MICROPLASTIC IN FRESH WATER FISH AT LUBUK YU RIVER, MARAN, PAHANG

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Abstract
Contamination of the river by microplastics is a worldwide significant problem. More or less, it is questionable whether this microplastic has already affected the freshwater organisms. Therefore, this study aimed to determine freshwater fish biodiversity and determine the abundance, distribution, composition of microplastics in fish from Lubuk Yu river, Maran, Pahang. Sampling was done at two different locations; middle and downstream, once a month for two months. Fishes were caught and identified. Digestive gut of fish undergone digestion, density separation and filtration. Through inspection of particles; each was identified, counted, and sorted out into categories based on shape, colour, and size. A total of 32 fishes were caught which represented 8 species. The most abundance species caught were Barbonymus schwanenfeldii, Cyclocheilichthys apogon, Hampala macrolepidota and Rasbora einthovenii with composition of 18.75% each. Shannon index (E), Shannon's equitability (E_H) for both locations; middle stream and downstream were 1.41, 0.55 and 1.74, 0.59 respectively. Out of the 8 species of fishes, only the samples from Hypsibarbus wetmorei and Rasbora einthovenii contained microplastics. Microplastic were found in filament form, with the size ranging between 1.01 mm and 2.00 mm and colours varied from blue, black and red. In conclusion, the study triggers an alarm that our freshwater fish biodiversity from Lubuk Yu river has already been exposed to microplastics.

Keyword: Biodiversity, freshwater fish, microplastic

Introduction
The word plastic represents the property of plasticity and the ability to deform without breaking. Plastics are a wide variety of malleable organic compounds that are synthetic or semi-synthetic and thus can be formed into solid objects (Anne & Helmenstine, 2020; Dris, 2017). History of plastic started with natural plastic and has expanded with the founding of synthetic plastic materials from celluloid, rayon, Polyvinyl Chloride, nylon to polystyrene (Britannica, 2020). After that, plastic industry faced its great expansion around World War II as it was used to make parachutes, ropes, body armors and helmet liners (Ostle et al., 2019). Throughout the 20th century, the developed world is exploded with consumer production allowing for plastics, alloys, and other composite materials to dominate production.
Plastics give us the possibility of producing beautiful, well-designed products from the many different types of plastics materials commonly available today (Andrady, 2015). Therefore, plastic are used almost everywhere in our daily activities. Most plastics are not biodegradable and persist in the environment for many centuries. Large visible plastic debris is defined as macroplastic, while smaller debris is defined as microplastic. Macroplastics represent plastics with the size of $\geq 5$ mm (Nihei et al., 2020; Sighicelli et al., 2018). Macroplastic is a large plastic in its original form. Whereas, microplastics commonly be described as plastics with a diameter of less than 5 mm (Jamieson et al., 2019; Ostle et al., 2019). Microplastics are classified into two categories. Primary microplastics are pellets used as feedstocks in the plastics industry or as abrasives in cosmetics and blasting media (Jamieson et al., 2019; Ostle et al., 2019). On the other hand, secondary microplastics are parts of larger damaged and broken plastics (Faure et al., 2015).

Despite of its usefulness and our dependency on plastic, now it is causing environmental pollution on a grand scale. Without proper management, most of these plastics even though from land-based sources, have the tendency to end up in water bodies such river and oceans. Major sources of litter are cities, industries, harbours and the tourism industry. This plastic debris has become a major treat to freshwater and marine ecosystems. Plastic is able to travel far away from its source since it is usually light in weight. Therefore, even the remotest beaches are found to accumulate vast amounts of plastic litter. Lahens et al. (2018) concluded that 80% of marine debris originates from land-based sources, even though 47% corresponds to unrecognizable pieces and 30% of coastal plastic debris is confirmed to originate from marine activities. Studies in two Malaysia islands namely Pulau Payar in Kedah and Pulau Tioman in Pahang indicated that plastic related items ranging from plastic bag, HDPE, and other plastics were the most abundant types which contributed 34.42% of the total marine debris (Fauziah et al., 2019).

River flows through continents and ends in to an ocean, lake, or another river. As this water flows, it will carry anything dumped intentionally or unintentionally with it. Therefore, it is clear that, rivers and sewages are important routes for terrestrial plastics and cause high rates of microplastic contamination in lakes, estuaries and ocean. River is the ecosystem that will be affected directly with waste pollution. Much more studies on ocean plastic waste have been conducted compared to river, which reflect a significant research gap in the field (Mani et al., 2015). Microplastic has been accumulated for decades in the freshwater system, thus, being a hotspot of water pollution (Fauziah et al., 2018). Microplastic ingestion has been documented frequently in the food chain by various species in the ecosystem and has increased exponentially over the past 7 years (Hamm et al., 2018). With this recent interest, this study attempted to provide information data on microplastic and fish biodiversity in fresh water ecosystem in Lubuk Yu river, Maran, Pahang. Therefore, the objectives are to determine the freshwater fish biodiversity and determine the abundance, distribution, composition of microplastics in fish from Lubuk Yu river, Maran, Pahang.

**Materials and Methods**

**Sampling Area**

Two sampling points of Lubuk Yu river, Maran Pahang were selected namely the downstream (3° 43' 37.200" N and 102° 38' 34.800" E) and the middle stream (3° 45' 10.800" N and 102° 39' 0.000" E) of the river. Sampling was done once a month for 2 months.

**Fish Collection and Identification**

Fish were caught using a fish net and sealed in a plastic bag then brought to the laboratory. Fish were first identified using common Malay names before being compared with available taxonomic
keys. Fish morphometric measurements including standard length (cm) and body width (cm) as well as weight (g) were recorded. The species diversity was calculated by using Shannon Index and Jaccard similarity index.

