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Baseline

Ingestion of microplastics by the estuarine polychaete, *Namalycastis* sp. in the Setiu Wetlands, Malaysia

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ABSTRACT

In this study, the ingestion of microplastics by the deposit-feeding polychaete *Namalycastis* sp. in the estuarine area of the Setiu Wetlands, Malaysia was confirmed. Samples were collected from six stations, covering the wetland from the south to the north, bimonthly between November 2016 and November 2017. Microplastics were extracted from polychaete samples following digestion in an alkaline solution (10 M NaOH). They were identified by physical characteristics (i.e., shape and color under dissecting microscope and scanning electron microscope), and chemical analysis using a LUMOS Fourier Transform Infrared Microscope (μ-FTIR). A total of 3277 pieces were identified, which were dominated by filaments (99.79%) and with the majority transparent in color (84.71%). Most of the microplastics identified were polypropylene (PP) followed by polyamide (PA) based on their main peak in the of μ-FTIR spectrum. Principal component analysis demonstrated the dominance of microplastics at stations 3 and 4 of the sampling area, probably because of the influx from the open sea and from aquaculture. The findings of this research provide baseline information on microplastics ingested by benthic organisms and their fate in the estuarine food web.

Microplastics are synthetic polymer particles with a scale size below 5 mm (Arthur et al., 2009), and they are now considered as a global issue because of their impact on environmental pollution. Microplastics have been categorized into primary (microsized polymer products) and secondary (fragmentation of larger plastic pieces into microsizes; Cole et al., 2011). Numerous experiments have established the presence of microplastics in the environment, in both marine and freshwater ecosystems. Thus, aquatic organisms at each trophic level are susceptible to their presence, with possibilities for entering the food chain (Ma et al., 2020).

Many studies are acknowledging the impact of microplastic ingestion on marine organisms when these particles are mistaken as a food source because their appearance closely resembles prey (Farrell and Nelson, 2013; Ory et al., 2018; Su et al., 2019). Microplastics move through the organisms' digestive system and require a longer digestion time, thus slowing the rate of egestion, which facilitates the transfer of microplastics from a lower trophic level through the food chain.

A wide range of marine organisms have mistakenly ingested microplastics, including benthic organisms such as mussels, oysters, and polychaetes. Previously, microplastics have been categorized in relation to farmed and wild mussels, with a minimum of 0.53 particles per individual (Ding et al., 2018; Cho et al., 2018), whereas commercial bivalves, such as oysters, mussels, and clams, ingest microplastics at not less than 0.97 ± 0.74 particles per individual. The lack of good practice in waste management enhances the abundance of microplastic fragments found in the digestive system of mussels (Digka et al., 2018). Although microplastics have been reported in many benthic organisms, scientific evidence on the microplastic ingestion of Polychaeta or bait worms, which live in estuarine habitats, is still needed. Some species, such as *Hediste diversicolor*, are known as keystone species for estuarine habitats, and it has been proven that different species of polychaetes living in estuarine habitats, such as *Ophryotrocha labronica* (Hodgson, 2019), *Marphysa sanguinea* (Jang et al., 2018; Pequeno et al., 2021), and *Hediste diversicolor* (Revel et al., 2020), have ingested various types of microplastics. Estuarine polychaetes are therefore recommended as indicator species for observing the abundance of microplastics, as they are

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widely and spatially distributed in the estuarine area. In recent years, microplastics have become a subject of investigation among researchers worldwide, but limited studies have been conducted in Malaysia. Recent studies have presented the characterization of microplastics from marine organisms, such as the filter feeder bivalve *Scapharca cornea* and the fish *Lates calcarifer* (Ibrahim et al., 2016; Ibrahim et al., 2017), found in Malaysian wetlands. In the present study, we sought to report on the interaction of microplastics with the estuarine polychaete *Namalycastis* sp., which is widely distributed in the Setiu Wetlands and is a keystone species (Magesh et al., 2012). The presence of microplastics in *Namalycastis* sp. is essential for the estuarine food web because this may allow these particles to be transferred to the next trophic level.

This study was conducted from November 2016 to November 2017 (Fig. 1). The Setiu Wetlands ecosystem has been experiencing water quality degradation because of the presence of excessive nutrients (Suratman and Latif, 2015; Suratman et al., 2017), hydrocarbons (Suratman et al., 2012), and heavy metals (Talukder et al., 2021). This is particularly true of areas near agricultural and aquacultural activities (Mostapa and Weston, 2016). Thus, an investigation into the abundance of microplastics is imperative for understanding the influence of new contaminants of concern on estuarine ecosystems. The population of polychaetes in the Setiu Wetlands, including *Neanthes glandicincta*, has been documented in the literature (Ibrahim et al., 2019). For the present study, the estuarine polychaete *Namalycastis* sp. was collected by hand from the inside of rotten nypa fronds at six different sampling sites within these wetlands. A total of 95 individuals with three replicates were gathered from the stations. However, the number of specimens at each station was not consistent because of the limitations relating to their reproductive biology and the occurrence of rotten nypa. The specimens were then kept in 80% ethanol solution for preservation prior to analysis (Table 1).

The maximum body width of specimens excluding parapodia was measured. Specimens of *Namalycastis* sp. within a size range of 0.9–4.7 mm body width were selected. Before digestion, the outer body of each individual was rinsed with deionized water to remove any possible contamination. The specimen was then cut into smaller pieces for the digestion process. The cut samples were immediately transferred into a 250 ml beaker and mixed with a suitable adjustable volume of 10 M NaOH solution. The beaker was covered with aluminum foil and placed

Table 1
Coordinates of sampling stations in the Setiu Wetlands.

