Journal of Ecosystem Sciences and Eco-Governance (JESEG)
www.jeseg.carsu.edu.ph

Special Issue | PAMS16
PRINT ISSN 2704 4394 | ONLINE ISSN 2782 8522
DOI: https://doi.org/10.54610/jeseg/4.Special_Issue.2022.005

Preliminary study on Microplastics in Bivalves *Perna viridis, Crassostrea iredalei,* and *Venerupis philippinarum* Harvested from Bacoor Bay, City of Bacoor, Cavite

Laurence C. Insigne[*,1,4] *Ulrica Loisse D. Salivio*[2], *Janella Buenavente*[3], *and Anne Corinne F. Sagun*[4]

[1]The Marine Science Institute, University of the Philippines- Diliman, Quezon City, Metro Manila, Philippines

[2]Department of Communication Research, Polytechnic University of the Philippines- Manila, Manila, Philippines

[3]Institute of Animal Science, College of Agriculture and Food Science, University of the Philippines- Los Baños, Laguna, Philippines

[4]Senior High School in San Nicholas III, Bacoor City, Cavite, Philippines

*Corresponding Author
*Email: lcinsigne@up.edu.ph

Received: July 26, 2021
Revised: January 28, 2022
Accepted: October 12, 2022
Available Online: November 30, 2022

Cite this article: Insigne, L.C., Salivio, U.L.D., Buenavente, J., & Sagun, C.F. (2022). Preliminary study on Microplastics in Bivalves *Perna viridis, Crassostrea iredalei,* and *Venerupis philippinarum* Harvested from Bacoor Bay, City of Bacoor, Cavite. *Journal of Ecosystem Science and Eco-Governance*, **4**(Special Issue):38-46.

ABSTRACT

An increasing number of studies have reported the presence of microplastics in marine organisms such as bivalves. Ingestion of microplastics can affect organisms and transport pollutants such as organic chemicals and heavy metals. In this study, the presence, quantity, and size of microplastics ingested by the three commercially important bivalve species *Perna viridis, Crassostrea iredalei,* and *Venerupis philippinarum* harvested from Bacoor Bay, Philippines, were investigated. The microplastics were extracted from the soft tissues of the bivalves using base digestion, recovered by filtration using 150 µm wire mesh, and analyzed microscopically. The results demonstrated that the quantity of recovered microplastics from the soft tissues of mussels, oysters, and clams are 0.93±0.12 items/g, 0.42±0.07 items/g, and 1.71±0.34 items/g, respectively. One-way analysis of variance (ANOVA) shows that the quantity of microplastics ingested by the three bivalve species was significantly different ($\alpha = 0.05$, $p = 0.0031$). The average size of microplastics ingested by mussels, oysters, and clams was 1.72±0.13 mm, 1.26±0.18 mm, and 1.21±0.1 mm, respectively. A slightly significant difference between the sizes of microplastics ingested by the three bivalve species ($\alpha = 0.05$, $p = 0.049$) was observed. This study presents preliminary data on the quantity and size of microplastics ingested by bivalves in Bacoor Bay. The results of this study further proved the necessity of determining programs and projects to reduce the potential risks of microplastics on the environment, economy, and, more importantly, on the safety of human food consumption. Further studies are needed to identify the specific polymers of plastics ingested by the three bivalve species harvested in Bacoor Bay.

Keywords: *clams, ingestion, microplastic, mussels, oysters*

1 Introduction

The Philippines is considered one of the top contributors of plastic debris in the ocean, with an estimated annual input of 0.28-0.25 million metric tons (MT) (Jambeck et al. 2015). The continuous release of plastic debris into the environment leads to its accumulation in marine and coastal regions (Possatto et al. 2011). The improper disposal of plastic waste and its growing production leaves no doubt about its potential impact and threat to these environments. Plastic contamination of aquatic ecosystems has been an increasing concern because of their persistence and ecological effects on the environment (Thompson et al., 2009), especially given the occurrence of smaller fragments of plastics that are bioavailable for ingestion by a wide range of marine organisms, called *microplastics*.

Microplastics, defined as plastic materials or fragments <5 mm, are likely the ocean's most numerically abundant plastic debris today (Law and Thompson 2014). The quantities of marine microplastics will inevitably increase due to the degradation of plastic items, ultimately breaking down into millions of microplastic pieces (Cózar et al. 2014). The widespread use and persistent nature of plastic have made microplastics ubiquitous in marine waters, sediments, organisms, and even sea salts (Yang et al. 2015). They can also enter the ocean directly by releasing cosmetic powders, pellets, fibers, and microbeads in facial cleansers into the marine environment (Fendall and Sewell 2009; Cole et al. 2011; Browne et al. 2015; Thompson 2015). Due to their small size, microplastics can be potentially ingested through the food chain (Van Cauwenberghe and Janssen 2014). Therefore, ingestion of microplastics could have significant impacts on the organisms. Research has established that these effects mainly revolved around decreased food uptake (Besseling et al. 2013), reduction of energy reserves (Wright et al. 2013), inflammation (von Moos et al. 2012; Avio et al. 2015), changes in immunological responses (Avio et al. 2015) and declines in broodstock fecundity and larval growth (Sussarellu et al. 2016). Other impacts may be caused by the toxicity of the concentrations of persistent organic pollutants (POPs) and heavy metals absorbed in the surrounding water by microplastics, as well as the residual toxic additives from the manufacture of plastics (Andrady 2011).

