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ASSESSMENT ON MICROPLASTICS CONTAMINATION IN FRESHWATER FISH: A CASE STUDY OF THE UBOLRATANA RESERVOIR, THAILAND

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ABSTRACT: Microplastic pollution is now a global issue. Reservoirs are an enclosed aquatic environment at risk from accumulation of microplastics. Few studies have used fish species as bio-indicators to monitor microplastic contamination in reservoirs. Freshwater fish were caught by local fishermen from 10 stations around the Ubolratana Reservoir, Thailand in October 2018 and the abundance, size, color and shape of microplastic particles in their stomachs and intestines were investigated. Fourteen fish species were examined. Results showed that 96.4% of the fish had ingested microplastics at mean abundance of 2.92±1.30 particles per fish, with significant differences of abundance between species. Microplastic abundance was highest in carnivorous fish Parambassis siamensis (4.11±1.08 particles per fish). The most common size of ingested microplastics was over 0.5 mm (66.4%), with 51.0% as blue color and 98.2% fiber shaped. High levels of microplastics were ingested by fish located in the middle and lower parts of the reservoir. Results revealed that blue fiber microplastics dominantly observed in fish were derived from the breakdown of nets used for fishing activities.

Keywords: Microplastic pollution, Freshwater fish, Riservoir, Microplastic ingestion, Plastic litter

1. INTRODUCTION

In 1950, global plastic production was 2 million tons. This increased to 381 million tons in 2015. Seventy-five percent of primary plastic production ends up as waste [1], while over half of the polymers, synthetic fibers and additives produced are buried as landfill or discarded and washed into rivers and oceans [1]-[2]. Degradation or fragmentation of plastic waste generates microplastic pollution of the environment [2]-[3].

Microplastics are defined as small particles of plastic less than 5 mm in length, and further categorized according to their origin as primary and secondary. Primary microplastics are manufactured as small size for a specific purpose such as microbeads in cosmetics, exfoliating personal care or toothpaste [4], while secondary microplastics result from the breakdown of larger plastic items by sunlight, wind or waves [5]. Types of microplastics most commonly found on coastal beaches are low-density polyethylene (LDPE), polypropylene (PP) and polystyrene (PS) [5].

The direct effects of microplastics on aquatic animals are both physical and nutritional. Plastic ingredients and additives are indirectly transferred through aquatic food webs and impact food safety and human health [5]-[9]. Chemical substances added in plastic products to enhance polymer properties include diethylhexyl phthalate, dibutyl phthalate, diethyl phthalate and benzaldehyde [10]-[11]. These substances leach out from discarded microplastic waste into the environment as both aquatic and terrestrial pollution [12].

Microplastic contamination occurs in the oceans and also in freshwater. Nevertheless, research concerning microplastic pollution in freshwater systems and aquatic animals remains limited. High levels of microplastic pollution in lakes [13]-[14] and riverine fish [15]-[16] have been reported. Reservoirs are watershed areas that collect water from streams and rivers. They are closely connected to the land as important sources of plastic waste and microplastics [17]. Moreover, reservoirs comprise important fishery production habitats in South East Asia [18]. Therefore, pollution from microplastics may impact fisheries' products as a consumer health risk [18].

Here, the abundance, size, color and shape of microplastics ingested by freshwater fish in the Ubolratana Reservoir were quantified and assessed. Results will be useful for biomonitoring microplastic contamination in reservoirs and provide a database for fisheries to improve food security.

2. MATERIAL AND METHODS

Table 1 Fish species habitats, feeding habits and abundance collected from 10 stations around the Ubolratana Reservoir.