Sampling station	Latitude	Longitude
1	5°43′21.08″N	102°40′13.87″E
2	5°41′30.77″N	102°41′54.98″E
3	5°40′54.46″N	102°42′33.25″E
4	5°39′49.27″N	102°43′57.69″E
5	5°39′03.80″N	102°44′55.32″E
6	5°37′10.34″N	102°47′31.51″E

in an oscillation water bath at 60 °C until completely digested. The digestion process depended on the size of each sample, with a maximum of 48 h required to complete the process.

All equipment was rinsed three times with deionized water to avoid any contamination while analyzing the samples (Van Cauwenberghe et al., 2013). Additionally, a cotton lab coat and latex gloves were worn when handling the samples, and only glass bottles were used throughout the experiment. Before inspecting the samples, the microscope and bench were cleaned, and the observation of microplastics was conducted in a clean and closed chamber. To avoid contamination and over-quantification, Petri dishes containing filter papers were used during the microscopic observation and μ-FTIR analysis.

The digestion samples were transferred into a clean Petri dish, and deionized water was added to elucidate the samples for identification under a dissecting microscope (Olympus SZX-ZB7, Olympus Corp., Tokyo, Japan), as the digested product was likely to be brown in color. Microplastics were classified and sorted into different shapes (i.e., filaments and fragments) and colors (i.e., transparent, red, blue, green, black, and brown; Hidalgo-Ruz et al., 2012). All sorted microplastics were transferred into glass bottles containing deionized water. Observation of the plastics by color is crucial as each color is representative of their specific polymer (Wright et al., 2013). Additionally, an image of the representative sample of microplastics was captured by a camera (DinoCapture 2.0 version 1.5.19). A detailed image of the surface morphology of the microplastics was analyzed using a scanning electron microscope (SEM; Model JEOL JSM-636 OLA, JEOL Ltd., Tokyo, Japan). The microplastic samples were mounted on aluminum stubs, coated with a thin layer of gold, and then visualized at various magnifications.

Following the microscopic observation, two representative samples

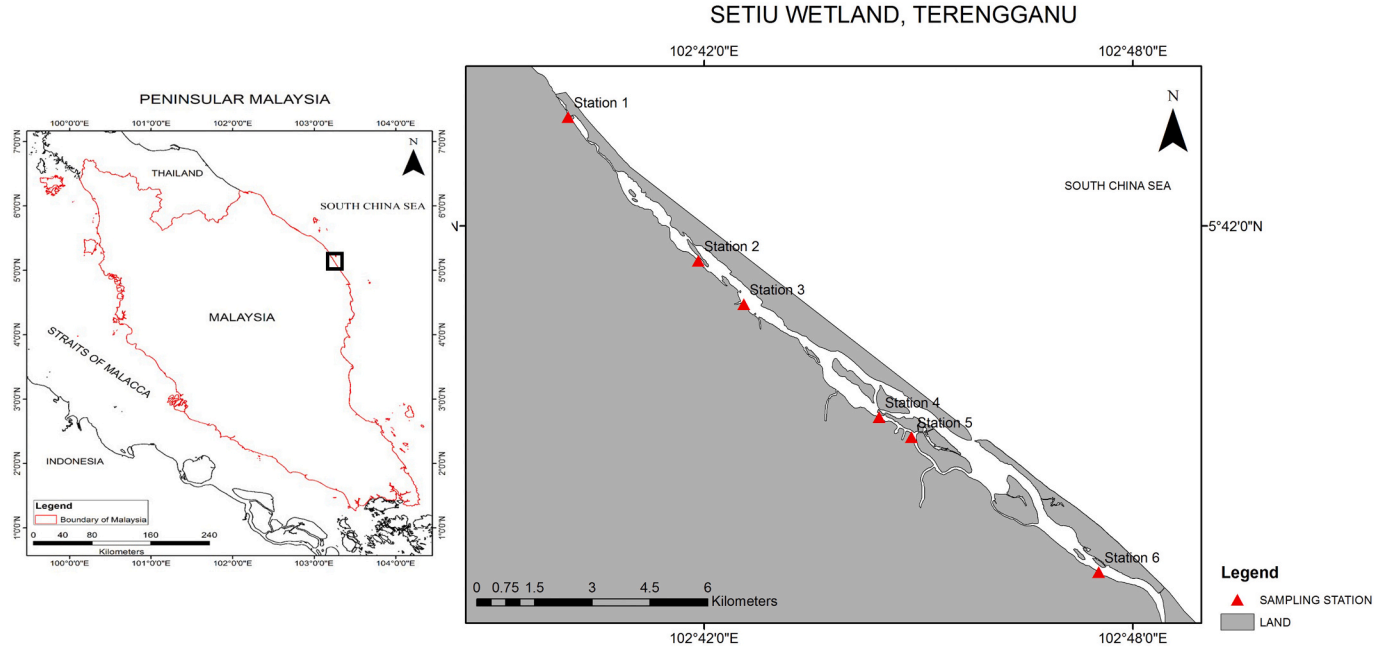

Fig. 1. Map of sampling stations in the Setiu Wetlands.

of the deionized water samples from each station were selected and subsequently filtered through clean filter papers. The filter papers were then dried in a closed and clean desiccator for 2 days. Sample drying is necessary to avoid the disturbance of hydroxides in the spectra. Fourier transform infrared microscopic spectroscopy (LUMOS μ-FTIR, Bruker) in attenuated reflectance mode was used to analyze individual microplastic particles in each sample for polymer identification with less interference from moisture and instrumental background. This analysis was conducted in a spectra range between 600 and 4000 cm^{-1} with 4 cm resolution and a rate of 64 scans for every sample. Spectroscopy software (OPUS, Bruker Corp., Massachusetts, USA) was used for spectral interpretation and chemical mapping for the μ-FTIR. The detection limit of μ-FTIR is 20 μm.

