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Presence and Characterization of Microplastics in Coastal Fish around the Eastern Coast of Thailand

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Abstract: Marine microplastic has been in the limelight recently. This study aimed to describe microplastic types ingested by 274 fish from Thailand's eastern coast in 2020 and to compare the microplastic content among different feeding traits. The microplastics in the gastrointestinal tracts and gills were extracted, analyzed, and identified using FT-IR spectroscopy. Approximately 13.14% of the total specimen ingested microplastics, with an average of 0.14 items per individual. The detection frequency of microplastics was relatively high compared with other regions in Thailand but relatively low compared to global standards. Of the microplastic contaminated specimens, 56.41% had at least one piece of microplastic in their gastrointestinal tract. Pelagic (14.47%) species were found to have ingested more microplastics than the demersal (12.63%) group. Dominant aspects found included PET (as in polymers), fiber (as in shape), and black (as in color). However, microplastic numbers fluctuated with the size, weight, and feeding behavior of fish. This result suggested that the pelagic has a higher exposure risk and microplastic ingestion in relatively small quantities in a range of fish species. Our results indicated that the occurrence of microplastics in fish is not influenced by organism habitat or trophic level, although the characteristics of pelagic fish might significantly increase the chance of exposure to microplastics in pelagic species.

Keywords: trophic level; Thailand; microplastics; fish; fiber; FT-IR

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1. Introduction

Recently, marine debris has been found sporadically in various locales such as floating on the river and the sea surface, as well as being embedded on shorelines and seafloor from a direct consequence of industrial activities, consumption habits, and poor waste management [1–4]. Several studies have demonstrated that low-density plastics (e.g., polypropylene or polyethylene) are dominant in the top layers of the water column, and high-density plastics (polyvinylchloride (PVC), polyester, or polyamides) usually sink to the sea bottom [5–8]. During the past decade, this form of contamination has been of concern due to a threat presented to wildlife and could impact economically on fisheries [9–13].

Plastics are degraded in oceanic and coastal environments into smaller pieces, mainly by mechanical erosion (the action of winds and waves), by physical abrasion against particles of sediment, or by solar radiation, whereas chemical degradation processes are slower [14,15]. Microplastics (MPs) were first described as any pieces of plastics in the size of five millimeters or smaller particles in the environment [16]. The abundance of MPs has increased rapidly over the past decades in a variety of habitats, including the aquatic system (freshwater, estuarine, and marine) and within aquatic organisms [17–23]. Globally, there have been various studies that specifically examined the contamination of MPs in fish [24]. Due to their characteristics (i.e., buoyancy, colors, sizes, and shapes), some plastic particles in the water column or on the seafloor may appear physically as natural food sources, resulting in accidental ingestion by fish [25,26]. Therefore, the ingestion of microplastics in aquatic organisms is of concern due to the physical and chemical harm to their bodies [27–30]. MPs can also be found in the food web, either in zooplankton or in the higher ingestion hierarchy and food supply, such as in humans [31–33].

During the past few years, several studies on microplastics in marine ecosystem have been conducted in Thailand, with the focus on marine organisms [34,35], estuarine environments [36,37], and ocean sediment [38]. Despite this, the accumulation of ingested microplastics in fish has been less emphasized [39].

Contamination of MPs in fish should be a priority concern due to many fishing grounds also being within the boundary of numerous urban areas and industrial estates, especially the eastern coast of Thailand. Map Ta Phut is also one of the essential fishing grounds in Thailand. Consequently, this area should be of the most concern in terms of marine microplastic pollution. However, research on the extent of microplastic contamination in fish around the eastern coast of Thailand is still unknown. Hence, this study's primary aim was to describe the types of microplastic ingested by fish collected from the eastern coast of Thailand. The secondary aim was to determine whether there were differences in microplastic frequency between feeding traits (pelagic and demersal fish).

