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Microplastic Depuration on *Asaphis Detlorata*

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Abstract

Depuration is an effort to reduce/eliminate contamination including microplastics, which one is using a water circulation system. This study aims to determine the effective depuration time to reduce the microplastic content in *Asaphis detlorata*. This study used a quantitative approach with experimental research design with a completely randomized design. There are 450 shells used as an experimental animal where the treatment consisted of four depuration times, namely 1;2;3; and 4 days with 3 repetitions of each treatment, while the control shells were without depuration. Analysis of variance (ANOVA) was used to see the effect of depuration treatment on the microplastic content. If the effect of the treatment was significantly different, then the post hoc test was continued to determine the differences between treatments. The results showed that *Asaphis detlorata* obtained from the mouth of the Lakatong river estuary were contaminated with microplastics ranging from 0.6 to 8.1 MPs/shellfish and an average of 3.96 MPs/shellfish. Depuration time significantly affected the microplastic content in shellfish depuration effectiveness. There is a tendency that the longer depuration time is decreased microplastic content in shellfish. The effective depuration time to reduce the microplastic content in *Asaphis detlorata* was 3 and 4 days. Further research is needed for a more effective depuration for cleaning microplastics in shellfish.

Keywords: Depuration, *Asaphis Detlorata*, Microplastic, Water Circulation System

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Introduction

Global production of plastics currently exceeds 320 million tonnes (Mt) per year, more than 40% of which is used as single-use packaging which results in plastic waste. (Wright & Kelly, 2017). Indonesia's position as the country with the second longest coastline in the world also has a record of being the second largest contributor to marine debris in the world after China (Jambeck et al., 2015). The large amount of plastic waste in Indonesia's oceans will threaten the marine life in it. Data on the presence of microplastics in seafood from Indonesian waters is still very minimal, whereas on the other hand, the level of plastic pollution in Indonesia is high (Jambeck et al., 2015).

Several studies provide evidence that small plastic particles accumulate in marine food chains. Scientists have also detected microplastics in human food, it is also suspected that humans have been orally exposed through food consumption. (Liebmann et al., 2019). According to Hantoro et al (2019), the estimation of microplastic intake through consumption of seafood shows a very large variation. In samples of blood clams, sediment and water, it

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was proven that 100% of the samples contained microplastics (Birnsteal et al, 2019). This has raised concerns about the potential economic impact and risk of exposure to microplastics on food, especially for coastal communities (Hantoro et al., 2019).

Shellfish is a biota that has the potential to be contaminated by contaminants such as heavy metals due to its filter feeder properties (Yennie & Murtinim, 2005). One way to provide a sense of security to consumers related to food consumption, especially shellfish, is by eliminating or reducing some of the hazardous materials from the biological, chemical or physical hazards sector (Sulmartiw, 2019). In principle, the depuration method is the step of purification of biota such as shellfish which are caught in polluted waters and then the cleaning or depuration process is carried out. The purpose of this depuration process is to reduce the risk of bacterial contaminants and several heavy metals that are harmful to human health (Gabr & Gab-Alla, 2008). Depuration has the potential to reduce the heavy metal content of Cadmium in shellfish (Ma'rifatul, 2017).

In addition to heavy metals, depuration can be used to remove microplastics (Lusher et al, 2017). Depuration can be done with a variety of cleaners, including recirculating systems (Ma'rifatul, 2017). Good depuration is very useful in reducing and even removing contaminants including microplastics (Cauwenberghe & Janssen, 2014), and can significantly reduce microplastic contamination in shellfish (Birnsteil et al., 2019).

