Journal of Sustainability Science and Management
Volume 18 Number 3, March 2023: 1-23
eISSN: 2672-7226

MICROPLASTIC UPTAKE BY MUD CREEPERS (*Cerithidea obtusa*) AT KUKUP, JOHOR

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Submitted final draft: 31 July 2022 *Accepted: 25 October 2022* http://doi.org/10.46754/jssm.2023.03.001

Abstract: Microplastics are ubiquitous in the aquatic environment. This study was conducted to determine the microplastic accumulation in *Cerithidea obtusa* obtained from Larkin Central Market in Johor, Malaysia. The mean concentration of microplastics was 0.444 ± 0.111 - 0.852 ± 0.513 particles/individual. The most ingested microplastic was sized between 0.250 mm 0.593 ± 0.694 particles/individual and the least ingested ranged in size between 0.501 mm and 1.000 mm and between 1.001 mm and 5.000 mm (0.333 ± 0.555 particles/individual). As for the colours of the microplastics ingested, black microplastics were the most ingested at 68.09%, while the least ingested was green at 2.13%. Fibres were the dominant microplastic shape found in this study at concentrations of 0.568 ± 0.670 particles/individual. The fibres ingested by *C. obtusa* were of polyethylene terephthalate (PET) origin. Based on the non-parametric test (Spearman's Correlation test), both specimen body length and soft tissue weight showed no correlation to microplastic ingestion rate (p = 0.273, p = 0.174). The results from this study will contribute to baseline knowledge on microplastic contamination of *C. obtusa* found in Johor, Malaysia. The study also showed that microplastics can find its way into humans through seafood consumption.

Keywords: Microplastic, mangrove, mud creeper, seafood.

Introduction

Plastic products are widely used around the world daily. Statistics show that the amount of plastic produced has increased over time. According to Plastics Europe (2020), global plastic production has increased by 2.79 percent from 358 million tonnes in 2018 to 368 million tonnes in 2019, with the packaging sector contributing the most to the uptick. Plastic waste can travel from the coast to the ocean, contributing to marine pollution. There are 99.5 million tonnes of coastal plastic waste created annually (Richie & Roser, 2018). Additionally, there are issues of plastic pollution in marine environments across the globe. This is because the seaside and coastal communities often dump garbage in rivers and the sea. The impact of plastics on the marine environment is as yet unknown and it is difficult to estimate how much plastic has entered and is retained in the ocean (Khalid *et al.*, 2021). However, according to Andrady (2011), as much as 75 percent of land-based plastic litter enters the ocean annually. Compared with large-sized plastics, these small-sized plastics, known as microplastics, pose more severe environmental and health issues (Richie & Roser, 2018).

Microplastic pollution is a common environmental issue. Microplastics have become a ubiquitous part of marine environment and are defined as plastic particles with a size of between 0.001 mm and 5.000 mm (Coppock *et al.*, 2017; Frias & Nash, 2019; Nor *et al.*, 2021). When plastic is dumped in a river or the sea, it faces environmental conditions and natural processes such as photodegradation, waves, and mechanical abrasions over time and it even interacts with ocean biota and becomes smaller plastic particles known as microplastics (Frias & Nash, 2019; Wu *et al.*, 2019; Thushari & Senevirathna, 2020). Microplastics are classified into two types, namely primary and secondary. Primary microplastics are microscopically-sized plastic fragments produced as by-products of particle emissions and emissions from industrial production, intermediate plastic materials, dust and fibers, and plastic-based materials (Group of Experts on the Scientific Aspects of Marine Environmental Protection, 2016). Typically, cosmetics, personal care products, cleaning products, sun protection products, pharmaceuticals as well as products for children are examples of microplastics used in domestic households and industries (Andrady, 2011; Cole *et al.*, 2011; Duis & Coors, 2016; Kuk-Dzul & Díaz-Castañeda, 2016). Secondary microplastics are formed in seas through mechanical deterioration (e.g., wave movement), UV degradation, and microbiological degradation (Khalid *et al.*, 2021). These primary and secondary microplastics are present in high volumes in the marine environment, potentially adversely affecting marine habitats and ecological processes.

Microplastics are global pollutants found in every marine environment across the globe, the increase in the amount of microplastics in marine environments has toxic effects on marine organisms. Microplastics can travel up the food chain and sometimes carry sediment and even biota. Microplastics enter marine ecosystems through various means, including river transport, sewage and wastewater, ports, atmospheric deposition and direct discharge, such as air blasting and cosmetics use (Schmid *et al.*, 2020; Thushari & Senevirathna, 2020; Huang *et al.*, 2021).

Microplastics can be ingested by marine life irrespective of their size (Lehtiniemi *et al.*, 2018; Nelms *et al.*, 2018). According to Richie and Roser (2018), microplastic emission levels were estimated at two million tonnes in 2020 and will exceed 2.5 million tonnes by 2050. Environmental ecology will face severe risk from absorbed chemicals and toxins, which could adversely affect human health (Carbery *et al.*, 2018).

It is reported that microplastics can accumulate and magnify at trophic levels (Nelms *et al.*, 2018), which may potentially impact humans as apex predators. The digestive systems of organisms at higher trophic levels contain more microplastics than those at lower trophic levels due to the predator-prey relationship (Nelms *et al.*, 2018; Wang *et al.*, 2021). Microplastics are in living organisms, as evidenced by reports of 693 species of marine animals where 76.5 percent of the reports detailed marine animal encounters with plastic in marine environments (Gall & Thompson, 2015).

The first incident of plastic ingestion in a marine environment was that of a juvenile harbour porpoise, which was found dead on a beach near Pictou in Nova Scotia, Canada, having ingested black plastic litter (5 cm x 7 cm) (Baird & Hooker, 2000). Since then, there have been numerous documented cases of the ingestion of microplastics by marine animals, particularly those usually consumed by humans as food. For example, in a study by Curren *et al.* (2020), imported shrimp species (*Litopenaeus vannamei*, *Pleoticus muelleri*, and *Fenneropenaeus indicus)* sold at a wet market in Singapore contained microplastics ranging between 13.4 and 7,050 particles with different type of microplastic identified such as fibers, films, fragments and granules.

Other popular studies on seafood focused on fish and bivalves. Fish species (*Epinephelus coioides*, *Platycephalus indicus*, and *Liza klunzingeri*) purchased from a local fishmonger at Bushehrport in the Persian Gulf, Iran contained microplastics at 0.158 - 0.931 particles/g w.w (Akhbarizadeh *et al.*, 2019). For bivalve species cultured for consumption by humans, *M. edulis* had an average of 0.36 ± 0.007 particles/g w.w whereas *C. gigas* contained an average of 0.47 ± 0.016 particles/g w.w and as a result, shellfish consumers in Europe may be exposed to 11,000 microplastic particles a year throughout their diets, according to research (Cauwenberghe & Janssen, 2014).

However, studies on the ingestion of microplastics by marine gastropods is still lacking. They are useful as bioindicators because they possess several advantageous characteristics, including reasonable sizes for analysis and repeatable sampling, less mobility (which allows them to accumulate pollutants at higher concentrations than background contaminates), and low or undetectable enzyme activity levels, which are responsible for metabolising pollutants. Furthermore, certain gastropods can be consumed as seafood, which contributes to the development of major health repercussions in humans (Edward *et al.*, 2010). The latest study on *C. obtusa* was reported at Pangkal Babu Mangrove Forest Area in the Tanjung Jabung Barat District of Jambi in Indonesia where microplastic concentrations in the species were 67 ± 16.01 particles/individual and film-type microplastics were dominant (Fitri & Patria, 2019)

Mangrove swamp forests are ecosystems that have a tidal nature between estuaries in the tropics and subtropics. An estuary is a semi-closed environment that receives water from both the land and the sea and can store a variety of pollutants (Zaki *et al.*, 2020). Additionally, mangroves' roots can trap sediment and suspended solid wastes in the water column (Govender *et al.*, 2020; Hilaluddin *et al.*, 2020. As a result, it can potentially accumulate a large number of microplastics. Aside from anthropological activities, natural factors such as ocean currents, tidal amplitudes, current velocities, the mangrove forest's overall nature (e.g., height) and the sediment composition, affect the microplastic deposition and accumulation in the mangrove ecosystem (Deng *et al.*, 2021). Most importantly, mangrove forests support highly diverse species (Govender *et al.*, 2020; Hilaluddin *et al.*, 2020; Zaki *et al.*, 2020).

