International Journal of Agricultural Technology 2023 Vol. 19(1): 83-98
Available online http://www.ijat-aatsea.com
ISSN 2630-0192 (Online)

Accumulation of microplastics in stomach, intestine, and tissue of two shrimp species (*Metapenaeus moyebi* and *Macrobrachium rosenbergii*) at the Khlong U-Taphao, southern Thailand

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Jitkaew, P., Pradit, S., Noppradit, P., Sengloyluan, K., Yucharoen, M., Suwanno, S., Tanrattanakul, V., Sornplang, K., Nitiratsuwan, T. and Geindre, M. (2023). Accumulation of microplastics in stomach, intestine, and tissue of two shrimp species (*Metapenaeus moyebi* and *Macrobrachium rosenbergii*) at the U-Taphao Canal, southern Thailand. International Journal of Agricultural Technology 19(1): 83-98.

Abstract The findings revealed that fibre was the most prevalent type of microplastic found in *Metapenaeus moyebi*, while fragment was the most prevalent type in *Macrobrachium rosenbergii*. The majority of MPs found in *Metapenaeus moyebi* was less than 100 µm in size, whereas *Macrobrachium rosenbergii* are larger than 1 mm. This study discovered MPs in a variety of colours, including blue, black, deep blue, red, and green. The amount of microplastics found in the three organs of two shrimp species did not differ significantly ($p > 0.05$). There was no correlation between microplastic and *Metapenaeus moyebi* length ($p > 0.05$), but there was in *Macrobrachium rosenbergii* ($p < 0.05$; $R > 0.5$).

Keywords: Microplastic, River, Shrimp, Seafood

Introduction

Plastic products are majorly produced to increase consumer convenience. These include carrier bags, trash bags, portable glasses, straws, book covers, and a myriad of other products. Plastic debris, marine wastes and microplastic are currently important problems of Thailand, ASEAN and the world When

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plastic products are no longer needed and reached the end of their lifecycle, they are being disposed in trash bins, although some can be recycled. The volume of plastic waste is increasing and only 14% of plastic packaging is currently recycled, while a huge number of plastic products are discarded into the ecosystem (Pradit *et al*., 2020). Large pieces of plastic waste including bottles, glasses, fishing nets and even shoes can be found in freshwater ecosystems, such as rivers and canals. As time passes, this plastic waste decomposes from environmental factors such as wave action and sunlight which becomes much smaller (< 5 millimetres) and this is known as "microplastics" (Andrady, 2011).

Microplastics are small pieces of plastic which are usually less than 5 millimetres in size (Arthur *et al*., 2009). It can be divided into two types which are primary and secondary microplastics. Primary microplastic is tiny particles designed for commercial use. Secondary microplastic is particles from the breakdown of larger plastic items into smaller plastics less than 5 millimetres in size. It is considered a type of marine debris when it contaminates the ocean in widespread and is difficult to manage. Currently, microplastic contamination is found in almost every kind of marine organism, including shrimps (Pradit *et al*., 2021), bivalves (Li *et al*., 2015), crabs (Wang *et al*., 2021), and fish (Azad *et al*., 2018).

Microplastics contamination in river and marine habitats is a severe management issue that is challenging to eliminate. The real cause of secondary microplastic contamination derives from wastewater discharged that usually comes from communities, agricultural land, and industrial facilities (Honingh *et al*., 2020). This microplastic pollution has prolonged accumulation in the environment for a long period of time and transfers the contaminants from freshwater sources to the marine ecosystems. This will later accumulate in the marine organisms that humans consume for food, such as mackerel (Herrera *et al*., 2019), and freshwater shrimps (Nan *et al*., 2020). By entering through the food chain, microplastics affect both living organisms and humans (Daniel *et al*., 2021).

