

Microplastic in Sediments and Ingestion Rates in Three Edible Bivalve Mollusc Species in a Southern Philippine Estuary

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Abstract Plastics are now a major environmental concern worldwide with their widespread contamination and accumulation. Microplastic particle (< 5 mm) is an emerging pollution issue as it is being detected worldwide in aquatic and terrestrial ecosystems, but relatively little is known in tropical regions. This study determined the (1) abundance of microplastics in sediment and (2) in situ and laboratory ingestion rates of microplastics in three scarcely studied tropical bivalve mollusc species (*Donax* sp., *Meretrix meretrix*, and *Katelysia hiantina*) in Panguil Bay, Southern Philippines. A total of 2258 microplastic particles (62.72 \pm 18.31 items/m²) were found on the sediment samples. Filament/fiber is the most abundant

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Chemistry Department, College of Arts and Sciences, Negros Oriental State University, 6200 Dumaguete City, Philippines type of microplastic in terms of morphology, while black and blue are the dominant colors of microplastic particles. There were 1495 microplastic particles $(4.15 \pm 3.37 \text{ particles/clam})$ present in the clam tissues, of which polypropylene (PP) and rayon (RY) polymers are the most common, whereas K. hiantina (707 particles) showed the highest amount of microplastics. The number of ATR-FTIR-confirmed polymer types in the wild clams is greater than that in the sediments. The study reveals abundant microplastics in sediments and in the three species of bivalve individuals from the wild. All clams ingested low-density polyethylene (LDPE) microplastic particles in the laboratory. The mean number of LDPE microplastic particles ingested by clams is 4.62 ± 2.40 particles/clam/7days, with the highest value observed in K. hiantina. Additionally, Donax sp., M. meretrix, and K. hiantina could ingest high densities of 40-60µm microplastic particles.

Keywords Bivalve mollusc · ATR-FTIR analysis · Microplastics · Panguil Bay · Sediments · Ingestion rate

1 Introduction

Plastics are widely used in different applications because they are lightweight, durable, and economical. According to PlasticsEurope (2020 as cited in Mai et al., 2022), global plastic production reached 368 million tons in 2019. Moreover, due to the market demand for plastic products, it is projected that plastic production may reach 600 million tonnes by 2025 and may exceed 1 billion tonnes by 2050 (FAO, 2017). Around 6300 metric tonnes (MT) of plastic waste is generated annually, out of which 9% is recycled, 12% is incinerated, and the remaining 79% gets accumulated in natural environments and ultimately ends up in seas and oceans (Geyer et al., 2017). The COVID-19 pandemic may have increased microplastic pollution due to the global production of personal protective equipment (PPE), gloves, face masks, and face shields in billion pieces (Lee and Kim, 2022). The common sources of plastic pollution in the terrestrial and marine environments come from the packaging materials that we use every day, which are made up of polyethylene (PE), polypropylene (PP), polystyrene (PS), polyethylene terephthalate (PET), polyvinyl chloride (PVC), and polyamide (PA) (Andrady, 2011; Avio et al., 2016).

Plastic wastes are potential threat to marine organisms. For example, ingestion of plastic debris or entanglement has been recorded in 44–50% of all seabirds, sea snakes, sea turtles, penguins, seals, sea lions, manatees, sea otters, fish, crustaceans, and half of all marine mammals (Kühn et al., 2015). Moreover, these plastic wastes contribute to the formation of a new contaminant called microplastics. Reports indicate a global increase and widespread distribution of microplastics in the environment, thereby increasing microplastic's bioavailability to various organisms. This scenario suggests that microplastics potentially threaten wildlife, natural ecosystems, and human health (Endo et al., 2005; Teuten et al., 2009; Rocha-Santos and Duarte, 2015; Arp et al., 2021).

