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CHARACTERIZATION AND QUANTIFICATION OF MICROPLASTICS IN SLIPPER-CUPPED OYSTER, *Crassostrea iredalei* (Faustino, 1932) FROM CAÑACAO BAY, CAVITE CITY, PHILIPPINES

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ABSTRACT – Microplastics are plastic particles that measure less than 5mm and pollute the marine environment and aquaculture areas. Microplastics pose risk on human health due to their capacity to adsorb heavy metals and retention in various trophic levels. This research aims to characterize and quantify the microplastics present in slipper-cupped oyster (*Crassostrea iredalei*) in Cañacao Bay, Cavite City, Southern Luzon, Philippines. The oyster shell lengths and widths were measured using Vernier caliper. The total shell and meat weights were determined using an analytical balance. The extracted soft tissues of the samples were digested using 10% Potassium Hydroxide (KOH) for 24 hours at 60˚C. The solutions were filtered using Whattman Grade 1 Quantitative Filter Paper (pore size: 11 micron). Microplastics were photographed, classified, and characterized using a stereomicroscope. Eight hundred twenty-seven (827) microplastics were collected and classified based on their appearance and characteristics; 817 were classified as microfibers and 10 were microplastic fragments. Microbeads and microfoams were not found in the collected samples. No significant relationship between the shell length, width and weights with the number of microplastic present ($p>0.05$). Results indicate that microplastics can be assimilated by the oyster regardless of their sizes and weights. This study confirmed the presence of microplastics in Philippine oyster, *C. iredalei* and important management strategies are recommended to reduce or prevent the microplastic inputs in the marine ecosystem and trophic levels.

Keywords: Crassostrea iredalei, microplastics, oyster

INTRODUCTION

Plastic is a multipurpose item that is widely present in everyday life. They are used in packaging, building sector, transportation, agricultural industry, and household. Their attributes such as durability, malleability, low weight and cost allow its use in various applications (Thompson et al. 2009; Andrady and Neal 2009). The annual plastic production increased from 1.5 million tons in the 1950s to 322 million tons in 2015 (Wright et al. 2013; Plastic Europe 2015). Every year, there is an estimated input of 9.5 million

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tons of new plastics (Boucher and Friot 2017). Plastic debris becomes brittle over time and fragments into smaller microplastics.

Microplastics are defined as small plastic particles with a diameter of < 5 mm (National Oceanic and Atmospheric Administration, 2015). Their presence in the marine environment is distributed through the currents (Lusher 2015). The classification of microplastics is based on whether they are produced to have a diameter of less than 5 mm or resulted from the breakdown of larger plastic debris (Kershaw 2015). Microplastics may also be classified as microbeads, fibers, fragments, sheets, foams, and granules based on their appearance and characteristics (Hidalgo-Ruiz et al. 2012; Virsek et al. 2016).

Microplastics have various industrial, commercial and pharmaceutical applications. Microplastics from microbeads are used in personal care products such as exfoliants in face scrubs. Microplastic fibers are shed from synthetic clothing and rope and from particles used in processes to clean boat hulls and large machinery (Thompson et al. 2004; Browne et al. 2007). Microplastics are also used to deliver drugs in some medical and are small enough to pass through wastewater treatment plants and enter aquatic ecosystems (Browne et al. 2007).

