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Microplastics in farmed oysters (*Crassostrea iredalei*) from Capiz, Philippines

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Abstract

Microplastics (plastic particles <5 mm in size) have been increasingly abundant, especially in filter-feeders. Oysters are commercially farmed and highly consumed in the Philippines. This study determined the presence of microplastics in commercial oysters (*Crassostrea iredalei*) sold in public markets from Cagay, Culajao, and Ivisan, Capiz. Thirty oysters yielded 47.6 g of meat, which were homogenized, divided into nine portions for KOH digestion, and incubated at 40 °C for 48 h. The digestates were passed through 25 µm Whatman filter paper. The retained particles were viewed under a microscope, identified by GESAMP descriptors, photographed, counted, and measured. Three residues were analyzed by FTIR spectroscopy to identify the chemical origin of the microplastics. The study confirmed the presence of microplastics in the oysters. The 38 microplastic particles were mostly fibers with some sheets. Their sizes ranged from 109 µm to 3.3 mm, and did not significantly differ among the three source locations. Only the sheet particle had a 49% match with cellophane.

Introduction. - Microplastics are plastic particles with sizes less than 5 mm that have become the most abundant pollutant in the aquatic environment [1,2]. Two types of microplastics exist: primary and secondary. Primary microplastics are those manufactured to size and secondary microplastics are tiny fragments from degraded larger plastics. Microplastics enter the seas directly as marine litter from shipping and fishing, or indirectly as solid wastes from land through rivers and estuaries [3, 4]. Encounters and interactions between microplastics and marine organisms are inevitable and problematic and such interactions will continue to increase as microplastics continue to accumulate over time [1].

Microplastics are imminent threats to marine biota because they are ingested and as a result, they have been found in the bodies of various marine animals including fishes [5,6] and bivalves [7,8], consequently causing digestive blockage, organ damage, low birth and growth rates, and reproductive failure [9,10]. Over time microplastics extend their effects to humans through the consumption of seafood [11] as a result of bioaccumulation]. The effects of microplastics on human health are unclear but the severity of adverse effects can depend on the toxicity of the chemicals used to produce the plastic [12].

There is huge potential for microplastics to adversely impact populations of marine animals and the Filipinos who consume them. Fish and other seafood account for 15.5% of the total protein intake in the Philippines [13] and the per capita consumption of crustaceans and mollusks is about 3 kg/year [14]. In 2019, Capiz is the top producer of fish and marine products in Western Visayas, including 31% of the aquaculture products from the region [15]. In Roxas City alone, mariculture areas produce about 513 metric tons of grouper, oysters, and mussels annually [16]. Thus, due to the considerable role of Capiz in producing commercial seafood, it is important to obtain data on microplastics found in farmed and marketed oysters (*Crassostrea iredalei*) from the aforementioned locale.

In summary, this study aims to identify and assess microplastics in commercial oysters (*C. iredalei*) from selected areas in Capiz. Specifically, it aims to:

(i) extract microplastics from oysters from three locations in Capiz;

(ii) describe, photograph, count, and measure the extracted microplastics;

(iii) identify the chemical origin of the microplastic particles; and

(iv) compare the microplastics found in oysters from the three locations.

The results of this study will benefit future studies that aim to further investigate and assess the

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presence of microplastics in marine environments and organisms. Furthermore, this study may also be a baseline for future studies concerning the occurrence of microplastics in the food chain. The use of KOH digestion adapted from Thiele et. al. [7] can be used as a reference for future bivalve tissue digestion for microplastic extraction.

Methods. - This study aims to identify and assess the presence of microplastics in *C. iredalei* through visual inspection, FTIR analysis, and statistical analysis.

Site Selection. Site selection was done through purposive sampling. Various seafood restaurants in Iloilo were contacted and inquired about their oyster supply, and most referred to Capiz as the source. Study sites were selected based on (1) the presence of oyster collectors or oyster farms; (2) availability and abundance of marketable oysters; (3) the oyster species was *C. iredalei*; and (4) presence of local sources of pollution. Three locations were then chosen- Cagay, Culajao, and Ivisan, Capiz (Figure 1) with the following latitude longitude coordinates: 11°35'47.8"N 122°46'39.2"E, 11°34'49.9"N 122°45'20.2"E, and 11°31'00.4"N 122°41'37.7"E respectively. All three locations had high levels of human populations, settlements, and commercial activities including fishing and fish farming.

