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Microplastic Content in the Digestion of Freshwater Mussels (*Pilsbryoconcha exilis*) at Different Depths in Koto Panjang Reservoir, Riau

Kandungan Mikroplastik dalam Pencernaan Kerang Air Tawar (Pilsbryoconcha exilis) pada Kedalaman Berbeda di Waduk Koto Panjang, Provinsi Riau

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Abstract

Microplastics are small pieces of plastic that can pollute the environment. This study aims to determine the types of microplastics in the digestion of freshwater kijing based on different stocking densities and water depths. This research was carried out from February to April 2024. Using the experimental method with a one-factor Group Random Design. Sampling of the kijing was observed after 2 weeks of laying according to the specified depth, and then the abundance of microplastics in the digestion of freshwater kijing was examined. Water quality parameters are measured by temperature, brightness, and pH. The study found five microplastic types: fiber, fragments, films, granules, and pellets. The most common type of microplastic found at all three different depths is the type of film. The microplastic abundance found at all three depths in order is 5.2, 5,4 and 6 particles/ind. From the ANOVA results, the depth has a p-value of 0.00850, which means that the p-value > 0.005 does not show any difference between depth data. While the p-value of the treatment is 0.00084, this means that the p-value of <0.005 is the difference between the treatment data. Treatments consisting of 1kg, 2kg, and 3kg had different abundance data in each treatment. This means that there are significantly different results by the treatment of microplastic abundance data in freshwater mussel.

Keywords: *Pilsbryoconcha exilis*, Microscope, Digestion.

Abstrak

Mikroplastik adalah potongan kecil plastik yang dapat mencemari lingkungan. Penelitian ini bertujuan untuk mengetahui jenis mikroplastik dalam pencernaan kijing air tawar berdasarkan padat tebar dan kedalaman air berbeda. Penelitian ini dilaksanakan pada bulan Februari hingga April 2024. Menggunakan metode eksperimen dengan Rancangan Acak Kelompok (RAK) satu faktor. Pengambilan sampel kijing diamati setelah 2 minggu peletakkan sesuai kedalaman yang ditentukan dan kemudian menghitung kelimpahan mikroplastik dalam pencernaan kijing air tawar. Parameter kualitas air yang di ukur suhu, kecerahan, dan pH. Hasil penelitian ditemukan 5 jenis mikroplastik yaitu fiber, fragmen, film, granulle, pellet. Jenis mikroplastik yang paling banyak ditemukan di ketiga kedalaman yang berbeda yaitu jenis film. Jumlah kelimpahan mikroplastik yang ditemukan di ketiga kedalaman secara berurut yaitu 5,2; 5,4 dan 6, partikel/individu. Dari hasil ANOVA yang diperoleh bahwa kedalaman memiliki nilai p-*value* 0,00850, nilai ini berarti p-*value* > 0,005 tidak terlihat perbedaan antar data kedalaman. Sedangkan p-*value* perlakuan bernilai 0,00084, ini berarti nilai p-*value* <0,005 terlihat perbedaan antara data perlakuan. Perlakuan yang terdiri dari 1kg, 2kg, dan 3kg memiliki perbedaan data kelimpahan di tiap-tiap perlakuannya. Hal ini berarti terdapat hasil berbeda nyata oleh perlakuan terhadap data kelimpahan mikroplastik di kerang air tawar.

Kata kunci: *Pilsbryoconcha exilis*, Mikroskop, Pencernaan

1. Introduction

Microplastics have become a global problem that pollutes the aquatic environment (freshwater and sea). This pollutant is in the form of plastic particles measuring <0.5 mm which come from the results degradation object known macroplastics as microplastic secondary in the form of pieces, parts, or fragmentation plastic (Fachrul et al ., 2021), including fragment, fiber, and filament or film types as the most environmentally hazardous types (Sari et al., 2021). On the other hand, primary microplastics, such as microbeads, are intentionally specially engineered as a base material for making pellets or mixtures of many cosmetics (Rocha-Santos & Duarte, 2015; Yona et al., 2021).

