Science of the Total Environment 786 (2021) 147466

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Microplastics in seawater and zooplankton: A case study from Terengganu estuary and offshore waters, Malaysia

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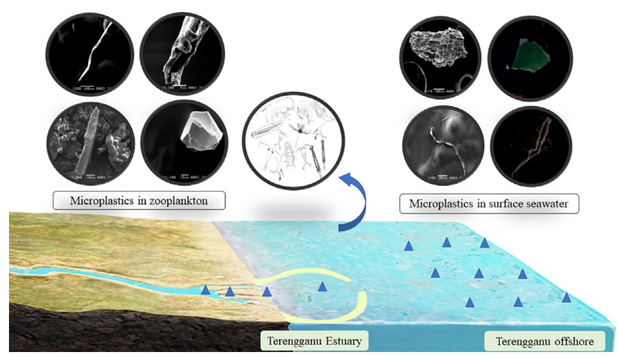
HIGHLIGHTS

- Microplastic abundance in seawater increased in estuary with increasing human impact.
- The majority of microplastics detected in seawater and zooplankton were fibres.
- Polymers found in surface seawater were identified as polyamide, polyethylene, and polypropylene.
- Zooplankton ingested an average of 0.104 particles ind.$^{-1}$ of microplastics.
- There is no significant relationship between microplastic ingestion and microplastic concentration in surface water.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 9 January 2021
Received in revised form 22 April 2021
Accepted 27 April 2021
Available online 1 May 2021

Editor: Dimitra A Lambropoulou

Keywords:
Estuary
Ingestion
Microplastics
Offshore
South China Sea
Zooplankton

ABSTRACT

Widespread accumulation and distribution of microplastics at the sea surface raise concerns as the habitat is a feeding ground for zooplankton. As primary consumers, these organisms are closely connected to microplastic input in the marine food chain. Little comparative information currently exists about this problem in estuary and offshore systems. This study investigates microplastic distribution in the surface water and the potential ingestion of microplastics in selected taxonomic groups of zooplankton from the Terengganu Estuary to offshore waters, Malaysia. In the surface water, three types of microplastics were found (fibres, fragments and pellets). Fibres made up the highest percentage, comprising 80.8% and 73.8% of microplastics in offshore waters and estuaries, respectively. The highest total density of microplastics was found in the Terengganu Estuary (545.8 particles m^{-3}). Microplastics sampled from the offshore waters were identified as polyamide, polyethylene, and polypropylene, which possibly originated from secondary microplastic sources. Two types of microplastics were detected in zooplankton: fibres and fragments. Fibres were the most commonly ingested microplastic type in zooplankton collected from offshore waters (94%) and estuaries (77.7%). The average sizes of ingested fibres and fragments were 361.7 ± 226.8 μm and 96.8 ± 28.1 μm, respectively, with a wider range of sizes ingested observed in offshore waters than in estuaries. The concentration of microplastics in seven zooplankton groups varied from 0.01 ± 0.002 particles ind.$^{-1}$ (Harpacticoida) to 0.2 ± 0.14 particles ind.$^{-1}$ (Aphragmophora). Notwithstanding the conformity of our results (increased anthropogenic activities led to greater plastic pollution within the estuary), no significant correlation was observed between the levels of microplastic ingestion and

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https://doi.org/10.1016/j.scitotenv.2021.147466

microplastic concentration in the surface water within both areas. Our results provide an important baseline reference on microplastic pollution from estuary to offshore waters, as well as proving that zooplankton act as a repository for microplastic in the marine ecosystem.

1. Introduction

Plastics are by far the most numerically abundant marine debris (Napper and Thompson, 2020; OSPAR, 2007; Thompson et al., 2009) and account for about 60–80% of marine waste and 90% of floating particles in the marine environment (Anjana et al., 2020; Andrady, 2011). In 2010 alone, 4.8–12.7 million tonnes of plastic were released into the ocean. This amount is predicted to increase by an order of a magnitude by 2025 (Jambeck et al., 2015). Asia has the most densely populated coasts in the world and will continue to be the most populated in the future (Nazarnia et al., 2020). These coasts, including estuarine areas, are affected by tropical cyclones, floods, accretion/erosion processes, and shifting water flow and levels, which contribute to releasing pollutants in estuaries and coastal area and dispersing them to points offshore (Cham et al., 2020). In Malaysia, plastics make up the highest proportion (24%) of the total municipal solid waste composition (Liang et al., 2021), with a 2–3% increase per year due to a growing population, changes in consumption patterns, and expansion of trade and industry in urban centres (Ibrahim and Noordin, 2020). Terengganu, Kelantan, and Pahang, the eastern states of Peninsular Malaysia, generate 0.71 kg/capita/day, with Terengganu recording the highest waste production among these states (Badgie et al., 2012; Ghani et al., 2020).

Initial studies have demonstrated an abundance of microplastic pollution in estuarine, coastal, and offshore environments (Hajbane and Pattiaratchi, 2017; Moore et al., 2011; Rodrigues et al., 2020). Estuarine and coastal ecosystems exposed to multiple sources of plastic debris are likely most at risk due to the high abundance of microplastics (Auta et al., 2017; Cole et al., 2011). Proximity to particular activities such as wastewater treatment, industry, agriculture, and urban areas appears to be important factors in determining the extent of microplastic pollution (Yonkos et al., 2014). The increase in marine plastic has been found to have a significant negative impact on the function of all marine ecosystems (Beaumont et al., 2019; Bidegain and Paul-Pont, 2018). Well-known impacts include ingestion (Liboiron et al., 2018), chemical contamination (Karami et al., 2016), entanglement (Zhao et al., 2016), and dispersal of invasive species (Kirstein et al., 2016).

Plastic debris can be defined and described by shape (spheres, beads, pellets, foams, fibres, fragments, films, and flakes; Napper and Thompson, 2020; Zhang et al., 2017), original usage, origin, polymer type, size, and colour (Napper and Thompson, 2020). Size is the most reported descriptor, as this includes all plastic types. The three categories that are typically used to describe the size of plastic pollutants are macroplastic (>20 mm), mesoplastic (5–20 mm), and microplastic (<5 mm; Anjana et al., 2020; Mbachu et al., 2020). There are two main classifications based on the source of the microplastic: primary and secondary. Primary microplastics directly enter the environment in a microscopic size (<5 mm; e.g., in cleaning products, in cosmetics, and as air-blasting media) while secondary microplastics result from the breakdown of larger plastic items (e.g., tyre wear or fibres from clothing; Anjana et al., 2020; Campanale et al., 2020; Caputo et al., 2021).

Zooplankton (0.2–20 mm) that live suspended in the water column are the most abundant and widespread organisms on Earth, and they typically feed in the microplastic size range (Telesh and Khlebovich, 2010). Several studies including one recent study in Malaysia showed that zooplankton of varying taxa and sizes could ingest different types of microplastics (Desforges et al., 2015; Md Amin et al., 2020; Sun et al., 2018a, 2018b). Microplastic ingestion affects the zooplanktons' growth and reproduction as microplastics are indigestible and aggregate within their digestive system, ultimately affecting their intracellular activities (Cole et al., 2019; Renzi et al., 2019; Wang et al., 2019). Also, due to zooplanktons' vital position as a link between two trophic levels (Laurenceau-Cornec et al., 2015), microplastics can be moved up the food chain and cause adverse effects in other marine animals (Cole et al., 2019).