In Singapore, seven (7) mangrove habitats had high levels of fibrous microplastics (<20µm) of polyethylene (PE), polyvinyl chloride (PVC), polypropylene (PP) and nylon origins at a mean concentration of 9.2 ± 5.9 particles/250 g of dry sediment (Nor & Obbard, 2014). In Southern Iran, mangrove sediment contained a total of 2,169 particles/kg where dominant microplastics were fibrous and white (Maghsodian *et al.*, 2021). Meanwhile, the authors determined that mudskippers (*Periophthalmus waltoni*) at the study location contained a microplastic mean abundance of 1 - 9 particles/kg and that the dominant microplastic type was fibrous, black and consisted of polystyrene (PS), polypropylene (PP) and polyethylene terephthalate (PET).

Overall, there is a lack of research regarding microplastics in Johor, Malaysia. The current study on microplastic contamination in marine organisms consumed as seafood in Johor, which considered three (3) fish species (*Drepane punctata*, *Hexanematichthys sagor* and, *Plotosus canius*) at the Melayu River in Johor, Malaysia found that they contained microplastic particles with a mean abundance of 2.00 ± 0.00 particles/individual, 2.00 ± 1.41 particles/individual and 3.92 ± 4.17 particles/individual, for each species researched respectively. Blue, fibrous PET and PE microplastics were dominant. In green mussels (*Perna viridis*) found at Kampung Pasir Putih in Johor, the microplastic abundance was found to be 1.95 ± 1.14 particles/individual where blue, fibrous microplastics were the most abundant type found (Maha, 2019).

Although *C. obtusa* is a widely commercialised species in Malaysia, it has limited documentation. Thus, it is crucial to study the ingestion of microplastics in *C. obtusa* as one of the microplastic pathways to humans is through the consumption of microplastic-contaminated food. Although the effects of microplastic ingestion in humans is limited, current microplastic studies globally focus on the adsorbent properties of microplastics, especially towards toxic chemicals such as heavy metals (Bowley *et al.*, 2021; Sharma & Ghosh, 2020). Upon entering the digestive system of humans, they may desorb the toxins, which pose more of a threat than the ingestion of the microplastic itself. The main purpose of the study is to identify the presence of microplastics in *C. obtusa* in Johor, Malaysia. The research objectives for this study were to (1) establish the microplastic ingestion rate in *C. obtusa*, (2) discover the characteristics (size, shape, colour, origin) of ingested microplastics in *C. obtusa* and (3) determine the association between body length and microplastic ingestion and between soft tissue weight and microplastic ingestion in *C. obtusa*.

Materials and Methods

Location of Study

Figure 1 shows the study location. The map was generated using MapWindow5 and OpenStreetMap database. The mud creepers (*C. obtusa*) were obtained from Larkin Central Market (1°29'47.2" N, 103°44'32.9" E) in Johor Baru. *C. obtusa* is a deposit-feeding organism that prefers mangroves as a habitat (Fitri & Patria, 2019). The species is categorised as a non-selective bottom feeder and forages for food in the mud (Hassan *et al.*, 2021). In addition, the species are a common Malaysian ingredient in seafood dishes.

The mud creepers were originally captured at Kukup village in Johor. The Kukup village is located in the Pontian District of Johor in Malaysia. The village is near the Straits of Malacca and is surrounded by muddy mangrove shorelines. Kukup village also connects to Tanjung Balai in Indonesia through regularly scheduled ferries. It is a small fishing community that is known for its open-air seafood restaurants on stilts over the water. To the west of the village is Kukup Island which is surrounded by 647 hectares of mangrove forest (Johor National Parks, 2019). Similarly, to the southeast of Kukup is Tanjung Piai, which is an important cape that is surrounded by mangrove forests. The mangrove ecosystem is a significant sink for various anthropogenic-based pollutants, including microplastics. The mangrove ecosystem also acts as a filter medium for litter (Fitri & Patria, 2019; Deng *et al.*, 2021).

Sample Preparation

Cerithidia obtusa obtained from the Larkin Central Market were stored in an iced-cold storage box during transport to the Environmental Engineering Laboratory at Universiti Teknologi Malaysia (UTM). Figure 2 shows the specimen obtained from Larkin Central Market. Distilled water was used to rinse off excess particles on the snail. The snail's total weight was taken to the nearest 0.01g using an analytical balance (Model: Sartorius BT 224 S) and a digital Vernier caliper was used to measure the shell length and

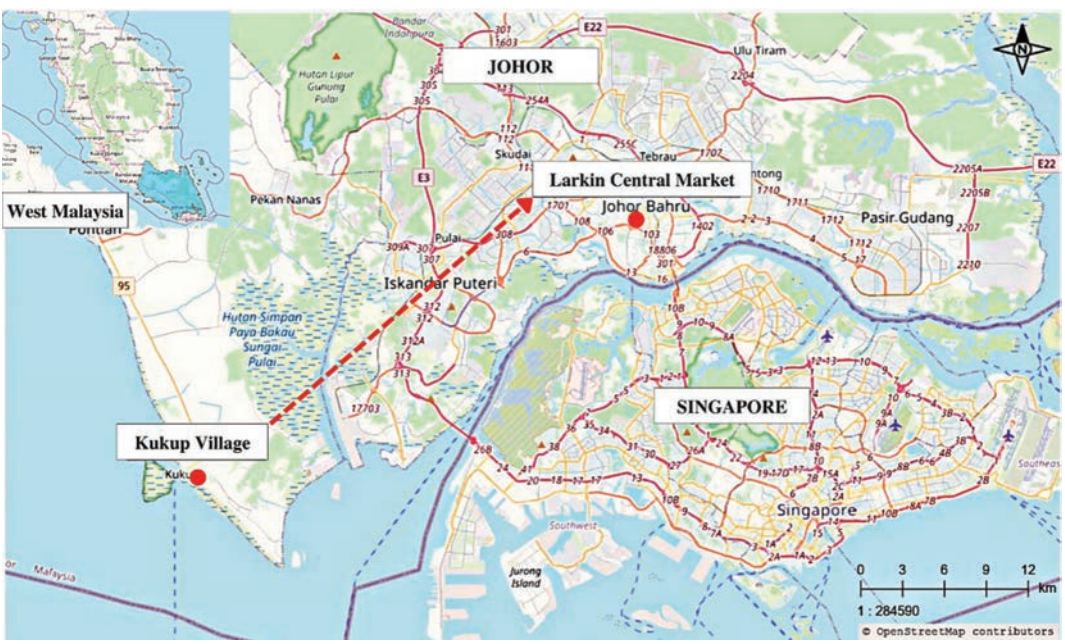


Figure 1: Sampling location

Figure 2: *Cerithidea obtusa* obtained from Larkin Central Market

width to the nearest 0.01mm (Cob *et al.*, 2008). The shell was carefully crushed using a mortar and pestle (Doyle *et al.*, 2020). Distilled water was used to remove excess shell pieces on the soft tissue. Similarly, the weight of soft tissue was measured. Three (3) sizes of *C. obtusa* were chosen for study and categorised as small (S), medium (M) or large (L). Three (3) replicates were prepared, where each replicate contained a pooled sample of nine (9) snails. The samples were put into 250 mL pre-cleaned conical flasks.