Bivalves play a significant role in the food web, for they serve as food to various marine species and humans across the globe (Davidson and Dudas 2016). Bivalves are of particular interest in microplastics research because of the extensive filter-feeding activity that exposes them directly to microplastics present in the environment. Bivalves can be a potential bioindicator of microplastic pollution (Li et al. 2016). Many studies have proven the presence of microplastics in the tissue of shellfish (e.g., Van Cauwenberghe and Jansenn 2014; Davidson and Dudas 2016; Dela Torre et al. 2019; Teng et al. 2019). The Slipper cupped oyster (*Crassostrea iredalei*), Asian Green Mussel (*Perna viridis*), and Manila Clam (*Venerupis philippinarum*) were chosen because of their economic importance, especially in Bacoor City. According to the Fisheries Statistics of the Philippines 2017 – 2019, the country produced 61,615.22 metric tons of the abovementioned species amounting to 1,267,214,810 Php.

The number of microplastics ingested by marine organisms can help determine the degree of impact of microplastic pollution and help assess the potential risk in the marine organisms. A qualitative study regarding microplastics was conducted in Bacoor Bay (Argamino and Janairo 2016), and another quantitative study (Bilugan et al. 2021) was conducted in Cañacao Bay, Cavite City. However, studies regarding the ingestion of microplastics by bivalves for human consumption in the Philippines are still limited (Galarpe et al. 2021). With the country being a top contributor to marine plastic debris and the ecosystem services provided by marine environments, research regarding microplastic pollution is needed (Abreo 2018). This study was conducted to measure the size and quantity of the microplastics ingested by three commercially important bivalve species, oysters (*C. iredalei*), mussels (*P. viridis*), and clams (*V. philippinarum*) cultured for human consumption in Bacoor City, Cavite, and determine the relationship between microplastic size and wet weight of the bivalves. This study aims to provide data on the quantity of microplastics in bivalves cultured for human consumption. Monitoring microplastics will hopefully contribute significantly to minimizing potential risks of microplastics in the environment, economy, and human health.

2 Materials and Methods

Collection of Samples

Bacoor Bay is a 957.25 ha.-long body of water located north of the city of Bacoor, which includes areas for municipal fishing (453 ha), habitat enhancement and management (40 ha), and eco-tourism (81 ha) (PEMSEA and Provincial Government of Cavite, Philippines, 2017). The samples were obtained from the bivalve culture sites harvested by local mussel farmers. Forty-five samples were obtained for this study (15 individuals per species). Only live bivalves that are in "harvestable" sizes are gathered. Following the protocol of other studies (e.g., Davidson and Dudas 2016; Catarino et al. 2018; Phuong et al. 2018), the bivalves were kept in cloth bags after collection, transported in an icebox with ice, and kept frozen until analysis. The bivalves were thawed one hour before the digestion of tissues.

Contamination Mitigation

Contamination with airborne plastic particles has been widely reported in microplastics research (Davidson and Asch 2011; Foekma et al. 2013). All equipment used in the experiment was thoroughly rinsed with filtered distilled water and dried at room temperature avoiding contact with the ambient air to mitigate airborne and laboratory contamination (Phuong et al. 2018). The reagents (distilled water, salt water, NaOH, and Sodium Dodecyl Sulfate solution) were filtered with a 150 μm sieve before usage. Laboratory coats and gloves were worn all the time. During the analysis of microplastics, the glass bottles were covered when not in use to avoid contact with the digestion solution with the ambient air. A procedural blank was also prepared. This contains filtered distilled water and undergoes the same procedure as the soft tissue samples.

Base Digestion of Tissue samples

The bivalves were rinsed using filtered freshwater, and the shells were opened. The shell length was measured using a metal ruler, while the wet weight of each bivalve was measured using a digital balance. The whole soft tissues of three individuals were emptied from the shell into a single 250 mL Erlenmeyer flask and regarded as replicates. Ten milliliters (10 mL) of 1 M NaOH (Sodium Hydroxide) and 5 mL of SDS (Sodium dodecyl disulfate) per g. of soft tissue were added to each container to digest the tissue. The bivalves were left in the solution for 24 h at room temperature to achieve optimum digestion. The digested samples were then subjected to 60°C incubation in a warm bath for 24 h. Subsequently, the samples were diluted to 100 mL warm (70°C) filtered distilled water, followed by filtration using a 150 μm wire mesh.

