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Abundance and characteristics of microplastic in cultured green mussels *Perna viridis* in Sorsogon Bay, Philippines

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Abstract

Microplastic ingestion by marine organisms is becoming an emergency threat to seafood industry, with farmed mussels as of particular interest. The context of trophic transfer accords that humans are at large into exposure to microplastic through its consumption. In the province of Sorsogon, Philippines, green mussel *Perna viridis* is sorted into various 'grading label': *Small* (5.0-6.9 cm), *Medium* (7.0-8.9 cm) and *Jumbo* (≥ 9.0 cm) and are marketed within and outside the province. Total microplastic varied from 0.31 to 2.5 items/ individual. Mussel size ranged from 5.0-6.9 cm showed the highest microplastics (2.57 items/ individual) while mussels below 2.9 cm has the least microplastic (0.31 item/individual). The majority of ingested microplastics were lines, while their colors and sizes varied. Fourier Transform Infrared Spectroscopy (FT-IR) indicated *organosiloxane* and *polyethylene terephthalate* as the most common polymer type identified. The results suggested that microplastics detected in the mussels are relatively within the narrow range with no significant differences of its distribution across its categorical sizes. With the results can be used as a baseline contribution for the risk assessment of microplastic pollution in Sorsogon bay.

Keywords: microplastic, FTIR, mussel, Sorsogon

Introduction

Previous studies have implicated the Philippines as one of the highest contributors of plastics to the marine environment ranking 3rd, contributing 0.28–0.75 million metric tons of marine plastic per year (Jambeck *et al.*, 2015; Lebreton *et al.* 2017) ^[1, 2]. Microplastics, defined as plastic materials or fragments <5 mm, are most likely the most numerically abundant plastic debris items in the ocean today (Law and Thompson, 2014) ^[3]. The quantities of microplastics will inevitably increase due to the degradation of large, single plastic items, ultimately breaking down into millions of microplastic pieces (Cozar *et al.*, 2014) ^[4].

One of the primary environmental risks associated with microplastics is their bioavailability to marine organisms (Wright *et al.*, 2013; Desforges *et al.*, 2015) ^[5, 6]. Green Mussel *Perna viridis* are of particular interest because they are cosmopolitan species, sedentary and have extensive filter-feeding nature exposing them directly to chemicals and pollutants present in the water column. Microplastic effects to different marine organisms have been already recorded, compromising them into physiological consequences. And into context of trophic transfer, the humans are at large into exposure to microplastic through the consumption of these seafood (Farrell and Nelson, 2013; Watts *et al.*, 2014) ^[7, 8].

This bivalve species is also not new in the province of Sorsogon, having its fisheries industry for several decades. Its cultivation is considered to be as one of the country's top mussel producers. Its market in the province are sorted into various 'grading label': *Small* (5.0-6.9 cm), *Medium* (7.0-8.9 cm) and *Jumbo* (≥ 9.0 cm). And is mostly exported outside the province while the rest of its production is locally consumed. Sizes below 5.0 cm are likewise being retailed to fishpond operators as trash feed for their mangrove crab culture and to fishermen as bait to their fishing. Suggesting that the size of the shell is usually important, and not how much mussel meat is in. Thus, this study was proposed to identify characterize and quantify

microplastics found in different sizes of cultured green mussels *P. viridis* in Sorsogon Bay, Philippines.

Materials and Methods

Sample Collection and Sampling Sites

A total of one-hundred fifty mussel *P. viridis* were collected from three sites along the coastal water of Sorsogon bay from January to March 2021 (Table 1). The samples were directly

collected from the sites using stainless steel scalpels and forceps (Figure 1). In order to provide an ample representation of the bay, sampling sites were haphazardly selected from three mussel farm sites. Fifty mussels from each farm site (ten individuals per size category) were collected and was transferred to the laboratory for microplastic analysis.