Filter feeding marine organisms such as bivalves are primarily susceptible to microplastic ingestion due to feeding and ventilation mechanisms of the gills (Moore et al. 2001; Wright et al. 2013; Avio et al. 2017). Ingestion of plastic debris has also been seen in wild seafood bivalves (Van Cauwenberghe and Janssen 2014). The ingested microplastics were reported to be translocated and accumulated to bivalve tissues (von Moos et al. 2012; Vermeiren et al. 2016). Microplastics were also found to move through aquatic food web (Farrell and Nelson 2013; Setala et al. 2014). Studies showed that microplastics consumed by farmed and wild oysters and other bivalves affected their growth, disrupted food passage in their guts and altered filter-feeding activities (Browne et al. 2008; Li et al. 2015; Green 2016). Microplastics in marine organisms have the adsorptive capacity for heavy metals (Ashton et al. 2010; Tien and Chen 2013; Rochman et al. 2014; Turner and Holmes 2015) which may pose risk to human health and marine ecosystem functions. Additionally, microplastics can be potential vectors of persistent organic pollutants (POPs) in marine fishes and bivalves and subsequently in humans (Lusher et al. 2017; Smith et al. 2018; Rodrigues et al. 2019). The potential health effects of microplastic on human health are particle and chemical toxicity which are dependent on microplastic size, shape, polymer type, density, chemical composition and absorptive dynamics (GESAMP 2015; Smith et al. 2018; Rodrigues et al. 2019). Microplastic might cause local effects on the gut epithelium, cell oxidative stress, enhanced inflammatory responses and fibrosis (Bouwmeester et al. 2015; GESAMP 2015; Schirinzi et al. 2017; Smith et al. 2018). Although, human health effects of microplastic are not well documented and fully understood (WHO 2019; GESAMP 2015).

Crassostrea iredalei, also known as slipper-cupped oyster, is the most common oyster species to be cultured and harvested for human consumption in the Philippines. This commercially important oyster is restricted to the Philippine archipelago and Malaysia and may reach a maximum shell length of 15 cm with an average of 8 cm (FAO 2016). *C. iredalei* has a large filtration capacity and filters almost 250 ml/h at 35 ppt (Chang et al. 2016) that may allow it to ingest microplastics and other toxic substances present in the marine environment. These oysters may serve not only as bioindicator of marine pollution but also important in addressing public seafood safety.

Studies on microplastic contamination in seafood for human consumption are vital in preventing and mitigating potential health impacts to the consuming public. In addition, microplastic researches provide information for aquaculture communities and local government in formulating and implementing guidelines for efficient plastic waste management especially in aquaculture areas.

In the Philippines, Argamino and Janairo (2016) confirmed the presence of microplastic in Asian green mussel, *Perna viridis* cultured in Bacoor Bay and provided management strategies of waste to mitigate the impacts of microplastics in the marine environment. Studies on microplastic contamination on Philippine bivalves and other seafood available for human consumption are lacking. Thus, this study was conducted to characterize and quantify the microplastics present in slipper-cupped oyster, *Crassostrea iredalei*, from Cañacao Bay oyster farm in Cavite City, Southern Luzon, Philippines. This study also determined the relationship of the shell dimensions (shell length and width) and weights (total weight and oyster meat weight) with the number of microplastics found in *C. iredalei* samples.

MATERIALS AND METHODS

Description of the Sampling Area

The sampling area is located in Cañacao Bay in Cavite City, Southern Luzon, Philippines where oyster farming is common. Cañacao Bay has mariculture farms that provide livelihood and seafood supply to local residents. Oyster farms are located in 17 provinces of the Philippines with major production areas in Cavite province which border Manila Bay (FAO 1989). Cañacao Bay is a waterway between Sangley Point and Cavite Point and constitutes a part of the larger Manila Bay. The province had earned the highest annual production of 1,578 metric tons of oyster in 2007 (Cayabyab and Reyes 2008). The coastal and marine waters of Cañacao Bay can be classified Class SA, for “waters suitable for the propagation, survival and harvesting of shellfish for commercial purposes” as per DENR (1997) Administrative Order No. 97-23.

