Study on the potential of sea urchin *Tripneustes gratilla* (Linnaeus, 1758) as a bioindicator dangerous plastic pollution in environment of gunungkidul beach Yogyakarta

S D Pratita¹, M B Gavintri¹, A N Rizkyta¹, L U Khasanah¹, F A Ponkiyawati¹, B Retnoaji²

¹Faculty of Biology, Universitas Gadjah Mada
²Laboratory of Animal Structure and Development, Faculty of Biologi, Universitas Gadjah Mada
³Corresponding author: salmadewi00@mail.ugm.ac.id

Abstract. Yogyakarta has a lot of beaches, especially in the Gunungkidul district. Sepanjang and Ngloang are tourist beaches in Gunungkidul, while Porok is a research station for the Faculty of Biology UGM. One common types of waste in the ocean is microplastic. The presence of microplastics in aquatic ecosystems in high numbers can threaten the population of benthic organisms and the stability of the ecosystem. *Tripneustes gratilla*, one of the sea urchins, can be used as an indicator of environmental pollution because it eats algae and detritus at the bottom of the water. This study aims to determine the microplastic content in seawater, beach substrates, and *Tripneustes gratilla* organs. Sampling by purposive random sampling, Analysis of microplastics in water, substrate, intestinal and gonads organs. The results obtained that there were four types of microplastics found: fragments, films, fiber, and monofilaments. The most common type of microplastic found is film. The abundance of microplastics on the substrate is higher than that of water. The abundance of microplastics in the intestinal organs of *T. gratilla* was higher than its gonads. Based on these results, *T. gratilla* has the potential as a bioindicator of microplastic pollution in the coastal environment of Gunungkidul, Yogyakarta.

1. Introduction

Plastic waste is the most dominant type of marine waste and can found from the poles to the equator. About 10% of the plastic used every day will become garbage in the oceans, both in Indonesia and in the world [5]. The plastic constituent structure in the form of stable polymers make plastic difficult and take a long time to decompose. Plastic can undergoes degradation to a smaller form to below 5 m which are called microplastics. Microplastics are so small that they can be easily easily enter the body and can harm the organs in the body. The harmful compounds present in these microplastics can pollute the ecosystem [6, 21]. Research on microplastics has been carried out at several locations. Suwartiningisih et al., (2020) mention that the microplastic that is often found on Baron Yogyakarta beach is fiber (53%), films (36.97%), and fragments (9.89%).

Sfriso, et al., (2020) stated that the highest abundance of microplastics in invertebrates was found in filter feeders and grazers. Sea urchins (*T. gratilla*) are one of the invertebrates with the main food, namely macroalgae and seagrass, and can be detritus eaters [13]. The distribution of *T. gratilla* is quite...
wide in Indonesia. In aquatic ecosystems, sea urchins are generally known as biological indicators of marine pollution because of their wide distribution, benthic behavior, high abundance, able to respond quickly and have high sensitivity to contaminants [16]. This type of sea urchin also has high commercial value because its gonads can be used for consumption. This is because the gonads of *T. gratilla* have beneficial bioactive components and high protein content. The presence of microplastics around sea urchins can cause sea urchins to be exposed to microplastic contamination that can harm organs, especially the sea urchins' gonads [19]. The diet of *T. gratilla* as a grazer and detritus feeder, coupled with the movement of *T. gratilla* whose range is not too wide, causes *T. gratilla* to have the potential to accumulate microplastics in a habitat. This accumulation can be a bioindicator of the type and abundance of microplastics in a habitat. Lololo and Nugraha (2019) found 155 particles/0.0757 g of microplastic in sea urchins on the Coral Reef of Gili Labak Island, Sumenep and dominated by fiber.

