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MICROPLASTIC OCCURRENCE IN THE DIGESTIVE TRACT OF MARBLE GOBY (*Oxyeleotris marmorata*) FROM JENEBERANG RIVER, MAKASSAR, INDONESIA

EGA ADHI WICAKSONO[1]*, SHINTA WERORILANGI[2] MUH AFDAL[3], ROBBY NIMZET[4] AND AKBAR TAHIR[2]

[1]Department of Fisheries, Faculty of Agriculture, Universitas Gadjah Mada, Jl. Flora, Bulaksumur, Sleman, 55281 Yogyakarta, Indonesia. [2]Department of Marine Science, Hasanuddin University, Jl. Perintis Kemerdekaan, KM 10 Tamalanrea, 90245 Makassar, Indonesia. [3]Department of Marine Science, Faculty of Fisheries and Marine, Lambung Mangkurat University, Banjarmasin, Indonesia. [4]Environmental Management Study Program, Graduate School, Hasanuddin University, Jl. Perintis Kemerdekaan, KM 10 Tamalanrea, 90245 Makassar, Indonesia.

**Corresponding author: ega.a.w@ugm.ac.id*
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Abstract: Microplastic pollution has been reported in various aquatic environments. Microplastics can accumulate in fish through active and passive uptake. Marble goby (*Oxyeleotris marmorata*) is a freshwater fish that act as an ambush predator. This feeding habit suggests *O. marmorata* tend to accumulate microplastics from the aquatic environment through passive uptake. This fish is also one of the consumption commodities utilised in the Jeneberang River. This study aimed to measure the microplastic abundance from the digestive tract of *O. marmorata* from the Jeneberang River. The *O. marmorata* individuals were captured using fish traps during the wet and dry seasons. Microplastics from the gastrointestinal tract of *O. marmorata* were extracted using the alkaline digestive method. The results showed that microplastic was found in *O. marmorata*'*s* digestive tract with an average abundance of 1.11 ± 0.19 MPs/Individual. There is a tendency for microplastic abundance in *O. marmorata* during dry season (1.58 ± 0.39 MPs/Individual) to be higher compared to the wet season (0.94 ± 0.23 MPs/individual). Blue and line microplastics were the most abundant found in the samples. Based on their shape, the microplastics found in the sample are suspected to be secondary MPs. These results indicate that microplastics have been uptake in high trophic-level fish.

Keywords: Pollution, feeding habit, season, trophic level, uptake.

Introduction

Plastic has now become one of the global environmental problems. Although plastic as a synthetic material was produced to benefit humans, it can also have a considerable negative impact on the environment. Among the 8300 million Mt of plastic produced as 2015, it is estimated that only about 10% is recycled. Then it is estimated that almost 80% is leaked and ends up in the environment (Geyer *et al.*, 2017). Plastics released into the environment cannot be decomposed naturally and will be persistent. Those plastics ultimately receive environmental stressors such as temperature fluctuations and UV-B exposure and are broken down into smaller sizes called "microplastics" (Cole *et al.*, 2011).

In general, microplastics (MPs) in the environment will be carried by the run-off and end up in the aquatic environment (Dris *et al.*, 2015). These MPs can negatively impact aquatic biota because of their toxic material and their ability to absorb other pollutants from the environment (Rochman, 2015). In addition, the physical presence of these MPs can also have impacts, such as digestive tract blockage and false satiety in the aquatic organism (Dris *et al.*, 2015). Several organisms, including fish, shellfish, and plankton, are known to have been exposed by MPs in their environment (Rochman *et al.*, 2015; Wicaksono *et al.*, 2020; Hossain *et al.*, 2020; Ramli *et al.*, 2021; Wicaksono, *et al.*, 2021a).

