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Bioaccumulation of Microplastics in Fish and Snails in the Nam Pong River, Khon Kaen, Thailand

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Abstract

This study investigated microplastic accumulation in two fish species, *Barbonymus altus* and *Laides longibarbis,* and two snail species, *Filopaludina martensi* and *Pomacea canaliculata,* were the most dominant species of fish and snails found and consumed in the Nam Pong River. Twenty-four samples from each species were collected. The results showed that the average amounts of microplastic accumulation in the gastrointestinal (GI) tracts of fish was 25.42 ± 26.50 pieces/fish (*B. altus*) and 7.60 ± 17.70 pieces/fish (*L. longibarbis*), and in snail soft tissue was 26.33 ± 33.30 pieces/snail (*F. martensi*) and 11.50 ± 16.80 pieces/snail (*P. canaliculata*). The average size of microplastics found in *P. canaliculata* was a minimum of 1 µm. The most common polymer type of microplastic was high-density polyethylene (HDPE) (26.00%), of which 55.47% was fragment-shaped and 22.29% was black. The accumulation of microplastics in the fish GI tract was found to be significantly higher in *B. altus* than in *L. longibarbis* at 17.83 pieces/fish (95% CI: 4.72 – 30.95, p = 0.008). One interpretation of this observation is that the consumption of different types of fish may affect microplastic acquisition.

Keywords: *Microplastic accumulation; Bioaccumulation; Gastrointestinal tract, Snail soft tissue*

1. Introduction

In an environment where contamination and accumulation can occur from waste disposal, the decomposition of plastics into microplastics is observed. The National Oceanic and Atmospheric Administration (NOAA) defined microplastics as plastics smaller than five millimeters and can be classified into two types: primary microplastics, which are miniscule when produced, and secondary microplastics, which become miniscule from the disintegration, peeling or decomposition of larger plastics (World Health Organization [WHO], 2019). According to previous studies, microplastic contamination and accumulation are currently found in sediment (Zhang *et al.,* 2018; Fuller and Gautam, 2016) in seawater, fresh water and wastewater (WHO, 2019), in the air (Dris *et al.,* 2017), in foods and beverages such as drinking water (WHO, 2019), and in fish, snails, honey and sea salt (EFSA CONTAM Panel, 2016), among others. Microplastic contamination can occur through the alimentary and respiratory systems of organisms in the food chain (Handy *et al.,* 2008). Microplastics can contaminate the food chain through the ingestion of zooplankton, which are low-level organisms in the food chain and are transferred to animals at the next level of the chain, such as fish, snails and humans. Therefore, microplastics can reach every level of the food chain (Nara, 2019). Many studies have reported microplastic accumulation in fish (Bessa *et al.,* 2018, and Van Cauwenberghe and Janssen, 2014). In Thailand, contamination has been reported through microplastic accumulation in mackerel (Azad *et al.,* 2018) and two types of bivalves: *Donax Sp.* and *Paphia Sp.,* on Chaolao Beach and Kungwiman Beach (Tharamon *et al.,* 2016). Microplastics can pass through either the GI tract or the gills (respiratory system) of fish via transcellular uptake or paracellular diffusion until they can enter body fluids (Handy *et al.,* 2008). Microplastic accumulation in the food chain can cause health effects in humans as well as chemical contamination. However, there are no factual data on the effect of microplastics on human health (Rist *et al.,* 2018). Only in vitro studies have been performed, for example, on the reproductive effects in oysters exposed to polystyrene microplastics (Sussarellu *et al.,* 2015); endocrine effects were found in mature freshwater fish exposed to polyethylene microplastics (Rochman *et al.,* 2014). The health effects depend on prolonged exposure to microplastics through different contact channels with various densities (16 – 2200 kg/m^3) (Nizzetto *et al.,* 2016) and shapes such as spheres, fibers and films (Carlos *et al.,* 2018).

