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First Evidence of Microplastics Ingestion by Freshwater Fishes from Nong Luang Wetland, Chiang Rai, Thailand

(Bukti Pertama Ketidakhadaman Mikroplastik oleh Ikan Air Tawar dari Tanah Bencah Nong Luang, Chiang Rai, Thailand)

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ABSTRACT

Microplastic (MP) ingestion has been reported in several marine species but knowledge regarding MPs in freshwater species is limited, with only a few studies on the occurrence of MPs in freshwater fish, particularly in Thai freshwater ecosystems. The aim of this study was to assess the occurrence of MPs for the first time in the gastrointestinal tracts (GITs) of freshwater fishes from the Nong Luang Wetland, Chiang Rai, Thailand. A total of 100 fish specimens representing 15 species from 8 families were examined. Basic fish measurements as total length and total weight were used to calculate Fulton's condition index (F). The GITs were subsequently removed and MPs were extracted by visual inspection and digestion solution. Qualitative attributes such as color and MP types (fibers, rod-shaped and fragments) were also recorded. Results showed relatively low MP prevalence at 21.0%, with 25 MP particles removed from the GITs of specimens representing 7 species. The number of ingested MP particles ranged between 1 and 4 per fish, with an average of 1.19±0.68. MP particles were categorized as fibers (92.0%), rod-shaped (4.0%) and fragments (4.0%) with blue fibers the most prevalent. No significant correlations were observed between the number of MP particles and the body length, weight, and F value of fish. These findings provide the first evidence of MP contamination in fishes from a Thai wetland as a baseline for future studies and risk assessments on the biota of many aquatic systems spread across the country.

Keywords: Blue fibre; fishing gear; freshwater fish; microplastic; wetland

ABSTRACT

Penghadaman mikroplastik (MP) telah dilaporkan dalam beberapa spesies marin tetapi pengetahuan mengenai MP dalam spesies air tawar adalah terhad dengan hanya beberapa kajian mengenai kejadian MP dalam ikan air tawar, khususnya dalam ekosistem air tawar Thailand. Matlamat kajian ini adalah untuk menilai kejadian MP buat kali pertama dalam saluran gastrousus (GIT) ikan air tawar dari Tanah Bencah Nong Luang, Chiang Rai, Thailand. Sebanyak 100 spesimen ikan mewakili 15 spesies daripada 8 famili telah diperiksa. Ukuran asas ikan sebagai jumlah panjang dan jumlah berat digunakan untuk menghitung indeks keadaan Fulton (F). GIT kemudiannya dikeluarkan dan MP telah diekstrak melalui pemeriksaan visual dan penyelesaian pencernaan. Atribut kualitatif seperti warna dan jenis MP (gentian, berbentuk batang dan serpihan) turut direkodkan. Keputusan menunjukkan kelaziman MP yang agak rendah pada 21.0% dengan 25 zarah MP dikeluarkan daripada GIT spesimen yang mewakili 7 spesies. Bilangan zarah MP yang dihadam adalah antara 1 dan 4 setiap ikan, dengan purata 1.19±0.68. Zarah MP dikategorikan sebagai gentian (92.0%), berbentuk batang (4.0%) dan serpihan (4.0%) dengan gentian biru yang paling lazim. Tiada korelasi yang ketara diperhatikan antara bilangan zarah MP dan panjang badan, berat dan nilai F ikan. Hasil ini memberikan bukti pertama pencemaran MP dalam ikan dari tanah bencah Thailand sebagai garis asas untuk kajian masa hadapan dan penilaian risiko ke atas biota kebanyakan sistem akuatik di seluruh negara.

Kata kunci: Gentian biru; ikan air tawar; mikroplastik; peralatan menangkap ikan; tanah bencah

INTRODUCTION

Due to high production levels and improper waste management, plastics are now increasingly discarded into the aquatic environment. Accumulation of plastic debris caused by anthropogenic activities is polluting aquatic ecosystems but few studies have addressed this aspect. Plastics generate remarkable societal benefits, with outstanding features of light weight, durability and versatility at low production cost (Andrady & Neal 2009; Hammer, Kraak & Parsons 2012; McDevitt et al. 2017). Plastic particles less than 5 mm in diameter are termed microplastics (MPs). These comprise a heterogeneous group of particles differing in size, shape, chemical composition and specific density that originate from diverse sources. MPs can be categorized into primary MPs and secondary MPs. Primary MPs are commonly defined as micro-sized particles released into the environment as resin pellets (raw material for the production of plastic products) or as personal healthcare product ingredients (e.g., peelings and shower gels), while secondary MPs emanate from fragmentation of large plastic materials by biodegradation, photodegradation, thermooxidative degradation, thermal degradation or hydrolysis (Duis & Coors 2016; Thompson et al. 2009; Wagner et al. 2014).