Characterization and quantification of microplastics

Visual inspection of the microplastics was done under a digital microscope (Digital Blue Computer Microscope) at 60x magnification. Microphotographs were captured using the software provided by the digital microscope. Identification of the microplastics was performed based on the criteria given by Noren (2007). All the microplastics extracted have no organic materials that are visible, clear, and have a homogeneous color. The particles were also subjected to a hot needle test to confirm that they were microplastics (Devriese et al. 2015). A needle is heated until visibly red when in contact with the microplastic particle. The plastic particle should move and be deformed, while non-plastics will show no movement. Moreover, the particle should be equally thick throughout their entire length, specifically in the case of fibers (Hidalgo-Ruz et al. 2012). The quantity of microplastics was recorded. The number of microplastic per species is divided by the wet weight of the samples to obtain the number of items per gram (items/g). The length of the microplastic samples was analyzed and measured using Image J Analysis software.

Statistical Analysis

In all the analyses, an alpha level of 0.05 is used. Shapiro-Wilk's test was used to determine the normality of data, and Levene's test was used to assess the homogeneity of variances. One-way analysis of variance (ANOVA) was used to identify whether there is a significant difference in the abundance of microplastics ingested by the three bivalve species and the sizes of the microplastics obtained. Pearson's r was used to determine the relationship between body size (soft tissue weight) and the size of ingested microplastic. Data were analyzed using Paleontological Statistics Version 3 (PAST3) software.

3 Results and Discussion

Presence of microplastics in the three bivalve species

Visual inspection of the particles from the digested tissues of the three bivalve species shows microplastics, as confirmed by the hot needle test. The microplastics recovered are all classified as plastic fragments because of their "small irregular", "flat," or "cracked morphology" (Mateos-Cardenas et al. 2020; Figure 1). Fragment morphotypes result from mechanical stress by water aided by sand and other sediments (Julienne et al. 2019).

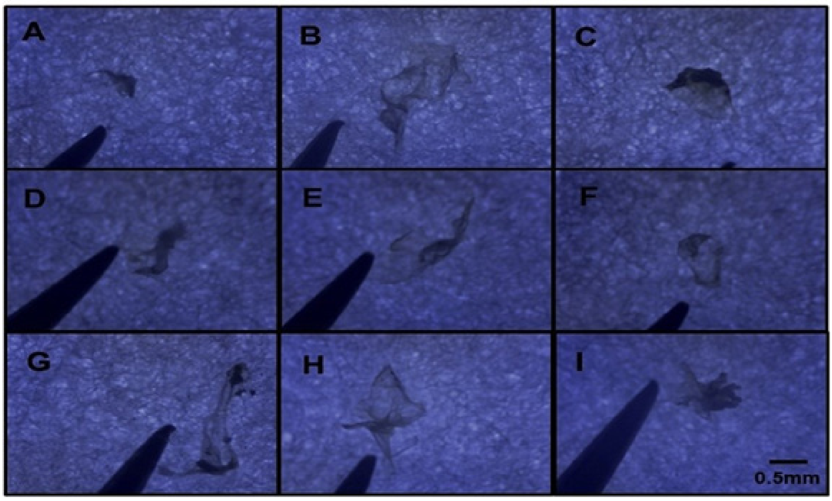

Figure 1. Microphotographs (60x magnification) of some of the microplastics obtained from *P. viridis* (A-C), *C. iredalei* (D-F), and *V. philippinarum* (G-I).

Quantity of microplastics from the three bivalve species

A total of 43 microplastics were obtained from 45 individuals in this study. The three species of bivalves, Asian Green Mussel (*P. viridis*), Slipper-cupped Oyster (*C. iredalei*), and Manila Clams (*V. philippinarum*) contained 14, 13, and 16 items, respectively. The number of microplastics per species is divided by the total wet weight of the samples to obtain the number of items per gram (items/g). Asian Green Mussels (*P. viridis*) contained 0.93±0.12 items/g, Slipper-cupped Oysters (*C. iredalei*) contained 0.42±0.07 items/g, and Manila Clams (*V. philippinarum*) contained 1.71±0.34 items/g. One-way analysis of variance shows that the number of items per gram per species has a significant difference ($\alpha = 0.05$, $p = 0.0031$). Table 1 shows the bivalves' shell length and wet weight, the number of microplastics obtained from the samples, and the number of items per gram of wet weight. The average quantity of microplastics ingested by commercially important bivalves in Bacoor City, Cavite, is 1.02±0.38 items/g.