Digestion of Snail

In most cases, remaining tissue and non-plastic particles following digestion can obstruct the visual detection of microplastics. As a result, it is crucial to break down tissue as quickly as possible during digestion without compromising the microplastics' original properties (Xu *et al.*, 2020). In this study, potassium hydroxide (KOH) (Brand: Merck) was used to digest the soft tissue. Based on a study by Thiele *et al.* (2019), 10% of KOH provides the most effective digestion as it allows for filtration using 1.2µm pore-size filter papers, which fulfils the size definitions of microplastics which is between 0.001 mm (1.0 µm) to 5.000 mm (Frias & Nash, 2019). To prepare a 10 percent KOH solution, 100 g of potassium hydroxide (KOH) pellets were dissolved in 1000 mL of distilled water. Then, 10 percent KOH was added to each conical flask containing pooled samples as much as three (3) times the tissue volume (Thiele *et al.*, 2019). Similarly, procedural blanks with no snail were prepared for each size and run in parallel with the digestion process (Akindele *et al.*, 2019; Xu *et al.*, 2020). The conical flasks were covered with aluminium foil to prevent airborne microplastic contamination (Maha, 2019) and left for 48 hours at 40°C in an orbital shaker (Model: IKA 3510001 KS 4000i) (Thiele *et al.*, 2019) at 150 rpm (Xu *et al.*, 2020).

Density Separation using Hypersaline Solution

The density separation using a hypersaline solution is used to recover microplastics from samples. As saline solutions are denser than water, more particles (those with a lower density than the solution) can be suspended on the surface of the solution after shaking and be removed, leaving heavier particles to settle in the bottom after some time (Hidalgo-Ruz *et al.*, 2012; Thiele *et al.*, 2021). Commonly, a saline solution of 1.2 g/cm^3 sodium chloride (NaCl) is used as it is cost-effective, easy to access, and does not pose a risk to the environment as opposed to denser saline solutions (> 1.5 g/cm^3) such as zinc chloride ($ZnCl_2$) and sodium iodide (NaI) (Quinn *et al.*, 2017). Digested samples in the conical flasks were filled with 150 mL of 1.2 g/cm^3 NaCl (Brand: Merck). The conical flasks were shaken vigorously before being left overnight and covered with aluminium foil to prevent airborne microplastic contamination. After 24 hours, the solution was filtered using a glass vacuum filtration set (Model: Gast) through a 1.2 µm GF/C Whatman® pore-sized filter. The filtered particles were then stored in a dry petri dish for microscope observation (Akindele *et al.*, 2019).

Stereomicroscopy for Observation of Microplastics

A stereomicroscope is a tool to identify microplastics with diameter of a few hundred microns or less (Mariano *et al.*, 2021). Particles filtered after the digestion process were observed under a stereomicroscope. The stereomicroscope used was a Huvitz-600 stereomicroscope located at the Environmental Laboratory in Universiti Teknologi Malaysia. The stereomicroscope was equipped with *i-solution* Premier software. Using the stereomicroscope, the presence of microplastics and the physical characteristics of the microplastics present in the digested snail samples were quantified and identified. The tool has a maximum magnification of up to 45x. Microplastics can be categorised by shape, colour and size (Lusher *et al.*, 2017).

In many studies, the dominant shapes of microplastics were fibres, fragments and beads. A fragment is defined as a piece that is torn or split from an object, while fibre is defined as thread-like material, while a bead is a rounded, solid particle. Colours could be reported in various shades. However, colour separation is subjective, and visual identification of microplastics cannot be made only based on colour (Lusher *et al.*, 2017).

Characterisation of Microplastics

Potential microplastics were verified by using ATR-FTIR (Model: PerkinElmer) to determine the polymer. In the course of the FTIR analysis technique, samples were brought into contact with infrared radiation (IR). The impact of the IR radiation on the atomic vibrations of molecules in the sample, energy was specifically absorbed and/or transmitted depending on the sample. As a result, FTIR was effective in identifying specific chemical vibrations present in the material (Nandiyanto *et al.*, 2019). The instrument used to collect spectra from 4000 cm^{-1} to 650 cm^{-1}. The spectrum can be used to generate data such as "absorption versus wavenumber" or "transmission versus wavenumber." In this study, the spectrum was displayed as "transmission versus wavenumber" curves. Recorded spectra were compared with absorption bands described in available literature that was derived from a spectral library for each polymer (Jung *et al.*, 2018). Acceptable identification requires a minimum of four (4) matching absorption bands. A description of bonds for each recorded absorption band was also depicted.

Quality Control (QC)

To prevent airborne microplastic contamination, a lab coat and nitrile gloves were worn at all times. All unused solutions were covered with aluminium foil. Beakers and conical flasks were rinsed thoroughly using distilled water before using and were covered with aluminium foil. Filter papers with filtrate were kept in a pre-labelled petri dishes after oven-drying. In addition, procedural blanks were run in parallel with the replicates to quantify and correct for microplastic abundance due to airborne microplastic contamination. Finally, a petri dish with wet filter paper was placed beside the vacuum pump filtration set to measure airborne microplastic contamination during the filtration process. Microplastic particles obtained from procedural blanks and the wet filter paper were quantified and subtracted from the total microplastics obtained.

Statistical Analysis

All calculations of data and graphs were conducted using OriginPro 2021 and Microsoft Excel 2019. The body length and soft tissue weight measurements of the gastropods (reported as mean ± standard deviation) and the number of microplastics ingested (reported as mean ± standard deviation) were used in the statistical analysis. To determine whether the data follows a normal distribution, Shapiro-Wilk and Anderson-Darlings tests were used (significance level was set at 0.05). As the data were not normally distributed, the correlation between body length and microplastic ingestion as well as soft tissue weight and microplastic ingestion was investigated using a 2-tailed Spearman's Correlation (significance level was set at 0.05). Reported values were adjusted to three (3) decimal places when necessary.

Results and Discussion

81 individual samples of *C. obtusa* of different sizes were obtained from the Larkin Central Market in Johor, Malaysia. *C. obtusa* is characterised by its brown or purplish-brown shell with a brighter zone below the spiral grooves (Rusnaningsih & Patria, 2020). To achieve the objectives of this study, all individual sizes of *C. obtusa* were sorted into different size categories during the snail sampling process. The three (3) categories for *C. obtusa* sample sizes were small, medium or large. Sample selection by size was to compare microplastic uptake by the size of *C. obtusa.* Table 2 shows the summary of physical parameter data for all snails.

Based on Table 2, the minimum and maximum body length for small-sized *C. obtusa* collected was 20.40 mm and 29.40 mm, respectively with a mean length of 23.569 ± 2.018 mm. For medium-sized *C. obtusa*, the minimum and maximum body length was 27.30 mm to 32.50 mm, respectively with a mean length of 30.622 ± 1.418 mm. For large snails, the minimum and maximum body lengths were 30.60 mm and 37.80 mm, respectively with a mean length of 34.582 ± 1.629 mm. From a study by Fitri & Patria (2019) on *C. obtusa* microplastic contamination in Pangkal Babu Mangrove Forest, the collected snail samples have a body length of between 18 mm and 44 mm, which means that the small-sized and large-sized *C. obtusa* in Johor, Malaysia were smaller and bigger, respectively than their counterparts at the Pangkal Babu Mangrove Forest.

