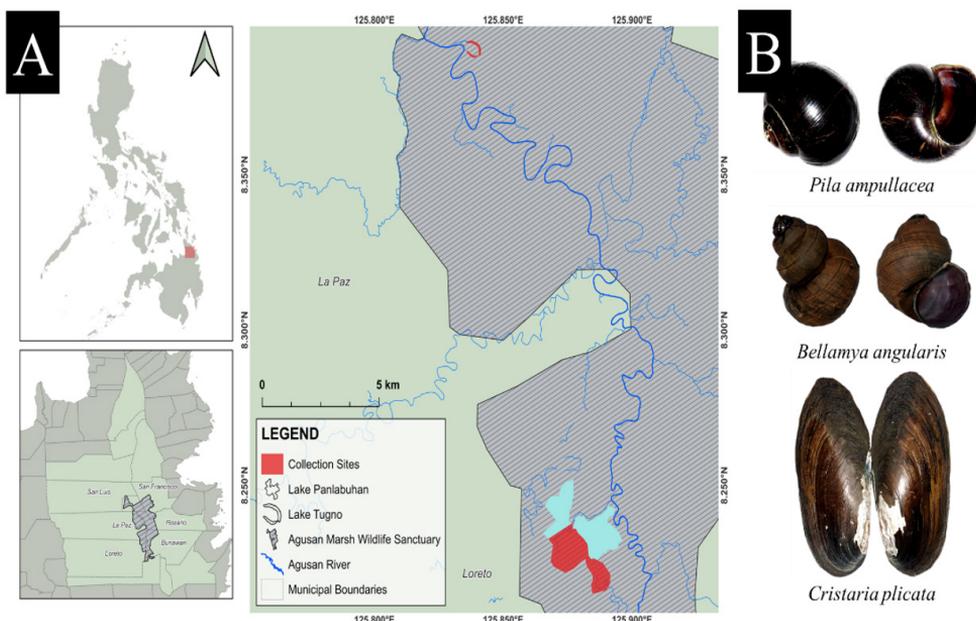
The Agusan Marsh, the largest wetland ecosystem in the Philippines, is a protected area and a catch basin for mining drains, agricultural pollutants, and plastics from neighboring regions (Jumawan and Seronay, 2017). Given that the marsh serves as a drinking water source for many residents and fishing is the primary source of income and food, there is concern about the potential bioaccumulation of harmful particles and components in these resources (Papellero *et al.*, 2019). Communities currently use Lake Panlabuhan and Lake Tugno in the Agusan Marsh for both invertebrate and vertebrate fishery resources.

In this study, three economically important mollusk species *Bellamya angularis*, *Pila ampullacea*, and *Cristaria plicata* were selected as bioindicators to assess MP contamination in a representative wetland ecosystem. These species are commonly consumed and traded, making them relevant for understanding the potential health risks associated with MP exposure. By quantifying MP abundance and characterizing the types of MPs found in these mollusks, this study provides critical insights into MP pollution in wetland ecosystems, contributing to the broader understanding of how MPs impact aquatic food webs and human health.

## 2. Methodology

### 2.1 Study Area

Two strategically located lakes inside Agusan Marsh were chosen as sampling sites. Lake Panlabuhan, a floodplain lake in the municipality of Loreto, is where the Panlabuhan Floating Village is located. At certain times of the year, particularly March–June, when the water is low, it emerges to form four lakes: Lake Dinagat, Lake Bukugon, Lake Kubasayon, and Lake Kanimbaylan. Lake Tugno, a lake in Sitio La Flora, Talacogon, north of Panlabuhan, is an oxbow lake cut off seasonally from the main river during the summer. Each lake had fifteen equally distributed sampling points, with three points at each lake's entrance connected to the Agusan River (Figure 1).



