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Microplastics Ingestion by *Scapharca cornea* at Setiu Wetland, Terengganu, Malaysia

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Abstract: Filter feeding bivalves are among the keystone species in marine environment that maintains or improves water quality and nutrients cycling between the water column and bottom dwelling organsims. Ingestion of microplastics by these organisms provides a possible step for the transfer of pollutants and plastics additives. Ingestion of microplastics in *Scapharca cornea* were determined and quantified at its natural habitat in Setiu Wetland, Terengganu, Malaysia. A total of 120 wild *S. cornea* bivalves were handpicked from three stations. Microplastics were extracted by alkali digestion (10 M of NaOH) and then identified visually according to their physical characteristics: shape, colour size and density. Most of the microplastics particles present were filaments. Fourier Transform Infrared Spectroscopy analysis of the microplastics showed the presence of strong peaks associated with polyethylene and polyamide.

Key words: Bivalves · Filter Feeder · FTIR · Microplastic Pollutants

INTRODUCTION

Marine invertebrates, especially bivalves tend to be selected as ideal test organisms in monitoring environmental programs [1]. These are due to their occurrence where they could be found in greater abundance and distribution, easy to sample, resistance to any stress condition, widely known as benthic filter feeder and more importantly, they are sessile organisms [2]. These bivalves can be found in marine environment, muddy and sandy condition with depth reaching 20 m [2]. The bivalve, *Scapharca cornea*, is found in great abundance in Setiu Wetland, Terengganu, Malaysia. These are collected regularly from sub-tidal and intertidal areas and are sold by the local community. According to Nakisah and Faizah [3], Setiu wetland is known as the only wetland in Malaysia that consists of nine inter-connected ecosystems: beach, sea, mudflat, river, lagoon, estuary, island, coastal forest and mangroves. In addition, the wetland is not just a unique by itself but is also rich with the vast number of flora and fauna living in it.

Based on Denuncio *et al.* [4], small organisms like invertebrates tend to ingest pollutants such as microplastics, from which they absorb toxic chemicals particularly phthalates [5]. Microplastics are defined as plastic materials less than 5 mm in size and are considered as one of the most hazardous marine debris to marine organisms [6, 7]. The accumulation of microplastic will eventually result in the blockage of the digestive tract and internal abrasions, starvation and physical deterioration that could be fatal to the organism. Ingestion of microplastics could also lead to blockage of enzyme production, nutrient depletion, diminished feeding stimulus and reduced growth rates [8] of some marine species. According to Ivar do Sul *et al.* [9], accumulation of microplastics could be transferred along the food web, from the smallest organisms like plankton to the largest fish. The ingestion of microplastics in vertebrates [10] and even now in scleractinian corals [11] have been described elsewhere, where the presence of microplastics have caused physical changes to their habitat particularly in coral reefs [12]. There are limited studies on the ingestion of microplastics by marine inverterbrates, despite their being commonly known as tools for bio-monitoring programs. Among the studies on the uptake of microplastic were on blue mussels (*Mytilus edulis*) and oysters (*Crassostrea gigas*) [13, 14], as both organisms are filter feeders and suspension feeders; thus, the

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possibility of ingesting microplastics is very high. Microplastics (<1 mm) were found in the tissues and faeces of mussels after being left overnight [14]. These showed that these organisms [15] have mistaken microplastics as natural food source. It also showed that microplastic particles are persistent polymers as these were retained inside the body without being damaged and translocation might also occur. However, the accumulation potential for microplastics, especially through ingestion by tropical marine invertebrates, is yet to be known and recorded. The current study describes the ingestion and accumulation of microplastic in a wild bivalve (*S. cornea)* of Setiu Wetland and their identification by Fourier Transform Infrared Spectroscopy (FTIR).

