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Microplastic Contamination in the Tissue of Giant Freshwater Prawns *Macrobrachium rosenbergii*, Thailand

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Abstract

Microplastics are everywhere and may be harmful to the biota environment. This study investigated microfiber contamination in giant freshwater prawn tissues, one of Thailand's economic species. The prawn samples were taken from the Trang River in southern Thailand in September 2022. Three tissue sections (head, middle, and tail) of 60 prawns were studied and the samples were digested with 10 % potassium hydroxide (KOH) solution. We utilized a stereomicroscope to study the microplastics and Fourier transform infrared spectroscopy (FT-IR) to determine the types of polymer. There were 3.50 ± 0.34 microfibers in the head per 5 g weight, 2.87 ± 0.29 per 5 g weight in the middle, and 3.10 ± 2.28 per 5 g weight in the tail. Fiber appearance is obvious, the predominant color is blue, the most common size is in the range of 101-500 µm, and the polymer type found is cotton rayon polypropylene (PP). The correlation analysis of microfiber content (in the stomach and intestine), carapace length, abdomen length, stomach weight, and body weight in giant freshwater prawns was not correlated with tissue microfiber content ($p > 0.01$). It was found that the number of microplastics in giant freshwater prawns was not significantly different ($p > 0.05$) among the 3 sections of prawn tissue (head tissue, middle tissue, and tail tissue).

Keywords: Contamination, Microfiber, Microplastics, Giant freshwater prawns, Polymer, Potassium hydroxide, Tissues

Introduction

Plastic is a popular material to create products. It can replace limited natural materials and is widely used all over the world. Plastic is a material with special properties and is superior to other materials in terms of strength, toughness, and resistance to acid-alkaline conditions. It is lightweight, can serve as an electrical insulator, and can also be used as a replacement for glass, wood, or paper [1]. However, the control of plastic waste is a problem which has not yet been resolved. As a result, plastic waste is released from the waste disposal process [2]. When these plastics are broken down into particles or fragments in the environment by processes such as physical, thermal, oxidation, or photodegradation or take the form of small plastic from the beginning, such as plastic pellets from plastic factories, microbead pellets used as ingredients in cosmetic products, or fibers from ropes, nets, and clothes, microplastics are created, which refers to plastic waste that is sized between 1 µm and 5 mm [3,4].

When microplastics are small, they can spread easily into the environment, making them difficult to collect and eliminate, thereby increasing contamination, diffusion, accumulation, and residue in the marine environment, sediments, and organisms. From the coast to the deep sea, microplastic is everywhere and is also toxic due to the plastic itself and its additives, while it can also absorb, release, and transport environmental toxins [5] such as PCBs, heavy metals, and pathogens. This directly affects marine life, such as obstructing the digestive tract resulting in less food consumption, reducing immunity, and harming the growth system and reproductive system, resulting in changes in population structure and ecosystems including economic and social impacts [6-10]. Microplastics are the topic of increasing numbers of studies by researchers into many organisms, yet few studies have investigated how microplastics affect organisms living in the Trang River, a major source of fishing and important aquaculture area of Trang province. The

Trang River flows through many districts starting from upstream at Huai Yot district (in the northern direction) and flowing downstream to Kantrang district before entering the Andaman Sea.

Giant freshwater prawns are an important economic animal in the region with a high price and popularity among consumers. Many traditional foods in Thailand use prawns, such as tom yum, curry, and grilled prawns, which are popularly consumed by both domestic and international consumers. Giant prawns are marketable due to their firm texture and large volume [11] and live in lakes, rivers, canals, marshes, and flooded grassland areas. The feeding behavior of giant freshwater prawns means they can eat plants, animals, and the carcasses of living things on the surface of the soil. The food intake of giant freshwater prawns varies according to their age. The larvae feed mainly on plankton while the post-larva feed mainly on zooplankton, and began to eat dead animals since shrimp at this stage prepare to adapt to living on the soil surface, while the juvenile stage is a juvenile shrimp. However, it can eat both plants and animals in addition to debris by accident [12,13]. Microplastics have been found to have spread everywhere in different environments, whether it is sediment or mangrove soil [14]. Several microplastics have been reported to be ingested by animals such as zooplankton and mammals [15,16]. In addition, regional shrimp studies have been reported [17-26]. The aim of this study was to examine microplastic contamination in the tissues of giant freshwater prawns. The information obtained from this study can be used as baseline data in the region.