Takalar Regency is one of the areas in the South Sulawesi Province of Indonesia with the potential for coastal resources, namely various types of fish and shellfish (Liebbman et al, 2019). The results of the marine debris identification study show that several beaches in Takalar Regency have been contaminated with plastic waste (Zulkarnaen, 2017). Mangarabombang District Takalar Regency has dirty and cloudy water conditions (Ulum, 2020). The Lakatong River, which empties into the waters of Mangarabombang Subdistrict, is a habitat for shellfish, where some of the surrounding communities depend on their livelihoods as dry collecting fishermen. The resulting shellfish is usually sold and partially consumed. Many community activities are found around the Lakatong River Estuary such as ponds, bathing tours and densely populated settlements with low levels of sanitation which are indicated by the large amount of plastic waste. This anthropogenic activity has an impact on the heap of plastic waste in coastal areas and can affect marine life. Information on the microplastic depuration process in sea shells is still very limited, while the potential for microplastic contamination in shellfish is very high. It is hoped that the depuration process on shellfish can improve food safety so that the shellfish is suitable for public consumption. In connection with this, research on microplastic depuration in shellfish is very important to do.

Methods

The research was carried out at the BRPBAPPP experimental pond installation located in Punaga Village, Mangarabombang District, Takalar Regency for depuration activities. Meanwhile, microplastic analysis was carried out at the Marine Ecotoxicology Laboratory, Faculty of Marine Affairs and Fisheries, Hasanuddin University.

The test animal in the form of *Asaphis detlorata* was obtained from fishermen who collect shells from the waters of the estuary of the Lakatong river, in Topejawa Village, Mangarabombang District, Takalar Regency (Figure 1). A total of 450 *Asaphis detlorata* were used as test animals consisting of 90 control (without depuration) and 360 for depuration treatment. The test animals were selected based on the relatively uniform size of the approximately 600 shells obtained from fishermen.

The experimental container for depuration is a glass aquarium measuring 50 cm long, 40 cm wide and 40 cm high, totaling 12 units equipped with an aeration system and running water. The source of sea water with a salinity of 35 ppt is taken from Punaga waters, then collected in a reservoir. Water sterilization using 60 ppm chlorine, then neutralized with thiosulfate and aerated to remove the effect of chlorine. Before being used as a test medium, seawater is first filtered using a filter bag with a mesh size of 300 microns. The experimental container is also equipped with a 1000 m³ volume of seawater reservoir which functions as a water source in the circulation system.

This study used a quantitative approach with a completely randomized experimental research design. The treatments tried were depuration time, namely 1; 2; 3 and 4 days, each treatment 3 replications. The experimental container was in the form of an aquarium filled with 60 liters of seawater, then aerated. The experimental container is also equipped with a flowing water system with a flow rate of 3.5 L / min. A total of 450 selected shellfish were used as test animals, 90 of which were used as the initial (control) sample without depuration to describe the condition of the microplastic content in shellfish in the study location. The morphometry of the initial samples was also measured to describe the length, width, height and weight of the shells. The morphometric shells have a length between 5.2-6.6 cm (average 5.8 cm ± 0.4 cm), a width between 3.9-6.4 cm (average 4.5 cm ± 0, 4 cm), thickness between 3.1-3.8 cm (mean 3.4 cm ± 0.3 cm), and weight between 4.8-14.4 g (mean 10.2 ± 2, 1 g).

A total of 360 shells were used as test animals for depuration treatment. In each experimental container, 30 shells were taken randomly, then water was flowed continuously into the experimental container with a flow rate of 3.5 L / minute. The depuration process runs for 1; 2; 3; and 4 days according to the depuration time treatment ¹¹ the test shells were not fed. During the depuration process, onsite quality measurements were taken in the morning (07:00-08:00) and evening (17:00-18:00). At the end of the study, all shellfish were harvested and 10 of them were separated to take their meat as samples for microplastic analysis.