Minitab Version 18 (Stat Inc., USA) was used to analyze and interpret data sets. Correlation and regression analyses were run to determine if there was any possible relationship between the different body sizes of *Namalycastis* sp. and total microplastic ingestion, which were verified as significant when $p < 0.05$. The spatial difference was identified via the ANOVA test and verified as significant when $p < 0.05$. Further analysis by post hoc comparison using the Tukey test was conducted if any significant difference existed. Principal component analysis (PCA) through the Eigen decomposition method was conducted to identify the spatial distribution of different microplastic categories. To differentiate between the principal component values, the Kaiser criterion was also used, and an eigenvalue of >1 was considered a significant principal component. A total number of 3277 pieces of microplastics were identified in the *Namalycastis* sp., with approximately 20.00–46.79 pieces ingested per individual (Table 2). Table 2 shows that individual microplastic ingestion at station 2 was the highest followed by stations 4, 3, 5, 6, and 1, in the order from highest to lowest. Microplastic distribution in the area around station 2 was more concentrated compared with the other areas, which had more openings to the sea and a larger volume of water. This is similar to a previous study in which Su et al. (2018) found that different hydrological conditions in the water system eventually affect the microplastic concentration in the aquatic ecosystem. For instance, a smaller volume of water in the river may result in a higher microplastic concentration. In the present study, station 2 had an area that was visibly smaller than others, which was reflected in the number of microplastics ingested by the polychaete.

Fig. 2a illustrates that filaments (99.79%) dominated the microplastics found ingested in the samples (stations and months, $N = 3277$); only a small number of microplastic fragments were identified. The proportion of microplastic filaments from the different stations ranged from 98.5%–100%. Filaments were also recorded as the dominant type of microplastics ingested by other species of polychaete, such as *Phragmatopoma caudata* (da Costa et al., 2021). As shown in Fig. 2b, in terms of color, transparent microplastics were recorded as the highest percentage at 84.71%, followed by black (4.67%), blue (3.78%), green (2.93%), brown (2.47%), and red (1.43%). The proportion of transparent microplastics from the different stations ranged from 77.12% to 94.81%.

In this study, the statistical analysis demonstrated that specimen size influenced the total number of microplastics ingested by *Namalycastis* sp. (Fig. 3). The data set was not normally distributed even after data transformation; hence, a nonparametric Spearman correlation was performed to identify the relationship between total microplastics ingested and microplastics in the estuarine area. There was no correlation found between microplastics in *Namalycastis* sp. and sediment ($r_s = 0.114$, $p > 0.05$) or estuarine water ($r_s = 0.171$, $p > 0.05$). However, a positive and medium correlation existed between body size and microplastics ingested by *Namalycastis* sp. ($r_s = 0.349$, $p < 0.05$). The amount of microplastics ingested by Polychaeta could be driven by their habitats, such as estuaries, and feeding behavior (Hodgson, 2019). However, based on the data, body size (mm) is the dominant factor in relation to microplastic ingestion; the correlation value clearly demonstrates that microplastic ingestion is not dependent on microplastics in the estuarine environment. The result of the linear regression model was significant ($F(1,93) = 16.15$, $p = 0.000$, and $R^2 = 0.148$), indicating that approximately 14.8% of the variance in microplastic ingestion is explained by the body size (mm) of *Namalycastis* sp. This is in contrast to many previous studies, which have reported that specimen size does not affect the total number of microplastics; for instance, farmed and wild mussels ingest a similar amount of microplastics although the mussels are different sizes (Digka et al., 2018).

Table 3 shows the classification (shape and color) of microplastic ingestion at different stations. No spatial interaction existed between microplastic ingestion and *Namalycastis* sp. ($F = 2.31$, $p = 0.064$). Additionally, there was no significant relationship found between filament distribution and the stations ($F = 2.35$, $p = 0.061$), and no significant relationship was found between the distribution of transparent microplastics and the stations ($F = 2.45$, $p = 0.052$). Thus, the results demonstrate that microplastic distribution remained constant throughout the Setiu Wetlands.

The first PCA showed two acceptable principal components with an eigenvalue of >1. The biplot below shows the spatial distribution of microplastic ingestion by *Namalycastis* sp. (Fig. 4). The total variance from both components was 72.7%, with the first principal explaining 48.7% and the second principal 24.0%.

The highest number of microplastics ingested by *Namalycastis* sp. were from station 2 (Table 2). However, compared with the other stations, stations 3 and 4 were most affected by microplastic ingestion based on different characteristics (Fig. 4). As can be seen on the map (Fig. 1), stations 4 and 5 are located near the opening to the South China Sea; although station 6 is closer to the opening than station 3, historically, station 3 was closer to the old sea opening. The presence of microplastics in the waters of the South China Sea has been reported previously in a separate study (Khalik et al., 2018), and the main input of microplastics into the wetland is likely from sea flows, which distribute the microplastics throughout the mangrove area. Additionally, note that small-scale aquaculture activities are present near station 3 and microplastic quantities ingested by the polychaete *Perinereis aibuhitensis* from the aquafarm site were found with filaments accounting for 23% of total particles (Jang et al., 2020). Hence, fragmentation has occurred and contributed to the abundance of microplastics. A previous study in this area revealed that estuary fish (*Lates calcarifer*) were likely to ingest broken-down plastic from the aquaculture site itself, resulting in higher microplastic intake compared with that of wild L. *calcarifer* (Ibrahim et al., 2017). The Fig. 4 shows that microplastic filaments correlated with transparent pieces, which both comprise the most abundant microplastics ingested.