**Microplastic analysis**
The digestive gut of fish was cut and put in a container and treated with 10% of potassium hydroxide (KOH) and homogenized using blender. Homogenize samples were then mixed with NaCl solution with a density of 1.2 g/cm$^3$. The mixtures were mixed for 20 minutes and then were left to settle until the supernatant was clear of sediment (Wang & Wang, 2018). After that, the supernatant was poured, filtered through a multi-tier sieve with pore size varied from 10 mm, 0.5 mm and lastly filtered with Whatman 1 filter paper. Sample which retained on the filter paper was air dried and observed under the microscope. Plastics show distinguishable characteristic such as being shiny, colored, weird shape and slippery surface. Microplastics were counted and its shapes and colour were recorded.

**Data Analysis**
Correlation analysis was performed to evaluate relationship between weight, length and width of the fish. Furthermore, analysis was also done to compare the differences between the two sampling locations and species in term of the microplastic in fish caught. All statistical analyses were performed in SPSS v 23. The level of significance was set to $p < 0.05$.

**Result and Discussion**

**Location background**
The Lubuk Yu river is one of the rivers that flow into Sungai Pahang with a length of 24.63 km. This river flows through Pahang’s reserve forest and the development area allocated for the ecotourism covers approximately 5 hectares which is located in Maran (Jabatan Perhutanan Negeri Pahang, 2020). Two sampling points of Lubuk Yu river were selected. The first sampling point, middle point is the recreational area and this river flows through human settlement and oil palm estate where the second sampling point is labelled as downstream. High population density has a direct effect on the presence of plastic pollutant (Mani et al., 2015). The downstream can be described as small stream with about 4-10m wide, slow flowing and turbid water with mud-sandy bottom. Meanwhile middle point is a small stream about 4-10m wide, moderate flowing and clear water with rocky bottom.

**Fish Biodiversity**
The most abundance species caught were *Barbonymus schwanenfeldii*, *Cyclocheilichthys apogon*, *Hampala macrolepidota* and *Rasbora einthovenii* with composition of 18.75% for each species (Figure 1). Shannon index ($E$), Shannon's equitability ($E_H$) for both locations; middle stream and downstream were 1.41, 0.55 and 1.74, 0.59 respectively. Those represented the diversity and evenness at downstream which were slightly higher and displayed greater number of species rather than in the middle stream. However, $E_H$ had proven that individuals were not evenly distributed among the different species in both locations. The Jaccard similarity index count to compare similarity between the two locations and 4 species were shared for both locations. Therefore, it can be concluded that the two locations are 50.00% similar.
Figure 1 Percentage of fish species sampled from Lubuk Yu river, Pahang

Figure 2 displays the 8 species of fishes that can be found in Lubuk Yu river, Maran, Pahang. Fish morphometric measurements indicated that there was a positive correlation between weight and length, weight and width as well as length and width of the fish with r, 0.840, 0.898, 0.640; n=32, p=0.00. Multicellular organisms were increased in size (length, weight) during development. Length-weight relationship (LWR) parameters are important to estimate the health of fish populations (Oliveira et al., 2020).
Ingestion of Microplastic by Fish

Results indicate only *Hypsibarbus wetmorei* and *Rasbora einthovenii* species contain microplastic in their gastrointestinal tract. Microplastics are graded as filaments counted for 67% whereas fragments are 33% and appeared in various size fractions. Filaments and fragments are the most frequent shapes of microplastics found in fish (Tanaka & Takada, 2016). The size of ingested microplastic length ranges between 0.65 mm to 4.68 mm. Previous studies have recorded that the size of microplastics ranged from 500 to 5000 μm and could give aquatic animals high incidence of ingestion (Sarijan et al., 2018).

Microplastic found in fish varies in colour. There were 1 blue, 1 red and 1 black microplastic. Previous studies have shown that many marine species mistakenly eat plastic waste for food (Gomiero et al., 2019). One out of 2 counted as 50% of *Hypsibarbus wetmorei* contained microplastic in its gastrointestinal tract. Whereas, a total of 33.3% individuals of *Rasbora einthovenii* that ingested microplastic displayed weight and length measured as 63.8g with 19.1cm and 69.5g with 20cm. The probability of species ingesting microplastics depends on the organism's physiological and behavioral characteristics. The ingestion of microplastics by both species might due to their foraging behavior. *Hypsibarbus wetmorei* and *Rasbora einthovenii* showed a benthic foraging behavior where they search for food between the midwater to the bottom depths of streams (Mohamad Radhi et al., 2017; Jiwyam, 2014). Their natural diet is feeding on worms,
crustaceans and insects which can be found in water and sediment of the river thus increasing the chances of microplastic ingestion by these species compared to other species sampled for this study. However, statistical analysis run does not display any significant differences in terms of different species, size and location against ingestion of microplastic by fish. An adequate number of samples will give better result and heterogeneity (Vermaire et al., 2017). However, in this study only a total of 32 small size of fishes were successfully caught and examined as samples.

**Conclusion**

Middle stream of the river can be considered unpolluted whereas downstream is polluted with high turbidity that causes it to be murky. The diversity of freshwater fish in Lubuk Yu river can be considered as low. Microplastics studies are alarming us that our freshwater ecosystem has already been affected by those pollutants. Therefore, it is suggested that the usage of plastic should be avoided in our daily activities because somehow this material will end up polluting our environment.

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**Conflict of interests**

The authors declare that there is no conflict of interest.

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