MATERIALS AND METHODS

Sample Collection: Samples of *Scapharca cornea* were collected in October 2014 from three different sampling stations in Setiu Wetland (station 1: N 05°41'144', E 102°42'629"; station 2: N 05°41'423", E 102°42'258"; and station 3: N 05°41'668", E 102°42'022" (Fig. 1). The coordinates of the sampling stations were determined using Garmin Global Positioning System (GPS) and distances between the stations were kept relatively similar (approximately 625 □ 850 m) to ensure the samples were general representatives of the area. A total of 40 samples of wild *S. cornea* (3.0 □ 4.0 cm in length) were handpicked from each station during low tide. The samples later kept in airtight containers and kept frozen until analyis.

Sample Preparation: Height (mm), length (mm) and width (mm) of *S. cornea* were measured using a vernier caliper (Fig. 2). All tissues of *S. cornea* were removed from its shell by cutting its adductor muscles. The wet tissue was weighed (g) using digital balance [fresh weight] and then dried in the oven (63°C) for 10 hr until completely dried. The dried tissue was weighed [dry weight] and recorded.

Sample Digestion: Microplastics were extracted by alkali digestion technique [16]. Briefly, tissues of *S. cornea* were treated with 10 M NaOH at 60 °C for 24 hrs. The sample was 90% digested after 24 hr treatment. The residues were then filtered twice using 500 µm filter. The residues later were kept in the oven at 60°C for 3 hrs for complete drying and digestion efficiency measurement was calculated.

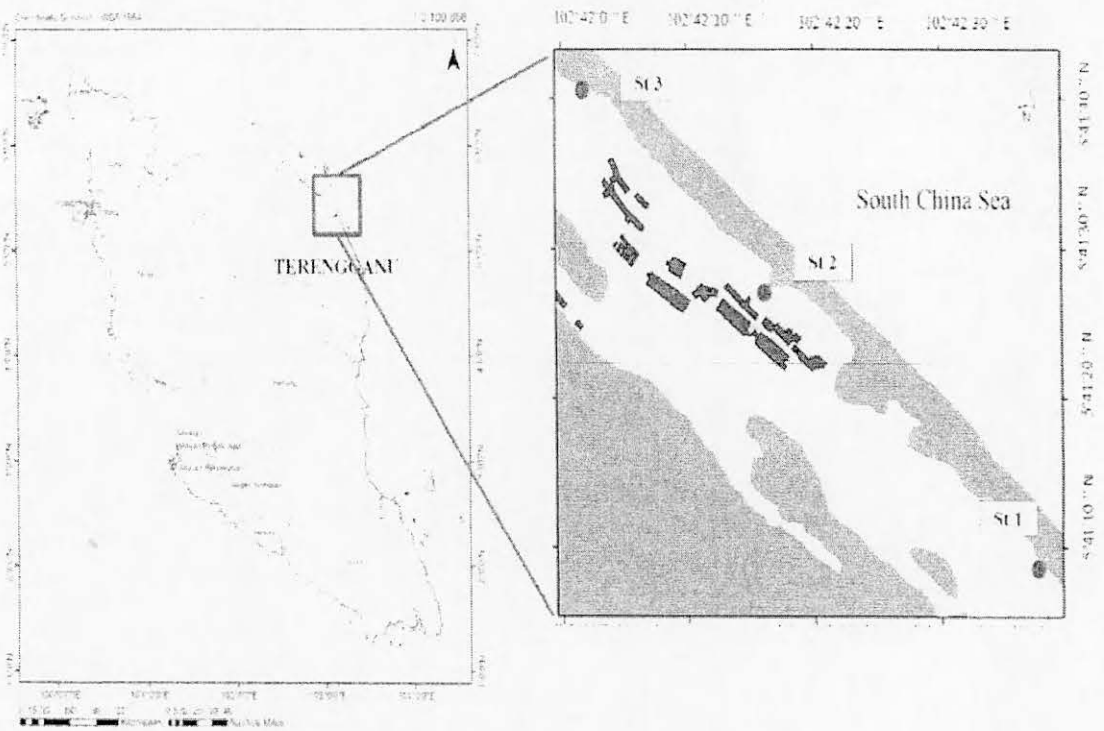

Fig. 1: Geographical location of three sampling sites at Setiu Wetland, Terengganu, Malaysia