Namalycastis sp. inhabits littoral and supralittoral areas in association with rotten plants (Glasby, 1999). Polychaete worms from this particular family are widespread and distributed throughout the wetland's estuarine area. There are different types of feeding habits among

Table 2
Total number of *Namalycastis* sp. in relation to alkaline digestion and microplastic ingestion (spatial variation).

Sampling site	Station 1	Station 2	Station 3	Station 4	Station 5	Station 6
Total of *Namalycastis* sp. digested	18	14	19	15	19	10
Microplastics abundance						
a) Total number microplastic ingestion by *Namalycastis* sp.	449	655	692	674	607	200
a) Number of microplastic ingestion per individuals (average)	24.94	46.79	36.42	44.93	31.95	20.00

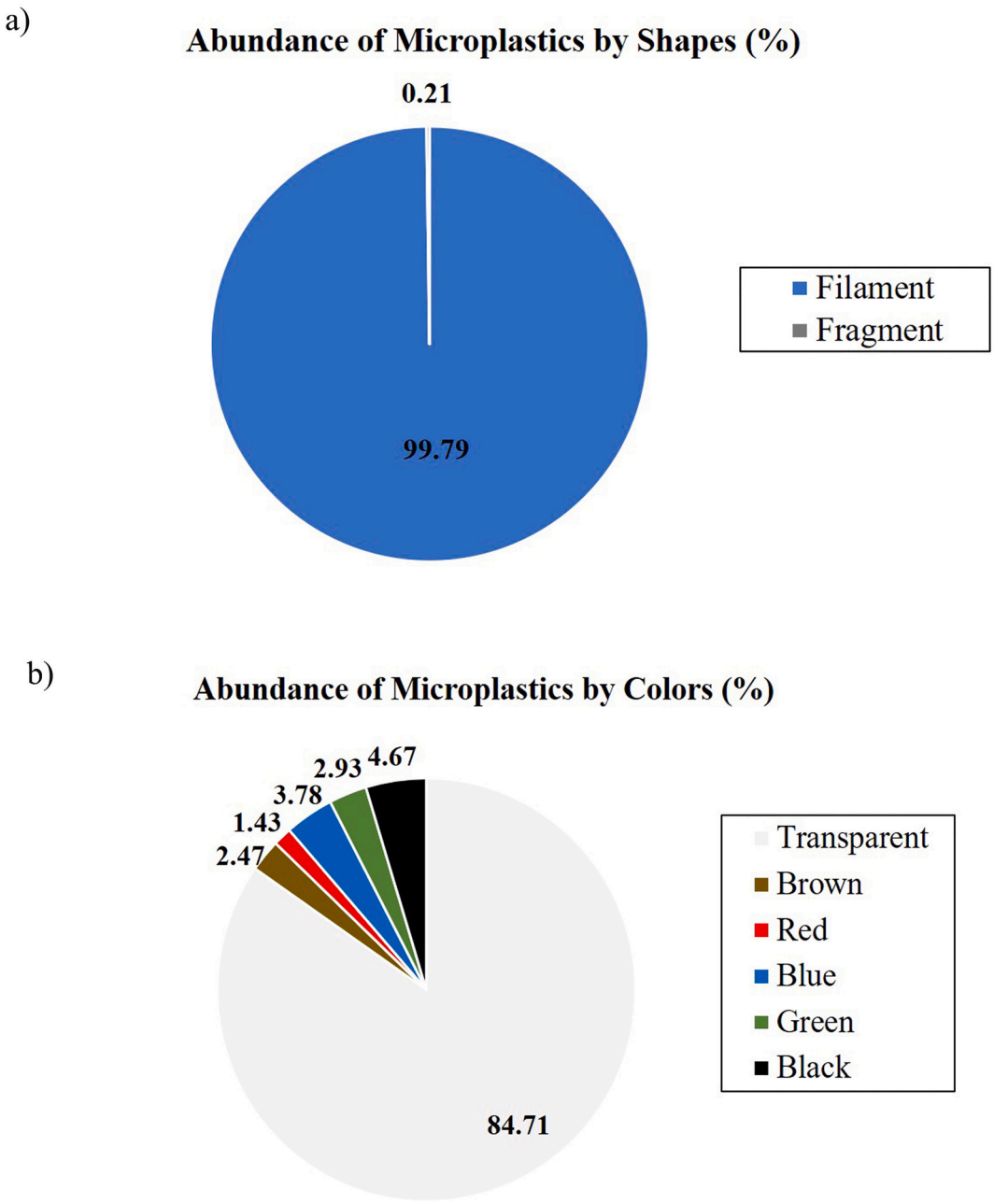

Fig. 2. Microplastic ingestion by *Namalycastis* sp.: a) abundance of microplastics by shape; b) identification of microplastics by color (N = 3277).