**Figure 1.** Map of Agusan Marsh and its lake systems Tugno, Panlabuhan, entrance of Panlabuhhan (A) and mollusk samples (B)

## 2.2 Sample Collection

Two univalve species, *Bellamya angullaris* (Viviparidae) and *Pila ampullacea* (Ampullariidae), and one bivalve, *Cristaria plicata* (Unionidae), were collected from each lake (Figure 1). The gastropod *B. angullaris*, *P. ampullacea*, and bivalve *C. plicata* are all shared resources for commercial and artisanal fishers and listed in the International Union for Conservation of Nature (IUCN) as “Not Evaluated” and “Least Concern” status, respectively. Permits and endorsements from Philippine’s Department of Environment and Natural Resources (DENR) Caraga Office (Protected Area Management Office, PAMO Resolution No. 2022-013) and the Protected Areas Management Board (PAMB) of Agusan Marsh to collect these mollusks were secured before the actual field collection. Similar-sized samples per species were handpicked or scooped from the lake’s bottom using a dip net. Shell length (cm) and whole-body wet weight (g) were measured using a Vernier caliper and analytical balance. Specimens were individually wrapped in aluminum foil and stored in iced coolers before MP analysis (Jin-Feng et al., 2018).

## 2.3 Quality Assurance and Quality Control

During collection and analysis, the use of cloth and any plastic material was minimized. The glassware used was washed and rinsed with distilled water. Prepared solutions and distilled water for final filtrations were filtered (Whatman No. 2, 8  $\mu\text{m}$  pore size, Cytiva, China) before use. Potential airborne contamination was prevented by covering the samples with glass or aluminum foil, thus maintaining the integrity of the samples and preserving the accuracy of the analysis (Banda et al., 2024; Inocente et al., 2023). Method blanks were processed alongside samples. Only organic microparticles were detected in the blanks.

## 2.4 Isolation of Microplastics

Before dissection, the wet weights of the collected samples exceeded half of the total weight, indicating a significant proportion of soft tissue within the specimens. The shells were cleansed to ensure an accurate assessment of microplastic (MP) content relative to weight, and the water obtained during dissection was included in the MP

extraction process. Dissection was performed to isolate soft tissue, which was then weighed precisely using an analytical balance and transferred to a beaker. After that, potassium hydroxide (KOH, 10%, JT Baker, Avantor, Sweden) solution (1:4, tissue:KOH) was added to the beaker. The lid was then sealed with aluminum foil, and digested at 60 °C for 48 hours to digest the organic matter. The resulting digested solution underwent density separation utilizing a saturated sodium chloride solution (1:2 sample:NaCl, Duksan Pure Chemicals Co. Ltd., South Korea). The solution was then filtered through a Whatman GF/C filter within a vacuum filtration apparatus. The filters were flushed with distilled water and dried in an oven at 40 °C (Jin-Feng et al., 2018).

### 2.5 Abundance and Morphological Analyses of Microplastics

Filters from the previous step were observed under a stereo microscope (Koppace, China) using 10x – 40x magnification. The suspected MPs taken out were put on a clean glass slide labeled for FTIR (Fourier Transform Infrared Spectroscopy, PerkinElmer *Spectrum Two* Inc, Waltham, MA, USA) analysis, and a compound microscope (Kern, Germany) was used to measure their sizes. The MPs were identified and measured based on physical characteristics (Hidalgo-Ruz et al., 2012). The MP morphology, such as shape (fiber, film, fragment), color, and size, was recorded. MPs were classified into five sizes: 0.25 – 0.5 mm, 0.5 – 1 mm, 1 – 5 mm, and > 5 mm. The units of MP abundance were expressed as the number of particles per individual (MP/Individual) and number of particles per gram (MP/g) (w/o shell, wt) for a consistent comparison to other species. Expressed as total number of microplastics per species divided by the total number of samples per species and MP/Individual per species divided by the Average weight per species,

### 2.6 Polymer Type Analysis of Microplastics

Attenuated Total Reflectance-Fourier Transform Infrared Spectroscopy (ATR-FTIR) (PerkinElmer *Spectrum Two* Inc, Waltham, MA, USA) was employed to determine the

type of polymer present in the suspected MP. The Bruker ATR Library of Polymers, the Bruker ATR Library for Chemicals, the Bruker ATR Library for Pharma, and the Bruker ATR Library for Forensics were utilized as references in comparing the FTIR spectra for verifying the origin of polymers (Wootton et al., 2021). FTIR analysis results that were roughly 20% identical were recognized as confirmed MPs.

### 2.7 Statistical Analysis

Means, standard error of the mean (SEM), and median values were computed for each sampling area and species. Microsoft Excel was used in the computation.