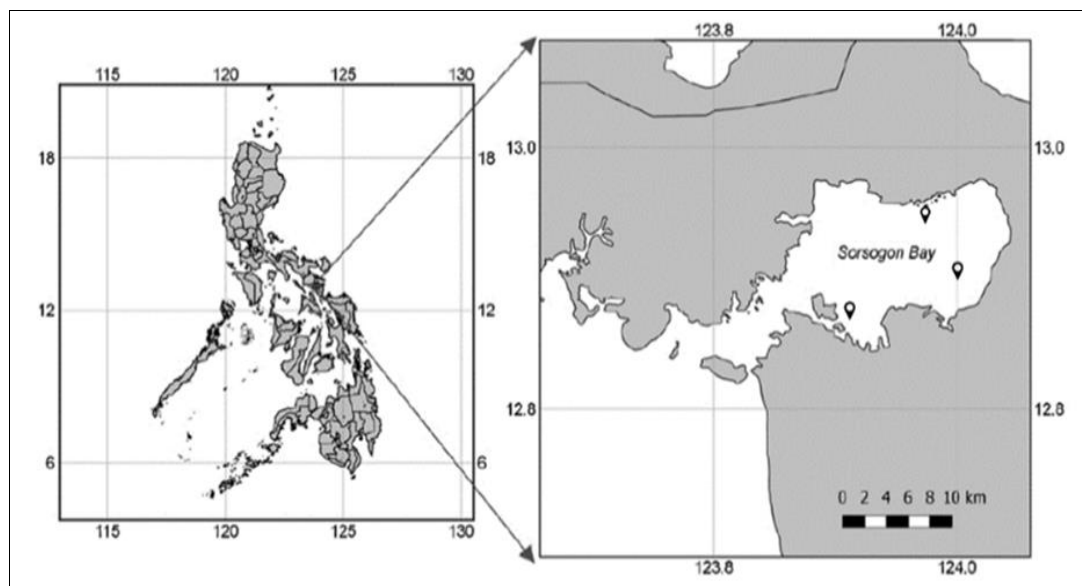


Fig 1: Sorsogon Bay located within the Philippines and zoomed in showing the sampling sites in Sorsogon City, in the municipality of Casiguran and in the municipality of Juban, Sorsogon, Image from Yñiguez *et al.*, 2018 ^[9].

Table 1: Length and weight and microplastic abundance in *P. viridis*

<i>P. viridis</i> size category (cm)	Number of individuals	Shell length (cm)	Soft tissue weight (g/individual)	Number of items/individual	Number of items/g wet weight
<2.9	30	2.37 ± 0.07	0.37 ± 0.03	0.3056 ± 0.03	0.8351 ± 0.16
3.0-4.9	30	3.93 ± 0.09	1.88 ± 0.13	2.0833 ± 0.29	1.1454 ± 0.26
5.0-6.9	30	5.65 ± 0.11	4.58 ± 0.32	2.5033 ± 0.35	0.4217 ± 0.06
7.0-8.9	30	7.27 ± 0.07	6.45 ± 0.30	1.9667 ± 0.29	0.2760 ± 0.04
>9.0	30	9.39 ± 0.06	9.85 ± 0.12	2.3333 ± 0.39	0.2313 ± 0.04

Quality assurance and quality control

To avoid contamination, all of the liquid (freshwater, saltwater and hydrogen peroxide) were filtered with 1 mm filter paper prior to use. All of the containers and beakers were rinsed three times with filtered water. The samples were immediately covered if they were not in used. All of the experimental procedures were finished as soon as possible.

Hydrogen peroxide treatment

The shell length and weight of each bivalve were recorded. Each sample was pooled into ~5 g of soft tissue each (Li *et al.*, 2015 and Li *et al.*, 2016) ^[10] ^[11]. Approximately 200 mL of 30% H₂O₂ was added to each bottle to digest the organic matter. The bottles were covered and placed in a drying oven at 65 °C for 24 h and then at room temperature for 24-48 h to extend the digestion effect of the soft tissue.

Floatation and filtration with saline (NaCl) solution

A concentrated saline solution was prepared to separate the microplastics from dissolved liquid of the soft tissue via floatation. Approximately 800 mL of filtered NaCl solution was added to each bottle. The liquid was mixed and retained overnight. The overlying water was directly filtered over Whatman filter paper using a vacuum system. Then the filter

were placed into clean petri dishes with a cover for further analysis.

Observation of microplastic

The filters were observed under OPTIKA LAB-20 stereomicroscope and OPTIKA B-290TB digital inspection microscope powered by PROVIEW v.4.815674.20191008 for imaging, particle size and shape identification and quantification. Colors were identified using Colors.exe software (Otaka *et al.* 2002) ^[12] adopting the 12 basic color terms of the ISCC-NBS (Inter-Society Color Council National Bureau of Standards) System of Color Designation as recommended by Kershaw *et al.* (2019) ^[13]. A proportion of 10% from all of the samples visually examined were validated through Attenuated Total Reflectance (ATR) Fourier Transform Infrared (FTIR) Spectroscopy (Perkin Elmer FT-IR Spectrometer Frontier).