Microplastics, typically defined as plastic pieces that are < 5 mm in size (Barnes et al., 2009), although a recent definition suggests to consider fragments smaller than 1 mm (Browne et al., 2015), have been documented in aquatic systems on all seven continents, in both freshwater and marine environments (Barnes et al., 2010), along beaches (Browne et al., 2011, Yabanlı et al., 2019), in sediments (Claessens et al., 2011; Reed et al., 2018), in biota (bivalve molluscs, zooplankton, and fish) (Rashid et al., 2021; Ding et al., 2021; Wootton et al., 2021), and in the water column itself (Eriksen et al., 2013). Microplastics are barely visible microlitter in the form of small fragments, fibers, and granules (Desforges et al., 2015), and their small dimension makes them available for ingestion by a wide range of marine organisms. Besides entanglement and ingestion of macro debris by large vertebrates, microplastics are accumulated by planktonic and invertebrate organisms, being transferred along food chains through the consumption of lower trophic level prey (Avio et al., 2016; Farrell and Nelson, 2013). Primary sources of microparticle-sized plastics refer to the pellet form of plastics used as feedstock in the plastic industry and/or personal care products. In contrast, microplastics derived from secondary sources are a result of fragmentation and degradation of larger plastic materials (Cole et al., 2011; Arp et al., 2021). The majority of microplastics in the oceans are secondary products derived from the degradation and fragmentation of mesoplastics or larger fragments (Gregory and Andrady, 2003).

In general, the bioavailability of microplastics and nanoplastics 0.001 to 0.1 μ m (FAO, 2017) may depend on their size, density, shape, and surface charges which will affect their behavior in seawater, leading to agglomeration, resuspension, and settling. Moreover, their uptake, disposal, and bioaccumulation by marine organisms are influenced by their feeding behavior, with benthic detritivores and suspension feeders representing more susceptible target species (Wright et al., 2013). Laboratory studies conducted show that microplastics can be ingested by a range of marine organisms when particles are mistaken for food (Browne et al., 2008; Boerger et al., 2010; Lusher et al., 2013).

In addition to direct physical impacts from the microplastic itself, ingested plastic debris may act as a medium to concentrate and transfer chemicals and persistent, bioaccumulative, and toxic substances (PBTs), such as polychlorinated biphenyls (PCBs) to organisms (Eerkes-Medrano et al., 2015). Different particles polymers, like PVC, PE, PP, and PS, were shown to have a high sorption capacity for dichlorodiphenyltrichloroethane (DDT), polycyclic aromatic hydrocarbons (PAHs), hexachlorocyclohexanes (HCH), and chlorinated benzenes (Bakir et al., 2012; Lee et al., 2014). This diversity of synthetic substances places human beings at high risk for cancer and debilitating diseases (Dewailly et al., 1989; Lohman and Muir, 2010).

The estuarine water of Panguil Bay, an important fishing ground in Southern Philippines, supports three species of exploited bivalve molluscs, namely, the wedge clam, *Donax* sp., Asian hard clam, *Meretrix meretrix*, and the surf clam, *Katelysia hiantina* (Jimenez et al., 2009). Microplastic contamination in habitats and ingestion in bivalve mollusc food species are poorly studied in the tropical region (Espiritu et al., 2019). Thus, this study aimed to estimate the distribution and abundance of microplastic materials in the sediment habitat of the three bivalve species, quantify the abundance of microplastics in three bivalve mollusc species (= in situ ingestion), and determine the ingestion rates of the three species on low-density polyethylene microplastic in the laboratory.

2 Materials and Methods

2.1 Sampling Area

Sediment and bivalve mollusc samples were collected from four different sites (Baroy [site 1], Tangub [site 2], Kapatagan [site 3], Cabgan [site 4]) in the estuarine Panguil Bay (Fig. 1). Panguil Bay is about 41 km long and is flanked by 10 municipalities and two cities belonging to three provinces (Lanao del Norte, Zamboanga del Sur, and Misamis Occidental) and three administrative regions (Regions 9, 10, and 12). There are 11 towns and 78 coastal barangays surrounding Panguil Bay (Jumawan et al., 2020).