One of the major pathways for plastics entering the oceans is by rivers and estuaries (Rech et al., 2014). Currently, little is known about the differences in microplastics ingested by zooplankton in estuaries or in the ocean. Thus, the present study aims to investigate microplastic ingestion by zooplankton using a similar method as Md Amin et al. (2020), but extending from the Terengganu Estuary to the offshore waters of the north-eastern coast of Peninsular Malaysia. This is the first study to shed light on microplastic presence from the estuary to offshore waters in Malaysia and clarifies the ingestion incidence of particles between different taxonomic groups of zooplankton. This project assumed that different zooplankton taxa ingest various microplastic shapes and sizes in these two areas, with microplastic ingestion expected to be highest at the Terengganu River Estuary compared to the South China Sea due to anthropogenic activities such as tourism, restaurants, fishing activities, and construction works surrounding the estuarine area. Therefore, the present study aims to answer several crucial questions regarding the non-seasonal differences between the Terengganu River Estuary and South China Sea (Terengganu waters), namely: (i) determining the density of microplastics in water; (ii) investigating the differences in microplastic ingestion by different zooplankton groups; (iii) determining the relationships between ingested microplastics with those found in water columns.

2. Materials and methods

2.1. Sampling and study area

Sampling was conducted in two areas. One is a shallow shelf area with a water depth of less than 80 m (Table 1). This shallow shelf is located south of the South China Sea, on the north-eastern coast of Peninsular Malaysia, in the coastal water of Terengganu. The second area includes estuaries (3.5–9 m depth) located in the Terengganu province, Kuala Terengganu which flow into the southern front of the South China Sea. Several anthropogenic activities such as restaurants, tourism, small-scale seafood processing industries, recreation, sand mining, boat manufacturing, and construction surround this area.

In the present study, samples were collected from these two areas between 28 August 2018 and 15 September 2018 on board the Universiti Malaysia Terengganu's RV Discovery. The offshore area consisted of nine stations denoted as North: C2, C4, C6; Middle: B1, B3, B5; and South: A1, A3, A5; Fig. 1(a), while the Terengganu Estuary area consisted of four stations (ST1, ST2, ST4, ST6; Fig. 1(b)). The distance from the coastline was estimated to be the closest at station A1 and farthest at station C6 (Table 1). For this study's purpose, we adopted the definition from Ferreira et al. (2014) to identify offshore as an area with considerable exposure to wind and wave action.

Details on the procedure from sampling to data analysis are summarized as in Fig. 2. At each station, samples were collected to determine microplastic levels in seawater and in zooplankton, with one replicate each. Microplastics from seawater samples were collected using a mobile water pump (pump rate calibrated to 2.041 L sec^{-1}) at

Table 1
Stations in offshore and estuary, coordinates of each sampling station, actual depth (m), distance of the offshore stations from the coast (km) and from estuary (km).

Location	Stations	Long (°)	Lat (°)	Actual depth (m)	Distance from coast (km)	Distance from estuary (km)
Offshore	A1	103.49639	5.52389	36	57	44
	A3	103.74722	5.52389	60	85	70
	A5	104.25944	5.52389	62.8	140	125
	B1	103.37083	5.775	37.8	80	94
	B3	103.62167	5.775	56.1	108	71
	B5	103.87278	5.775	62	135	54
	C2	103.49639	6.02611	51.5	117	85
	C4	103.74722	6.02611	58	145	100
	C6	104.25944	6.02611	66	202	145
Estuary	ST1	103.14873	5.3434	6	0.2	–
	ST2	103.13348	5.33909	9	0.2	–
	ST4	103.12984	5.33121	4	0.2	–
	ST6	103.11726	5.32416	3.5	0.2	–

approximately 1–2 m below the surface water level for ~10 min of pumping and filtered through a 20 μm mesh sized net. Samples were then stored in glass bottles and cooled at 4 °C for further laboratory analysis. For zooplankton, samples were collected using a similar approach but filtered through a 200 μm mesh sized net at each station. Filtered water volume was calculated by multiplying the pump rate and pumping period. All water samples were kept inside glass sample bottles and preserved with 4% formaldehyde for laboratory analysis.

2.2. Microplastic analysis in seawater

As a precautionary measure against microplastic contamination during analysis, all equipment used was first cleaned and washed several times with deionised water. Then, water samples were filtered through glass fibre filters (GF/F 0.7 μm 47 mm Ø [Whatman, Kent, UK]). Each filter paper was placed into a glass Petri dish and covered with aluminium foil to avoid further contamination (Khalik et al., 2018; Md Amin et al., 2020). Samples were dried in an isolated glass container at room temperature for 24 h before sorting.

Samples were later analysed under a dissecting microscope (Olympus SZX7, Japan, 8×–56× magnification). Identification was made under a dissecting microscope covered with a modified glass box to isolate the samples from the foreign environment. Microplastic particle size was measured and classified based on the shape using a microscope eyepiece camera (Dyno eye AM4023X, 2.0 software) and a scanning electron microscope (SEM). Microplastic particles were stored in distilled water in 200 mL glass vials (Van Cauwenberghe et al., 2015) for further polymer analysis using a Fourier Transform Infrared microscope (μFTIR; LUMOS, Bruker Optics, Germany). Throughout the analysis, four control filter papers were left open during the preparation and analysis stages to ensure no airborne contamination occurred.

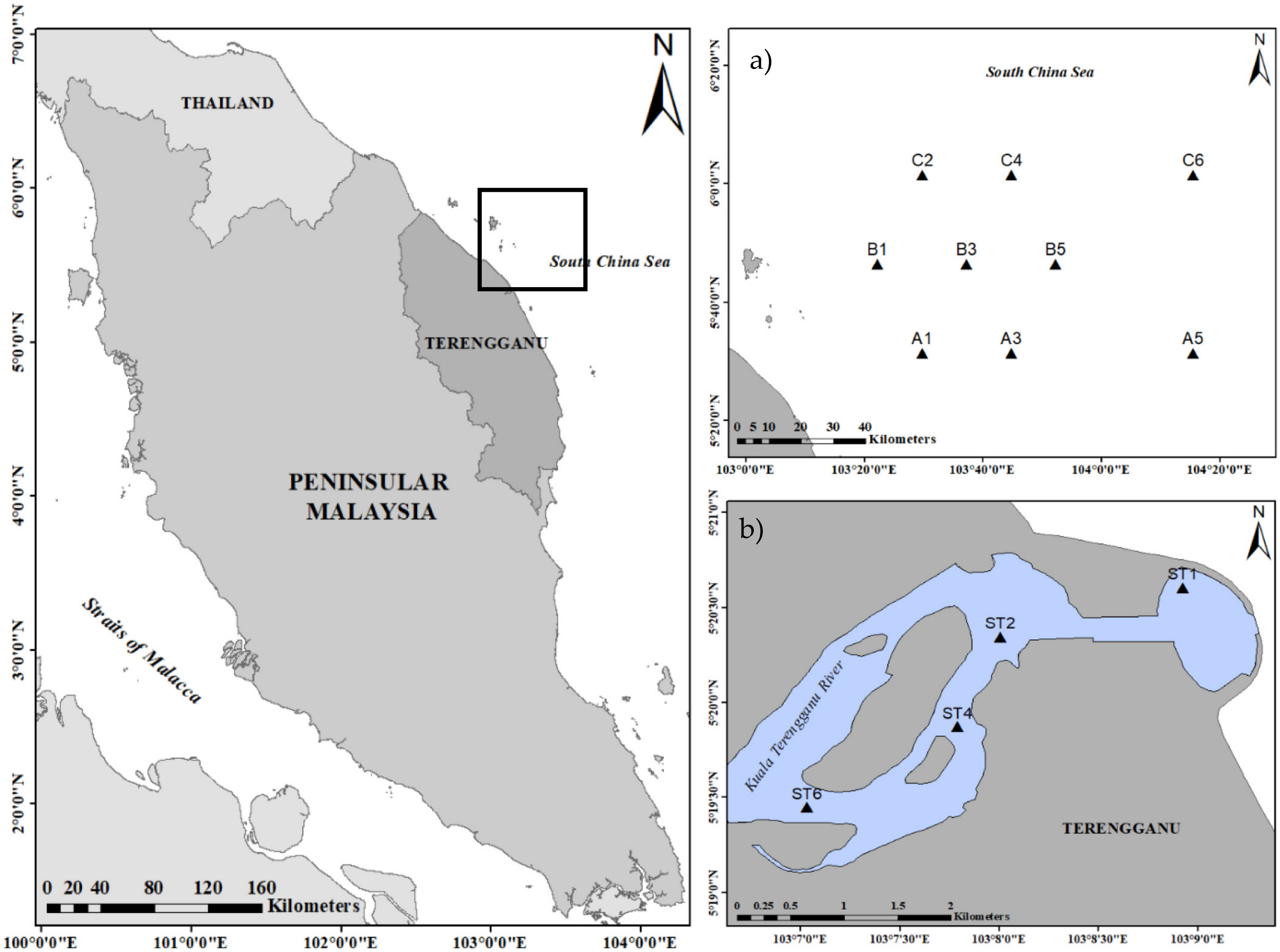


Fig. 1. Zooplankton and microplastic samples were collected from (a) three transects consisting of nine stations at the Terengganu offshore, and (b) four stations at the Terengganu Estuary. Box indicates the location of the study area.

