GSJ: Volume 9, Issue 3, March 2021
ISSN 2320-9186

Global Scientific JOURNALS

GSJ: Volume 9, Issue 3, March 2021, Online: ISSN 2320-9186
www.globalscientificjournal.com

MICROPLASTICS IDENTIFICATION ON THE DIGESTIVE TRACK CRUSTACEA FROM PANGANDARAN WATERS, WEST JAVA

Rizky Ghena Oktafira[1], Herman Hamdani[2], Wahyuniar Pamungkas[1] and Mochamad Rudyansyah Ismail[1]
[1] Majoring in Marine Science, Padjadjaran University
Jl. Raya Bandung-Sumedang KM. 21 Jatinangor, Sumedang, Indonesia
[2] Majoring in Fisheries, Padjadjaran University
Jl. Raya Bandung-Sumedang KM. 21 Jatinangor, Sumedang, Indonesia
E-mail: oktafiraghena@gmail.com

ABSTRACT

Microplastics that settle on the bottom of the waters can be dangerous if accidentally swallowed by benthic biota that feeding on the sediments (deposit feeder). Some examples of benthic biota that have the potential to consume microplastics are included in the crustacean subphylum, namely white shrimp (Penaeus merguiensis) and crab (Portunus pelagicus). The purpose of this study was to visually determine the abundance of microplastics present in the digestive tract of white shrimp and crabs and to determine the ratio of the abundance of microplastics in the body of the two types of crustacean biota with the average body weight. The research station was determined by using a purposive sampling method. Microplastics extraction in digestive track of crustaceans was carried out using a 10% KOH solution. The results showed that the microplastics found in the digestive tract of the crustacean samples were 1 μm – 2 mm in size and consisted of fibers, fragments, films, and pellets with the predominant colors being black and transparent. White shrimp accumulated more microplastics in their digestive tract than crabs with an average abundance of microplastics in the shrimp body of 0.21 particles/g in an average body mass of 35.45 g while the average abundance of microplastics in the crab body was 0.03 particles/g in the average body mass is 183.17 g.

Key words: Benthos; crustacean; deposit feeder; microplastics; Penaeus merguiensis; Portunus pelagicus

INTRODUCTION

Plastics with a diameter of 1 nm to 5 mm are defined as microplastics (Prasath and Poon, 2018). Microplastic particles found in the environment are divided into two types, which are primary microplastics and secondary microplastics. Primary microplastics are plastic particles which are micro-sized once they reach the environment and are manufactured for cosmetic, industrial, and household purposes (Moore, 2008). Example of primary microplastics includes microbeads which used in beauty products. Meanwhile, secondary microplastics define as plastic particles that can be micro-sized or macro-sized when they contaminate the environment, but degrade into micro-plastic pieces due to the effect of waves, microorganisms, human activity, ultraviolet light, and other factors (Arthur et al., 2009). Examples of secondary microplastics include the degradations of fishing nets, aqua bottles, food packages, and others.

Microplastics found in the waters consist of several forms, which are fibers, fragments, films and pellets (Chubarenko et al., 2016). Through its shape, it can be seen the characteristics of microplastics such as fibers in the form of thin fibers, generally coming from fishing nets, fragments in the form of hard, jagged and irregular plastic pieces, films in the form of transparent, soft and thin plastic and pellets in the form of hard plastic cylinders .The colors contained in microplastics vary depending on the type of polymer constituent, some of the colors that are often found are white, blue, black, red, yellow, green, purple, gray, and transparent (Zhou et al., 2018).

The existence of microplastics in the ocean comes from anthropogenic activities on land and sea which include tourism, household activities, industrial activities, fishing and shipping. Microplastics that contaminate the marine environment are initially on the water surface but due to biofouling by microorganisms, it causes microplastic particles increasing the density and sinking into the column to the bottom of the water (Egbeocha et al., 2018). As a result, microplastics are known to be contaminants that can be found on the water surface, in the water column, and also at the bottom waters. The abundance of microplastic particles was present in the highest concentration in sediments, followed by the abundance of microplastics in water surface and the water column (Hidalgo-ruz et al., 2012).