The resulting quantity of microplastic in this study is on par with the results of other research on microplastic ingestion (Table 2). In terms of local studies on microplastics from bivalve soft tissues, this study shows the presence of microplastics in the mussels as with the study of Argamino et al. 2016. The quantity of microplastics from the bivalves in this study is slightly higher compared to the previous research done by Bilugan et al. (2021) on microplastics in *Perna viridis* in different locations in Bacoor Bay. The variation in the results of this study may be attributed to the difference in the physiology of each species (Phuong et al. 2018). The number of microplastics found in an organism is proportional to its size, and it may also vary because of the differences in the filtration rates of each species (Li et al. 2015). The procedures can also affect the number of recovered microplastics from the samples. This study uses alkaline digestion (1M NaOH). Dehaut et al. (2016) suggested that an alkaline solution is the most effective in recovering the gut contents of M. edulis. Catarino et al. (2016) have also used a strong base to digest the soft tissues of mussels and found that the use of NaOH is effective in clearing tissues and the use of both NaOH and has minimal damage to the microplastic particles. In a study on microplastics in fish guts, the use of NaOH and SDS as the digestive agent had no noticeable effect on the shape of the plastic particle (Budimir et al. 2017) compared to the more corrosive acid digestion (Claessens et al. 2013).

Size of the microplastics obtained

The average size of the microplastics obtained in this study is 1.4±0.16 mm. Mussels have the largest particles measuring an average of 1.72±0.13 mm, followed by oysters with an average of 1.26±0.18 mm, and lastly, samples from clams measure an average of 1.21±0.1 mm. The values for

Table 1. Size measurements of bivalves and the quantity and size of microplastics in the bivalves (n=45)

Commercially Important Bivalve Species	Average Shell Length (mm±SE)	Average Weight (g±SE)	Total Number of Microplastics	Average Size of the Microplastic particles (mm±SE)	Number of microplastics per wet weight (items/g±SE)
Asian Green Mussels *(Perna viridis)* (n=15)	5.43±0.26	3.11±0.27	14	1.72±0.13	0.93±0.12
Slipper- cupped Oysters *(Crassostrea iredalei)* (n=15)	7.98±0.09	6.06±0.39	13	1.26±0.18	0.42±0.07
Manila Clams (*Venerupis philippinarum)* (n=15)	2.94±0.06	1.93±0.12	16	1.21±0.1	1.71±0.34

Table 2. Other studies regarding microplastic contamination in different bivalve species

Species	Locality	Reagent used in Digestion	Quantity	Reference
Mytulis edulis and *Crassostrea gigas*	Germany and France	69% HNO3	0.07-0.24 particles/g (mussels) 0.05-0.35 particles/g (oysters)	Van Cauwenberghe and Janssen (2014)
Mytulis edulis	Belgium	HNO3/HClO4	0.26-0.51 items/g	De Witte et al. (2014)
Nine species of marine bivalves	China	30% H2O2	2.1-10.5 items/g	Li et al. (2015)
Mytulis edulis and *Mytilus galloprovincialis*	Europe	HNO3/HClO4	0-0.32 items/g	Vandersmech et al. (2015)
Venerupis philippinarum	Canada	69–71% HNO3	0.07 – 5.47 items/g	Davidson and Dudas (2016)
Mytulis edulis	China	30% H2O2	0.9-4.6 items/g	Li et al. (2016)
Mytulis edulis	France	10% KOH	0.24 items/g	Phuong et al., (2017)
Chlamys farreri and *Mytilus galloprovincialis*	China	10% KOH/ 30% H2O2	3.2- 7.1 items/g	Ding et al. (2018)
Crassostrea gigas, Crassostrea angulate, Crassostrea hongkongensis and *Crassostrea sikamea*	China	10% KOH and 30% H2O2	0.14-2.35 items/g	Teng et al. (2019)
Venerupis philippinarum and *Crassostrea gigas*	Canada	10% KOH	0.03-0.05 items/g	Covernton et al. (2019)
Crassostrea gigas, Mytilus edulis, Venerupis philippinarum, and *Patinopecten yessoensis*	South Korea	10% KOH	0-1.08 items/g	Cho et al. (2019)
Argopecten purpuratus	Peru	10% KOH	0.03-0.13 items/g	Dela Torre et al. (2019)
Crassostrea gigas and *Siliqua patula*	U.S.A.	10% KOH	0.16 and 0.35 items/g	Baechler et al. (2019)
Perna viridis and *meretrix meretrix*	India	10% KOH	0.18-1.84 items/g	Dowarah et al. (2020)
Ruditapes decussatus, Cerastoderma spp. and *Polititapes* spp.	Portugal	1.8 M KOH	10.4- 18.4 items/g	Cozzolino et al. (2021)
Perna viridis, Crassostrea iredalei, and *Venerupis philippinarum*	Philippines	1M NaOH and SDS	0.42-1.71 items/g	In this study.

the average size of microplastics per species have a slightly significant difference (α = 0.05, p =0.049). Table 1 shows the data for the average microplastic size. Pearson's R correlation shows the relationship between the size of the bivalves (wet weight) and the size of microplastics has a weak negative correlation (r=-0.102) and is not significant (α = 0.05, p =0. 717563) as shown in Figure 2.

