Existence of Plastic Debris in the Stomach of Some Commercial Fish Species in the Lower Gulf of Thailand

S. M. Oasiqul Azad

A Thesis Submitted in Fulfillment of the Requirements for the Degree of
Master of Science in Marine and Coastal Resources Management
Prince of Songkla University
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I hereby certify that this work has not been accepted in substance for any degree, and is not being currently submitted in candidature for any degree.

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ABSTRACT

In present society, plastic has reached a decisive position, with extensive industrial, medicinal, municipal and commercial application. It has turned into a key global environmental issue in the current decade because of their marine ubiquity, bioavailability and capability of carrying toxic chemicals. It's very crucial and imperative to investigate about microplastic pollution not only in Thailand but also for the world because of its harmful effects on marine biota as well as for human well-being. The study emphases, for the first time, on the occurrence of plastic offal in the gastrointestinal contents of some commercially significant marine fishes caught from the lower Gulf of Thailand during August, 2017 to May, 2018. Size and weight range of the samples were 7.6 to 37.1 cm and 4 to 133 g. outcomes of the investigation emphasised on the ingestion of plastics in the 63.33% samples (171 out of 270 discrete fish samples). The plastics ingested were microplastics (53.40%; <5 mm), mesoplastics (45.18%; 5-25 mm) and macroplastics (1.42%; >25 mm). Transparent color plastics (38.24%) were the most dominant color found in the stomach of fishes examined. Fibres were the major types of plastics found during this study. There was no relationship found between size of plastics and different biological features of the investigated fishes. This present investigation emphasise the omnipresence of microplastics in the lower Gulf of Thailand marine biota, as well as the water column where fish lives and feed. Therefore, it denotes an urgency to diminish the use of plastics or to ensure the appropriate recycling of it. These preliminary findings signify an imperative phase in exploring eco-toxicological perspectives such as the occurrence and effects of plastic

offal on the food chain; the probable impacts associated to the transmission of toxins on human health etc. Furthermore, operative controlling programs in the study zone and neighbouring extents for the plastic contamination are instantaneously obligatory.

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S. M. Oasiqul Azad

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This thesis is based on the following papers:

1. Azad, S. M. O., Towatana, P., Pradit, S., Patricia, B. G., Hue, H. T. T. and Jualaong, S. (2018): First evidence of existence of microplastics in stomach of some commercial fishes in the lower Gulf of Thailand. - Applied Ecology and Environmental Research. 16(6): 7345-7360.

2. Azad, S. M. O., Towatana, P., Pradit, S., Patricia, B. G. and Hue, H. T. T. (2018): Ingestion of microplastics by some commercial fishes in the lower Gulf of Thailand: a preliminary approach to ocean conservation. - International Journal of Agricultural Technology. 14(7): 1017-1032.

1. INTRODUCTION

Plastics are fabricated organic polymers, which are obtained from the poly-merisation of monomers obtained from gas or oil (Derraik, 2001; Rios et al., 2010; Thompson et at., 2009). From the 1950s up to 2018, an approximated 6.3 billion tons of plastic has been manufactured globally, of which a projected 12% has been ignited and additional 9% has been recycled. The rest has been abandoned in landfills or the natural environment. As of 2018, around 380 million tons of plastic are manufactured worldwide every year (The Economist, 2018). Plastic production by human are high due to its stability, light weight, attractive appearance and low cost (Hester and Harrison, 2011; Thompson et al., 2004; Jambeck et al., 2015). In modern society, with widespread industrial, medicinal, municipal and commercial applications, plastic has attained a crucial status.