Referring to Table 1, the mean soft tissue weight of small-sized *C. obtusa* was 0.264 ± 0.048 g with the minimum soft tissue weight and maximum soft tissue weighing 0.20 g and 0.36 g, respectively. On the other hand, the medium-sized snail had a mean soft tissue weight of 0.367 ± 0.074 g with the minimum soft tissue weight and maximum soft tissue weight at 0.27 g and 0.49 g, respectively. The mean soft tissue weight of large-sized snails was 0.450 ± 0.057 g with the minimum soft tissue weight and maximum soft tissue weight at 0.33 g and 0.55 g, respectively. The total body weight (shell + soft tissue) of *C. obtusa* in Pangkal Babu Mangrove Forest was between 0.38 g and 1.80 g which was smaller than the total body weight of the same species in the current study at between 1.00 g and 4.20 g. The weight of soft tissue was not reported in the study by Fitri & Patria (2019) but it can be assumed to be lower than in the current study due to the lower total body weight.

Abundance of Ingested Microplastics

A total of 81 snails from three (3) size groups were collected and pooled into nine (9) small-sized snail individuals, nine (9) medium-sized individuals and nine (9) large-sized individuals per replicate. There were microplastics found in the soft tissues of *C. obtusa*. Each size category ingested a total of 23 particles, 12 particles and 12 particles for small-sized snails, medium-sized snails and large-sized snails, respectively. Therefore, the current study proved that the species could ingest microplastics. Since there are many sources of water that flow from

Table 1: Summary of physical characteristics of *C. obtusa*

Size Category	Min. Length (mm)	Max. Length (mm)	Mean. Length (mm)	Min. Soft Tissue Weight (g)	Max. Soft Tissue Weight (g)	Mean Soft Tissue Weight (g)
Small	20.40	29.40	23.569 ± 2.018	0.20	0.36	0.264 ± 0.048
Medium	27.30	32.50	30.622 ± 1.418	0.28	0.49	0.367 ± 0.074
Large	30.60	37.80	34.582 ± 1.629	0.33	0.55	0.450 ± 0.057

various directions and locations, the origin or source from which these microplastics cannot be identified. Some of the factors that can potentially contribute to microplastic pollution are industrial and commercial activity and effluent disposal. Table 2 outlines the detected microplastics in *C. obtusa* for the current study whereas Table 3 presents microplastic ingestion studies by aquatic gastropods. Figure 3 shows the mean abundance of microplastics ingested by *C. obtusa* according to individual size. The total amount of microplastics extracted from *C. obtusa* tissue was 47 particles. As shown in Figure 3, the mean abundance of microplastics for small-sized snails was 0.852 ± 0.513 particles/individual, 0.444 ± 0.193 particle/individual for medium-sized snails and 0.444 ± 0.111 particles/individual for large-sized snails. In the study by Fitri & Patria (2019) for the same gastropod species, the mean microplastic abundance was 67 ± 16.01 particles/individual which was a lot higher than the current study. The study also noted that the highest abundance of microplastics was in *C. obtusa* obtained from Station 1 (190 ± 37.70 particles/individual) which was the nearest to the river mouth. The lowest microplastic abundance in the species was in Station 4 (153.3 ± 21.58 particles/individual) which was the furthest from the river mouth this showed that mangrove forests trapped marine-based plastic litter. It is not possible to compare the results of that study with the current study as the specimens for this study was obtained from the local market. However, it can be safely assumed that the background microplastic content at the study site was exceptionally low.

Small-sized snails contained a significantly higher amount of microplastic than medium-sized snails and large-sized snails. Currently, there is no data on microplastic ingestion in *C. obtusa* by specimen size. Organisms will accumulate microplastics when their uptake rates exceed the rate of egestion (Woods *et al.*, 2018; Fitri & Patria, 2019). However, this may be due to a small-sized snails having a low egestion rate for microplastics. Further study should be done on this. Although Akindele *et al.* (2019) proved that bigger aquatic gastropod species contained more microplastics due to having higher nutrients requirements, the study also showed that the microplastic load ratio to wet weight in smaller species was higher. A statistical analysis was also done in the study to determine the correlation between size and microplastic ingestion rates. Table 4 shows the current studies on microplastic ingestion by aquatic gastropods.

Based on the results of this study, the accumulation of microplastics may be due to the size of the snails. In this study, researchers do not know what the level of microplastic contamination at Kukup village in Malaysia is as there are no preexisting microplastic studies in that area. In addition, although the samples were said to be captured at Kukup village and then sold at the Larkin Central Market, researchers are unable to ascertain the actual origins of the mud creepers as other places in Johor have mangrove ecosystems as well. Having a measure of accessible microplastic data might contextualise current findings on the mud creepers. However, we can be certain that microplastic contamination does occur in Johor (Sarijan *et al.*, 2018; Maha, 2019; Primus & Azman, 2022).

Overall, there are more studies on microplastics in gastropods at the international level as compared with at the local level. Nevertheless, these studies on aquatic gastropods prove that aquatic gastropods can ingest microplastics and therefore can become bioindicators of pollution levels in their habitat. The samples taken from the Larkin Central Market showed that 50 percent of the snails had ingested microplastics. These results validate the presence of microplastic contamination in mud creeper snails taken from the Larkin Central Market, which originate from the Kukup village in Malaysia. As mud creepers are a popular local seafood, consumption of seafood containing microplastics exposes humans to the same, that directly or indirectly affects human health and food security. In a similar study by Chen *et al.* (2020), microplastics were found in popular local seafood in Taiwan namely clams (*Meretrix lusoria*), oysters (*Crassostrea gigas*) and squids (*Loliginidae spp.*) which comprised mostly of fragments of PP, PE and PET.

Organisms at lower trophic levels such as small fish, crustaceans and bivalves contained more microplastics per gram of net weight (Walkinshaw *et al.*, 2020). Therefore, a trophic transfer is an effective pathway of microplastic ingestion from seafood as prey to humans as apex predators as long as the top predator consumes the prey as a whole (Nelms *et al.*, 2018). The author mentions the effects of microplastic ingestion on prey at lower trophic levels, which include a reduced food intake, reduced reproduction rate and lower energy levels as well as increased mortality rates. It is worth mentioning that microplastics are vectors for hazardous pollutants such as heavy metals and pathogenic microbes (Sharma & Ghosh, 2020; Bowley *et al.*, 2021). However, a study by Cunningham *et al.* (2021) on a marine predator, shore crab (*Carcinus maenas*) towards its common prey, the blue mussel (*Mytilus edulis*), suggested that microplastic ingestion does not alter the predator's feeding rate but increases in the number of prey did decrease the feeding rate, which the authors attributed to hyperbolic Type II functional responses (high predation rates at low prey densities).

Physical Characteristics of Ingested Microplastics

Most studies reported microplastic contamination findings in terms of the physical properties of microplastics such as shape, size and colour. Microplastics can be categorized into several shapes such as fibres, fragments, films, pellets and beads (granules) (Hidalgo-Ruz *et al.*, 2012). Fibre-shaped microplastics have a thin structure, while beads have fragment-like characteristics but are round in shape. By comparison, fragment-shaped microplastics have an incomplete shape created by greater material degradation. Microplastics can be categorised by a broad spectrum of colours. Additionally, microplastics can also be

Table 2: Detected microplastics in *C. obtusa*.

Size Category	N	Fibre (particle)	Fragment (particle)	Mean abundance (particle/individual)
Small	27	22	1	0.264 ± 0.048
Medium	27	12	0	0.367 ± 0.074
Large	27	12	0	0.450 ± 0.057

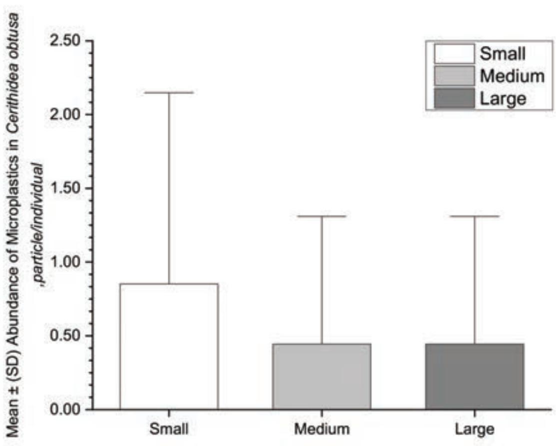


Figure 3: Mean abundance of microplastics in *C. obtusa*

Table 3: Studies investigating microplastic ingestion by aquatic gastropod species.