2. Materials and Methods

2.1. Study Area and Sample Collection

Fish samples were collected around the eastern coastal water of Thailand from Rayong to Trat province in two periods during the routine of Rayong Marine Fisheries Research and Development Center standard haul trawls, as shown in Figure 1. Trawling operations were carried out during daytime at a speed of 2.5–3 knots trawl gears. Samples were obtained in January and May 2020 at depths from 20 to 50 m. Nineteen species of fish used in this analysis comprised 10 pelagic species and 9 demersal species. These were selected on the basis of data from previous trawls at the nine stations and the likelihood that sufficient numbers (≥5 individuals per species) would be obtained for analysis. The number of individuals of each species was not controlled; hence, the number of individual species was different (Table 1).

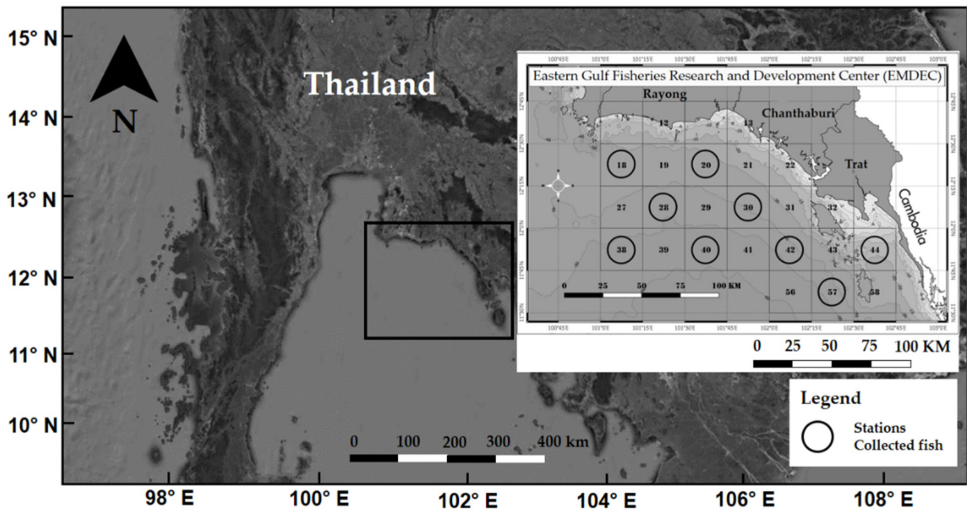

Figure 1. Sampling area in the eastern coast of Thailand and sampling site for fish.

2.2. Microplastic Extraction

Fish samples were frozen within 2 h of capture, then transported to the laboratory and stored at −20 °C until further analysis. Subsequently, after defrosting, the skin was washed with distilled water to remove large matters. Basic measurements (body length and wet weight) were recorded for each fish. Then, each individual was dissected in a metal tray using a scalpel, forceps, and scissors. The skin was first peeled off during the anatomy to prevent contamination, and then the gastrointestinal tract and gills were separated. The separated tissues were immediately placed into clean beakers and covered with aluminum foil to minimize the risk of contamination.

Table 1. A summary of fish traits and plastics distribution in fish.