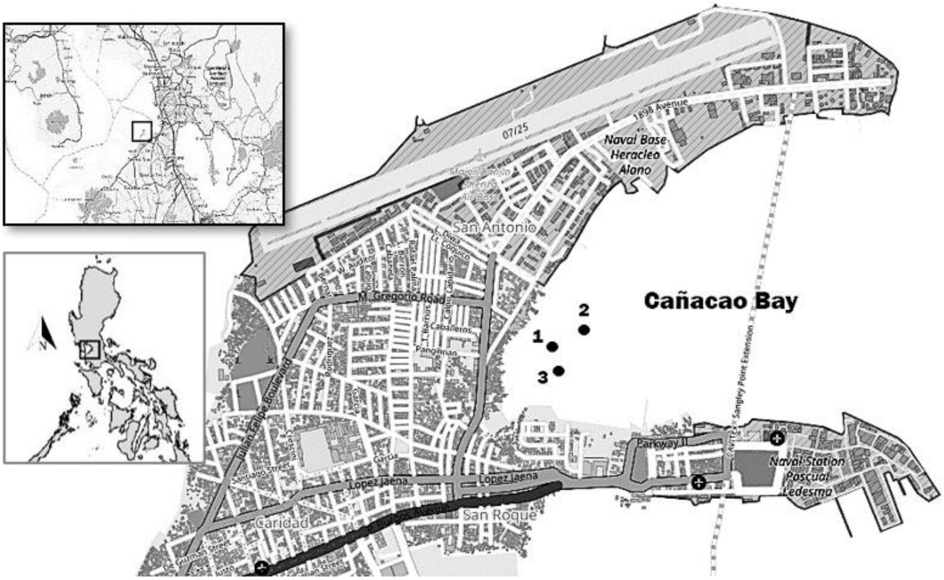


Figure 1. Map of the Cañacao Bay sampling area. Three sampling points were indicated with dots. (Source: OpenStreetMap).

Sampling, Collection and Identification of Oysters

Slipper-cupped oyster, *Crassostrea iredalei* were collected from three sampling points within the oyster farm of Cañacao Bay (Figure 1). A total of fifty (50) oyster samples were collected within 1-meter depth from the water surface. Seventeen (17) oyster samples were collected in sampling points 1 ($14^0$29’16”N, $120^0$54’21”E) and 2 ($14^0$29’12”N, $120^0$54’26”E), respectively. Sixteen (16) oyster samples were collected in sampling point 3 ($14^0$29’11”N, $120^0$54’19”E).

Immediately, the collected samples were covered with foil and placed on glass jars with metal lid and then placed in iceboxes during transport to the laboratory for analysis (Foekema et al. 2013). The collected samples were identified using morphological characters and available taxonomic keys (Harry 1985; Nawawi 1993; Leal 2002; Lam and Morton 2003; MolluscaBase 2018).

Precautionary Measures and Preparation of Samples for Acid Digestion

Precautionary measures were observed to prevent contamination by the airborne microplastics or by small plastic particles that may come from external sources. The extraction and filtration processes were conducted under the laminar flow cabinet pre-cleaned with ethanol. During the experiment, nitrile gloves were used in the cleaning and handling of the specimens. Equipment and glass wares used in the entire experiment were washed with deionized water three times (Foekema et al. 2013; Li et al. 2015). Furthermore, 100% cotton laboratory gowns were worn by the researchers in order to prevent plastic particles from clothes to contaminate the samples (Karami et al. 2017b). The collected oyster samples were cleaned using filtered deionized water (Karami et al. 2017a).

Measurement of bivalve morphometrics

Oysters’ shell length and width, total shell weight and meat weight were determined. A digital Vernier caliper was used to measure shell length and width. Total shell weights were weighed using a digital balance (OHAUS Explorer ® Analytical Balance) to the nearest 0.01g. The oyster meat was removed using a knife, weighed using a digital balance and sealed immediately with aluminum foil to minimize the risk of contamination from airborne microplastics (Foekema et al. 2013).

Acid Digestion of Oyster Samples

The extracted oyster meat inside the glass containers was soaked in 10% KOH solution. The amount of KOH added is three times the volume of the biological materials (Foekema et al. 2013). The oyster samples were subjected to 60 ºC incubation for a 24-hour period to an efficient digestion of biological tissues with no significant degradation on all tested polymers (Dehaut et al. 2016).