¹⁵ Water quality parameters including temperature, salinity, pH and dissolved oxygen were measured in situ in the morning and evening using a YSI ProfesionalPro DO-meter. At each depuration time treatment was completed, the test shells were removed from the experimental container. A total of 10 shells from each container or experimental unit were taken randomly, then the morphometry was measured and the shells were opened to take the meat as samples for microplastic analysis. The shellfish obtained was weighed and put into a sample bottle containing 20-50 mL of 10% KOH solution. The microplastic analysis technique in shellfish meat refers to the method Rochman et al (2015). The clam meat sample was then put into a cool box with a cold temperature and taken to the Marine Ecotoxicology Laboratory, Faculty of Marine Affairs and Fisheries, Hasanuddin University

The next step is extracting the shellfish to separate the microplastics from the shellfish. Each sample pot containing 10 grams of clam meat was filled with ¹% (20-50 ml) KOH (Potassium Hydroxide) solution up to 3 times the tissue volume in ultrapure water and incubated overnight (24 hours) at 60 ° C to digest organic matter. To avoid cross-contamination between samples, all tools and glassware were rinsed with ultrapure ¹ water 3 times between samples. Furthermore, the plastic debris is extracted and measured. For each sample, the digested material was carefully sorted and examined under a surgical microscope using glass petri dishes that had been rinsed with ultrapure water three times. ⁹ chunks of plastic debris larger than 0.5 mm were recorded. Plastic debris photos are used for final confirmation i.e. to identify the type of plastic debris and to determine ¹⁷ size of the material. Identification and counting of microplastics were carried out using optical analysis

Using a Carl-Zeiss binocular microscope with a magnification of up to 80 times (Hidalgo-Ruz et al., 2012).

Microplastics are broadly classified according to morphological characters, namely size, shape, color (Velzeboer et al., 2014). The forms of microplastics include fragments, characterized by irregular particles, crystals, feathers; fiber forms characterized by filaments, microfibers, strands, yarns; bead shape characterized by powder, granule, cut, flake; foam form; and granular shape (Hidalgo-Ruz et al., 2012). Depuration effectiveness was calculated by comparing the difference in the number of microplastics in each treatment with the control treatment divided by the microplastic content in the control treatment. To determine the effectiveness of depuration, the following formula is used. Depuration effectiveness = $\{(\text{Number of MPs in depuration treatment} - \text{Number of MPs in control treatment}) / \text{Number of MPs in control treatment}\} \times 100$ (Suhendra & Mitra, 2008).

To determine the effect of the depuration time treatment, the data on the microplastic content in shellfish were analyzed using One Way Anova, and if it was proven that there was a significant / significant effect, a Post Hoc Test was carried out to determine the differences between treatments. Water quality data is displayed in tabular form and analyzed descriptively to assess the appropriateness of the water media for test animals during the depuration process.

Results and Discussion

Microplastic Depuration of *Asaphis detlorata*

Microplastics <5 mm in size were detected in analyzed *Asaphis detlorata* shells. In the control clam group, 100% *Asaphis detlorata* contained microplastics ranging from 1 - 19 MPs / shellfish with an average value of 8.1 ± 5.7 MPs / shellfish. After depuration, the microplastic content in shellfish was less than those that were not depurated (Table 1). The pattern of decreasing MPs content in shellfish during the depuration process decreased on the second day, but rose again on the third day and decreased on the third and fourth days. Overall the depuration process that was tried was able to reduce the MPs content in shellfish meat. This shows that the longer the depuration time (days), the less amount of microplastic contaminants in *Asaphis detlorata* will be. Depuration time significantly affected MPs content in shellfish meat. In the control treatment, 1 and 2 days, the three were not significantly different, but significantly different from the 3 and 4 days treatment, while the 3 and 4 day depuration treatments were not significantly different (Table 1).

Table 1. Average microplastic content in shellfish meat at each depuration time treatment

Treatment	Minimum	Maximum	Average	Sd
Control	6,7	9,2	8,1 ^a	1,3
1 day	1,4	9,4	4,3 ^a	4,9
2 days	1,7	9,0	6,1 ^a	3,9
3 days	0,4	1,1	0,7 ^b	0,4
4 days	0,1	1,1	0,6 ^b	0,5

Note: Different superscripts in the same column show a real difference

The size of the microplastics found in shellfish ranged from 0.1 to 2.0 mm (Table 2). Based on the analysis of variance, the depuration time treatment did not significantly affect the MPs size in shellfish meat. This is indicated by the average MPs size that is relatively the same in the range 0.9-1.6 mm.