MP wastes pollute a wide range of natural terrestrial, freshwater and marine habitats (UNEP 2018). MPs enter aquatic environments by many pathways and cause physical and toxicological effects on organisms at different trophic levels (Anbumani & Kakkar 2018; Horton et al. 2017; Law & Thompson 2014). MPs are difficult to identify and readily ingested by aquatic organisms because of their microscopic size. They can absorb and transport more organic matter than large plastic particles because of their greater relative surface area (Bakir, Rowland & Thompson 2013, 2012). Anbumani and Kakkar (2018) and Ha and Yeo (2018) presented a generic overview on the exposure and toxicity of MPs, and determined growth impairment, behavioral impairment, reproductive impairment, feeding impairment, reduced survival and increased mortality in aquatic organisms. The toxicity profile of MPs in short-term exposure does not have a major biological effect but numerous studies have identified toxicity from long-term exposure (Anbumani & Kakkar 2018; Foley et al. 2018; Ha & Yeo 2018; Li, Liu & Chen 2018; Wagner et al. 2014).

Recent research on MPs has focused on marine and coastal environments rather than freshwater waterbodies, and these ecosystems require detailed investigation (Li, Liu & Chen 2018; Wagner et al. 2014; Yu et al. 2018). Several studies have reported on the ingestion of MPs by marine species; however, information for freshwater species, especially freshwater fish is scarce. The first evidence on the occurrence and detection of MPs in freshwater fishes was reported by Campbell, Williamson and Hall (2017) and Silva-Cavalcanti et al. (2017), with most previous studies concentrated in Europe, North America and East Asian regions (Ajith et al. 2020). Few studies have been conducted in Southeast Asia, particularly in Thailand, with only a few studies on the occurrence of MPs in freshwater fish (Kasamesiri & Thaimuangphol 2020; Kasamesiri et al. 2021). Kasamesiri and Thaimuangphol (2020) reported on MP ingestion by freshwater fish in the Chi River as the first observations on Thai river ecosystem biota. Recent studies conducted in Thai reservoir (Ubolratana reservoir) also showed that the freshwater fish had ingested MPs (Kasamesiri et al. 2021).

MPs have been studied worldwide in terrestrial and freshwater ecosystems, particularly in wastewater and other water bodies (Elkhatib & Oyanedel-Craver 2020; Rochman 2018). However, only a few studies have focused on the occurrence of MPs in freshwater systems such as lakes, rivers and wetlands (Horton et al. 2017; Kumar, Sharma & Bandyopadhyay 2021). Wetlands provide many ecosystem services, and also provide for human livelihoods, including aesthetic and recreational values (Mitsch, Bernal & Hernandez 2015; Scholte et al. 2016). Wetlands also act as a reservoir (i.e., both sink and source) of MPs (Kumar, Sharma & Bandyopadhyay 2021). This study was conducted in the Nong Luang Wetland, Chiang Rai Province, Northern Thailand. This small-scale wetland in the Chiang Saen Valley is surrounded by low mountains and hills. The Nong Luang Wetland has been designated as wetland of international importance. It is located near the Nong Bong Kai wetland, designated as a Ramsar site in 2001 (TISTR 2011). Many people live in and around this wetland and depend on the wetland's resources for their livelihoods. Nong Luang Wetland is a well-renowned area with developed agriculture, aquaculture, and animal husbandry (Masa 2015; ONEP 2004; Panboon, Soe-been & Sukkasem 2017). Wetland investigations based on fishes have limited impacts on the understanding of pollutant effects on fish populations but provide valuable information about resident biota. Freshwater biota research is lacking in Thailand, and this investigation was conducted to determine baseline pollution levels of MPs in the gastrointestinal tracts (GITs) of fishes from the Nong Luang Wetland. Study findings will fill the current knowledge gap as the first scientific record of the occurrence of MPs in the GITs of freshwater fish captured in Nong Lung wetland, Chiang Rai Province, Northern Thailand.

Materials and Methods

STUDY AREA

This study was conducted in the Nong Luang Wetland, a small-scale wetland area in the Chiang Saen Valley of

Chiang Rai Province, Northern Thailand surrounded by low mountains and hills. Nong Luang Wetland covers an area of 14.71 km^2 and has been designated as a wetland of international importance (TISTR 2011). This wetland stores rainfall and water runoff from the highlands and consists of marshes and swamps. Many major tributaries as the Mae Lao River, Huai Sak Stream, Rong Ber Stream and Cham Tong Stream drain to this wetland and exit through the narrow northern channel to the Kok and Mekong Rivers, respectively (Masa 2015). The Nong Luang Wetland area encompasses the Wiang Chai and Don Sila Sub-districts of Wiang Chai District, as well as areas in the Huai Sak Sub-district of Mueang Chiang Rai District in Chiang Rai District in Chiang Rai Province (19º50'55.1"N, 99º56'32.6" E; Figure 1), located near the Nong Bong Kai wetland (designated as a Ramsar site in 2001). This area forms part of an ecologically connected network comprising wildlife, water birds and migratory birds (ONEP 2004). Many people living in and around this wetland depend on the wetland's natural resources for their livelihoods.