## 3. Results and Discussion

### 3.1 Species morphology of samples

*Bellamya angularis* from Panlabuhan (average length 35.88 mm ± 1.12) are shorter than those from Tugno (average length 45.19 ± 0.80 mm). Conversely, *Pila ampullacea* from Panlabuhan with average lengths of 65.02 mm (±0.92) are longer than their counterparts in Tugno (Table 1). The bivalve *Cristaria plicata* was only found in Tugno, precluding size comparison. The weights of samples before dissection indicated that soft tissue constituted a substantial portion of total weight. Shells were washed, and water from dissection was included in MP extraction for accurate MP count relative to weight (g) (Table 1).

Benthic invertebrates generally prefer shallow, calmer waters due to the stress of higher flow rates, which can dislodge snails. This might explain the larger sizes of *P. ampullacea* in Panlabuhan, aligning with Crowl and Schnell (1990) who noted increased snail size in higher flow rates. High flow rates in Panlabuhan likely limit vegetation and algal blooms, important food sources for benthic organisms, thus potentially affecting snail sizes (Stromberg et al., 2007). However, *B. angularis* were larger in Tugno, possibly due to their smaller size being more advantageous in high flow conditions, or the abundance of prey species supported by higher vegetation and algal blooms in tranquil waters.

Flow rates of both lakes were not measured but observed, necessitating further tests. The high flow rates, turbidity, and deeper waters of Lake Panlabuhan may explain the absence of *C. plicata*, which was found only in Lake Tugno (Wen et al., 2006). Lack of morphological studies on *B. angularis* complicates the determination of recommended collection size ranges. Additionally, the sizes of *P. ampullacea* and *C. plicata* samples were below the ranges presented by Broto et al. (2020) and Klishko et al. (2014).

3.2 Quantification and Characterization of Microplastics

There are 81 confirmed MPs extracted from the species samples (n = 70) of both lakes (Lake Panlabuhan = 35; Lake Tugno = 46). PET has the highest number of MP particles and is the most abundant polymer type from both lakes (Panlabuhan = 23; Tugno = 25),

followed by LDPE (Tugno = 8) followed by EVA (Panlabuhan = 6; Tugno = 5). Cellulose acetate (CA) and polypropylene (PP) are unique to Tugno (Table 2).

Only 81 out of 375 (21.60%) suspected MPs were confirmed as polymers using FTIR-ATR. 35% of the collected samples (25 of 70 mollusk samples) did contain MPs. The difference in the mean abundance of MPs per individual could be attributed to feeding habits leading to increased likelihood of MP ingestion. *B. angularis*, suspected to be a predatory species, contained whole morphologically different species in the digestive system of the gastropod upon dissection. Some species of snails predate smaller species. However, this feeding habit still needs to be confirmed due to the lack of biological studies specific to this species. Multiple studies have indicated that an omnivorous diet is consistent with a higher count of MPs (Mizraji et al., 2017). *P. ampullacea*, a gastropod species

Table 1. Morphological information of biota samples from both lakes

Site	Species	n	Length (mm) ± SEM	Width (mm) ± SEM	Weight w/ shell (g) ± SEM	Weight w/o shell (g) ± SEM
PAN <sup>†</sup>	<i>B. angularis</i>	15	35.88 ± 1.12	24.04 ± 0.30	9.04 ± 0.33	4.14 ± 0.22
	<i>P. ampullacea</i>	15	65.02 ± 0.92	50.05 ± 0.54	60.43 ± 1.92	30.06 ± 1.18
TUG <sup>±</sup>	<i>B. angularis</i>	15	45.19 ± 0.80	30.12 ± 0.40	15.3 ± 0.66	8.05 ± 0.31
	<i>P. ampullacea</i>	15	61.94 ± 0.86	50.83 ± 0.59	61.59 ± 2.43	26.09 ± 1.02
	<i>C. plicata</i>	10	108.7 ± 1.63	52.13 ± 1.12	58.21 ± 3.82	21.74 ± 1.13