Khlong U-Taphao in Songkhla Province in the Southern Thailand is a waterway which belongs to a sub-watershed of the Songkhla Lake Basin, located in the west of the city Hat Yai. This is where many branches of canals flow into the watershed and the border of high mountain ranges from the west, south, and east to Songkhla Lake in the north. Khlong U-Taphao watershed is used as a water-distribution canal from Khlong Sadao Reservoir to Songkhla Lake. Khlong U-Taphao is very meandering in the upper part of the watershed but becomes straight when entering into the lower basin. Most of the watershed areas are plain land which are suitable use for agriculture, industry, commercial businesses, and urban communities. It is most likely a source of water for consumption and industry usage but this watershed receives wastewater which is discharged from Hat Yai City Municipality. Therefore, the untreated discharged wastewater may cause the release of toxic contaminants and pollutants into the freshwater environment and this will lead to sensitive changes to the ecosystem. Besides that, Khlong U-Taphao plays an important role by preventing flooding to occur in Hat Yai district. There are plenty of economic aquatic animals found throughout the year, including Moyebi shrimps (*Metapenaeus moyebi*) and giant freshwater prawns (*Macrobrachium rosenbergii*) in this watershed. They can live in both estuaries and freshwater, and these two species are the most important sources for fishermen to make a decent living.

This study focused on microplastic contamination in Moyebi shrimp (*Metapenaeus moyebi*) and giant freshwater prawns (*Macrobrachium rosenbergii*) living downstream of Khlong U-Taphao. The study investigated the accumulation of microplastics in the organs of both types of shrimp and raised awareness to the public about microplastic pollution.

Materials and methods

Sample collection

Samples were randomly collected in February 2022 from fishermen living in the downstream areas of Khlong U-Taphao, Songkhla Province (662429E, 786694N) (Figure 1). The samples were consisted of *Metapenaeus moyebi* and *Macrobrachium rosenbergii* (Figure 2), and were stored in a freezer at -20°C for subsequent laboratory analysis.

Microplastic analysis

The frozen *Metapenaeus moyebi* and *Macrobrachium rosenbergii* samples were thawed by leaving them at room temperature. The shrimp and prawn samples were measured to ascertain their total length, carapace length and total weight. Their heads and bodies were separated and divided into three parts, stomach, intestine, and tissue. Each part was digested by 50 ml of 10% potassium hydroxide solution for 12 hours. During digestion, aluminium foils were used as a lid cover to cover the samples. The obtained solution from the digestion was filtered by a 20 µm nylon mesh cloth and oven dried at 50°C to remove moisture.

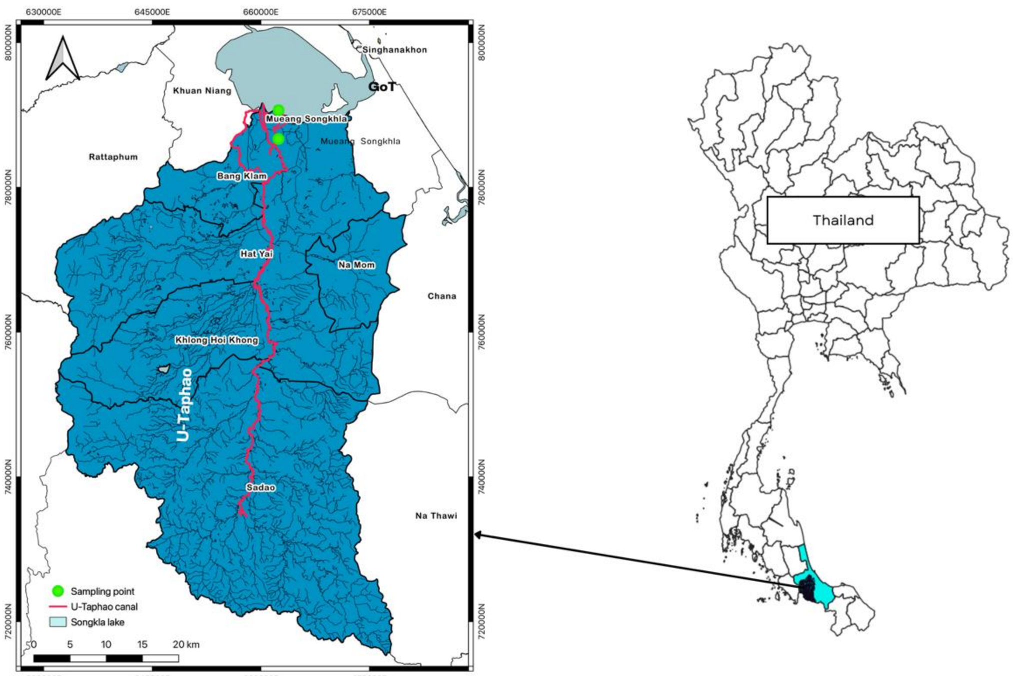

Figure 1. The study area of Khlong U-Taphao, Songkhla Province, Southern Thailand

