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MICROPLASTICS INGESTION BY FRESHWATER FISH IN THE CHI RIVER, THAILAND

*Pattira Kasamesiri[1] and Wipavee Thaimuangphol [2]

[1,2]Faculty of Technology, Mahasarakham University, Thailand; [1,2]Research Unit of Excellence for Tropical Fisheries and Technology, Mahasarakham University, Thailand

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ABSTRACT: Microplastic pollution mainly emanates from terrestrial sources but studies of plastic contamination in freshwater ecosystems remain limited. Consumption of freshwater fish is widespread throughout all regions of Southeast Asia. Contamination of microplastics in fish is an important issue which leads to human health risk. Common freshwater fish in the Chi River, Thailand were caught by local fishermen and investigated for abundance, size, color and shape of microplastics. Eight fish species were investigated. Results showed that 72.9% of the collected fish were polluted with microplastics at mean abundance of 1.76±0.97 particles per fish and was no significant difference of abundance between species. Percentage occurrence of microplastics was highest in omnivorous fish *Puntioplites proctozysron* (86.7%) with the most common size of microplastics ingested by fish at over 0.5 mm (47.5%), of which 56.9% were blue color and 86.9% were fiber shaped. Results revealed that fishing nets and fish cages were major sources of microplastic contaminants in the Chi River.

Keywords: Microplastics Pollution, Freshwater fish, Stomach content, Aquatic Environment

1. INTRODUCTION

Plastic production has continually increased since 1950, reaching 332 million tons in 2015 [1]. Increasing plastic debris has become a serious pollution problem in the aquatic environment. The amount of plastic debris is now 10 times greater than a decade ago on the shores of South Atlantic islands [2]. Plastic debris is widely distributed over ocean shorelines and has also become a major component of riverine pollution [3]-[5], which impacts to aquatic animals such as zooplankton, aquatic invertebrates, fish, marine reptiles and mammals by ingestion [6]- [10].

Microplastics are small plastic items with particle size <5 mm which can be classified into primary microplastics (pellets, plastics used in cleansing, cosmetic products and manufactured plastic products) and secondary microplastics [11]. Secondary microplastics originate from degradation and fragmentation of large plastic items and enter the aquatic environment through different pathways [1], [12]. Microplastics are easily spread with wide distribution because of their small size, light weight and durability [13]. Oceans and many urban rivers are contaminated with microplastics [14].

Direct effects of pollution by microplastics on aquatic organisms hamper their ability to ingest natural prey by obstruction of the digestive tract [6] and reduction of swimming velocity which affects resistance time of fish when swimming against the water flow [15]. Microplastic ingestion also leads to neurotoxicity and oxidative damage in marine fish [16] and reduction in photosynthetic activity and chlorophyll *a* of algae [17]. Moreover, various chemical substances are contained in all plastic products such as styrene, toxic metals, polychlorinated biphenyls (PCB), bisphenol A (BPA), polycyclic aromatic hydrocarbons (PAHs) and phthalates for improving polymer properties [18]-[19]. Microplastics deliver these chemical substances to aquatic organisms and act as mediators for other chemical contaminants in aquatic environments [20].

Microplastics transfer from primary consumers to higher trophic levels via a trophic food web and, finally, may contaminate humans [12], [19], [21]. Thus microplastic contamination is a crucial issue for food safety and human health [19]. Reports concerning microplastic contamination in fish have mainly concentrated on oceans and shorelines with the minority assessing the effects in freshwater environments [22]-[27]. Thus, this study assessed the abundance, size, color and shape of microplastics ingested by freshwater fish in the Chi River. Results will provide useful data for fishery and aquaculture food security.