SEM – Standard Error of the Mean; <sup>†</sup> Panlabuhan; <sup>±</sup> Tugno

Table 2. Checklist showing the occurrence of each polymer from lakes and fishes

Polymer Type	Panlabuhan	Tugno	<i>B. angularis</i>	<i>P. ampullacea</i>	<i>C. plicata</i>
Polyethylene terephthalate (PET)	+	+	+	+	+
Polyvinyl chloride (PVC)	+	-	-	+	-
Low-density polyethylene (LDPE)	-	+	+	+	-
High-density polyethylene (HDPE)	+	+	+	+	+
Polyamide (PA)	+	+	+	+	-
Ethylene-vinyl acetate (EVA)	+	+	-	+	+
Cellulose acetate (CA)	-	+	-	+	-
Polypropylene (PP)	-	+	-	+	-

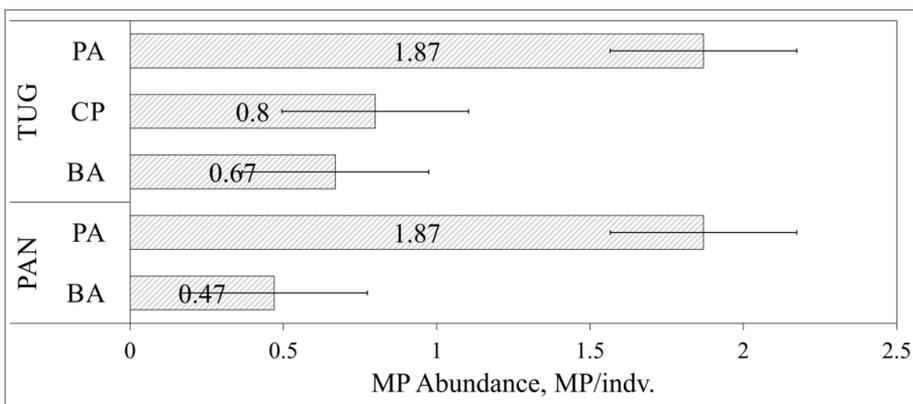
commonly present in shallow waters grazing in underwater vegetation and algae as well as any dead animal matter has a higher MP count per individual when compared to both species of *B. angularis* and *C. plicata* with an average mean abundance of 1.87MP/Individual in both lakes. This result is consistent with the presumption that an omnivorous diet contributes to a higher MP count (Mizraji et al, 2017). Furthermore, *C. plicata*, a filter feeder, had a higher MP/Individual ( $0.8 \pm 0.33$  MP/Individual) compared to *B. angularis* in both lakes but is lower than *P. ampullacea* (Figure 2).

Despite its small size, *Bellamya angularis* exhibited a significantly higher accumulated MP (MP/g) compared to *P. ampullacea* and *C. plicata* from the two lakes (Figure 2). This finding suggests that the MP/Individual metric may not accurately reflect the relative abundance of MP particles when considering the weight of each species. Moreover, the variation in MP particles per g of tissue observed among species may be dismissed, as suggested by Wu et al. (2022), since it does not align with the biometry of the species, indicating a lack of correlation between species' characteristics and MP abundance.

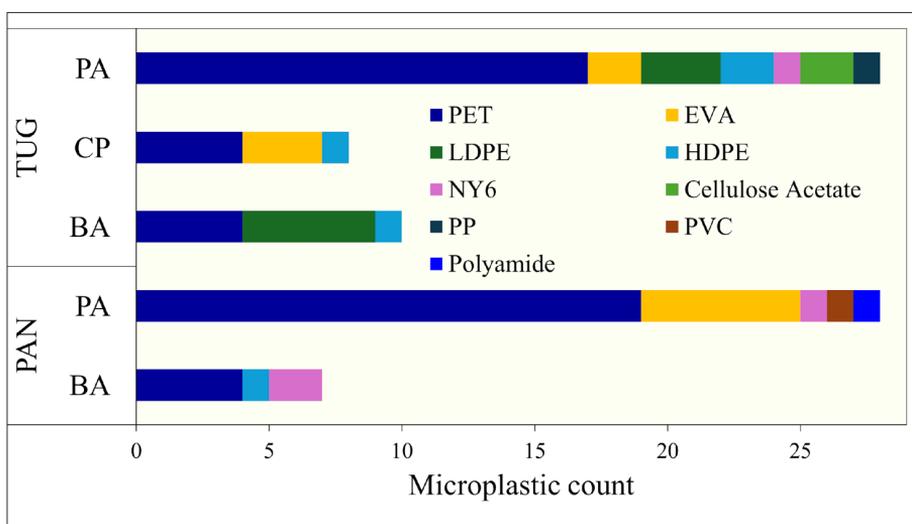
Tugno appears to have a higher mean MP abundance (1.27 MP/ Individual.) than Panlabuhan (1.17 MP/Individual.). However, to avoid bias, these values are only limited to the species (*B. angularis* and *P. ampullacea*)