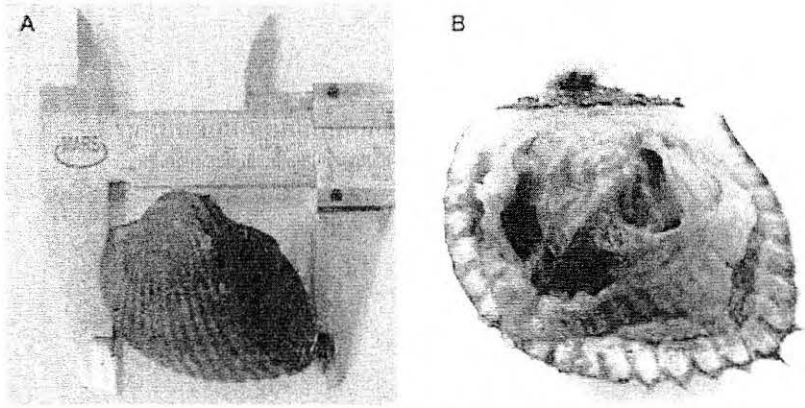

Fig. 2: (A) External and (B) internal morphology of *Scapharca cornea*.

Microplastics Sorting and Identification: Microplastics particles were sorted by observing their physical characteristics (shape, colour and size) using a dissecting microscope (Olympus, SZX7). Images of all microplastic particles found were taken using digital camera (Stylus Olympus camera, VG-165). The microplastics found were put into different solutions (35 ppt saltwater and 0 ppt distilled water) to estimate their density. Isolated microplastics particles were filtered using 0.45 μm cellulose nitrate membrane filter papers (Whatman-0401170) to remove the solution before being further analyzed using Fourier Transform Infrared (FTIR) spectroscope (Perkin Elmer; Spectrum 100) for polymer identification purposes. Microplastics particles were analyzed in the FTIR with the mid-IR range of 4000 □ 400 cm^{-1} and 16 scans per analysis.

RESULTS

Description of Microplastics: Digestion with 10M NaOH was the most efficient in the extraction of microplastics from the tissues of *Scapharca cornea*. Most of the microplastics found were filaments, length ranging from 0.12 to 9.5 mm and were either transparent, blue, green, white, black, orange, red, purple, grey and brown (Fig. 3). Figure 4 shows the color distribution for microplastic particles isolated from the bivalve tissues. Transparent filaments were the most common microplastic particles in the tissues from all three sampling stations, followed by blue- and black-colored filaments.

Density Separation: The density of the microplastic particles were estimated by putting these in two different density solutions at room temperature (35 ppt saltwater and 0 ppt distilled water). Density values are used to tentatively identify the chemical components making up the microplastic. Stations 2 and 3 had similar proportions of microplastics having similar densities as seawater and distilled water, saltwater: 66; distilled water: 62 for station 2 and saltwater: 47; distilled water: 41 for station 3. Station 1 had distinctly more microplastics with density similar to seawater than to distilled water (saltwater: 422; distilled water: 242) (Fig. 5).

Microplastics Quantification Based on the Average Weight of Dry Tissue: Total number of microplastics found in the tissue samples from three stations showed large difference (Fig. 6). The microplastics particles in St. 1 was 557.98 particles/g dry weight tissues; St. 2: 261.22 particles/g dry weight tissues; and St. 3: 86.27 particles/g dry weight tissues. Station 1 had the highest number of microplastics particles (664) which is proportional to the average weight of dry tissues (1.19 g). However, stations 2 and 3 had similar values and were much lower than station 1.

FTIR Analysis: FTIR spectrum of possible microplastic presence in *Scapharca cornea* is shown in Figure 7. The compounds show a strong peak in the region of 3433 cm^{-1}, which may attributes to N-H stretching vibrations of terminal amine groups. The absorption band at 2925 cm^{-1} corresponds to C-H aliphatic stretching modes. This peak give us information of the presence of alkyl chin in microplastic obtained in *S. cornea*, respectively. The band at 1635 cm^{-1} corresponds to the C-N amide stretching vibration, while the transmittance peak at 1420 cm^{-1} matches with the bending of CH_2 vibrations. The absorption band at 1262 cm^{-1} and 1111 cm^{-1} show the CH_2 wagging vibrations in the microplastic sample.

