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Occurrence of Microplastics in Lanchester's Freshwater Prawns *(Macrobrachium lanchesteri)*

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Abstract

Microplastics (MPs) are a contemporary societal concern that have been identified as a growing environmental hazard as a result of improper disposal. The goal of this research was to study the MPs found in Lanchester's freshwater prawn, *Macrobrachium lanchesteri*, in three different sites along the irrigation canal in central Thailand. In each site, twenty individuals (n = 20) of *M. lanchester* were examined for MP content. A total number of MPs in site 1 was 1093 items/20 ind. (range 35-80), with an average of 60.72 ± 10.54 items/individual. Site 2 was 1001 items/20 ind. (range 34-71), with an average of 50.05±8.71 items/individual, and site 3 was 1039 items/20 ind. (range 33-71), with an average of 51.95±10.62 items/individual. Among the various shapes, types, and colors of MPs identified, fiber and fragment, as well as blue, were the most common. The prawn had the greatest MPssize range of 200-250 µm in each point, followed by MPssize ranges of 250-500 µm and <100 µm. FT-IR data confirmed 1 particle of ethylene propylene rubber (EPR), 4 particles of polyethylene glycol (PEG), 1 particle of polyethylene terephthalate (PET), 1 particle of bis (2-ethylhexyl) phosphate, 1 particle of cellulose acetate, 2 particles of glyceryl polypropylene glycol ether, 1 particle of polyester (PE), and 1 particle of hydroxyethyl cellulose (HEC). The findings of this study provide detailed and useful information for a better understanding of MP contamination in the region, and *M. lanchesteri* is proposed as an appropriate species for monitoring MPs in freshwater ecosystems.

Introduction

Microplastics (MPs) are pervasive pollutants of emerging concern that have recently received a lot of attention due to their widespread presence in the environment and potential negative effects on living biota and human health. Plastic production has risen dramatically worldwide, rising from 1.5 million tonnes in the 1950s to 335 million tonnes in 2016 (Kumar et al., 2021). Plastics degrade into smaller pieces in the aquatic system as a result of weathering processes such as photo-degradation, oxidation, and mechanical abrasion

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(Andrady, 2011), and particles that have a length of less than five millimeters are known as microplastics (Frias and Nash, 2019). Aquatic ecosystems have been identified as being threatened by microplastics (Anderson et al. 2016). Wright et al. (2013) showed that MPs are accessible to a wide range of aquatic organisms because they are similar in size to planktonic organisms and other suspended particles. As particle size decreases, the possibility of bioaccumulation increases (Law and Thompson 2014). Contaminants that adsorb to MPs can increase their toxic effects (Rochman et al. 2013).

The genus *Macrobrachium* (Bate, 1868) is a genus of freshwater prawns of the family Palaemonidae, which are decapod crustaceans. Except for Europe, they can be found on all continents in the tropics and subtropics (Holthuis, 1993). Freshwater Prawns *Macrobrachium lanchesteri* is a small but hardy Thai native prawn that can be found in a variety of freshwater habitats, including rivers, streams, rice fields, lakes, ponds, and reservoirs. This prawn is a good swimmer, so it is less confined to the bottom than many palaemonids, and it is commonly found in large numbers in these habitats. This species has been found in temperatures ranging from 25.5 to 36.0°C and is capable of living in shallow waters with relatively high water temperatures for several hours. *Macrobrachium lanchesteri* is one of the top five economic species in Thailand and many other Southeast Asian countries. *M. lanchesteri* is a valuable economic resource for people living in rural areas of northeastern Thailand (Uraiwan & Sodsuk, 2004). They are consumed as native foods and used in a variety of forms, including shrimp paste, crispy shrimp, koi kung, and kung jom, a Northeastern Thai delicacy that generates approximately 240,000 baht per year (Rottanapradap, 2013). *M. lanchesteri* also has economic value in the aquatic environment and plays an important role in the food chain ecosystem (Thongmee et al. 2021).

Aquatic organisms can absorb MP particles easily (Li et al., 2016). The consumers or predators in the freshwater food chain, such as shrimp, can ingest MPs through prey items such as small crustaceans and arthropods, as well as fish larvae. As a result, the presence of MPs in freshwater organisms' gastrointestinal tracts has raised global concern, as freshwater food may be a major source of MPs in humans (Strungaru et al., 2019). The present study aims to assess the presence of MPs in the Lanchester's freshwater prawn (*Macrobrachium lanchesteri*) in the irrigation canal at Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom Province, Thailand. The findings will be the first of their kind, revealing microplastics in freshwater prawns.

Material and method

1. Sampling and preparation of samples

The freshwater prawn sample was collected in November 2020 from three different sites along the irrigation canal at Kasetsart University's Kamphaeng Saen Campus in Nakhon Pathom Province, Thailand (N 14°02′16, E 099o57′49). In each site, there are different types of vegetation coverings, and water passes through different areas. The water for this canal is supplied by the Mae Klong Dam. Irrigation canal water is a valuable water resource for the university, serving primarily as a source of drinking water and agricultural activities. Lanchester's freshwater prawns were collected in waterbodies using an aquatic dip net near macrophytes (edge habitats). There were a total of 60 prawns caught. The samples were transported in a cooler to the laboratory and stored at -20°C pending processing and analysis.