Filtration, Characterization, and Quantification of Microplastics

After acid digestion of the organic material in the samples, the resulting solutions were filtered using Whattman Quantitative Filter Paper Grade 1 (11-micron pore size). Visual inspection and characterization of microplastics under stereomicroscope (Olympus SZ661) was performed and microplastics were photographed for classification, characterization and quantification (Karami et al., 2017a). The microplastic classification and quantification were based on the Guide to Microplastic Identification by Hidalgo-Ruz et al. (2012). Characterization were described according to their visual features as set by Virsek et al. (2016) on Protocol for Microplastics Sampling on the Sea Surface and Sample Analysis.

Statistical Analysis

Linear regression analysis using IBM SPSS Statistics 20.0 (IBM, Armonk, NY) was used to analyze the relationship of shell morphometrics with the amount of microplastics in *Crassostrea iredalei*.

RESULTS AND DISCUSSION

Crassostrea iredalei oysters (n=50) were examined for the presence of microplastics (Figure 2). The shell length ranges from 52.78 – 77.31 mm (mean 62.12 ± 5.42), while shell width ranges from 33.28 – 64.17 mm (mean 45.33 ± 5.98). The shell weight ranges from 22.93 – 58.54 grams (mean = 35.51 g ± 8.27) and the meat weight ranges from 3.08 – 9.37 grams (mean = 5.51 g ± 1.46) (Table 1).

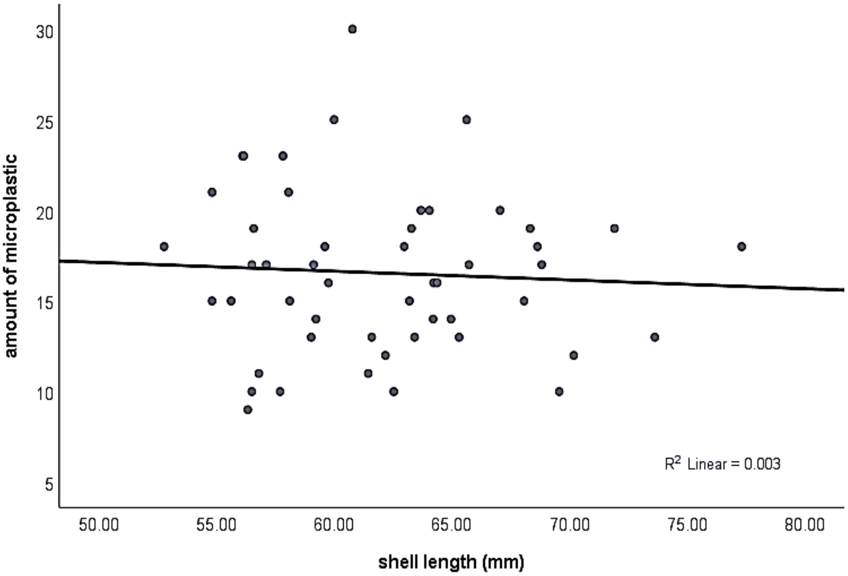
Table 1. Descriptive statistics of shell dimensions, weights, and amount of microplastics.

	Mean	**Std. Deviation**	**N**
Amount of microplastic	16.54	4.487	50
Shell length (mm)	62.1168	5.41573	50
Shell width (mm)	45.3294	5.97622	50
Total weight (g)	35.5104	8.26637	50
Meat weight (g)	5.5884	1.46148	50

Shell dimensions (length and width) and weights (total weight and meat weight) are positively correlated ($p< 0.05$). However, shell dimensions (length and width) and weights (total weight and meat weight) of *Crassostrea iredalei* samples were found to have no significant relationship with the amount of microplastics ($p > 0.05$). Regression analyses revealed that shell length ($F=0.164$, $df = 49$, $p = 0.687$) and shell width ($F = 0.298$, $df = 49$, $p = 0.588$) are not correlated with the amount of microplastic found in *C. iredalei*. Figures 3A and 3B depict the poor linear relationships of shell sizes (length and width) and the amount of microplastic present in the oyster samples.

Figure 2. *Crassostrea iredalei* oyster samples from Cañacao Bay. The upper right photo shows the oyster's visceral organs and the lower right photo shows the oyster's posterior adductor scar.