Data on the amount of waste generated in the northeast region of Thailand show that Khon Kaen Province was ranked second and first for the amount of hazardous waste from communities (Department of Pollution Control of Thailand, 2020). The Nam Pong River is one of the most important rivers of Khon Kaen. Rivers are considered the main water source for rice cultivation and human consumption, among other uses. The river is also used as a drainage area by local communities and industry for municipal and industrial wastewater and community waste. The most dominant species of fish and snails found and consumed in the Nam Pong River include *B. altus*, *L. longibarbis* (Vidthayanon, 2017), *F. martensi* and *P. canaliculata* (Neeratanaphan *et al.,* 2008). From a preliminary study that collected aquatic animals (*B. altus* and *L. longibarbis*), microplastic accumulation was found in all samples collected in the shape of spheres, fragments, filaments and rods. The polymers were classified as polyethylene, polystyrene, polyethylene terephthalate and polyvinyl (Yasaka, 2020). Research on the effects of microplastic contamination in the environment on freshwater biota is a key matrix to determine the contamination of an area. (Karlssona *et al.,* 2017). While many international studies have been conducted on microplastic contamination and bioaccumulation, there have been few studies in Thailand, especially on bioaccumulation in freshwater animals. Current evidence shows that plastic particle toxicity depends on the concentration, particle size, exposure time, particle condition, shape and polymer type (Kogel *et al.,* 2020). For example, size and surface charge also influence the ability of microplastics to cross the GIT mucus gel layer (Behrens *et al.,* 2002). Smaller sizes and negative surface charge are most likely to lead to the increased uptake of microplastics to other organs and dissemination around the body, if released. Additionally, existing studies highlight that the immunological response is dependent on the chemical composition of the plastic, with PET being more harmful than PE (Wright and Kelly, 2017).

The aim of this study is to determine (a) the presence of microplastics in different species, and feeding habit of aquatic animals in Nam Pong River by examining the amount, shape, size, and color of microplastics, (b) polymer type and their possible sources of microplastics to determine the pathways.

2. Materials and methods

2.1 Sampling sites

This study investigated microplastic accumulation in 4 species of aquatic animals of the Nam Pong River, Muang District, Khon Kaen Province. Eight samples per spot from three collecting spots along the Nam Pong River were collected for each species (Figure 1). The samples were collected within a 1.5 km radius of each water collecting station: 1)

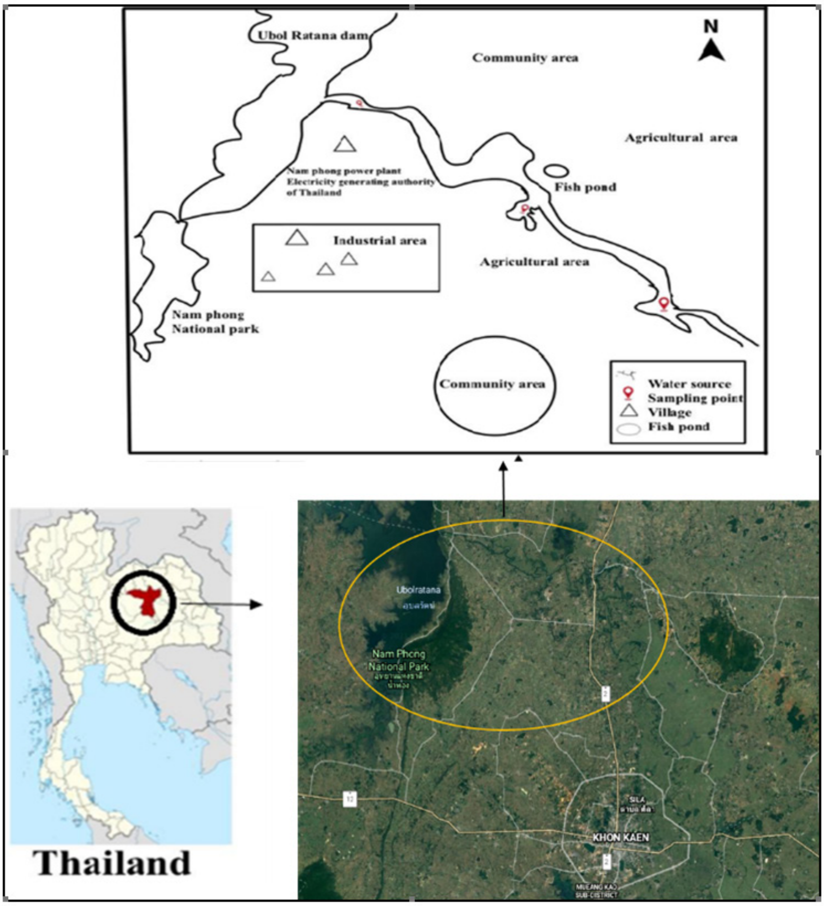

Figure 1. Location of the study area: three collecting spots along the Nam Pong River

Huay Sai bridge, highway 2109, Ubonrat District, latitude: 16° 46'29.11" north, longitude: 102° 37'26.34" east; 2) the Bueng Jode reservoir, the middle of the Ubonrat Dam and Nam Pong River, latitude: 16° 43'37.63" north, longitude: 102° 45'4.38" east, which is 20 km away from the first sampling area; and 3) the Nongwai reservoir, the end of the Nam Pong River, Nam Pong District, latitude: 16° 43'30.84" north, longitude: 102° 48'12.30" east, 55.70 km away from the first sampling area.