Species	Habitat	Feeding habits	Total length (cm)	Weight (g)	Numbers (Individuals)
Puntioplites proctozysron	Benthopelagic	Omnivore	7.77±3.53	13.82±7.57	37
Cyclocheilichthys repasson	Benthopelagic	Omnivore	9.45±2.06	8.18±3.25	14
Parambassis siamensis	Demersal	Carnivore	4.81±0.64	1.40±0.61	18
Henicorhynchus siamensis	Benthopelagic	Herbivore	13.52±4.84	27.14±17.22	22
Pristolepis fasciatus	Demersal	Carnivore	12.43±1.13	44.28±10.53	7
Labiobarbus leptocheilus	Benthopelagic	Planktivore	11.00±0.71	10.80±0.84	5
Barbobymus goniontus	Benthopelagic	Omnivore	9.33±1.15	11.33±3.79	3
Rasbora aurotaenia	Benthopelagic	Planktivore	8.91±0.55	5.35±0.80	10
Clupeichtys aesarnensis	Pelagic	Planktivore	3.84±0.39	0.47±0.13	10
Mystacoleucus marginatus	Demersal	Carnivore	5.83±0.98	2.73±1.42	15
Osteochilus vittatus	Benthopelagic	Omnivore	18.41±4.53	45.72±21.16	8
Paralaubuca harmandi	Benthopelagic	Carnivore	10.64±3.45	4.86±2.29	7
Mystus mysticetus	Demersal	Carnivore	12.87±1.28	19.83±4.02	6
Hemibagrus spilopterus	Demersal	Carnivore	17.10±2.43	38.00±14.53	5

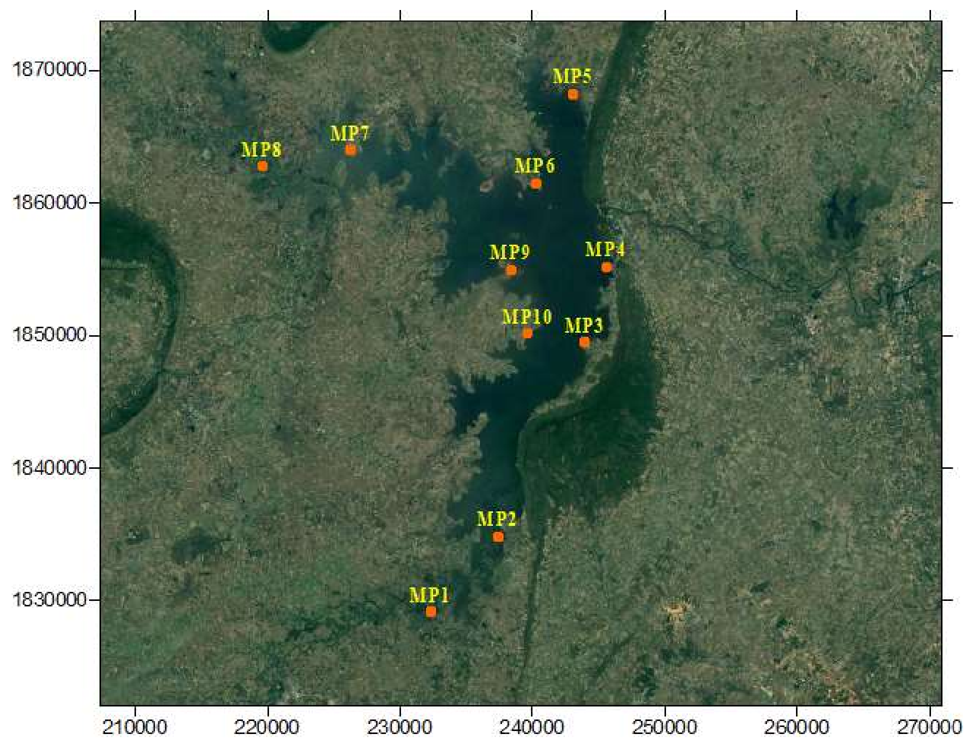


Fig. 1 Location (UTM; 48Q) of the sampling stations in the Ubolratana reservoir, Thailand.

2.1 Study sites and Samples Collection

Study sites were located at the Ubolratana reservoir, northeast of Thailand. This man-made lake was opened in 1966 for electricity generation, flood control, irrigation, etc. The reservoir aggregate water from the Nam Phong River, covered an area of two provinces and flow into the Chi River (the longest river of Thailand) with capacity 263,000 m^3 and 12,104 km^2 of catchment area. The multi-purpose reservoir was used for fisheries activity for longtime. The maximum yield in fisheries have been up to 2,480 ton/year in 1976 [19].