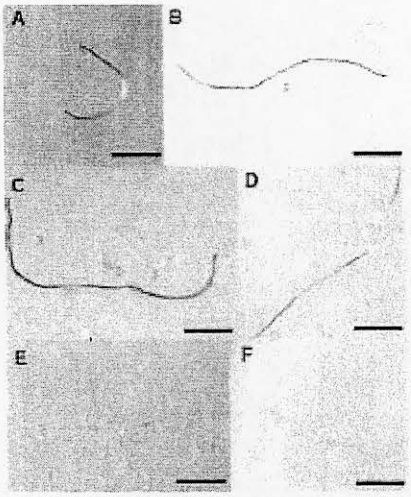

Fig. 3: Microplastics found in the *Scapharca cornea* tissues (A) purple filament (B) brown filament (C) black filament (D) blue filament (E) transparent filament (F) green filament. Scale bars: 0.1 mm (A); 0.2 mm (B, C, D, E); 0.5 mm (F)

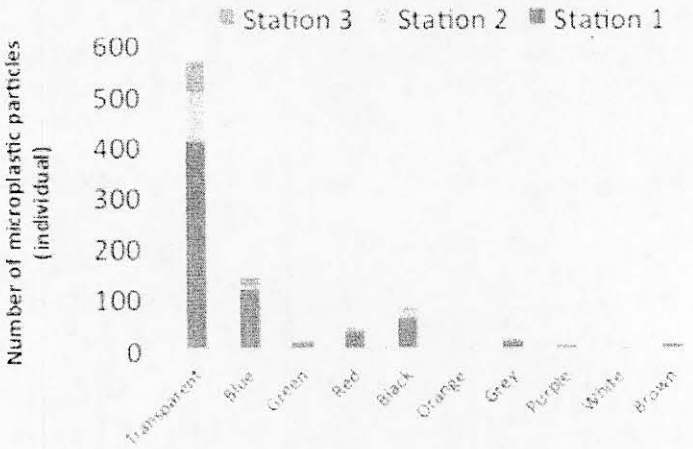

Fig. 4: Amount of microplastics particles found in three stations based on its color

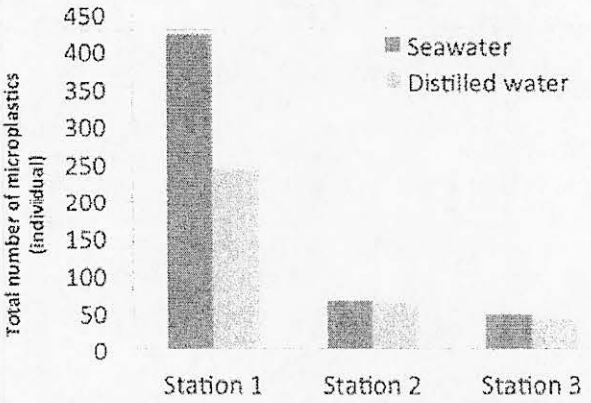

Fig. 5: Proportion of microplastics tested with two different density solutions; saltwater and distilled water