in both lakes, excluding *C. plicata*, as the species is only available in one site (Tugno). This difference could be attributed to the distinct properties of both lakes. Since the species of this study are benthic organisms, stagnant and shallower waters are the ideal habitats for these species. As such, with lower hydrodynamic conditions, MP particles are more likely to settle into the sediments where both bivalve and univalve species filter feed and graze, resulting in higher counts of MP particles in Tugno as opposed to the higher hydrodynamic activity of lake Panlabuhan resulting in lower MP/Individual (Petersen and Hubbart, 2021).

Eight MP polymer types have been identified using the FTIR analysis, with Polyethylene terephthalate (PET) being the most abundant out of all the polymer types. PET is then followed by Ethylene-vinyl acetate (EVA), Low-density polyethylene (LDPE), High-density polyethylene (HDPE), Polyamide (PA), cellulose acetate (CA), Polyvinyl chloride (PVC), Polypropylene (PP), respectively (Figure 3). PET is the most prevalent thermoplastic polymer resin of the polyester family, and it is used in garment fibers, containers for beverages and foods, thermoforming for manufacturing, and engineering resins combined with glass fiber (De Vos et al., 2021). Extracted PET MPs from collected species are generally fibrous.



**Figure 2.** Mean abundance of MPs per mollusk species: *Cristaria plicata* (CP), *Bellamya angularis* (BA), *Pomacea ampullacea* (PA) in the two lakes (Panlabuhan: PAN; Tugno: TUG).



**Figure 3.** Polymer types of the mollusk species *Cristaria plicata* (CP), *Bellamya angularis* (BA), *Pomacea ampullacea* (PA) from the two lakes (Panlabuhan: PAN; Tugno: TUG).

High EVA counts in the obtained samples could be attributed to using foams and plastic wraps for boats, fish cages, and aquaculture operations near the marsh and upstream area of the river. The most common fishing method is fish cages built of mesh nets, which are most likely made of EVA due to their strength. These polymer-based fishing gears contribute to MPs, with data indicating that an estimated 380 tons of plastic-based fishing equipment are lost (Deshpande *et al.*, 2020). Parvin *et al.* (2021) predicted high EVA volumes. Due to its durability and low cost, PVC is one of the most popular polymers used today to make various items (van Nieuwenhuysse, 2018). Despite being one of the most frequent polymer types utilized in product manufacturing, these MP polymers had the fewest MP particles detected from the mollusks assessed (Figure 3). By visual categorization, three different characterizations: fiber, a thin filament-like particle with three-dimensional bends; filaments (large rod-like structures); and fragment (particles with jagged edges and film-like appearance) were described (Figure 4).

### 3.3 Morphotypes of Confirmed Microplastics

Fiber is the dominant morphotype in both lakes, followed by filament with 16 particles but only present in Tugno (Figure 4). These