2.2 Sample collection and preparation

2.2.1 Sample collection

Eight samples were collected from each aquatic animal species (Fish: *Barbonymus altus, Laides longibarbis ; Snails: Filopaludina martensi, Pomacea canaliculata)* per collecting spot from each of three collecting spots. Purposive sampling was used. The aquatic animal species were selected because they are the most dominant species of fish and snails found and consumed in the Nam Pong River (Vidthayanon, 2017; Neeratanaphan *et al.,* 2008). Fish and snail sampling was conducted by collecting mature fish and snails in November and December 2019. Fish with body lengths of 20 – 30 cm (Ngor, 2018) were collected by netting or trawling. All fish samples were wrapped in foil, placed over wet ice and then stored at -20°C. Snail samples were collected from mature snails by using frames of 100x100 centimeters at a depth of 15 centimeters; the samples were separated by sifting with a 2x2 mm sieve. Then, the snails were classified by weight and measurement. The snail samples were kept at a temperature of -20°C (Peters and Bratton, 2016).

2.2.2 Sample preparation

Fish samples were prepared by dissection and cleaned with distilled water. The body weight in grams and length in centimeters of the fish were then recorded. The soft tissue and gastrointestinal (GI) tract were separated. Then, the GI tract weight and soft tissue weight (grams) prior to soft tissue digestion were recorded. The tissue of snail samples was separated.

2.2.3 Microplastic separation

GI tract digestion was modified following the method of Gago *et al.* (2019). After rinsing the sample with distilled water, digestive solution (10% potassium hydroxide (KOH) filtered through a 0.45 - µm nitrocellulose membrane filter) was added to a 1:3 volume sample to digest the biological material. The mixture was placed in a temperature-controlled oven at 40°C until all visible organic material was digested or up to a maximum of 48 hours. Then, the digested organic material was filtered out with a 0.45 - µm nylon membrane. Saturated sodium chloride (NaCl) was added to the filter. The microplastic density was separated by shaking for two minutes by using a shaker (Model No. MI0103002, Guangzhou Four E's Industrial Co., Ltd., Guangzhou, China). Then, the precipitate and the solution were separated into layers. The clear solution was filtered through 0.45 µm filter paper. Then, the filter paper was dried at 50°C for four hours. The samples were analyzed for microplastics.

2.3 Inspection of microplastics and identification

Microplastic size, shape and color were examined with a NIKON SMZ745T stereomicroscope and scanning electron microscopy (SEM), and the microplastic polymer type was examined by Fourier transform infrared spectroscopy (FT-IR) (Model TENSOR27). The investigation was conducted according to the six microplastic size ranges: 1 – <100, 100 – <350, 350 – <500, 500 – <1000, 1000 – <3000, 3000 – <5000 µm and plastics from 5000 µm (mesoplastics).

2.4 Statistical analysis and data calculation

Descriptive statistics (in terms of percentage, mean and standard deviation) and inferential statistics were obtained by using the Stata program, version 14. Analytical statistics were used to test the difference in microplastic sizes, 95% CI of the mean difference and P-value using a *t*-test. This study showed a nonnormal distribution; thus, the mean microplastic numbers among species were analyzed by a nonparametric test (Kruskal-Wallis) with statistical analysis at a 0.05 significance level.

3. Results and discussion

3.1 Microplastic accumulation

In this study of microplastic accumulation in two species of fish, *B. altus* and *L. longibarbis*, and two types of snails, *P. canaliculata* and *F. martensi,* the four species were found to have a mean length and mean body weight as follows: 19.82 ± 0.62 cm and 349.43 ± 17.90 g (*B. altus*), 28.38 ± 1.78 cm and 428.07 ± 18.22 g (*L. longibarbis*), 3.31 ± 0.26 cm and 116.82 ± 1.24 g (*P. canaliculate*), and 1.21 ± 0.11 cm and 56.06 ± 2.05 g (*F. martensi*), respectively (Table 1). The mean GI tract weights of 9.37 ± 1.71 g, 11.08 ± 1.61 g, 2.85 ± 0.24 g and 1.58 ± 0.24 g are displayed (Table 2). The data show that microplastic accumulation was not found in the soft tissue of either species of fish. The accumulation of microplastics was found in the GI tract. The accumulation rate of microplastics was high in *B. altus* at 70.83%, while microplastic was found in nine *L. longibarbis* fish at 37.5%. There was microplastic accumulation in *B. altus* [610 pieces/24 fish (25.42 ± 26.50 pieces/fish)] and 182 pieces/24 fish (7.60 ± 17.70 pieces/fish) in *L. longibarbis*. A comparison of microplastic accumulation between the 2 fish species found that the amount of microplastics in *B. altus* was significantly higher than that of 17.83 pieces of *L. longibarbis* (95% CI: 4.72 – 30.95, p = 0.008). Based on their feeding habits, omnivorous fish were found to have higher microplastic accumulation than both herbivorous and carnivorous fish