Panguil Bay description, hydrography, and water circulation were reported by Canini et al. (2013) and Metillo et al. (2015). As reported, "Panguil Bay is a south-western inlet of the greater Iligan Bay, and with an average of 5 m, the depth gradually decreases from the mouth to the inner portion, but a narrow and deep lengthwise channel in the middle occupies threequarters of the total length of the bay. The volume of exchanged water daily is about 22 % of the total volume of 1.1 km³. The remaining mangrove forest has an area of 27.3 km², mostly concentrated in the inner parts of the bay. Rainfall on the catchment area brings runoff into Panguil Bay via 29 major rivers and 46 minor tributaries. Variation of hydrographic parameters is primarily influenced by monsoon. Strong north-east (NE) monsoon winds prevail over Panguil Bay from November to March but are strongest from January to February. Weak, variable winds typify the intermonsoon months of April and May. Salinity is generally higher (10-31.7) in the cooler (24-32 °C) NE monsoon months (from October to March), but lower (3.3–29.7) in the warmer (30–32 °C) south-west (SW) monsoon months of June through to September. Higher mean dissolved oxygen values (7.1-7.9 mg L^{-1}) occur in November and August, compared with those $(6.5-6.6 \text{ mg L}^{-1})$ in February and May. The pH (8.8-9.2) is typical of normal seawater in February, but slightly acidic, typical of a mangrove estuary, in other months. Dissolved nitrate was generally lower $(0.4-0.7 \text{ mg L}^{-1})$ in rainy SW monsoon months compared with the warmer months of February through to May (0.4–0.9 mg L^{-1}). The lowest concentrations of dissolved orthophosphate were recorded in November $(0.1-0.2 \text{ mg } \text{L}^{-1})$, but higher in rainy months during SW monsoon (0.1–0.4mg L^{-1}). Heavy rainfall and strong winds during NW monsoon coincided with highest concentrations $(0.8-2.9 \text{ x } 10^2 \text{ mg } \text{L}^{-1})$ of total suspended solids throughout the bay." The most abundant commercially exploited species in the bay are invertebrates with molluscs contributing to 10% (Jumawan et al., 2021).

Plastic materials like fishing net, rope, plastic bag, adhesive tape, styrofoam, tire cord, wire, and cable insulation, and food and drink wrappers were found in the sampling area which could be some of the possible sources of plastic pollutants in Panguil Bay (Bonifacio, 2021).

2.2 Collection and Quantification of Microplastics in Sediments

Sampling of sediments was conducted at three transect lines with three quadrats located 10 m away from each other for each area, specifically in the intertidal zone. The sampling quadrat was 1×1 m. A metallic shovel was used to collect sediments at 5 cm depth. The sediments were sealed in a plastic container which was rinsed in the laboratory with seawater before use. Sediments were transported back to the laboratory, dried, and kept at room temperature until extraction (Barasarathi et al., 2014; Besley et al., 2016). Dried sediment samples were sieved through 0.45 mm mesh to separate macroplastics. Microplastics in 2 kg sediment samples were extracted using the method adapted from Besley et al. (2016) with minor modifications and additional changes. A fully saturated salt solution was prepared by dissolving 358.9 g of sodium chloride (NaCl) in 1 L of demineralized water. The salt solution was filtered to remove





impurities. Microplastic was extracted by density separation, where dry sand was combined with a saturated salt solution. One hundred (100) g of dried sand was added to 400 mL of fully saturated salt solution and was stirred for 5–10 min. The sediments were then allowed to settle for not less than 3 days or until the water became clear. The supernatant was poured into a vacuum filtration system and filtered through Whatman filter paper grade 1 (11 μ m pore size). Procedures were repeated two times for each sample. Collected particles in the filter paper were examined using a stereo microscope, compound microscope, and ATR-FTIR spectroscopy. Blanks consisting of salt solution only were handled the same way as samples to assess laboratory contamination.

2.3 Source of Polyethylene Microplastic

Low-density polyethylene (LDPE) particles were obtained from Beijing Yan Lin Fu Chemical Sales Center (China) and were processed and size-sorted into 40–60- μ m microplastics through grinding and sieving by milling machine, blender, and metallic sieves. LDPE microplastic sizes were verified under a compound microscope.