Sepanjang, Nglolang, and Porok beaches are beaches in Gunungkidul, Yogyakarta. Sepanjang and Nglolang Beach is a tourism sector, while Porok Beach is a non-tourist beach. The three beaches have a substrate structure in the form of coral reefs and macroalgae that are suitable for the habitat of *Tripneustes gratilla*. The abundance of sea urchins *T. gratilla* itself is quite a lot to be found on the Sepanjang and Nglolang beaches [8, 24]. The different potentials of the Sepanjang, Nglolang, and Porok beaches in the tourism sector cause the accumulation of pollution to be different. Research on the analysis of microplastic pollution on the three beaches and the development of research on the potential of *T. gratilla* sea urchins as bioindicators of microplastic pollution have never been carried out. Based on this background, this study aims to determine the types of microplastics in the coastal environment and organs of *T. gratilla*, analyze the abundance of microplastics in the coastal environment and organs of *T. gratilla*, analyze the effect of microplastics on the organs of *T. gratilla* sea urchins, and study the potential of *T. gratilla* sea urchins. gratilla as a bioindicator of microplastic pollution. This research is expected to be a reference for further research and recommendations to the local government about harmful plastic pollution in the Gunungkidul Beach environment of Yogyakarta, especially for tourist beaches.

2. Material and Method

This research is empirical research. The method used is online-offline blended. The online method includes determining sampling points using Google Earth, searching literature (journals) as a reference for research conducted through open access databases such as Google Scholar, etc. and data analysis using Microsoft Excel. The Offline Method includes several things, namely: Sampling, Microplastic Testing with a Microscope, and Observation of Body Tissues exposed to Microplastics with the Paraffin Method.

The tools used in this study include: ziplock, tweezers, bucket, toolbox, scalpel and blades of various sizes, analytical balance, 100 mL sample bottle, 50 mL Erlenmeyer tube, stereo microscope, Whatman filter paper. sizes of 125 mm and 250 mm, cotton, ice box, and 50 mL and 100 mL flakon bottles.

The main materials in this study were sea urchins (*T. gratilla*), substrate sediment at each sampling station, and sea water. Sea urchins are taken to a minimum to maintain the diversity of the existing ecosystem. Other materials used include: KOH 10%, NBF solution, saturated NaCl, distilled water, graded alcohol (70%, 80%, 90%, and absolute), HE dye.

The variables used include independent and dependent. The independent variables are: water samples, sediment samples, and samples of *T. gratilla* at each station. The dependent variable, namely: the content of existing microplastics. The sampling locations used included three coastal environments, namely Sepanjang Beach, Nglolang Beach, and Porok Beach. At each beach, two points were taken as data collection stations. The location for processing the samples taken was carried out at the Laboratory of Animal Histology and Embryology, Faculty of Biology, Universitas Gadjah Mada, Yogyakarta. The workflow is:
2.1. Determination of Sampling Point
Determination of the sampling point is done by observing using Google Earth. In this study, the existing sampling points include four stations. The research stations include three beaches, namely Sepanjang Beach, Nglolang, and Porok. Research Stations on Porok and Nglolang Beaches consist of: one station per beach, but the research stations on the Panjang Coast include two stations. This is because the beach is longer than the two beaches previously. This makes the entire area of the Sepanjang Beach into the research coverage.

![Figure 1](image.png)

*Figure 1. Sampling point of Porok, Nglolang, and Sepanjang Beach Gunungkidul Yogyakarta*

2.2. Microplastic Sampling and Analysis
Sampling includes sediment samples, seawater samples, and samples Sea urchin (*T. gratilla*). The methods of these three samples have the main difference in the microplastic analysis. Sampling was carried out in three environments Gunungkidul beach, Yogyakarta. The microplastic analysis was carried out in Histology and Embryology Laboratory, Faculty of Biology, Universitas Gadjah Mada. Sediment samples taken were sediments located about 5 cm from the water surface. The sediment sample was dried, then filtered with paper 5 mm sieve to separate the microplastics from the others. Next Sediment and microplastics were separated using the principle of density separation. Microplastics have a lower density than sediment so that microplastics will be above the sediment. In this separation, saturated NaCl is used which is mixed with sediment in a ratio of 1:3. Then stirred for 2 minutes and allowed to stand 2 minutes. 1/3 of it was taken and filtered with 125 mm filter paper and observed under a microscope. Sampling of habitat water was carried out by taking 100 ml of seawater to be taken to the laboratory. The sample was placed in an Erlenmeyer and stirred for 2 minutes. The sample was filtered with 250 mm filter paper. Furthermore, observations were made on a microscope to analyze the type of microplastic [11]. The sampling method is *Tripneustes gratilla* with purposive random sampling method. Furthermore, the digestive tract and gonads were taken. The sample was mixed with 10% KOH in a ratio of 3:1, then heated on a hot plate at 40°C for 24 hours. The sample was allowed to stand for 24 hours at room temperature and filtered through filter paper. Furthermore, observations were made on a microscope to analyze the type of microplastic [11].