The ocean floor, which is littered with microplastics, is quite large and could be consumed by crustaceans that live as benthos and feeder deposits. This is due to the inability to distinguish between plastic and food, besides that the tiny size of microplastics can also accidentally enter his body when eating (FAO, 2017). Microplastics that have been in the biota for a long time will clog the digestive tract, causing the biota a false sense of fullness and reducing the amount of food consumed (Lusher et al., 2013).

One of the coastal areas in Indonesia that is affected by microplastics is the coastal area of Pangandaran Regency. According to Ismail et al. (2019), Layur fish (*Trichius sp*.) and Gulamah fish (*Johnius sp*.) from Pangandaran Beach had as many as 193 microplastic particles in their digestive tracts. The Bojong Salawe waters in Karangjaladri Village are also suspected of being polluted by microplastics. This is due to the fact that the waters of Bojong Salawe are one of Pangandaran Regency's largest fishing and aquaculture areas. Fishing and cultivation activities in the waters of Bojong Salawe potentially contribute microplastics to the sea which will then be consumed by biota, especially benthos such as white shrimp (*Penaeus merguiensis*) and crab (*Portunus pelagicus*).

In light of this, research on the microplastic content in the digestive tracts of crustaceans from Bojong Salawe waters, Karangjaladri Village, Parigi District, Pangandaran Regency, West Java Province, is needed in order to determine the abundance of microplastics based on the form, color, and size contained in the digestive tract of white shrimp (*Penaeus merguiensis*) and crab (*Portunus pelagicus*). It can also calculate the ratio of microplastic levels in the digestive tracts of white shrimp (*Penaeus merguiensis*) and crabs (*Portunus pelagicus*) to their overall body weight. The objectives of this research is to discover more about the circumstances of microplastic contamination in crustaceans in the Bojong Salawe waters.

RESEARCH METHODS

This research was conducted on July 19, 2020 and is located in Bojong Salawe Beach, Karangjaladri Village, Parigi District, Pangandaran Regency, West Java Province. Purposive sampling was utilized to determine the location of one data collection station, which is near to the river mouth and serves as a fishing ground. The location of the coordinates of the data collection is at 7º42 "58.007" LS and 108º30 "30,438" East Longitude. Data obtained in the form of water quality measurements, including white shrimp (*Penaeus merguiensis*) and crab (*Portunus pelagicus*) and sediment substrate.

There are several measurements to measure water quality, including: temperature measurement using a thermometer, salinity using a refractometer, water pH using a pH meter and DO using a DO meter. Measurement of water quality for each was carried out three times. Sampling of white shrimp (*Penaeus merguiensis*) and crab (*Portunus pelagicus*) was carried out using shrimp nets on fishing boats measuring 3 GT, each type was taken as many as 30 and stored in a coolbox filled with ice. Sediment sampling was conducted by diving and using a shovel to obtain sediment samples at a depth of ± 3-5 meters. The sediment samples are then stored in a ziplock bag.

Samples of white shrimp (*Penaeus merguiensis*) and crabs (*Portunus pelagicus*) were taken to the Biogeochemical Laboratory, 2nd floor, 3rd building, Faculty of Fisheries and Marine Sciences, Padjadjaran University. Meanwhile, the sediment substrate samples were taken to the Marine Conservation Laboratory on the 1st floor of building 3, Faculty of Fisheries and Marine Sciences, Padjadjaran University.