(a) (b)

Figure 2. Prawn and shrimp samples **(a)** Giant freshwater prawn (*Macrobrachium rosenbergii*) and **(b)** Moyebi shrimp (*Metapenaeus moyebi*)

Observation and detection of microplastics

Microplastic samples left in the nylon mesh cloth were counted and measured. The colours and shapes were identified under a Leica EZ4 W Stereomicroscope (Leica, Germany). It operates with a Leica Application Suite to record images of microplastic samples. Microplastic polymer measurement was performed by randomly selecting microplastic samples from the organs of each types of shrimp, and the polymer type analysed using Fourier Transform Infrared Spectroscopy (FTIR), with 4000 to 400 cm^{-1} wavelength and 4 cm^{-1} resolutions. The microplastic spectrum found in the shrimp samples was compared to the spectrum standard of each type of polymer.

Quality control of experiments

A blank test was conducted in a laboratory by placing Petri dishes containing distilled water in a laboratory. At the end of the experiment, no microplastic was found in the Petri dish.

Data analysis

Data were analyzed on microplastics abundance, size, colour, and shape using Microsoft Excel (Office Professional Plus 2019, Microsoft, USA) and data presented as mean ± standard error (items/individual). An independent t-test was performed to compare the differences of microplastics between the moyebi shrimps and giant freshwater prawns. One-way ANOVA statistical testing was compared each organ of both shrimp types. Pearson correlation was confirmed the association between microplastics abundance in the shrimps' stomach, intestine, tissue, TL (Total Length), BW (Body Weight), and LI (Length of Intestine). A significance level was set at $p < 0.05$.

Results

Abundance of microplastics in stomach, intestine, and tissue of shrimps

According to the analysis of 102 samples (stomachs = 34; intestines = 34; tissue = 34) from 17 *Metapenaeus moyebi* and 17 *Macrobrachium rosenbergii*, microplastics were found in all organs of two shrimp species. The average length and body weight in *Metapenaeus moyebi* were 7.94 ± 0.19 cm and 4.48 ± 0.29 g, respectively. They contained microplastics with an average of 14.76 ± 1.98 items / individual. Microplastics were found in their stomachs, intestines, and tissue with an average of 6.35 ± 1.37, 4.12 ± 0.69, and 4.29 ± 0.83 items/individual, respectively. The average length and body weight in *Macrobrachium rosenbergii* were 18.47 ± 0.69 cm and 71.66 ± 9.52 g, respectively. They contained microplastics with an average of 11.24 ± 1.74 items/individual. Microplastics were found in the stomachs, intestines, and tissue with an average of 3.00 ± 0.73, 2.94 ± 0.77, and 5.29 ± 0.93 items/individual, respectively. The data of microplastics accumulation in the stomach, intestine, and tissue of both types of shrimp were presented in Table 1. There were no statistically significant differed ($p > 0.05$) in the quantity of microplastics in each type of shrimps. The quantity of microplastics found in each organ was based on statistical analysis.

Table 1. Data of the shrimp size measurement and the quantity of microplastics found in each organ of the shrimps

Species of shrimp	Measure shrimp size			MPs (items/organ)			MPs (items/ind)
	TL (cm)	BW(g)	LI (cm)	ST	IN	TI	
Moyebi shrimp *(Metapenaeus Moyebi)*	7.94 ± 0.19	4.48 ± 0.29	4.38 ± 0.18	6.35 ± 1.38	4.12 ± 0.69	4.29 ± 0.83	14.76 ± 1.98
Giant freshwater prawns (*Macrobrachium rosenbergii)*	18.47 ± 0.69	71.66 ± 9.52	7.98 ± 0.46	3.00 ± 0.73	2.94 ± 0.77	5.29 ± 0.93	11.24 ± 1.74

TL = Total length, BW = Body weight, LI = Length of intestine, ST = Stomach, IN = Intestine, TI = Tissue.