2.4 Collection and Laboratory Maintenance of Samples

A total of 840 bivalve mollusc individuals were collected in Panguil Bay. The three bivalve mollusc species (the wedge clam, *Donax* sp.; Asian hard clam, *Meretrix meretrix*; and the surf clam, *Katelysia hiantina*) were collected in the same four sampling sites of the Panguil Bay. After collection, 360 mollusc individuals for acid digestion were refrigerated, and 480 mollusc individuals intended for polyethylene ingestion rate study were transferred to the laboratory, where they were acclimatized in filtered seawater for 7 days at constant aeration, with a photoperiod of 12 h light and 12 h darkness.

2.5 Laboratory Feeding

Live individuals were scrubbed to remove any organisms from their shells. The laboratory exposure method was adapted from Avio et al. (2015) and Ribeiro et al. (2017) with minor modifications.

Individuals of three species were randomly placed in individual and identical 500-mL glass beakers with 400 mL of the microplastic-seawater mixture following a setup of three bivalve mollusc species \times 10 replicate individuals of each species. Microplasticseawater mixture was prepared by mixing 1.5 g of 40-60-µm LDPE microplastics and 1 L of filtered seawater. No sediments were added, and portable oxygen pumps were used to provide aeration. The use of plastic material was avoided while experimenting. The exposure ran for 7 days. The water was changed in each glass beaker every 24 h with LDPE microplastics. Clams were not fed to avoid any interaction of microplastics and food, but animals remained alive through the microplastic exposure period. After 7 days, ingestion rates of clams were measured using the same acid digestion protocol for the wild-based clams. Usually, whole organisms (Lusher et al., 2017b) are digested or analyzed in laboratory studies.

2.6 Acid Digestion of Clams from the Wild and Laboratory Experiment

Microplastics from wild clams and LDPE synthetic microplastics from clams in the laboratory experiment were determined using the digestion protocol adapted from Claessens et al. (2013) with minor modifications. Clams were refrigerated (1 °C) before and during preliminary processing and were frozen until acid digestion could take place. During freezing, clams gape, creating a potential route for contamination. Clams were bound with elastic bands while in the freezer to maintain pressure on both sides of the shell to avoid contamination (Davidson and Dudas, 2016). Clams were not depurated in order to include microplastics that had been both recently ingested and possibly translocated to tissues (Mathalon and Hill, 2014; Li et al., 2015). Clamshells were thoroughly rinsed with water and then removed using a scalpel and forceps. The soft tissues were weighed separately. Clam tissues were digested in 10-15 mL of 69% nitric acid overnight at room temperature in a beaker/flask, followed by ~2 h of boiling (~100 °C). Digestions were complete when there was no visible organic material remaining, and the solution was clear and yellow. The resulting mixture was diluted (1:10 v/v)with warm (~80 °C) deionized water and vacuum filtered over 3-µm pore-sized filter paper immediately after dilution. All beakers and Buchner funnels were rinsed with deionized water before digestion and throughout to reduce the amount of sample lost during transfer stages. Filter papers were then examined under a stereomicroscope (10–40× magnification) for microplastic particles. Individual microplastic samples were further examined under a compound microscope (10–100× magnification) when necessary.

2.7 Microplastic Identification

The simplest method in the identification of microplastics is visual identification, a time-consuming but the most appropriate technique when dealing with a high volume of samples and limited resources (Crawford and Quinn, 2017). Microplastics were identified by color, structure, and type after clearing from debris, salt crystals, and sand grains. Most microplastics were flexible, will not break, and often spring back when prodded. A fine tweezer was used when prodding without damaging the microplastics. Microplastics could be covered by debris, salt crystals, and sand grains, making them difficult to see and identify. The chemical composition of microplastic samples from sediments and bivalves was determined using Fouriertransform infrared spectroscopy (Perkin Elmer Spectrum Two, USA) attenuated total reflectance mode (8 to 14 scans, 4 cm^{-1} resolution, 4000 cm⁻¹ to 450 cm⁻¹ wave number range), a standard analytical technique for identifying the chemical composition of samples larger than 0.5 mm (Biginagwa et al., 2015). The following criteria were used to define a plastic particle through visual examination (Noren, 2007):

- a. No cellular or organic structures are visible in the plastic particle/fiber.
- b. If the particle is a fiber, it should be equally thick, not taper towards the ends, and have a threedimensional bending (not entirely straight fibers, which indicates a biological origin).
- c. Clear and homogeneously colored particles (blue, red, black, and yellow).
- d. If it is not obvious that the particle/fiber is colored, i.e., if it is transparent or whitish, it shall be examined with extra care in a microscope under high magnification.