Crustacean samples, including white shrimp (*Penaeus merguiensis*) and crab (*Portunus pelagicus*), were processed according to Rochman *et al* (2015). Processing of biota samples begins by measuring the body weight of each individual first before taking the digestive tract in the form of the gut using surgical scissors, then the gut is weighed. The gut of each entity is then labeled as a marker. Before being placed in a 100 ml Schott bottle, each gut sample was crushed with a mortar and pestle. The 10% KOH solution is applied to the Schott bottle as a separating solution between organic matter (gut wall) and microplastic particles, up to 3x the body weight of the sample or until the gut is fully submerged. The schott bottle was then incubated at 60ºC for 1x24 hours, after which 10 ml of saturated NaCl was added and filtered through 0.45 µm filter paper. A microscope is then used to examine the residue left on the filter paper.

Furthermore, sediment sample processing refers to the research of Hidalgo-ruz *et al.*, (2012). The sediment is first dried under the sun for 3x24 hours until it is completely dry. The next step is to add 3 liters of saturated NaCl to 1 kg of dry sediment in order to make the microplastic particles float on the surface of the solution. The floating microplastics were filtered with 0.45 µm filter paper and observed under a microscope.

There may be as many as 20 microplastic particles > 800 µm in size or visible to the eye were found from crustacean and sediment samples were collected into one to see the type of polymer using FTIR (Fourier Transform Infra Red). The abundance of microplastics in the crustacean gut was calculated using the formula Jabeen *et al.*, (2016), entailed:

Microplastic abundance =

$$\frac{\text{Number of microplastics particles}}{\text{Crustacean gut mass (g)}}$$

RESULTS AND DISCUSSION

Research Location Conditions

Water quality parameters utilizing dissolve oxygen (DO), pH, temperature and salinity levels. Water quality data is taken in the morning at 07.30 - 08.00 WIB in sunny weather. Through water quality data collection, the results obtained for the average DO content of 12.63 mg / L, a pH of 7.13, a temperature of 28ºC and a salinity of 34.6 ppt. Referring to the Decree of the State Minister of Environment (Kepmen LH) No. 51 of 2004 concerning Sea Water Quality Standards, attachment number three for marine biota, it appears that the data on the quality of the Bojong Salawe waters obtained are in accordance with the quality standards listed in attachment number three of the Decree of the State Minister of Environment No.51 of 2004. According to this, the waters of Bojong Salawe are still in a good category for the survival of marine life.

Bojong Salawe waters receive a runoff that flows through the Bojong Salawe river mouth. Plastic waste floating on the surface of the waters was not found at the sampling station. At low tide, there is also no visible plastic waste depositing at the bottom of the sediment. Plastic waste was discovered in the roots of mangrove trees growing along the coast of Bojong Salawe. Garbage reaches the mangrove area when the tide is high or when a sea storm occurs which goes beyond the low shore, according to Silmarita *et al*. (2020). Since the mangrove area is blocked by the beach, trash would be left in the mangrove area when the water recedes.

Microplastic Identification by Shape

The total number of microplastic particles found in the digestive tract of white shrimp, crab, and sediment were 430 particles. The results revealed that there were only four forms of microplastics in the crustacean sample and the sediment substrate, including fiber, fragments, film and pellets. According to Chubarenko *et al.*, (2016) microplastics in the form of fibers, fragments, films and pellets are forms of microplastics which commonly appeared in the waters.

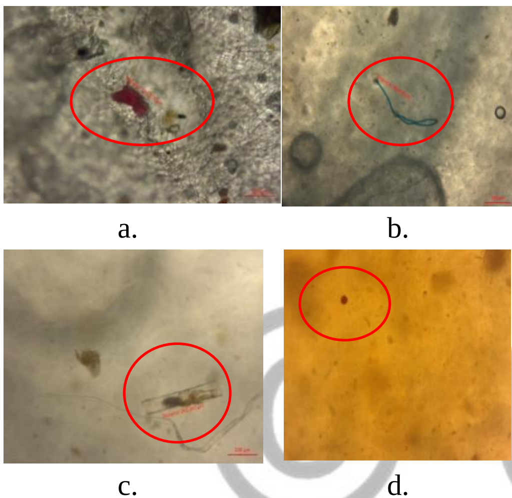

Figure 1. The form of microplastics found (a. Fragments, b. Fiber, c. Films and d. Pellets)