Plastic contamination is the gathering of plastic substances in the environments which have several hostile effects on wildlife, wildlife habitat as well as on human beings (Moore, 2017; Parker, 2018). Plastic has become a pervasive and dominant component of marine debris because of its lightweight, cumulative global production, durable nature and continuing inappropriate dumping (Derraik, 2002; Gregory, 2009; Moore, 2008; Thompson et al., 2009). Plastic waste has been gathered in the environment at a rigid rate through unintentional release and indiscriminate abandonment. Plastic contamination can anguish waterways, land and ocean. Numerous living organisms, typically marine organisms, can be exaggerated either through expose to chemical toxicants within plastics that intervene with their physiology or by mechanical effects, for example, troubles related to ingestion of plastic garbage or tangle in plastic substances. The impacts of large plastic objects (i.e., macroplastic) on marine life were widely reported. Nearly 92% of all adverse encounters between marine litter and organisms occurs because of plastic waste (Gall and Thompson, 2015). Above 660 marine species were identified to be exaggerated globally by plastic litter directly or indirectly (Dias and Lovejoy, 2012). It can cause various problems for fish and wildlife, such as ingestion, entanglement and death (Gall and Thompson, 2015).

According to various studies, plastic litter is divided into three categories such as macroplastics, mesoplastics and microplastics (Browne, 2010; Fendall and Sewell, 2009). Following to a recent investigation, size range of microplastics are <5 mm to 0.1 µm (Lippiatt et al., 2013). Plastic matters gradually breakdown into minor trashes due to oxidation, ultraviolet (UV) radiation and mechanical forces, which is lower than 5 mm in diameter, termed microplastic (Barnes et al., 2009; Cole et al., 2011; Lippiatt et al., 2013). Microplastics are widely distributed, in deep sea sediments and surface water (Song et al., 2015; Woodall et al., 2014), from lakes to open sea water (Eriksen et al., 2014; Imhof et al., 2013), and in numerous marine organisms through the trophic levels Van Cauwenberghe and Janssen, 2014; Van Franeker et al., 2011). Ingestion of the minute particles can avert animals, together with lugworms, from devouring their natural kill, leading to starvation and even demise. A distinct report, from Plymouth University, indicates that ingesting microplastic can also lessen the health of lugworms by carrying dangerous chemicals, including hydrocarbons, antimicrobials and flame retardants of them. Equally benthic (de Sá et al., 2015) and pelagic (Rummel et al., 2016) fish may possibly consume microplastics directly, or indirectly (i.e. consume them in prey). Birds (Herzke et al., 2016) and mammals (Fossi et al., 2012) feeding on aquatic organisms or existing in aquatic environs are also acknowledged to ingest microplastics. Microplastics are found in nearly all marine and freshwater environments and have been tracked out in protected and remote areas (Claessens et al., 2013) creating their possible malicious impacts a worldwide crux. Microplastics are not only problematic for aquatic organisms but also detrimental for human health as well as human being. The possible health complications from microplastics can be understood of in the similar way we consider about silicosis or byssinosis from naturally arising inorganic and organic particulates. Excluding the understandable dissimilarity with microplastics is there is nothing natural about them.

Numerous studies focused on the introduction of plastic and other anthropogenic debris in marine habitats and food web through digestion by diverse marine organisms, ranging from zooplankton to vertex predators (Fosso et al., 2014, 2012; Ivar Do Sul and Costa, 2014). In the stomach of Mediterranean organisms such as elasmobranches, turtles, teleosts and some invertibrates, plastic debris was also recorded (Deudero and Alomar, 2014; Lazar and Gračan, 2011). The zones with convergence currents and where anthropogenic debris was accumulated, the consequence of debris assimilation by marine wildlife was more obvious (Moore et al., 2001).