2. Extraction of microplastics from Macrobrachium lanchesteri

Before use, all sample processing equipment was rinsed with filtered deionized water. Within a week of being collected, prawn samples were processed. The frozen prawns were thawed in a stainless steel tray. The body weight of each specimen was measured and recorded. Individual prawns from each site was analyzed to assess MP ingestion in the prawns' whole body. To remove any microplastics from their exoskeletons, the prawns were rinsed with filtered deionized water. Individually, the prawn bodies were placed into 30 mL beaker. Following that, 20 mL of a hydrogen peroxide solution (30% H_2O_2) was added to each individual beaker to digest tissue (Ehlers et al., 2019). All beakers were then wrapped in parafilm and placed in an ES-20 environmental shaker-incubator at 150 rpm for 7 days. Microplastics with density separation were separated after 7 days. Each sample was transferred to a glass separation funnel, which was then filled with 16 g of potassium formate (99% HCO_2K). Using vacuum filtration, the solution was drained and filtered onto nylon membrane filters (pore size of 0.45 μm; diameter 47 mm). The filters were placed in small aluminum bowls, covered with aluminum foil, and dried in a drying cabinet (50°C) for two days.

3. Visual observation of the microplastics

The microplastics on each filter paper were observed and photographed using a stereomicroscope (Leica EZ4E). A visual examination was also performed to identify suspected microplastics based on morphological characteristics such as color and shape (Hidalgo-Ruz et al. 2012). Microplastic particles were classified based on their shapes, such as fiber, sphere, film (a thin and small layer) and fragment (part of a larger plastic item) (Su et al. 2016; 2018). For corroboratory FTIR analysis, representative suspected particles that were visually identified as potential plastics were chosen.

4. FT-IR analyses of microplastics found in *Macrobrachium lanchesteri*

The 20 microplastic particles were selected and identified using a Perkin Elmer Spectrum-Fourier transform infrared spectrometer (FT-IR). The spectrum ranged from 4000 to 500 cm^{-1}, with an 8 cm^{-1} spectral resolution. Polymer type analysis and functional group characterization were compared with spectra databases and instrument libraries, and the characteristic peaks of the functional groups were combined to determine the polymer type. As in previous studies (Bergmann et al. 2017), only particles with a hit quality of more than 700 were considered microplastics.

Fig. 1 depicts a summary of the protocol.

5. Statistical analyses

The abundance of microplastics was expressed as MP/individual±SD. Levene's test was used to verify the normal variance structure. One-way analysis of variance (ANOVA) was used to compare MP concentrations, types, and sizes between sites. MP concentration, type, and size were the dependent variables, and site was the independent variable. A post hoc HSD Tukey test (THSD) was used to identify significant differences between conditions that were established at $p < 0.05$. Statistica 20.0 software was used for all statistical analyses.