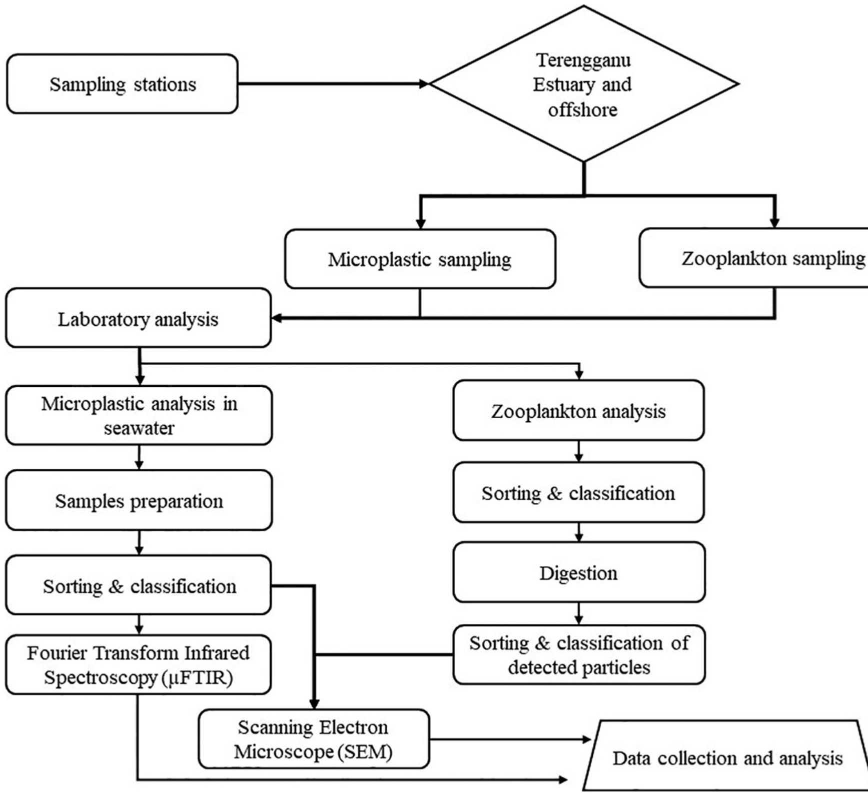


Fig. 2. Flowchart of the experimental design.

2.3. Microplastic characterization using Fourier transform infrared spectroscopy

Confirmation of the functional groups of the microplastic polymers was performed using μFTIR under attenuated total reflectance (ATR) single mode analysis. We were only able to examine offshore samples because the glass Petri dishes containing the estuary samples broke while shipping. Therefore, no polymer analysis was conducted for samples from the estuaries. This spectroscopic method is the primary method for polymer identification, owing to its accuracy for particle sizes less than 1 mm and being non-destructive for the sample (Abdullah and Ibrahim, 2016; Shim et al., 2017). In our case, particles (microfiber) larger than 100 μm in diameter were used for identification due to their higher in number and suitable size for IR analysis. The spectrum was recorded in the mid-FTIR spectral range of 4000–500 cm^{-1}, where the compound was identified based on functional group and fingerprinting. The FTIR spectra obtained during this study were compared with ATR-Complete Spectral Library (Bruker) with at least 75% matching index and also with previous work by Sun et al. (2017), Jung et al. (2018), and Tofa et al. (2019).

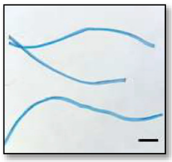
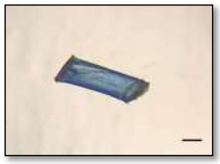
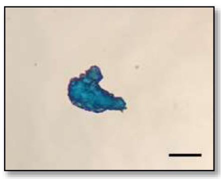
2.4. Microplastic analysis in zooplankton

In order to prevent airborne contamination, all analyses were done in an isolated environment. The working area was covered with a modified glass box to prevent any possible contamination. Samples were analysed and identified under a dissecting microscope (Olympus cx21, Japan 8×–56× magnification) equipped with a microscopy eyepiece camera (Dyno eye; Dino Capture 2.0 software). All zooplankton (≥200 μm) were individually measured and sorted using a loop and needle and classified manually at the order level according to Al-Yamani et al. (2011), Conway (2012), and Boxshall et al. (2017). Zooplankton group densities were calculated by dividing the number of each zooplankton group in each sample by the volume of filtered seawater.

Microplastic analysis in zooplankton was done by sieving selected taxonomic groups at the order level through a 20 μm sieve and checking individuals for external microplastic particles using a dissecting microscope (Olympus SZX7, Japan 8×–56× magnification). Any external microplastics were removed using tweezers. After that, 15–20 individuals from each taxonomic group were digested by putting them in a glass cavity block (4 cm × 4 cm, 3 cm diameter) and filled with 65% nitric acid (HNO_3) until samples were submerged (0.02–0.1 mL; Md Amin et al., 2020). Samples were then covered and incubated in a water bath (80 °C for 30 min) until samples were fully digested (Desforges et al., 2015; Md Amin et al., 2020; Sun et al., 2017). Desforges et al. (2015) tested different protocols to measure microplastic in zooplankton and mentioned the possible effects of nitric acid on microplastic particles after digestion. Therefore, preliminary pre- and post-digestion evaluations using the same method as above were performed on nylon and polyethylene to justify further the results. The preliminary experimental results showed no morphological changes in microplastics, except colour (Table 2).

After zooplankton digestion, each cavity block was carefully examined under a dissecting microscope (Olympus SZX7, Japan, 8×–56× magnification). Samples were further verified under a compound light microscope (Olympus cx21, Japan, 100× magnification) to determine microplastic particle presence. Microplastics that were detected were counted and measured. The particles found were sorted into two categories according to shape: fibres or fragments. The ingested microplastic particles were carefully deposited into 20 mL glass scintillation vials containing ultrapure water for further analysis using an SEM. Due to small number of microplastics detected in this study for polymer analysis using the FTIR, the SEM (model: JSM-6610LV) was used to verify microplastics by their morphological characteristics, specifically a lack of visible cellular or organic structures (Wang et al., 2017). To detect any microplastic contamination, four cavity blocks containing HNO_3 were placed during work stages to detect microplastic contamination around the work area. The microplastic ingestion incidence in

Table 2
Paired images showing examples of plastic particles. (a) A fraction of fishing net; nylon and (b) microbead from face cleansing product, Garnier, containing polyethylene; before and after digestion in HNO_3 incubated in water bath at approximately 80 °C for 30 min. [scale bar = 200 μm].