2. MATERIAL AND METHODS

2.1 Samples Collection

Fish samples were collected from four sampling stations along the Chi River in the northeast of

Table 1 Species and number of fish samples caught in the Chi River

Species	Habitats	Feeding features	Total Length (cm)	Weight (g)	Total Number
Labiobarbus siamensis	benthic	Detritivore	9.6±0.7	8.0±2.3	15
Puntioplites proctozyson	midwater – benthic	Omnivore	11.1±3.2	20.4±17.7	6
Cyclochelichthy repasson	midwater – benthic	Omnivore	11.8±2.6	18.7±11.4	15
Henicorhynchus siamensis	midwater	Omnivore	12.4±1.2	20.1±6.1	27
Labeo chrysophekadion	benthic	Detritivore	17.0±2.8	57.5±23.7	14
Mystus bocourti	benthic	Carnivore	14.9±2.7	21.1±9.2	20
Hemibagrus spilopterus	benthic	Carnivore	20.4±3.9	56.7±27.2	6
Laides longibarbis	pelagic-benthic	Detritivore	15.8±1.3	22.5±2.4	4

Thailand (Fig. 1). The Chi River is the longest river flowing within Thailand at 765 kilometers and runs through seven provinces. The four study sites were located along the middle region where the river flows through urban areas of Maha Sarakham Province. Sources of plastic pollution were composed of fishing activity, aquaculture, agriculture, and sewage from industrial factories and residential areas. Fish were caught by local fisherman using gill nets during the late rainy season (October 2018) and stored at -20°C prior to examination for microplastics.

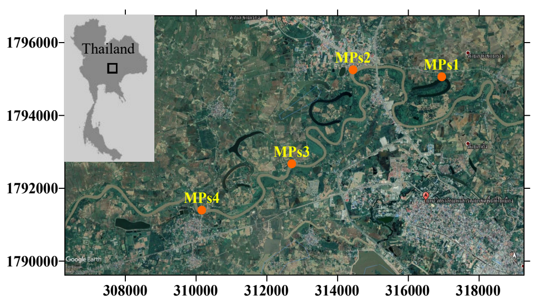

Fig.1 Location (UTM; 48Q) of the sampling stations at Chi River, Maha Sarakham Province, Thailand

2.2 Microplastic Extraction and Analysis

Fish samples were weighed and measured for total length to an accuracy of 0.1 g and 0.1 cm, respectively. After dissection, fish stomachs and intestines were placed in 50 ml Erlenmeyer flasks and 30% H_2O_2 was used to digest the organic matter (Jabeen 2017). Volume of H_2O_2 was based on weight of stomach and intestine samples (approximately 30 ml/sample). Extracted samples were placed in an incubator at 65°C for 24 h. A saturated NaCl solution (approximately 300 g/L) was filtered and added into the flasks to separate microplastics by flotation. The saline solution was kept for 12 h at room temperature and then the supernatant was pipetted and filtered through a glass microfiber filter (Whatman GF/C 1.2 µm pore size). The filter paper was placed on Petri-dishes to record the numbers of microplastic particles, and colors and physical characteristics were divided into fibers, rods, fragments, and pellets under a stereomicroscope (Nikon SMZ745/745T). Microplastics were measured for their longest dimension

2.3 Statistical Analysis

Significant differences of microplastic abundance in fish gut among sampling stations and fish species were analyzed by Independent-Samples Kruskal-Wallis Test. Significant difference was set as p-value less than 0.05.

3. RESULT AND DISCUSSION

3.1 Abundance of Microplastics Ingested by Fish

A total of 107 individual fish were identified into 8 species (Table 1). We observed microplastics inside the gastrointestinal tract of 78 individuals (72.9% of total fish samples). Percentage occurrence of microplastics in each species ranged between 50.0-86.7% with the highest in *Puntioplites proctozysron* (Smith's barb) (Fig. 2). All feeding types (omnivore, carnivore, detrivore) showed high occurrence of microplastics (>50%) but less than planktivorous fish (77%) from Tokyo Bay, Japan [30]. Proportion of ingested microplastics was higher than fish in the English Channel (36.5% from 10 fish species) [22], islands