2.8 Contamination Control

Contamination control measures were strictly implemented and followed all throughout the experimentation. The use of plastic materials was minimized and all equipment were rinsed with distilled water before every test. Container walls were washed frequently with distilled water during the extraction of microplastics to avoid losing samples. Laboratory room was cleaned before and after daily experimentation. Cotton coats were worn and the number of people was minimized inside the laboratory. Control samples were constantly checked for contamination.

2.9 Statistical Analysis

Plastic particle concentrations were represented as mean values \pm standard deviation (SD). Kruskal-Wallis *H* Test was used to evaluate microplastic abundance in sediments and clams among sampling sites, and LDPE exposed clams among sampling sites and mollusc species since data did not meet the assumption of normality (Shapiro-Wilk *W* Test) and/ or homogeneity of variance (Levene's Test). Statistical significance was defined at p < 0.05. All statistical data analyses were performed using the IBM SPSS Statistics version 23.

3 Results and Discussion

a. Abundance and distribution of microplastics in sediments and wild clams

Microplastics found in the sediments and wild clams were categorized into fragments, filament/fiber, and foam (Fig. 2). A total of 2258 microplastic particles in the sediments were visually identified under the microscope. Filament/fiber (56%) was found to be the predominant type of microplastic, followed by fragments (42%) and foam (2%) being the lowest. On the other hand, a total of 1495 microplastic particles were found on clams in which filament/fiber (65%) was the predominant type of microplastic particles, followed by fragments (35%). No styrofoam was found in all the clam samples. Filaments/fibers are the most abundant microplastics since apart from plastic bags and bottles, packaging straps and fishing lines in





oceans are the most common plastic pollution items (Blettler et al., 2018). Additionally, polyolefins (PE and PP) and nylons are primarily used in fishing gear applications and are commonly detected in coastal areas (Klust, 1982; Timmers et al., 2005).

Different colored microplastic filaments/fibers were found in the sediment and wild clam samples (Fig. S1). Most of the filaments/fibers found were \leq 1 mm in size, measuring them along their longest dimension. Colors of microplastic particles in sediments and wild clams were black, blue, brown, green, orange, red, transparent, violet, white, and yellow (Fig. 3). In sediments, black (28%) and blue (31%) particles are the predominant colors. The same dominant colors were found in clams-black (37%) and blue (14%). In this study, black and blue accounted for more than half of the colors of microplastics in both sediment and clams, but color and shape, when describing and quantifying microplastics, are still in question as they introduce subjectivity due to the process of visual sorting with microscopy (Baruah et al., 2022). Colored microplastics are the most common in water, accounting for 50.4-86.9% of the total microplastics (Wang et al., 2017). Studies in China indicated that transparent and blue microplastics are the most abundant microplastics detected in biota (Fu et al., 2020). Recently, grouping microplastics into four obvious colors (transparent, black, white, and colored) instead of evaluating other more controversial colors (e.g., yellow, green, blue) has been suggested by Jiang et al. (2019). However, the variations in size, shape, and color in microplastics are a particular concern since they could easily be mistaken for food by marine organisms and seabirds (Thompson et al., 2004; Browne et al., 2008; Gregory, 2009).