SAMPLE COLLECTION

Before sampling, all tools and containers were cleaned with filtered pure water. Fifteen species of fish as 100 individuals (Table 1) were collected from local markets situated nearby Nong Luang Wetland. Sources of plastic pollution included fishing activities, aquaculture, agriculture and sewage from residential areas. All the fishes were caught by local fisherman during the rainy season (August-September 2020). Criteria for choosing different species included their importance in the fishing activities of the area and also their diverse feeding features and habitats. Samples were transported in iceboxes to the laboratory and stored at -20 °C before examination. Plastic material was avoided throughout the whole sampling process.

SAMPLE PROCEDURE AND MICROPLASTIC IDENTIFICATION

In the laboratory, freshwater fishes were identified to species level, then weighed and measured for body length to an accuracy of 0.01 g and 0.1 cm, respectively. Body length was measured from the tip of the snout to the end of the caudal fin. Fulton's condition index (F) was calculated for each fish using the formula:

$$F = 100 \times W/L^3$$

where W is the weight in grams and L is the length in cm (Nash, Valencia & Geffen 2006).

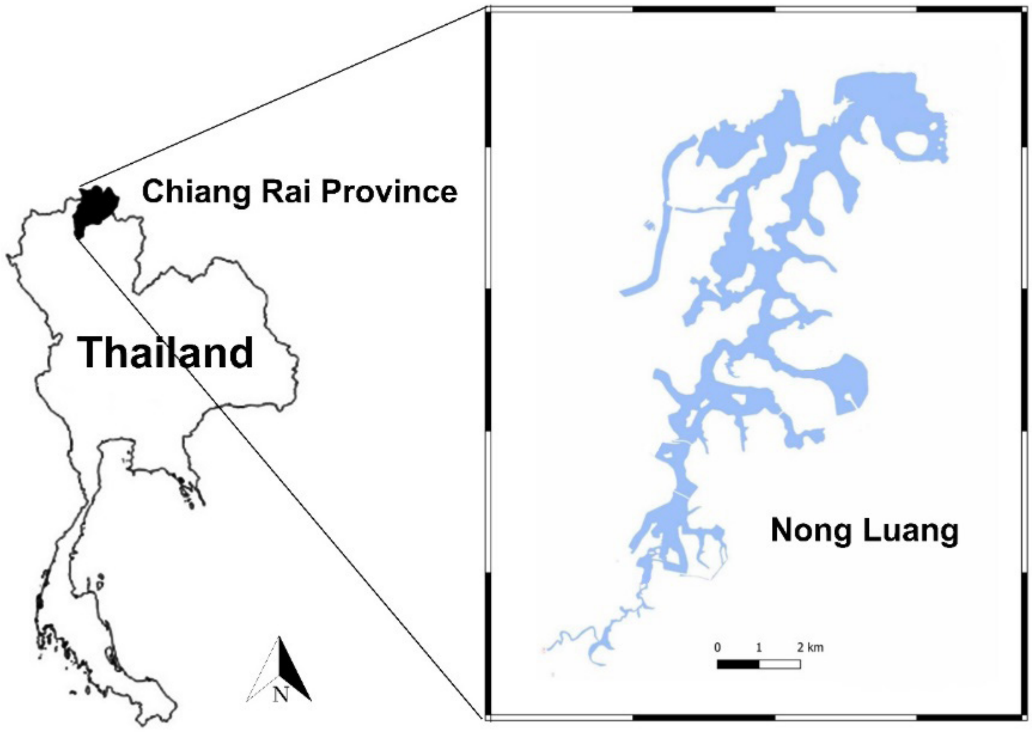

FIGURE 1. Geographic position of Nong Luang Wetland (Chiang Rai Province, Thailand)

Lab coats and disposable latex gloves were worn to prevent sample contamination with MPs from other sources. All laboratory materials (Petri dishes, scalpels, scissors and tweezers) used during the sample processing were also cleaned with filtered purified water before use following previous methods (Bessa et al. 2018; Calderon et al. 2019; Yuan et al. 2019). The samples were analyzed in a laboratory with restricted access to prevent airborne contamination during the entire sampling procedure (visual inspection and digestion of solutions).