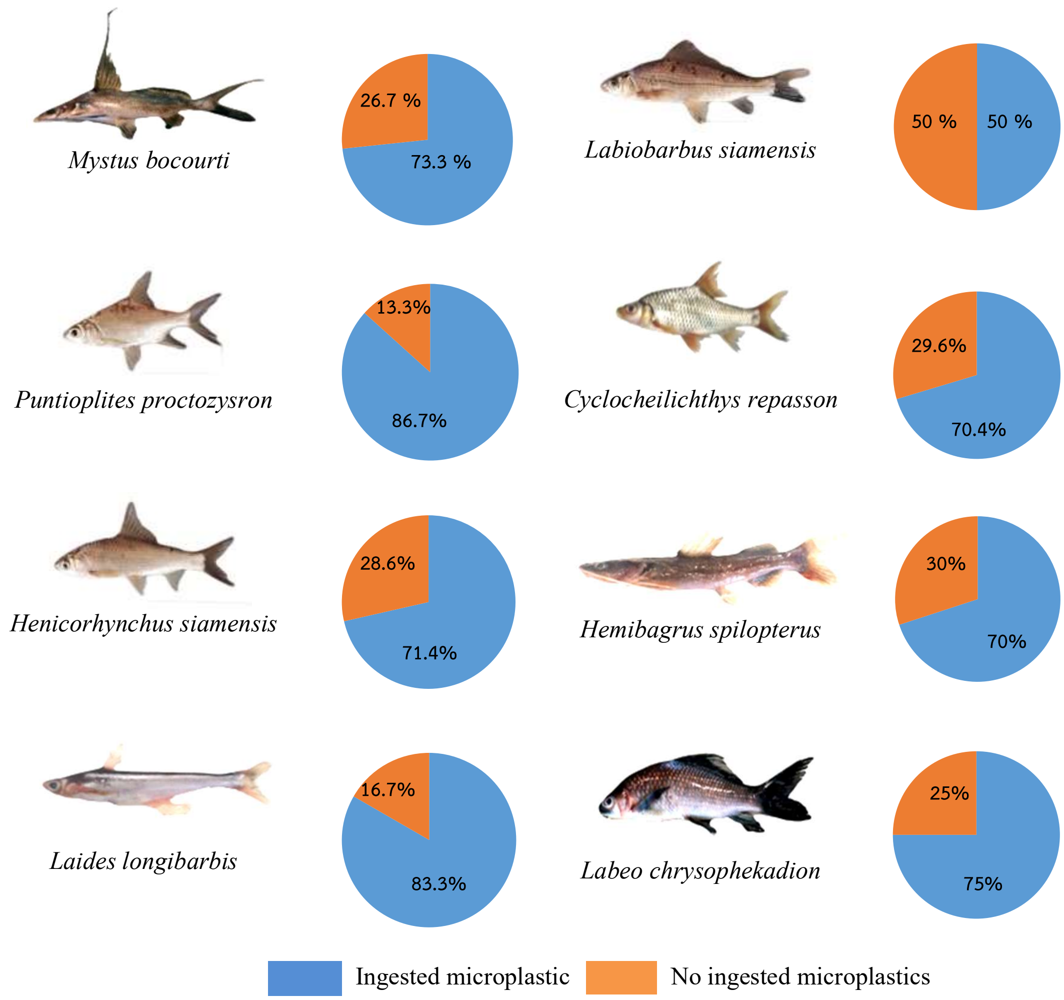

Fig.2 The percent occurrence of microplastics ingested by fish in each species

in French Polynesia (37.2% from 4 fish species) [23] and estuaries of Brazil (9% of 69 fish species) [25]. Microplastics ingested by the fish samples varied from 1 to 2 particles per fish with an average of 1.76±0.58 (Fig. 3). There was no significant difference in abundance of microplastics ingested between species (Kruskal-Willis Test, p-value=0.849). Omnivorous fish in intertidal zones of Chile recorded significantly higher numbers of microplastics than herbivorous and carnivorous fish [26], showing that occasions to ingest microplastics of feeding types were similar in present study. Abundance of microplastics was relatively high compared with fish from tropical estuaries of Brazil that affected from the anthropogenic pressures (0.12±0.37 particle per fish) [25]. Conversely, abundance of microplastics was lower than fish in the Pajeu River of Brazil (3.6 particles per fish) [7], possibly due to different flushing efficiency from river catchments and diverse microplastic sources [5], [12].