(Mizraji *et al.,* 2017). *B. altus* eats plants, zooplankton, insects and organic matter, while *L. longibarbis* eats insects and zooplankton. This result is consistent with the results of Kasamesiri and Thaimuangphol's (2018) studies on the accumulation of microplastics in various fish species in the Chi River region. *L. longibarbis* was found to have less microplastic accumulation than *Puntioplites proctozone,* which eats all types of food.

The results of microplastic accumulation in the two types of snails showed that microplastic accumulation in *P. canaliculata* was higher than that in *F. martensi* for 24 samples (100%) and 20 samples (83.88%), consistent with the findings of Panebianco *et al.'s* (2019) study, in which more than 50% microplastic accumulation in snails was found. This study found microplastic accumulation reaching 632 pieces/24 snails (26.33 ± 33.30 pieces/snail) and 276 pieces/24 snails (11.50 ± 16.80 pieces/snail) (Figure 2). The microplastic accumulation in *P. canaliculata* was 14.83 pieces/snail more than that in *F. martensi* (95% CI: -0.48 - 30.15, p = 0.057). However, there was no significant difference.

Based on feeding habits, the higher abundance (Figure 2) and rates (Table 2) of microplastic accumulation were found in omnivorous aquatic animals rather than in carnivorous aquatic animals. Significant differences in microplastic accumulation were found between *B. altus* and *L. longibarbis*, which may be explained by habitat and appetitive behavior. *B. altus* occurs at midwater depths in large- and medium-sized rivers and floodplains. Various plantations and livestock farms are commonly found near villages where organic detritus is disposed of by humans (Rainboth, 1996). This observation is consistent with the findings of Mochamad's research (2018), who found that omnivorous feeding habits had greater potential for microplastic ingestion.

Table 1. Feeding habits, habitats and physical measurements of aquatic animals: body weight (BW) in grams and length in centimeters

Aquatic animal species	N	Feeding habit	Habitats	BW* (g)	Length* (cm)
B. altus	24	Omnivorous	Midwater	349.43 ± 17.90	19.82 ± 0.62
L. longibarbis	24	Carnivorous	Bentho-pelagic	428.07 ± 18.22	28.38 ± 1.78
F. martensi	24	Omnivorous	Benthic	56.06 ± 2.05	1.21 ± 0.11
P. canaliculata	24	Omnivorous	Bentho-pelagic	116.82 ± 1.24	3.31 ± 0.26

* The values are expressed as mean ± SD.

Table 2. GI tract in grams and basic descriptions of microplastic accumulation

Aquatic Animal species	Organ	Weight (g) (Mean ± SD)	Found		Dominant		
			N	%*	Shape	Color	Polymer type
B. altus	GI tract	9.37±1.71	17	70.83	Sphere	Transparent	HDPE
	Soft tissue	179.18±17.90	0	0	-	-	-
L. longibarbis	GI tract	11.08±1.61	9	37.50	Fragment	Blue	PETE
	Soft tissue	204.73±18.22	0	0	-	-	-
F. martensi	Soft tissue	1.58±0.24	20	83.33	Filament	White	LDPE
P. canaliculata	Soft tissue	8.55±0.72	24	100	Fragment	Black	PS

* % Microplastic accumulation

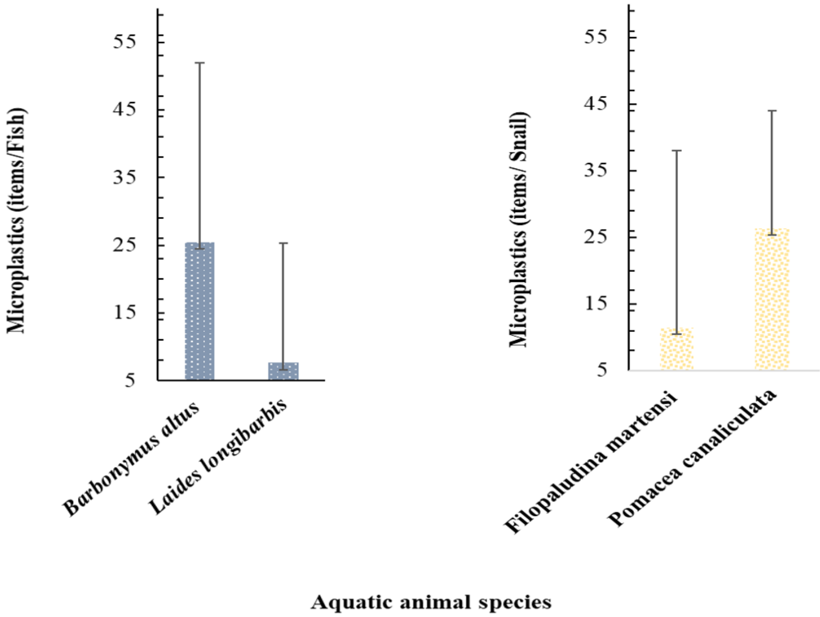

Figure 2. Average number of microplastics accumulated according to aquatic animal species