Shape, size and colour of microplastics in shrimp

The characteristics of microplastics in both of shrimps species were presented in Figure 3. Microplastics were found in *Metapenaeus moyebi* which comprised of 122 items of fibre (48.61%) and 129 items of fragments (51.39%). Microplastics were found in *Macrobrachium rosenbergii* which comprised of 103 items of fibre (53.93%) and 88 items of fragments (46.07%) as illustrated in (Figure 3).

The quantity and size of microplastics found in *Metapenaeus moyebi* resulted in 101 items (40.24%) of microplastics which were smaller than 100 microns (µm), 80 items (31.87%) were 100 µm – 500 µm sized, 31 items (12.35%) were 500 µm to 1 mm sized, and 39 items (15.54%) were microplastics bigger than 1 millimetre (mm). Whereas, the quantity and size of microplastics found in *Macrobrachium rosenbergii* resulted in 39 items (20.42%) of microplastics which were smaller than 100 µm, 58 items (30.37%) of 100 µm – 500 µm sized, 33 items (17.28%) were between 501 µm – 1 mm, and 61 items (31.94%) of microplastics were bigger than 1 mm.

According to analysis of microplastic size, those smaller than 100 µm were found in greater numbers in *Metapenaeus moyebi* than in *Macrobrachium rosenbergii.* Other sizes were 100 µm – 500 µm, 500 µm – 1 mm, and greater than 1 mm in both shrimps and prawns, as illustrated in Figure 4. Figure 5 depicts the colors of microplastic discovered in this study. Blue microplastics were the most commonly discovered, followed by black and other colors.

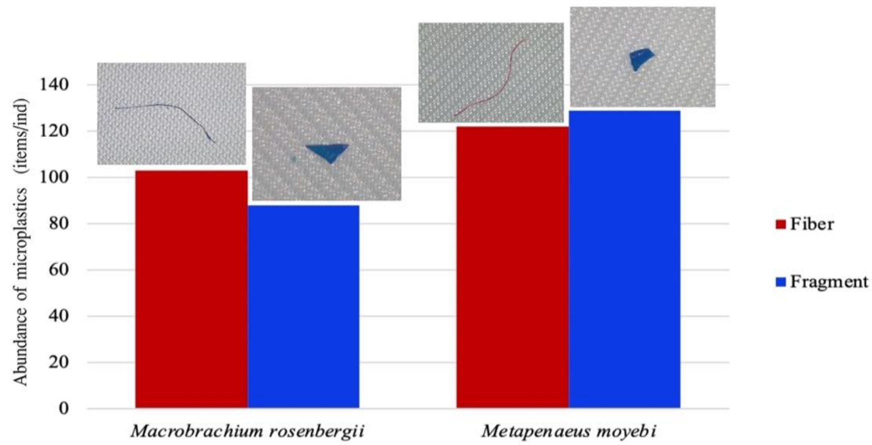

Figure 3. Fiber and fragment types of microplastics found in Moyebi shrimps (*Metapenaeus moyebi*) and giant freshwater prawns (*Macrobrachium rosenbergii)*