Statistical analysis

Statistical test for normality was performed using the SPSS version 25.0 and a confidence level of 95% ($p=0.05$). Shell length (cm), shell weight (g) and soft tissue weight (g) were presented as mean ± standard error. One-Way ANOVA test were performed to determine the significant differences of the

items/ individual and items/ g_{wet} weight among the categories of *P. viridis* sizes.

Results

Characteristics of microplastic in *P. viridis*

Multiple types of microplastic, including fragments, foams, films, lines and pellets, occurred in the tissues of *P. viridis*.

Lines were the most popular microplastic shape and consisted 67% of the total microplastic found on the different class sizes of green mussel. Pellet were the least common shape and were not observed at all except for mussels with sizes 3.0 – 4.9 cm. Lines were only found exclusively in the mussels with sizes below 2.9 cm (Figure 2).

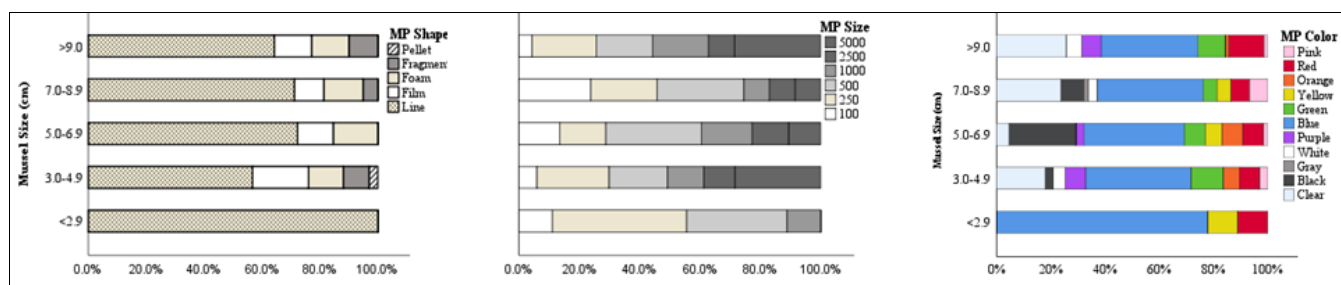


Fig 2: Characteristics of microplastic found in *P. viridis* of Sorsogon bay in terms of (a) shape; (b) size; and (c) color.

The most diverse colors were observed in lines followed by films. With the most popular colors blue, transparent, red, green and black. Overall the lines were usually blue and transparent. The size of the microplastics ranged from 100 μ m

to 5 mm in the bodies of the green mussel were examined. Microplastics with sizes 250 μ m and 500 μ m were the most common in green mussels, each consisting 25% of the microplastics calculated (Figure 2).

