Density of Microplastics in Philippine Cupped Oyster (Crassostrea iredalei)

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Abstract — Oysters have enemies that cannot be seen. Oyster farmers and consumers do not even know of the enemy's existence without the aid of a microscope. The purpose of this quantitative study was to investigate the accumulation of microplastics in the soft tissue of the Philippine Cupped Oyster (C. iredalei) as well as quantify the density, characterize the isolated microplastics in terms of color, size, and shape, and determine the composition and abundance for each type. Anchored on the concept of environmental health, and the theories of bioaccumulation and biomagnification, this study is concerned on the possible effects of microplastic ingestion by C. iredalei as an organism, and when consumed, its possible deleterious effects on other aquatic organisms and the risks it poses to human health. Findings revealed the presence of microplastics in the soft tissue with 100% frequency of occurrence and a mean density of 11.80 ± 9.69 microplastic particles/oyster. Diversified colors of microplastics were observed with transparent being the most abundant (54.80%). Irregularly shaped microplastics were dominant (56%) followed by thread-like microplastics (41%). The particles recovered varied in size with smaller particles being overwhelmingly frequent (91.31%) while larger particles (0.38%) were less frequent. Microplastic particles with a size range of 1.10 to 30.31 µm (68.36%) were most abundant. Isolated particles were mostly composed of fragments (54%), microfibers (40%), and a number of films (5%). Primary microplastics such as pellets were rarely found (1%). This investigation recommends water and sediment microplastic analysis to establish a relationship between the presence of microplastics in oysters and its environment. Research on the transport and fate of microplastics in oysters to assess the ecological risks of the negative effects of microplastics in estuarine environments and the exposure risks to human health is also recommended. Furthermore, it is recommended that a study on the extrusion through depuration should be conducted to evaluate whether this process aids in the elimination of microplastics from the soft tissues of oysters. Existing policies/programs/strategies related to plastic pollution and proper waste management for good aquaculture practices may be revisited and be given priority by concerned government agencies for oyster sustainability.

Keywords — Microplastics, Philippine Cupped Oyster, Organic Content, Accumulation, Composition, Concentration, Abundance, Bioaccumulation, Biomagnification

I. Introduction

Microplastics are tiny ubiquitous plastic particles present in marine environments, some of which, cannot be seen with the naked eye. They are not an individual entity, but constitute a cocktail of polymers and additives that has the ability to absorb substances from the surrounding environment, including living substances, nutrients and marine pollutants (Guzzetti, Sureda, Tejada, & Faggio, 2018). Marine debris such as microplastics is a serious threat to marine animals. While it has been documented that large pieces of litter can have dramatic impacts on marine animals, the dangers of plastics measuring less than five millimeters in size up to 1 millimeter, known as "microplastics" are less obvious (Betts, 2008; Hidalgo-Ruz, Gutow, Thompson, & Thiel, 2012; Jambeck et al., 2015; NOAA, 2016; Sharma & Chatterjee, 2017; Ritchie & Roser, 2018).

Today, it is an issue of increasing scientific concern because these microparticles which are very small in size are easily accessible to a wide range of aquatic organisms and ultimately transferred along the food web (Sharma & Chatterjee, 2017). Plastic pollution is considered a crucial environmental problem at present (UNEP, 2014; Blettler, Ulla, Rabuffetti, & Garello, 2017) and has been named as "one of the biggest environmental challenges of this lifetime" because of the persistent qualities and high pollution rates of plastics to the environment (UN Environment, 2018; Schwarz, Ligthart, Boukris, & Van Harmelen, 2019). Plastic pollution has been identified alongside climate change as an emerging environmental issue that might affect biological diversity and human health (Sutherland et al., 2010; Blettler et al., 2017).

According to Jambeck et al., (2015), in 2015, the global primary production of plastic was 381 million tonnes from 2 million tonnes in the 1950s indicating that plastic production has increased exponentially nearly 200 folds over the years and is expected to continue to increase year after year. Previous studies have implicated the Philippines as one of the highest contributors of plastics to the marine environment alongside China and Indonesia (Jambeck et al., (2015) contributing 1.88 million metric tons of plastics per year to the world's ocean plastic pollution (Jambeck et al. 2015; Ritchie & Roser, 2018).