Source	Before digestion	After digestion
 A. Nylon		
Cleansing product (Brand: Garnier) B. Polyethylene		

each zooplankton group at each station was determined by dividing the number of particles found in zooplankton by the number of digested zooplankton (Md Amin et al., 2020). Finally, to infer the potential intake of plastic in zooplankton community, the amount of microplastics ingested by predominant groups per cubic meter was estimated by multiplying the zooplankton density (no. of zooplankton/m^3 seawater) by the ingested microplastic concentration (no. of particles/zooplankton).

2.5. Scanning electron microscopy analysis

The surface morphology of ingested microplastics and in seawater was determined using an SEM (JOEL JSM-6360LA, Massachusetts, USA). Samples were prepared by mounting particles on a stainless-steel stub. The mounted samples were coated with a thin layer of gold before being placed into a holder and transferred into the SEM chamber. The SEM was operated at 10 kV with magnifications of 140× and 4300×.

2.6. Statistical analysis

All data analysis was conducted in SPSS 24.0. Nonparametric one-way ANOVAs (Kruskal-Wallis tests; KW) was used to compare microplastic densities and ingested particles between the Terengganu Estuary and offshore waters. The Spearman correlation coefficient was used to examine relationships between ingested microplastic and zooplankton size and between ingested microplastic density and microplastic density in water. This nonparametric test was conducted after the invalidation of parametric assumptions. Plots were created using Microsoft Excel 2016 and ArcGIS 10.3.

3. Results

3.1. Microplastics in seawater

Of the total microplastics extracted (1687 particles m^{-3} and 1900 particles m^{-3} in estuary and offshore, respectively), the majority were fibres (73.8% and 80.8%), followed by fragments (22% and 18.6%) and pellets (4.2% and 0.17%; Fig. 3a).

In offshore water, Station C4 at the north recorded the highest density for fibres (300.4 particles m^{-3}), while fragments were highest at station B5 (163.3 particles m^{-3}; Fig. 3b). In the estuary, ST1 recorded the highest density for fibres (387.5 particles m^{-3}) and ST2 the highest density for fragments (129 particles m^{-3}). Pellets were found only at stations A5 (13.064 particles m^{-3}), ST1 (45.8 particles m^{-3}), and ST4 (25.0 particles m^{-3}). Our results indicate that fibre and fragment densities in the Terengganu Estuary were significantly higher than those in offshore areas (KW, H = 4.68 and H = 4.06, for fibre and fragment, respectively, $p < 0.05$), although this was determined from a limited number of samples and only one sampling campaign, while no differences were observed for pellets (KW, H = 1.5, $p > 0.05$).

3.2. Polymer identification of microplastic samples

In this study, the μFTIR spectra of fibre microplastic of sizes >100 μm showed primarily nylon, polyethylene, and polypropylene in Terengganu offshore waters (Fig. 4). Functionalities and important vibrational characteristics of the polymers identified are presented in Table 3. The functional group of nylon (polyamide polymer) was represented in a sharp 3350 cm^{-1} for N—H bending, followed by bending at 1450 cm^{-1} (Fig. 4a). Another polyamide band characteristic is attributed to strong C—N and C═O carbonyl stretches at the 1100 cm^{-1} and 1750 cm^{-1} wavelengths, respectively. In contrast, the polyethylene and polypropylene polymers FTIR spectra (Fig. 4b and c) recorded all similar major peaks related to CH stretching (2890–2995 cm^{-1}), CH_2 bending (1480–1510 cm^{-1}), and CH_2 rocking (700 and 880 cm^{-1}; Table 3). Additionally, the bending and rocking for CH_3 of polypropylene can be attributed to the vibration bands at 1380 cm^{-1} and 1100–1200 cm^{-1}, where they overlapped with C—H bending and C—C stretching.

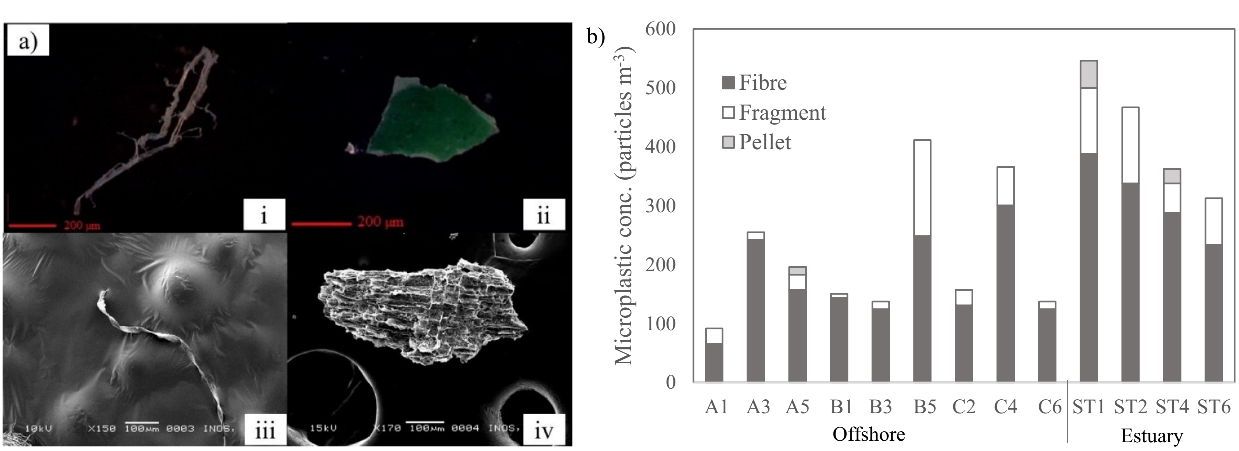


Fig. 3. (a) Examples of microplastics found in seawater under stereomicroscope; (i) fibre, (ii) fragment and scanning electron microscope (SEM); (iii) fibre and (iv) fragment. Note that examples are from various samples. (b) Total microplastic concentration for nine and four stations in the surface water of offshore and estuary, respectively.

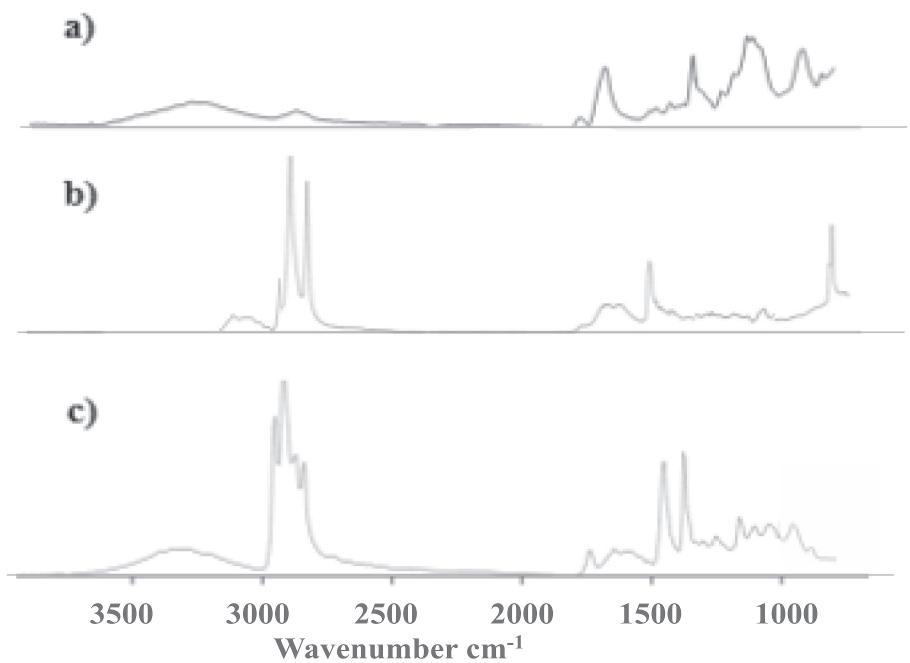


Fig. 4. Spectra produced from microplastic particles consisting of (a) nylon (polyamide), (b) polyethylene, and (c) polypropylene identified by ATR-FTIR.