2.3. Histological Observations with Paraffin Method
Determination Histological observations observed three organs, namely the digestive organs, respiratory organs and gonads. *T. gratilla* was dissected and the organs separated observed, then fixation with NBF
solution for 12 hours, washing with 70% alcohol, dehydrated with 70%, 80%, 90%, 96% alcohol to absolute alcohol, then clearing with Toluol. Next infiltration and embedding with paraffin. Then cut tissue with a thickness of 5 μM, after which the attachment and staining of the tissue with HE (Haematoxylin eosin) or MAF (Mallory acid fuchsin) dyes. Proceed with Mounting, after which the network can be observed below microscope.

2.4. Analysis method
The analysis section is carried out by observing the preparations and calculating microplastics through a Leica microscope. This calculation is done manually based on the images obtained under the microscope. After that, the data that obtained will be analyzed for the abundance of microplastics. Abundance analysis microplastics was carried out using Spreadsheet and Microsoft Excel. As for the formula used are:

\[ K = \frac{n}{A} \]

Information:
K: Abundance of microplastics
n: number of microplastics
A: Volume of solution

3. Result and discussion
In this research that we have done, the result of this research are type of microplastic, amount of microplastic, abundance of microplastic, and histology section of intestinum and gonad *Tripneustes gratilla*.

![Figure 2](image)

**Figure 2.** Type of Microplastic in water, sediment, intestine, and gonad *T. gratilla* are fragment (A), film (B), monofilament (C), and fiber (D)

The type of microplastic that we found in substrat, seawater, and organ samples of *T. gratilla*, namely, fragments, films, monofilaments, and fibers. Microplastic fragments has a shape such as gravel and its colored or darker than film. Microplastic film has small and transparent. Monofilament has shape such as strands aof thin colored thread. Fiber-type has a strand shape but wider than monofilament and has transparent color [23].

The types of microplastics found can identify the types of plastics located at the research site. The type of microplastic fragment comes from waste such as pipes, bottles, and hoses. Film type microplastics are derived from plastic bag waste, mica or other clear plastic, then Monofilament microplastics are derived from cloth waste or nylon thread [12]. Meanwhile, fiber type microplastics come from fishing line or fishing nets. The sources of these microplastics come from degradation products plastic carried by rivers, run off, tides, and carried away from activities humans such as fishing gear, cultivation equipment, and clothes that come from waste household [9].
Table 1. Amount of microplastic that found in each sampling location

| Seawater sample | Type of microplastic | Amount |
|-----------------|----------------------|--------|
|                 | Fragment | Film | Monofilament | Fiber |
| No   | Sample Point |  |  |  |  |
| 1    | Porok | 20 | 45 | 3 | 26 | 94 |
| 2    | Ngloolang | 118 | 71 | 0 | 17 | 206 |
| 3    | Sepanjang Timur | 44 | 129 | 3 | 59 | 235 |
| 4    | Sepanjang Barat | 52 | 47 | 4 | 22 | 125 |