polychaetes, such as subsurface, surface deposit, and suspension feeders (Jumars et al., 2015). These different feeding habits may result in the uptake of microplastics by marine organisms. This assumption has been proven when the nonselective feeder *Arenicola marina* was found to ingest higher microplastics than the selective filter feeder *Mytilus edulis* (Van Cauwenberghe et al., 2015). By contrast, no microplastics were observed in the surface and subsurface deposit feeder *Marenzelleria* sp., but this species assisted in the bioturbation movement of microplastics into marine sediment (Näkki et al., 2017). Nonetheless, there is limited information on the feeding habits of the estuarine polychaete *Namalycastis* family. It has been reported before that most estuarine polychaetes inhabit and feed on rotten plants and detrital materials (Glasby et al., 2007). This is found to be similar to the *Namalycastis* sp. used in the present study. *Namalycastis* sp. generally inhabits and prefers to feed on the soft structure of rotten nypa. However, the physical structure of the microplastic filaments, which resemble the fibrous structure of rotten nypa, may be mistaken by *Namalycastis* sp. as a food source. This factor may be a reason for the higher number of microplastic filaments ingested compared with fragments (Fig. 2a).

Moreover, in terms of color, Fig. 2b shows the number of transparent microplastics to be the highest, followed by black, blue, green, brown, and red. It is noteworthy that transparent microplastics were the most ingested by this species, and it can be speculated that *Namalycastis* sp. incidentally ingest microplastics, mistaking them as rotten nypa, their natural food. Many marine species are visual predators; hence, it is suggested that the color of the microplastics plays an important role in ingestion. It has been widely reported that the misidentification of specific microplastic colors increases the possibility of microplastic ingestion as a food source (Crawford and Quinn, 2017). Additionally, transparent microplastics are less visible than those of other colors; hence, *Namalycastis* sp. is prone to ingest these microplastics when feeding. Fig. 5 shows some of the colors of the microplastic filaments

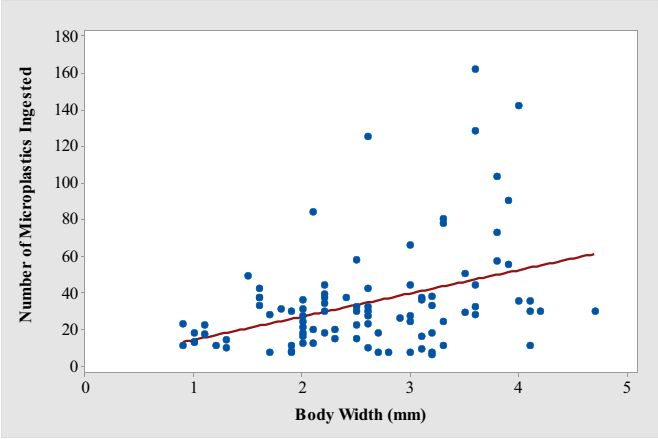

Fig. 3. Regression line of body width (mm) and number of microplastics ingested by *Namalycastis* sp. The blue circles represent the number of microplastics. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

found in *Namalycastis* sp. under stereomicroscopic observation.

Microplastics found in estuarine polychaetes are expected to be transferred to the next trophic level. The polychaetes are less likely to feed on their natural food because of the proportional uptake of microplastics, which may consequently reduce the energy of *Namalycastis* sp. As an example, weight loss and low energy were reported in *Arenicola marina* (L.) due to microplastic uptake (Besseling et al., 2013). Thus, the presence of microplastics within their habitat may affect the population of the estuarine polychaetes as well as the food chain.

In further microplastic characterization, SEM was used to visualize the direct observation of crack morphology and biofouling attachment on microplastics surfaces. The fragmentation of microplastics can be caused by thermooxidative degradation (heat) and photooxidative degradation (light; Crawford and Quinn, 2017). The roughness structure on the overall surface of the fragment in Fig. 6b indicates a long exposure toward oxidative degradation. This is in line with Tiwari et al. (2019), who reported that high oxidative and weathering conditions influence the surface roughness of the plastic sample. Consequently, plastics were oxidized and fragmented (secondary) into irregular shapes (Wang et al., 2017).

The morphology of the filament surfaces observed under SEM is shown in Fig. 6c–f. Fig. 6d shows a piece of microplastic fragmented into smaller pieces, thereby decreasing the size and potentially increasing the susceptibility of marine organisms to microplastics. It can be seen that one part of the microplastic filament has a fragmented structure, whereas the rest is smooth without any signs of disintegration, thereby indicating the poor resistance of microplastics toward heat and ultraviolet light. Fig. 6f shows the enlarged visual of the attachment of unknown microorganisms to microplastic filaments or biofouling. Biofouling is defined as the accumulation of microorganisms on the wetted surface of microplastics. Without considering the specific density of polymer type, biofouling increases the density and deposition of microplastics onto the seabed/sediment (Kaiser et al., 2017).

As shown in Fig. 7, the associated polymers for microplastic filaments were interpreted as polypropylene (PP; Fig. 7a) and polyamide (PA; Fig. 7b). Their presence may be the result of anthropogenic activities in the coastal and wetland systems, as these polymers are widely used in ropes, fishing nets, and fishing lines. A similar PP interpretation via FTIR has been conducted as part of previous research (Ibrahim et al., 2021; Tiwari et al., 2019; Jung et al., 2018; Castillo et al., 2016). In detail, the peaks at 2875–2964 cm^{-1} relate to the C—H stretch mode, whereas peaks at 1468 and 1383 cm^{-1} refer to the C—H bending of the methylene (CH_2) group and methyl group (CH_3), respectively. For PA, a peak at 3269 cm^{-1} is in the region of the N—H stretch mode and interpreted as the secondary amine group. Furthermore, C—H stretch and N—H bend peaks were observed at 2922–2852 and 1510 cm^{-1}, respectively. A small peak at 1269 cm^{-1} was assigned as the C—N stretch mode belonging to this polymer. Additionally, a peak at 1637 cm^{-1} was identified as the C═O carbonyl group for PAs. Similar PA interpretation has been identified in other studies (Md Amin et al., 2020; Jung et al., 2018; Ibrahim et al., 2017; Neves et al., 2015). FTIR spectra for microplastic fragments were not available because of their limited number and sample loss during handling. Moreover, there were difficulties in interpreting individual particles in the samples, as the spectrum obtained frequently included filter paper (background) spectrum.