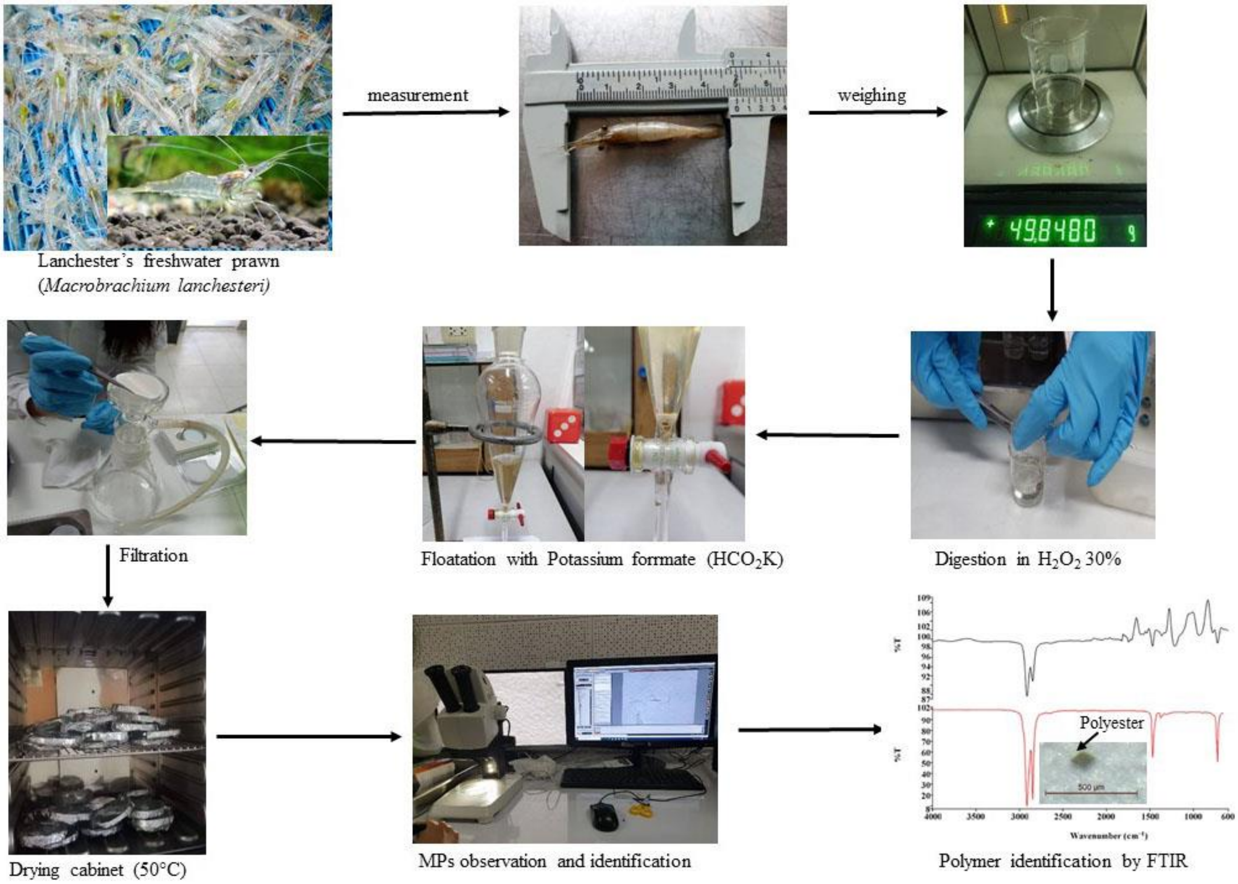

Fig. 1 Steps in the extraction of microplastics from the whole body of *Macrobrachium lanchesteri*

Results

1. Abundance of microplastics

Microplastic concentrations in Lanchester's freshwater prawn, *Macrobrachium lanchesteri* De Man, 1911, were determined for the first time. A total of n = 60 prawns were processed. The average body length of 20 prawns in site 1 was 2.96±0.82 cm, with an average weight of 0.27±0.08 g. The average body length of 20 prawns in site 2 was 3.18±0.61 cm, with an average weight of 0.30± 0.13 g. The average body length of 20 prawns in site 3 was 3.07±0.71 cm, with an average weight of 0.28±0.10 g. In site 1, the total number of MPs in prawns were 1093 item/20 ind. (range 35-80), with an average of 60.72±10.54 item/individual. In site 2, the total number of MPs in prawns were 1001 items/20 ind. (range 34–71), with an average of 50.05±8.71 items/individual. In site 3, the total number of MPs in prawns were 1039 items/20 ind. (range 33–71), with an average of 51.95±10.62 items/ individual (Table 1).

Table 1 Microplastic particle mean values and standard deviations in prawns in three irrigation canal sites. Asterisks indicate significant differences in the size, type, and color of microplastics in each prawn ($p < 0.05$; one-way ANOVA followed by the Tukey HSD test)

Microplastic properties	Site 1 (N =20)	Site 2 (N=20)	Site 3 (N=20)
Mean body length (cm)	2.96±0.82	3.18±0.61	3.07±0.71
Mean body weight (g)	0.27±0.08	0.30±0.13	0.28±0.10
Total number of MPs (item)	1093 (range 35-80)	1001 (range 34-71)	1039 (range 33-71)
	(60.72±10.54)	(50.05±8.71)	(51.95±10.62)
Size			
<100 µm	11.64±4.18^{a}	6.94±1.58^{a}	6.40±3.18^{b}
200-250 µm	22.66±4.78^{a}	20.50±2.21^{a}	21.45±5.04^{a}
250-500 µm	19.11±4.89^{a}	16.90±5.31^{a}	15.70±4.20^{a}
>500 µm	7.94±4.00^{a}	7.11±3.77^{a}	8.84±4.07^{a}
Type			
Fiber	22.16±5.07^{a}	18.50±5.91^{a}	27.05±5.67^{b}
Fragment	21.66±4.24^{a}	19.60±4.27^{a}	21.10±5.56^{a}
Sphere	16.88±6.41^{a}	11.95±3.60^{b}	6.06±3.47^{c}
Color			
Blue	20.61±4.77^{a}	18.45±4.13^{a}	18.65±3.92^{a}
Violet	10.27±3.12^{a}	7.16±3.11^{b}	7.06±3.64^{b}
Red	13.77±4.13^{a}	11.55±5.11^{a}	12.15±4.71^{a}
Transparent	15.61±4.25^{a}	15.52±6.56^{a}	15.50±6.74^{a}

Remark: *Values with different letters indicate significant mean difference following Turkey post hoc tests ($P<0.05$)

2. Microplastic particle size

The MPs were identified and categorized into four sizes: <100 µm, 200–250 µm, 250–500 µm, and > 500 µm (Table 1, Fig. 2). The prawns in site 1, 2, and 3 had the greatest size MPs range of 200-250 µm, followed by MPs size range of 250-500 µm and MPs size range of <100 µm. The largest MPs size ranged over 500 µm in site 3. In site 3, the MPs size ranged from <100 µm was significantly different (Table 1, Fig. 2).