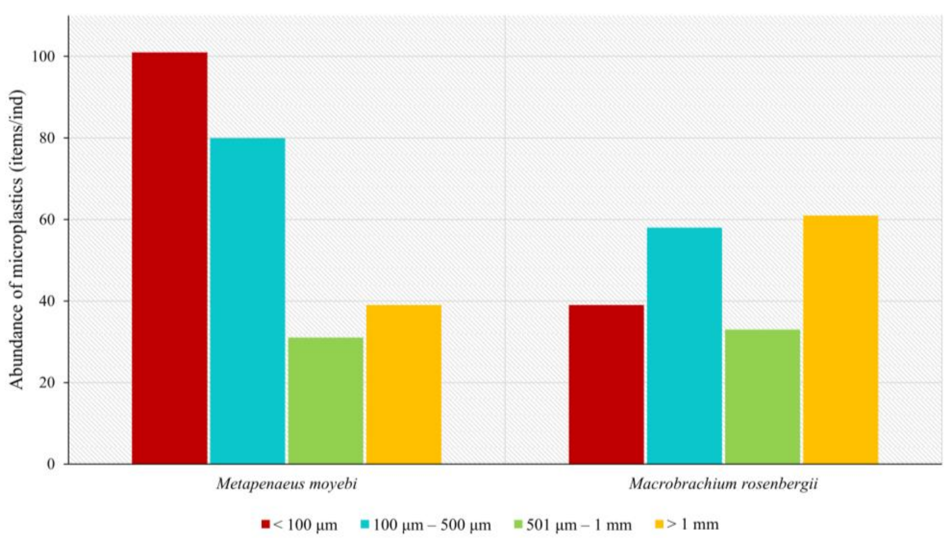

Figure 4. Quantity of microplastics in varied sizes found in moyebi shrimps (*Metapenaeus moyebi*) and giant freshwater prawns (*Macrobrachium rosenbergii*)

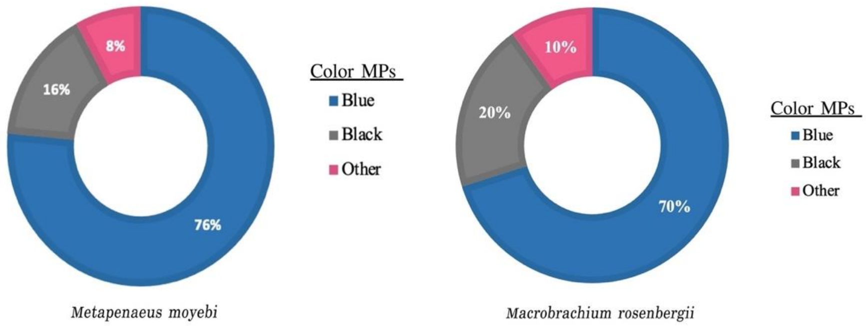

Figure 5. Proportion of colours of microplastics found in moyebi shrimps (*Metapenaeus moyebi*) and in giant freshwater prawns (*Macrobrachium rosenbergii*)

Polymer identification in shrimp

The identification of microplastic polymers using FTIR resulted in five types of polymers identified according to the analysis of microplastic samples randomly selected from the stomach, intestine, tissue of *Metapenaeus moyebi* and *Macrobrachium rosenbergii*. In this study, the polymers found were Rayon 32%, Polypropylene (PP) 27%, Polyethylene Terephthalate (PET) 18%, Poly (Ethylene: Propylene) 18%, and Polyester 5% as shown in Figure 6 and Figure 7. PP, Poly (Ethylene: Propylene), PET, and rayon were found in both shrimps whereas polyester was found only in giant *Macrobrachium rosenbergii*.

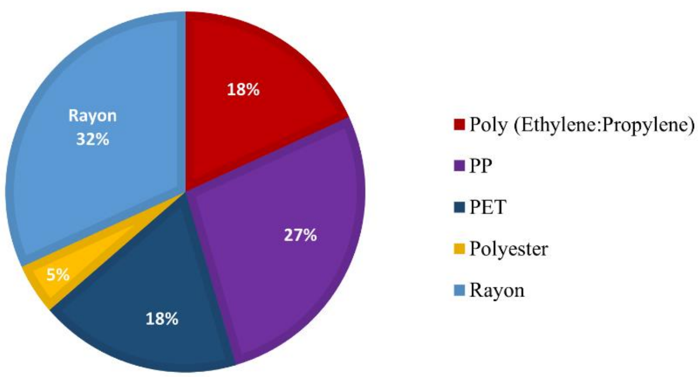

Figure 6. Microplastic polymers detected in moyebi shrimps (*Metapenaeus moyebi*) and giant freshwater prawns (*Macrobrachium rosenbergii*)