Fish samples were collected from ten sampling stations around the Ubolratana reservoir, Thailand (Fig. 1). Fish were caught by local fisherman using gill nets during the rainy season of August and October 2018. Fish samples were weighed and measured for total length to an accuracy of 0.1 g and 0.1 cm and then dissected to separate stomachs and intestines and stored at -20°C prior to examination for microplastics in laboratory.

2.2 Microplastic Extraction and Analysis

Fish stomach and intestine was defrosted and digested with 30% H_2O_2 in 50 ml Erlenmeyer flasks [20]. Volume of H_2O_2 was based on weight of stomach and intestine samples (approximately 30 ml/sample) and placed in an incubator at 65°C for 24 h. The separation of microplastics from extracted solution used a saturated NaCl solution (approximately 300 g/L) which filtered and added into the flasks then kept for 12 h at room temperature. Microplastics were floated by saline solution then the supernatant was pipetted and filtered through a glass microfiber filter (Whatman GF/C 1.2 μm pore size).

The numbers, colors, sizes and physical characteristics of microplastic particles on the glass fiber filter were observed under a stereomicroscope (Nikon SMZ745/745T). Microplastics were measured for their longest dimension and characteristics were divided into fibers, rods, fragments, and pellets.

The abundance of microplastics between fish species and sampling stations were analyzed by Independent-Samples Kruskal-Wallis Test with significant difference was set as p-value less than 0.05.

3. RESULT AND DISCUSSION

3.1 Percent of Occurrence and Abundance of Microplastics in Fish

Fish sample 167 individuals were identified into 14 species (Table 1). A total of 488 microplastics particles were observed inside the stomach including intestine with 96.4% of fish samples (163 individuals). Ten fish species had 100 percent occurrence of microplastics in their gastrointestinal tract (Fig. 2). The percentage of occurrence microplastics ingested by fish was higher than the Chi river of Thailand (72.9% from 8 species, n=107) [16] and the Pajeu river, Brazil [22] (83% from 1 species, n=40) but similar to that the high level and biological risks of microplatics in Poyang Lake, China (90.9% from 1 fish species, n=11) [23].

The abundance of microplastics ingested by fish varied from 0 to 6 particles per fish with an overall

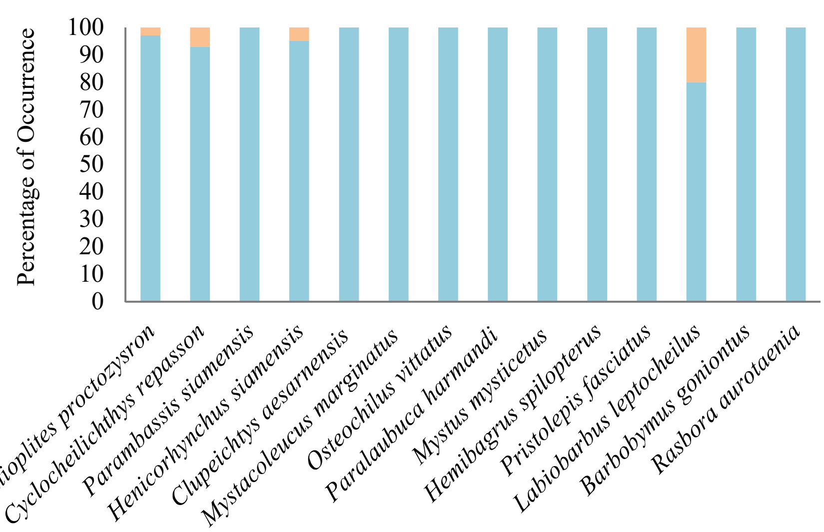
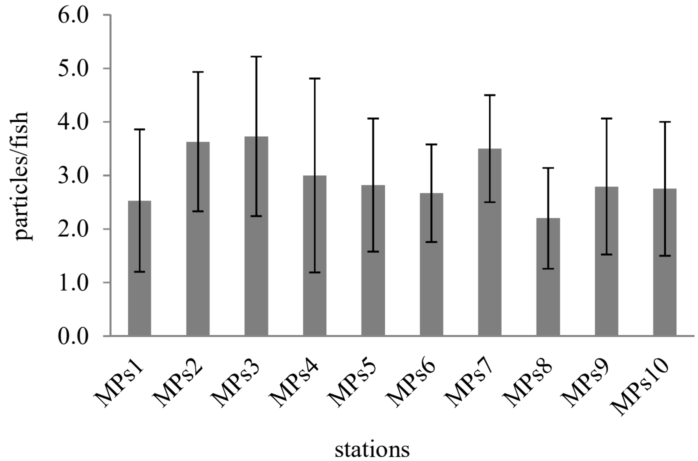