The influence of microplastic assimilation on diverse marine entities with miscellaneous feeding mechanisms has been studied in different parts of the world (Thompson et al., 2004; Leslie et al., 2013; De Witte et al., 2014). Furthermore, these earlier investigations indicated that the eco-toxicological situations of certain species were associated to the environmental stress levels in their territories (Nayar et al., 2004)

From earlier studies, noteworthy amounts of plastics amassed into the marine environment and coastal ecosystems were mainly from Asian countries including Thailand which had moderately high financial growth rates (Jambeck et al., 2015). Plastic has also been acknowledged as one of the vital constituents in Municipal Solid Waste (MSW) composition of Thailand (Chiemchaisri et al., 2007; Kaosol, 2009). Therefore, land-based plastic can be the principal cause of plastic pollution in coastal waters (Jambeck, et al., 2015). Furthermore, Thailand is one of the five countries which dump more plastic (60%) into the oceans than the rest of the world collectively and the other countries are China, Indonesia, the Philippines and Vietnam (GlobalPost, 2016). Even so, there was no investigation done on microplastic pollution in marine fish, especially, in the lower Gulf of Thailand.

Considering the threat related to the plastic pollution, this investigation provides an obligatory engrossment to the knowledge and understanding of plastic existence in the commercial fishes.

2. OBJECTIVES

In view of the above discussion, this study has been undertaken to investigate the following specific objectives:

1. To explore, for the first time, the existence of microplastic debris in the stomach content of various commercially important marine fishes in the lower Gulf of Thailand.
2. To investigate the amount, frequency and form of plastics ingested by some commercial and abundant fishes in the lower Gulf of Thailand.

3. MATERIALS AND METHODS

3.1 Study site

The study location (Sathing Phra District; 7º28'24”N, 100º26’18”E) was selected at Songkhla Province, the lower Gulf of Thailand (Fig. 1) to symbolize the coast with different anthropogenic activities. Neighboring districts of Sathing Phra are Singhanakhon of Songkhla Province, Pak Phayun of Phatthalung Province, Lrasae Sin and Ranot of Songkhla Province. To the east of Sathing Phra is the Gulf of Thailand. Sathing Phra is a coastal fishery community with commercial fishing and culture performance. However, the area is presently affected by adjacent fishing settlement and some tourism activities.