In another study done in Bacoor Bay by Bilugan et al. (2021), the dominant size of the microplastic particles collected from *P. viridis* ranges from 10 to 50 μm which is smaller than the microplastics observed in this study. In the other research done by Argamino & Janairo (2016) regarding microplastics in *P. viridis* in Bacoor Bay, the particle size ranges from 10 to 30 μm in white and red particles,

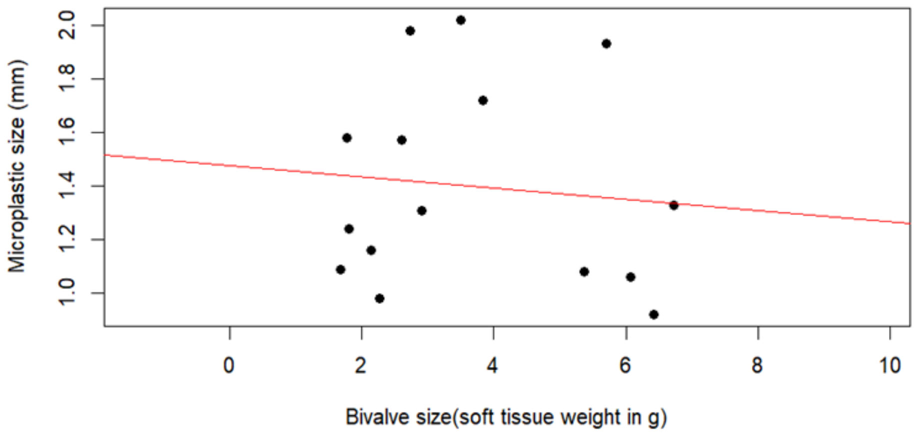

Figure 2. Relationship between bivalve size (soft tissue weight in grams) and microplastic size (mm) (R= -0.1026, p=0.72)

while blue particles are described to be around less than 5 mm in length. The difference in the size of obtained microplastics from bivalves can be attributed to the difference in the filtration method. This study used a sieve with a 150 μm mesh size. In contrast, the survey by Bilugan et al. (2021) used a filter paper with a pore size of 0.45 μm and the survey by Argamino & Janairo (2016) used filter paper with 11 μm pore size, allowing smaller microplastic particles to be recovered. Different studies regarding the ingestion of microplastics have demonstrated the size-dependent toxicity of the particle. In smooth nut clams, *Enncula tenuis*, energy reserves significantly decreased with higher doses and larger-sized (125-500 μm) microplastic particles (Bour et al. 2018). In another study done by Tang et al. (2020) regarding the toxicity of microplastics and persistent organic pollutant (POPs) when ingested by *Tegillarca granosa*, toxicity was enhanced in smaller-sized nano plastics compared to microplastics which is attributed to size-dependent interaction between the plastic particle and the POPs.

Numerous studies have revealed the negative effects of microplastic ingestion on bivalves. Ingested microplastic can be translocated and accumulate in different tissues of bivalves, which can cause various negative consequences such as neurotoxic effects, DNA damage, and alterations of immunological responses (Browne et al. 2008; Von Moos et al. 2012; Avio et al. 2015; Ribiero et al. 2017). The ingestion of microplastics can also cause mechanical effects like macroplastics. Microplastics might block the feeding appendages, hinder food passage through the intestinal tract (Tourinho et al. 2010), or cause pseudo-satiation resulting in decreased food intake (Derraik 2002; Thompson 2006). Exposure to microplastics can also reduce the quantity and quality of the gametes produced by oysters (Sussarellu et al. 2016). The impact on bivalve reproduction can eventually harm the production of bivalves in Bacoor Bay. Aside from these, microplastics can also be a vector of persistent organic pollutants (POPs), heavy metals, and microorganisms (Bakir et al. 2014; Koelmans 2016; Ma et al. 2020). Consumption of these pollutants is very harmful and can negatively affect human health. Knowing the hazardous effects of microplastics, the local government of Bacoor City should act proactively and implement scientific and research-based policies to effectively mitigate the present microplastic pollution in the locality.

4 Conclusion and Recommendations

The presence of microplastic ingestion in three commercially important bivalve species *(P. viridis, C. iredalei,* and *V. philiipinarum)* has been reported in this study. This study is the first preliminary report on the quantity of microplastics ingested by the three bivalve species. The quantity of microplastics recovered from mussels, oysters, and clams are 0.93±0.12 items/g, 0.42±0.07 items/g, and 1.71±0.34 items/g, respectively. The average size of microplastic ingested is 1.4±0.16 mm and is not correlated to the bivalves' body size (soft tissue weight). The presence of microplastic in the three commercially important bivalve species in Bacoor Bay should alert the local government of Bacoor City to investigate further microplastic pollution in its marine waters and its organisms. It is necessary to establish programs and projects to reduce the potential risks of microplastics to the environment, economy, and, more importantly, food safety. This study should be an eye-opener for policymakers, researchers, and individuals to act on this emerging environmental problem. The researchers suggest further monitoring microplastic ingestion in bivalves to determine its spatial and temporal variation. In addition, the microplastics should be verified with instruments like Fourier-Transform Infrared Spectroscopy and Raman Spectroscopy to strengthen the validity of the identified microplastics. More importantly, rigorous research should investigate the potential for humans to ingest microplastics and the extent of impacts of the toxicity associated with these contaminants. However, to be able to do that, researchers should focus first on standardization and harmonization protocols in extracting microplastics in organisms because, currently, there is no standardized and harmonized method for extracting microplastics in marine organisms such as bivalves.