Potential sources for the PP and PA polymers are primarily related to aquaculture and fisheries (Md Amin et al., 2020; Ibrahim et al., 2017). In the Setiu Wetlands, small-scale cage and pond cultures are growing as economic activities among villagers in and around the wetland. Hence, fishery tools, such as ropes, fishing lines, and nets, are easily found fragmented and dispersed in the aquatic environment because of sunlight and other biological processes, such as fouling and microbial degradation. Numerous studies have demonstrated similar PP interpretations, such as in mangrove areas in Singapore and Shanghai (Mohamed Nor and Obbard, 2014; Peng et al., 2017). According to the World Wildlife Fund, 2019, Malaysia is expected to generate USD 186

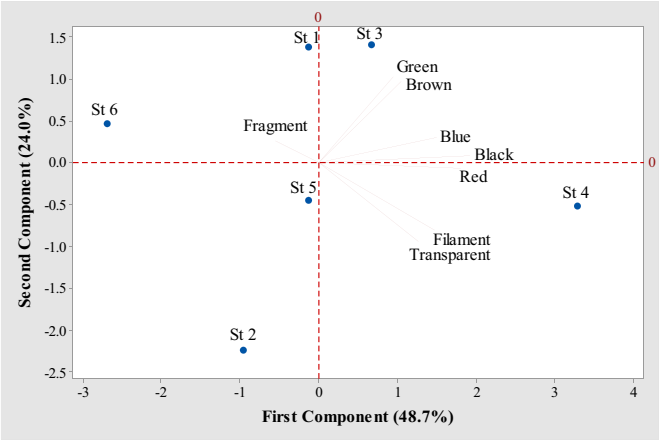

Fig. 4. PCA of interaction between microplastic characteristics and samples. Biplotted interaction between different samples (dots) and different microplastic characteristics (vectors): shape and color.

Table 3
Spatial variation of microplastic ingestion by *Namalycastis* sp. (particles/individual).

Station	Shapes and color of microplastic ingestion (particles/individual)							
	Filament	Fragment	Transparent	Brown	Red	Blue	Green	Black
1	178.00	1.17	138.17	7.83	3.00	10.83	7.33	12.00
2	327.00	0.50	310.50	1.50	2.00	2.50	4.50	6.50
3	246.33	Null	205.00	12.17	2.50	6.33	9.67	10.67
4	360.17	0.50	305.00	6.00	5.83	16.33	7.50	20.00
5	213.33	Null	179.17	4.67	3.00	10.50	4.67	11.33
6	98.00	0.83	80.50	2.17	1.83	6.00	5.33	3.00