Fig. 2 The percentage of microplastics occurrence in fish gastrointestinal tract. Blue bar: ingested microplastics; Orange bar: not ingested microplastics.

average of 2.92±1.30 particles per fish, which lower than the Poyang Lake (0-18 particles/fish) [23] and Qinghai Lake (2-15 particles/fish) [24], China but higher than riverine fish of The Chi river, Thailand (1.76±0.58 particles/fish). There was significant difference in abundance of microplastics ingested by fish between sampling stations (Kruskal-Willis Test, p=0.016). The abundance of microplastics per fish varied among stations, the highest number of particles per fish was at MP3 (average 3.79±1.25 particles/fish) (Fig. 3A). The tourist spot (Bang Saen II Beaches) was located at MP3 (Fig. 1) that may cause source of microplastics. Meanwhile, station MP8 was located at upper part of reservoir had the lowed abundance of microplastics (average 2.20±1.24 particles/fish). The number of particles per fish between species was also significant difference (p=0.001) with the highest ingested microplatics in *Parambassis siamensis* (average 4.11±1.08 particles/fish) (Fig. 3B). *P. siamensis* is a demersal and carnivorous fish. There was significant difference between habitats (p=0.016) and feeding habits (p=0.002) related to abundance of imcroplastics ingested. The results showed that demersal and carnivorous had the highest abundance of microplastics particle of 3.49±1.32 and 3.29±1.33 particles per fish, respectively. The carnivorous fish ingested microplastics than other feeding habits, this difference to the riverine fish in Skudai River, Malaysia that herbivorous fish had highest microplastic ingestion (1.50±1.73 particles/fish) [25].



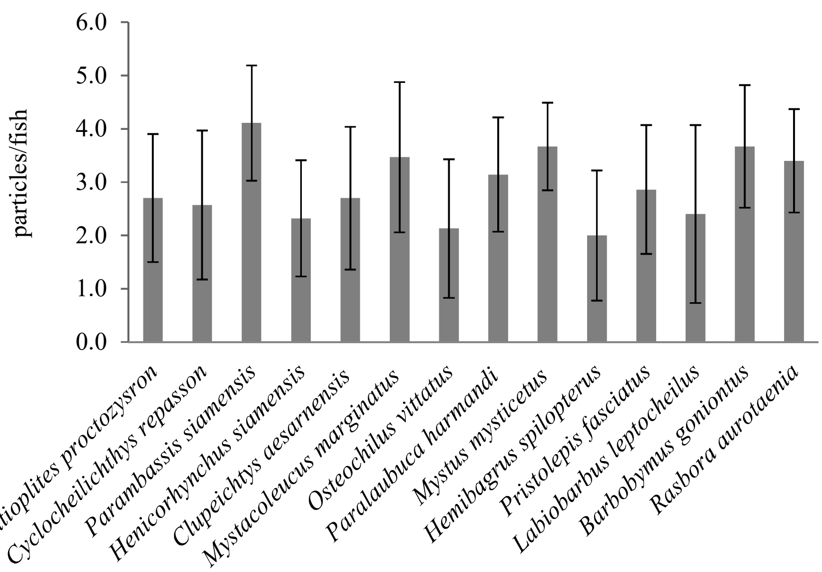


Fig. 3 The mean abundance of microplastics between sampling stations around Ubolratana reservoir (A) and mean abundance microplastics in fish digestive tract by species (B). Error bar is ± SD.