Fish samples were cut open with a scalpel and the gastrointestinal tracts (GITs) were immediately placed in clean glassware to minimize the risk of contamination. The hydrogen peroxide (H_2O_2) solution digestion method was conducted as previously described (Jabeen et al. 2017; Pazos et al. 2017) with some modifications. Briefly, each dissected GIT was placed in an Erlenmeyer flask and digested using 30 mL of 30% H_2O_2 (Merck, Germany). The volume of liquid did not exceed 50% of the total volume of the flask. The flasks were then covered with tinfoil and placed in an incubator at 65 °C for 24 h. Three blanks with only 30% H_2O_2 were run for each batch of 20 samples to correct for potential airborne MP deposition in the laboratory (no contamination was found in the blanks during MP extraction). After 24 h of digestion, saturated NaCl solution (density 1.20 g cm^{-3}) was filtered and added into the flasks to separate the MPs by floatation. The mixtures were manually stirred for 2 min and allowed to settle for 12 h at room temperature. The supernatant was then pipetted and filtered through 8 µm Whatman paper.

The filter paper was stored in a glass Petri dish and subsequently observed under a stereomicroscope (Nikon SMZ-2B, Japan). Suspected MPs were photographed and analyzed following previously established criteria (Hidalgo-Ruz et al. 2012; Lusher, McHugh & Thompson 2013; Nor & Obbard 2014). Particles were classified as MP-like if they had the following characteristics: (i) no cellular or organic structures visible, (ii) fibers equally thick throughout the length, (iii) homogeneously colored, (iv) if fibrous, all fibers had 3-dimensional bending and (v) particles were shiny and not matte. The MPs were categorized as fibers, rod-shaped and fragments according to Tanaka and Takada (2016). The number, shape and color of the MPs were recorded.

DATA ANALYSIS

The frequency percentage of MP occurrence within the GITs was calculated using the formula:

$$FO\% = (Ni/N) \times 100$$

where FO% is the frequency of occurrence of MP particles; Ni is the number of GITs containing MPs and N is the total number of GITs examined (Pegado et al. 2018).

Spearman correlation analysis was used to assess possible relations between the number of MPs and body length, weight and Fulton's fish condition index. Statistical tests were only conducted on specimens with MP particles in the GIT and performed using SPSS 28.0.1.0. Statistical tests were considered significant at P value < 0.05.

ETHICS APPROVAL

Fieldwork, including collection of fishes in the field and specimen preparation in the laboratory, were managed according to the guidelines approved by the Institute of Animals for Scientific Purposes Development (IAD), National Research Council of Thailand, and the relevant document (no. U1-03137-2559) was approved by the committee. The guidelines for animal care and use of animals for research, testing, biological production and teaching in the kingdom of Thailand were used according to the Animals for Scientific Purposes Act 2015. This study complied with all the relevant national regulations and institutional policies for the humane care and use of animals.

RESULTS

A total of 100 fish specimens were collected and classified into 8 families, 11 genera, 15 species and four feeding habits as carnivore, detritivore, omnivore and zooplanktivore (Table 1). Body length averaged 12.1±3.97 (6.0-24.0) cm, with average body weight 46.96±31.90 (8.96-163.35) g. Fulton's condition index ranged from 1.25 to 5.06 (average 2.73).

Numbers of MPs in fish GITs were analyzed and are listed in Table 2. Overall, 21.0% (21/100) fish retained MPs, with 25 MPs removed from the 7 fish species *Trichopodus microlepis*, *Oreochromis niloticus*, *Cyclocheilichthys armatus*, *Gymnostomus siamensis*, *Labiobarbus siamensis*, *Oxyeleotris marmorata,* and *Pristolepis fasciatus* that each contained at least one MP. The percentage of occurrence of fish species with MPs ranged from 15.79 to 100, while MPs/fish ranged from 1.0 to 2.0. Among the 7 different species, *L. siamensis* recorded the highest MP prevalence (FO% = 100) followed by *C. armatus* (FO% = 50.0), *G. siamensis* (FO% = 28.57), *P. fasciatus* (FO% = 27.27), *Or. niloticus* and *Ox. marmorata* (FO% = 25.00) and *T. microlepis* (FO% = 15.79). No fish was found with more than four MPs in the GIT tract. Proportions of fish with different amounts of MPs in the GIT are shown in Figure 2(A).

TABLE 1. Information regarding the fish species collected from the Nong Luang Wetland