Figure 1. Satellite image of the three sample locations taken using Google Maps.

Oyster Sampling. *C. iredalei* is easy to identify by its cup-shaped or slipper-shaped shell and the purple adductor muscle scar. The species identification was confirmed by the Southeast Asian Fisheries Development Center (SEAFDEC) in Tigbauan, Iloilo. Oyster samples were collected during the cool dry season (January, 2020) from a fish farm in Cagay and from the wet markets in Culajao and Ivisan, Capiz. A total of 15–20 oysters per site, all alive with shells shut tight. They were transported to PSHS-WVC and refrigerated at 3–6 °C for processing within 3 days. Ten live oysters of shell height 5–10 cm were chosen from each site sample for microplastic extraction. Oysters of this size were considered adults with well-developed digestive tracts, active feeding behavior, and high potential for ingestion of microplastics. The oysters were washed clean with tap water, blot-dried, then dissected in a laminar-flow cabinet to avoid contamination. To remove the oyster meat, the shell was opened by sliding a knife or scalpel between the valves and cutting the adductor muscle. The meat from 10 oysters from each site were pooled and weighed on an analytical balance. Oyster sizes (shell heights) did not differ significantly by location), but the larger oysters from Ivisan, Capiz yielded more meat.

Extraction of Microplastics. The pooled oyster meat from each source location was homogenized by combining the meat and divided into three equal weights in three beakers. The homogenates in nine beakers were chemically digested in a 10% w/v KOH solution at three times the meat volume [17]. The stock solution 10% KOH solution was prepared with 20 g KOH pellets dissolved in 200 ml distilled water. The homogenates were then incubated without agitation at 40°C for 48 h in an incubator (Biobase). Subsequently, the resulting digestates were poured on Whatman No. 4 qualitative filter paper with a pore size of 20–25 µm. The papers with residues were then placed in separate petri dishes, air-dried for 6 h and examined for microplastics. A blank sample, 10% KOH solution without oyster meat, was processed through all procedures to quantify possible microplastics contamination in the laboratory.

Visual Assessment by Microscopy. The residues were first subjected to visual assessment through microscopy at 40x magnification as the microplastic particles cannot be seen by the naked eye. The residues were viewed under a digital microscope connected to a laptop (Dell Inspiron 14 8th generation). Microplastics were identified and sorted by type according to the Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection (GESAMP) [2] guidelines. The computer program Image Focus 4 was used to take images of the microplastics on filter papers. The microplastic particles were counted on 5x5 mm grids. The ImageJ and AmScope affiliate software were then used to measure the particle sizes.

FTIR Spectroscopy. Three of the extracted microplastics, named 'single fiber', 'fiber cluster', and 'sheet microplastic' were sent to the University of the Philippines Regional Research Center (UP-RRC) for Fourier Transform Infrared spectroscopy with a Thermo Scientific Nicolet iS5). This is in order to identify what type of plastic the microplastics originated from. The resulting FTIR spectra were compared or matched with the polymer database in the FTIR spectra library of UP-RRC. The analysis of the spectra was verified by a PSHS-WVC chemistry teacher.

Data Analysis. Data on oyster sizes and microplastic particle sizes were tabulated by source location and the means ± standard deviation were computed. The data were tested for normality via Shapiro-Wilk test. Furthermore, the means were compared by source location (one-way ANOVA at a 95% significance level). When the data were not normally distributed, the non-parametric Kruskal-Wallis test was used. All computations were done using the IBM® SPSS® Statistics v27 and RStudio© v3.6.1 software. The results were verified by a Statistics teacher from PSHS-WVC.