3A



3B

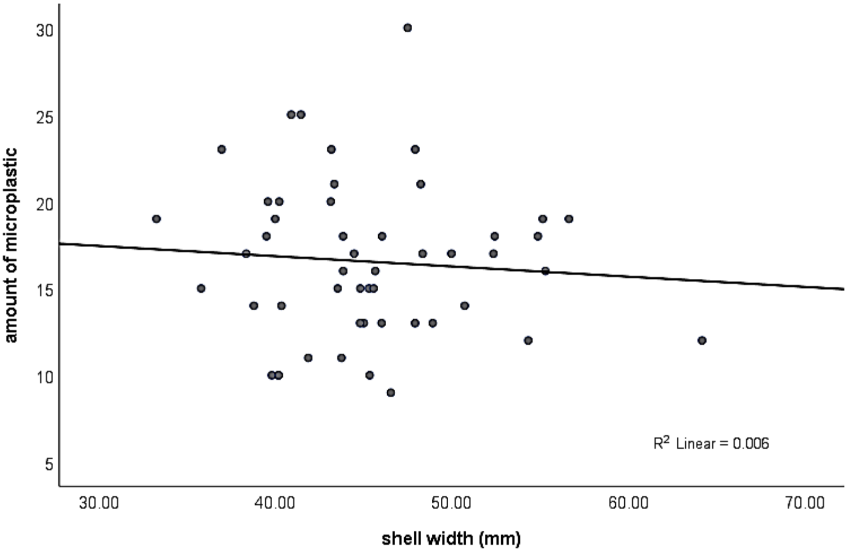


Figure 3. Scatterplots showing the poor linear relationships of A) shell length and the amount of microplastics ($R = 0.058$, $R^2 = 0.003$); and B) shell width and amount of microplastics ($R = 0.079$, $R^2 = 0.006$).

According to Ridgway et al. (2010), shell length is an indicator of age in oyster *Crassostrea gigas* rather than its capacity to retain microplastic in its system. Powell et al. (1992) also established no significant relationship between shell sizes (length and width) and filtration rates among oyster species they examined. Factors like temperature, salinity and concentration of suspended particle in the water affected filtration rates rather than shell sizes. Xu et al. (2017) suggests that the filtering process limits the retention of microplastics within the clam, *Atactodea striata* and may account for the quantities of microplastics not certainly proportional to oyster size.

Similarly, total shell weight (F = 0.215, df = 49, p = 0.645) and meat weight (F = 1.289, df = 49, p = 0.262) are not significantly correlated to the amount of microplastic found in the oyster samples. Linear regression analyses revealed that there are poor linear relationships between weights and the amount of microplastic present in the *C. iredalei* oyster tissues (Figures 4A and 4B).

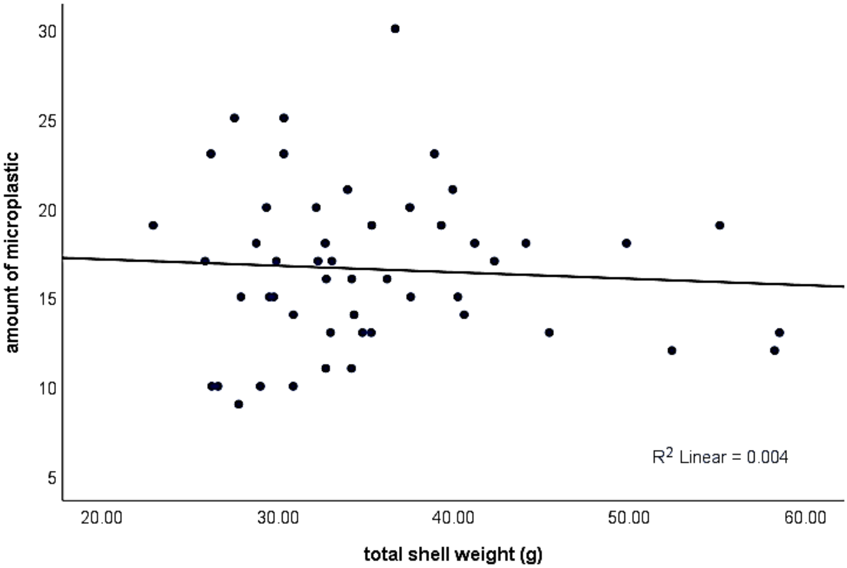
Baechler et al. (2020) revealed that no significant correlations were found in Pacific oysters and razor clams' shell length and number of microplastics. Same correlation results were found between body weight and the number of microplastics. Other factors such as filtration and particle retention are involved the ingestion and accumulation of microplastic in bivalve tissues. Oysters filtering large amount of water only means that they are supposed to be more exposed to more microplastics leading to a greater bioaccumulation per individual (Barille et al. 1997). Filtration of water was found higher in smaller oysters than larger ones that may result to higher microscopic yield (Shumway 1996). Mechanism of particle retention was basically of fluid mechanical nature (Jørgensen 1990) and not related to oyster weights.