Materials and methods

Sample collection

A total of 60 giant freshwater prawns (about 5 kg) were randomly purchased from local fisherman who fished in the Trang River, Kantrang district, Trang province, southern Thailand, in September 2022 (**Figure 1**). All 60 prawns were used for the analysis.

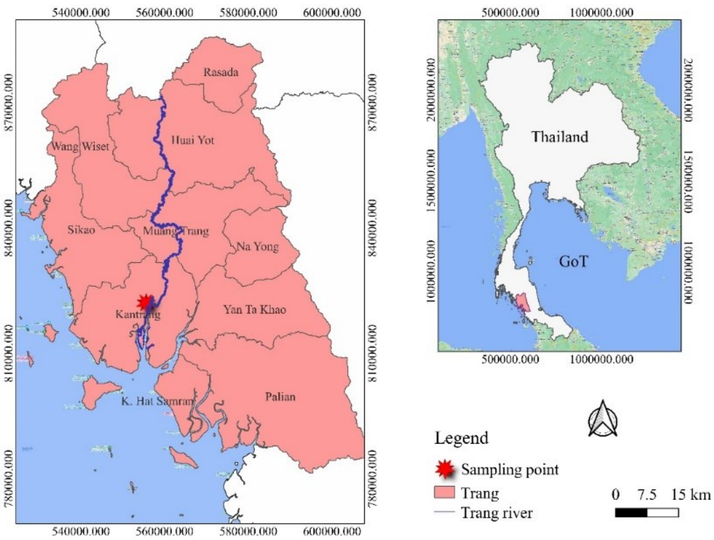


Figure 1 Map of the study area.

Analysis of microplastics in giant freshwater prawns

Before analyzing microplastics, the lengths of the carapace and abdomen were measured **(Figure 2)**. The measurement standards of the Food and Agriculture Organization of the United Nations (FAO) were used. Microplastics were analyzed from the tissues of giant freshwater prawns which were divided into 3 sections (head tissue, middle tissue, and tail tissue; 5 g in each segment) **(Figure 3)**. The samples were digested with 30 mL of 10 % potassium hydroxide (KOH) solution, stirred with a glass stirring rod continuously for 1 min, covered with foil paper to prevent contamination of the substance with foreign matter from the air, heated at 60 °C for 5 min, and then left at room temperature for 12 h. After that, the samples were filtered through a 20 µm filter cloth and dried in a hot air oven at 55 °C for 5 h. The microplastics were then counted and visually observed under a stereo microscope, Olympus brand, model SZ61, with a light-emitting diode base.

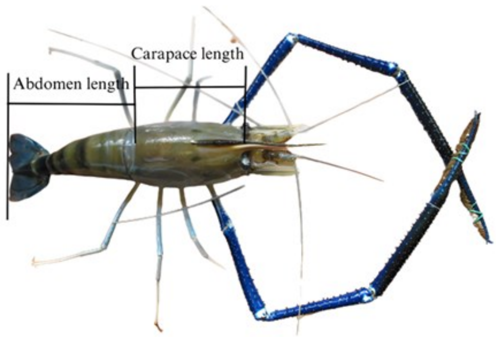


Figure 2 Giant freshwater prawns (Note: from N = 60, abdomen length about 4.61 cm, carapace length about 8.74 cm, body weight about 66.55 g/individual).

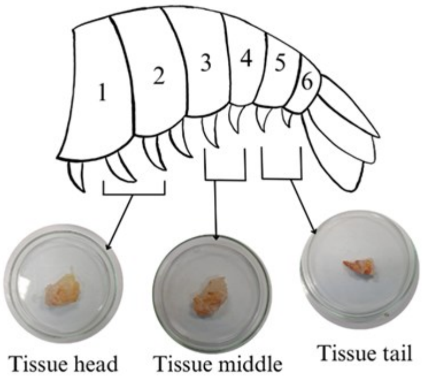


Figure 3 The tissues of giant freshwater prawns as divided into 3 sections in this study.

Polymer identification

Polymer types of microplastics were analyzed by a Fourier transform infrared spectrophotometer (FTIR); spotlight 200i; Perkin Elmer. The wavelength in the analysis ranged from 4,000 to 400 cm^{-1}. The obtained spectrum was compared to the reference library spectrum of each polymer type. A matching score higher than 70 % guarantees the identification of the plastic polymer.

Microplastic prevention

There were no disturbances in the laboratory such as wind. The researchers wore gloves, gowns, hygienic shower caps, and surgical masks throughout the operation. A blank test was performed by placing a 250 mL beaker with distilled water in a laboratory; after the experiment, no microplastics were found in the beaker. Laboratory work took place at the Faculty of Environmental Management, Prince of Songkla University, Thailand.