Safety Procedure. Use of personal protective equipment was observed during all laboratory work. Practice trials were conducted of all procedures from dissection of the oysters to extraction of microplastics

to microscopy. Furthermore, the empty oyster shells were bagged for disposal by the PSHS-WVC COOP personnel. Then, the used KOH solution was stored in plastic bottles and turned over to the Science Research Assistant at PSHS-WVC for proper disposal. Lastly, the researchers cleaned the work areas and organized the glassware after every use.

Results and Discussion. - This study aims to identify and assess microplastics in commercial oysters from selected areas in Capiz. Specifically, it aims to: (1) extract microplastics from oysters by means of KOH digestion and filtration; (2) describe, photograph, count, and measure the extracted microplastics under a digital microscope; (3) identify the chemical origin of the microplastic particles by spectroscopy; and (4) compare the microplastics found in oysters from three locations in Capiz.

Occurrence of Microplastics. A total of 38 microplastic particles were extracted from 47.6 g of meat from 30 oysters from the three sites in Capiz (Table 1). The data confirmed the occurrence of microplastics in farmed and marketed *C. iredalei* in Capiz — at least one microplastic particle per oyster regardless of source location. In other words, one microplastic particle was extracted for every 1.5 g of oyster meat.

Table 1. Counts and types of microplastic particles in oysters from three sites in Capiz, Philippines. (NO - Number of Oysters, TW - Total weight of oyster meat, TMPs - Total microplastics (NT + NS), NT - Number of threads, NS - Number of sheets)

Site	NO	TW (g)	TMPs (NT+NS)	NT	NS
Cagay	10	13.84	12	8	4
Culajao	10	15.48	15	13	2
Ivisan	10	18.28	11	10	1

This study confirmed the presence of microplastics in commercial oysters *C. iredalei* from raft farms and wet markets in Capiz, Philippines. Most particles were blue fibers, which are possibly remnants of fishing nets. Some were sheets that matched cellophane, a common packaging material. Cellophane is a polymeric cellulose film produced from processing cellulose from wood, cotton, hemp, or other sources. Some cellophane are coated with polyethylene or other polymers to make it heat sealable for automated wrapping machines. Though labeled as "biodegradable plastic," cellophane will break down completely only when being subjected to prolonged temperatures above 50 °C [10].

Particle Sizes of Microplastics. All the 38 (sum of all TMPs) extracted particles were <3.3 mm in their greatest dimension (Table 2) and thus were microplastic by definition. The smallest particle was 109 µm, which is much larger than the 20-25 µm pore size of the Whatman No. 4 filter paper that was used. The particle sizes apparently differed among locations, with mean sizes highest in Cagay and lowest in Ivisan (Table 2). However, the differences were not statistically significant (Kruskal-Wallis test, $p = 0.28$). Furthermore, a non-parametric test was used because the size data were not normally distributed (Shapiro-Wilk test, $p < 0.05$).

Table 2. Particle sizes (greatest dimension) of microplastics in oysters from three sites in Capiz. (NP - Number of Particles)

Sites	NP	Particle size (mm)			
		Min	Max	Mean	SD
Cagay	12	0.165	3.207	1.506	0.961
Culajao	15	0.123	2.928	1.266	0.967
Ivisan	11	0.109	3.260	0.951	0.975

The microplastics found in oysters in this study had particle sizes of 0.11–3.25 mm, larger than those (0.1–0.3 mm) found in green mussels in Bacoor Bay [18], but similar to those (0.56–4.58 mm) found in the waters of Pasig River [19]. Much smaller microplastics of sizes 2–6 µm adversely affected reproduction in the oysters studied by Sussarellu et. al. [20]. It remains to be determined whether smaller microplastics, and even the larger microplastics that were found, would also be harmful to *C. iredalei.*

Types and Composition. The microplastics left on the filter paper were mostly thin blue fibers but some were sheet-like and white or transparent. The 38 microplastic particles consisted of fibers (81.58%) with some sheets (18.42%) (Table 1). Digital images of the extracted microplastic particles are shown in Figure 1.