Eight hundred twenty-seven (827) microplastics were identified from the fifty (50) samples of *Crassostrea iredalei*. Only two types of microplastics were quantified and classified: microfibers and microfragments (Figures 5 and 6). Microfibers were the most common type of microplastics with a total of 817 and microfragments with only 10 counts. The microplastic mean count is 16.54 ± 4.49 per organism.

This high concentrations of microplastics in bivalves are closely related to the plastic contamination of the living environments of bivalves (Li et al. 2015). The oyster farm sampling area is near a coastal residential community where domestic wastes are disposed in water bodies including diapers, cigarette butts and water used in cleaning and washing clothes. Browne (2015) suggested that microplastic fibers found in marine habitats may be derived from sewage as consequence of washing clothes. Synthetic fibers that are usually found in fabrics contributed in microplastic contamination in the marine ecosystem. Aside from domestic sources of microplastics, materials used in maricultures including nets, ropes and floaters can contribute to the microplastic pollution.

The increase in coastal populations has been observed to increase the amount of pollution entering waterways (Jambeck et al. 2015; Lebreton et al. 2017). Several macroplastics floating along the water bodies and covering the surface of the water were found at the location of the sampling area. These are the stakes made by the residents which the oysters and mussels cling onto for culturing. The presence of microplastics in the marine environment is predicted to rise in the future, due to increased production and fragmentation of existing plastics in the oceans (Bergmann et al. 2015). Production trends, usage patterns and changing demographics resulted to an increase in the incidence of plastics debris and, eventually, microplastics in the ocean environment. The study of Claessens et al. (2011) showed that elevated microplastic concentrations appeared to be primarily linked to the geophysical characteristics of the area (i.e. enclosed areas in harbours). The Cañacao Bay oyster farm is situated in an enclosed bay of the Cavite Peninsula which may be contributory factor to the high microplastic contaminations.

4A



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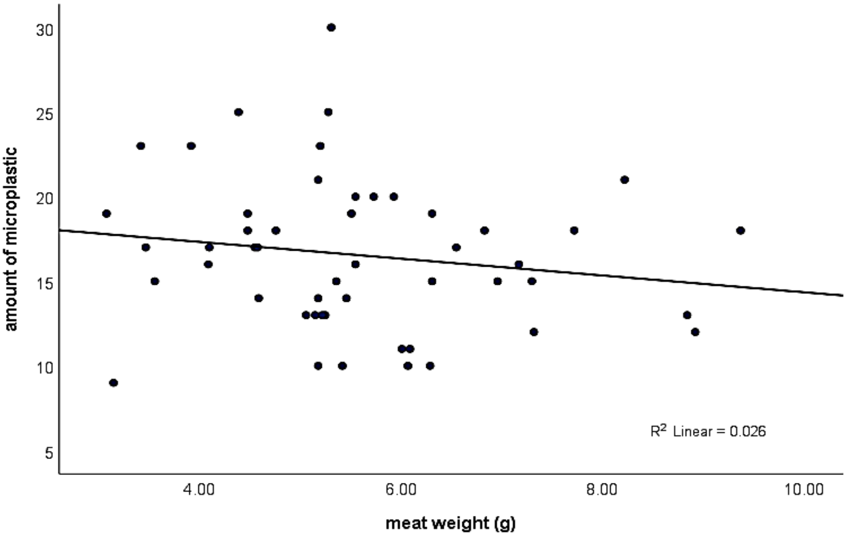