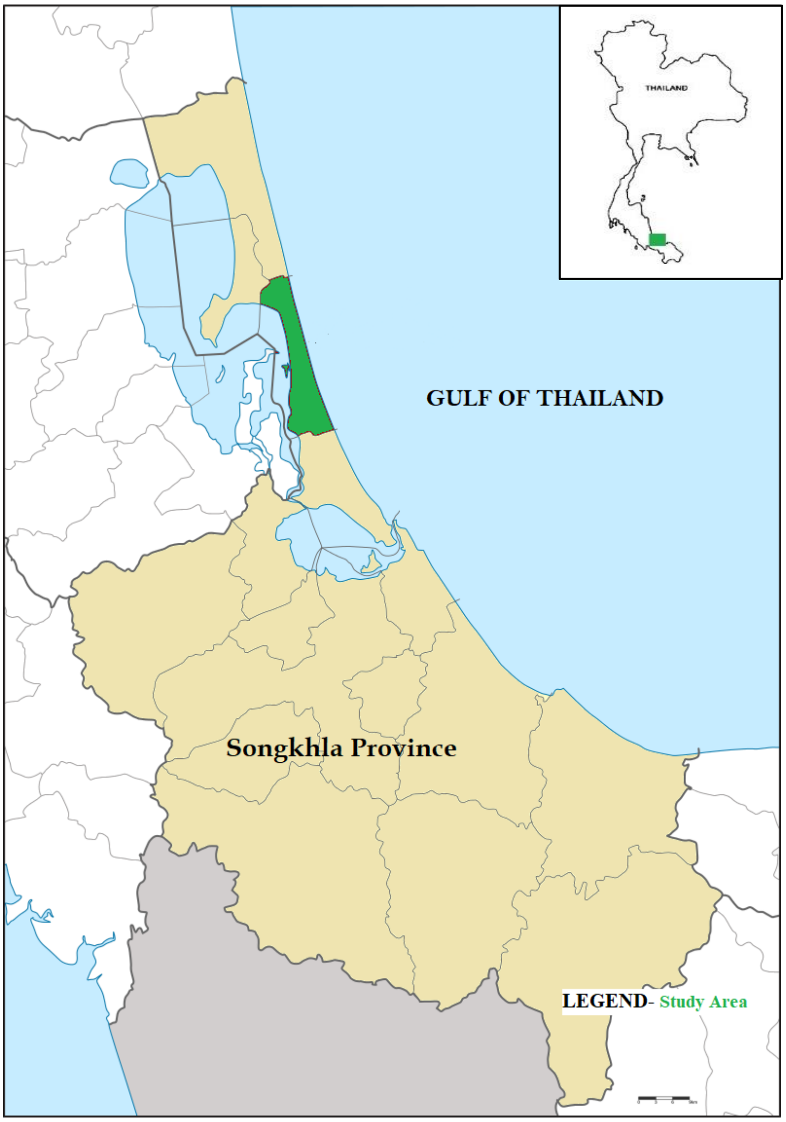


Figure 1. Map of the study site in Songkhla Province, the lower Gulf of Thailand.

3.2 Sampling and identification of fishes

Fish samples were randomly collected from August, 2017 to May, 2018 from the lower Gulf of Thailand. Fish samples were purchased from local fishermen. Then the fish samples were kept in an icebox with adequate ice and transported to the laboratory where they were immediately frozen and kept at -20ºC until further analysis.

Species of fish samples were primarily identified by the local fishermen and then their habitat, trophic level, sex and details about the species were assigned and verified according to the standard taxonomic keys of Talwar and Jhingran (1991); Froese and Pauly (2017); SEAFDEC (2014). Fishes were recognized to species where likely and pictures were taken of discrete fish for succeeding identification.

3.3 Analytical methods

In the laboratory, each fish was defrosted subsequently and sampled fishes were thoroughly rinsed by filtered distilled water to remove sediments and impurities from the external veil. After thoroughly rinsing the samples, each specimen sample was measured (Total length, TL; Fork length, FL and mouth size) and Weighed (Total weight, W).

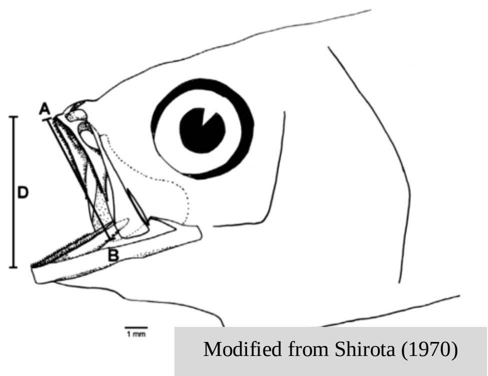


Figure 2. Measurement of mouth gape size of fish.

In order to measure the mouth size of fish, the method proposed by Shirota (1970) was applied, which is as follows-

<u>Mouth gap size of fish:</u>

$$D=\sqrt{2}AB \qquad \text{(Eq.1)}$$

Where, D= Mouth gape size,

AB= Measurement of upper- maxilla length

Eq. 1 was performed established on the endorsements found in Shirota (1970). Considering that the conceptual size of the maximum width of food (In this case, plastic particles) resembles to 50% of D (Shirota, 1970). Then consequently, each fish was dissected from the higher part of the oesophagus to take away the stomach according to the approaches published elsewhere (Claessens et al., 2013; Lusher et al., 2013; Rocha-Santos and Duarte, 2015). Stomach contents were then separately placed inside a petri dish. In research laboratory, stomach contents were observed in order to recognize plastic offal, which were then totaled, assembled by color and measured (length) under the Stereo Zoom Microscope (OLYMPUS SZ2-ILST). To define the length of each particle of debris, all snapped pieces were digitally measured by the software package ImageJ 1.4.3.6 (Public domain). The ingested plastics were characterized as microplastics (<5 mm), mesoplastics (5-25 mm) and macroplastics (>25 mm) after the method of Galgani et al. (2013). Quantification of microplastics in the marine environment were accomplished according to the recommendations of National Oceanic and Atmospheric Administration (NOAA) (Masura et al., 2015).