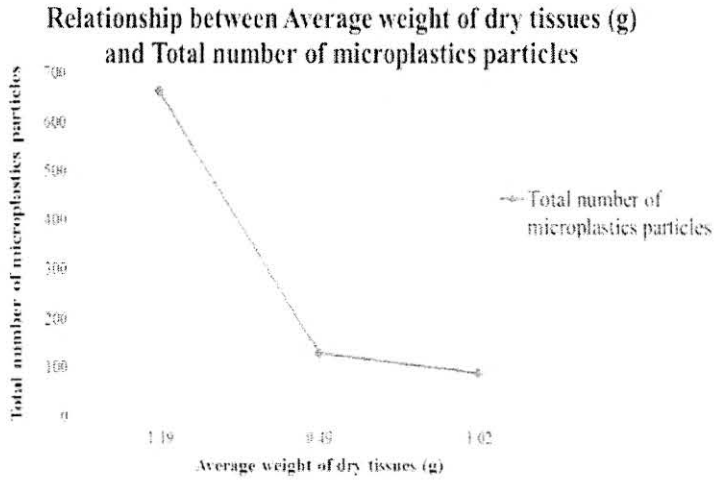

Fig. 6: Relationship between average weight of dry tissues (g) and total numbers of microplastics particles present in the bivalves tissues. (The line segments joining the points are meant as a guide to the eye)

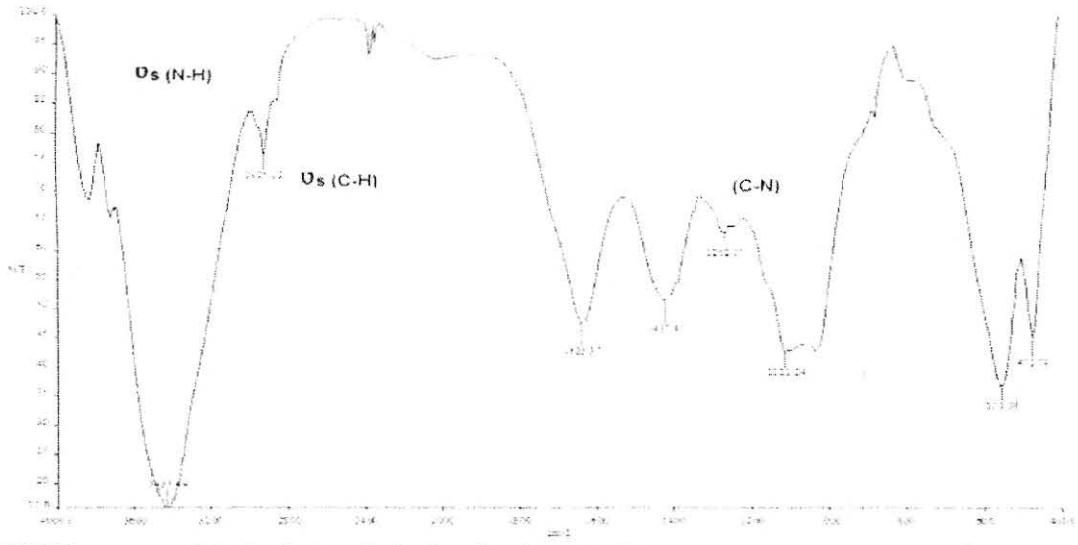

Fig. 7: FTIR spectrum of obtained microplastic from *Scapharca cornea*

DISCUSSION

Microplastics ingestion by the bivalve, *Scapharca cornea* was determined for the first time in a Malaysian wetland, in particular and in the Asian tropics, in general. Results of this study could be predicted through visual analysis as the colour shown means different types of polymer might be present. Based on Figure 3 and 4, there are several types of polymers may existed in *S. cornea* by looking at the various colour of microplastics particles, according to classification made by previous studies [18, 19]. The highest recorded microplastics in bivalve tissue was in the form of transparent filament, however the colour coated or additives do not really determine the types of microplastics [20], as most of the polymers are subjected to aging process that diminished their color, thus giving the transparent or opaque color when ingested by the organisms. Additionally, density separation also could give a greater information in analyzing the microplastics particles [21]. Based on Figure 5, most of the microplastics found in all three stations of Setiu Wetland area shows the specific density in the saltwater solution, compared to the distilled water. According previous studies reported by

Hidalgo-Ruz *et al.* [22], polymers such as polyethylene and polypropelene have density lower than water density, meanwhile polystyrene, polyamide and polyester will have specific densities slightly above the water. Thus this type of microplastics will be accumulated either in high salinity water or distilled water during the density separation. With specific density around 0.91–0.97 g cm^{-3} (low density and high density polyethylene) and 1.02 g cm^{-3} (polyamide) these microplastics can be sorted from other microplastics using this technique [21, 22]. Furthermore, possibility of polyethylene to present is high as it comprised majority of the world polymer production. Most of it produces as food wrapper, plastic bags, drinking straws, balloons, packaging of frozen food and many more [20, 21].