Microplastics may fall within the optimal prey range for a variety of aquatic animals. This microplastic's size range makes it bioavailable for ingestion (Browne, Dissanayake, Galloway, Lowe, & Thompson, 2008; Cole et al., 2013; Germanov, Marshall, Bejder, Fossi, & Loneragan, 2018; Lusher, Welden, Sobral, & Cole, 2017). Some predatory fish might mistake plastic for food and filter-feeders might ingest them unintentionally while feeding volumes of water (BLASTIC, 2018). The ingestion of microplastics is considered as the most frequent interaction between plastic debris and marine organisms because it concerns a broader range of marine species (Lusher, 2015; Hermsen, Mintenig, Besseling, & Koelmans, 2018).

Recently, scientists have found microplastics in over 114 aquatic species, and more than half of these aquatic species end up on our dinner plates (Royte, 2018). The ubiquity of microplastics combined with its associated negative effects has raised concerns regarding marine species, ecosystems, and eventually the impact it may have on human health (Sharma & Chatterjee, 2017; Hermsen et al., 2018).

A study conducted by Bucol et al., 2020, was able to quantify and characterize microplastics that have been ingested by the rabbitfish (Siganus fuscescens), a commonly sold commercial fish from the Philippines. With this discovery, studies regarding the current condition of our marine and estuarine organisms will promote the development of methodologies or strategies to address the problem that will lead to a substantial contribution to the local and global community.

The biomonitoring of microplastics in oysters which are considered as one of the most commonly eaten seafood, will be able to help monitor both trends in local marine plastic pollution, and potentially evaluate the risk it may pose to human health through the consumption of such an important local seafood.

Literature Review

The degradation of plastic is a slow process, often taking hundreds to thousands of years. They will persist in the environment for a very long time and the probability of it being ingested, be chemically and biologically contaminated, and the accumulation of it in the bodies and tissues of many organisms is very high. The toxic chemicals it contains that come from both the ocean and water runoff can also have high chance to biomagnify up the food chain. Communities with strong reliance on seafood will likely experience and feel the human health impacts of marine plastics. Seafood consumption places humans at risk for microplastic exposure. As of 2015, 6.7% of all protein consumed was represented by the global seafood and approximately 17% of animal protein consumption (FAO, 2018).

Filter feeding organisms such as the Philippine cupped oyster, are susceptible to ingest microplastics directly from plastic polluted water or indirectly through contaminated planktonic prey (Germanov et al., 2018). Studies have shown that the abundance of microplastics is generally higher in shellfish than in fish. The abundance of microplastics in shellfish can be attributed to the its feeding strategy since most of them are filter feeders. Filter feeders, such as bivalves, oysters, and clams, display a non-selective feeding behavior and, therefore (Wesch, Bredimus, Paulus, & Klein, 2016; Hantoro et. al., 2019), have high probability of ingesting these damaging particles called microplastics as they filter through large volumes of water daily to obtain adequate nutrition (Germanov et al., 2018; Wesch, Bredimus, Paulus, & Klein, 2016; Hantoro et. al., 2019).

Li et al., (2018), recommend oysters as bio monitors for the microplastic pollution in estuaries after conducting a study which investigated microplastic pollution in wild oysters Saccostrea cucullata from 11 sampling sites along the Pearl River Estuary in South China. The microplastic abundances in oysters ranged from 1.4 to 7.0 items per individual or from 1.5 to 7.2 items per gram tissue wet weight, which were positively related to those in surrounding waters. The oysters near urban areas contained significantly more microplastics than those near rural areas. Fibers accounted for 69.4% of the total microplastics in oysters. Microplastic sizes varied from 20 to 5000 mm and 83.9% of which were less than 100 mm. Light color microplastics were significantly more common than dark color ones.