According to Eq. 2, To figure the total magnification of an image, the power of the objective (1X, 2X, 3X, 4X) which were set according to the precision of the plastic debris were multiplied by the power of the eyepiece (10X).

Total magnification:

$$\text{Total magnification} = \text{Power of objective} \times \text{Power of eyepiece} \qquad \text{(Eq.2)}$$

3.4 Preclusion of contamination

Prominent maintenance was occupied to preclude sample adulteration during dissection, extraction, sorting and visual identification. Our method includes numerous steps such as (i) Implement personal hygiene program, (ii) Use seperate equipments, (iii) Clean and sanitize all work surfaces etc. to abstain procedural contamination, cross-contamination or/and misidentification of natural debris (e.g., algae, shells, and coral) as anthropogenic debris. To obviate cross-contamination, all glassware and utensils were rinsed three time with distilled water between samples.

3.5 Statistical analysis

All data analysis was done in Microsoft Excel for mean, minimum and maximum. The frequency of plastic debris occurrence (*F%*) in these fish was assessed by the quantity of the individuals observed where plastic debris were present in the gut contents. The R 3.2.0 (R Core Team, 2015) statistical software was used to analyze the data. One-way ANOVA was accomplished to compare results among the clusters. Differences at $p < 0.05$ were considered statistically noteworthy.

4. RESULTS AND DISCUSSION

Current investigations (Fossi et al., 2012) revealed data on the effect of microplastic on gigantic filter-feeding creatures such as baleen whales and sharks in the Mediterranean sea, which can possibly devour microplastic offal. No data has been reported on microplastic ingestion by fishes from lower Southern Thailand. This study provided the first published record of plastic debris in the stomach contents of some commercially important fishes from the lower Gulf of Thailand (Fig. 1). Size and weight of the examined fishes range from 8.5 to 37.1 cm and 8 to 133 g. respectively for manuscript-1. On the other hand, in manuscript-2, total length and body weight of the examined fishes range from 7.6 to 21.9 cm and 4 to 99 g respectively (Table 1).

Entirely, 270 discrete fishes (98 demersal, 111 pelagic and 61 reef- associated individuals) were perused for the presence of plastic debris throughout this investigation. Among them, 167 (61.85%) individuals have different size and shape of plastic debris in their stomach (Table 2).

Table 1 exhibits mean value and range of lengths, body weight and stomach weight along with the information on trophic level and sex ration of examined fishes.

Table 1. Mean values and range of fish lengths and weight for each fish species together with their habitat.