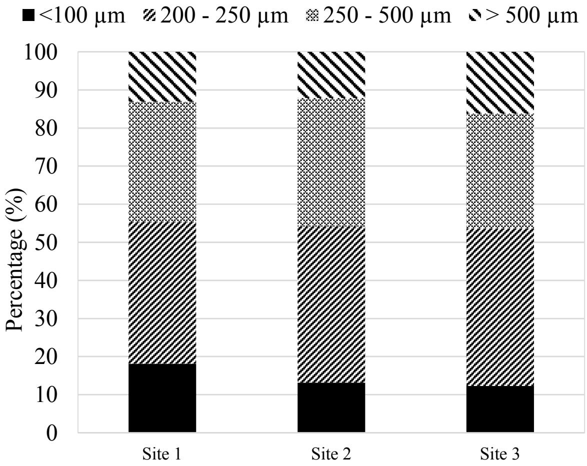

Fig. 2 Percentages of different sizes of microplastics in prawns at each sampling site

3. Color and type of microplastics

Fiber and fragment MPs were detected in the majority of prawns from all sampling sites (72–91%), with sphere MPs being less common (9–28%) (Table 1, Figs. 3-6). The relative abundance of MPs sphere shape was significantly different in all sampling sites, and the abundance of MPs fiber shape was significantly different in site 3 (Table 1).

All the prawns in each sampling site were found to be of five different colors, with blue being the most common, followed by white (transparent), red, violet, and green. The green color was detected in small amounts of prawns in site 1, while not appearing in prawns from sites 2 and 3. (Fig. 3, 5). The MPs' violet color varied significantly between prawns at each sampling site (Table 1, Fig. 7).

4. Identification of polymers by FT-IR

FT-IR spectroscopy was used to characterize MPs, and the 12 items analyzed were Ethylene propylene rubber (EPR) (1 particle), Polyethylene glycol (PEG) (4 particles), Polyethylene terephthalate (PET) (1 particle), bis(2-ethylhexyl) phosphate (1 particle), Cellulose acetate (1 particle), Glyceryl polypropylene glycol ether (2 particles), Polyester (PE) (1 particle), and Hydroxyethyl cellulose (HEC) (1 particle) (Figs. 8-10).

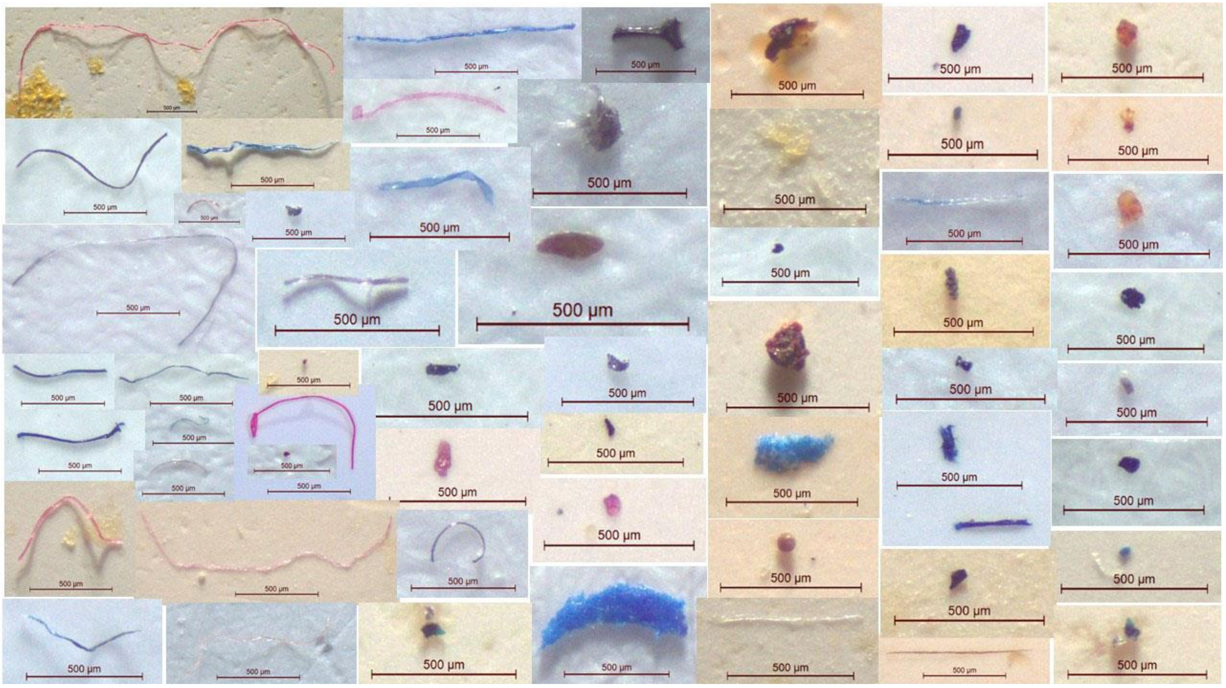

Fig. 3 An example of microplastic type, size, and color in Lanchester's freshwater prawn *(Macrobrachium lanchesteri)* in site 1