In the environment, entry routes can be via several routes, including disposal waste, stormwater runoff and degradation of rubbish macroplastics (Browne, 2015). Microplastics inside waters can cause these pollutants to be swallowed by aquatic biota so that they accumulate in the stomach and intestines, which can ultimately cause biomagnification through the food chain by predatory fish and even humans. Implications negative microplastics in fish biota from various published articles, including: (a) the occurrence of intestinal blockages and lack of nutrients absorbed by the intestines (Cole et al ., 2015); (b) transferring incoming pollutants or plastic additives and moving them to other tissues (Brennecke et al. 2015); and (d) moving into the food chain (Farrell & Nelson, 2013), including to the population man (Cole et al., 2015).

Polluter microplastic has been found in the waters of the Koto Panjang Reservoir. From the results of previous research, 3-6 types of microplastics (film, fiber, fragments, pellets, foam, granules) were found in the water column (Edy et al., 2021; Friadi et al., 2023). Factors influencing the abundance of gastropods in seagrass include environmental parameters such as temperature, salinity, pH, sediment fraction, nitrate, and phosphate. Other factors that influence the abundance of gastropods include food availability, predation, and competition. However, only three are known to be found in the digestive tract (stomach and intestines) of kapiek and carp fish (Margaretha et al., 2022; Ulfa et al., 2022), so the study of microplastics in other fish biota is still ongoing. Microplastics in the water column that continue to increase can threaten the sustainability of wild and farmed fish biota. Kijing can filter particles from the water column with a size between 0.1 - 50.0 nm, and the filtration efficiency reaches 100% at particle sizes >4.0 nm (Komarwidjaja, 2006).

When mussels filter water, food materials such as phytoplankton and other microorganisms are also filtered and then converted into body tissue so that mussels can function as water cleaners (Kadar, 1997), except for particles that are not filtered out, like microplastics will be held up in digestion. Research shows that fiber and film were found in mussels (*P. exilis*) in the Jembrana River, Bali (Yunanto et al., 2021), so the kijing can be used as a biofiltration for microplastics. This study aimed to analyze the type of microplastic in freshwater mussels placed based on different stocking densities and water depths in the Koto Panjang Reservoir.

2. Materials and Methods

2.1. Time and Place

This research will be conducted from February 2024 to April 2024, which will take place at the Koto Panjang hydropower reservoir. Microplastic analysis was conducted at the Waste Processing Laboratory of FPK, Universitas Riau. The location of each experimental unit was carried out at the damsite location of the Koto Panjang Reservoir.

2.2. Methods

The experimental method used in this study with a single-factor randomized block design (GRD), namely the amount of stocking density divided into three treatment levels, namely: (1) 1 kg, (2) 2 kg and (3) 3 kg. Different water depths are divided into groups consisting of 3 group levels, namely: In this experimental design, the factors are: (1) surface (0 m), (2) depth 4 m, and (3) depth 8 m. The response to stocking density treatment and water depth groups observed in mussels was (1) mussel survival, (2) the type of microplastic in the digestion mussels and (3) the abundance of microplastic in the digestion mussels.

2.3. Procedures

2.3.1. Preparation of Gravestone Container

Preparation of the gravestone container using 27 plastic baskets measuring 41 cm long, 28 cm wide and 15 cm high. Each basket will be divided into three vertically sections in every three repetitions. Each basket cover will be locked using a T table so the mussels do not come out of the container. When pulled and sunk into the water, the basket will be given a frame of wire aluminium for a stronger basket. During the research, the outer walls of the basket will be cleaned once a week so that the circulation of the incoming water flow into the basket becomes smooth from various dirt and moss.

2.3.2. Gravestone Preparation

Mussels were obtained from the Rumbai area of Pekanbaru City, as much as 60 kg (20 tails/kg), with a total of 1200 tails selected with relatively uniform sizes. Then the mussels were taken to the Koto Panjang Reservoir and adapted for 3 days. After the adaptation period, the mussels were divided according to the predetermined stocking density and a portion of the mussels, as much as 30% of the total mussel population. Before the adaptation, 10 mussel samples were taken randomly to determine the type and abundance of initial microplastics by taking the contents of the mussel's body before being affected by microplastics from the Koto Panjang Reservoir. The gravestones that have been selected and entered into the basket following treatment, then placed into the water according to the specified depth. Each experimental unit will be hung on the floating structure.