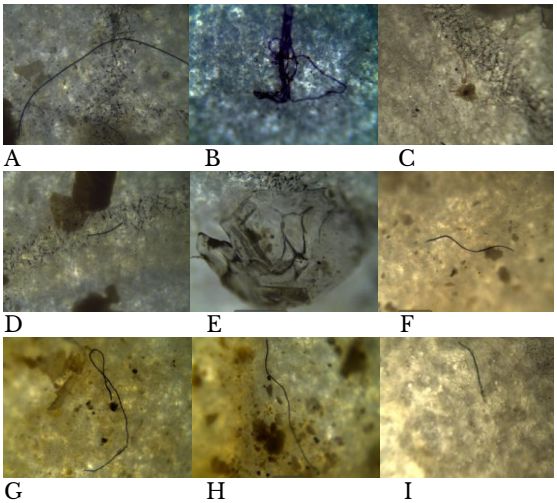


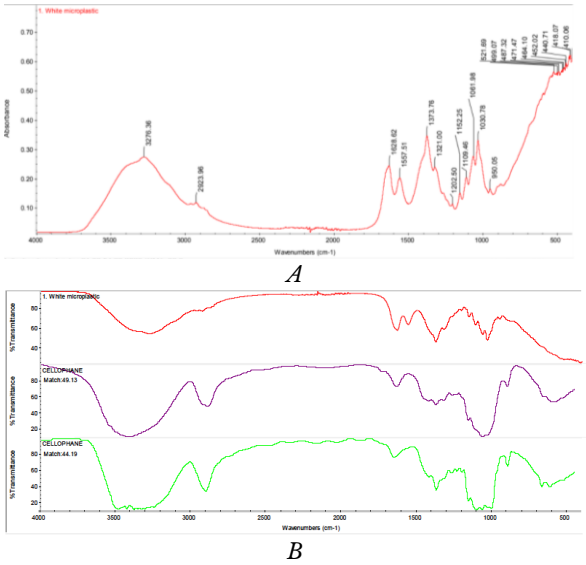
Figure 1. Microplastic particles extracted from farmed oysters from Capiz. Images taken by digital microscope of the residues on three filter papers per location. A-C Cagay; D-F Culajao; G-I Ivisan.

Indeed the study sites confirmed the extensive use of plastic nets, ropes, containers, and other gear in locations consume a variety of plastic products and packaging materials that were plainly visible as fishing and fish farming. Furthermore, it can be inferred that numerous households in the three

scattered litter: sachets of shampoo and coffee, single-use bags, wrappers, jars, etc. Markets and tourism and recreational activities were also sources of plastic pollution at the study sites.

Chemical Origin of Microplastics. The microplastics analyzed by FTIR spectroscopy are shown in Figure 5. 'Sheet microplastic' is image E. 'Single fiber' is image A and 'fiber cluster' is image G.

Figure 2 shows the FTIR spectrum of the 'sheet microplastic' together with the library matching, which yielded a 49% match with cellophane. A 49% match under normal conditions is not considered an ideal match rate, however, with the nature of these microplastic samples, it is deemed acceptable. The samples were dried insufficiently due to lack of supporting articles, and with this, saltwater residues and oxidized groups interfered in the results, thus producing a low match rate. In contrast, the FTIR spectra for 'single fiber' and 'fiber cluster' showed no match with any polymer in the database (Figures 3 and 4).

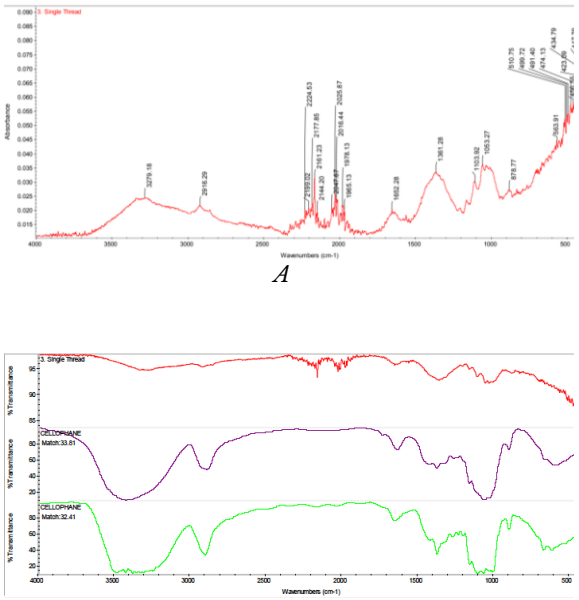


A

B

Figure 2. A The FTIR spectrum of sheet microplastic; B Library match with cellophane, 49%.