Fish Species	n	Average ± SD Weight (g)	Average ± SD Length (cm)	No. of Fish Found MPs (No. of MPs)	Average ± SD MPs Items/Ind
Pelagic					
Aurigequula fasciata (Lacepède, 1803)	5	64.80 ± 9.73	15.10 ± 0.89	1 (1)	0.20 ± 0.45
Leiognathus equulus (Forsskål, 1775)	6	78.23 ± 16.97	16.37 ± 2.23	0 (0)	ND [1]
Stolephorus indicus (Van Hasselt, 1823)	5	19.40 ± 1.52	13.25 ± 0.42	1 (1)	0.20 ± 0.45
Lutjanus lutjanus (Bloch, 1790)	6	20.92 ± 27.86	10.33 ± 3.33	0 (0)	ND [1]
Lutjanus madras (Valenciennes, 1831)	5	47.60 ± 10.50	14.40 ± 1.34	1 (1)	0.20 ± 0.45
Sphyraena obtusata (Cuvier, 1829)	6	56.82 ± 15.82	21.32 ± 1.98	1 (1)	0.17 ± 0.41
Rastrelliger kanagurta (Cuvier, 1816)	15	50.34 ± 41.85	15.20 ± 4.94	2 (2)	0.13 ± 0.35
Amblygaster clupeoides (Bleeker, 1849)	9	19.89 ± 2.32	12.89 ± 0.60	1 (1)	0.11 ± 0.33
Atule mate (Cuvier, 1833)	9	32.15 ± 15.73	12.39 ± 3.93	1 (2)	0.22 ± 0.67
Gerres erythrourus (Bloch, 1791)	10	87.76 ± 32.07	16.60 ± 1.98	3 (3)	0.30 ± 0.48
Demersal					
Nemipterus hexodon (Quoy & Gaimard, 1824)	40	75.71 ± 34.79	18.38 ± 6.02	0 (0)	ND [1]
Scolopsis taenioptera (Cuvier, 1830)	54	58.01 ± 27.02	15.87 ± 2.67	6 (6)	0.11 ± 0.32
Saurida elongata (Temminck & Schlegel, 1846)	35	65.34 ± 40.78	19.91 ± 3.87	13 (15)	0.43 ± 0.65
Saurida undosquamis (Richardson, 1848)	17	49.59 ± 23.40	18.45 ± 2.63	1 (1)	0.06 ± 0.24
Upeneus vittatus (Forsskål, 1775)	21	21.30 ± 11.68	16.75 ± 22.59	2 (2)	0.10 ± 0.30
Upeneus tragula (Richardson, 1846)	5	49.24 ± 23.48	15.74 ± 3.57	1 (1)	0.20 ± 0.45
Upeneus sulphureus (Cuvier, 1829)	12	30.83 ± 9.70	12.65 ± 1.45	0 (0)	ND [1]
Platycephalus indicus (Linnaeus, 1758)	5	24.00 ± 2.45	15.16 ± 0.63	1 (1)	0.20 ± 0.45
Priacanthus tayenus (Richardson, 1846)	9	53.42 ± 56.46	14.47 ± 5.95	1 (1)	0.11 ± 0.33

ND [1] = not detected.

In order to degrade organic matter and enable detection of microplastic particles, both gastrointestinal tracts and gills were subjected to hydrogen peroxide digestion according to [40–42] with minor modifications. At first, each sample was transferred into a 500 mL clean glass beaker. About 20 mL 10% KOH solution was added, and the mixture was stirred in a water bath at 80 °C with 60 rpm oscillator for 1 h to increase the organic matter digestion rate. Then, 20 mL of 30% H_2O_2 was added, and the mixture was heated on a hot plate at 60 °C until H_2O_2 was evaporated. If the organic matter was visually observed, 1–5 mL of H_2O_2 was added, and this was repeated until no organic matter was in the beaker. After 24 h, around 100 mL of the filtered NaCl solution was added, and the mixture was stirred for 5 min for density separation. After settling for 4 h, the sample was filtered under vacuum through filters (20 µm pore size; 47 mm diameter). This process was repeated three times to increase the recovery rate of microplastics. The filter was placed in Petri dishes and dried in an oven at 40 °C while being covered in aluminum foil. Finally, all samples were kept in the desiccator for further analysis. During the digestion procedures, three procedural blanks were also run without samples in parallel with samples containing the digestion solutions, and any particles detected in these blanks were characterized as contamination.

2.3. Prevention of Microplastic Contamination

Lab coats and gloves were always worn throughout the laboratory work. All workstations were cleaned with 70% alcohol before working on any samples, and all glassware was rinsed three times and covered with aluminum foil to prevent contamination from the air. For filtering process, the distilled water and saturated NaCl (density 1.2 g/cm^3) solution were filtered by a vacuum pump with WHATMAN® GF/C filter (1.2 µm pore size, 47 mm diameter). No microplastics were found in blank test filters, indicating that microplastic contamination from the laboratory environment or reagent solutions was negligible.

2.4. Identification of Microplastics

All possible microplastic particles were subjected to visual examination by stereomicroscope (Olympus Zeiss SZ51) identified by their colors and shapes following [43]. All the plastic items from the samples were sorted and quantified by color (blue, black, white, yellow, red, and transparent), shape (fragments—irregular pieces; pellets—spherical and ovoid debris; fibers—thin and elongated pieces). Polymer types of the samples collected were identified using a micro-Fourier transform infrared spectrophotometer (Spectrum Two with Spotlight 200i, PerkinElmer) in the reflection mode. Spectra were produced with wavenumbers 600–4000 cm^{-1}. The spectra were compared with a database of reference standard compounds to identify the polymers present in the particles. The number of microplastics in the gastrointestinal tract and gills was expressed as the number of microplastic items per individual (MP items/individual).