3.3. Microplastic in zooplankton

Individual zooplankton identified belonged primarily to Arthropoda, representing up to 98.1% of the total zooplankton studied. Six orders of Arthropoda were identified: Calanoida (44.2%), Cyclopoida (24.0%), Harpacticoida (7.2%), Mysids (2.5%), Decapoda (1.6%), and Cladocera (18.6%). One order of Chaetognatha, Aphragmophora, represented 0.2% of the total samples analysed. In the offshore waters, station B3 recorded the highest total density of zooplankton individuals (291.2 ind. m^{-3}) while the lowest was at the farthest south station, A5 (50.6 ind. m^{-3}). Meanwhile, the highest total density of zooplankton in the Terengganu Estuary was recorded in station ST1 (812.5 ind. m^{-3}) and the lowest total density was recorded in station ST6 (262.5 ind. m^{-3}; Table 4).

Two types of microplastics—fibres and fragments—were extracted from zooplankton at all sampling stations (Fig. 5a). Fibres and fragments represent 94% and 6%, respectively of the total ingested microplastics in offshore waters and 78% and 22%, respectively from the Terengganu Estuary (Table 4). The typical sizes of fragments and fibres ranged from 68.0–144.0 μm and 400.1–500 μm, respectively in offshore waters and from 90.0–92.0 μm and 80.0–99.0 μm, respectively in the Terengganu Estuary (Fig. 6a, b). No clear relationship was observed between zooplankton size and ingested microplastic size in the study area (Spearman correlation: $r^2 = 0.355$, p-value $= 0.175$).

The average concentration of ingested microplastics varied with different zooplankton taxa, ranging from 0.01 ± 0.002 particles ind.$^{-1}$ (Harpacticoida) to 0.20 ± 0.14 particles ind.$^{-1}$ (Aphragmophora; Fig. 5b). In the Terengganu offshore waters, microplastics were mainly accumulated in Calanoida, followed by Cladocera, Cyclopoida, Aphragmophora, Harpacticoida, Mysida and Decapoda, at 47%, 2%, 11%, 9%, 6%, 2%and 1%, respectively. For the Terengganu Estuary, microplastics were mainly accumulated in Calanoida, Cladocera, and Mysida, at 54%, 26%, and 20%, respectively, whereas no microplastic was detected in Cyclopoida and Harpacticoida (Fig. 7a). The estimated microplastic ingested per cubic meter from offshore and estuarine waters varied depending on the zooplankton density, which was highest at Stations A3 and ST2 (Table 4). Stations B5 and ST6 recorded a lower abundance from offshore waters and estuaries, respectively. However, no significant difference was found between the estuarine and offshore areas (KW, H = 5, $p > 0.05$).

The total microplastics ingested in the zooplankton community in offshore water were highest in Calanoida (16 particles m^{-3}), followed by Cyclopoida (8.7 particles m^{-3}), Mysid and Cladocera (1.6 particles m^{-3}), Harpacticoida and Aphragmophora (0.11 particles m^{-3}), and Decapoda (0.5 particles m^{-3}), while the total microplastics ingested were highest in Calanoida (20.8 particles m^{-3}), followed by Cladocera (4.2 particles m^{-3}), and Mysid (4.1 particles m^{-3}) in the estuary water (Fig. 7b).

The microplastics concentration in seawater in relation to the abundance of most common zooplankton varied between locations. The ratio was between 0.5:1 to 4.5:1 in offshore stations and between 0.8:1 to 1.4:1 in the estuary stations (Fig. 8). No significant correlation was observed between microplastic density in water and ingested microplastic in the study area (Spearman correlation: $r^2 = 0.235$, p-value $= 0.421$).

Table 3
IR assignments and vibrational characteristics of microplastic polymers using μFTIR (ATR mode).

Spectrum	Wavenumber (cm^{-1})	Functional groups	Polymer
A	3350	N—H stretching	Polyamide
	1450	N—H bending	
	2800–2995	C—H stretching	
	1400	CH_2 bending	
	1750	C=O stretching	
	1100	C—N stretching	
B	**3200**	O—H (*weathering*)	Polyethylene
	2920, 2830	C—H stretching	
	1510, 1480	CH_2 bending	
	1720	C=O (*weathering*)	
	700	CH_2 rocking	
C	**3200**	O—H (*weathering*)	Polypropylene
	2800–2980	C—H stretching	
	1720	C=O (*weathering*)	
	1490	CH_2 bending	
	1380	CH_3 bending	
	1100–1200	C—H bending, CH_3 rocking, C—C stretching	
	880	C—H bending, CH_2 rocking, C—C stretching	

Table 4
Details on the total number of individuals per station, ingestion incidence, total zooplankton density, relative proportion of particle types and ingestion incidence in zooplankton community from both study locations.

Location	Station	No. of ind.	Ingestion incidence (particles ind^{-1})	Zoo. Density (ind. m^{-3})	Fibre (%)	Fragment (%)	Ingestion in zoo. community (particles m^{-3})
Offshore	A1	326	0.015	177.5	80.0	20.0	2.72
	A3	435	0.028	236.8	100.0	–	6.53
	A5	93	0.043	50.6	100.0	–	2.18
	B1	408	0.022	222.1	100.0	–	4.90
	B3	535	0.019	291.3	77.8	22.2	5.44
	B5	242	0.012	131.7	100.0	–	1.63
	C2	108	0.028	58.8	100.0	–	1.63
	C4	148	0.000	80.6	–	–	0.00
	C6	217	0.023	118.1	100.0	–	2.72
Estuary	ST1	195	0.015	812.5	66.7	33.3	12.50
	ST2	108	0.019	450.0	100.0	–	8.33
	ST4	82	0.037	341.7	66.7	33.3	12.50
	ST6	63	0.016	262.5	100.0	–	4.17

4. Discussion

4.1. Microplastics in seawater

This is the first study to report differences of microplastic abundance between estuary and offshore water in Malaysia. A higher microplastic density in the Terengganu Estuary (421.8 ± 110 particles m^{-3}) compared to offshore waters (211.2 ± 104 particles m^{-3}) was observed, with fibres making up the highest percentage in the study area. The presence of microplastics in all samples collected is in accordance to those reported in three Australian estuaries (98–1032 particles m^{-3}; Hitchcock and Mitrovic, 2019) and the Yangtze Estuary (67.5 particles m^{-3}; Li et al., 2020) but far exceeds the reported microplastic pollution in a Portuguese estuary (0.45 ± 0.52 particles m^{-3}; Rodrigues et al., 2020), the English Channel (0–1.5 particles m^{-3}; Maes et al., 2017), the Gulf of Mexico (4.8–8.2 particles m^{-3}; Di Mauro et al., 2017), or Indonesian waters (0.04–0.9 particles m^{-3}; Germanov et al., 2019). Conversely, the concentration of microplastics in the present study was more than one order of magnitude lower than those recorded in polluted estuaries in the USA, Germany, and China (>2000 particles m^{-3}; Gray et al., 2018; Stolte et al., 2015; Yan et al., 2019). Similarly, this abundance here did not exceed 9×10^3 particles m^{-3}, which is the highest record in the Terengganu coastal water by Md Amin et al. (2020). Comparisons between studies however, remain a challenge and must be carried out carefully as no standard protocol has been previously highlighted. Spatial and temporal variations of local environmental conditions, anthropogenic pressure levels, units inconsistency and methodologies used can vary between studies (Li et al., 2020). One should be noted that insufficient sampling volume (<20 L) may lead to vague results (Tamminga et al., 2018; Li et al., 2020). There is a clear pattern in decreased abundance values against increased sampling volume and mesh size (McEachern et al., 2019). In this study, we have taken a large volume of samples (>1000 L) using smaller mesh size (20 μm) to reduce the variations adequately.