Family	Fish species	Habitat	Feeding habit	n[a]	Weight (g)	Length (cm)	F[b]
					Mean±SD	Mean±SD	
Osphronemidae	*Trichopodus microlepis*	Pelagic-benthic	Zooplanktivore	19	17.36±5.33	8.4±1.05	2.93
	Trichopodus tricopterus	Pelagic-benthic	Zooplanktivore	2	12.50±3.54	8.6±0.71	1.97
Clariidae	*Clarias batrachus*	Benthic	Carnivore	1	126.00	20.9	1.38
Cichlidae	*Oreochromis niloticus*	Pelagic-benthic	Omnivore	12	30.25±5.65	8.9±0.37	4.29
	Cyclocheilichthys lagleri	Pelagic-benthic	Zooplanktivore	2	43.00±11.31	10.0±0.42	4.30
	Cyclocheilichthys armatus	Pelagic-benthic	Zooplanktivore	2	39.5±0.71	12.3±1.20	2.12
	Cyclocheilichthys repassan	Pelagic-benthic	Omnivore	1	29.00	10.9	2.24
Cyprinidae	*Gymnostomus siamensis*	Midwater	Omnivore	7	63.85±12.62	13.2±0.77	2.78
	Gymnostomus lineatus	Midwater	Omnivore	1	28.00	9.8	2.97
	Labiobarbus siamensis	Benthic	Detritivore	1	35.00	12.3	1.88
	Mystacoleucus obtusirostris	Midwater-benthic	Omnivore	1	25.00	9.6	2.83
Bagridae	*Mystus albolineatus*	Benthic	Carnivore	2	60.00±7.07	14.8±0.25	1.85
Butidae	*Oxyeleotris marmorata*	Benthic	Carnivore	32	72.84±64.35	13.3±3.44	3.10
Pristolepididae	*Pristolepis fasciatus*	Benthic	Omnivore	11	24.00±22.62	7.8±1.82	5.06
Notopteridae	*Notopterus notopterus*	Pelagic-benthic	Carnivore	6	98.17±65.18	19.9±4.11	1.25
Total				100	46.96±31.90	12.1±3.97	2.73

[a] Total number of fishes, [b] Fulton' s condition index

Most fish contained one MP, and only one species of fish (*Ox. marmorata*) contained three or more MPs in the GIT. MPs ingested by the fish samples varied from 1 to 4 particles per fish, with an average of 1.19±0.68. Correlation analysis showed no significant association ($P > 0.05$) between the number of MPs and the body length, weight and Fulton's condition index.

The most common MP morphotype was fiber, followed by rod-shaped and fragment, accounting for 92.0%, 4.0% and 4.0% of the total, respectively (Table 2 and Figure 3). The proportion of MP types varied with fish species although fibers were dominant in all species. The percentage of fiber reached 100% in most of the fishes (Figure 2(B)), except for *T. microlepis* and *P. fasciatus* where fibers accounted for 66.7% of recovered MPs. Rod-shaped MPs were only found in *P. fasciatus*, accounting for 33.3% of the total number of items in this species, while MP fragments were only found in *T. microlepis* at 33.3%.

Colored MPs accounted for 85% of the total recorded in the Nong Luang Wetland. Five MP colors were found in the GITs as black, green, red, blue and transparent. Color distribution of ingested MPs was generally uniform across all fish species analyzed, with blue as the most prominent (39%) followed by red (23%), black (15%) and transparent (15%), while green was less frequent (8%). The variety of colored MPs was higher in Ox. marmorata (Figure 2(C)).