The highest number of microplastics in sediments was found in site 4 (75.89 \pm 22.88 items/m²), followed by site 1 (63.78 \pm 10.65 items/m²), and site 2 $(61.67 \pm 17.19 \text{ items/m}^2)$ while site 3 (49.56 ± 11.93) items/ m^2) had the lowest number (Fig. 4A). The abundance of microplastic particles observed in sediments was similar within and among sampling sites. Hence, we can assume that the abundance of microplastic particles in sediments in the sampled areas and perhaps the entire Panguil Bay is homogenous. While prevailing currents and waves may cause differences in the distribution of macroplastics, processes involved in microplastic distribution are less well-known. Still, they can be influenced by particle distribution in the water column, settling rate, particle redistribution via hydrodynamics, and particle aggregation or animal activities (Browne et al., 2010). The mean abundance of sediment microplastics in the sampling sites is 62.72 ± 18.31 items/m². This is higher compared to that reported in a Brazil estuary with 60 items/m² (Ivar do Sul et al., 2009) but lower compared to those in Portugal with 133.3 items/m² (Martins and Sobral, 2011); South Korea with 913 items/m² (Heo et al., 2013); St. Lawrence River, Canada with $13,832 \pm 13,677$ items/m² (Castañeda et al., 2014); and Pearl River, Hongkong with 5595 items/ m^2 (Fok and Cheung, 2015).

Microplastic particles present in wild clams (N = 30 of each of the species of clams: *Donax* sp. \leq

Fig. 3 Abundance of microplastic particles in sediments (A) and wild clams (B) from Panguil Bay, Southern Philippines. The number of microplastic particles from each sampling site (N = 30 per species) was visually determined. (C) The number of microplastics per clam ranged from 0 to 21 particles (Table S1)



1 g, *Meretrix meretrix* 3–5 g, and *Katelysia hinatina* 3–7 g per site) are presented in Fig. 4B. The highest total number of microplastic particles per individual clam was observed in *K. hiantina* (707), followed by *M. meretrix* (499), and the lowest in *Donax* sp. (289). Meanwhile, sites 2 and 3 showed the highest number of microplastic particles ingested by one individual of *M. meretrix* (19) and *K. hiantina* (21), respectively. The lowest number of microplastics was found in *Donax* sp. (8) from site 4. All bivalve mollusc species have samples that have zero microplastics found after digestion. Comparison of mean values of microplastic

particles per clam (Fig. 4C) reveals that the highest mean value was recorded in *K. hiantina* (5.89 \pm 4.23), followed by *M. meretrix* (4.16 \pm 2.86) and, lastly, *Donax* sp. (2.41 \pm 1.47). Considering all the clam species (N = 360), the grand mean of particles per clam is 4.15 \pm 3.37, with filament/fiber being the most abundant shape. The abundance of microplastic particles observed in clams was statistically significant within and among sampling sites and mollusc species. Hence, we can assume that the abundance of microplastic particles among bivalve mollusc species varies, which could be attributed to contrasting Fig. 4 Types of microplastic polymer found in sediments (A) and wild clams (B) of Panguil Bay, Southern Philippines. Polymer types: acrylonitrile butadiene styrene (ABS), cellulose acetate (CA), polyethylene (PE), polyethylene terephthalate (PET), polypropylene (PP), polystyrene (PS), polyvinyl chloride (PVC), azlon (AZ), ethylene vinyl acetate (EVA), nylon-6 (PA6), phenoxy resin (PR), polystyrene (PS), and rayon (RY). Unclassified particles have a correlation of < 0.7



No. of Microplastic Particles

feeding mechanisms and rates (ability to reject inedible particles) among species (Van Cauwenberghe and Janssen, 2014).

Out of the 2258 visually identified particles from the four sediment sampling sites, 56 particles (14 particles \times 4 sites) were randomly selected and further validated via FTIR spectroscopy (Fig. 5A). The identified polymer types include acrylonitrile butadiene styrene (ABS), cellulose acetate (CA), polyethylene (PE), polyethylene terephthalate (PET), polypropylene (PP), polystyrene (PS), and polyvinyl chloride (PVC) (Fig. S2). The top three polymers are PP (23), PVC (12), and PE (10). A total of 175 particles were successfully identified as plastic polymers from the three wild clam species (Fig. 5B). The number of FTIRconfirmed polymers is greater in the wild clams than in sediments. These polymers are azlon (AZ), ethylene vinyl acetate (EVA), nylon-6 (PA6), polyethylene (PE), polyethylene terephthalate (PET), polypropylene (PP), phenoxy resin (PR), polystyrene (PS), polyvinyl chloride (PVC), and rayon (RY). The top three polymers found in clams are RY (62), PET (37), and PP (21), while PS (1), and PVC (1) are present in the smallest amount. Of the 175 samples, 22 were unclassified polymers due to poor correlation values.