Fish, as aquatic organisms, can accumulate MPs in the environment through at least two main mechanisms, active and passive MPs uptake (Roch *et al.*, 2020). Fish can uptake MPs actively due to the MPs that resemble plankton in the water as their natural prey. So, the fish actively uptake the MPs from the environment. Microplastic active ingestion often occurs in fish with a feeding habit that relies on the visual sense. For example, common goby (*Pomatoschistus microps)* and amberstripe scad (*Decapterus muroadsi)* generally prefer to ingest MPs that visually match the colour of their natural prey in the environment (de Sá *et al.*, 2015; Ory *et al.*, 2017). Meanwhile, passive mechanisms occur due to the MPs accumulation of other pathways, for example, through food webs (Roch *et al.*, 2020). Fish preying on the smaller fish containing MPs can indirectly uptake the MPs so that the MPs can be transported to the higher trophic level fish in a passive mechanism.

Marble Goby (*Oxyeleotris marmorata*) is a freshwater consumption commodity widely utilised in Indonesia. In contrast to fish that actively seek prey visually, *O. marmorata*, tend to act as ambush predators (Jiwyam, 2008; Lim *et al.*, 2020). This fish tends to find prey by hiding in a burrow and ambushing prey that passes. This fish is also a nocturnal fish that looks for prey at night in the absence of light. With this feeding habit, the possibility of *O. marmorata* uptake MPs actively from the environment will be less. So the presence of MPs in *O. marmorata* can reflect the MPs uptake from the indirect mechanism. Marble Goby is one of the common freshwater commodities in the Jeneberang River. This river, located in the south part of Makassar City, that suspected to be the MPs source to its surrounding aquatic environment (Afdal *et al.*, 2019; Wicaksono *et al.*, 2020; Wicaksono *et al.*, 2021). Microplastic pollution has been reported to contaminate both Jeneberang River's water and sediment (Wicaksono *et al.*, 2021b).

This study aimed to determine the abundance and characteristics of MPs present in *O. marmorata* as a high tropic fish at the Jeneberang River. The MPs abundance will also be compared between two different seasons, which, based on a preliminary study, shows an influence of seasonal variation on MPs abundance in the same region (Wicaksono *et al.*, 2021). While the MPs characteristic (shape, size and colour) could provide more information regarding the origin of microplastic as well as the implication to its bioavailability to the aquatic organism. This information is essential considering that the *O. marmorata* has unique feeding habits, so it is suspected that it can be used as an initial estimate of the level of indirect MPs transfer through the food chain.

Materials and Methods

The *O. marmorata* individuals were collected from the Jeneberang river (Figure 1.) in March and August 2019 to represent the wet and dry seasons. Samples collection was carried out using fish traps as the main fishing gear. Four traps were placed in the river's littoral zone (1-2.5 m in depth). Trap setting was done in the afternoon (3-4 pm), and the hauling time was done in the following morning (8-10 am). The live fish samples were then put into the coolbox and transported to the Marine Ecotoxicology Laboratory, Hasanuddin University.

The fish samples were kept in cold storage (-20°C) for preservation. Each of the samples was weighed, measured, and followed with the dissection process. The dissection was done to collect the Gastrointestinal Tract (GIT) organ of *O. marmorata*. The cut was made from the fish' anus to the upper part of fish operculum to open the fish' abdominal cavity. The GIT was then transferred to the bottle samples for the digestion process. The GIT samples of each *O. marmorata* individual were then added by the potassium hydroxide (20%) for alkaline digestion, about three times the sample volume (Foekema *et al.*, 2013; Rochman *et al.*, 2015). The samples were then incubated at room temperature (≈ 27°C) for 14 days to let the organic matter of the sample digested.

Microplastic identification was done using visual sorting. The sample was observed using a stereomicroscope (Euromax, SB-1902, Netherland) with a magnification of 45×.