Species	Location	Microplastic Abundance	Dominant Microplastic	Reference
Laevistrombus turturella	Bintan Island, Indonesia	360 ± 118.43 - 492 ± 107.68 particles/ind.	Fibre	Al-Hamra & Patria, 2019
Cerithidia obtusa	Pangkal Babu Mangrove Forest, Indonesia	167 ± 16.01 particles/ind.	Film	Fitri & Patria, 2019
Lanistes varicus, Melanoides, tuberculata and Theodoxus fluviatilis	Osun River and Rhine River, Nigeria	6.1 ± 1.05 - 1.71 ± 0.46 particles/g w.w	PE and PP fibre	Akindele *et al.*, 2019
Littoraria scabra	Pramuka Island, Indonesia	86.88 particles/ind.	Fibre	Patria *et al.*, 2020
Nerita articulata, Nerita polita, and Chicoreus capucinus	Klang River estuary, Malaysia	0.25 - 0.88 particle/ ind.	Black-coloured fibre of PE-PDM and polyester origins	Zaki *et al.*, 2020
Cerithidia obtusa	Larkin Central Market, Johor, Malaysia	0.444 ± 0.111 - 0.852 ± 0.513 particle/ind.	Black-coloured PET fibre	This study

classified by their degree of ageing, which are primary microplastics (pristine) and secondary microplastics (weathered). Microplastics can also be grouped by their size in terms of the size range. Microplastics can also be sorted by their chemical composition to determine their point of origin through Attenuated Total Reflection-Fourier Transform Infrared (ATR-FTIR). FTIR spectroscopy is a straightforward, efficient, and non-destructive approach for detecting and differentiating most plastic polymers (Jung *et al.*, 2018). Figure 4 shows microplastics found in *C. obtusa* during microplastic identification using a stereomicroscope. The shape and colour of the microplastics ingested by *C. obtusa* in this study were fibres and fragments in multivariate colours.

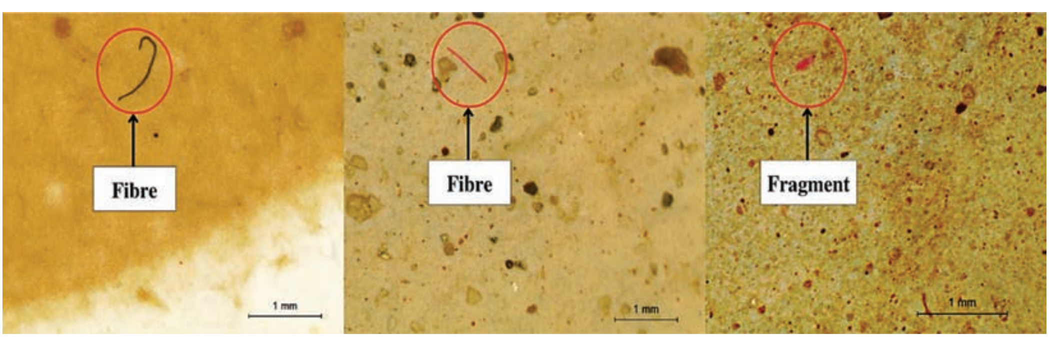


Figure 4: Ingested microplastics in *C. obtusa*

Shape of Ingested Microplastics

The microplastic shapes found in *C. obtusa* soft tissues in all (3) three sizes of specimens is shown in Figure 5. Based on the observations under the stereomicroscope, almost all of the shapes found in *C. obtusa* were fibrous, and one (1) sample was in the shape of a fragment. All microplastics found could be generated from fragmented larger plastic items, which are commonly classified as secondary sources of microplastics. Figure 5 (a) shows the mean abundance of microplastics in *C. obtusa* by microplastic shape. Fibres accounted for between 0.568 ± 0.670 particles/individual. All size categories of *C. obtusa* ingested fibres at 48 percent for small-sized snails, and 26 percent for both medium-sized and large-sized snails. Figure 5 (b) shows the percentage of microplastic by shape. Only one (1) fragment-shaped microplastics (0.012 ± 0.111 particles/individual) was determined in this study which was found in small-sized *C. obtusa*. Common microplastic shapes are fibres, fragments, films and beads. However, both films and beads do not exist in this study. Because of their low densities, fibres are known to float for a longer period on the top of the water, but fragments and granules with higher densities sink (Patria *et al.*, 2020). In the study by Fitri & Patria (2019) on *C. obtusa* in the Pangkal Babu mangrove forest, the dominant microplastic shape was film. However, in a study of another mangrove-dwelling marine gastropod (*L. scabra*) on Pramuka Island at Jakarta Bay in Indonesia, 68 percent of the total microplastic content in the specimen was fibrous which is likely to originate from garments (Patria *et al.*, 2020).

Similarly, in other parts of the world, the dominant microplastic shape in mangrove forests was also fibrous (Govender *et al.*, 2020; Maghsodian *et al.*, 2021). In Johor rivers, film type microplastics was found (Sarijan *et al.*, 2018). However, fibres were dominant in surface water and *P. viridis* at Kampung Pasir Putih, which is near the Straits of Johor in Malaysia (Maha, 2019). In the adjacent country, which is Singapore, most microplastics in their mangrove ecosystems were also fibrous (Nor & Obbard, 2014) which proved the prevalence and distribution of fibre-shaped microplastics in the Straits of Johor. The particle morphologies of the microplastics found in Singapore suggested they were of secondary origin, generated from degraded marine plastic litter. The microplastic concentration in mangrove-based, benthic animals varied depending on their environment as mangrove sediment was determined to be a microplastic sink, which explained the lower levels of microplastics in the surface water at the Red Sea and the Arabian Gulf mangrove forests (Martin *et al.*, 2020). In addition, since the start of the Covid-19 pandemic, the use of face masks rose significantly and discarded masks can be seen littering the streets and in the seawater until they become biofouled and reach the sea bottom (Ardusso *et al.*, 2021; Morgana *et al.*, 2021).

Fibres have a low density, allowing them to travel long distances over water. Microplastics in mangrove sediment were higher during the dry season than in the wet season (Govender *et al.*, 2020) which may be due to the microplastics in the sediment being resuspended by the water column during the wet season. However, buoyant particles can sink to the bottom as sediment due to a biofouling process (Martin *et al.*, 2020) which may explain the high number of ingested fibres. Microplastic-contaminated benthic invertebrates, notably filter feeders and deposit feeders feed on organic material in sediment, hence polluted sediment can affect the organism's microplastic intake.