Chemical composition is the most basic criterion for defining microplastic pollution (Zhang et al., 2020). Plastics are synthetic polymers made from



31%

Fig. 5 Proportion of microplastics in terms of color in sediments (A) and wild clams (B) found in the habitat of the three bivalve mollusc species in Panguil Bay, Southern Philippines

various compounds with different characteristics, and FTIR and Raman spectroscopy are the highly recommended equipment to identify them (Hidalgo-Ruz et al., 2012). Larger microplastic particles (> 500 µm) can be analyzed by ATR-FTIR (Tagg et al., 2015; Ivleva et al., 2017). For smaller particles, FTIR must be combined with an optical microscope, the so-called micro-FTIR technique (Imhof et al. 2012; Zhang L. et al. 2020), which can analyze 10 µm sizes via reflection and transmission mode (Simon et al., 2018). In this study, background scans were done before analysis, and each spectrum was evaluated manually based on the peaks' position. Samples with low correlation (< 0.6) were excluded from the interpretation of the final results (Bucol et al., 2020). In the present study, a total of 12 plastic polymers were identified via ATR-FTIR spectroscopy: ABS, AZ, CA, EVA, PA6, PR, PE, PET, PP, PS, PVC, and RY, half of which is included in the most common types of polymers found in the marine environment. These plastic polymers are commonly used in food packaging and fishing activities. The most common plastic polymers in the marine environment are PE, PP, PS, PVC, PA, PVA, and PET (Hidalgo-Ruz et al., 2012; Avio et al., 2016; Lusher et al., 2017a).

7%

Comparisons of microplastics have been difficult and limited to a few studies due to the difference in units used in other reports, a drawback observed in current microplastic research (Wright et al., 2013; Van Cauwenberghe et al., 2015), as well as plastics in general. Some units cannot be transformed between each other as they are measured in different dimensions (Yu et al., 2020). Additionally, there are no universally accepted methods for quantifying microplastic contamination since the widely used microscopy, photography, and spectroscopy may still result in unrealistic contamination estimates (Kroon et al., 2018). In fact, not all microplastics were sent for FTIR analyses since some were too small in size for FTIR-ATR analysis. If not careful, the number identified and the number analyzed may have discrepancy due to the particles being lost during the inspection, microscopic photography, and transferring of the microplastics to containers for spectroscopy. There is always an element of subjectivity and potential bias during visual identification. The effectiveness of visual identification is dependent on the size of the item being scrutinized and becomes more considerably difficult as the size of the item decreases. Microscopy techniques though, which were done in this study, can confirm the absence or presence of cellular structures and improve visual identification skills (Crawford and Quinn, 2017).

5%

5%

Water Air Soil Pollut (2022) 233:455

14%

b. Laboratory feeding experiment

All individuals (100%) of *Donax* sp., *Meretrix meretrix*, and *Katelysia hiantina* had ingested lowdensity polyethylene (LDPE) microplastics after 7 days of exposure period (Table 1). The highest number of LDPE microplastics ingested by *Donax* sp., *M. meretrix*, and *K. hiantina* after 7 days were 6, 14, and 13, respectively. The highest total number of LDPE microplastics was ingested by *K. hiantina* (740) followed by *M. meretrix* (608), and the lowest in *Donax* sp. (316). The mean LDPE microplastic particle ingested by the three clam species was 4.62 \pm 2.40 particles/clam/7days. A total of 1664 LDPE