| Substrate Sample | Type of microplastic | Amount |
|------------------|----------------------|--------|
|                 | Fragment | Film | Monofilament | Fiber |
| No   | Sample Point |  |  |  |  |
| 1    | Porok | 212 | 172 | 10 | 7 | 401 |
| 2    | Ngloolang | 147 | 236 | 10 | 15 | 408 |
| 3    | Sepanjang Timur | 97 | 108 | 16 | 54 | 277 |
| 4    | Sepanjang Barat | 120 | 220 | 5 | 51 | 396 |

| Intestinum sample | Type of microplastic | Amount |
|-------------------|----------------------|--------|
|                   | Fragment | Film | Monofilament | Fiber |
| No    | Sample point |  |  |  |  |
| 1    | Porok | 65 | 71 | 3 | 26 | 165 |
| 2    | Ngloolang | 71 | 122 | 3 | 26 | 222 |
| 3    | Sepanjang Timur | 105 | 85 | 1 | 19 | 210 |
| 4    | Sepanjang Barat | 126 | 200 | 6 | 51 | 383 |

| Gonads Sample | Type of microplastic | Amount |
|---------------|----------------------|--------|
|               | Fragment | Film | Monofilament | Fiber |
| No    | Sample point |  |  |  |  |
| 1    | Pantai Porok | 4 | 14 | 2 | 55 | 75 |
| 2    | Pantai Ngloolang | 22 | 65 | 6 | 89 | 182 |
| 3    | Pantai Sepanjang Timur | 37 | 21 | 0 | 16 | 74 |
| 4    | Pantai Sepanjang Barat | 26 | 19 | 2 | 7 | 48 |

The amount of microplastics found in each sample was different at each location. This amount of microplastic can determine the type of waste the most abundant plastic found at each sample point. On the table It is known that the most common types of microplastics found are films, so that the type of plastic waste that is most commonly found on beaches in Gunungkidul Indonesia comes from plastic bags, mica, or other clear plastic. Meanwhile, the type of microplastic that was rarely found was the monofilament type. Types of microplastic films were found in each sample, types of microplastics. It is lighter than other types of microplastics. Microplastic film is easily carried away by water currents and enters the organs *Tripneustes gratilla* in both intestine and gonads. Film type microplastic has a lower density so it tends to float in the column waters are easier to transport [7]
Figure 3. Diagram of the abundance of microplastic in seawater and substrate samples in each sample point.

Figure 3 shows the abundance of microplastics on the substrat sample is higher than the seawater sample. Highest abundance of microplastics in the substrate sample higher than the water sample. The highest abundance of microplastics in the seawater sample, there is a Sepanjang Beach 1 (east) which is 117.5 particles/m3, while the lowest is at the sample point of Porok Beach at 47 particles/m3. The abundance of microplastics in the highest substrate samples was Nglolang Beach which is 163.2 particles/m3, while the lowest abundance of microplastics was found at the Porok Beach which is 80.4 particles/m3. Factors of human activity, hydrodynamics and geography become a limiting factor for the presence of microplastics in a waters. Sepanjang Beach as it is a tourist destination, in this area human activities higher. Nglolang Beach is a tourist area which is located in west of Sepanjang Beach and is one of the few beaches yet known to tourists. Porok Beach is the research station of Gunungkidul where human activity is lower than in the Sepanjang Beach and Nglolang Beach. High human activity is capable of causing the use of plastic material increases. This allows the microplastics found in Sepanjang Beach has the highest abundance. In general sea waves on the southern coast of Gunungkidul have energy characteristics because it is directly facing the high seas. Wave height suspected to play a role in the carrying of microplastics in the Gunungkidul Beach area.
Figure 4. The abundance of microplastics in intestinum and gonad samples *T. gratilla* at each sample point.

The highest abundance of microplastics was found in intestinal samples in Sepanjang Beach 2 (west) by 127%. While the lowest abundance of microplastics is Porok Beach at 55%. Then the highest abundance of microplastics in gonadal samples is Ngololang Beach by 60.67%. While the lowest abundance of microplastics was found in the Pantai Panjang 2 (west) is 16%.