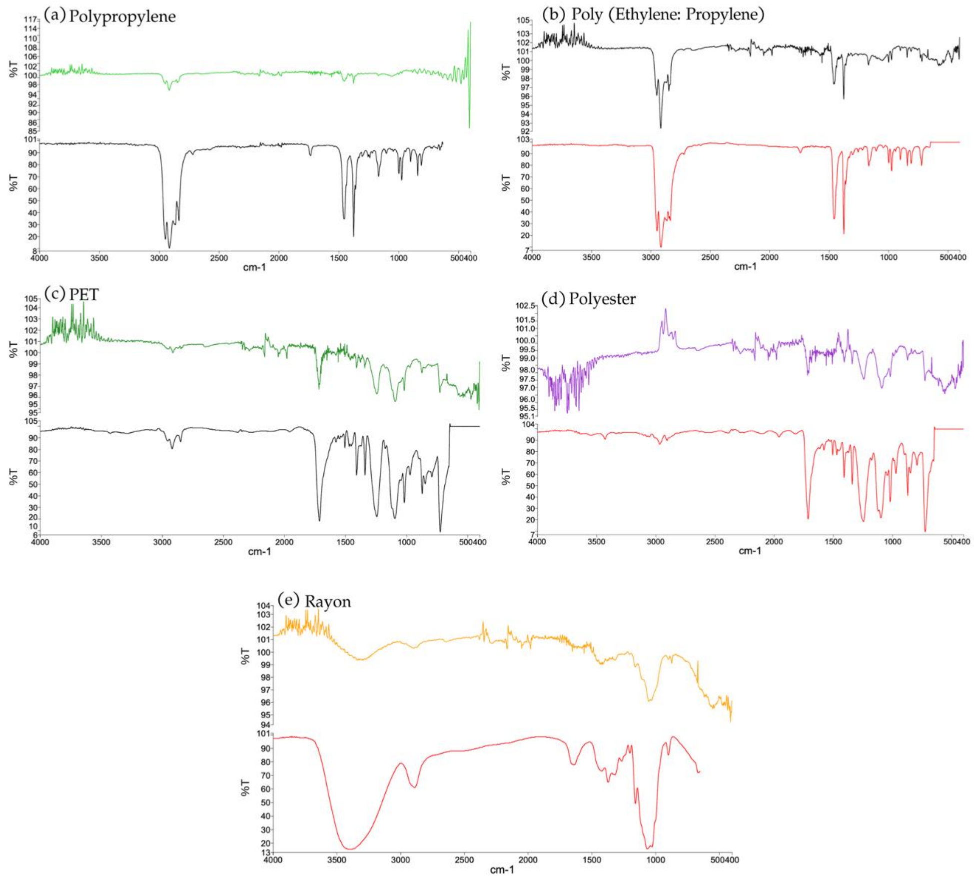

Figure 7. Spectrum of polymers: (a) PP, (b) Poly (Ethylene: Propylene), (c) PET, (d) Polyester and (e) Rayon. Above the line: Sample shrimp. Below the line: spectrum reference

Discussion

Generally, invertebrates have the capacity to ingest microplastics which can accumulate in their body. Therefore, microplastic contaminants have accumulated in the stomachs, intestine, and tissue of both shrimp and prawn species. The microplastic shapes found in this study were mostly fibres and fragments in both types of shrimp and this result is consistent with a study done by Hossain in 2020, which found fragmented microplastics in brown shrimp (small shrimp) and fibre microplastics in tiger prawn (large shrimp) (Hossain *et al*., 2020). The study area's potential sources of microplastic include industrial and domestic effluent, agricultural practices, and fishing, all of which have been widely reported (Azizi *et al*., 2021; Kelly *et al*., 2019; Pradit *et al*., 2021).When this study is compared to others (Table 2), the incidence of microplastic varies slightly from location to location, which could be due to the different species of shrimp and their environment. The color of microplastics in this study showed a preference for blue and black in both shrimp species, which is similar to what has been reported by Ogunola *et al.* (2022), Daniel *et al.* (2020), Gurjar *et al.* (2021), Yan *et al.* (2021), and Hossain *et al.* (2020). Colors in microplastics can be derived from original materials such as blue clothing lint and blue fishing nets. The size of microplastic varied between shrimp species and organisms. Microplastics are typically less than 500 m in size. This demonstrates that the size of ingestion is most likely determined by shrimp species, habitat, and feeding habits.