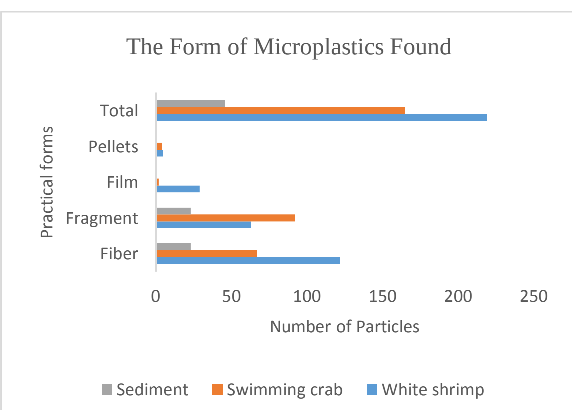

Figure 2. The number of microplastics based on the shape in each sample

In terms of identification outcomes, fiber microplastic becomes the most common form found in the digestive tract of white shrimp with a total of 122 particles. Microplastic fiber has physical characteristics in the form of long thin fibers (Zhou et al., 2018), when observed using a microscope, fine fibers will be seen on the surface of the fiber. One of the organism type that shrimp enjoys is sea worms or *polychaeta* (Gutiérrez et al., 2016). It is suspected that the shape of the fiber has similarities to the *polychaeta* which is cylindrical in shape with a lot of hairs on its body surface (Yusron, 1985).

The inability of shrimp to distinguish between food and fiber microplastic seems to be the cause of the many forms of fiber microplastic accumulating in their digestive tract. The source of microplastic fiber at the research location comes from anthropogenic activities of fishing and settlements that bring fiber through river mouth from the mainland (Kapo et al., 2020).

Microplastic fragments were the most common form in the small crab gut which was 92 particles. Crabs are familiar as omnivorous crustaceans that are selective in choosing their food and can eat the same food for quite a long time (De Oliveira et al., 2015). Plankton is the primary diet of crabs, according to Erlinda et al., (2016). Tanaka and Takada discovered plankton that accumulates large amounts of microplastic fragments (2016). The type of fragments that have accumulated in large amounts in the small crab gut is believed to have come from plankton, which is often eaten by crabs over a long period of time. The most abundant form of microplastics in nature, according to Kovac Virsek et al., (2016), is fragments. Since there are various fragments in nature, they have a high probability of being swallowed up by small marine biota like plankton, which is eaten by biota like crabs. The origin of the microplastic fragments is unknown, but their existence in the waters is linked to the degradation process that occurs in the ecosystem (Tanaka and Takada, 2016).

The amount of film and pellet microplastics found in this sample was not excessive, and these two types of microplastics were not found at all in the sediments. The film takes the form of a microplastic, and is made from very thin and low-density material that has degraded. Microplastic films float on the surface or in the water column due to their low density, and are more frequently carried by currents (Hastuti et al., 2019). Crustacean sampling was carried out when the current in the Bojong Salawe waters was very strong. Since the sediment has been transported by the current, it is believed that the current is the source of the lack of film type contained in the sediment. The film that accumulated in the guts of shrimp and crabs is believed to have come from species that had previously accumulated film microplastics in the crustacean guts. Pellet microplastics are generally found in waters close to plastic factories since pellets are used as raw material in the plastic manufacturing process (Dewi et al., 2015). Moreover, the research location is far from the plastic factory so there is little possibility of pellets in Bojong Salawe waters. This can be seen from the small pellet content in the shrimp and crab bodies and was not found in the identified sediments.

Microplastic Identification by Color

According to the results, there are eight different forms of color in microplastics detected in sediment and the digestive tracts of white shrimp and crab. Black, transparent, gray, green, purple, yellow, red, and blue are among the colors available.