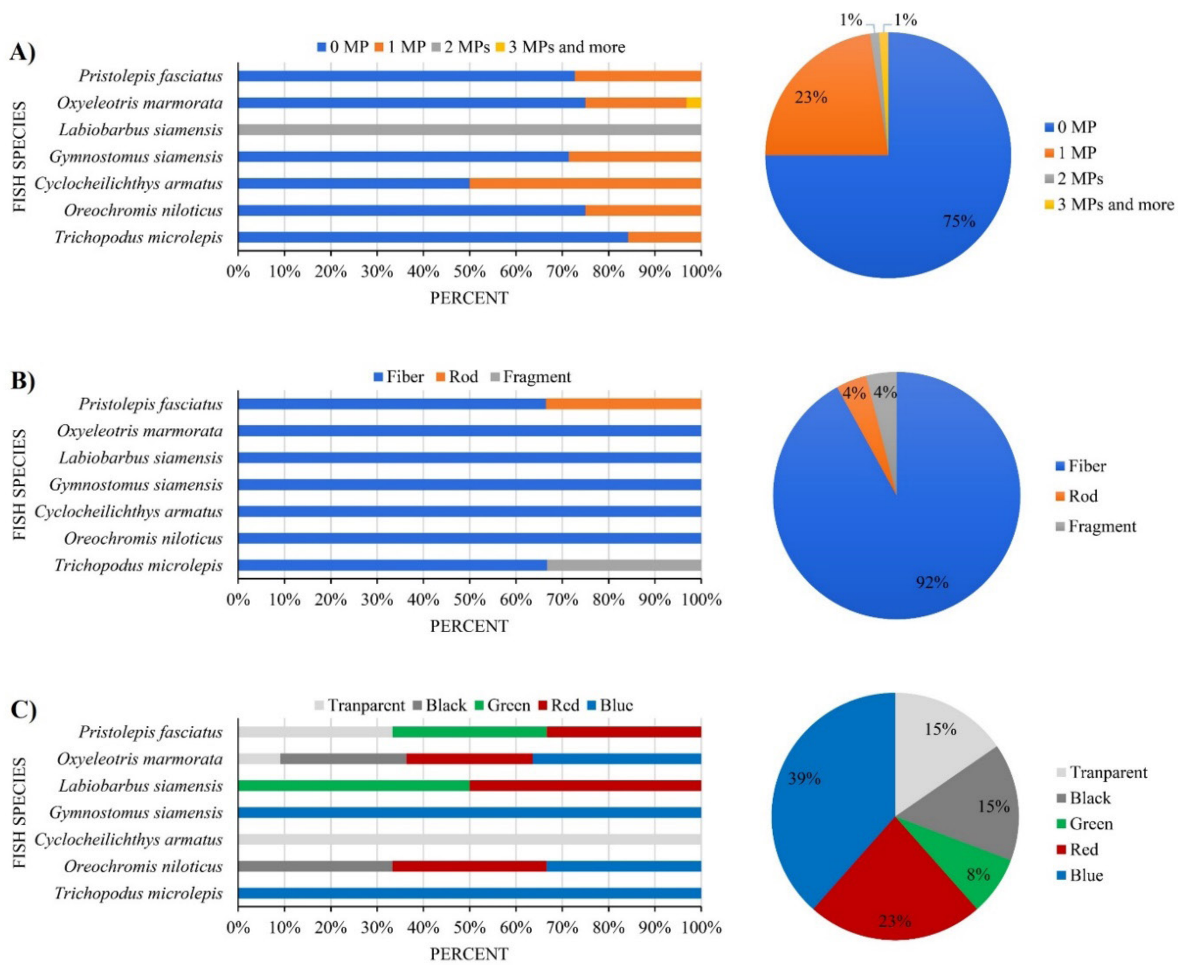

FIGURE 2. Composition of microplastics by number (A), type (B) and, color (C) in the gastrointestinal tract of fishes from Nong Luang Wetland

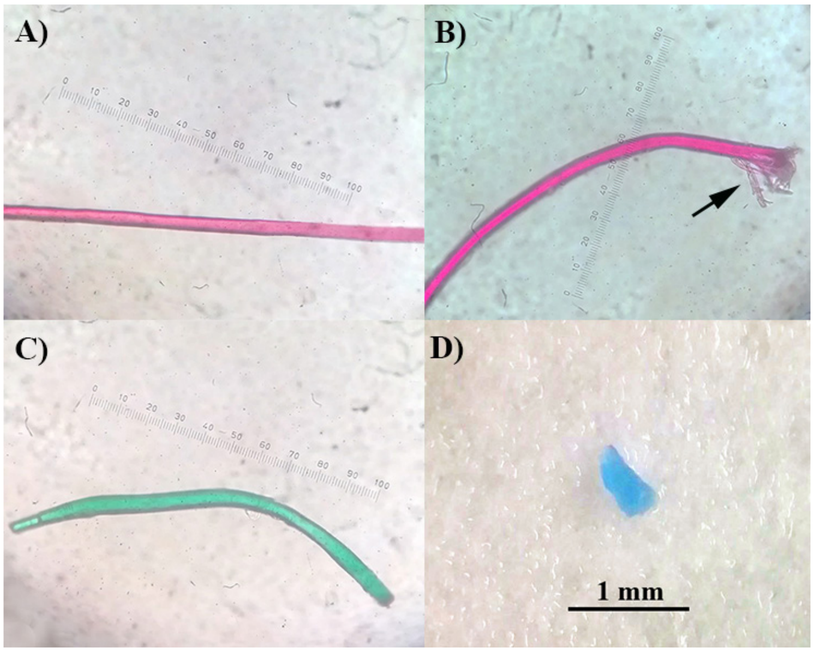

FIGURE 3. Examples of microplastics found in the gastrointestinal tract of fish: A-B) fibers are isolated from filter paper exhibiting fraying (arrows), C) rod-shape, and D) fragment. (A-C, compound microscope, 10x magnification; scale bar represents 2.5 mm)