Table 3. The median, minimum and maximum sizes of microplastics

Aquatic animal species	Size of Microplastic (μm)			
	Minimum	Maximum	Median	Mean±SD
B. altus	11	3909	91	397.40 ± 638.10
L. longibarbis	19	4226	132	450.70 ± 715.70
F. martensi	2	4318	116	481.30 ± 811.50
P. canaliculata	1	4545	75	446.90 ± 703.0

3.2 Size, shape and color of microplastics

The results of the microplastic size were within the small size range of microplastics (1 to < 100 μm), which were the predominant size in both aquatic animals (Figure 3). The average size of microplastics that accumulated in *L. longibarbis* was larger than that in B. altus at 450.70 ± 715.70 (11-3909) μm and 397.40 ± 638.10 (19 - 4226) μm, respectively (Table 2), corresponding to the average length of the B. altus body, which is longer than the body of *L. longibarbis.* However, the mean size of microplastics in both fish species was not significantly different (p = 0.087). In addition, this study investigated only microplastics up to 20 μm, which is the size of microplastics reported to be translocated into organs in the body (Barbozaa *et al.,* 2018). *B. altus* accumulated more than *L. longibarbis*, with 22 pieces (0.92 ± 1.61 pieces/fish) and seven pieces (0.29 ± 0.86 pieces/fish), respectively. *B. altus* presented a greater risk of microplastic exposure than *L. longibarbis*. The mean size of microplastics accumulated in *F. martensi* was larger than that in *P. canaliculata* at 481.30 ± 811.50 (2 - 4318) μm and 446.90 ± 703.0 (1 - 4545) μm, respectively (Table 2). The difference was not significant (p = 0.777); in contrast, the smallest microplastic that accumulated in *P. canaliculata* was 1 μm. However, the mean sizes of the microplastics in Table 2 present high SDs; therefore, the results are reported with minimum, maximum and median sizes of microplastics (Table 3).

As mentioned above, four shapes of microplastics were found: spheres, fragments, filaments and rods (Figure 4). The most common microplastic shape from all fish species is fragments at 23.86%, which is consistent with the reports of previous studies (Merga *et al.,* 2020; Cannon *et al.,* 2016; and Rummel *et al.* (2016). However, in *B. altus*, the most common shapes were spheres, followed by fragments, filaments and rods (Figure 4). Rod shapes were found only in *B. altus.* Filaments were the largest microplastics (3909 μm), and spheres were the smallest (11 μm). Qiao *et al.* (2019) observed that the shape-dependent accumulation of microplastics in the gut was in the order of fibers (filament) > fragments > beads (spheres), and microplastic fibers resulted in more severe intestinal toxicity than microplastic fragments and beads; therefore, the result of the most common shape in our study may indicate that the possible greater accumulation of microplastics follows the order of *F. martensi* > *L. longibarbis* = *P. canaliculata* > *B. altus*, and the opportunity of intestinal toxicity in *L longibarbis* is greater than the opportunity of intestinal toxicity in *B. altus*. An important source of fiber microplastics is the degradation of fishing gear, fish cages or nylon ropes (Thushari *et al.,* 2017) and sewage from washing clothes (Browne *et al.,* 2011).

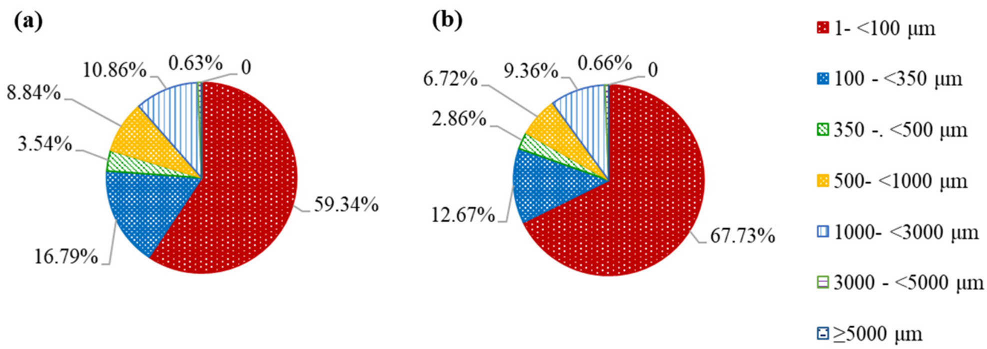

Figure 3. Percentages of microplastic accumulation based on size range:(a) Fish and (b) Snail