2.3.3. Observation and Sampling of Mussel

Microplastics were observed after 7 days, destroying all parts of the mussel's digestive system. This procedure follows what has been recommended as an effective way to extract microplastics that do not damage polymers during digestion but still destroy organic matter in organisms. After 7 days, the sample was dropped onto a glass object with a dropper, then covered with a cover glass and placed under a CX 21 microscope with the sweep method. Identification process of microplastic is done with the method of Optical Microscopy, namely determining the form of the microplastic particle using a microscope. Object microplastic obtained under a microscope, then photographed, and the colour recorded according to the references in Table 1

Table 1. Classification type microplastics

No.	Type Microplastics	Source Microplastics	Reference
1.	Film	The film is thin, not regular, soft, transparent, and more flexible than the fragments.	Ebere et al. (2019)
2.	Fiber	Thin in shape, can be long or short, and can clump or form knots.	Ebere et al. (2019)
3.	Fragment	Fragments are thick, irregular, rugged, jagged, and have sharp, curved edges.	Ebere et al. (2019)
4.	Pellets	Pellets have regular properties, are hard, have various shapes (discs, ovals or cylinders), and are colored from clear to white and yellowish.	Al-Shawafi et al. (2018); Boucher & Friot (2017); Zhou et al. (2018)

2.3.4. Water quality measurement

Water quality measurements were carried out twice at the beginning and end. Research following the time and location of sampling of mussel biota placed in Koto Panjang Reservoir. Measurement of water quality parameters such as temperature, brightness and pH.

2.4. Data Analysis

Data on the number of measurements of length, width and weight of the mussels, as well as the type and abundance of microplastics, water quality (temperature, pH, brightness) are presented in the form of images and tables using Microsoft Excel 2020. Then discussed descriptively by comparing it with the lake/reservoir water quality standards and experimental analysis results. Furthermore, for data on the number of types and abundance of microplastics, the F analysis of variance (ANOVA) test was conducted on a randomized block design with a significance level of 99%. Calculation abundance of microplastics (particles/ind), according to Boerger et al. (2010), is as follows: Abundance (Particles/Ind) = $\frac{\text{Total of microplastics}}{\text{individual}}$.

3. Result and Discussion

3.1. Type Microplastics in the Channels Freshwater Mussel Digestion

. Several types of freshwater mussels, kijing (*P. exilis*), as research samples that have been analyzed, were obtained with Type 5 microplastics in the stomachs of freshwater mussels or clams. Type microplastics obtained are fiber, film, fragments, granules, and pellets seen in Figure 1.