5 Acknowledgement

The researchers would like to thank SHS in San Nicholas III, Bacoor City for sponsoring the study, the local mussel farmers in Bacoor Bay for assisting in collecting samples, and Dr. Deo Florence Onda for helping us with the initial version of the manuscript. The authors thank the reviewers for their insightful comments and suggestions that improved the manuscript.

Statement of Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

6 Literature Cited

Abreo, N. A. S. (2018). Marine plastics in the Philippines: a call for research. *Philippine Science Letters,* **11**(1), 20-21.

Argamino, C. R., & Janairo, J. I. B. (2016). Qualitative Assessment and Management of Microplastics in Asian Green Mussels (*Perna viridis*) Cultured in Bacoor Bay, Cavite, Phillipines. *EnvironmentAsia*, **9**(2).

Andrady, A. L. (2011). Microplastics in the marine environment. *Marine Pollution Bulletin*, **62**(8), 1596-1605.

Avio, C. G., Gorbi, S., Milan, M., Benedetti, M., Fattorini, D., d'Errico, G., Pauletto, M., Bargelloni, L., Regoli, F. (2015). Pollutants bioavailability and toxicological risk from microplastics to marine mussels. *Environmental Pollution*, **198**, 211-222.

Baechler, B. R., Granek, E. F., Hunter, M. V., & Conn, K. E. (2019). Microplastic concentrations in two Oregon bivalve species: Spatial, temporal, and species variability. *Limnology and Oceanography Letters,* **5**(1), 54-65.

Bakir, A., Rowland, S. J., & Thompson, R. C. (2014). Transport of persistent organic pollutants by microplastics in estuarine conditions. *Estuarine, Coastal and Shelf Science,* **140**, 14-21.

Besseling, E., Wegner, A., Foekema, E. M., Van Den Heuvel-Greve, M. J., & Koelmans, A. A. (2013). Effects of microplastic on fitness and PCB bioaccumulation by the lugworm *Arenicola marina* (L.). *Environmental Science & Technology,* **47**(1), 593-600.

Bilugan, Q., Limbago, J., & Gutierrez R. (2021). Detection and quantification of microplastics from cultured green mussel *Perna viridis* in Bacoor Bay, Cavite, Philippines. *Sustinere: Journal of Environment and Sustainability,* **5**(2), 64-145.

Browne, M. A., Dissanayake, A., Galloway, T. S., Lowe, D. M., & Thompson, R. C. (2008). Ingested microscopic plastic translocates to the circulatory system of the mussel, *Mytilus edulis* (L.). *Environmental Science & Technology,* **42**(13), 5026-5031.

Browne, M. A. (2015). Sources and pathways of microplastics to habitats. *Marine Anthropogenic Litter,* 229-244.

Bour, A., Haarr, A., Keiter, S., & Hylland, K. (2018). Environmentally relevant microplastic exposure affects sediment-dwelling bivalves. *Environmental Pollution*, **236**, 652–660.

Budimir, S., Setälä, O., & Lehtiniemi, M. (2017). Effective and easy to use extraction method shows low numbers of microplastics in offshore planktivorous fish from the northern Baltic Sea. *Marine Pollution Bulletin,* **127**, 586-592.

Catarino, A. I., Thompson, R., Sanderson, W., & Henry, T. B. (2016). Development and optimization of a standard method for extraction of microplastics in mussels by enzyme digestion of soft tissues. *Environmental Toxicology and Chemistry*, **36**(4), 947–951.

Cho, Y., Shim, W. J., Jang, M., Han, G. M., & Hong, S. H. (2019). Abundance and characteristics of microplastics in market bivalves from South Korea. *Environmental pollution*, **245**, 1107-1116.

Claessens, M., Van Cauwenberghe, L., Vandegehuchte, M. B., & Janssen, C. R. (2013). New techniques for the detection of microplastics in sediments and field collected organisms. *Marine Pollution Bulletin,* **70**(1-2), 227-233.

Covernton, G. A., Collicutt, B., Gurney-Smith, H. J., Pearce, C. M., Dower, J. F., Ross, P. S., & Dudas, S. E. (2019). Microplastics in bivalves and their habitat in relation to shellfish aquaculture proximity in coastal British Columbia, Canada. *Aquaculture Environment Interactions,* **11**, 357-374.

Cózar, A., Echevarría, F., González-Gordillo, J. I., Irigoien, X., Úbeda, B., Hernández-León, S., ... & Duarte, C. M. (2014). Plastic debris in the open ocean. *Proceedings of the National Academy of Sciences,* **111**(28), 10239-10244.