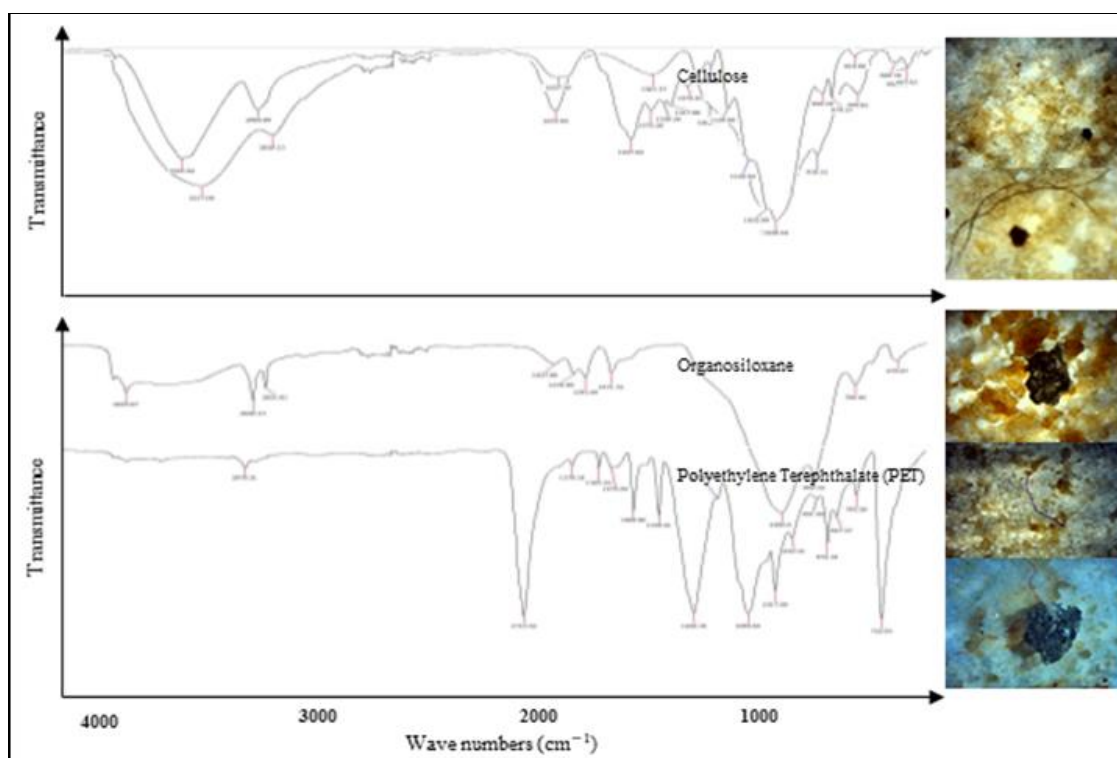


Fig 3: (a) Identification of micro plastics with Attenuated Total Reflectance - Fourier transformed Infrared Spectroscopy (ATR-FTIR). The measurements in the figure were performed under the transmittance mode. (b) Photographs of different types of microplastic found the *P. viridis* of Sorsogon bay.

Of the visually identified microplastic, 10% of these particles were validated for ATR-FTIR analysis. Of this subsample, only 62% were identified as microplastic composing 24% organosiloxane and 33% polyethylene terephthalate (PET) or polyester fiber. Natural fiber identified were cellulose materials composing 43% of the total samples.

Abundance of microplastic in *P. viridis*

The number of total microplastic varied from 0.31 to 2.5 items/ individual and from 0.23 to 1.15 items/ g_{wet} weight. Mussel ranging the size of 5.0-6.9 cm showed the highest

detected microplastics (2.57 items/ individual) while mussels with sizes below 2.9 cm has the least (0.31 item/individual). One-way ANOVA revealed that there was no significant differences found on the number of items per individual across its class sizes $F(4,91)=0.95$, $p=0.44$ but not with the number of items per tissue weight $F(4,91)=15.32$, $p=0.00$. (Figure 4). A Tukey post hoc test revealed that the microplastic item in soft tissues of green mussels was significantly lower in class size 5.0-6.9cm (0.42 ± 0.06 item/ g_{wet} weight, $p=0.00$), class size 7.0-8.9cm (0.28 ± 0.04 item/ g_{wet} weight, $p=0.00$) and class size >9.0 cm (0.23 ± 0.04

item/g_{wet weigh}, $p=0.00$) compared to class size 3.0-4.9cm (1.14 ± 0.26 item/g_{wet weigh} $p=0.77$). There is no statistically significant difference between the class size 3.0-4.9cm and

class size <2.9cm (0.83 ± 0.23 item/g_{wet weigh} $p=0.77$) (Figure 4).