The bioaccumulation of microplastics in marine creatures' guts depends on the microplastic shape. In a study by Qiao *et al.* (2019), the effect of different shapes of microplastics on zebrafish (*Danio rerio*) was observed in laboratory settings. Fibres accumulated the most in the gut of the species with beads being the least. Non-spherical shape of fibre requires more time to pass through the gut. The current study indicated that the common shape of microplastics found in the Kukup village was fibrous. Thus, this study proved the widespread transport of fibrous microplastics from the river or sea into mangrove areas. Fibre is derived from synthetic materials such as

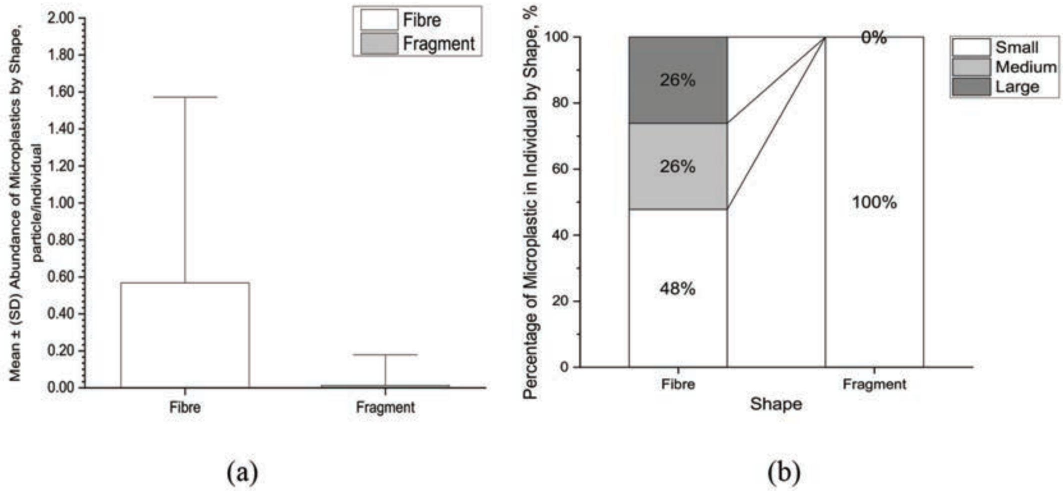


Figure 5: Abundance of microplastics found (a) by type and, (b) percentage of microplastics in an individual by shape

acrylic, nylon, polyester, and rayon, often used in clothing. The presence of fibres in the river, or seawater, is probably from human activities such as washing clothes and may also be from the garment production industry (Falco *et al.*, 2019).

Size of Ingested Microplastics

Microplastics were classified into four (4) size categories: < 0.250 mm, 0.251 mm - 0.500 mm, 0.501 mm - 1.000 mm and 1.000 mm - 5.000 mm as shown in Figure 6(a). In *C. obtusa*, the dominant microplastic size was of those in the < 0.250 mm 0.593 ± 0.694 particles/individual size category followed by the 0.251 mm - 0.500 mm (0.444 ± 0.577 particles/individual size category, then the 0.501 mm - 1.000 mm 0.333 ± 0.555 particles/individual size category and the 1.001 mm - 5.000 mm 0.333 ± 0.555 particles/individual size category. These results prove the microplastic size range does not affect the accidental ingestion rate of *C. obtusa* snails obtained from the Larkin Central Market.

In addition, the results indicated that microplastics with a size of < 0.250 mm were the most widely ingested microplastic size range by *C. obtusa*, which may be related to the common size of its natural prey. The size of the ingested microplastics according to the size of snails is shown in Figure 6 (b). According to the graph, small-sized snails ingested the most microplastics at 58.33 percent, in the 0.251 mm to 0.500 mm size range and the least microplastics at 33.33 percent in the 1.001mm to 5.000 mm size range. Meanwhile, 31.25 percent of medium-sized snails ingested microplastics in the 0.250 mm size range and 22.22 percent of the microplastics in the 0.501 mm to 1.000 mm size range and another 22.22 percent consumed microplastics in the 1.001 mm to 5.000 mm size range. In contrast, large-sized snails consumed the most microplastics in the 1.001mm to 5.000 mm size range at 44.44 percent and the least microplastics in the size range of between 0.251 mm and 0.500 mm at 16.67 percent.

According to the study by Zaki *et al.* (2020) on tropical estuary gastropods (*N. articulata, N. polita and C. capucinus*) found in Klang River, the size of microplastics ingested by the (3) three species was between 30 µm and 1850 µm with the majority of ingested microplastics in the 300 µm and 1000 µm size range. It can be assumed that the marine animals consumed microplastics that are similar in size to prey. Although the sizes of microplastics is an extremely broad subject, most microplastics in the mangrove ecosystem adjacent to Johor which is in Singapore were < 0.04 mm.

Similarly, in the Melayu River at Johor in Malaysia, the most dominant microplastics were < 0.05 mm in size. Small microplastics were found in mangrove sediment. This is most likely owing to the increased vulnerability of small plastic materials to being entrained by vertical mixing and sinking (Martin *et al.*, 2020). Overall, the characteristics of microplastics depended on the background contamination of the area itself. Even large microplastics can undergo further fragmentation and weathering to become smaller in size. In a study by Lehtiniemi *et al.* (2018), the model organism, mysid shrimps were not affected by the colour of the provided coloured microplastics, which suggests that mysid shrimps are mechano-reception feeders rather than visual predators. The mysid shrimps were most likely to encounter the microplastics by accident and choose whether to reject or ingest the particle based on the size. However, *C. obtusa* is a non-selective feeder (Hassan *et al.*, 2021) which means that the species ingests whatever particles fit in its mouth.

Although there is a lack of studies on microplastic ingestion and mouth size in smaller species, *Holothuria floridana* (sea cucumbers) consumed more nylon line pieces than any other material, but no pellets in a laboratory setting (Plee & Pomory, 2020). The authors concluded that pellets may have been too big for the tentacles to grip or fit into their mouth. Additionally, Xiong *et. al* (2019) observed that goldfish (*Carassius auratus*) expelled large microplastics after ingestion which means that microplastic sizes can affect fish during ingestion, making smaller microplastics more easily swallowed. The authors also noted that although PE microplastics were used in their study, the shape may be detected differently by goldfish. Films are thin and can be easily deformed, making them difficult for goldfish to detect. Due to the nature of fibre that is flexible and easy to fit into the mouth, the size of ingested fibres might be possibly irrelevant. The majority of the microplastics discovered in this study were particles with a < 0.250 mm diameter, the size distribution of microplastics is important because it defines the possible impact of these contaminants on ecosystem biota. Microplastics < 0.250 mm is similar in size to most planktonic marine copepods (< 1 mm) (Peralta & Yusoff, 2015) and have a higher potential for ingestion by a diverse variety of species.

Colour of Ingested Microplastics

Several types of microplastic colours were ingested by *C. obtusa*, namely black, red, blue, and green. Figure 7 shows the percentage

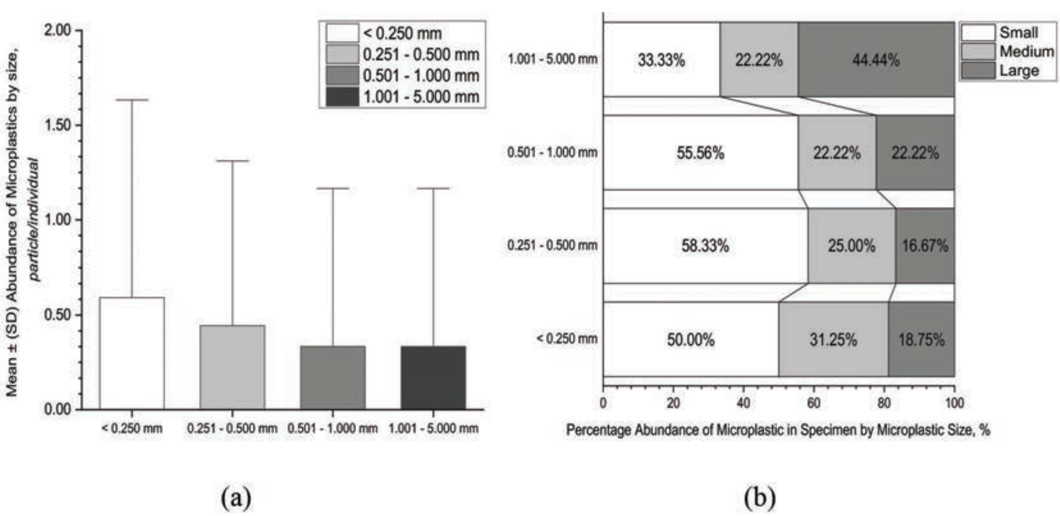
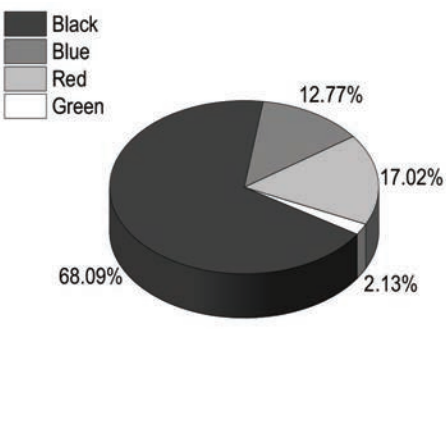