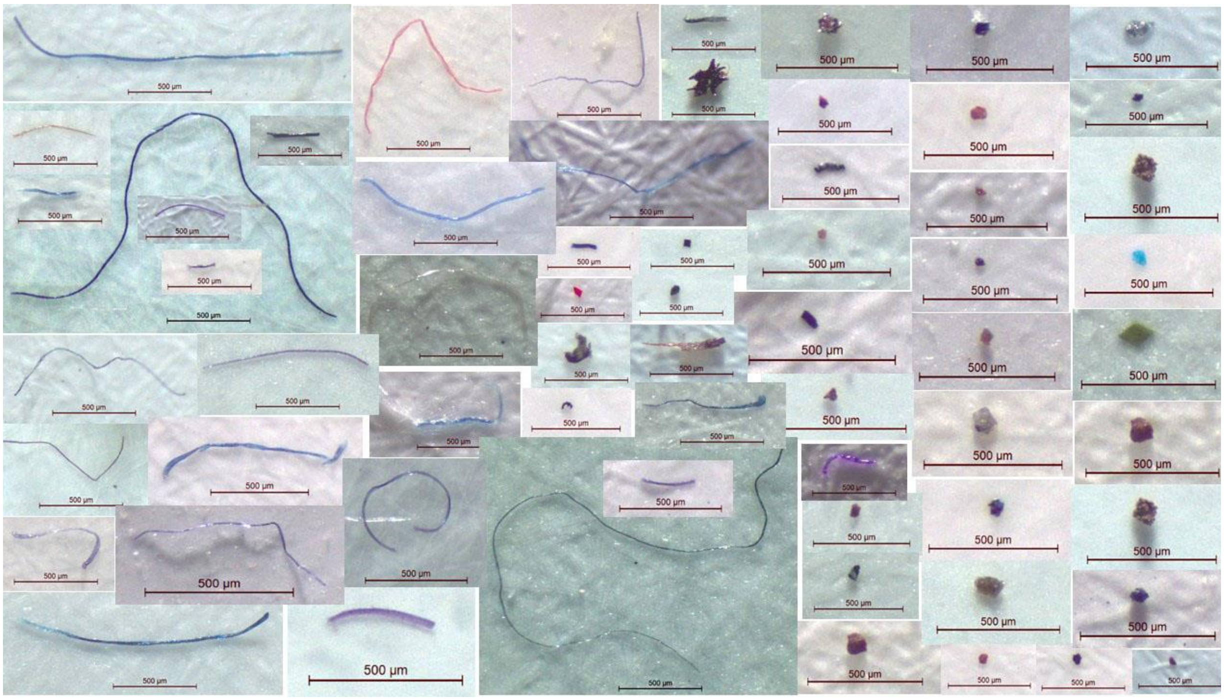

Fig. 4 An example of microplastic type, size, and color in Lanchester's freshwater prawn *(Macrobrachium lanchesteri)* in site 2

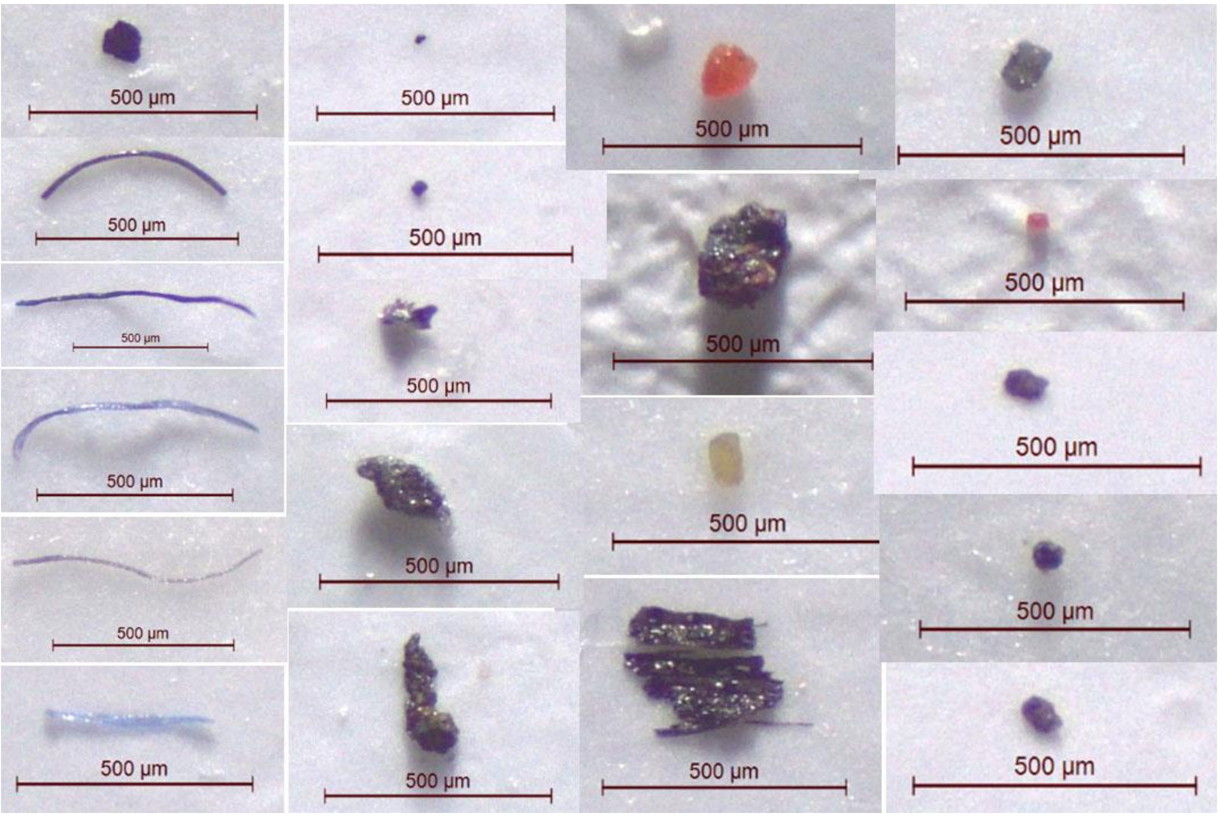

Fig. 5 An example of microplastic type, size, and color in Lanchester's freshwater prawn *(Macrobrachium lanchesteri)* in site 3