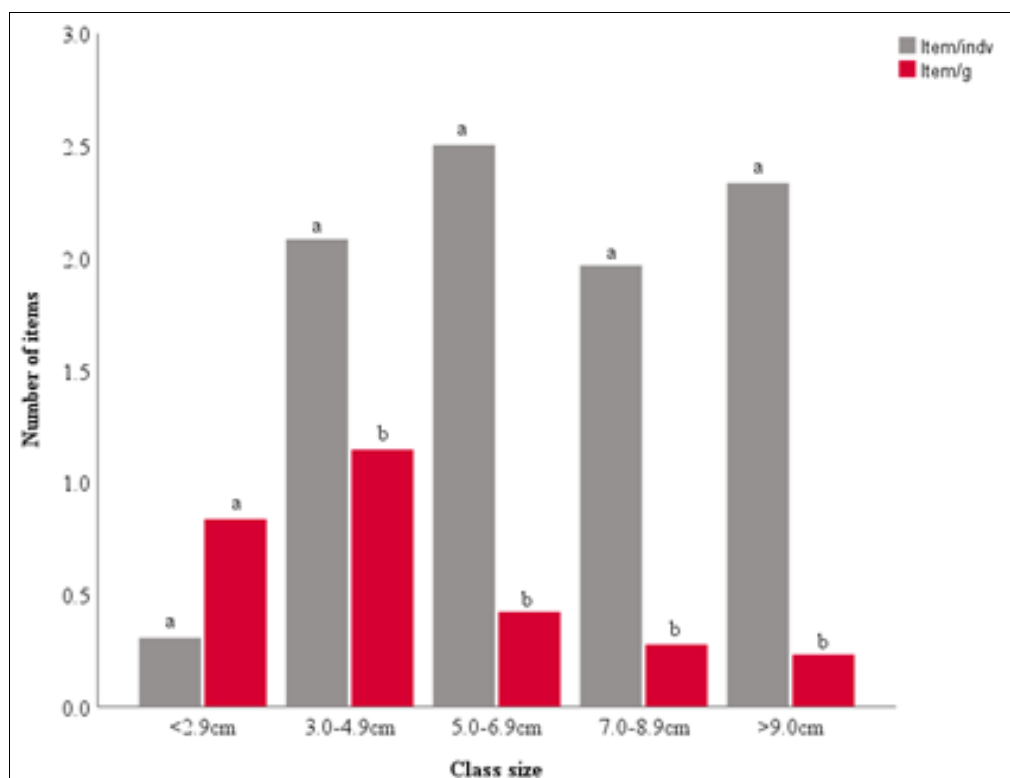


Fig 4: Mean abundance of microplastics. The letters (a and b) indicate the results of One-way ANOVA. Bars with the same letters indicates no significant difference.

Discussion

We found that microplastic pollution was detected in different aquaculture farms in Sorsogon bay. Compared to other published literature, the level of microplastic in Sorsogon Bay were approximately lower (Table 2). In terms of variation, we predicted more microplastic in larger mussels. As expected microplastic abundance (item/ individual) in the larger mussels were relatively higher than the smallest sizes. In contrast, when microplastic abundance were considered as

(items/ g_{wet weight}), the highest microplastic abundance was in 3.0-4.9cm size class and was low in the largest mussel (>9cm). This was because the mean number of microplastic per mussel was relatively consistent (~0.3 items/ individual) across the largest mussels (5.0-6.9, 7.0-8.9, 9.0+mm), even as mass was higher in the larger mussels. Moreover, there is no correlation both on the items found per individual $r_s(94)=0.048$, $p=0.646$ and on the number of items per soft tissue weight $r_s(94)=-0.070$, $p=0.496$.

Table 2: Comparison of microplastic pollution in bivalves n the present study vs the previous studies.

Species and sources	Treatment method	Identification method	Types of microplastic	Levels of microplastic	References
<i>Perna viridis</i> Philippines	30% H ₂ O ₂	Visual identification and verified with ATR-FTIR	Lines (fibers)	0.3 – 2.5 items/ individual	This study
<i>Perna viridis</i> Philippines	30% H ₂ O ₂	Visual identification and verified with ATR-FTIR	Lines (fibers)	0.23 – 1.15 items/ g _{wet weight}	This study
<i>Perna viridis</i> Philippines	30% H ₂ O ₂	Visual identification	Fibers	0.27 – 0.41 items/ g _{wet weight}	Bilugan <i>et al.</i> , 2021 [14]
<i>Perna viridis</i> Vietnam	10% KOH	Visual identification and verified with an μ-FT-IR	Fibers	2.6 items/ individual	Nam, P.N. <i>et al.</i> , 2019 [15]
<i>Perna viridis</i> China	30% H ₂ O ₂	LUMOS microscopy ATR mode	Fibers	0.77 - 8.22 items individual	Qu <i>et al.</i> , 2018 [16]
<i>Mytilus edulis</i> United Kingdom	30% H ₂ O ₂	Visual identification and verified with an μ-FT-IR	Fibers	1.1 - 6.4 items/individual	Li <i>et al.</i> , 2018 [17]
<i>Mytilus edulis</i> China	30% H ₂ O ₂	Visual identification and verified with an μ-FT-IR	Fibers	1.5 - 7.6 items/individual	Li <i>et al.</i> , 2016 [11]
<i>Mytilus galloprovincialis</i> Northern Ionian Sea	30% H ₂ O ₂	Visual identification and verified with ATR-FTIR	Fibers, fragments	1.95 ± 1.14 items/individual	Digka <i>et al.</i> , 2018 [18]