2.5. Statistical Analysis

All results are presented as mean $\pm$ standard deviation of the mean (SD). All fish individuals were mixed by fish type, pelagic and demersal fish, without considering their species, and location was collected before data analysis. ANOVA tests were used to calculate the differences in the number of plastic items/individuals found between demersal and pelagic fish. *t*-test was used to determine whether there was a significant difference in the frequency of microplastics shape, color, and polymer type between demersal and pelagic fish. Relations between body length and microplastic number were tested in this study. Moreover, we decided to determine whether length and weight were linearly related by using simple linear regression. The significance level was set at an alpha of 0.05. Statistical analyses were performed with SPSS Statistics 27.0.

3. Results

3.1. Characteristic of Fish

Specimens caught in this study consisted of 10 pelagic species and 9 demersal species (total of 19 species, 274 individuals). Pelagic species included planktivore species (*Stolephorus indicus*, *Rastrelliger kanagurta*, and *Amblygaster clupeoides*) and predatory species (*Aurigequula fasciata*, *Leiognathus equulus*, *Lutjanus lutjanus*, *Lutjanus madras*, *Sphyraena obtusata*, *Atule mate*, and *Gerres erythrourus*). The body length of the total fish ranged from 3.10 to 24.00 cm, and the weight ranged from 6.00 to 150.00 g. All demersal species were predatory, including *Nemipterus hexodon*, *Scolopsis taenioptera*, *Saurida elongata*, *Saurida undosquamis*, *Upeneus vittatus*, *Upeneus tragula*, *Upeneus sulphureus*, *Platycephalus indicus*, and *Priacanthus tayenus*. The body length of the total fish ranged from 9.00 to 115.00 cm, and the weight ranged from 3.37 to 203.37 g (Table 1).

3.2. Abundance of Microplastics in Fish and Habitat

Thirty-nine microplastic pieces were discovered among eight pelagic species and seven demersal species. Out of the 76 pelagic specimens, only 11 individuals (14.47%) contained ingested microplastics, and out of the 198 demersal specimens, 25 individuals (12.63%) contained ingested microplastics. For pelagic species, *Gerres erythrourus* had the highest number of microplastics (0.30 $\pm$ 0.48 item/individual) found, while *Saurida elongata* had the highest number found in the case of demersal species (0.43 $\pm$ 0.65 item/individual) (Table 1). The abundance of microplastics was not statistically significant different between pelagic and demersal species ($p > 0.05$). The occurrences of MPS were higher in medium fish, both in pelagic and demersal species (Figure 2A,B).