In addition, studies comparing between estuaries and offshore waters are scarce. Zhao et al. (2014) reported that the density of microplastics in the Yangtze Estuary was much higher than in the East China Sea. Likewise, in Australian waters, the concentrations of plastic pollution were higher in estuary samples than in nearshore and offshore samples, and the results varied temporally (Hajbane and Pattiaratchi, 2017). The surrounding environment, which can be influenced by the intensity of anthropogenic activities, is expected to relate to the concentration of plastic pollutants found. Aquatic environments in close proximity to urban areas have been found to have a high abundance of microplastic pollutants (Eriksen et al., 2013; Md Amin et al., 2020; Rodrigues et al., 2020). In sub-catchments of the Chesapeake Bay, USA, Yonkos et al. (2014) reported microplastic abundance to be strongly related to population density and urbanisation. Suratman and Latif (2015) pointed out that low water quality and high amounts of pollutants observed in the Terengganu Estuary were due to increased population densities and industrial activities in the region. In fact, diverse activities such as restaurants, tourism, fishing, seafood processing industries, recreation, sand mining, boat manufacturing, and construction actively surround this lower estuary area. These activities may influence the abundance of microplastics pollution, subsequently affecting the marine organisms in this area. On the other hand, the unevenness of the

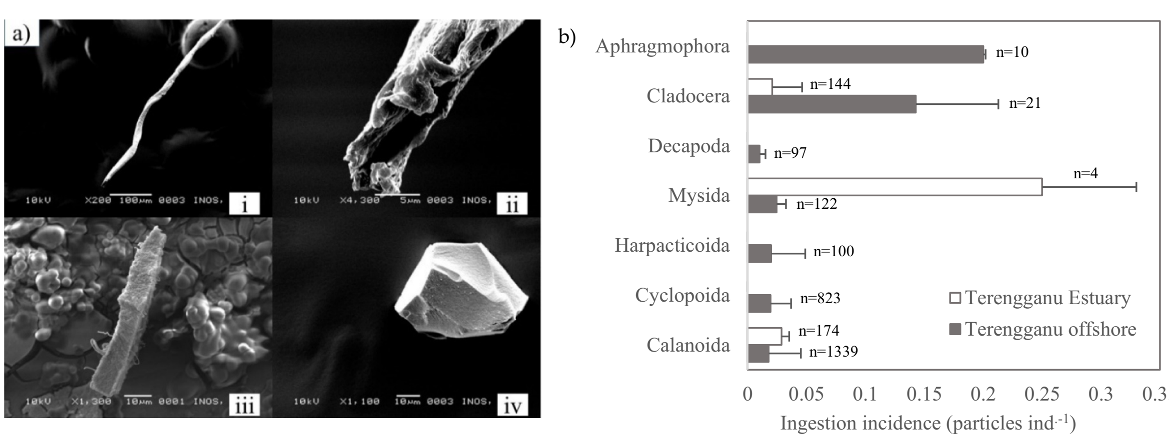


Fig. 5. (a) Examples of ingested microplastic under the scanning electron microscope (SEM); (i, ii) fibres; (iii, iv) fragments; (b) Ingestion incidence (mean ± SD) in different zooplankton groups (n: total number of individual).

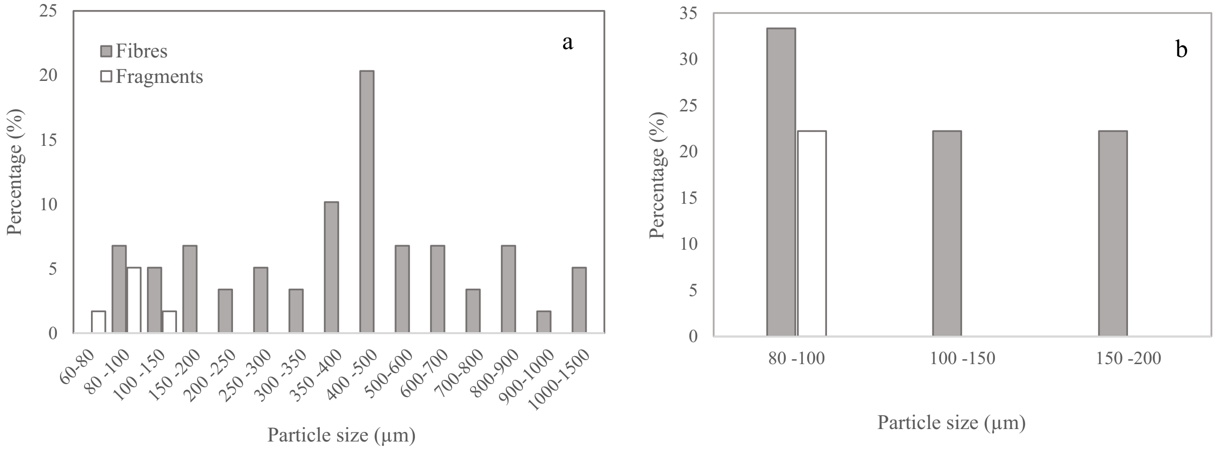


Fig. 6. Size composition of ingested microplastics by zooplankton in the (a) Terengganu offshore and (b) Terengganu Estuary.

microplastic distribution in the offshore water is probably related to more variant microplastic origin at the sea. According to Zhao et al. (2019), microplastic in this area may come from both the land and sea due to the high dispersion. Hydrodynamic factors (e.g., wind, tidal currents, wind, vertical mixing, and wave action) may have contributed to the increases in microplastic abundance at some sampling sites, resulting in spatial patches of floating microplastic (Schmidt et al., 2017; Zhao et al., 2019).

4.2. Polymer diversity in seawater

In plastic pollution studies, polymer identification of particles is important to validate the visual identification processes (Löder and Gerdts, 2015). The variety of polymers available helps to classify possible local sources and allow authorities and stakeholders to resolve this global issue by introducing successful preventive measures. Although we are unable to compare the different polymers between locations, the