In this study, microplastics quantification based on the average dry tissue is really important (Fig. 6). It could be used as a prediction of the amount of microplastics particles in one bivalve corresponding to human body. Based on the figure, total number of microplastics found in the samples shown a huge gap between them as St. 1: 557.98 particles/g dry weight tissues; St. 2: 261.20 particles/g dry weight tissues; and St. 3: 86.27 particles/g dry weight tissues whereby the finding of microplastics particles resembled the existence of microplastics in the three sampling stations. The quantifications of microplastics collected shown are decreasing drastically. This might happened because of tides or affected by winds or water movements. In station 1, the location of *S. cornea* is really suitable with the sufficient amount of foods and less water movement. It seems that microplastics particles may retained for a long period in the location. Meanwhile in station 2, the location is unfavourable where the water movement was very fast due to the tides coming in. During sampling was done at station 3, the tides were slowly settled down. This might be the reason on why the amount of microplastics is differed between each of the stations. On the other hand, the quantification of microplastics for overall sampling stations was considered high. There are several factors where they have been identified to be a highly potential for contributing into the plastics contaminant. As facing in front of the Setiu wetland is a South China Sea. This is a passage where majority of ships and boats are entering the wetland. It is a place for fishermen busying with fishing and collected marine invertebrates such as bivalves, crabs and shrimps. Moreover, there are agricultural activities that are handling by local people in order to fulfill the demand of customers like oysters and groupers. Even though they are being cultured, the organisms are left freely in the cages with the flowing water moving in and out. This shows that the wetland is a busy place with the activities of fishermen coming in and out. Thus, the probability for fishing gears or nets to be dumped or abandon in waterways are higher. These fishing gears and nets would be degraded and eventually settled down on the bottom floor. Furthermore, the existence of placements by local people besides of estuary has contributed a large volume of microplastics wastes particularly from washing cloth. Microfiber extracted from the cloths are likely to be ingested by marine organisms especially invertebrates. The smaller the size of microplastics, the higher the chance for organisms to ingest it. These explained why there are so many similar types of polymer (filament type) found in the organisms.

Collected microplastic suspensions that were further identified by FTIR spectroscopy analysis suggested that the micropalstic found in the bivalve tissue in this study are mostly polyethylene and polyamide origins (Fig. 7). This can be seen by the strong absorption band of N-H stretching for terminal amine group that corresponds with 3433.49 cm^{-1} and C-H aliphatic stretching modes correspond with 2925.22 cm^{-1} in the tested microplastics particles, which possibly presenting the polyamide (nylon) polymer [22]. The C-N amide stretching of polyamide is presented by the peak at 1635.37 cm^{-1} that comes together with the bending of CH_2 vibrations peak at 1420 cm^{-1}; while the absorption band at 1262.07 cm^{-1} and 1111.24 cm^{-1} of C-H_2 wagging vibrations give us information about the crystalline and amorphous structures of the microplastic compounds. Polyamides are mainly produce for nylon substances, cloths and fishing gear like nets and ropes [21, 23].

CONCLUSION

To date, our understanding on microplastics regarding the accumulation, dynamics of transport and associated distribution has been extremely limited in Malaysia especially on their path and fate in the food web. The results of the microplastics particles found are widely distributed in the wetland, where both polyethylene and polyamide from nylon are suspected to be the most abundance. The higher number of it is leading to concern as they consist of potential impact on environmental health. This is due to the ability of microplastics to absorb and desorb persistent organic pollutants (POPs) from the surrounding. Thus, with the

higher numbers of microplastics present in the *S. cornea* shows that the level of contamination in-situ is a bit high. Therefore, actions should have been taken in measured in order to minimize the levels of contamination from being worst.

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