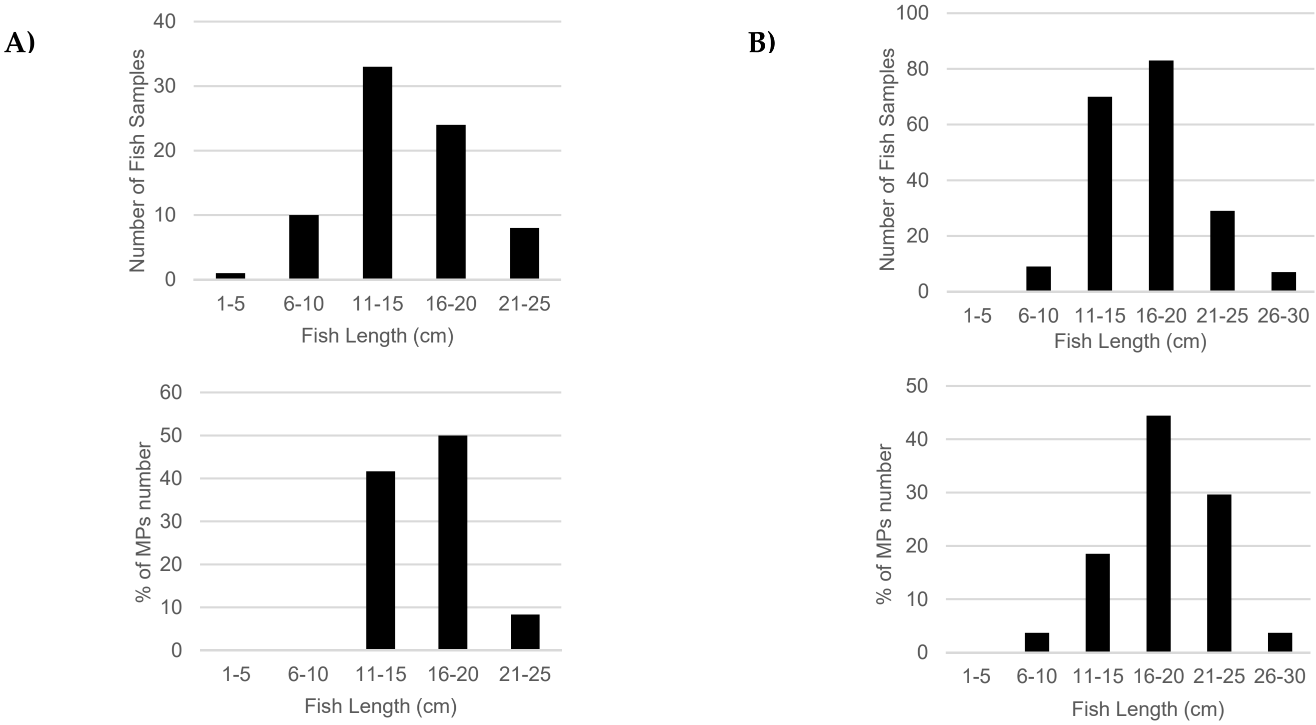

Figure 2. Occurrence percentage of microplastics number sorted by fish size between (**A**) demersal and (**B**) pelagic fish.

A total of 39 plastic particles were recovered from the gills and gastrointestinal tract of all fish species (Figure 3). The size of microplastics in the pelagic fish ranged from 0.33 to 5.00 mm (with an average 1.77 m $\pm$ 1.85 mm), whereas it varied between 0.10 and 5.00 mm (with an average 1.53 $\pm$ 1.24 mm) in demersal fish. Three shapes of microplastics were observed in fish samples, including fibers, fragments, and film. Different morphotypes of microplastic were observed in diverse fish species. The fiber was the most abundant form in both pelagic and demersal species. The proportions of microplastics found were fiber (83.33%) and film (16.67%) for pelagic and fiber (88.89%), fragments (7.14%), and film (3.57%) for the demersal group. The proportion of microplastic shape categories was comparable among species (pelagic and demersal) and between fish. The shape of microplastics was significantly different among pelagic species ($p < 0.05$) and demersal species ($p < 0.05$).

The color of microplastics can be classified into six categories: black was the most abundant microplastics color category, both in pelagic and demersal species, with the proportion of 50% black, 33.33% red, and 16.67% green in pelagic species, while in demersal species, the proportion shifted to black (66.67%), blue (22.22%), and others (11.11%) (Figure 3b).

Further analysis on polymers was conducted via FT-IR microscopy, and five types of polymers were found, namely, polyethylene (PE), polypropylene (PP), polyethylene terephthalate (PET), polystyrene (PS), and nylon (Figure 3). PET was the most abundant polymer type both in pelagic and demersal species (Figure 3d). The proportions of polymer found were different among fish types: in the case of pelagic species, PET was the most abundant (83.33%), followed by PE (8.33%) and PP (8.33%), while in demersal species, the sequence was PET (66.67%), nylon (22.22%), PE (3.57%), PP (3.57%), and PS (3.57%). The ingestion of microplastics varied in species with different food sources, such as omnivores and planktivores. Types of microplastic polymers were significantly different among both pelagic species ($p < 0.05$) and demersal species ($p < 0.05$).

The ratio of microplastic found in guts of pelagic specimens (25% of total individuals) and demersal specimens (70% of total individuals) was quite varied, while the ratio of microplastics found in gills of pelagic specimens (75% of total individuals) and demersal specimens (30% of total individuals) differed, as shown in Figure 3e.