Statistical analysis

Data analysis of microplastic abundance, size, color, and shape was performed using Microsoft Excel (Office Professional Plus 2016) and the data were presented as mean ± standard error (pieces per 5 g weight). Analysis of variance was performed to compare microplastic differences between the 3 tissue sections of the giant freshwater shrimp. Pearson correlation was used to confirm the association between microplastic abundance in the giant freshwater prawns' stomach, intestine, carapace length, length of abdomen, body weight, and stomach weight.

Results and discussion

A total of 60 giant freshwater prawns were randomly sampled at Bo Nam Ron Sub-district, Kantang District, Trang Province. Carapace length, abdomen length, body weight, and stomach weight ranged from 3.40 - 7.00 cm, 6.90 - 6.33 cm, 28.94 - 175.25 g and 0.22 - 6.33 g, respectively (**Table 1**). The number of microplastics in the tissue head was 210 pieces per 5 g weight, in the middle tissue there were 172 pieces per 5 g weight, and in the tail tissue there were 186 pieces per 5 g weight (**Figure 4**). Of the 3 tissues, the highest microplastic count was found in the head tissue, followed by the tail tissue and the middle of the body (**Figure 4**). It was found that the numbers of microplastics in giant freshwater prawn tissue were not significantly different (p = 0.340) among the 3 sections of tissue (head tissue, middle tissue, and tail tissue). The correlation coefficients (r) of microfiber content in prawn tissues and carapace length, abdomen length, stomach weight, and body weight in giant freshwater prawns were not correlated with tissue microfiber content ($p > 0.01$) (**Table 2**).

Table 1 The information on carapace length, abdomen length, stomach length, body weight, and stomach weight of giant freshwater prawns.

Macrobrachium rosenbergii (n = 60)	**Measure size**											
	Carapace length (cm)			**Abdomen length (cm)**			**Body weight (g)**			**Stomach weight (g)**		
	min	max	mean ± SE	min	max	mean ± SE	min	max	mean ± SE	min	max	mean ± SE
	3.40	7.00	4.61 ± 0.09	6.90	6.33	8.74 ± 0.16	28.94	175.25	66.55 ± 30.96	0.22	6.33	0.93 ± 0.16

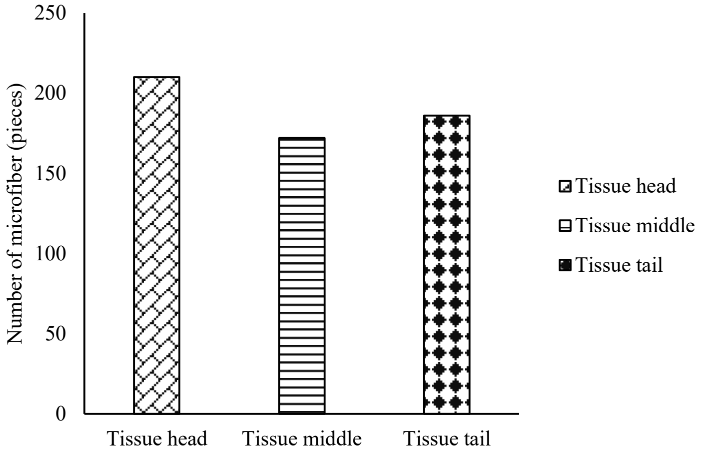


Figure 4 Number of microfibers in each organ of giant freshwater prawns.

Table 2 Correlation coefficients for the amount of microplastic in prawn tissue and other parameters.

	MPs Tissue Head	**MPs Tissue Middle**	**MPs Tissue Tail**	**Carapace length (cm)**	**Length of abdomen (cm)**	**Stomach weight (g)**	**Body weight (g)**
MPs Tissue Head	1	−0.045	−0.023	−0.252	−0.248	−0.129	−0.252
MPs Tissue Middle		1	0.136	−0.032	−0.055	−0.044	0.004
MPs Tissue Tail			1	0.089	0.009	0.039	0.052
Carapace length (cm)				1	0.866**	0.491**	0.924**
Length of abdomen (cm)					1	0.509**	0.878**
Stomach weight (g)						1	0.540**
Body weight (g)							1