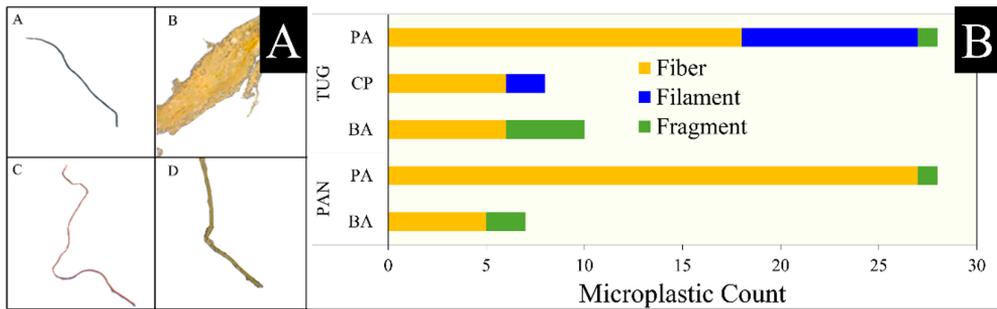
morphotypes identified could be baseline data for freshwater mollusks. For univalve species like *P. ampullacea*, which are omnivorous grazers, their primary exposure to MPs is through ingestion by grazing on sediments with vegetation and algae contaminated with MPs. This method of ingesting vegetation could explain the presence of fragments in these species and the absence of fragments in filter feeders. The lack of fragment MPs in *C. plicata* is due to their feeding habit, which filters out algae and biomatter. This filtration process prevents larger MPs from entering the digestive systems of the bivalves, which is consistent with the results in *C. plicata* only having fiber and filament MPs, as these morphotypes can quickly enter through the filters due to their smaller and thinner width compared to other morphotypes despite their variations in length. Fibers are long, thin, thread-like particles that are typically flexible, while filaments are also long, thread-like particles but are often flatter compared to fibers (Chubarenko *et al.*, 2024). Fibers appear to have a three-dimensional structure, while filaments are flat and two-dimensional.

Jin-Feng *et al.* (2018) documented  $0.39 \pm 0.50$  mm MP fragment MPs in *C. plicata* from the local markets of Qingdao, China suggesting that *C. plicata* could still ingest bigger sized MP fragments. *Bellamya angularis*, with similar feeding habits to

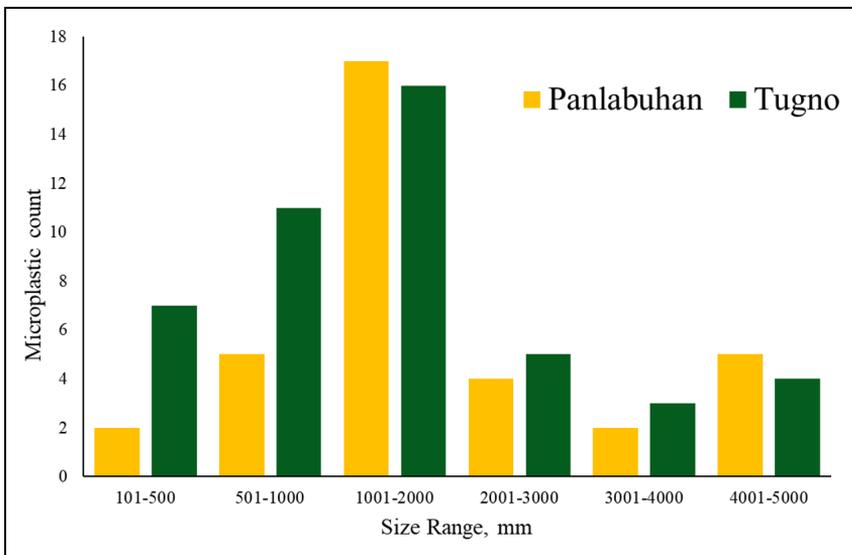
*P. ampullacea*, could bioaccumulate MPs differently due to their suspected predatory habits, feeding on smaller snails, on which these snails could already have accumulated MPs. The nature of prey-predator in MPs and the level of retention and the extent of accumulation through predation in snails currently need studies. However, studies suggest that predators could ingest more MPs than omnivores (Sequeira *et al.*, 2020), which is consistent with the results of MP/g (Figure 2).

In terms of size, particles ranging from 1001-2000  $\mu\text{m}$  (1-2 mm) are the most abundant in both lakes (Figure 5). The extracted microplastic (MP) particles in the

studied lakes display a variety of colors, with black (22%) being the most prevalent, followed closely by blue (20%), and then brown and red each accounting for 15%. Other colors include transparent (12%), green (9%), grey (5%), and yellow (2%) (Figure 6). Despite black being the most common color overall, distinct differences in color distribution exist between the two lakes. In Lake Panlabuhan, black MPs predominate, whereas in Tugno, brown, blue, and red MPs are equally prevalent, with each color noted in nine instances (Figure 6). Green and yellow MPs are exclusive to Lake Panlabuhan, while grey is only found in Tugno.