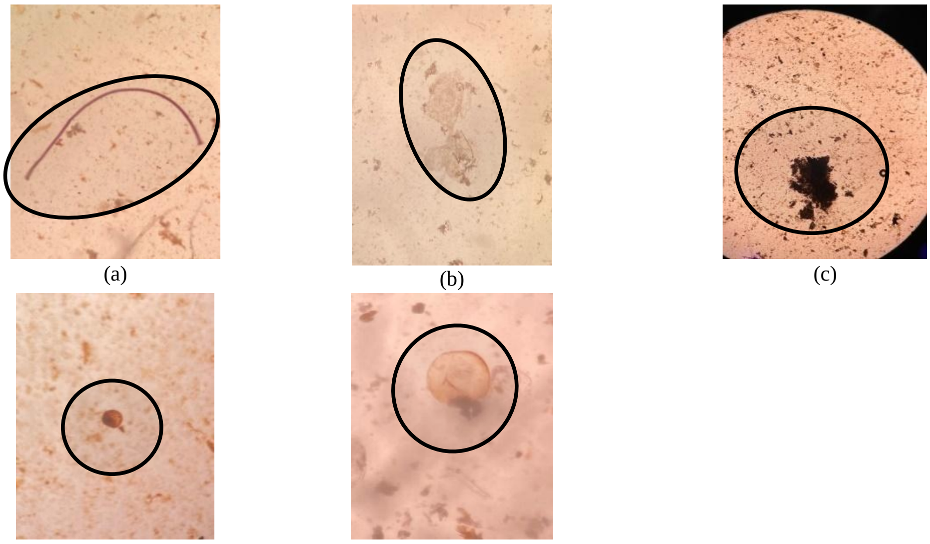

Figure 1. Microplastics found in the digestive system of freshwater mussels; a) Fiber; b) Film; c) Fragment; d) Pellet ; e) Granule

3.2. Abundance of Microplastics in the System Freshwater Mussel Digestion

Abundance is the number or density of a particle found in an organism. In research, abundance refers to the number of microplastic particles in an organism, such as freshwater mussels. Abundance measurements are important for understanding the level of exposure and potential impacts. Ecology from microplastic to the organism is said. The taller the abundance, the more microplastics found, the greater the possibility of negative impacts on the health of organisms, including disorders of the digestive system, decreased biological function, and even death.

In this study, the abundance of microplastics in the digestion of freshwater mussels will be calculated based on the number of particles found in the digestive system per unit mass or tissue volume. This parameter will be one of the crucial indicators in assessing the level of contamination by microplastics in freshwater mussels and ecologically implications (Li et al., 2019). The number of abundance microplastics in the system. The digestion of freshwater mussels can be seen in Table 2.

Table 2. The abundance of microplastics in the freshwater mussel's digestion system

Depth (m)	Type	Abundance (Particles/Individuals)		
		1 kg	2 kg	3 kg
0	Fiber	5.9	6.3	7.2
	Fragment	5.1	5.7	6.3
	Film	6.6	8.3	9.7
	Granule	0.7	0.9	1.4
	Pellets	0.8	0.7	1.2
Average		3.8	4.4	5.2
4	Fiber	5.2	6.3	7.3
	Fragment	4.8	6.0	6.8
	Film	6.6	8.2	9.9
	Granule	0.8	0.7	1.1
	Pellets	0.5	0.8	1.6
Average		3.6	4.4	5.4
8	Fiber	6.0	6.6	7.7
	Fragment	6.5	7.2	8.2
	Film	8.3	9.6	13.3
	Granule	0.7	0.8	1.2
	Pellets	0.6	0.8	1.4
Total		4.4	5.0	6.4

Based on the data in Table 2 at a depth of 0 meters, the value abundance of microplastics in the system freshwater mussel digestion with highest in the 3 kg treatment, with a type of microplastic film of 9.7 particles/individual. Based on observation, microplastics found on the surface of waters are caused by the presence of activity population, tourism and fishing. Public habits influence the type of Microplastic film about using plastic bags and packaging others (Azizah et al., 2020). At a depth of 4 m abundance of microplastics is highest film film-type microplastics amounting to 9.9 particles/ind in the 3 kg treatment. Film microplastics are secondary plastic polymers derived from fragmentation pocket plastic or plastic packaging and have low identity (Kingfisher, 2011).

At a depth of 8 meters abundance of microplastic was highest obtained in the 3 kg treatment with type microplastic film, totalling 13.3 particles/ind. The depth of 8 m is the deepest depth reached in this study.

Microplastics at a depth of 8 m come from plastic waste originating from waste disposal by residents and floating net cages (KJA) that are degraded in the depths of the water body. The plastic waste will then disintegrate into small particle fragments called microplastics (Galgani, 2015). For abundance data, microplastics can be seen in Figure 2.