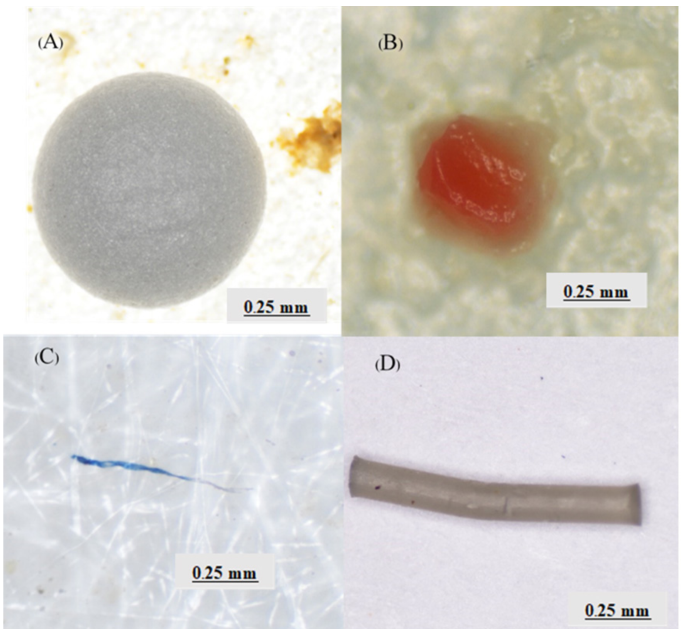

Figure 4. Typical photographs of microplastics: (a) sphere, (b) fragment, (c) filament, and (d) rod

The most common shapes of microplastics in *L. longibarbis* were fragments of spheres and fibers (Figure 5). The rod shape was not found in *L. longibarbis*. The filament shape was the largest microplastic, which was also found in *B. altus,* with a larger average size (4226 µm). The smallest microplastic shapes (19 µm) were spheres and fragments. The most common shapes of the microplastics in *P. canaliculata* were fragments of spheres and filaments (Figure 4). The largest filament microplastics accumulated in the fish with a size of 4545 µm, and the fragment with the smallest size was 1 µm. In *F. martensi,* filament fragments and spheres were found (Figure 5). Filament microplastics were the largest microplastics, similar to *P. canaliculata*. However, filaments were smaller than those in *P. canaliculata* (4318 µm), and the smallest shape was two fragments (2 µm).

Twelve colors of microplastics were found in this study: blue, pink, brown, black, green, red, orange, white, transparent, purple, yellow and gray. The three most common colors of microplastics accumulated in *B. altus* were transparent, brown and purple, accounting for 30.16%, 18.52% and 15.57%, respectively, which is consistent with the reported values by Azard *et al.* (2018). The most common colors found in *L. longibarbis* were blue, black and white at 24.73%, 14.84% and 10.99%, respectively, consistent with the findings of Sarijan *et al.* (2019). The three most common colors of microplastics accumulated in *P. canaliculata* were black, pink and blue, accounting for 44.46%, 12.34% and 8.39%, respectively, while *F. martensi* had the most common colors: white, blue and transparent, accounting for 30.49%, 16.30% and 14.86%, respectively (Figure 6). The color identification of microplastics can be used to characterize microplastics originating from waste in combination with the polymer type to more clearly identify the type of plastic waste or hazardous waste. Our results found that the variety of colored microplastics had similar color patterns between different feeding types and were consistent with the most dominant colors of gillnets to catch fish because local fisherman usually use transparent, black (Panchan *et al.,* 2013), white, blue, yellow, red and green (Balık *et al.,* 2001) nets.