Table 2. Average size of microplastics in shellfish meat at each depuration time treatment

Treatment	Microplastic size (mm)			
	Minimum	Maximum	Average	Sd
Control	0,9	1,2	1,1 ^a	0,1
1 day	1,3	2,0	1,6 ^a	0,4
2 days	1,2	1,7	1,5 ^a	0,3
3 days	0,6	1,5	0,9 ^a	0,5
4 days	0,1	1,6	1,1 ^a	0,8

Note: Different superscripts in the same column show a real difference

Microplastics in the form of fibers, fragments, films and monofilaments were detected in shellfish in all treatments (Table 3 and Figure 2). In the control clam group, the most microplastic forms found were fragments, while in the depurated shellfish group the most microplastic forms were found was fiber. After the depuration process, the microplastics detected on the fourth day of treatment were fibers and films with the least amount of microplastic contamination compared to other treatments. The results showed that the form of fragments and monofilament from the microplastic was released the fastest by the shells during the depuration process, compared to the form of fiber and film.

Table 3. Form and number of MPs in shellfish meat at each depuration time treatment

MPs Form	Depuration time treatment (days)				
	Kontrol	1	2	3	4
Fiber	60	114	80	11	4
Fragment	128	3	0	0	0
Film	50	12	13	10	11
Monofilament	3	0	3	0	0

Fiber microplastics found in marine **13** bitats can come from domestic waste (Browne, 2015). Fiber is a type of microplastic that comes from the fragmentation of monofilament fishing nets, ropes and synthetic fabrics. Fishery activity is a source of microplastic fiber because most fishing nets are made of fiber. The fragments are known to come from pieces of plastic products and breakdown of rigid plastics with a very strong synthetic polymer. The source of the fragments found in the sediment can come from plastic waste generated by the activities of local residents (Wright et al., 2013). Microplastic films were identified as those commonly used in **6** astic wraps and bags. These microplastics are easily destroyed and have a low density. film is a secondary plastic polymer that comes from fragmentation of plastic bags or plastic packaging and has a low density (Kingfisher, 2011).



Fiber



Fragment

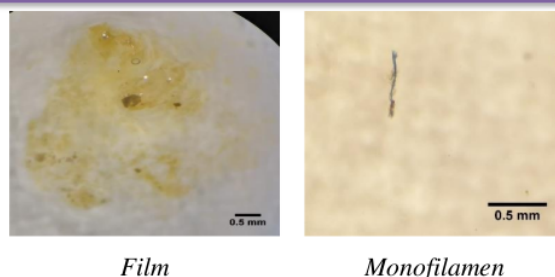


Figure 2. Microplastic shape in *Asaphis detlorata* (Carl-Zeiss binocular microscope with 80 times magnification)

The results of statistical analysis showed that the time of depuration had a significant effect on the value of the effectiveness of depuration. The three treatments, namely control, 1, and 2 days were not significantly different, but they were significantly different from the 3 and 4 day depuration treatment, while treatments 3 and 4 were not significantly different (Figure 3). Depuration on the 3rd day has an effectiveness value of 91.76% and the 4th day is 93%. This shows an increase in the value of the effectiveness of depuration along with the length of time for depuration.