3.2 Colors, Shapes and Size of Microplastics Ingested by Fish

Six colors of microplastics were found in fish gut as blue, red, black, white, transparent and brown. The most common color was blue 56.9% (Fig. 4A). Most abundant color results concurred with former studies [8], [13], [28]. Variety of colored microplastics was higher in *Mystus bocourti* and *Cyclocheilichthys repasson* (Fig. 5) as carnivorous and omnivorous species, respectively. Fish may ingest microplastics as a result of visual confusion between prey [28] but our results gave similar color patterns between different feeding types.

Four shapes of microplastics were presented in fish gut (Fig. 6). The most abundance shape was fiber followed by rod-shaped, pellet and fragment, respectively (Fig. 4B).

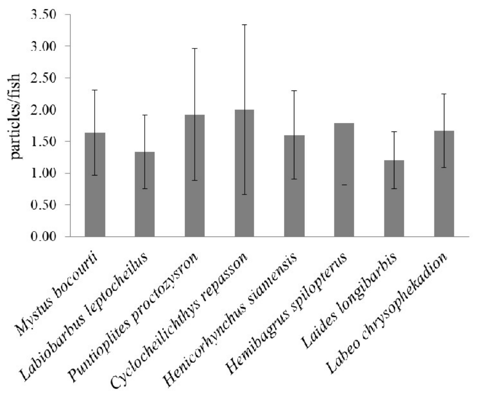

Fig.3 The average number of microplastic ingested by fish (particles per fish) in each species. (Mean±SD)

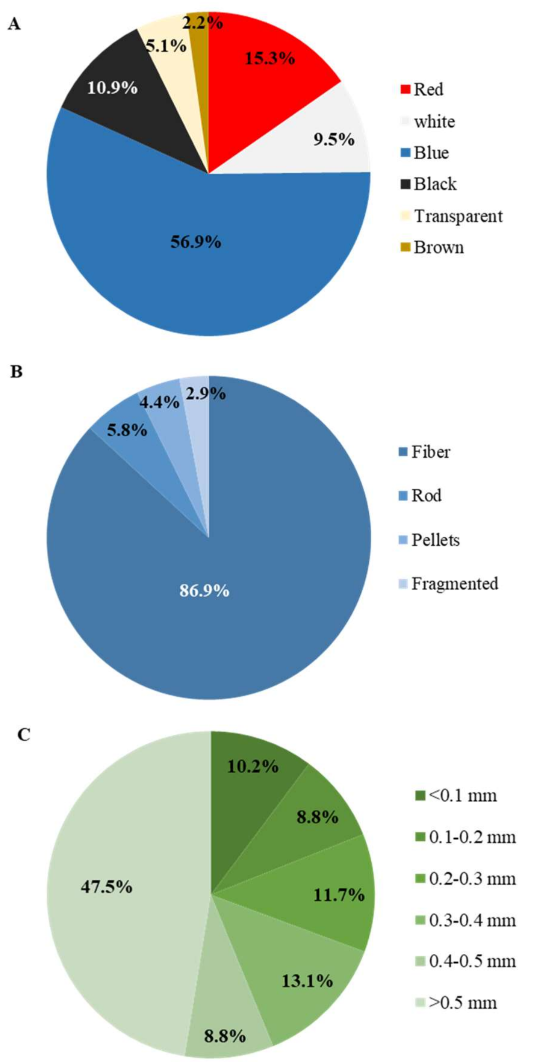

Fig. 4 The percentage of the total number of microplastics ingested by freshwater fish of color (A), shape (B) and size (C).