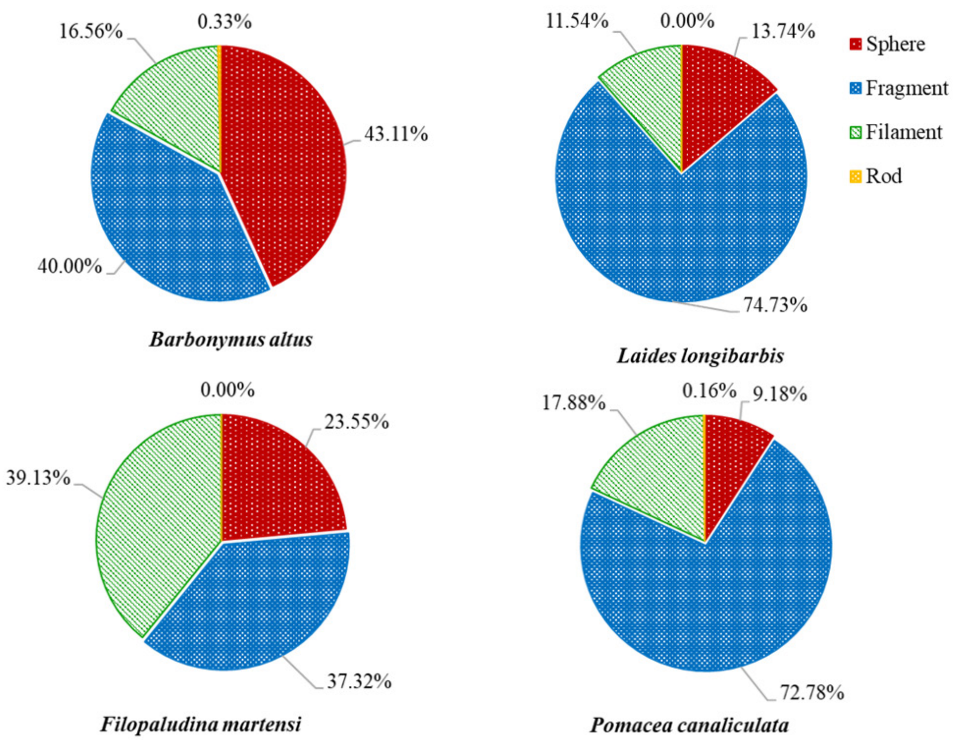

Figure 5. Percentages of microplastic shapes

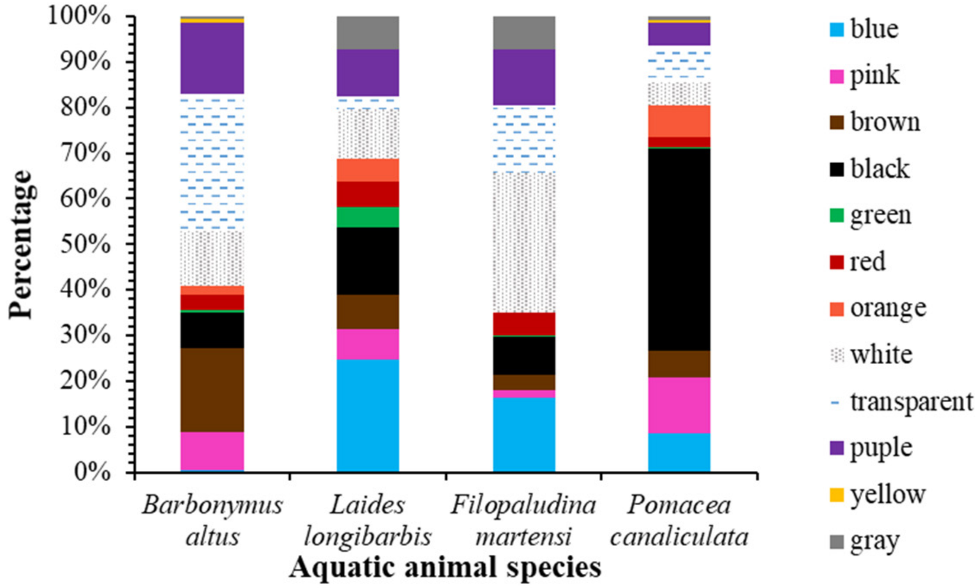

Figure 6. Percentages of microplastic colors in aquatic animals

3.3 Polymer type of microplastics

To identify the polymer types of microplastics found in the study, Fourier transform infrared (FT-IR) spectroscopy was used. A total of nine types were found: polyurethane (PU), nylon, polyethylene terephthalate (PETE), high-density polyethylene (HDPE), low-density polyethylene (LDPE), polystyrene (PS), polyvinyl chloride (PVC), and polypropylene (PP) and polycarbonate (PC).