Figure 4. Scatterplots showing the poor linear relationships of A) total shell weight and amount of microplastic (R = 0.067, R^2 = 0.004); and B) meat weight and amount of microplastic (R = 0.162, R^2 = 0.026).

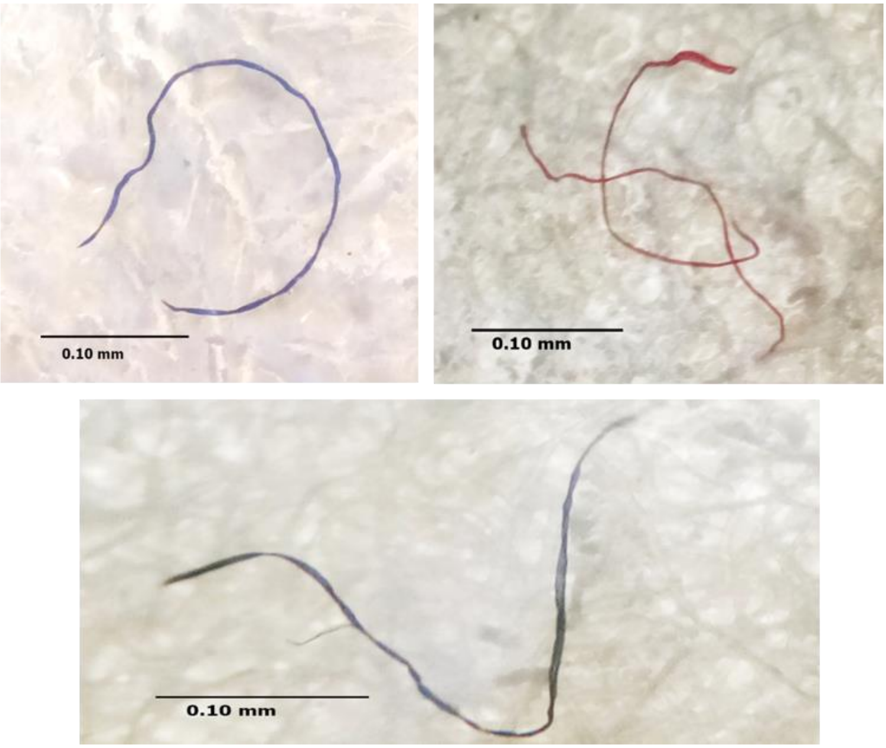

Figure 5. Microplastic fibers isolated from acid-digested *Crassostrea iredalei* tissues.

More than 98% of the total microplastic identified were characterized as microfibers (Figure 5) which is the most conspicuous among the three most abundant types of microplastics including microfragments and microbeads (Chubarenko et al. 2016). Sizes of the microplastic fibers that were found ranges from 0.3 mm to 1.0 mm in length. Similarly, Baechler et al. (2020) reported 99% of the microplastics that were quantified in Pacific oyster and razor clams are microfibers.

Microplastic beads and foams were not found in the samples which may be attributed to factors such as water density and accumulation and movement in the bottom sediments. Beads and foams may sink and accumulate in the bottom sediments, which allow it to be digested by benthic organisms (Claessens et al. 2011). Water density can also be a factor of the distribution of these microplastics. Microplastic particles commonly found in sediment had higher density than water, whereas low-density microplastic was commonly found on the surface of the water. In certain cases, low-density microplastic modified through the process of biofouling by prokaryotic, eukaryotic and invertebrates that increase the density of microplastic can also reach the bottom sediments (Andrady 2011; Reisser et al. 2013). These processes in the marine environment affected the absence of beads and foams in our samples. Although factors such as biofouling, turbulence and freshwater input may result in vertical mixing (Browne et al. 2007; Morét-Ferguson et al. 2010; Kukulka et al. 2012).