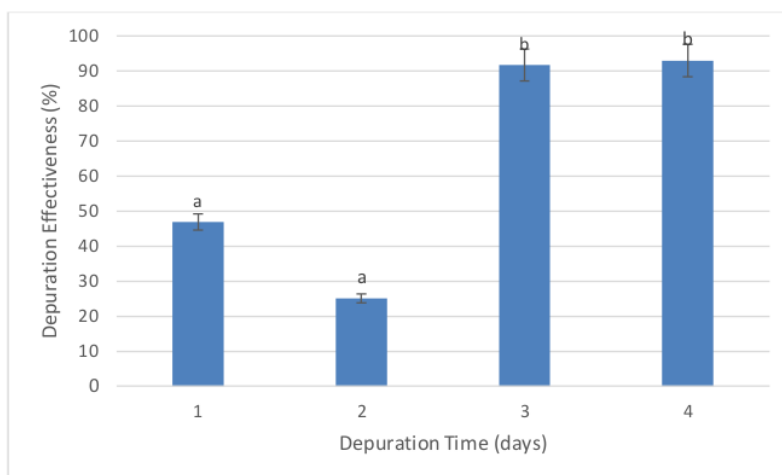


Figure 3. The effectiveness of microplastic depuration time in shellfish

The microplastic content of the depurated medium at the end of the experiment showed an increasing amount (Table 4). This proves the existence of the excretion process carried out by the dissociation shells by releasing contaminants. The long depuration time is also accompanied by an increase in the amount of contaminants, this indicates that the water circulation process in the experimental container allows contaminant deposits to occur, because they are not completely rinsed out even though the flow rate during the depuration process is 3.5 L / minute.

Table 4. Microplastics (MPs / L) in depurated media at the end of the experiment

Value	Depuration time treatment (days)			
	1	2	3	4
Minimum	5	12	11	11

Maximum	28	15	28	23
Average	14,3	13,0	17	18,3
sd	12,1	1,7	9,5	6,4

Depuration of Water Quality Media

The feasibility of water quality is an important factor for shellfish life. During the depuration process, the water temperature ranges from 24.6-26.4 oC (Table 5). The range of water temperature is below the criteria for the optimal range for marine biota, but is still in the proper range for tropical marine biota. The low water temperature is caused by the "bediding" season in the South Sulawesi region which lasts from July to September. The "bediding" season which occurs in the dry season is accompanied by strong winds, resulting in salinity in Punaga waters ranging from 35-36 ppt. While the salinity in the experimental unit is between 35.34-35.62 ppt, this value is high enough for the optimal salinity of marine life, but it is still in the feasible category. Meanwhile, the pH ranges from 7.73 to 9.06, and is in the proper pH range for marine biota. The dissolved oxygen content during the experiment was between 4.49-5.39 ppm and was considered suitable for marine life.

Table 5. Water quality parameters (range, mean ± sd) during depuration

Parameter	Treatment / Depuration (days)				Optimal Value
	1	2	3	4	
Temperature (°C)	25,4-26,2 25,78±0,42	24,6-26,3 25,64±0,60	24,9-26,4 25,68±0,56	24,8-26,3 25,69±0,54	28-32
Salinity (ppt)	35,39- 35,64 35,53±0,11	35,37- 35,61 35,45±0,09	35,34- 35,62 35,45±0,08	35,36- 36,61 35,39±0,32	<30
pH	8,19-8,86 8,55±0,35	8,21-9,03 8,75±0,32	7,73-9,06 8,70±0,38	7,91-9,01 8,51±0,43	7-8,5
Dissolved oxygen (mg / L)	4,63-5,39 5,10±0,27	4,71-5,28 5,10±0,20	4,49-5,17 4,93±0,20	4,62-5,36 5,06±0,22	>5

*) Standard Quality Standards Decree of the State Minister for the Environment No. 51 of 2004 concerning Sea Water Quality Standards for Marine Biota.

Asaphis detlorata obtained from the mouth of the Lakatong River show that they are 100% contaminated by microplastics. This means that the commodity *Asaphis detlorata* from these waters can be categorized as not meeting the "food safety" requirements as required for seafood for human health. The percent²⁴ of shellfish contaminated with microplastic will get smaller in line with the length of the depuration process. The value of the effectiveness of the depuration time reached 92% occurred in the 3-4 day depuration process. The depuration technique in a container with a running water system that was tried in this study was able to reduce the microplastic content in shellfish meat. The microplastic content at 2 days depuration showed an increased amount compared to the one day depuration time. This is presumably due to the adaptation process for shellfish after the shells were removed from their natural habitat. The adaptation time for organisms or biota that live in the research environment occurs to reduce the level of risk of death in biota at the beginning of maintenance, so that later the biota used is not stressful (Budiawan et al., 2019).