The most common type of microplastic polymer accumulated in *B. altus* was HDPE (Figure 7). The transparent filament PETE was the largest microplastic (3909 µm), while a brown sphere-shaped PS was the smallest microplastic (11 µm). In *L. longibarbis,* the most common type of microplastic polymer accumulated was PETE at 42.31%. PETE was the largest microplastic, the same as in *B. altus* (4226 µm in the shape of a blue filament). The smallest microplastics (19 µm) were black and blue fragments of PU, red spheres of HDPE, and black fragments of PS. The most common types of microplastic polymers accumulated in the two types of snails were PS at 24.63% and LDPE at 26.45% in *P. canaliculata* and *F. martensi,* respectively (Figure 6). In *P. canaliculata,* HDPE was the largest (4545 µm), while pink and purple fragments of PETE were the smallest microplastics (1 µm). The transparent filament of HDPE was the largest microplastic (4318 µm) found in *F. martensi;* a blue fragment of PETE and a blue filament of PP were the smallest microplastics (2 µm). However, our study found that PC accumulated only in *B. altus* and *P. canaliculata* at 0.16% (1 piece/24 sample).

Our polymer type results show that microplastics may come from household waste around the sampling site and float with water. HDPE polymers are often found in plastic containers, food packaging, and laundry products, while PETE is found in many types of bottled waste, clothing fibers, and cosmetic glitter. In addition, PS is often found in foam container litter and coffee mugs, while LDPE polymers are used mainly in manufacturing containers, dispensing bottles, tubing, and plastic bags.

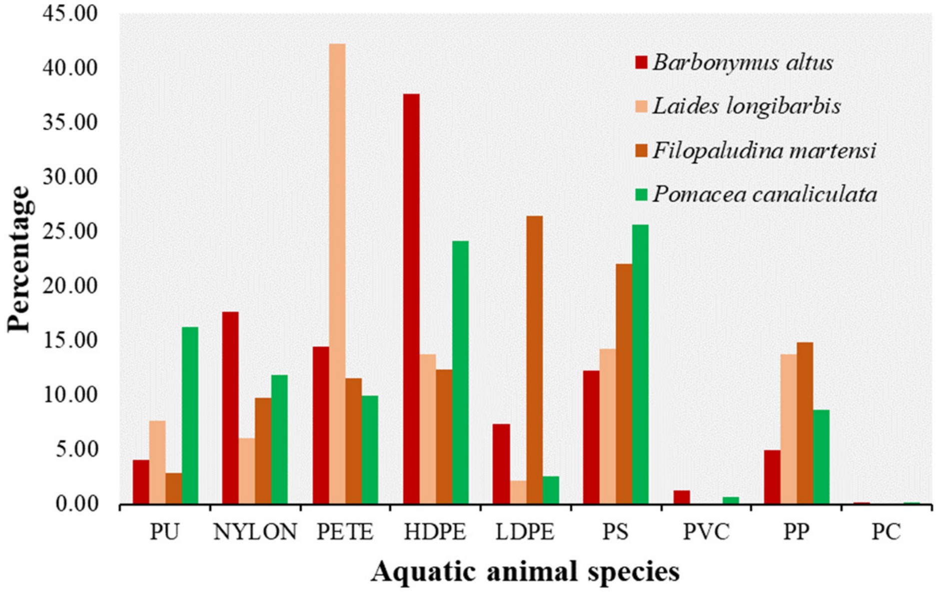

Figure 7. Percentages of polymer types of microplastics in aquatic animals

According to the results of the study, the size and polymer type of the smallest microplastics accumulated in *B. altus* were 11 µm of PS, which is the maximum size of microplastics that can be transported through the digestive tract (Barbozaa *et al.,* 2018) and is able to penetrate organs (Kogel *et al.,* 2020). According to a human cell-level study of the toxicity mechanism of PS polymers, PS with a diameter of less than 5 µm was able to induce 4% of the hemolysis process compared to the control group. However, PS greater than 10 µm in diameter was unable to pass through the erythrocyte membrane (Hwang *et al.,* 2020). According to the results of our study, the size and polymer type of the smallest microplastics found in *F. martensi* were 2 µm of PP, the size of microplastics that can be transported through the GI tract (Barbozaa *et al.,* 2018). It has also been reported that PP microplastics of 5 - 10 µm could be transferred through the human placenta (transplacental passage) (Ragusa *et al.,* 2021).

4. Conclusion

This is the first study to investigate the effect of microplastic accumulation in aquatic animals in the Nam Pong River, Khon Kaen Province. Microplastic accumulation studies are found in aquaculture; however, more fish species and fish collected from different communities should be considered to ascertain the potential impact of microplastics in freshwater ecosystems. Significant differences in the accumulated amounts of microplastics in different fish were found. This finding may be used as a preliminary assessment of the potential risks arising from the consumption of certain aquaculture species with different dietary characteristics.

Therefore, further studies of the health risks and the relevant factors, including microplastic size, concentration, and time to exposure, should be pursued, especially in aquaculture groups with greater numbers of species, more samples, multiple types of each species, and more specific microplastic polymer types, and the wastewater quality in the effluent channel of a sewage treatment plant should be monitored.

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