Table 1. Color on microplastic particles

Color	**Microplastic Particles**			
	White shrimp	**Crab**	**Sediment**	**Total**
Black	71	53	12	136
Grey	16	11	3	30
Green	2	1	5	8
Purple	1	10	2	13
Yellow	23	9	4	36
Red	17	37	12	66
Blue	22	24	7	53
Transparent	67	20	1	88
Total	**219**	**165**	**46**	**430**

In general, the results showed that black microplastics were the most common with 136 particles. Furthermore, there were 88 transparent colored microplastics, 66 red microplastics, 53 blue particles, 36 yellow particles, 30 gray particles, 13 purple particles and 8 green particles. Black microplastic particles have been shown to be the most dominant color in many prior studies, such as Hiwari et al., (2019), who found that black was the most dominant color of all the microplastic colors found in their sample. Furthermore, Hossain et al., (2020) investigated the microplastic content in penaeid shrimp, and the results of their research revealed that the color of the most dominant microplastic in the sample body was black. The amount of contaminants absorbed in the microplastic is indicated by the black color (GESAMP, 2015). In the aquatic environment, microplastics have the potential to adsorb chemical contaminants (Egbeocha et al., 2018). The fact that the color on the microplastic is still dense implies that it has not been discolored significantly (Hiwari et al., 2019). Overall, the black microplastics found in shrimp, crab, and sediment samples mostly came in the form of fragments, which were macroplastic pieces.

Instead of black, transparent is another dominant color in the sample. Transparent microplastics in samples consist of various shapes such as films and fragments. Some microplastic fibers have been discovered to be discolored or to change color from blue to transparent. Microplastics undergo a color shift that may suggest a longer exposure period in coastal waters. The longer microplastics are in the marine environment, the more likely they are to be oxidized (Frias et al., 2010), so the transparent color of microplastics can imply how long they have been UV photodegradable (Hiwari et al., 2019).

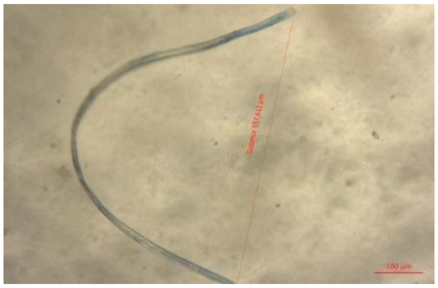

Figure 3. Discolouring on microplastic samples

Meanwhile, red, blue, yellow and purple microplastics are found mostly in the form of fibers. This microplastic is believed to have come from fishing activities in the Bojong Salawe waters.

Microplastic Identification by Size

The microplastics found ranged in size from 1 µm – 2 mm. The sizes are grouped into 9 groups, which are <20 µm, 20–40 µm, 40–60 µm, 60–80 µm, 80–100 µm, 100–500 µm, 500–1000 µm, 1000–2000 µm and 2000–5000 µm . Microplastic size grouping refers to the research of Nor and Obbard (2014).

Table 2. Size of Microplastic Particles

Size	**Microplastic Particles**		
	White Shrimp	**Crab**	**Sediment**
<20 µm	13	19	-
20-40 µm	25	43	2
40-60 µm	22	17	3
60-80 µm	15	7	2
80-100 µm	11	8	3
100-500 µm	105	54	22
500-1000 µm	25	16	12
1000-2000 µm	3	1	1
2000-5000 µm	-	-	1
Total	**219**	**165**	**46**

The size of microplastics that dominates in the guts of white shrimp, crab and sediment is in the size range of 100-500 µm with 105 microplastic particles in white shrimp, 54 in crabs and 22 in sediment. The concentration of microplastics in the guts of white shrimp and crab is based on the availability of microplastics in the sediment, as demonstrated by the amount of particles measuring 100-500 m, which is the largest size in the three samples.