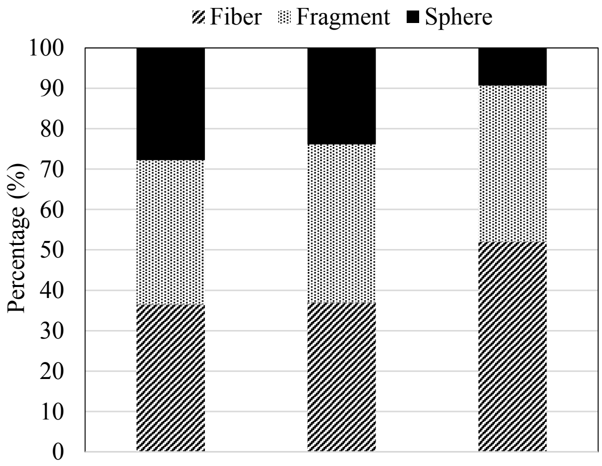

Fig. 6 Percentages of different microplastic types in prawns at each sampling site

Fig. 7 Percentages of different colours of microplastics in prawns at each sampling site

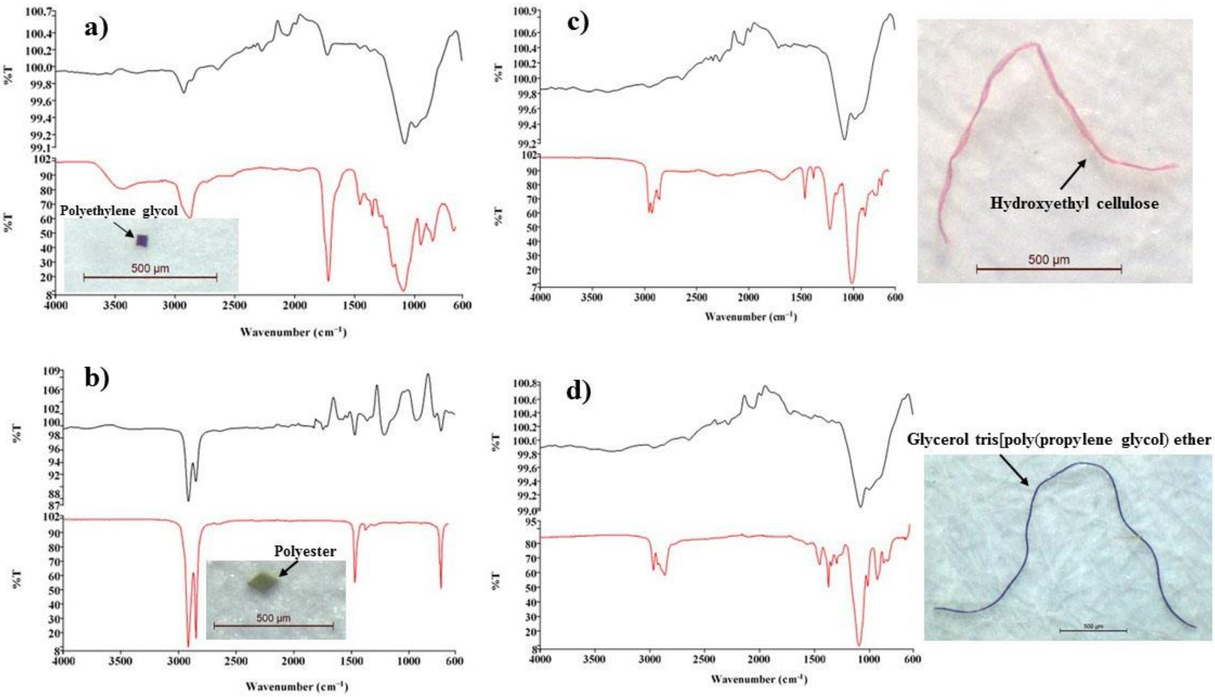

Fig. 8 An FT-IR spectra of representative microplastic polymers found in prawns in site 2