DISCUSSION

Nong Luang Wetland plays an important role as an ecologically connected network comprising wildlife and supports the livelihoods of the local people. The area has been designated as a wetland of international importance. This study contributed new knowledge as baseline data on the ingestion of MPs by freshwater fish in the Nong Luang Wetland by providing an initial snapshot of MP pollution levels as the first evidence of MP ingestion by freshwater fishes in a Thai wetland area. Investigations of MP ingestion by freshwater fishes are limited compared to similar studies of marine fishes. Recent studies conducted in Thai river and reservoir systems showed that the fishes have MPs in their GITs (72.9% and 96.4%, respectively) (Kasamesiri & Thaimuangphol 2020; Kasamesiri et al. 2021). Here, 21.0% of fish were found with MPs, while the number of MPs recovered per fish varied from 1 to 4 (overall average of 1.19±0.68 particles per fish), indicating a relatively low prevalence of MPs in freshwater fishes from this wetland. Comparisons of MP abundances recorded in the literature must allow for diverse sampling, sample preparation, extraction, purification, identification and quantification methodologies (Hidalgo-Ruz et al. 2012; Li, Liu & Chen 2018; Mai et al. 2018; Wang & Wang 2018). Previous studies using similar units for quantifying MPs in freshwater fish compared with our results indicated that the Nong Luang Wetland contained fewer MPs than the Taihu Lake, China (95.7%) (Jabeen et al. 2017), freshwater fish (*Hoplosternum littorale*) in the Pajeú River, Brazil (83.0%) (Silva-Cavalcanti et al. 2017) and in the Widawa River, Poland (53.9-54.5%) (Kuśmierek & Popiołek 2020), with comparable results to freshwater fishes of Southwestern Germany (18.8%) (Roch et al. 2019) and the common noxious fish (*Gambusia holbrooki*) in Australian urban wetlands (19.4%) (Su et al. 2019). In our study, the percentage occurrence of MPs in each species ranged between 15.79% and 100%, with the highest in *L. siamensis*. However, it was difficult to conclude a general trend of MPs in fish due to the small number of samples. Further evaluations require a larger sample size. Low intensity of MPs was detected at 1 to 4 particles per fish, concurring with Roch et al. (2019) who found low intensity of ingested MPs ranging between 1 and 4 particles per fish in Southwestern Germany. Similar results were also recorded in other freshwater systems (Jabeen et al. 2017; Kasamesiri & Thaimuangphol 2020; Silva-Cavalcanti et al. 2017).

TABLE 2. Amount and type composition of microplastics in the gastrointestinal tract of fish species from Nong Luang Wetland

Family	Fish species	FO%	MP/ all fish	MP/ fish with MP	Fiber (%)	Rod-shape (%)	Fragment (%)
Osphronemidae	*Trichopodus microlepis*	15.79	0.16	1.00	66.7	0	33.3
	Trichopodus tricopterus	0	0	0	0	0	0
Clariidae	*Clarias batrachus*	0	0	0	0	0	0
Cichlidae	*Oreochromis niloticus*	25.00	0.25	1.00	100	0	0
	Cyclocheilichthys lagleri	0	0	0	0	0	0
	Cyclocheilichthys armatus	50.00	0.50	1.0	100	0	0
	Cyclocheilichthys repassan	0	0	0	0	0	0
Cyprinidae	*Gymnostomus siamensis*	28.57	0.29	1.00	100	0	0
	Gymnostomus lineatus	0	0	0	0	0	0
	Labiobarbus siamensis	100.00	2.00	2.00	100	0	0
	Mystacoleucus obtusirostris	0	0	0	0	0	0
Bagridae	*Mystus albolineatus*	0	0	0	0	0	0
Butidae	*Oxyeleotris marmorata*	25.00	0.34	1.38	100	0	0
Pristolepididae	*Pristolepis fasciatus*	27.27	0.27	1.00	66.7	33.3	0
Notopteridae	*Notopterus notopterus*	0	0	0	0	0	0
Total		21.0	0.25	1.19	92.0	4.0	4.0

Nong Luang Wetland is a well-renowned area with developed agriculture, aquaculture and animal husbandry (Masa 2015; ONEP 2004; Panboon, Soebeen & Sukkasem 2017). The MPs observed in this study indicated that key environmental pressures from anthropogenic activities are now influencing MP distribution (Kumar, Sharma & Bandyopadhyay 2021; Vendel et al. 2017). Global studies showed that effluent discharge and urbanization increased the accumulation of MPs within environments and resident organisms (Du et al. 2020; Duis & Coors 2016; Li, Liu & Chen 2018; Wagner et al. 2014). Communities living around the Nong Luang Wetland have no wastewater treatment plant. Wastewater originating from the surrounding rural non-point sources discharges into river networks and wetlands without treatment, contributing plastics as MPs in the aquatic environment (Yuan et al. 2019). Thus, sewage wastewater discharges were considered as one source of contamination. MPs in wetlands are transported by surface runoff and associated with diffuse urban pollution sources, while recreational activities in urban wetlands increase the risk of MP pollution via unmanaged waste disposal and recreational fishing (Su et al. 2019).

Aquacultural activities also increase MP pollution. Poor waste management practices such as illegal waste accumulation and waste burning were observed in the wetland. MP concentrations in freshwater wetlands vary with space and time (Rodrigues et al. 2018; Stanton et al. 2020). Eriksen et al. (2013) concluded that the abundance of MPs in wetlands depended upon various vital factors including investigated locations, approaches, and human interference, while Meng, Kelly and Wright (2020) considered that flow regimes, freshwater topography and extent of biofouling determined microplastic transport at spatial scales, together with anthropogenic activities.