Physical factors in the environment are considered to degrade microplastics of various sizes found in the guts of white shrimp and crabs (Claessens et al., 2013). This is due to the lack of enzymes in the organism's body that can break down synthetic polymers from microplastics (Andrady, 2011).

Microplastics Abundance in Crustaceans

Using the formula of Jabeen et al., (2016) the abundance of microplastics in the white shrimp and crab gut samples was measured. According to the calculations, the transparent colored microplastic dominates the white shrimp gut with an abundance value of 13.76 particles/g, while the black colored microplastic dominates the crab gut with an abundance value of 2.66 particles/g. Fiber is the most abundant type in shrimp gut, with a value of 20.86 particles/g, while fragments are the most abundant form in small crab gut, with a value of 5.24 particles/g. The most popular microplastic size in shrimp and crab intestines is between 100 and 500 m, with an excess of 18.32 particles/g in shrimp and 2.79 particles/g in crabs.

The significance of microplastic abundance in shrimp and crab samples varies depending on where these crustaceans live in the water. Anthropogenic activities that occur near living biota often lead to the increase or decrease of existing microplastic levels. Microplastics have a high risk of contaminating oceans with a lot of anthropogenic activities (Kustiasih et al., 2017). Using the formula of Jabeen et al., (2016), the overall average abundance of microplastics in the body of white shrimp and crab was compared. The results obtained were the abundance of microplastics in the shrimp body of 0.21

particles/g with an average body mass of shrimp of 35.45 g whereas the average number of microplastic abundance in the crab was 0.03 particles/g with an average body mass of the crab was 183.17 g. Based on these two scores, it can be seen that the abundance of microplastics in the shrimp body is greater than the abundance of microplastics in the crab body.

Differences in the habits of the two groups of crustaceans in acquiring food are believed to be the source of the disparity in the abundance of microplastics in the bodies of white shrimp and crabs. White shrimp belongs to the penaeid shrimp family, which eats any species that sink to the ocean floor or rise through the water column (Williams 1981 in Gutiérrez et al., 2016). Crabs, on the other hand, always wait for the organisms that will become their food to approach by burying themselves in the sand (Effendy et al., 2006). It can be seen from the variations in foraging habits between white shrimp and crabs that shrimp are much more aggressive in the process of acquiring their food than crabs.

FTIR Analysis

The FTIR test was carried out using a microplastic sample in the form of fiber> 800 μm. The FTIR test showed that 88.52 percent of the microplastic fiber is close to fisher or fishing nets. Since the polymer form of the microplastic sample examined had never been detected before using this instrument, the absence of a detected polymer type was assumed. Several types of polymers commonly used in fishing nets include Polyamide (PA), Polyethylene (PE), Polypropylene (PP), Polyvinyl Chlorida (PVC) and Polyester (PES) (Thomas and Lekshmi, 2017).

CONCLUSION

According to research conducted in the Bojong Salawe Waters, Karangjaladri Village, Parigi District, Pangandaran Regency, West Java Province, the outcomes of microplastics found in white shrimp guts are dominated by transparent colors, microplastic forms in the form of fiber, and sizes ranging from 100 to 500 μm with an abundance of 18 to 32 particles per gram. In the microplastic crab gut, it was found to be predominantly black, the shape of the microplastic was in the form of fragments and ranged in size from 100-500 μm with an abundance of 2.79 particles/g. The abundance of microplastics in the shrimp body is greater compared to the abundance of microplastics in the crab body. It appears that the average abundance of microplastics in the shrimp body is 0.21 particles per gram with an average body mass of 35.45 g, while the average abundance of microplastics in the crab body is 0.03 particles per gram with an average body mass of 183.17 g.

SUGGESTION

From the research findings, it is advisable to carry out further research on the impact of microplastics on crustaceans. This is pivotal in response to a large number of microplastic particles in this two biota.

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