3.2 Colors, Physical Characteristics and Size of Microplastic Particles

Four colors of microplastics were found in gastrointestinal tract of fish which compose of blue, black, transparent and red. The main colors of microplastics in fish samples was blue (51.0%) (Fig. 4A), concurrent with the former studies such as fish in Chi River, Thailand [16] and Skudai River, Malaysia [25]. The color proportion of microplastics in each fish species were similar (Fig. 5). Although most of fish feed on resemble their prey [23],[26] but our results showed that blue microplastics was dominant color in all fish species. These may related to the microplastics abundance in fish environment [23] and there were unexpectedly ingested while fish feeding. Moreover, blue color was a favorite color of fishing net which was used in the Ubolratana reservoir.

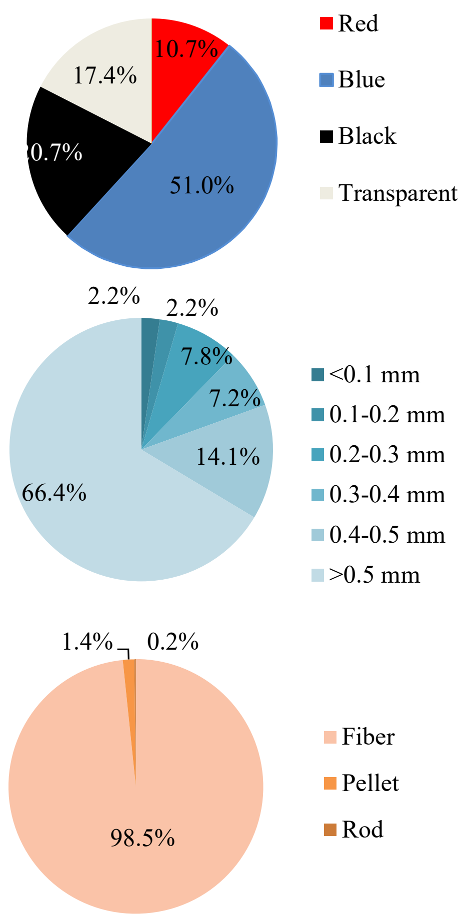


Fig. 4 Percentage of microplastics ingested by all fish samples base on color (A), shape (B) and size (C).

Three shapes of microplastics were found in fish. The common shape of microplastics ingested by fish was fiber (98.2%) followed by pellet and rod, respectively (Fig. 4B). Fiber character of microplastics were also a major shape in other reports such as in waters and sediments of Magdalena, Colombia [2] and Taihu Lake, China [14], in fish gut of Chi River, Thailand [16] and fish of Poyang Lake, China [23]. Source of fiber shape microplastics come from nylon rope, nylon line, fish cage, other fishing gear [27] and sewage from washing clothing made of woven synthetic textiles [2].

Ingested microplastics size ranged between 0.03-4.77 mm. The dominant size-class was larger than 0.5 mm (66.4% of total plastic particles) (Fig. 4C). Microplastics fiber shape are usually classified into large size over 0.5 mm [16]. These size rage were similar to study in Poyang Lake, China found microplastics in fish gut larger than 0.5 mm, accounting for 82.1% of total plastic particles [23].

The dominant of color, shape and size in present study were blue fiber and larger than 0.5 mm. The result showed impact of plastic fishing gear which used in fisheries around reservoir to the abundance and physical character of microplastics. Damaged or unusable fishing gears disposal, net repairing and other fishing related activities increased abundance of microplastics as PA nylon in aquatic environments [27], finally could transport to aquatic organism like fish [13].

Freshwater fish collected in this study are commercial fish in local market of Thailand and some species were made into fishery products such as pickled fish. Thus, the potential consumer health risk can be higher when microplastics comtaminated in fish [9]. However, the monitoring and mitigation of microplastics pollution must be continue investigated and including the detrimental impact to aquatic ecosystem and human health.