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

Characteristics of microplastics, fibers, and fragments were observed in this study. The results are as follows for the head tissue; 162 fibers (77 %), 48 fragments (23 %); middle tissue; 144 fibers (84 %), 28 fragments (16 %); and tail tissue; 156 fibers (84 %), 84 fragment (16 %) (**Figure 5).** The appearance of fiber was prominent in this study, consistent with the studies of [14,27,28] which found the most fiber characteristics in biota. Most fibers found were light and low density plastic fibers, which always float and are easily carried by waves and wind [29]. Possible sources of fiber found in prawn tissue could be from broken fishing nets, fishing equipment, or aquaculture cages and food packaging materials [26], as well as synthetic fibers from household laundry [30]. Microplastic contamination in household wastewater can be as high as 128 - 308 mg per kg of washed laundry or 640,000 - 1,500,000 pieces, which is estimated to be released depending on the type of fiber composition of synthetic fabrics, number of cycles, pattern, and stage of washing [31]. Polyester-type synthetic fiber laundry releases more than 1,900 high-density microfibers per wash or up to 100 microfibers per liter of washing wastewater [32].

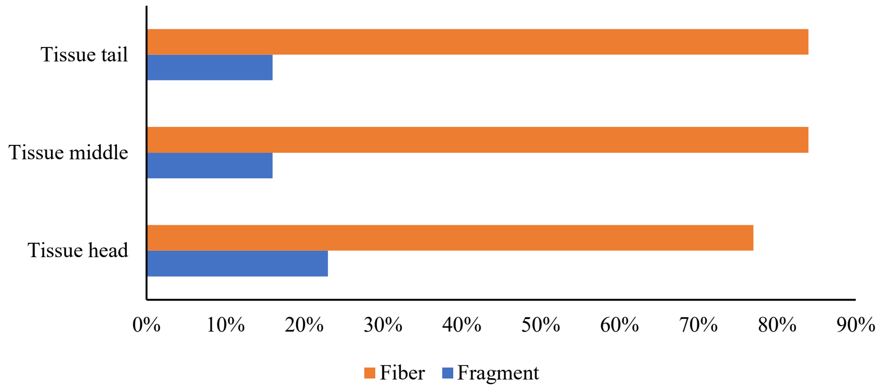
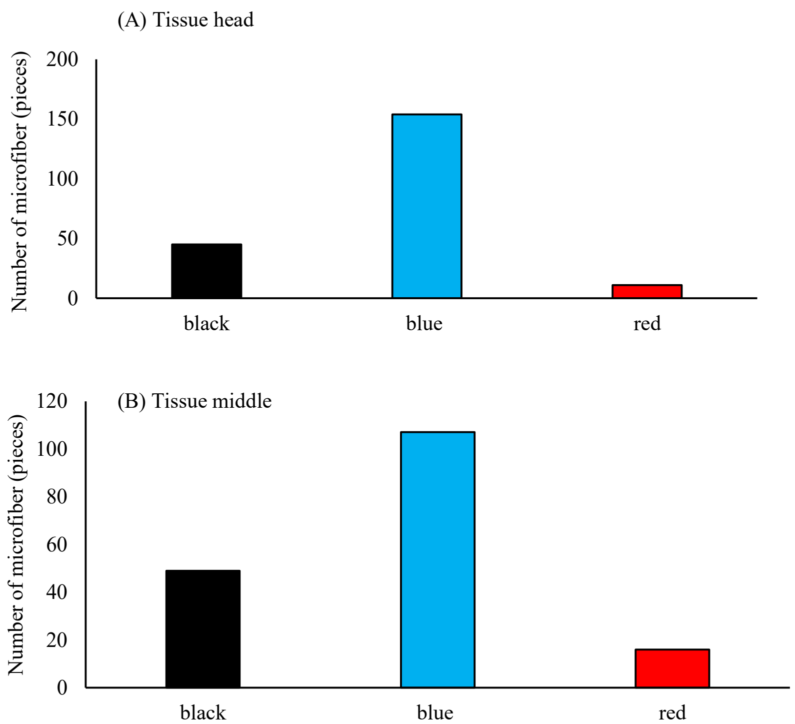


Figure 5 Characteristics of microplastic in prawns.

Three main colors were observed in this study. In all tissue sections, the order of colors found was blue > black > red **(Figure 6)**. Microfibers have a variety of colors that can indicate the origin of the plastic products that are dyed, such as clothes, plastic bags, drinking straws, or toys [33]. In this study the microfiber colors blue, black, and red probably come from clothing lint and fishing nets. However, the type of cleaning products had an effect on the shedding of microfibers [34].