Table 2. Shows microplastic accumulation in shrimps form various studies

Shrimp/organism	Location	Method	Abundance of microplastics	Color	Shape	Size	Polymer	Ref.
King Prawn, Tiger Prawn, Banana Prawn/gut and tissue	Australia	12-24 h with 10% KOH	0.8 ± 0.1 pieces/ind	Blue, black, transparent, and red	Fiber and fragment	38 µm – greater than 1 mm	Polyolefin, PS, PE, PP, polyamide, polyacrylate	Ogunola *et al.* (2022)
Parapenaeopsis hardwickii/ tissue *n = 10 - 20*	Xiangshan, China	24 h with 10% KOH and 30% H_2O_2	1.0 MPs/ind or 0.25 MPs·gWW^{-1}	No color range	Fiber and films	< 500 – 1000 µm	Cellulose, polyamide, acrylonitrile, PE, PP, PET	Wu *et al.* (2020)
Fenneropenaeus indicus, n = 330	oastal waters of Cochin, India.	24 h with 10% KOH	0.39 ± 0.6 microplastics/shrimp 0.04 ± 0.07 microplastics / g	Red, blue, black, transparent, green	Fiber, fragment	157 – 2785 µm	Polyamide, polyester, polyethene, PP	Daniel *et al.* (2020)
Metapenaeus monoceros / gastrointestinal tract	north eastern Arabian Sea	30 min with 69% HNO_3	7.23±2.63 items per individual. 78.48 ± 48.37 MPs per gram of the gut material	Blue, translucent, black, red	Fiber, fragment, pellet, film, beads	< 100 µm, - greater than 1000 µm	PE, PP, PA, nylon, PES, PET	Gurjar *et al.* (2021)

Shrimp/organism	**Location**	**Method**	**Abundance of microplastics**	**Color**	**Shape**	**Size**	**Polymer**	**Ref.**
Parapeneopsis stylifera (n = 50) / gastrointestinal tract			5.36 ± 2.81 items per individual. 64.79 ± 24.58 MPs per gram of the gut material					
Penaeus indicus (n = 70) / gastrointestinal tract			7.40 ±2.60 items per individual. 47.5 ± 38.0 MPs per gram of the gut material					
Litopenaeus vannamei/ Intestine, n = 54	Zhuhai City, China	KOH (10%, V/V)	14.08 ± 5.70 items/g w.w.	Blue, transparent, yellow, red	Fiber, film. fragment	<0.5 mm – 3 mm	Cellulose, PE, PP, PA, polyester	Yan *et al.* (2021)
Parapenaeopsis hardwickii, n = 18	Songkhla Lake, Southern Thailand	12 h with 10% KOH	4.11 ± 1.12 pieces/stomach	Black, blue, white, red	Fiber	0.5 – 1.5 mm	Rayon, polyester, polyvinyl alcohol, PE, paint	Pradit *et al.* (2021)
Metapenaeus brevicornis, n = 18			3.78 ± 0.48 pieces/stomach			0.5 – 5 mm		
P. monodon/ gastrointestinal tract, n = 50	Northern Bay of Bengal	30% H_2O_2	6.60 ± 2 items/ind	Blue, black, transparent, green, red	Fiber and fragment	250 µm – 5 mm	Rayon, polyamide	Hossain *et al.* (2020)
M. monocerous/ gastrointestinal tract, n =100			7.80 ± 2 items/ind	Blue, black, transparent, green		< 250 µm – 5 mm		
Metapenaeus moyebi	Khlong U-Taphao, Songkhla Thailand	30% H_2O_2	14.76 ± 1.98 items/ind	Blue, black, other	Fiber and fragment	< 100 µm – greater than 1 mm	Rayon, polyester, PET, PP, Poly (Ethylene:Propylene)	This study
Macrobrachium rosenbergii			11.24 ± 1.74 items/ind					

Rayon polymer was found frequently in recent and other studies. The most common source of rayon is clothing fiber, which can come from used clothes and nappies, according to Hossain *et al*. 2020 and Pradit *et al* (2021). Polymers such as PP, PET, and Polyester are widely used in the production of ropes, bottle caps, fishing gear, and drinking straws (resin type: PP), food packaging, bottles, strapping (resin type: PET), textiles, and abrasives in cleaning products (resin type: Polyester) (Coyle *et al*., 2020).Microplastics abundance was found in greater amounts in both shrimps, Metapenaeus moyebi and Macrobrachium rosenbergii, compared to the items per individual of 0.8 ± 0.1 items/individual in *Penaeus esculentus* (Ogunola *et al*., 2022), 0.39 ± 0.6 items/ individual in *Fenneropenaeus indicus* (Daniel *et al*., 2020), 7.23 ± 2.63 items/individual in *Metapenaeus monoceros* (Gurjar *et al*., 2021), 6.60 ± 2 items/ind in *P. monodon* and 7.80 ± 2 individual in *M. monocerous* (Hossain *et al*., 2020). Furthermore, when it came to microplastics in the stomach, *Metapenaeus moyebi* had the highest amount compared to other studies, which had *Parapenaeopsis hardwickii* at 4.11 ± 1.19 items/stomach and *Metapenaeus brevicornis* at 3.78 ± 1.12 items/stomach (Pradit *et al*., 2021). *Macrobrachium rosenbergii*, on the other hand, had the lowest amount of microplastics than *Parapenaeopsis hardwickii* and *Metapenaeus brevicornis*.