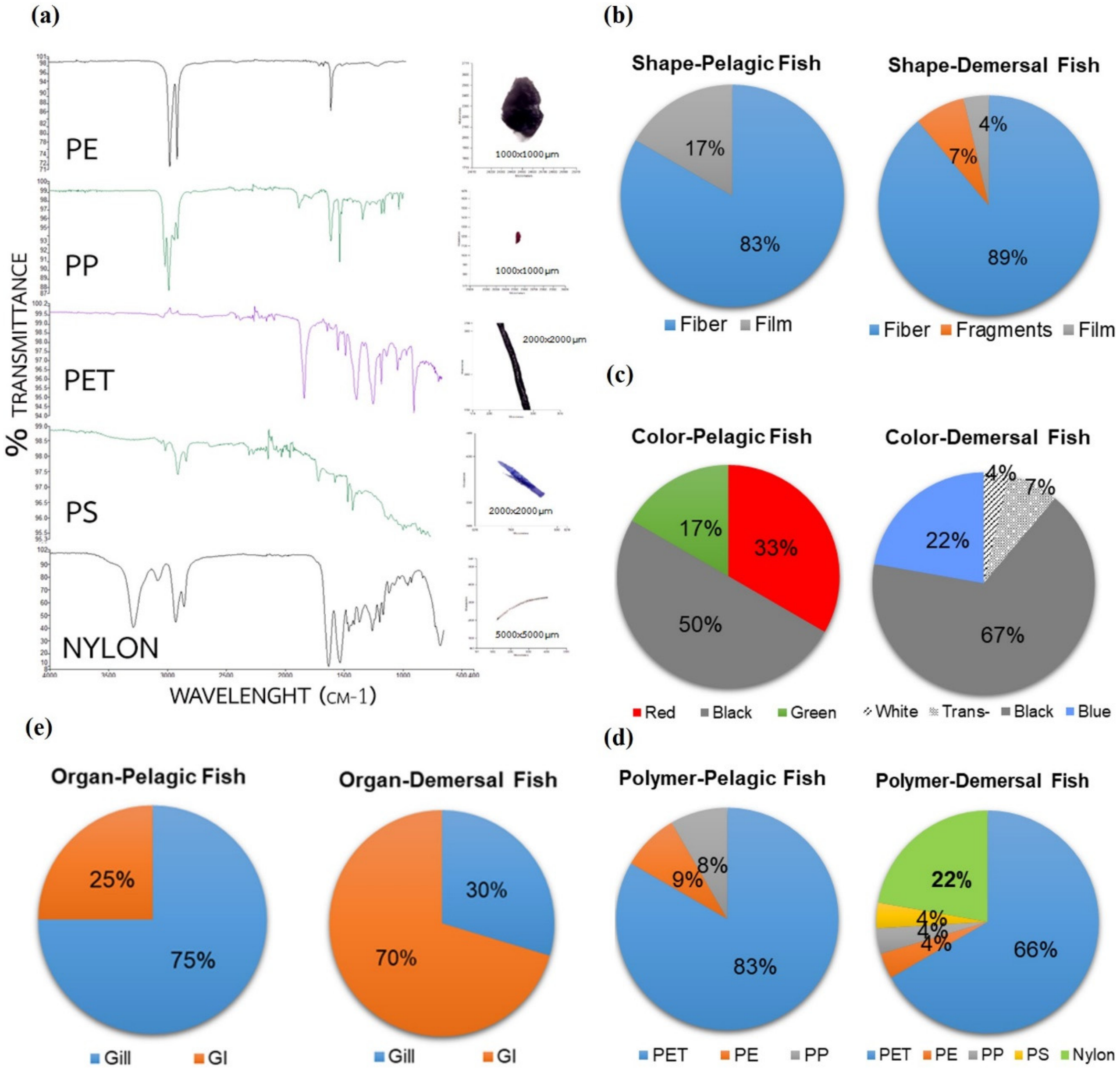

Figure 3. Characterization of microplastics in pelagic and demersal species samples from trawling fishery in the eastern Gulf of Thailand: (**a**) examples of microplastics with FT-IR spectrum and captured image under the optical microscope, (**b**) shape, (**c**) colors, (**d**) polymer type, and (**e**) fish organs of detected microplastics.