One of the main food of sea urchins is detritus found in sediments, so that sea urchins (*T. gratilla*) are suspected of eating microplastics that deposited and mixed with detritus. Food-like microplastics will be consumed by marine organisms so that it enters the intestine and gonads of sea urchin. Sepanjang and Ngololang beaches are tourism beaches so that the abundance of microplastics in the intestine with the highest number, while Porok Beach is a conservation beach as a conservation research station ecosystem so that the levels of microplastics are low.

Figure 5. Histology of gonad male *T. gratilla* (A) with magnification 40x and (B) magnification 100x.

Figure 5 is the result of observing the gonadal histology of male *T. gratilla*. The image shows no damage to the gonads. The presence of microplastics in the gonads is thought to be due to the microplastics present in the gonads water enters the body of *T. gratilla* through two routes, namely when eating and through the water vascular system. Microplastic toxicity in reproductive organs is closely related to oxidative damage [22]. The content of microplastics in the gonads very high levels can affect the reproductive success of sea urchins.
Studies on microplastics in oyster gonads showed a negative effect on index reproductive health, quality, and quantity of gametes [17]. A low reproductive rate will result in the vulnerability of a person’s survival organism. In addition, microplastics are able to absorb toxins and chemicals others from marine waters so that they have potential ecological impacts (through trophic transfers) [1]. Presence of microplastics in gonads of sea urchins cause a risk to human health when consumed [4].

Figure 6. Histology of intestinum T. gratilla (A) with magnification 4x10 and (B) with magnification 10x10. Part of intestinum consist of, (C) coelom, (EC) epithel columnar, (TM) tunica mucosa, (GC) goblet cell, (CM) connective muscle, (P) peritoneum, (TS) tunica serosa.

Figure 6 is the result of the intestinal histology of T. gratilla. These histological preparations showed no damage to the intestine of T. gratilla. Microplastics found in the intestine are thought to be due to T. gratilla feed on microplastics that have the same shape as detritus from algae or other organic matter. However, it can still be dangerous for T. gratilla. Microplastics can absorb toxins and other chemicals from the ocean so that Microplastics eaten by animals will be transferred into the food chain which indirectly [1]. If the amount of microplastic enter the body very much and in the long term animals, these animal can die because the microplastic particles cannot be digested by the body [2].

In this study, it can be seen that the presence of microplastics on the organ of T. gratilla. The greatest abundance of microplastics is in intestine with a type of microplastic in the form of a film. The same result was obtained in substrate and water samples so that sea urchins (T. gratilla) can be used as bioindicators harmful plastic pollution. Bioindicators are organisms or parts of organisms that are able to contain information about the condition of the quality of their environment, where this organism has a level of sensitivity or tolerance to various types of contamination and are able to respond to it [20]. Sea urchins have a higher average lifespan than other organisms such as, crustaceans, molluscs, and algae that allow accumulation more pollutants [10]. Ability in the process feeding on the algae and detritus allows these animals to accumulate pollutants or plastic in small sizes. T. gratilla tissue sensitivity on microplastic pollution needs to be studied more deeply because it has not tissue was found in damaged T. gratilla on the histological section.

4. Conclusion
Based on the research that has been done, it can be concluded that there are four types of microplastics found in the coastal environment of Gunungkidul Yogyakarta, namely fragments, films, fibers, and monofilaments. The highest abundance of microplastics in the water samples was at Sepanjang Beach 1, while the lowest was at Porok Beach. The highest abundance of microplastics in the substrate sample was at Nglolang Beach, while the lowest was at Porok Beach. The highest abundance of microplastics in intestinal samples was at Sepanjang Beach 2, while the lowest was at Porok Beach. The highest abundance of microplastics was in the highest gonadal samples at Nglolang Beach, while the lowest was at Sepanjang Beach 2. The Tripneustes gratilla organ samples did not show any damage due to microplastics. The type of microplastic with the highest abundance in water, substrate, and T. gratilla
showed the same results, namely the type of film. These results indicate that *T. gratilla* has potential as a bioindicator of microplastic pollution.

5. Reference

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