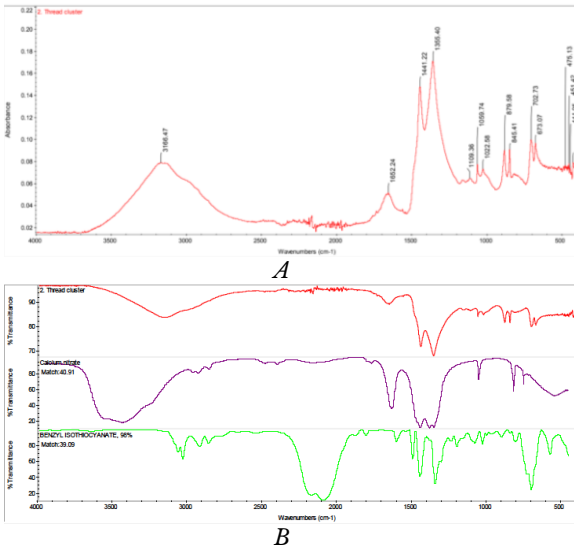
The fibers and sheets that were analyzed by FTIR spectroscopy could not be matched with any polymer in the UP-RRC spectra database. This result was probably due to insufficient drying of the fibers in the residue on the filter paper. Furthermore, the structural identity of the microplastic samples was also compromised given the changes in the chemical structures of the samples (e.g. bond breakage, etc.). Da Costa et al. [21] reported that the physical and chemical properties of a microplastic particle are affected by prolonged exposure to saltwater. The formation of oxidized groups from exposure to water results in noise when the spectroscopy test was conducted. While it is difficult to identify the primary causes of noise in spectroscopy, deficiencies in sample preparation/handling is considered a probable cause.



A

B

Figure 3. A The FTIR spectrum of 'single fiber' microplastic; B Library match not found.



A

B

Figure 4. A The FTIR spectrum of 'fiber cluster' microplastic; B Library match not found.

Limitations. Cellulose filter paper was unavailable during the conduct of the laboratory procedures and Whatman® Grade 4 qualitative filter paper was used instead. Thus, particle retention was limited to 25 µm. Secondly, the Fourier Transform Infrared (FTIR) spectra were only compared to pre-identified polymer databases. Thus, other non-polymer spectra were not subjected for comparison.

Conclusion. - Microplastic presence was confirmed in commercial oysters *C. iredalei* collected from three various locations in Capiz with Culajao yielding the highest number of microplastics among the three locations. The abundance of microplastic fiber found and the identification of cellophane in 'white microplastic' suggest that the source of microplastics come from maritime activities such as fishing and improper disposal of household waste in the area. Statistical analysis concludes that the

location of the collection sites do not directly affect the microplastic size.

Recommendations. - To improve the study, it is recommended that cellulose filter paper with pore size lower than 25 µm should be used during filtration to enable the extraction of microplastics that have a diameter less than 25 µm. Additionally, air-drying the filter papers used during filtration at room temperature can be used as an alternative to oven-drying, which will greatly enhance the generated IR spectra as water will no longer be factored in the FTIR analysis. It is also recommended that a library match with a more extensive polymer library should be conducted to yield a more specific polymer type. Furthermore, the microplastics extracted may be weighed using more precise weighing scales to calculate microplastic abundance in milligrams microplastic/sample mass or for sediment studies, milligrams microplastic/kg^{-1} sediment. Finally, it is recommended that the results of this study are communicated to local government units to allow oyster farmers to reevaluate the conditions of their farm and create appropriate measures to reduce microplastic pollution such as imposing strict guidelines on proper waste disposal.

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