According to Barboza et al., (2018), microplastics could be causing ecological effects such as stunting growth and depressing reproduction of aquatic organisms. Evidences were found indicating that in the wild, especially in heavily industrialized and urbanized areas with high concentrations of plastic debris, there is a decline of population and at least some of them could decrease in abundance over time, with potential adverse environmental health consequences and negative effects to biodiversity conservation, ecosystem services, and may affect human food security, reducing food availability for the human population. Thus, more studies on the effects of microplastics are needed to properly assess and manage future risks, with special focus on the long-term effects induced by the exposure and contamination to ecologically relevant concentrations of microplastics of marine organisms that are commonly found in the environment (Barboza et al., 2018).

II. Methodology

Laboratory Preparation and Isolation of Microplastics from the Organism

All materials, equipment, and laboratory surfaces were thoroughly washed and rinsed; afterward, all materials were kept under clean air conditions. Vessels and tools were rinsed three times with distilled water and were covered with aluminum foil before and after use. Used solutions and filters were checked for contamination before use; the same applied for the outside of the sample specimens. Cotton clothing which is 100% natural fiber were worn while working with the samples to avoid contamination. Laboratory gown was worn during processing of samples.

Sampling, Processing, and Storage

This study employed field sampling. A total of 45 individuals of the oyster, C. iredalei were collected from the Liboran River of Barangays Linabo, and Banonong, and Pulauan River of San Pedro. Each sample were placed inside a labeled zipped bag and were transferred to the laboratory on ice immediately and then kept frozen inside a freezer for 24 hours for subsequent analysis. After 24 hours of being kept in the freezer, oysters were thawed, shell length were measured using calipers, and shucked. Each soft tissue was weighed (in grams) to two decimal places using a portable balance (Scout Pro) and was placed into a labeled 100mL test tube and covered with aluminum foil immediately to avoid contamination.

A replicate of three positive and three negative controls were included for each batch of samples and treated in parallel to the sample treatment. Negative control was in place by placing clean Petri dishes next to the sample, and checked for any occurred air- borne contamination. Positive control was implemented by adding Six (6) microplastic particles of known type and of targeted sizes to "clean" samples, which were then treated and analyzed the same way as the actual samples. particles were intentionally added to each positive control samples to confirm whether the 10% KOH solution will dissolve the plastic particles. All 6 (six) particles from each positive control were recovered intact.

Following Foekema et al., (2013) and as adapted and confirmed effective by Kühn et al., (2017) and Munno, Helm, Jackson, Rochman & Sims (2018), to be able to extract and characterize microplastics without degrading plastic polymers in the soft tissue of the Philippine Cupped Oyster (C. iredalei), the entire organic specimen of each sample was left for digestion in 10mL 10% KOH solution and kept at room temperature for 3 weeks. Following complete digestion, 20 mL of saturated NaCl solution was added to each test tube to float the microplastics. After 24 hours of floatation at room temperature, the overlying water were vacuum filtered through a 20 mm pore size, 47 mm diameter filter. Each filter was placed into a clean petri dish with a cover for observation under a stereoscopic microscope with a digital camera (OMAX, MD827S30). Photographs for each suspected microplastic particle were taken and then identified according to their morphological characteristics using ScopeImage 9.0 v.9.3.3.426 (Sep 22 2016). Plastic particles were identified based on lack of cellular structure, uniformity of shape in the case of microfibers and microbeads, through their bright colors and smooth appearances and homogenous thickness and gloss across the particles. Microplastics were categorized based on its type, shape, and size. To measure the microplastic's size, the fibers were measured along their actual length, while the fragments, films and pellets were measured through their longest dimension. The sizes, shapes and colors of microplastics on filters were identified and recorded as described in a previous study (Li et al., 2015; Hermsen et. al., 2018).

III. Results and Discussion

The mean (± S.D.) shell length for all the forty-five (45) oysters collected from Barangay San Pedro, Barangay Linabo, and Barangay Banonong was 5.39 cm ± 1.04. At the same time, the mean (± S.D.) weight of soft tissue per oysters was 4.31 g ± 2.99 g (Table 1).