4. CONCLUSION

The assessment on microplastics contamination can be used fish species as bioindicators. The abundance of microplastics ingested by fish in the Ubolratana reservoir has nearly reached to high-level contamination in freshwater fish compared to results of previous studies in freshwater systems. Opportunities to convey microplastics through the food chain must be concerning and monitoring. Physical chracters of microplastics indicate their origin as mainly from damaged fishing gear such as fish cages and gill nets

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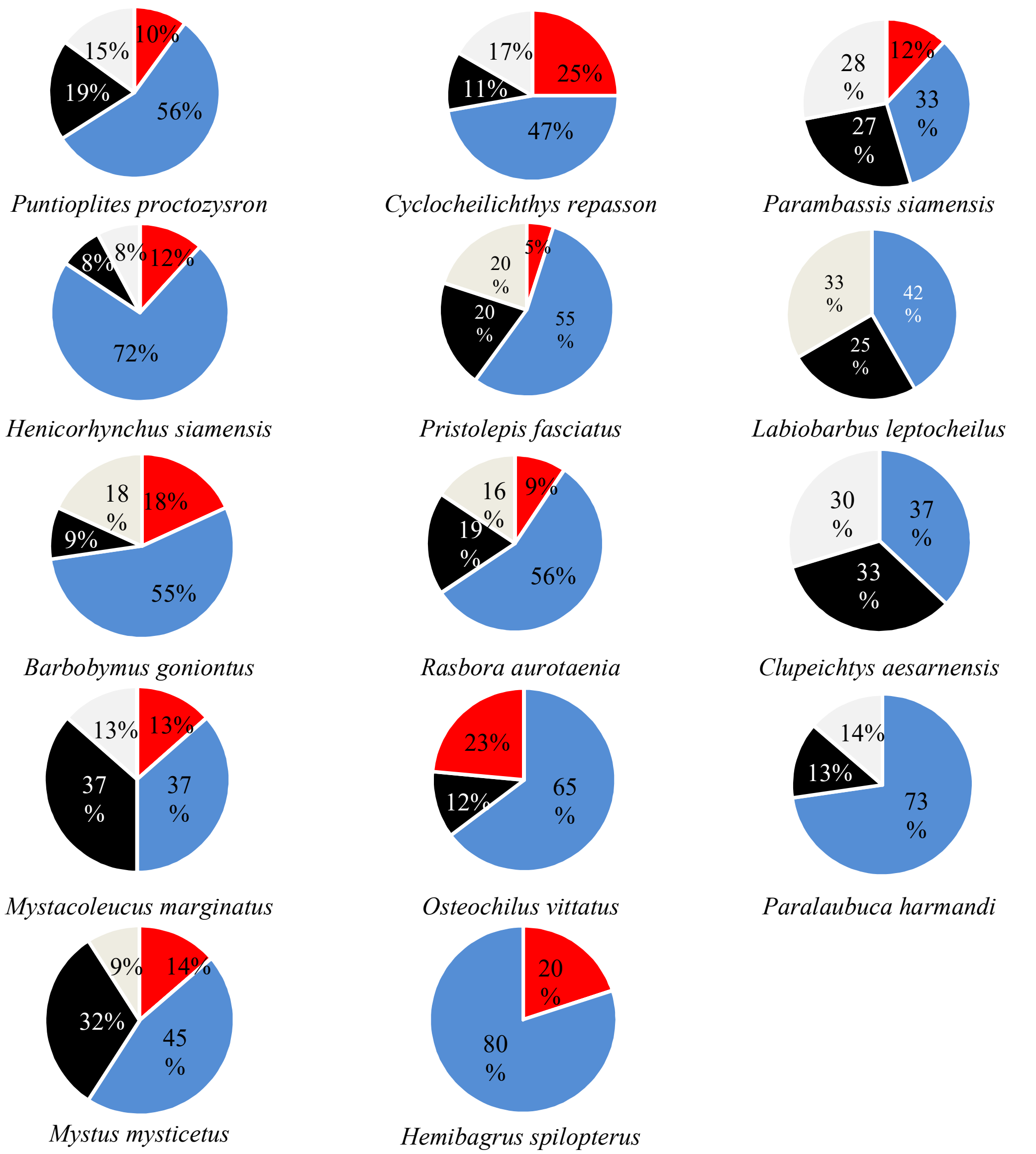

Fig. 5 Composition of microplastics particles colors in each fish species.

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