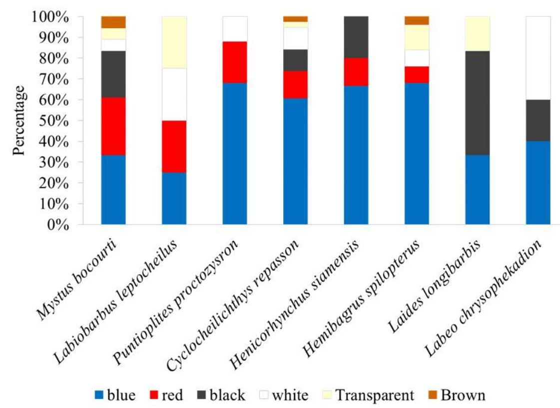

Fig.5 The percentage of microplastics colors in each fish species.

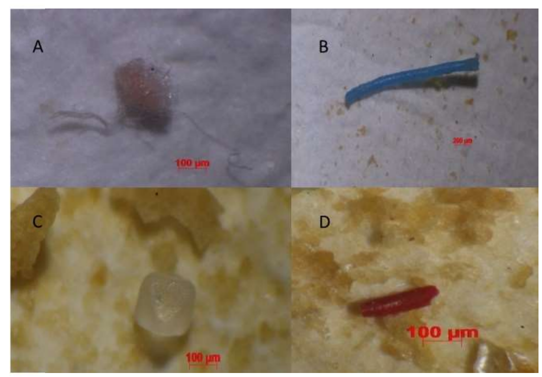

Fig.6 Shape of microplastics found in stomach and intestine of fish: fiber (A), rod (B), pellet (C) and fragment (D).

Fiber shape was a major type ingested by fish [7], [13], [28] and related to human activities [12]. An important source of fiber microplastics is from degradation of fishing gear, fish cages or nylon ropes [8] and sewage from washing clothes [29].

Size distribution of microplastics ranged between 0.03-3.84 mm. The most abundant size was over 0.5 mm (47.5% of the total number of plastics) (Fig. 4C). Large particles were common in fiber type. Our results were similar to a previous report on surface water in reservoirs of China which found microplastic sizes ranging from 1 to 5 mm as more abundant [31]. Fish from coastal and freshwater areas of China contained microplastics ranging from 0.04-5 mm (76.3%) [13], while in our study, particles smaller than 5 mm were 100% of total number of plastics ingested by fish.

Size of plastics larger than 0.5 mm are used in fishing ropes or lines [30], indicating that the source of microplastics in the Chi River emanated from fishing gear and equipment used in aquaculture. The Chi River is an important fishery resource in the northeast of Thailand with abundant fishing activities [32]. Local fisherman usually use transparent or black gillnets to catch fish [32]. Blue fiber fish cages in Nile tilapia aquaculture may also be an important source of microplastics ingested by freshwater fish in the Chi River. Plastic materials are used in fishing gear such as nets, traps, hooks and lines. These are commonly made from polyamide (PA) and polyethylene (PE) [1]. Polyamide is a low-density polymer that is found in both pelagic and demersal fish [22]. Results confirmed that the percentage occurrence of microplastics ingested by each fish species was not related to vertical habitat zonation but concerned feeding activities.

4. CONCLUSION

The abundance of microplastics ingested by fish in the Chi River indicated middle-level contamination in aquatic animals compared to results of previous studies in both freshwater and ocean fishes. Opportunities to convey microplastics through the food chain into the human body remain limited. Shapes of microplastics indicate their origin as mainly from fishing gear such as nets and synthetic fibers from clothing.

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