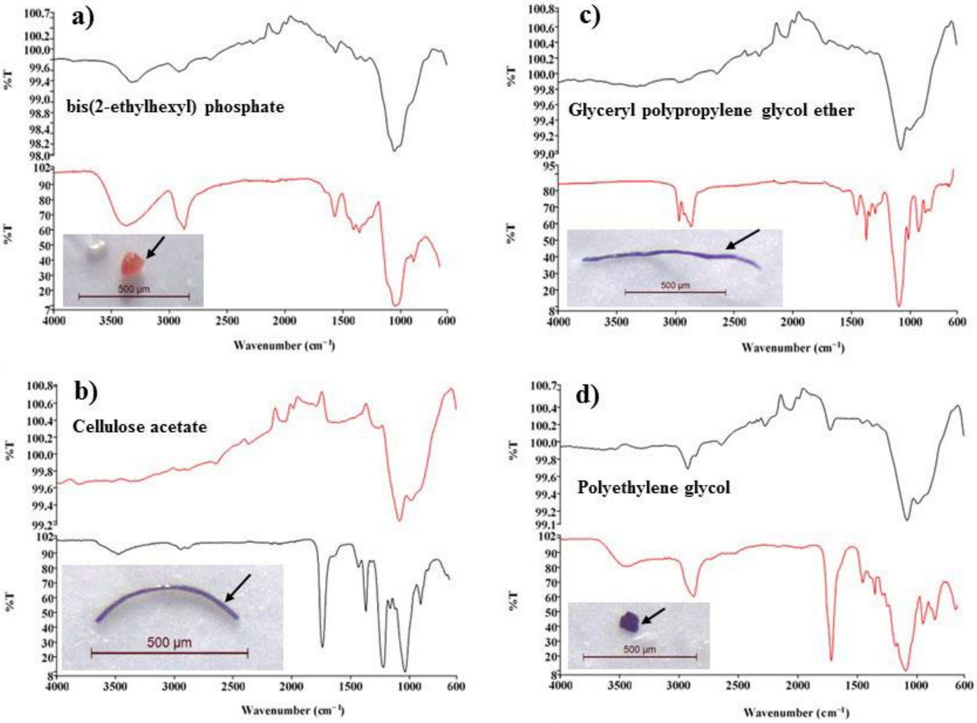

Fig. 9 An FT-IR spectra of representative microplastic polymers found in prawns in site 3

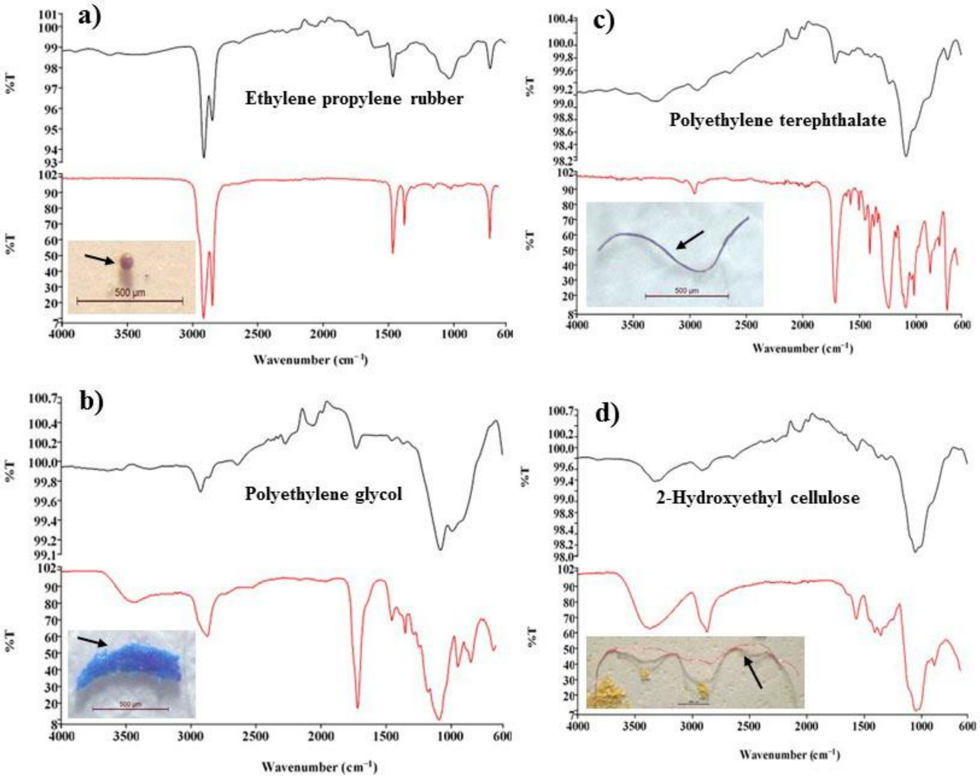

Fig. 10 An FT-IR spectra of representative microplastic polymers found in prawns in site 1

Discussion

This research offers a better understanding of microplastic contamination in Lanchester's freshwater prawns as well as a new approach to conducting microplastic biomonitoring investigations in the field with healthy animals. Some species of invertebrates have been identified as potentially good bioindicators of microplastic pollution in their individual ecosystems based on their life-history strategies (Abbasi et al., 2018; Avio et al., 2017; Ory et al., 2017; Sanchez et al., 2014). There is still a deficit in understanding freshwater creatures as compared to studies on microplastic pollution in marine organisms. It's the first time a freshwater crustacean has been used as a bioindicator to measure microplastic pollution. *Macrobrachium lanchesteri* De Man, 1911, is a common crustacean in Thailand's freshwater ecosystems and could be a good bioindicator for microplastic pollution monitoring.