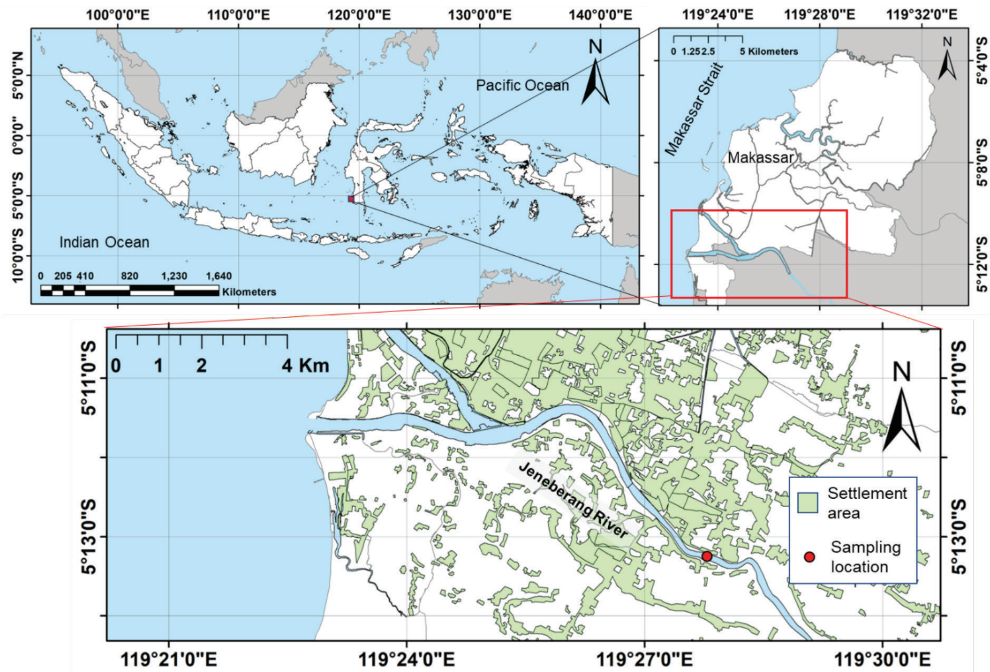

Figure 1: Map of sampling location

Microplastics were identified by following several key characteristics including bright-homogenous colour, light reflective, flexible texture, and the absence of cellular characteristics (Wicaksono, 2021). Microplastic found in each sample then counted and categorised by colour, shape and size.

Microplastic abundance in the *O. marmorata* sample is calculated by the following formula:

$$\text{Microplastic abundance} = \frac{\sum \text{microplastic found on sample}}{\text{number of samples}}$$

While the MPs contamination level is calculated by this formula:

$$\text{Contamination level} = \frac{\text{Number of the individual(s) with microplastic}}{\text{number of individuals}} \times 100\%$$

The abundance of MPs in the samples was then compared between the wet and dry seasons using Mann-whitney non-parametric test. The proportion of MPs shape, colour and size are presented in percentages.

Results and Discussions

The number of *O. marmorata* samples collected from the Jeneberang River was 45 individuals (Wicaksono, 2021), with 33 individuals caught during the wet season and 12 individuals caught during the dry season (Table 1). In general, marble goby in the Jeneberang River has an abundance of MP to 1.11 ± 0.19 MPs/Individual with contamination levels reaching 62.22% of the total sample. The average abundance of MPs in *O. marmorata* in the dry season (1.58 ± 0.38 MPs/Individual) was higher than that in the rainy season (0.94 ± 0.22 MPs/Individual) (Figure 2.). However, based on the non-parametric Mann-Whitney test, there was no significant difference in MPs abundance in *O. marmorata* between the wet and dry seasons (P = 0.06). In terms of contamination level, *O. marmorata* captured during the dry season tends to have a higher contamination level than individuals captured during the wet season.

Oxyeleotris marmorata belongs to the potamodromous fish, which have a primary habitat in the freshwater environment. In the Jeneberang river, marble goby is usually found in the weirs' upstream part, separated from the downstream, which tends to have brackish water. Based on previous studies, MPs in water and sediment on the Jeneberang river tend to accumulate in the upstream area of the weir (Wicaksono *et al.*, 2021b). This condition might result in *O. marmorata* being one of the species with a more significant potential to be affected by MPs in the Jeneberang River. So far, reports of the presence of MPs in goby fish have been reported, for example, in *Neogobius melanostomus*, which reaches 1.25 MPs/individual in the Rhine river, and the contamination level even can reach 100% in the Great lake (Roch & Brinker, 2017; McNeish *et al.*, 2018). Then the pelagic fish caught in the Jeneberang river has also been reported to be contaminated by MPs with abundance can reach 3.5 MPs/individual (Wicaksono *et al.*, 2020).