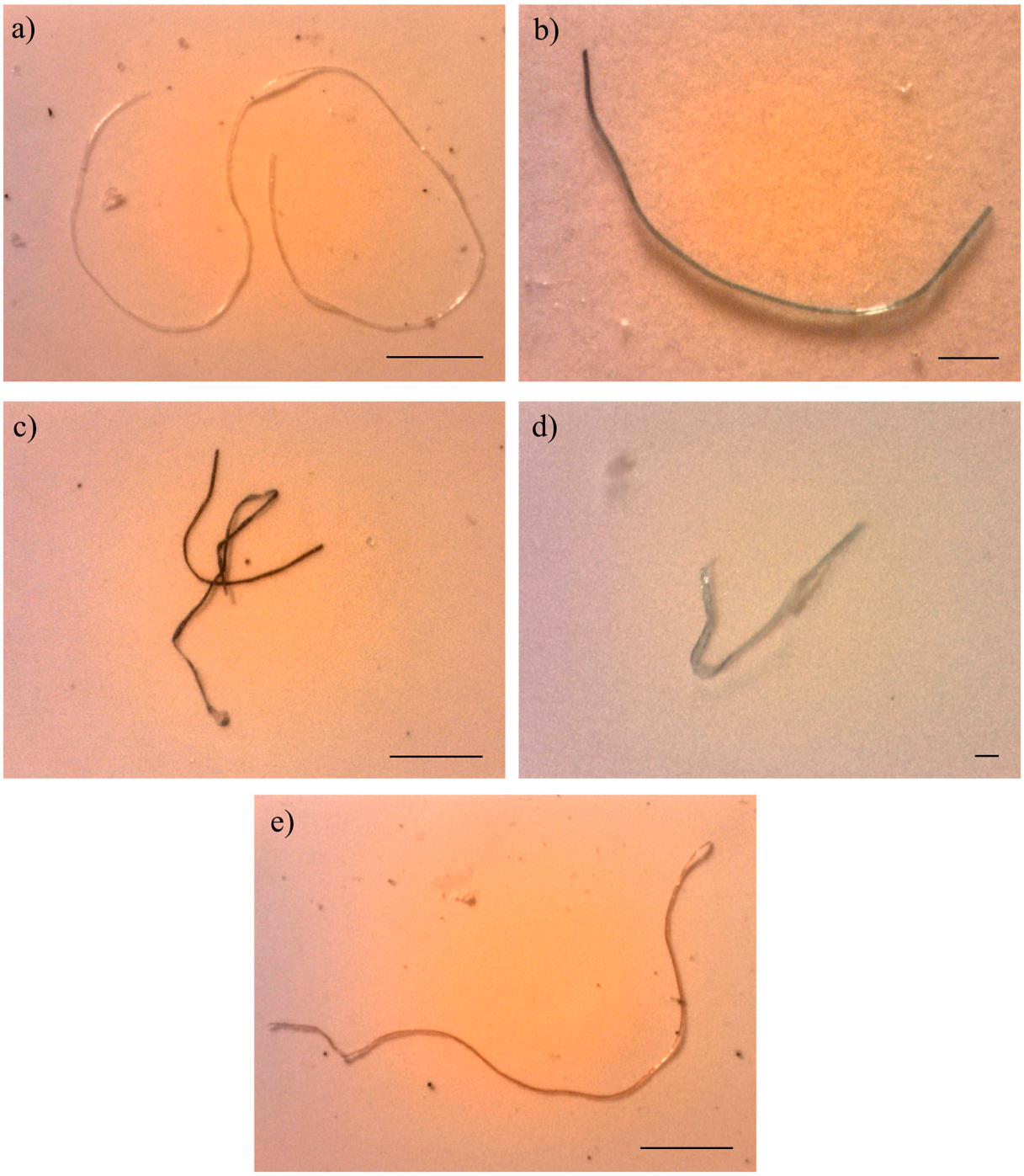

Fig. 5. Images of microplastic filaments with different colors: a) transparent, b) green, c) black, d) pale blue, e) red. Scale bars: 0.5 mm. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

million per year from mangrove activities and products, such as fisheries, timber, plant products, and tourism. The Setiu Wetlands are a natural protected area, but there remain small-scale economic activities, such as cage-cultured fish and pond culture.

Microplastic ingestion by estuarine polychaetes (*Namalycastis* sp.) has been proven in this study. The abundance of microplastic filaments and those that are transparent in color are mistaken by estuarine polychaetes because the structure closely resembles the structure of their natural food, rotten nypa. PP and PA are the main polymer types, reflecting their wide use in daily life and high concentration in the estuarine area. There was no significant difference found in microplastic ingestion in terms of the spatial distribution of microplastics in the Setiu Wetlands; however, the highest number of microplastics had been ingested by estuarine polychaetes from station 2. The main input of microplastics in the estuarine area is from the opening to the sea and degradation from aquaculture activities. This research provides the initial evidence for microplastic ingestion by estuarine polychaetes. Findings in this study can be used as a baseline for microplastic distribution in the estuarine area for future work.

CRediT authorship contribution statement

Siti Rabaah Hamzah: Sampling, carried out laboratory works, and writing original draft-preparation.