Table 1. Mean (± standard deviation) of Shell Length and Mean Weights of Soft Tissue for Philippine Cupped Oyster (C. iredalei) at Each Sampling Site

Sampling Site	Mean shell length (cm)	Mean weight of soft tissue (g)
San Pedro	4.93 ± 0.48	3.67 ± 0.75
Banonong	4.88 ± 0.65	3.17 ± 0.63
Linabo	6.33 ± 1.21	6.08 ± 3.01
Overall	5.38 ± 1.04	4.31 ± 2.99

[a] (n=15/site)

Upon microscopic evaluation, all forty-five (45) Philippine Cupped Oysters contained microplastics in their soft tissue thereby making the frequency of occurrence of microplastics 100% with a density of 11.8 particles per oyster ± 9.69 (Table 2). The highest density of microplastics came from samples collected from Barangay Banonong with a mean density of 13.3 particle/oyster ± 13.62 particle/oyster (Table 2). Diversified colors of microplastics observed were transparent, black, gray, and also colored microplastics (brown, blue, white, yellow, red, pink, and orange). Transparent microplastics were found to be the most abundant. Irregular shaped microplastics were the most dominant particle shape taking up 56% of all the shapes that were identified followed by thread-like microfibers (41%), elongated fragments and films (2%) and round fragments and pellets (1%). The microplastics recovered varied in size with the smaller particles being frequent (93.76%) and larger particles being less frequent (0.38%). Microplastic particles with a size range of 1.10 to 30.31 µm (68.36%) were the most abundant.

The composition of microplastics isolated from the soft tissue of the Philippine Cupped Oyster (C. iredalei) were mostly fragments, microfibers, films and pellets. Fragments were the most abundant type of microplastics comprising 54% of the total microplastics that were isolated from the samples, followed by microfibers (40%), films (5%) and pellets (1%).

Table 2. *Summary on the Composition and Density of Microplastics*

Category	**Type**	**Result**
Microplastic Types	Fragment	54.23%
	Microfiber	40.68%
	Film	4.71%
	Pellet	0.38%
Microplastic Colors	Transparent	54.80%
	Black	18.46%
	Gray	7.34%
	Brown	5.46%
	Blue	4.52%
	White	3.77%
	Red	2.82%
	Yellow	1.88%
	Pink	0.56%
	Orange	0.38%
Microplastic Shapes	Irregular	55.56%
	Thread-like	40.68%
	Elongated	2.44%
	Round	1.32%
Microplastic Sizes	Small (≤ 147.19 µm)	93.76%
	Medium (> 147.20 µm and is ≤ 293.29 µm)	5.86%
	Large (> 239.30 µm)	0.38%
	San Pedro	100.00%
Frequency of Occurrence	Banonong	100.00%
	Linabo	100.00%
Mean Density	San Pedro	11.06 ± 8.51
No. of microplastic	Banonong	13.33 ± 13.62
particles/oyster	Linabo	10.66 ± 3.33

IV. Conclusion

The Philippine Cupped Oyster (C. iredalei) is able to ingest and accumulate microplastics of varying types, colors, sizes, and shapes in its soft tissue. All oysters that are grown in the sampling sites of Barangay San Pedro, Barangay Linabo, and Barangay Banonong contain microplastics in their soft tissues. The result shows that oysters are more prone to ingest and accumulate smaller sized microplastics and diversified color of microplastic particles. It can also be concluded that irregularly shaped, thread like and round microplastics can be up taken by oysters. Among the different types, most of the plastic wastes in the estuaries of Dapitan City have gone through fragmentation as microplastic fragments are the most abundant type. Majority of the microplastics that were isolated from the soft tissue of the oyster samples were mostly secondary microplastics derived from the fragmentation of larger plastics. The presence of microplastics in the soft tissue of the Philippine Cupped Oyster (C. iredalei) indicates that the Dapitan City River is polluted with plastic wastes of varying types, colors, sizes, and shapes that may have been unintentionally or intentionally dumped or thrown in the river. Other marine organisms are at risk of ingesting these polymers thereby possibly damaging their health, growth, reproduction, and can be biomagnified by transferring from one trophic level to another. Human health may be at risk since human beings are known consumers of these bivalves which are eaten whole.

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