There was no significant difference in MPs abundance in *O. marmorata* individuals between the two seasons based on mann-whitney test (Figure 2). But, it can be seen that there is a trend in MPs abundance in *O. marmorata* where the MPs abundance and contamination level during the dry season are higher compared to MPs abundance in the wet season. This trend may happen due to the condition of the MPs abundance in water and sediments on Jeneberang river, which also tends to be higher during the dry season. The higher MPs abundance during the dry season also happens in the Tallo River environment in the same region as the Jeneberang river (Wicaksono *et al.*, 2021). During the rainy season, due to high rainfall, MPs will be diluted by the high volume of water and lead to lower concentrations in the environment (McNeish *et al.*, 2018; Wicaksono *et al.*, 2021). This condition could increase the possibility of MPs accumulation to the *O. marmorata* being higher during the dry season.

In general, there were no differences in the MPs colour, shape and size characteristics between the two seasons (Figure 3). Blue is the most common MPs colour found in *O. marmorata* individuals. Blue MPs is also the most abundant MPs colours often found in the aquatic environment around Makassar City (Afdal *et al.*, 2019). This include the water in Jeneberang river mouth, where blue MPs can exceeding 70% of other MPs colour (Wicaksono *et al.*, 2020). The probability of blue MPs ingestion will be higher in line with high presence of blue MPs in its environment. The same phenomenon also occurs in MPs shape trends, where line MPs are also the most abundant MPs shape found in the aquatic environment around Makassar City, so its abundance in *O. marmorata* also tends to be greater (Wicaksono *et al.*, 2020; Wicaksono *et al.*, 2021).

The MPs size could be used as an initial prediction of MPs impact. Microplastic in small size could be easier to translocate to organism tissue. In mammals, MPs with a size < 20 μm can penetrate the blood vessels and translocate to organs (Lusher *et al.,* 2017). Microplastic with size < 0.1 μm can be

Table 1: Characteristics of *O. marmorata* samples

Seasons	No. of Samples	Avg. Weight ± SE (g)	Avg. Length ± SE (cm)	No. Individual with MPs	Contamination Level
Wet	33	132.75 ± 9.68	21.41 ± 0.52	18	60%
Dry	12	129.03 ± 17.7	21.55 ± 0.65	10	80%

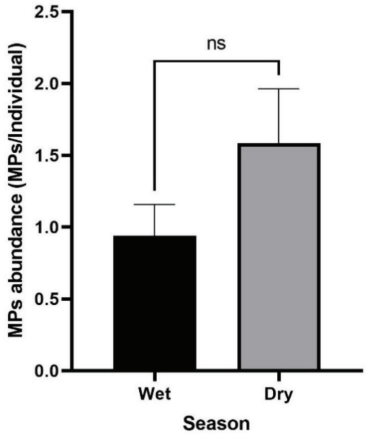

Figure 2: Microplastic abundance on *O.marmorata* during wet and dry season

accumulated in several organs of Nile tilapia (*Oreochromis niloticus)* (Ding *et al.,* 2018). While goldfish (*Carrassius auratus)* exposed to 2-4 mm MPs, tend to reject the MPs uptake (Jabeen *et al.,* 2018). The existence of Small MPs in the sample could indicate the potential of MPs translocation and toxicity to the *O. marmorata*. Therefore, further research regarding the effect of small MPs to *O. marmorata* fitness is needed to investigate further.