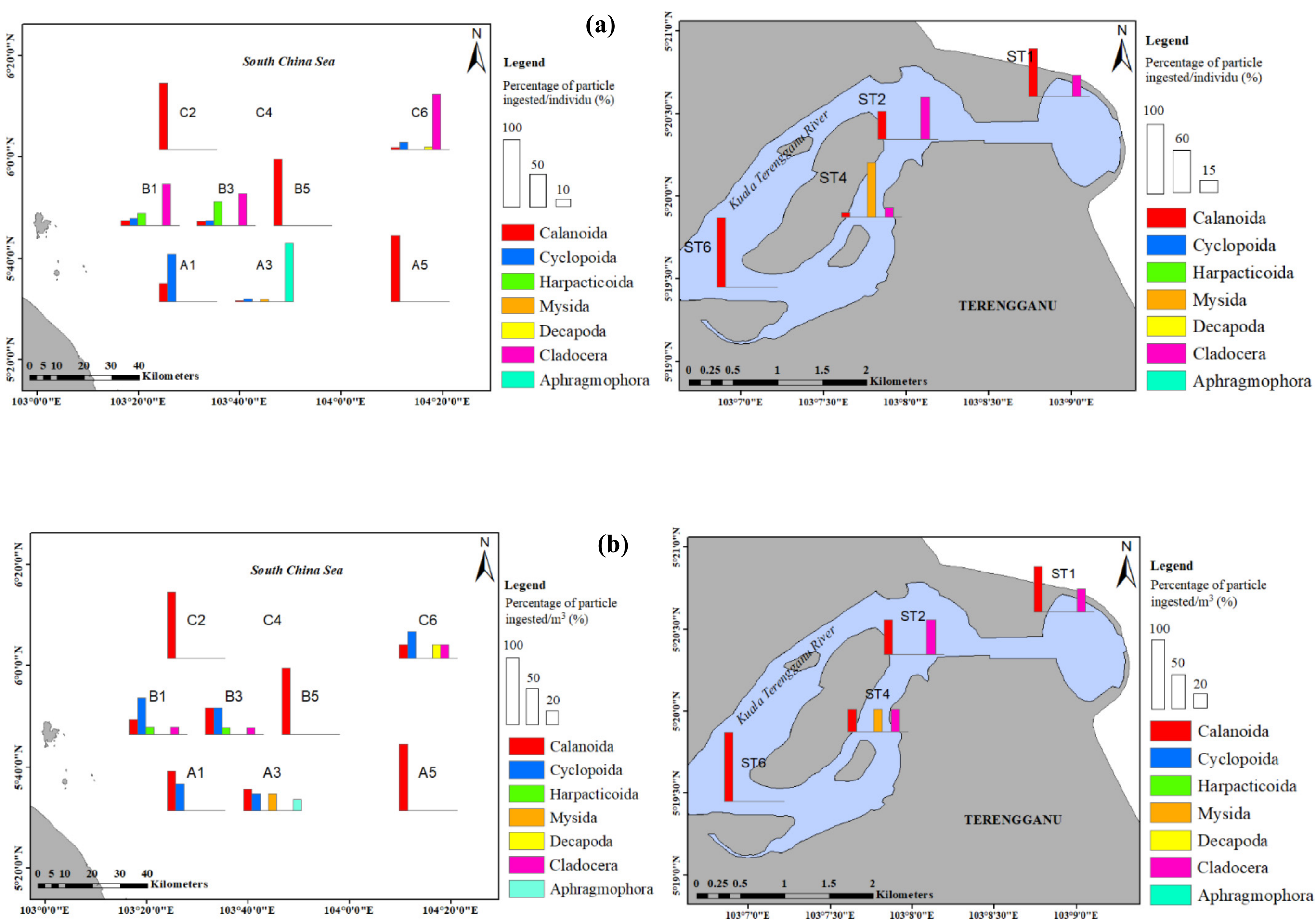


Fig. 7. Percentage map of (a) particle ind.$^{-1}$ and (b) ingested microplastics m^{-3} in different zooplankton taxa between Terengganu offshore water (left) and Terengganu Estuary (right) (Note the differences in scale applied to each map).

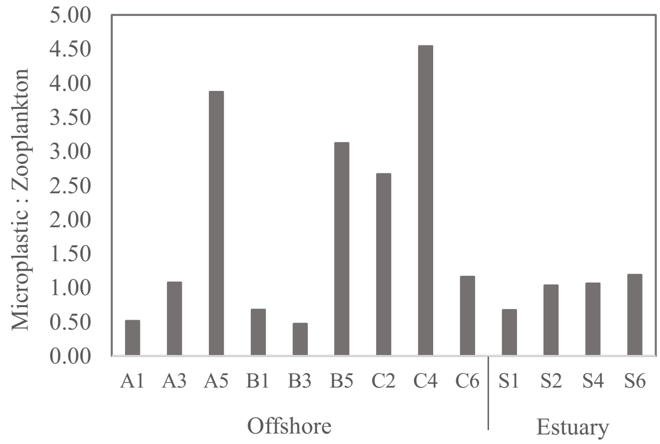


Fig. 8. Microplastic concentration (particles m^{-3}) to zooplankton abundance (ind. m^{-3}) ration in the surface offshore and estuary water.

predominance of fibres identified as polyamide, polyethylene, and polypropylene mirrors the diverse activities performed in the area. Polyethylene and polypropylene can be related to food storage, consumer goods, and single use items, and polyamide is predominantly used in fishing nets and in the automotive sector (Graziano et al., 2019). Similar to previous finding (Jung et al., 2018; Tofa et al., 2019), it is worth noting the presence of hydroxyl and carbonyl groups in the FTIR spectra in the present study, which might be due to the photooxidation process. This suggests that secondary sources of microplastics prevail, rather than primary sources, and they are linked to the numerous activities taking place in the surrounding urban area, including littering. Due to the lack of monitoring data, it is still unclear how much estuarine river input contributes to microplastics pollution. According to Hajbane and Pattiaratchi (2017), the offshore area diverged widely from the estuarine and nearshore samples and showed temporal and spatial variation in microplastic pollution. Through hydrodynamic modelling, Krelling et al. (2017) indicated that a higher proportion of fragmented or weathered microplastic items observed in open ocean are not necessarily directly derived from the internal area of the estuarine complex but might have originated from other regions. This is supposed to be affected by local current, particularly during events of high wave energy. In order to explain this, in situ monitoring studies must be performed to understand the potential impact of the oceanographic meteorological events on the abundance and distribution of marine debris.

4.3. Microplastic ingestion in zooplankton

The accumulation of floating microplastics on the surface of seawater raises concerns about the exposure of these synthetic particles on marine organisms. A growing number of studies have been carried out to determine the occurrence of microplastics in marine organisms (Lusher et al., 2013; Rochman et al., 2015; Sun et al., 2017; Welden and Cowie, 2016). However, limited study has been conducted on the microplastic in zooplankton in estuarine and ocean environments. In regard to the zooplankton community, calanoid is the dominant zooplankton from estuary to offshore areas of Malaysian water (Chew and Chong, 2011; Hwang et al., 2009; Kassim et al., 2008) which is in line with our current finding. As expected, our study indicates that the different zooplankton groups ingested microplastics in different amounts. There have been reports of similar incidences of ingestion in zooplankton groups from marine environment such as the Terengganu coast (0.003–0.14 particles ind.$^{-1}$; Md Amin et al., 2020), Portuguese coastal waters (0.04–0.14 particles ind.$^{-1}$; Frias et al., 2016), and Kenya's marine environment (0.16–0.46 particles ind.$^{-1}$; Kosore et al., 2018); and high ingestion incidence in the East China Sea (0.03–1.00 particles ind.$^{-1}$; Sun et al., 2018b), and the Yellow Sea (0.07–1.17 particles ind.$^{-1}$; Sun et al., 2018a).

Regarding the spatial distribution between estuary and offshore areas, a higher ingestion incidence in the estuary was anticipated, with an eventual decrease further offshore. Surprisingly, microplastic ingestion in zooplankton between these two areas did not differ evidently. The result was inconsistent with previous studies documented relatively high plastic ingestion by marine organisms in estuaries compared to other marine environments. For instance, over 70% (51) of fish studied in the River Thames examined by McGoran et al. (2017) ingested plastic fibres, and an average of 3.41 microplastics per individual was discovered in the fish *Diplodus vulgaris* from the Mondego Estuary (Bessa et al., 2018), which is high compared to previously published estimates of plastic ingestion in fish (Di Beneditto and Awabdi, 2014; Lusher et al., 2013; Romeo et al., 2015; Sigler, 2014). Several laboratory studies have reported that high abundance or concentrations of microplastics lead to increased ingestion (Cole and Galloway, 2015; Messinetti et al., 2017). Peters and Bratton (2016) explained that substantial interspecific variations in ingestion rates of microplastics in estuaries are anticipated due to dynamic environments, which are often influenced by hydrodynamics, the location of the sampling site, and levels of anthropogenic disruption (Nazarnia et al., 2020; Peters and Bratton, 2016). In the field, Frias et al. (2016) found the microplastic concentration to zooplankton abundance ratio was 0.04–0.12 in coastal waters off Portugal, while Hitchcock and Mitrovic (2019) documented the highest ration 0.7 in a Portuguese estuary. However, the ratio in this study (0.5–4.5) is comparable to that reported (0.009–3) in the surface water in three Australian estuaries (Rodrigues et al., 2020). This proportion leads to an assumption of the higher potential for microplastics to be ingested by zooplankton, which reflected by the higher microplastic concentrations in the study area. In contrast to our finding, previous studies have shown a link between the levels of microplastic ingestion by zooplankton and microplastic concentration in the surface seawater (Desforges et al., 2015; Md Amin et al., 2020). According to Dris et al. (2016) and Botterell et al. (2019), a number of biotic and abiotic factors other than abundance/co-occurrence can affect the bioavailability of microplastic to zooplankton. This includes selectivity in zooplankton, gut retention time, duration of microplastic availability and distribution (vertical and horizontal) of microplastic and zooplankton in the water column. The role of vertical transport concerning the abundance of microplastics in seawater is poorly understood. Based on a vertical transport model for microplastics in the oceans by Kooi et al. (2017), it was predicted that microplastic particles could float, sink, and vertically oscillate. However, their movements depend on the size and density of the particles. Nevertheless, to fully understand the difference in vertical zooplankton and microplastic abundance and other factors influencing ingestion incidence, further investigation must be conducted.