In our study, the correlation between the number of ingested MPs, fish length and fish weight was positive but weak, with no significant correlation observed between fish length and weight and number of MPs in GITs. Correlations between these attributes depend on fish species and feeding habits, fish trophic level, sample size and MP characteristics (Mai et al. 2018; Yuan et al. 2019). Our small sample size negatively impacted meaningful factor correlations and this aspect requires further evaluation with larger sample sizes and individuals from different size classes according to the scientific literature in freshwater habitats (Mai et al. 2018; Possatto et al. 2011). Previous studies confirmed that factors determining the levels of MPs ingested by fish are still not well understood; however, even small amounts of MP ingestion seriously impact fish health (Anbumani & Kakkar 2018; Du et al. 2020; Li, Liu & Chen 2018; Wagner et al. 2014).

Three MP morphotypes were presented in fish GITs as fiber, rod-shaped and fragment, concurring with previous studies (Jabeen et al. 2017; Kasamesiri & Thaimuangphol 2020; Kasamesiri et al. 2021; Pazos et al. 2017; Sun et al. 2019). Fibers were the dominant category of MPs ingested by fish from the Nong Luang Wetland at 92.0% of the total occurrence, similar to results in most field studies of MP ingestion in freshwater fishes (Andrade et al. 2019; Campbell, Williamson & Hall 2017; Collard et al. 2018; Horton et al. 2018; Jabeen et al. 2017; Kasamesiri & Thaimuangphol 2020; Kasamesiri et al. 2021; McGoran, Clark & Morritt 2017; McNeish et al. 2018; Silva-Cavalcanti et al. 2017; Sun et al. 2019). The dominance of fibers as the MP type originated from the peeling of plastic fishing gear, fish cages or nylon ropes and other equipment used in aquaculture (Cole et al. 2011; Kasamesiri & Thaimuangphol 2020; Kasamesiri et al. 2021; Welden & Cowie 2017). Domestic sewage containing fibers from laundry was also an important source of fibers (Browne et al. 2011). Lusher, McHugh and Thompson (2013) concluded that rayon made up over half of the polymers identified, with possible sources as clothing, furnishing, hygiene products and nappies as indirect input through sewage. In the same vein, Wagner et al. (2014) suggested that urban areas near water bodies are potential sources of MPs, while Jabeen et al. (2017) considered freshwater systems and estuaries (transitional systems) as potentially more prone to fiber contamination than the marine environment because these systems are closely located to potential fiber point source discharges.

Fibers observed in fish GITs in the Nong Luang Wetland varied in color, with the highest variety found in *Ox. marmorata*. Blue fibers were the most abundant followed by red, black, transparent and green. Previous studies also reported blue as the most abundant fiber color (Bessa et al. 2018; Kasamesiri & Thaimuangphol 2020; Kasamesiri et al. 2021; Lucas-Solis et al. 2021; Lusher, McHugh & Thompson 2013). The Nong Luang Wetland is an important fishery resource with abundant fishing activities. Local fisherman usually use transparent or black gillnets to catch fish, while blue fiber fish cages are used in aquaculture, with blue plastic materials used in fishing gear (nets, traps and lines) as sources of blue fibers ingested by fishes in this wetland. Blue fibers are also commonly found in freshwater systems affected by sewage spillage (Leads & Weinstein 2019). In our study, MP type and color played no relevant role, implying passive uptake. The distribution of MPs and color varied between habitats and feeding habits, with no evidence of active uptake. More detailed research is required to evaluate potential uptake pathways. Some previous studies hypothesized that the color of MPs and resemblance of MPs to food may contribute to the likelihood of MP ingestion in fishes as a result of visual confusion between prey (Alomar et al. 2017). Ory et al. (2017), Savoca et al. (2017), and Selleslagh and Amara (2015) highlighted that various fish species consider everything as potential prey, including MPs being mistaken as a food source.

In conclusion, our study sheds light on the occurrence of various microplastic morphotypes in fish GITs from the Nong Luang Wetland, with fibers being the most dominant type. While color variations were observed in the fibers, they did not significantly influence the uptake process. We recognize the limitations of our study, particularly the absence of microFTIR analysis, and propose future research to employ advanced analytical techniques, such as microFTIR, to enhance the accuracy and reliability of microplastic identification (Lin, Chiu & Kuo 2023, 2022).

Conclusions

This is the first report detailing MP ingestion by freshwater fishes from a wetland in Thailand. Results showed that MP pollution was relatively low compared to other investigated areas. MPs were ingested by freshwater fishes, indicating that this emerging pollutant is ubiquitous in the Nong Luang Wetland. The main MP type as fiber was found in the GITs of 7 fish species. Color compositions of MPs ingested by fish were diverse, with blue being the most abundant. MP types indicated their origin as mainly from fishing gear, such as nets and synthetic fibers from clothing. Wetlands provide for human livelihoods, and MP ingestion by biota that negatively impacts food webs must be investigated to maintain both structures and functions of wetland ecosystems. More attention must be paid to wetland pollution and other biological toxic hazards impacting aquatic animals and humans. The Thai Government should improve waste management facilities and monitor aquacultural activities in wetland areas. Data presented here provide a meaningful reference as the baseline pollution level for future MP research in the Nong Luang Wetland.

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