In this depuration process, shellfish feeding is not carried out to avoid contamination. According to Tremblay et al (2012), the food filtering process in shellfish takes place when the shells open their shells. While in a threatened condition, the clams will close their shells. The process of absorption of pollutants by shellfish is carried out simultaneously with the eating process taking place. The adductor muscle structure is used by this type of shellfish to respond to the opening and closing of the shell during this food filtering process. Tremblay et al (2015) stated that the ligament which is the attachment of the two shells will respond to the opening and closing of the shell based on conditions of pressure to open and pressure to relax.

This eating mechanism is an important consideration in the depuration treatment process in assault. This mechanism is one of the requirements for the functioning of shellfish metabolism to receive depuration treatment. The surviving shells excrete. The excretion of shellfish containing contaminants is then shed off by a depuration process with continuous purification of the water pumped by a circulation pump (Riyadi et al., 2016). However, it does not rule out that the contaminants that have been released can still be reabsorbed by the shellfish so that they can increase the contaminant content in the shellfish meat. This is reflected in the 2-day depuration treatment, where the microplastic content increases again. On the third day of depuration, it is suspected that the emptying process of the stomach contents began to occur so that there were enough contaminants left and fewer contaminants left in the digestive system. Depuration can reduce contaminants in shellfish more than without depuration. Depuration is an effective method of cleaning microplastics (Birnstiel et al., 2019)

Microplastics in the form of fibers, fragments, films and monofilaments were found in control shells and the dominant ones found were fragment-shaped microplastics. After the depuration process, the microplastics found were fibers and films. If you look at the results of the depuration analysis of the four treatments, the number of microplastics in the form of film did not change significantly and was different from the microplastics in the form of fibers, fragments and monofilaments which experienced changes where the number of microplastics in these forms was getting smaller with the time of depuration. Film form microplastics did not come out much after depuration, presumably because this microplastic has a low density and is easily destroyed so that this form of microplastic remains found in the shellfish meat.

In this study, the remaining media for depuration was taken and analyzed for its microplastic content so that there was an indication that the longer the depuration time was, the microplastic content left in the experimental container would increase. The presence of microplastics left in the experimental container indicates that there has been a release of microplastics from the shells to the media during the depuration process. The decreasing value of the microplastic content in shellfish and the increasing of microplastics in the depuration medium proved that the depuration treatment of *Asaphis detlorata* was able to successfully reduce microplastic contaminants in the shells, thereby increasing food safety for shellfish consumers. However, this depuration technique still needs to be improved in order to completely remove microplastic contaminants because there are still microplastics that are detected even after the depuration time of 4 days. Perhaps the depuration of 92 hours or 4 days is not long enough to completely remove microplastics (Von Moos et al., 2012).

When viewed from the aspect of food safety, seafood contaminated with microplastics is not safe for consumption. Microplastics contain chemical compounds that are added during their manufacture and absorb contaminants around the environment (Rochman, 2015). The discovery of microplastics in seafood makes it one of the new contaminants (novel food

contaminant). Thus the presence of plastic pollutants in seafood consumed by humans can pose a food safety risk that needs to be studied further (Wideanarko & Hantoro, 2018).

Conclusion

Asaphis detlorata originating from the waters of the Lakatong River Estuary, Takalar Regency, have been contaminated with microplastics in the form of fibers, fragments, films and monofilaments. The microplastic content in shellfish meat can be reduced by depuration using running water for 3-4 days with a depuration effectiveness rate reaching 92%. Because there are still microplastics after 4 days of depuration, further research is needed to study depuration techniques that are more effective in cleaning microplastics from shellfish meat so that they are safe for consumers.

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