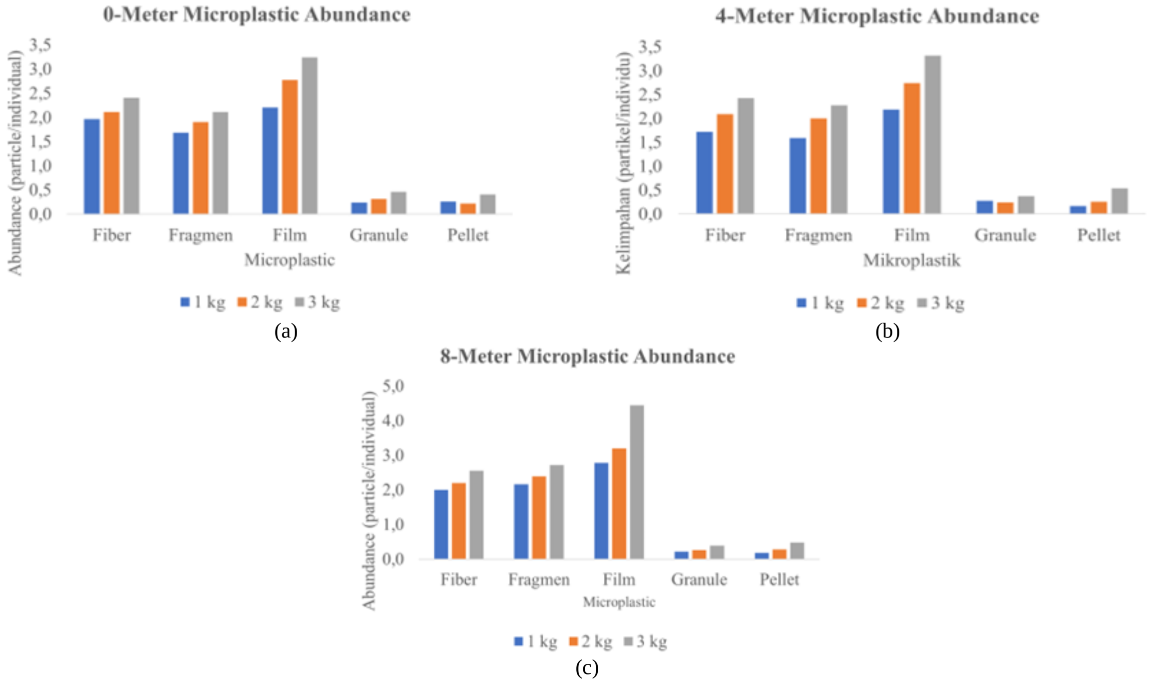

Figure 2. Abundance of microplastics in the digestive system of freshwater mussels; (a) 0 m, (b) 4 m, (c) 8 m

3.3. Water Quality Conditions

Water quality measurements were carried out in this study, with the averages in Table 3.

Table 3. Average Water Quality Measurements

No	Parameter	Depth (m)	Average
1	pH	0	6.12
		4	6.07
		8	6.11
2	Temperature (ºC)	0	29.4
		4	29.1
		8	28.9
3	Brightness	-	1.40

The optimal temperature for aquatic biota, including freshwater mussels, ranges between 11-29°C (Komarawidjaja, 2006). The water temperature in the Koto Panjang Reservoir during the study was recorded at 28.9-29.4°C, still within the range that supports the survival of mussels. Water temperature is influenced by air temperature and sunlight intensity. The average water clarity is 1.40 m, measured with the secchi disc (Sayekti et al., 2015), affecting sunlight penetration and microplastics distribution. Meanwhile, the water pH ranges from 6.07 to 6.12, which is suitable for the productivity of aquatic organisms (Kulla et al., 2020). A pH that is too low can reduce oxygen levels, increase fish breathing frequency, and reduce appetite (Safitri et al., 2021).

4. Conclusions

This study found five types of microplastics in the digestive system of freshwater mussels (*Pilsbryoconcha exilis)* at three different depths: fiber, film, fragments, granules, and pellets. At a depth of 0 m, the highest abundance of microplastics was found in the 3 kg treatment with a total of 4.3 particles/ind. At depths of 4 and 8 m, the highest amount was also in the 3 kg treatment with 4.5 and 5.3 particles/ind, respectively. The most abundant type of microplastic was film, with a range of 1.6-2.2 particles/ind. The ANOVA results showed that depth and treatment significantly affected microplastic abundance (p-value <0.05).

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