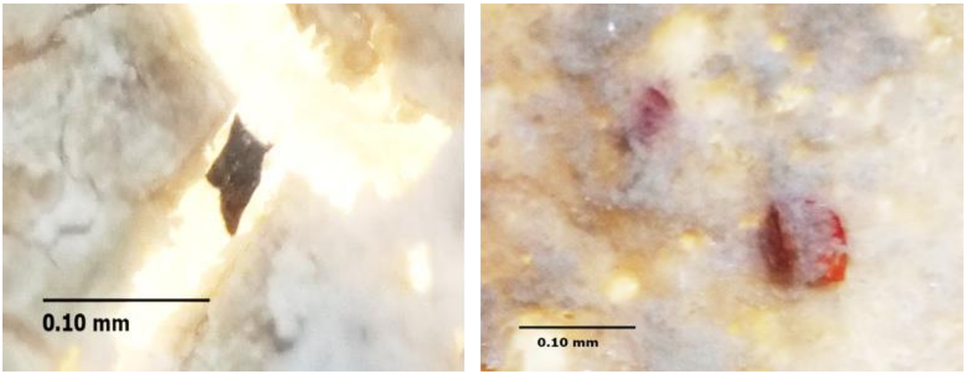

Figure 6. Microplastic fragments isolated from acid-digested *Crassostrea iredalei* tissue.

The identified microplastic fibers and fragments were characterized based on their distinct morphological features. Microfibers have string-like shape with irregular bends, soft and malleable texture, and with a wide range of color. Fragments have a thin and flat shape, hard and rigid texture, and color that is often black or red. The features of the microplastic fibers and fragments shown in the Figures 5 and 6 are consistent with the characteristics observed in the previous studies by Willis et al. (2017), Hidalgo-Ruz et al. (2017), and Virsek et al. (2016).

Virsek et al. (2016) characterized microplastics according to their visual features into: fragments, films, granules and foams. Fragments were rigid with sharp crooked edges and irregular in shape and in different colors. The films were thin and flexible and transparent. Granules were often smaller than pellets (less than 1 mm) and with natural colors. Foams have soft irregular shape with white to yellow color characteristics. Willis et al. (2017) characterized microplastic fiber as having string like shape with irregular bends and soft malleable texture and various color. Fragments have thin flat shape with hard and rigid texture and often black and red in color.

CONCLUSION AND RECOMMENDATION

This study confirms the presence of microplastics in the soft tissues of *Crassostrea iredalei* in Cañacao Bay oyster farm in Cavite City, Southern Luzon, Philippines. There is a total of 827 microplastics counts based on microscopic examination and characterization. Two types of microplastics were found: microfiber and microfragments. Microfibers were found to be the most dominant having 817 counts; microplastic fragments have 10 counts. Microplastic beads and foams were not seen but does not nullify their existence in the oyster farm sampling area.

The shell sizes (length and width) and weights (total weight and oyster meat weight) of the *Crassostrea iredalei* oyster do not show significant relationship with the amount of microplastic that can be ingested and bioaccumulated. *C. iredalei* could be used as bioindicator to confirm and monitor the presence of microplastic pollution in the marine environment.

It is recommended that proper aquaculture practices in the oyster farms must be observed to reduce microplastic contamination and appropriate and effective waste management (Argamino and Janairo 2016) must be established and observed in the residential communities and industries near Cañacao Bay, Cavite City, Southern Luzon, Philippines.

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STATEMENT OF AUTHORSHIP

The first author supervised the conduct of study, analyzed the data and contributed to the write-up and editing of the final manuscript. The second, third, fourth and fifth authors conducted the field sampling, laboratory experiments and write-up of the entire manuscript and the last author provided technical supervision and assistance.

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