There were no statistically significant differences in microplastic accumulation found in the stomach, intestine, or tissue of *Metapenaeus moyebi* and *Macrobrachium rosenbergii*, with $p = 0.591$ and $p = 0.064$, respectively. This study comparing the amount of microplastics found in shrimp organs is quite interesting because microplastics were found in tissues of both shrimp species. Consistent with the findings of Mehinto *et al.* (2022), smaller microplastics less than 83 m can easily move from the digestive tract to other tissues (Mehinto *et al.*, 2022). Microplastic contamination in shrimps has the potential to enter the food chain because shrimp can be food for larger fish in the ecosystem (Paul *et al.*, 2018; Thammatorn and Palic, 2022).This study looked at the relationship between shrimp length/weight and the amount of microplastics accumulated in their organs. As shown in Table 3, the length and weight of *Metapenaeus moyebi* were unrelated to the amount of microplastics accumulating in their stomach, intestine, or tissue, with a statistical significance level of $p > 0.05$. With regard to *Macrobrachium rosenbergii*, it was discovered that, at a statistical significance level of 0.05, the length and weight of the prawns were connected to the amount of microplastics accumulated in their stomach, intestine, and tissue. This suggests that the number of microplastics in tissue and the stomach increased in a manner that was exactly proportionate to the growth in shrimp length and weight. At a statistical significance level of $p > 0.05$, the length of the intestines was not related to the amount of microplastics accumulation in both shrimp species (p = 0.801 for *Metapenaeus moyebi* and p = 0.790 for *Macrobrachium rosenbergii*).

Table 3. Shows the relationship between the length/weight of Moyebi shrimps and the quantity of microplastics found their stomach, intestine, and tissues

		TL (cm)	BW (g)	IN (cm)	ST (items)	IN (items)	TI (items)
Metapenaeus moyebi	TL (cm)	1	.834**	.659**	0.169	0.409	-0.039
	BW (g)		1	0.47	-0.073	0.275	-0.06
	IN (cm)			1	0.318	-0.066	-0.142
	ST (items)				1	0.29	0.156
	IN (items)					1	-0.01
	TI (items)						1
Macrobrachium rosenbergii	TL (cm)	1	.972**	.648**	.612**	0.08	.653**
	BW (g)		1	.654**	.572*	0.017	.603*
	IN (cm)			1	0.436	-0.07	0.461
	ST (items)				1	0.145	.665**
	IN (items)					1	0.002
	TI (items)						1

** Correlation is significant at the 0.01 level (2-tailed).
* Correlation is significant at the 0.05 level (2-tailed).

The current study found microplastic in the stomach, intestine, and tissue of two shrimp caught in Khlong U-Taphao *(Metapenaeus moyebi* and *Macrobrachium rosenbergii*). It is concerning for future aquatic conservation as well as human health. Furthermore, the more species, sampling areas, and seasons studied, the better the understanding of the current microplastic situation. The findings of this study will be used to create a database for future research on microplastic contamination in aquatic organisms in southern Thailand. This study may increase the community's awareness of contaminated waste in the natural environment.

Acknowledgements

This research was supported by National Science, Research, and Innovation Fund (NSRF) and Prince of Songkla University (Grant No. ENV6505026M), PSU-TUYF Charitable Trust Fund under a project "Coral reef biodiversity conservation and connectivity in southern Gulf of Thailand to support reef resilience and sustainable use" and Coastal Oceanography and Climate Change Research Center (COCC).

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(Received: 30 September 2022, accepted: 30 December 2022)