4. Discussion

Much research has looked into the occurrence of microplastics in fish, and evidently there was a clear link found between numerous factors such as feeding regimes [44–46], habitat [47,48], activity areas [49], or water columns that distinguish the difference amongst benthic to pelagic species [48,50] and microplastic accumulation.

This study provided the first published characterization record of microplastics by FT-IR in the gastrointestinal tract and gills in both pelagic and demersal fish in the eastern coastal water of Thailand. The proportion of fish to have ingested plastics (13%) was less than those recorded by [25], showing 36.5% of 504 fishes (five demersal and five pelagic species) from the English Channel, 33% of 122 catfish from Brazil [51], 35.5% of commercially caught fish across southern Australia [28], 33% of six fish species of commercial importance in central Chile [52], and 85.4% of 29 of the commercial fish species from the Bohai Sea in China [53]. However, results of this study presented a higher frequency of microplastic in fish found compared to other areas of Thai water [39].

In this study in particular, demersal fish had ingested lower microplastics than pelagic fish, which corresponded with few previous studies [41,47,54]. While there were cases of other studies that reported no statistically significant differences in the occurrence of microplastic ingestion between pelagic and demersal fishes [25,45,48], it was evident that the pelagic fish species ingested more plastic debris than the demersal fish species. Due to microplastic density, the plastic debris appears to float in the marine water bodies. Some studies demonstrated a heavy load of terrestrial turned marine plastic debris, which in turn is broken down into smaller pieces. The particles remain in the water body for a long period of time, and some sink to the bottom. However, there was no significant difference between the abundance of microplastics ingested by the examined pelagic and demersal species. Microplastics are theoretically spread throughout the entire water column, resulting in a similar accumulation of microplastics in both groups. From another point of view, some results have also been reported in significantly higher abundances of microplastics in demersal than pelagic fish species [48,50,55], which may be associated with the ultimate sink and presence of plastic debris near the seabed in the marine environment. Our results revealed the different ingestion of microplastic rates because of various factors (plastic density, residence time, and water currents). Moreover, sampling site location and level of anthropogenic disturbances might be vital to each system [44].

The majority of microplastic shapes found were similar among both pelagic and demersal, this being fiber (83.33% and 88.89%, respectively). These results were consistent with many studies in fish from estuarine, marine, and freshwater environments, such as [56,57]. Fiber (varying in size) can enter into the ocean from domestic laundry discharges made of synthetic materials (polyester, polyethylene, or nylon) or fragments of fishing gear (e.g., ropes and nets) [58–60].

The dominant color found in this study was black, being inconsistent with surveys from the southern part of Thailand where transparent microplastics were the most frequent [61] and red-colored MPs in Thai water [39]. Therefore, the sampling locations with physical and biological factors might key to resolving the variation of microplastics distribution patterns, hence helping to determine the origin of MPs. Moreover, the authors of [62] reported that color of microplastics and the similarity to food might contribute to the likelihood of ingestion, especially planktivorous fish.

Our results showed different microplastic type distribution patterns between the water column and near bottom habitats for microplastic polymer. Polyethylene terephthalate (PET) was predominant with 64.29% in pelagic and demersal fish species. PET is widely used in fibers for clothing, plastic containers for liquid, and foods [63]. Polyamide (nylon), the second polymer, accounted for 22.22%, is only detected in demersal fish. Nylon is a common material for marine utensils and instruments, including fishing nets and rope [60,64]. Nylon (polyamide) was found only in fish feeding in demersal water due to its density, which most likely tends to sink to the sea bottom. Polyethylene (PE) was found only at 8.33% and 3.70% in pelagic and demersal species, respectively. PE is primarily used in packaging (plastic bags, plastic containers). This result suggested that pelagic fish has a higher chance of being exposed to microplastics. The buoyancy force of plastics in the marine environment might be an important factor for the distinctive number of microplastics between pelagic and demersal fish [31]. Microplastics are most likely to have been ingested from the water column along with the food by pelagic visual predators, also from the secondary plastic intake from their prey, or the presence of higher concentrations of bottom marine litters around this area [48].