Fibres and fragment shapes presented distinct patterns in zooplankton at the study area, with fibres making up the highest percentage of the ingested particles in the South China Sea (94%) and the Terengganu Estuary (78%). This finding is per previous reports in the East China Sea (Sun et al., 2018b) and the northern South China Sea (Sun et al., 2017) where fibres are ingested the most, at 54.6% and 70%, respectively. The density of fibres allows them to remain suspended in water columns, which increases their likelihood of zooplankton ingestion. Contrastingly, fragments rapidly sink to the seabed and are more likely to be incorporated into estuarine sediment deposits such as intertidal mudflats and the central muddy basins of wave-dominated estuaries and lagoons (Jones, 2019). The current study's findings differ from those of Md Amin et al. (2020), which reported higher fragment ingestion than fibres in the Terengganu coastal area. This variation could be due to food preferences, feeding habits, and feeding rates (Figueiredo and Vianna, 2018). The size, concentration, and quality of food can influence the zooplankton feeding rate (Md Amin et al., 2011). The specific zooplankton clearance rates suggest that predators would also ingest accessible microplastics when searching for prey. It has been estimated that fish larvae and chaetognath can ingest 0.01–0.10 and 0.001–0.006

microplastics per day, respectively, while searching for food at saturation food concentrations due to their different maximum ingestion rates (Figueiredo and Vianna, 2018). Justifiably, the composition of mechano-chemo receptors supports selecting appropriate prey items (Cole et al., 2013). Copepods are considered to be filter feeders and display varying selectivity patterns, including the ability to select a broad range of particle sizes (Benedetti et al., 2015; Gismervik, 2006), which may increase their chance of ingesting microplastic particles. A recent laboratory study by Botterell et al. (2019) reveals that shape can affect the ingestion rate of microplastics in *Calanus helgolandicus*, *Acartia tonsa*, and *Homarus gammarus* larvae, with each species ingesting significantly more fragments, fibres, or beads, respectively. Their findings also show that an infusion of info-chemicals can significantly increase the ingestion rates. However, in the field, copepod *Temora longicornis* showed no evidence of microplastic consumption in Chichester Harbour, UK, suggesting species-specific dietary selectivity. The potential for trophic transfer of microplastic pollution is likely to be low for this particular species. Moreover, egestion rate was also likely to have affected the finding. In the marine worm *Arenicola marina*, microplastic polyvinylchloride uptake resulted in gut resident times 1.5 times longer than that in control worms without microplastics (Wright et al., 2013). The duration of retention of microplastics in wild zooplankton is not known, but microplastics can likely be retained longer in wild zooplankton than in experimental ones (Egbeocha et al., 2018).

Distribution patterns of microplastics in zooplankton according to their size also varied between the areas. However, the abundance of smaller sized particles (90–100 μm) inside the estuary is uncertain. It has been reported that the limitations of ingested microplastic sizes are probably due to the gape sizes of the species' mouthparts, where it was found that larger mouthparts in some species contribute to the ingestion of larger microplastic particles (Botterell et al., 2019; Cole and Galloway, 2015; Vroom et al., 2017). For instance, smaller microplastics (15 μm) were ingested more frequently in the copepod *Calanus finmarchicus*, than larger microplastics (30 μm), suggesting that smaller microplastics had higher bioavailability for this species. (Vroom et al., 2017). Similar to our finding, when compared to the size of microplastics ingested by zooplankton in laboratory experiments (Cole et al., 2013), zooplankton in their natural environment appear to ingest much larger plastic fragments from secondary sources (Md Amin et al., 2020; Sun et al., 2017). Microplastics' ability to independently fold or twist, which reduces their sizes and makes them more available to zooplankton, may the cause of these differences. It has been reported that fibres with low density polymer and smaller size, such as polyethylene and polypropylene—less dense than water—are expected to be suspended, transported, and distributed in surface seawater from estuary to offshore water (Li et al., 2020), indicating a higher possibility of being ingested by marine organisms. In addition, turbulence in the lower estuary could increase the risk of certain zooplankton species interacting with microplastics (Botterell et al., 2019). Due to increased particle contact rates, in particular in species such as Calanoida with ambush and pause-and-travel feeding behaviours, moderate to high turbulence levels have been predicted to increase the ingestion rates of prey (Saiz et al., 2003). In addition, the lowered number of larger microplastic sized ingested could be related with the retention time spent in a lower estuary, which could enhance biofouling levels and consequently cause this particle to sink (Kaiser et al., 2017). Further explanations could also rely on the wave action at the lower estuary which may potentially enhance fragmentation of both microplastics and larger items by mechanical action against rocks (Cheshire et al., 2009), leading to increased zooplankton ingestion.

5. Conclusions

This is the first study to focus on microplastic pollution in areas of estuary and offshore waters in Malaysia. As expected, there were differences between both areas in microplastic abundance with the highest recorded at the lower estuary. The increased abundance is suggested to be due to high amounts of pollutants from diverse human activities. Fibres with different polymers, identified as polyamide, polyethylene, and polypropylene, were the most commonly detected microplastic in seawater. Similarly, fibres were the most commonly ingested by zooplankton. These fibres possibly originated from secondary microplastics which mirrors the multiple activities occurring in the estuary, namely restaurants, tourism, fishing, seafood processing industries, and marine traffic. However, no clear relationship was observed between microplastic concentration and zooplankton ingestion incidence, indicating that other biotic and abiotic factors can influence the bioavailability of microplastic to zooplankton. The size of ingested microplastics also varied, with a wider range of sizes observed in offshore waters than in the estuary. This suggest that the ability to ingest a broad range of size irrespective of zooplankton size. The findings of this study document the relation of locality towards the distribution and spread of microplastics and therefore, can act as a benchmark and lay strong fundamentals towards conducting future studies on the spread of microplastic pollution in the marine environment, particularly within zooplankton communities. This can bridge the gap between zooplankton health and the possibility of microplastic transmission through the food web. More extensive, regular monitoring of microplastic pollution and its effects on marine organisms are required to fully elucidate the environmental factors that could potentially drive variation in the effects of microplastic exposure on marine organisms, temporally and spatially. Sharing scientific findings with society is also necessary to raise public awareness about microplastic pollution and to inspire actions to prevent or minimise plastics entering the marine environment.

CRediT authorship contribution statement

Zakaria Daoud Taha: Conceptualization, data collection and analysis, interpretation of the result, writing and editing; **Roswati Md Amin**: Conceptualization, project administration, data analysis, interpretation of the result, writing, editing and funding; **Sabiqah Tuan Anuar**: Interpretation of the result, review and editing; **Ammar Arif Abdul Nasser**: Data collection and analysis; **Erqa Shazira Sohaimi**: Data analysis and editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The research was funded by grant (TAPE RG: 55187) from Universiti Malaysia Terengganu. We want to thank the Centre of Research & Field Service, UMT's research vessel (RV Discovery); the captain and crews onboard and Faculty of Science and Marine Environment, Universiti Malaysia Terengganu for supporting this research. We also acknowledge Assoc. Prof. Dr. Poh Seng Chee and team, Nurul Ummi Izzati Hamidon, Yuzwan Muhamad and Che Mohd Zan Husin for their assistance in field and laboratory works analyses.

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