In case of seafood safety, the number of microplastic is useful than total mass. Following exposure diet, consumers

purchasing mussels of grading label small to jumbo sizes (5.0- ≥9.0 cm) would likely to ingest ~44 to ~23 particles per 100g

portion. If accounting for a 57% representation for the actual microplastic found on this study, this results in to ~24 to ~13 microplastic particles per 100g. This number is comparably smaller to the ~70 microplastic particles per 100g portion of microplastic in UK supermarkets. According to EFSA statement, only microplastics smaller than 150µm may translocate across the human gut epithelium (EFSA CONTAM Panel, 2016) ^[19] which equates to an estimated 15% of the particles recovered from farmed mussels of Sorsogon Bay (figure 2) and the absorption of these penetrating organs may be limited to ≤0.3% (EFSA CONTAM Panel, 2016) ^[19]. Likewise, on the initial study of Catarino *et al.*, 2018 ^[20], the risk of plastic ingestion via mussel consumption is minimal when compared to fiber exposure during a meal via dust fallout in a household.

This detection of microplastic on farmed green mussels of Sorsogon bay, only suggest the increasing evidence of microplastic contamination to this known seafood, and its entry to our diet is a function of the waste that we disposes of. Despite majority of identified microfibers were cellulose, probably from biodegradable textile cottons, its effect to human ingestion is still lacking.

Organosiloxanes is an evidence of possible waste from silicon-containing products such as baking utensils and pans, baby nipples, and pacifiers, medical devices and implants, water-repellent windshield coating, construction lubricants and sealants as well as deodorant creams and moisturizers. The primary health concerns associated with siloxanes have focused primarily on D₄ and D₅ compounds that are toxic and bioaccumulative. Siloxane products can be avoided through reading product labels and purchasing toxic-chemical free cookware alternative like glass or ceramics (Meghan J, 2021) ^[21]. Polyethylene terephthalate (PET) detection are also daunting, which are mainly used in the plastic industry. PET has become widely used in the plastic industry as resin form for plastic bottles, food jars, food trays and as fiber form for textiles (known as polyester), monofilament, carpet, and films. While it is generally considered a "safe" plastic, and does not contain BPA, in the presence of heat it can leach antimony trioxide and phthalates. Both of these are dangerous to health. While antimony may contribute to menstrual and pregnancy issues, phthalates are endocrine disruptors (Filella M, 2020) ^[22].

With this findings, it indicates that there is a haphazard waste-mismanagement introduction of this plastic wastes into the bay. Its prevalence should be investigated and its waste management practices should be revisited both on land and in the shoreline.

Since human health consequences of different types of microplastic in seafood are still evolving, its detection in this kind of seafood is worth considering. Especially that public perception on the risk from microplastics are increasing as agitated in media. If consumers perceive that the seafood contain microplastics, there is a potential that their interpretation of the relative risks involve may result in behavioral change or in the reduction of seafood consumption. Clearly, this would result in the loss of income in the seafood industry, and loss of safe nutritious protein for consumers (Bergmann *et al.*, 2015) ^[23].

Though this marine pollution concern is ubiquitous and increasingly evident, with Sorsogon bay is clearly no exception to this paradigm, this evidence of microplastic occurrence in the farmed green mussels of Sorsogon bay of varying grading label on its market will hopefully drive

effective social perception and behavioral change work on supporting measures addressing the issue.

Conclusion and Recommendation

The study investigated the abundance and characteristics of microplastic in cultured green mussels in Sorsogon Bay. Commercially marketed into various 'grading label': small, medium and jumbo, the species was detected with microplastic. Primary results from the study were, (i) low abundance of microplastic, as compared to other published literatures with no significant differences on the microplastic items per individual on various 'grading label' but not on microplastic items per soft tissue weight (ii) lines or fibers dominated the microplastic shape, blue as the most popular color, 250µm to 500µm as the most common microplastic size and organosiloxanes and polyethylene terephthalate (PET) were the detected polymer types. Its detection to cultured green mussels in Sorsogon bay is an evidence that the bay is no exception to this ubiquitous type of marine pollution. Indicating that there is a haphazard waste-mismanagement introduction into the bay needing visitation and investigation. Since public perception on seafood-containing microplastic can lead to their behavioral change in seafood consumption, perception survey should be conducted in preparation for the risk-assessment of microplastic occurrence in commercial bivalves of Sorsogon bay.

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