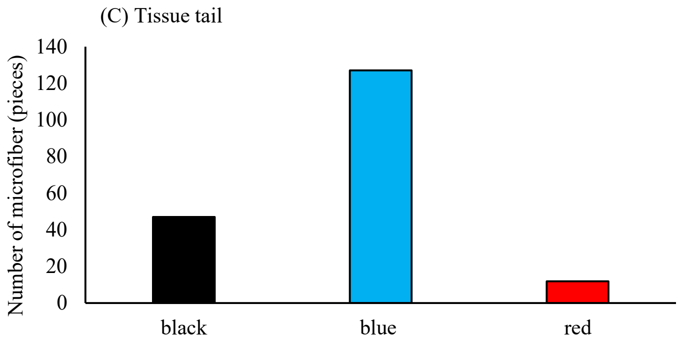
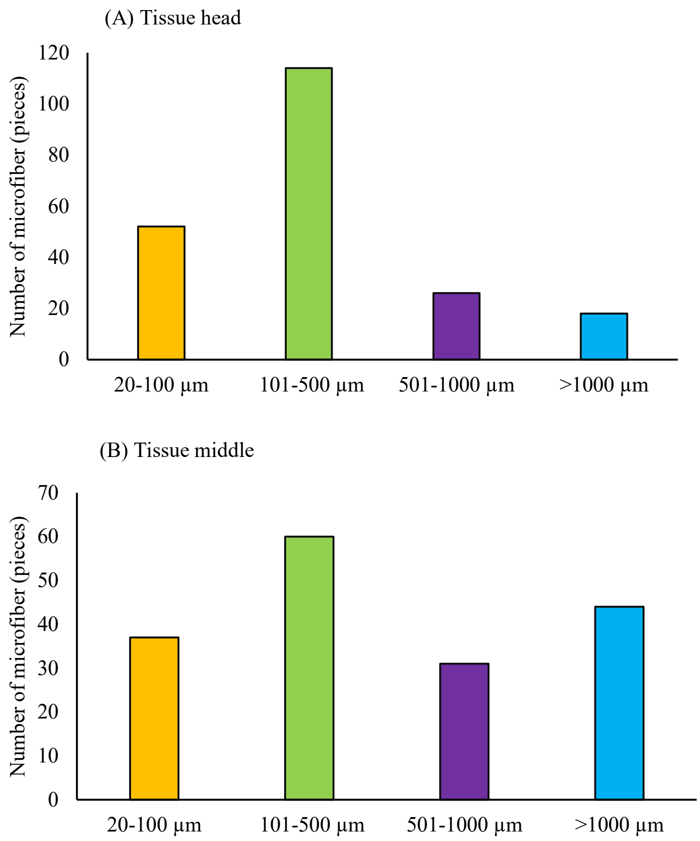


Figure 6 The color of microfibers in each organ**:** A**)** Head tissue; B**)** Middle tissue, and C**)** Tail tissue.

The size classes of microplastic found are shown in **Figure 7**. The most common size classes were 100 **-** 500 µm, similar to the study of **[**31**].**