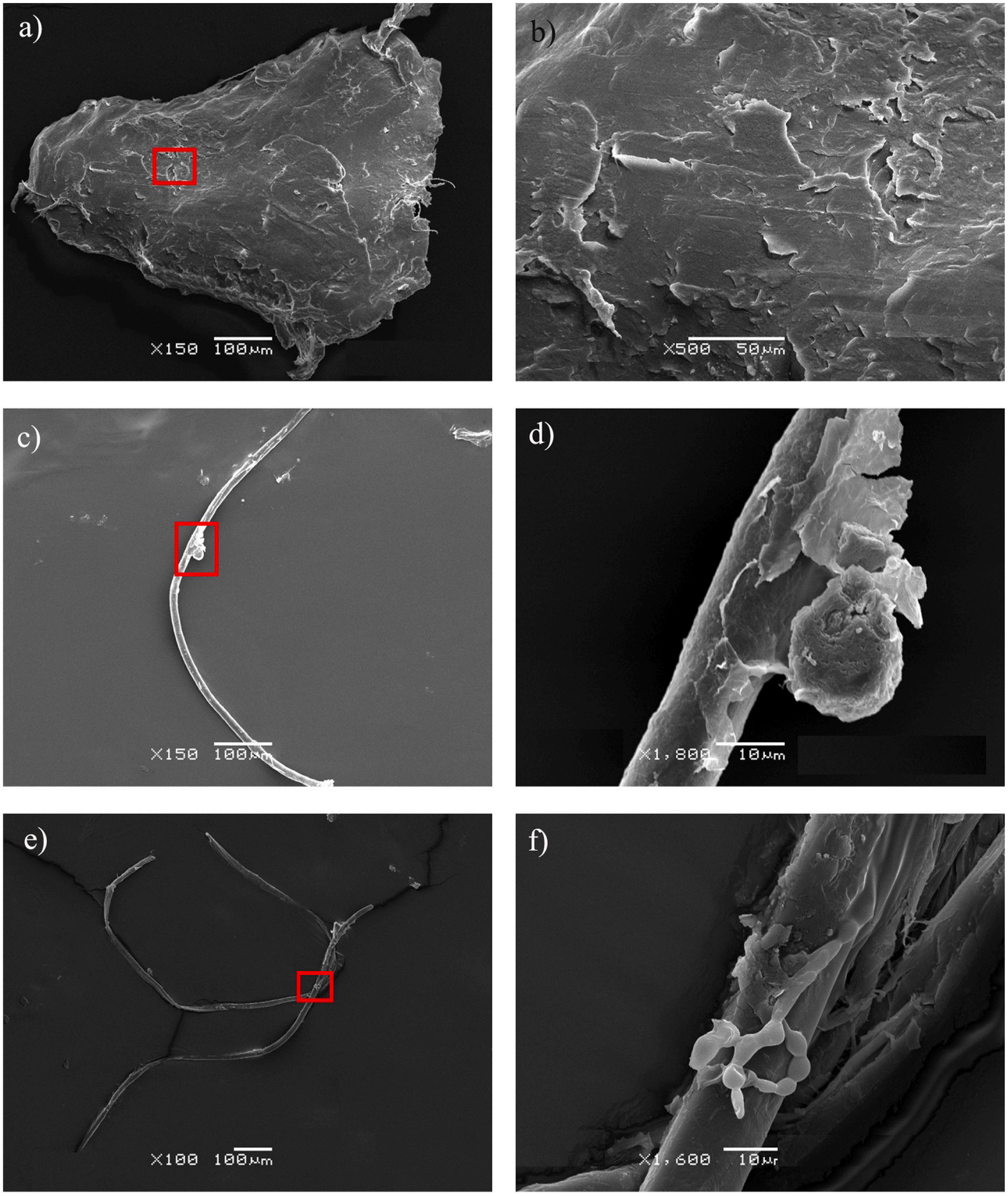

Fig. 6. SEM images of microplastics in *Namalycastis* sp.: a) fragment; b) enlarged view of red square in (a); c) filament; d) fragmented microplastics, enlarged view of red square in (c); e) filament with the attachment of microorganism; and f) enlarged view of red square in (e). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Raad Shaher Altarawneh: Sampling and carried out laboratory works.

Sabiqah Tuan Anuar: Methodology, data Interpretation, and reviewing and editing.

Wan Mohd Afiq Wan Mohd Khalik: Statistical analysis, reviewing and editing.

Prabhu Kolandhasamy: Reviewing and editing.

Yusof Shuaib Ibrahim: Designed the study, carried out the sampling, drafted and completed the manuscript, and acquired funding.

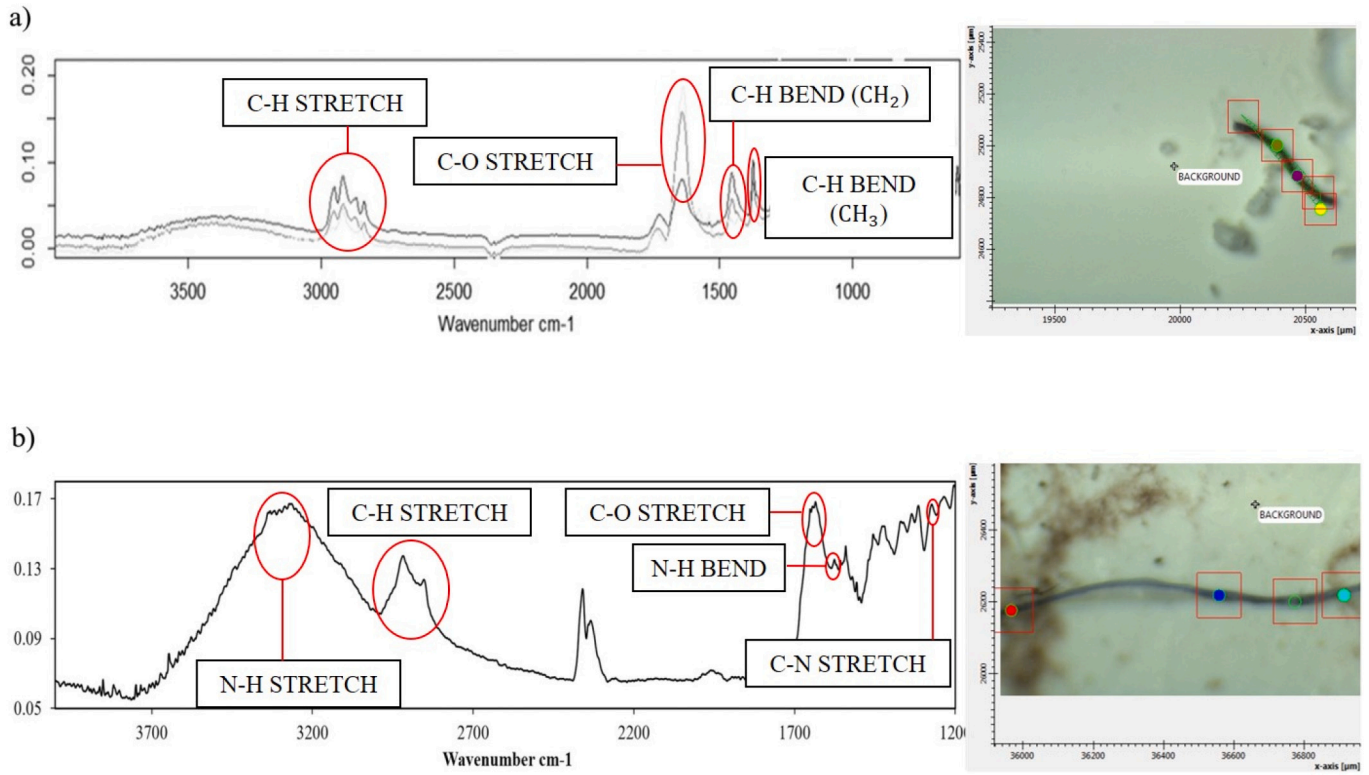

Fig. 7. μ-FTIR spectra for microplastic filaments: a) polypropylene and b) polyamide.

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.

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