Cozzolino, L., Carmen, B., Zardi, G. I., Repetto, L., & Nicastro, K. R. (2021). Microplastics in commercial bivalves harvested from intertidal seagrasses and sandbanks in the Ria Formosa lagoon, Portugal. *Marine and Freshwater Research.*

Davison, P., & Asch, R. G. (2011). Plastic ingestion by mesopelagic fishes in the North Pacific Subtropical Gyre. *Marine Ecology Progress Series,* **432**, 173-180.

Davidson, K., & Dudas, S. E. (2016). Microplastic ingestion by wild and cultured Manila clams (*Venerupis philippinarum*) from Baynes Sound, British Columbia. *Archives of Environmental Contamination and Toxicology,* **71**(2), 147-156.

Dehaut, A., Cassone, A. L., Frère, L., Hermabessiere, L., Himber, C., Rinnert, E., Riviére, G., Lambert, C., Soudant, P., Huvet, A., Duflos, G.,& Paul-Pont, I. (2016). Microplastics in seafood: Benchmark protocol for their extraction and characterization. *Environmental Pollution*, **215**, 223-233.

De-la-Torre, G. E., Mendoza-Castilla, L., & Laura, R. P. (2019). Microplastic contamination in market bivalve *Argopecten purpuratus* from Lima, Peru. *Manglar*, **16**(2), 85-89.

Derraik, J. G. (2002). The pollution of the marine environment by plastic debris: a review. *Marine Pollution Bulletin,* **44**(9), 842-852.

Devriese, L. I., Van der Meulen, M. D., Maes, T., Bekaert, K., Paul-Pont, I., Frère, L., Robbens, J.,& Vethaak, A. D. (2015). Microplastic contamination in brown shrimp (*Crangon crangon*, Linnaeus 1758) from coastal waters of the Southern North Sea and Channel area. *Marine Pollution Bulletin*, **98**(1-2), 179-187.

De Witte, B., Devriese, L., Bekaert, K., Hoffman, S., Vandermeersch, G., Cooreman, K., & Robbens, J. (2014). Quality assessment of the blue mussel (*Mytilus edulis*): Comparison between commercial and wild types. *Marine Pollution Bulletin,* **85**(1), 146-155.

Ding, Jing- Feng., Jing-Xi, L. I., Cheng-Jun, S. U. N., Chang-Fei, H. E., Jiang, F. H., Feng-Lei, G. A. O., & Zheng, L. (2018). Separation and identification of microplastics in digestive system of bivalves. *Chinese Journal of Analytical Chemistry,* **46**(5), 690-697.

Dowarah, K., Patchaiyappan, A., Thirunavukkarasu, C., Jayakumar, S., & Devipriya, S. P. (2020). Quantification of microplastics using Nile Red in two bivalve species *Perna viridis* and *Meretrix meretrix* from three estuaries in Pondicherry, India and microplastic uptake by local communities through bivalve diet. *Marine Pollution Bulletin,* **153**, 110982.

Fendall, L. S., & Sewell, M. A. (2009). Contributing to marine pollution by washing your face: microplastics in facial cleansers. *Marine Pollution Bulletin*, **58**(8), 1225-1228.

Foekema, E. M., De Gruijter, C., Mergia, M. T., van Franeker, J. A., Murk, A. J., & Koelmans, A. A. (2013). Plastic in north sea fish. *Environmental Science & Technology,* **47**(15), 8818-8824.

Galarpe V. R. K., Jaraula, C. M. B., & Paler, M. K. O. (2021). The nexus of macroplastic and microplastic research and plastic regulation policies in the Philippines marine coastal environments. *Marine Pollution Bulletin,* **167**, 112343.

Hidalgo-Ruz, V., Gutow, L., Thompson, R. C., & Thiel, M. (2012). Microplastics in the marine environment: a review of the methods used for identification and quantification. *Environmental science & technology*, **46**(6), 3060-3075.

Jambeck, J. R., Geyer, R., Wilcox, C., Siegler, T. R., Perryman, M., Andrady, A., Narayan, R.,& Law, K. L. (2015). Plastic waste inputs from land into the ocean. Science, **347**(6223), 768-771.

Julienne, F., Delorme, N., & Lagarde, F. (2019). From macroplastics to microplastics: Role of water in the fragmentation of polyethylene. *Chemosphere*, **236**, 124409.

Koelmans, A. A., Bakir, A., Burton, G. A., & Janssen, C. R. (2016). Microplastic as a vector for chemicals in the aquatic environment: critical review and model-supported reinterpretation of empirical studies. *Environmental Science & Technology,* **50**(7), 3315-3326.

Law, K. L., & Thompson, R. C. (2014). Microplastics in the seas. *Science*, **345**(6193), 144-145.

Li, J., Yang, D., Li, L., Jabeen, K., & Shi, H. (2015). Microplastics in commercial bivalves from China. *Environmental Pollution,* **207**, 190-195.