**Figure 4.** (A) Representative microplastic particles: A - Polyethylene terephthalate, fiber; B - High-density polyethylene, film; C - Ethylene-vinyl acetate, fiber; D - Polypropylene, filament; (B) Shape composition of MPs from the two lakes of Agusan Marsh



**Figure 5.** Size classification of microplastics (MPs) from the tissue samples

The variation in MP colors might influence their likelihood of ingestion by aquatic organisms, as different colors could attract or resemble natural prey. For instance, brightly colored MPs might be mistaken for food, as suggested by Wright *et al.* (2013). Nevertheless, the ingestion of MPs involves multiple factors, including particle size, shape, and surface characteristics, alongside the dietary habits and preferences of the organisms. Although further studies are required to elucidate the exact impact of MP color on ingestion, the primary feeding behavior of the target species in these environments; grazing and filter feeding; suggests that color may not play a significant role. These species tend to feed passively on algae, biofilms, and organic detritus, relying more on tactile and chemical cues rather than visual ones to locate their food sources.

Visual inspection and characterization of morphotypes are essential supplementary information to determine the possible causes of pollution and the likelihood of the MPs being bioaccumulated by different species, specifically their feeding method. As such, MP types contribute more to their chances of bioaccumulation, whether actively (through feeding) or passively (environment). The most abundant of the morphotypes recorded in this study are fibers (76%), filament (15%) and fragments (9%) (Figure 3). Fibrous and filamentous MPs, consistent with their higher proportion in the extracted MPs, could indicate that these morphotypes are more likely or more easily bio accumulated through ingestion (such as grazing for the gastropods and filter feeding for the bivalves).

Bivalves accumulate more microplastics than gastropods due to their filter-feeding behavior, higher feeding rates, stationary lifestyle in microplastic-rich environments, and greater biomass (Khanjani *et al.*, 2023). In contrast, gastropods use a radula to selectively scrape surfaces for food, reducing their interaction with suspended microplastics. Bivalves can accumulate up to 27 times more microplastics in their viscera compared to fish (Naji *et al.*, 2018; Claessens *et al.*, 2011), making them effective bioindicators. However, microplastic ingestion causes

physical damage to their digestive tracts, reduces feeding rates, and impacts growth and survival (Cole *et al.*, 2020; Bringer *et al.*, 2021). It also increases oxidative stress and DNA damage (Pedersen *et al.*, 2020).

The presence of microplastics in bivalves raises concerns for human health. Although the full effects on humans are not yet clear, evidence suggests they can cause inflammation, oxidative stress, and cellular damage (Sharifinia *et al.*, 2020; Schwabl *et al.*, 2019). A global assessment reported an average intake of 751 microplastic particles per capita per year from shellfish consumption (Ding *et al.*, 2022). Additionally, studies show that contaminants like PCBs can transfer from microplastics to the human digestive system, further increasing health risks (Mohamed Nor & Koelmans, 2019).

#### 4. Conclusion

This study is the first to report that edible wetland mollusks in Agusan Marsh contain microplastics in their system. The PET emerged as the predominant MP polymer, alongside LDPE, EVA, CA, and PP, with some polymers being unique to each lake. The study observed that fibers and filaments were the most common MP morphotypes, more likely to be bio accumulated by the grazers and filter feeders studied. The ingestion of MPs by freshwater mollusks may be influenced by feeding behaviors, while differences in MP abundance between the lakes were linked to hydrodynamic conditions and MP sedimentation. The findings highlight the potential of bivalves as bioindicators of microplastic pollution and the health risks to humans from consuming microplastic-contaminated mollusks from wetlands. To mitigate these effects and preserve the health of ecosystems like Agusan Marsh, comprehensive strategies combining environmental management, waste reduction, and regulatory measures are recommended. This could involve enhanced monitoring of MP levels, promoting sustainable practices, and fostering public awareness to reduce plastic pollution and its consequences on aquatic life.

## Acknowledgement

The authors gratefully acknowledge the financial support provided by the Philippine Department of Science and Technology-National Research Council of the Philippines (DOST-NRCP; grant number E-255) for this research. Our sincere thanks also go to the DENR Caraga and the Protected Areas Management Bureau (PAMB) of Agusan Marsh for granting permission to collect mollusk samples. Furthermore, we deeply appreciate the invaluable assistance provided by the local fishermen and guides of Lake Panlabuhan and Tugno, who supported our team throughout the fieldwork.

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