Line MPs might originate from various sources. For example, it could come from the rope that is often used in fishing and boat activities (da Costa *et al.*, 2017). Line MPs can also originate from the laundry waste effluent from the residential area. Research shows that more than 700,000 pieces of line MPs could be released into the environment in one washing process of about 6 kg of polyester clothing (Napper & Thompson, 2016). Fragment MPs are usually secondary MPs derived from larger plastic items in the environment. In the presence of several environmental stressors (ie. UV-b exposure, high temperature, abrasion) larger plastic items can break into smaller pieces in the form of MPs. The representative of MPs found in the sample is shown in Figure 4. The polymer identification, using several tools like Fourier-Transform Infrared Spectroscopy (FT-

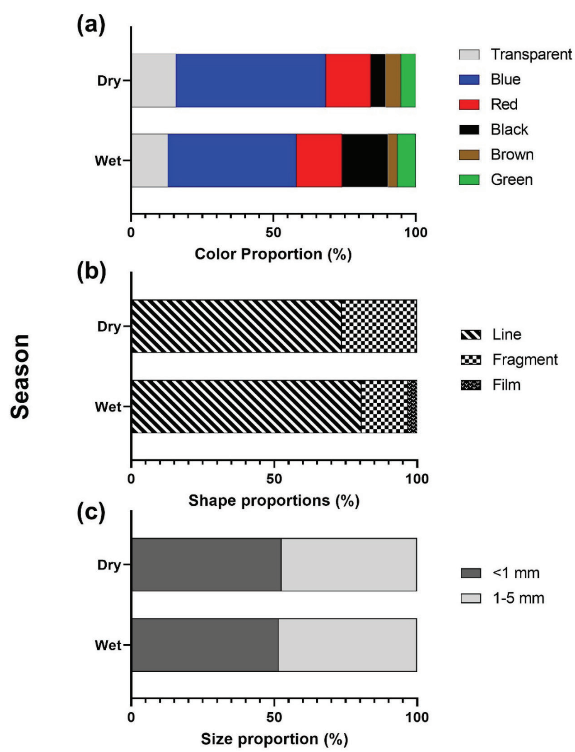

Figure 3: Colour (a), Shape (b), and size (c) proportions of MPs in *O. marmorata*

IR); Raman; and Gas Chromatography-Mass Spectrometry (GC-MS), could provide more robust information to predict the source of MPs in the environment.

Oxyeleotris marmorata is a piscivore, which was also indicated by the number of fish parts in their stomach contents. Piscivore fish tend to be less likely to accumulate MPs directly from the environment. Research from the Rhine river, shows that the abundance of MPs in goby tends to be influenced by feeding habits, instead of MPs abundance in its aquatic environment (Bosshart *et al.*, 2020). Thus, it is very likely that the MPs present in *O. marmorata* in this study were uptaken passively, for example transported through the food web. Therefore, the research on MPs ingestion from the lower trophic level organism in Jeneberang are also important in the future.

Conclusion

Microplastic was found in *O. marmorata* captured from Jeneberang river. There is a tendency for the MPs abundance and contamination level to be higher in the dry season. Blue MPs is the most abundance colour found in *O. marmorata* individuals. Based on their shape, the microplastics found in the sample are suspected to be secondary MPs. Microplastic polymer identification are needed for the further research.

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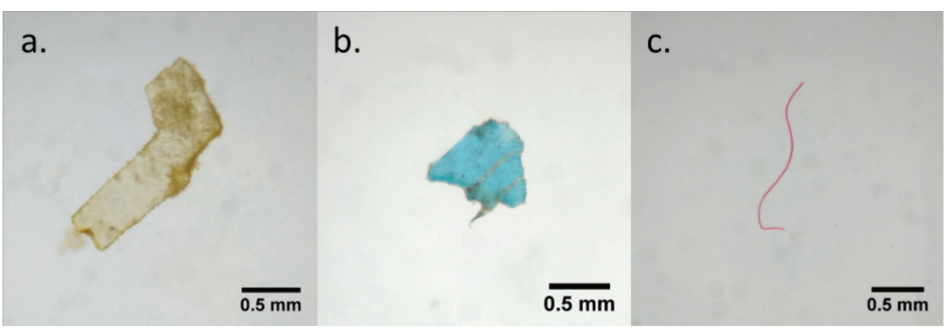

Figure 4: Representation of MPs found in samples. Fragment(a), film (b) and line (c)

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