Figure 6: Abundance of microplastics found in *C. obtusa* (a) by microplastic size and, (b) according to specimen size

of the number of black, red, blue and green microplastics for the specimens studied. According to the graphs, black was the most common microplastic colour found in *C. obtusa* at 68.09%. In comparison, green was the least ingested colour by the snails at 2.13%. For the rest of the microplastics, red was the second-most common colour ingested by the snails at 17.02%, followed by blue microplastics at 12.77%. According to Figure 7 (b), small-sized snails had ingested the most significant number of black microplastics, 18 (52.94%) microplastics, compared with medium and large-sized snails, 7 at 20.59% of the microplastics and 9 at 26.47% of the microplastics. Red was also the second most ingested colour by small-sized snails 4, at 57.14% of the microplastics followed by medium and large size snails 2 at 28.57% of the microplastics and 1 at 14.29% of the microplastics, respectively. In addition, the blue colour ingested by medium-sized snails and large-sized snails at 40.00% had the exact value of 2 microplastics while the small-sized snails were 1 at 20.00% of microplastics. The least ingested colour by *C. obtusa* was green, which was only 1 at 100% of microplastics in medium-sized snails. The plastic industry greatly influences the development of aquaculture and fisheries. Most fishing and aquaculture equipment is made of plastic due to its high durability. Often, fishing equipment and items such as fishing nets and polystyrene boxes are transparent and white. However, none of the colours was identified in this study. It can be said that the bright-coloured microplastics obtained in this study may have been transported from land-based sources.

Microplastics can be assumed to inherit colour from their parent plastic products. The colour variety of microplastics in this study was similar to other studies done in mangrove ecosystems (Govender *et al.*, 2020; Maghsodian *et al.*, 2021). However, due to the effects of weathering, the colour may change. These results are different/more or less from the study conducted at the Pangkal Babu Mangrove Forest Area in the Tanjung Jabung District of Jambi in Indonesia. Most of the microplastic found in *C. obtus*a was black (Fitri & Patria, 2019) Similarly, other aquatic gastropods, although not mangrove-dwellers, were found to ingest black microplastics (Akindele *et al.*, 2019; Zaki *et al.*, 2020) which suggests that certain gastropod species may have a colour preference for microplastics that may resemble their food.

Selective feeding based on colour has been well-documented in fish. In a study on microplastic ingestion by goldfish (*C. auratus*), the species ingested microplastics similar to its



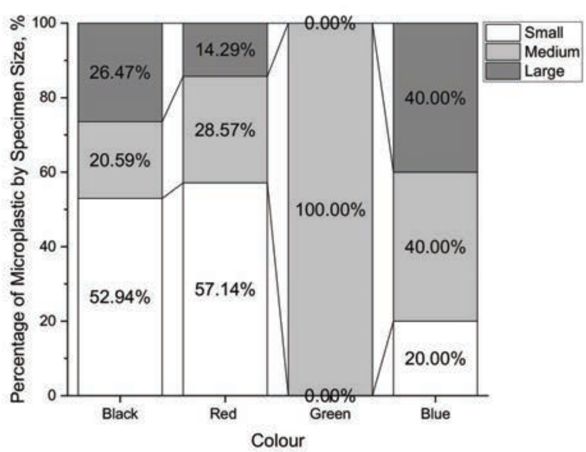


Figure 7: Total percentage of microplastic found in *C. obtusa* (a) by colour and, (b) according to different snail sizes

natural food which is green and black (Xiong *et al.*, 2019). On the other hand, blue microplastics were preferred by amberstripe scad (*Decapterus muroadsi*) as they resembled their natural prey, the blue copepod. These species, however, are visual predators and depend on an acute visual system to catch their prey (Ory *et al.*, 2017). As a result, fish grazing on elusive prey are likely to be vulnerable to mistakenly targeting non-dietary things that resemble their normal prey. In addition, a study was conducted on the gut content *C. obtusa* in Kuala Selangor Nature Park in Selangor and the Tanjung Piai National Park in Johor, Malaysia (Hassan *et al.*, 2021). The results showed that 91.05% of their gut contents contained vegetal detritus comprising leaf matter, diatoms, dinoflagellate, porifera and foraminifera. It is worth noting that vegetal detritus is dark-coloured and similar to the dominant colour in the current study. Although the authors have labelled *C. obtusa* a non-selective bottom feeder, the composition may change according to the environmental factors such as tidal effects.

Verification of Microplastics

Based on the spectra library provided by Jung *et al.* (2018), the spectrum obtained from the ATR-FTIR analysis on dominant fibres ingested by *C. obtusa* showed that PET microplastics were dominant in the study (refer to Figure 8). The dominant polymer was similar to other studies in the mangrove ecosystem (Nor & Obbard, 2014; Maghsodian *et al.*, 2021) which was claimed to be anthropogenically sourced except for the study by Govender *et al.* (2020) which identified PP and PE as dominant polymers. In addition, the polymers that are dominant in Johor waters were of the PET and PE variety (Primus & Azman, 2022). PET plastics were mainly used to produce textile and packaging materials (Carr *et al.*, 2020; Choong *et al.*, 2021; Sait *et al.*, 2021). PET plastics are produced through the polymerising ethylene glycol (EG) and terephthalic acid (TPA) (Syariffuddeen *et al.*, 2012; Carr *et al.*, 2020). As a packaging material, PET is often used to manufacture for single-use applications. Only a small portion of PET gets recycled back into its original form, with the vast majority being downcycled into a lower grade, non-recyclable plastics (Carr *et al.*, 2020). Table 4 details the assignment of bonds according to the adsorption bands for PET polymer identification.

Similar to findings by Jung *et al.* (2018) and Syariffuddeen *et al.* (2012), at 1714 cm^{-1} C=O stretch was created followed by C-O stretch at 1240 cm^{-1} and 1094 cm^{-1}. Out-of-plane (oop) bend of aromatic C-H was identified at 723 cm^{-1}. It is worth noting that as crystallinity decreases, the flexibility of the polymer increases. PET used to create bottles and textiles is typically associated with a high crystallinity at between 30 and 40%, which is common in the industry. However, for packaging material, PET is typically created with low crystallinities at 6% – and 7% (Carr *et al.*, 2020). In microplastic study, the crystallinity of polymer is associated with the adsorption capability of microplastics where higher crystallinity results in lower adsorption affinity