Li, J., Qu, X., Su, L., Zhang, W., Yang, D., Kolandhasamy, P., Li, D., Shi, H. (2016). Microplastics in mussels along the coastal waters of China. *Environmental* *pollution,* **214**, 177-184.

Ma, Y., Wang, L., Wang, T., Chen, Q., & Ji, R. (2020). Microplastics as Vectors of Chemicals and Microorganisms in the Environment. In *Particulate plastics in terrestrial and aquatic environments.* 209-230. CRC Press.

Mateos-Cárdenas, A., O'Halloran, J., van Pelt, F. N., & Jansen, M. A. (2020). Rapid fragmentation of microplastics by the freshwater amphipod *Gammarus duebeni* (Lillj.). *Scientific reports*, **10**(1), 1-12.

Noren (2007). Small plastic particles in Coastal Swedish waters. In N-Research Report KIMO Sweden.

PEMSEA and Provincial Government of Cavite, Philippines (2017). State of the Coasts of Cavite Province. Partnerships in Environmental Management for the Seas of East Asia (PEMSEA), Quezon City, Philippines.

Phuong, N. N., Zalouk-Vergnoux, A., Kamari, A., Mouneyrac, C., Amiard, F., Poirier, L., & Lagarde, F. (2017). Quantification and characterization of microplastics in blue mussels (*Mytilus edulis*): protocol setup and preliminary data on the contamination of the French Atlantic coast. *Environmental Science and Pollution Research*, **25**(7), 6135-6144.

Phuong, N. N., Poirier, L., Lagarde, F., Kamari, A., & Zalouk-Vergnoux, A. (2018). Microplastic abundance and characteristics in French Atlantic coastal sediments using a new extraction method. *Environmental Pollution*, **243**, 228-237.

Possatto, F. E., Barletta, M., Costa, M. F., do Sul, J. A. I., & Dantas, D. V. (2011). Plastic debris ingestion by marine catfish: an unexpected fisheries impact. *Marine Pollution Bulletin*, **62**(5), 1098-1102.

Ribeiro, F., Garcia, A. R., Pereira, B. P., Fonseca, M., Mestre, N. C., Fonseca, T. G., Ilharco, L., & Bebianno, M. J. (2017). Microplastics effects in *Scrobicularia plana*. *Marine Pollution Bulletin,* **122**(1-2), 379-391.

Sussarellu, R., Suquet, M., Thomas, Y., Lambert, C., Fabioux, C., Pernet, M. E. J., Goïc, N., Quillien, V., Mingant, C., Epelboin,, Y., Corporeau, C., Guyomarch, J., Robbens. J., Paul-Pont, I., Soudant, P., & Huvet, A. (2016). Oyster reproduction is affected by exposure to polystyrene microplastics. *Proceedings of the National Academy of Sciences*, **113**(9), 2430-2435.

Teng, J., Wang, Q., Ran, W., Wu, D., Liu, Y., Sun, S., Liu, H., Cao, R., & Zhao, J. (2019). Microplastic in cultured oysters from different coastal areas of China. *Science of the Total Environment,* **653**, 1282-1292.

Thompson, R. C. (2006). Plastic debris in the marine environment: consequences and solutions. *Marine Nature Conservation in Europe,* **193**, 107-115.

Thompson, R. C., Moore, C. J., Vom Saal, F. S., & Swan, S. H. (2009). Plastics, the environment and human health: current consensus and future trends. *Philosophical Transactions of the Royal Society B: Biological Sciences,* **364**(1526), 2153-2166.

Thompson, R. C. (2015). Microplastics in the marine environment: sources, consequences and solutions. In *Marine Anthropogenic Litter.* 185-200. Springer, Cham.

Tourinho, P. S., do Sul, J. A. I., & Fillmann, G. (2010). Is marine debris ingestion still a problem for the coastal marine biota of southern Brazil? *Marine Pollution Bulletin,* **60**(3), 396-401.

Van Cauwenberghe, L., & Janssen, C. R. (2014). Microplastics in bivalves cultured for human consumption. *Environmental Pollution,* **193**, 65-70.

Vandermeersch, G., Van Cauwenberghe, L., Janssen, C. R., Marques, A., Granby, K., Fait, G., Kotterman, M., Diogéne, J., Karen, B., Robbens, J.,& Devriese, L. (2015). A critical view on microplastic quantification in aquatic organisms. *Environmental Research,* **143**, 46-55.

Von Moos, N., Burkhardt-Holm, P., & Köhler, A. (2012). Uptake and effects of microplastics on cells and tissue of the blue mussel Mytilus edulis L. after an experimental exposure. *Environmental Science & Technology*, **46**(20), 11327-11335.

Wright, S. L., Thompson, R. C., & Galloway, T. S. (2013). The physical impacts of microplastics on marine organisms: a review. *Environmental Pollution*, **178**, 483-492.

Yang, D., Shi, H., Li, L., Li, J., Jabeen, K., & Kolandhasamy, P. (2015). Microplastic pollution in table salts from China. *Environmental Science & Technology,* **49**(22), 13622-13627.