Table 1Amount oflow-density polyethylene(LDPE) microplasticparticles ingested bythe four clam species inlaboratory experiment for aduration of 7 days	Mollusc	Sampling site	Ν	Min	Max	Number of microplas- tic	Sum	Mean ± SD (particles/clam/7days)
	<i>Donax</i> sp.	Baroy	30	2	5	89	316	2.63 ± 0.99
		Cabgan	30	1	3	63		
		Kapatagan	30	1	4	77		
		Tangub	30	1	6	87		
	Meretrix meretrix	Baroy	30	1	9	134	608	5.67 ± 2.37
		Cabgan	30	2	7	139		
		Kapatagan	30	2	13	169		
		Tangub	30	3	14	166		
	Katelysia hiantina	Baroy	30	4	9	170	740	6.17 ± 2.05
*The minimum and maximum values are the numbers of particles obtained in each clam sample		Cabgan	30	3	10	144		
		Kapatagan	30	4	8	182		
		Tangub	30	6	13	244		
	Total		360				1664	4.62 ± 2.40

microplastic particles were ingested by 360 bivalve mollusc individuals. Meanwhile, the mean number of LDPE microplastic particles ingested by the three clam species was 4.62 ± 2.40 beads/clam/7days. The study revealed that Donax sp., Meretrix meretrix, and Katelysia hiantina could ingest 40-60-µm microplastic particles. In the study of Van Cauwenberghe et al. (2015), the mussel Mytilus edulis can retain in their tissues particles ranging from 10 to 30 µm. It means that organisms will most likely ingest when microplastic particles are readily available in their present environment, especially in high amounts. Results showed a significant difference within and among sampling sites and mollusc species exposed to LDPE microplastic particles for 7 days. Such difference reinforces our main finding on the differences in microplastic accumulation among the three clam species, invoking a similar explanation of contrasting feeding behavior among bivalve filter-feeding species.

Other bivalve mollusc studies focus on the microplastic accumulation, translocation, and effects on the organisms' tissues (liver, gut, gills, and brain) by carrying out different biological analyses (e.g., Browne et al., 2008; von Moos et al., 2012; Avio et al., 2015; Sussarellu et al., 2015; Van Cauwenberghe et al., 2015). Additionally, ingestion and egestion in marine organisms are important processes that can potentially influence the cycling and transformation of secondary pollution by microplastics (Browne et al., 2011). However, there is still limited knowledge on the relationship between plastic uptake and egestion up to date (Van Cauwenberghe and Janssen, 2014). In the study of Graham et al. (2019), it was reported that Pacific oysters showed an efficient egestion rate, egesting $84.6 \pm 2\%$ of the microplastic particles taken up, which is slightly higher compared to the 74.5% egestion rate post-depuration in a study conducted by Van Cauwenberghe and Janssen (2014), while only the $15.4 \pm 2\%$ of beads taken up were retained within the shell cavity. Ingestion and egestion rates of microplastics vary among bivalve mollusc species as both are influenced by particle selectivity (Ward et al. 2019), food or particle abundance, and other environmental conditions (Chae and An, 2020).

4 Conclusion

The abundance, distribution, and characteristics of microplastic particles in the marine environment and the aquatic organisms' ingestion capability are essential for evaluating microplastics' potential effects and impacts on ecosystems. Sediment and clam samples obtained from the different sites of Panguil Bay, Southern Philippines, were positive for microplastics, predominantly black and blue colored filament/fiber and fragment particle type. Using ATR-FTIR spectroscopy, 7 polymer types were identified in sediments— ABS, CA, PE, PET, PP, PS, and PVC, whereas 10 polymers were identified in clams—AZ, EVA, PA6,

PE, PET, PP, PR, PS, PVC, and RY. These polymer materials came from fishing nets, rope materials, and food and drink wrappers/packages. All clams exposed to low-density polyethylene (LDPE) microplastic particles were found to have ingested the microplastic. The highest number of LDPE microplastic particles ingested within 7 days of laboratory exposure is 14, while the lowest is 1. Further studies in microplastics, especially in the Philippines, are needed to establish standardized sampling, extraction, and identification techniques. Also, food-related effects from microplastic ingestion are needed, especially for different food and microplastic exposure patterns. Bioaccumulation and biomagnification processes that might explain the pathways of microplastics in organisms' bodies should be of priority. Future research on the ecological and physiological impacts of microplastics should consider the physical characteristics of microplastics because they can significantly modulate how they are encountered and processed by biota.

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Data Availability All data are available in the manuscript and in the supplementary materials

Code Availability Not applicable

Declarations

Ethics Approval Not applicable

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