Moreover, our study also aimed to differentiate the possibilities to expose microplastics by comparing the frequencies of found microplastics in the gastrointestinal tract and gills in pelagic and demersal fish. Overall, the percentage of fish that had microplastics in the gastrointestinal tract (57.90%) was higher than in gills (42.10%). However, microplastics were primarily found in the gill of pelagic fish (72.73%). In contrast, microplastic remains mainly in the gastrointestinal tract of demersal fish (70.37%). These results suggest that ecological regimes are also important in uptake microplastics either by chance or intention. The microplastics found in gills resulted from their retention during water filtration. This process and the uptake of microplastics through gills depend on the microplastic size and the morphology and efficiency of the filtering apparatus [65]. Microplastics presented in the gastrointestinal tract were swallowed through the mouth, and thus both large and very small particles were able to enter. After ingestion, some microplastics were likely internalized; others may have been retained in the gastrointestinal tract, whereas the remaining ones were rejected with feces. Microplastic accumulation in gills and the gastrointestinal tract may lead to the death of fish. Considering the pollution by microplastics, the toxicity in animals, and the potential risks to humans, more research on human exposure and toxicity of microplastics to humans is urgently needed. Moreover, the sink-source of microplastics in the marine environment needs to be considered for further research for a sustainable management plan for each location.

In order to preliminarily evaluate the transfer of microplastics between trophic levels, the selected fish species were analyzed considering their position in the trophic hierarchy. Results showed that planktivore (*R. kanagurta*, *S. indicus*, and *A. clupeoides*) had 13.79% detection frequency of microplastics compared with carnivores with 17.02% (Table 1). On the other hand, the detection frequency of microplastic in carnivores among demersal species was low (12.63%), as shown in Table 1. However, our results contrasted with some previous studies, such as important commercial fish in central Chile [51]. The study by [66] also suggested that planktivores, filter feeders, and suspension feeders are likely to encounter low-density plastics. Moreover, the occurrence of microplastics in some marine organisms is not influenced by habitat or trophic level but is affected by the feeding mode of organisms [67]. However, these results are not properly determined the transfer of microplastics through the marine food web due to the low number of fish samples and detected microplastics.

There was no statistically significant correlation between the number of ingested particles and fish length or weight, and fish samples. However, it has been obvious that microplastic particles has been observed in most fish. This might lead to results of sub-lethal or lethal health consequences in a variety of marine organisms [68], gut blockage, false satiety sensation, physical injury [17,66], hepatic stress [69], effects on fish reproduction and growth [70], and indication of neurotoxicity and oxidative damage in wild fish [71].

Notably, the Eastern Coastal Ocean would be an essential source of microplastics due to its proximity to the high degree of anthropogenic activities such as industry, fishing, and marine tourism. This study represents the first report evaluating the relationship between microplastics content and the feeding type. It is now widely recognized that to assess the magnitude of microplastic contamination in aquatic ecosystems, we must crucially identify their composition in order to understand potential sources, sinks, pathways of distribution, and the cycle through the food web. The final goal is to improve the management and protection of coastal environments.

5. Conclusions

This is the first study to report the microplastic abundances of in fish around the eastern coast of Thailand. This study indicated that microplastics were detected about 13.14% in gills and gastronomical tracts from fish. We observed no statistically significant difference in the frequency of microplastic occurrence or abundance between the pelagic and demersal species. Fibrous microplastics were the most dominant shape in this area. The average size of microplastics was 1.77 ± 1.85 mm and 1.53 ± 1.24 mm in pelagic and demersal fish, respectively. The polymer type showed PET, PE, and nylon as the main polymer types of this study. Moreover, we found no significant effect on body condition (weight or length) or behavior on the microplastic accumulation in fish species. Overall, this area is moderately contaminated with microplastics compared with other areas.

On the basis of the number of microplastic in the individual species, we found that *S. elongata* is a candidate species for monitoring microplastics. Due to the heavy influx of inland pollution in the area and the specific shallow water topography connecting terrestrial and marine realms, further research is needed to determine the processes and pathways responsible for releasing microplastics in coastal environments and evaluate the risks for fish health.

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