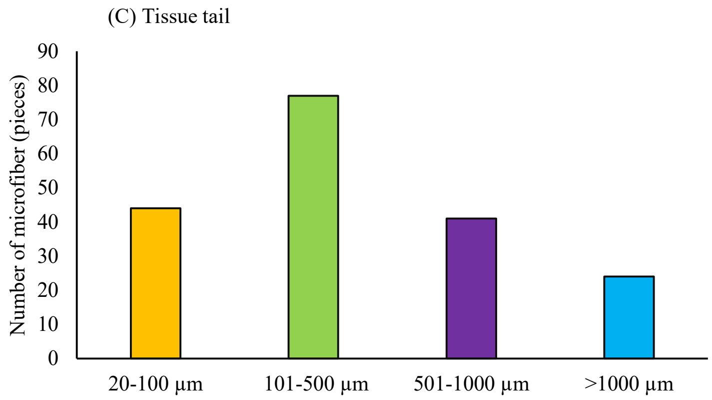
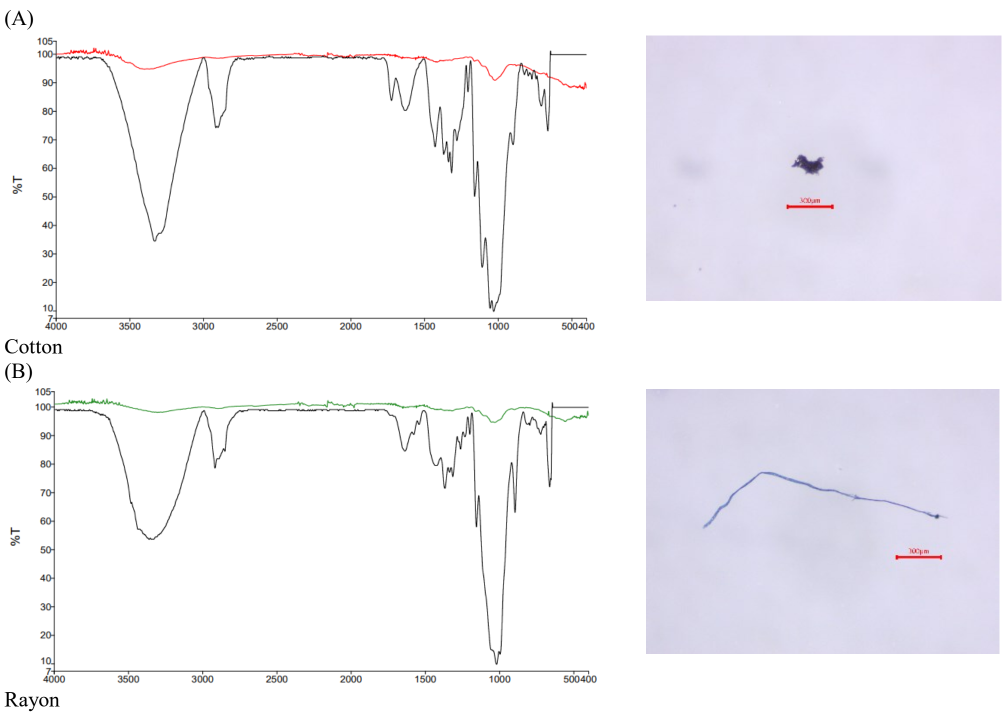
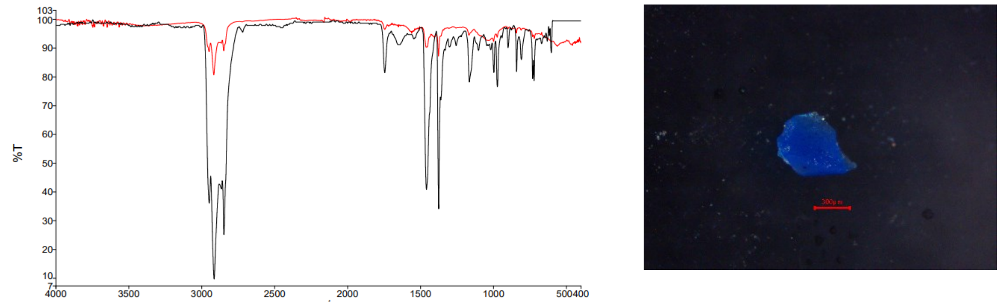


Figure 7 Sizes of microfibers in each organ**:** A**)** Head tissue; B**)** Middle tissue, and C**)** Tail tissue**.**

The types of polymers found in this study consisted of cotton rayon and polypropylene **(**PP**).** Polypropylene **(**PP**)** **(Figure 8)** has a thermoplastic plastic density of 0**.**85 **-** 0**.**93 g/cm^{-3} which is less than water so it can float in water **[**35**]**, while most of it is used to make food packaging, ropes, and bottle caps**.** Since PP is a type of plastic that can be recycled **(**Recycle**)**, it is used in the production of a large number of products **[**36**].** These microfibers can enter the body and accumulate in the bodies of aquatic animals via ingestion **[**37**]** and are then transferred through the food web to reach humans as a top consumer **[**38**].** Most plastics contain additives which are added in the production process, some of which are toxic **[**9**].** These toxins can be transferred through the food chain, including to humans where consumption may be toxic **[**39**].**



(C)



Polypropylene (PP)

Figure 8 Polymer types found in giant freshwater prawns**.** Note the spectral lines from the sample **(**top line**)** and FTIR spectral lines **(**bottom line**).**

Conclusions

This study determined microplastic abundance in giant freshwater prawns caught from the Trang River. We found microfiber contamination in the tissues of giant freshwater prawns, with fibrous type microplastics found to be the most common. Three types of polymer were found in giant freshwater prawns: Cotton, rayon, and polypropylene (PP). The sources of microplastic found in this study are likely to be wastewater, household laundry, and fishing activities. Future studies should be performed to assess the risk of microplastic contamination.

Acknowledgements

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