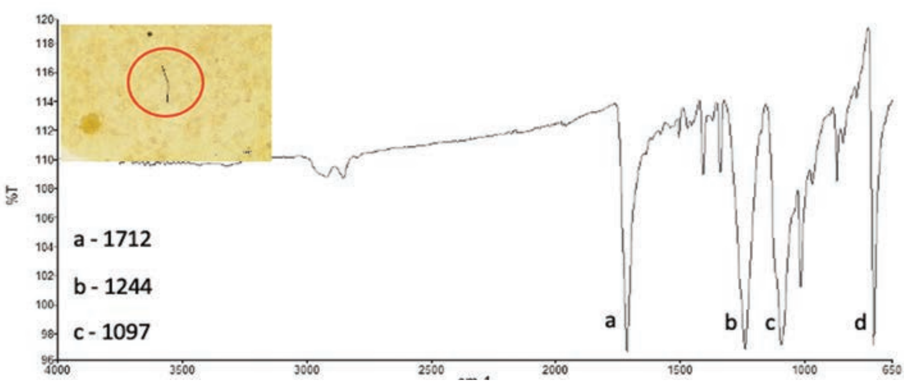
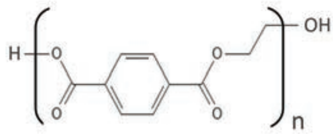


Figure 8: Spectrum obtained from ATR-FTIR analysis of fibre

Table 4: Description of PET polymer

Polymer	Chemical Structure	Adsorption Band (cm^{-1})	Characteristic Bond	References
Polyethylene terephthalate (PET)		a) 1714 b) 1240 c) 1094 d) 723	C=O stretch C-O stretch C-O stretch Out-of-plane (oop) aromatic C-H bend	Jung *et al.*, 2018; Syariffuddeen *et al.*, 2012

towards organic pollutants (e.g., PAH, PCB) (Fu *et al.*, 2021). Thus, ingested microplastics that contain these harmful pollutants may pose more harm to animals and humans as they can desorb upon entering the gastrointestinal tract (Nor *et al.*, 2021).

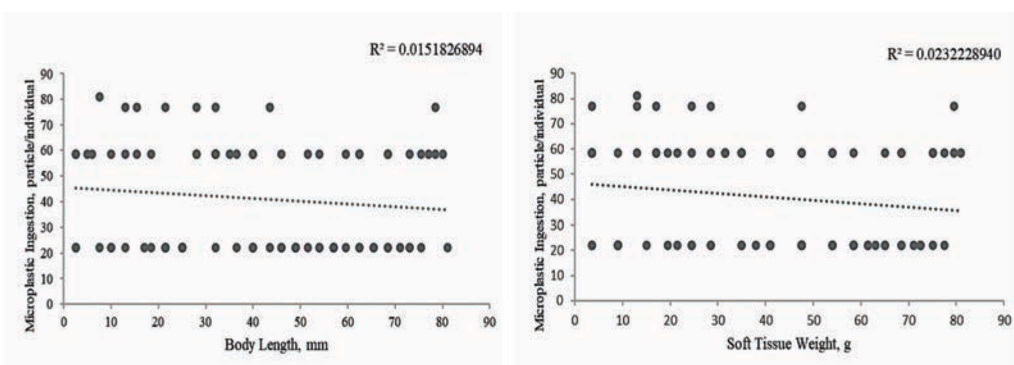
Influence of Microplastic Ingestion on Body Length and Soft Tissue Weight

A statistical approach was conducted to determine the correlation between specimen size and microplastic ingestion rate. Shapiro-Wilk and Anderson-Darling tests were conducted to determine the normality of data. Due to the data not being normally distributed, a non-parametric test (Spearman's Correlation test) was conducted using a significant level, $\alpha = 0.05$ (refer to Table 5). Based on Spearman's Correlation test, specimen body length and microplastic ingestion rate do not correlate (Spearman's Rho = 0.123, p = 0.273). Similarly, there was no correlation between specimen soft tissue weight and the microplastic ingestion rate (Spearman's Rho = 0.152, p = 0.174). Figure 9 shows the linear regression analysis for a) body length and, b) soft tissue weight that confirmed there was no correlation between the variables and microplastic ingestion rate.

In a study by Akindele *et al.* (2019) on African gastropods species, *L. varicus* and *M. tuberculata* ingested more microplastics than

Table 5: Spearman's correlation data

Variable	N	Spearman's Rho	T statistic	DF	p-value (2 - tailed distribution)
Body length	81	- 0.1232	1.1036	79	0.2731
Soft tissue weight	81	- 0.1524	1.3705	79	0.1744



(a) (b)

Figure 9: Spearman's correlation plot for (a) body length and, b) soft tissue weight

T. fluviatilis due to it being the larger species. In addition, a study by Patria *et al*. (2020) on periwinkle snails (*L. scabra*), reported the size of snails showed a significant correlation between ingested microplastics and snail body weight. However, the microplastic abundance ratio by wet weight of snail in the small-sized specimen was higher (Akindele *et al.*, 2019). The study is also supported by Wu *et al.* (2022) where there was a negative correlation between shell size of hard clams (*M. meretrix*) and microplastic count per gramme wet weight. Thus, authors suggested to eat larger bivalves to reduce microplastic intake through bivalve consumption. Additionally, in a study about microplastic ingestion in fish, the amount of plastic consumed has also no relation to body size, condition factors, or stomach fullness (Vries *et al.*, 2020). Microplastic consumption, retention, and excretion is most likely be random, due to the fact that no correlation was seen. In addition, as the snails were obtained randomly at the Larkin Central Market, there is no distinct size range as some of the snail sizes overlapped. Therefore, it may be possible that small-sized snails did not ingest most microplastics.

Conclusion

Microplastic pollution is an emerging issue around the world. Microplastics are prevalent in the environment and can exist in the sediment, water column, the air and in living organisms. The rapid production and use of plastics is making this problem much worse. This study provides baseline information on the accumulation of microplastics by *C. obtusa* obtained from the Larkin Central Market of Johor in Malaysia. The body length of *C. obtusa* in the Malaysian state was between 20.40 mm and 37.80 mm with a mean of between 23.200 ± 1.380 mm and 34.744 ± 1.553 mm whereas the animals' soft tissue weight was between 0.20 g – and 0.55 g with a mean of between 0.258 ± 0.050 g and 0.454 ± 0.055 g. This study confirmed the presence of microplastics in *C. obtusa* at between 0.444 ± 0.111 and 0.852 ± 0.513 particles/individual, which was predominantly fibrous and were related to the widespread of fibrous microplastics in the marine environment in Johor, Malaysia. Black-coloured microplastics were the most abundantly found microplastic in this study, which may be related to the common colour of the microplastics available in the habitat, which may resemble vegetal detritus, a source of food for *C. obtusa*.

In addition, the dominant microplastic was of < 0.250 mm size in this study may harm other micro- and nano-organisms (e.g., phytoplankton) living in the same mangrove ecosystem as the *C. obtusa* and may likely disrupt the trophic levels as small microplastics cause small organisms to die after ingestion and larger microplastics may cause entanglement. The fibrous microplastics were mainly made up of PET polymers, which can be traced to plastic packaging and textiles. These microplastics can be safely assumed to be of originate anthropogenic origin. The body length and soft tissue weight of *C. obtusa* showed no correlation with microplastic ingestion rates, which could mean that microplastic ingestion, retention and egestion in *C. obtusa* were random. In conclusion, microplastics can be ingested by *C. obtusa* snails of various sizes as they are non-selective feeders and will consume almost everything that can fit in its mouth. This study showed that *C. obtusa* can be a vector for the transport of microplastics from the sediment and the water column into the food chain. This may raise concerns as microplastics also can become vectors to transport harmful pathogens, addictives, heavy metals and organic pollutants from one biota to the other. Although studies on the effects of microplastics in humans is limited one can assume that the effects are chemical-level (desorption of harmful chemicals and pathogens into the gut) and not physical-level (pseudo-satiation).

Acknowledgements

We would like to sincerely thank Universiti Teknologi Malaysia (UTM) for supporting this study. This study was funded by a Universiti Teknologi Malaysia Fundamental Research Grant bearing Cost Centre No. Q.J130000.2551.21H72.

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