The results show that prawns living in the irrigation canal had MP particles in their entire body burden. This is consistent with previous reports of MP pollution in organisms from different climate zones. Microplastics, for example, were found in 63% of brown shrimp from shallow water habitats in the southern North Sea (Devriese et al., 2015). MPs were discovered in 36% of the glass shrimp *Paratya australiensis* (Family Atyidae) found in fresh waterbodies in eastern Australia (Nan et al., 2020). This study found MPs in sampling site 1 were 1093 items/20 ind., MPs in sampling site 2 were 1001 items/20 ind., and MPs in sampling site 3 were 1039 items/20 ind., indicating that prawns in the irrigation canal are not free of MP pollution. Recently, Reunura & Prommi (2022) detected microplastics in the gastrointestinal tract (GT) of *Litopenaeus vannamei* (Penaeidae) and *Macrobrachium rosenbergii* (Palaemonidae) in a cultured pond in the central part of Thailand. Female and male *M. rosenbergii* and *L. vannamei,* in particular, had an average of 33.31±19.42, 33.43±19.07, and 11.00±4.60 MP items/individual in their GTs. Furthermore, they had 32.66±5.10, 32.14±4.85, and 10.28±1.19 MP items/ gram of intestinal material, respectively.

M. lanchesteri is one of Thailand's top five economic species, as well as beingsignificant in many other Southeast Asian countries. *M. lanchesteri* is an important economic resource for local people in rural areas in Northeastern Thailand (Uraiwan & Sodsuk, 2004). *M. lanchesteri* is a valuable economic resource for those living in rural areas of northeastern Thailand (Uraiwan & Sodsuk, 2004). It is eaten as a native cuisine and is utilized in a variety of dishes, including shrimp paste, crispy shrimp, koi kung, and kung jom, a local delicacy in Northeastern Thailand that generates about 240,000 baht per year (Rottanapradap, 2013). The gastrointestinal tract is not removed when these prawns are consumed because they are typically consumed fresh. *M. lanchesteri* also plays a vital part in the aquatic food chain environment, which has economic significance (Thongmee, 2012).

M. lanchesteri feeds on diatoms, small aquatic insects, and detritus. The majority of these prey animals ingest MPs and contribute to trophic transfer to prawns and other predators, including fish, cetaceans, seabirds, and humans (Guebert-Bartholo et al., 2011; Devriese et al., 2015; Amelineau et al., 2016; Teng et al., 2019). At sampling site 1, MP abundance in prawns was 60.72±10.54 items/individual, 50.05±8.71 items/individual at sampling site 2, and 51.95±10.62 items/individual at sampling site 3, which is comparable to MP abundance in two types of shrimp (*Metapenaeus monocerous,* and *Penaeus monodon*) (Hossain et al., 2020).

Fiber and fragment were the most common shapes in the ingested MPs, accounting for 72–91% of all prawns. In Bangladesh's northern bay of Bengal, tiger shrimp were found in 57% of occurrences and brown shrimp in 32% of occurrences (Hossain et al., 2020). According to other results (Browne et al. 2011; Claessens et al. 2011), fishing nets, ropes, and lines, laundry, and urban debris are all likely sources of fibers and fragments in an irrigation canal.

In this investigation, five different colors of MPs were detected in prawn samples, including blue, white (transparent), red, violet, and green, which is similar to Hossain et al. (2020). Other studies have reported that MP items are black (Bellas et al., 2016), white/transparent (Boerger et al., 2010), and blue (Ory et al., 2017). Fiber form MPs were also found in both shrimps (57–58%), which is consistent with previous findings in brown shrimp (Devriese et al., 2015) and decapod crustaceans (Murray and Cowie, 2011).

All MPs in the <100 μm, 200-250 μm, 250-500 μm, and > 500 μm size ranges were found in all prawns. The variance in MP size could be explained by food selectivity and irrigation canal habitat variables.

Based on FT-IR examination, 12 of the 20 randomly selected particles were found to be plastic, while the remaining 8 were found to be non-plastic; of the 12 MP particles, four were polyethylene glycol (PEG), two were glyceryl polypropylene glycol ether, and one was each of ethylene propylene rubber (EPR), polyethylene terephthalate (PET), bis (2-ethylhexyl) phosphate, and cellulose acetate, polyester (PE) and hydroxyethyl cellulose (HEC) polymers. Polyethylene glycol, a common raw material in the production of facial foam, skin cleaners, soap, and dishwashing liquid, may contribute to the accumulation of freshwater organisms. According to the findings of Steer et al., rayon particles can be found in used clothing, furniture, feminine hygiene items, and nappies (2017). However, because prawns are commonly consumed without having the gastrointestinal system or exoskeletal structures removed, MPs detected in prawns may be passed to humans through the food chain. The presence of MPs in prawn species means that future research into the effects of MPs in Thailand's freshwater environment would need to include a wider range of species and habitats.

Conclusions

This study provides preliminary information on the occurrence of microplastics (MPs) in *Macrobrachium lanchesteri,* a Thai native prawn species. All sampled prawns individually were found to have microplastics in varying abundances. The findings indicate that microplastics were widely distributed throughout Thailand. The prawns in this study were found to ingest primarily microplastics of a specific type and color, as well as a wide range of microfiber sizes. As a result, they may be a viable technique for future biomonitoring of microplastic pollution in the biota of the environment. The data from this first study could be used as reference or baseline data for future extensive research.

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