Manuscript-1								
TL	**Fish species**	**Sample, n (M:F)**	**Fork length (cm)**		**Total length (cm)**		**Body weight (g)**	
			Mean±SD	**Range**	**Mean±SD**	**Range**	**Mean±SD**	**Range**
Demersal	*Alepes apercna* (Grant, 1987)	3 (2:1)	12.5±0.4	13.0-12.2	14.5±0.6	15.2-14.0	38.3±3.2	42.0-36.0
	Dasyatis zugei (Müller & Henle, 1841)	3 (2:1)	-	-	36.6±0.5	37.1-36.2	86.0±3.6	89.0-82.0
	Dendrophysa russellii (Cuvier, 1829)	3 (1:2)	-	-	12.9±1.7	14.0-11.0	32.7±4.2	36.0-28.0
	Leiognathus berbis (Valenciennes, 1835)	8 (2:6)	8.5±0.9	9.8-7.5	9.6±1.0	10.9-8.5	13.3±4.3	20.0-8.0
	Leiognathus fasciatus (Lacepède, 1803)	3 (2:1)	8.0±0.2	8.1-7.8	9.5±0.1	9.6-9.5	13.3±1.2	14.0-12.0
	Leiognathus splendens (Cuvier, 1829)	10 (3:7)	8.7±0.3	9.3-8.2	9.9±0.5	11.0-9.3	13.2±3.2	19.0-8.0
Pelagic	*Alepes melanoptera* (Swainson, 1839)	8 (3:5)	15.4±0.8	17.1-14.4	17.2±0.8	18.2-16.0	57.1±8.5	73.0-49.0
	Alepes vari (Cuvier, 1833)	3 (0:3)	17.2±0.3	17.4-16.9	19.0±0.8	19.6-18.1	83.7±4.0	88.0-80.0
	Anodontostoma chacunda (Hamilton, 1822)	14 (8:6)	11.0±1.4	13.2-9.3	13.1±1.6	16.0-11.1	32.6±13.6	59.0-17.0
	Johnius borneensis (Bleeker, 1851)	3 (3:0)	-	-	12.7±0.3	12.9-12.4	24.7±1.5	26.0-23.0
	Johnius carouna (Cuvier, 1830)	20 (9:11)	-	-	16.1±3.1	22.7-12.0	53.9±29.4	129.0-15.0
	Opisthopterus tardoore (Cuvier, 1829)	3 (0:3)	10.3±0.4	10.7-10.0	11.7±0.4	12.1-11.4	11.0±2.6	14.0-9.0
	Rastrelliger brachysoma (Bleeker, 1851)	3 (0:3)	15.2±0.3	15.5-14.9	17.3±0.4	17.6-16.9	43.3±2.1	45.0-41.0

	Sardinella gibbosa (Bleeker, 1849)	3 (3:0)	13.9±1.1	14.6-12.6	15.6±1.1	16.3-14.3	32.7±7.5	37.0-24.0
	Sardinella jussieu (Lacepède, 1803)	8 (4:4)	10.8±0.6	11.5-10.0	12.7±0.7	13.7-12.1	22.5±4.3	32.0-19.0
	Scomberomorus commerson (Lacepède, 1800)	4 (1:3)	21.1±3.7	26.4-18.6	23.5±4.3	29.8-20.5	98.3±56.4	182.0-61.0
	Scomberomorus guttatus (Bloch & Schneider, 1801)	5 (5:0)	17.0±0.6	17.9-16.3	19.9±0.8	21.1-19.0	52.4±6.1	62.0-47.0
Reef- associated	*Alepes kleinii* (Bloch, 1793)	4 (1:3)	12.4±0.6	12.8-11.5	14.0±0.5	14.3-13.3	29.5±3.1	32.0-25.0
	Drepane longimana (Bloch & Schneider, 1801)	3 (1:2)	-	-	15.6±0.8	16.3-14.7	116.7±14.7	133.0-105.0
	Megalaspis cordyla (Linnaeus, 1758)	29 (25:4)	16.3±1.1	18.3-13.8	18.0±1.4	20.7-15.3	53.7±12.3	77.0-36.0
	Sardinella albella (Valenciennes, 1847)	14 (6:8)	12.5±1.2	13.8-10.2	14.2±1.3	15.5-11.6	30.9±7.4	40.0-19.0
	Scomberoides tata (Cuvier, 1832)	3 (1:2)	16.2±1.5	17.1-14.4	18.0±2.4	19.4-15.2	51.0±17.3	62.0-31.0
	Scomberoides tol (Cuvier, 1832)	3 (0:3)	15.2±0.3	15.5-14.9	16.9±0.7	17.6-16.2	47.3±3.1	50.0-44.0
	Terapon theraps (Cuvier, 1829)	5 (4:1)	13.4±0.9	14.8-12.4	14.1±0.9	15.4-13.2	49.6±13.5	73.0-40.0
	Total	165 (86:79)		**Range**		8.5 – 37.1 cm		8 – 133 g
				Manuscript-2				
TL	**Fish species**	**Sample, n (M:F)**	**Total length (cm)**		**Body Weight (g)**		**Stomach Weight (g)**	
			Mean±SD	**Range**	**Mean±SD**	**Range**	**Mean±SD**	**Range**
Demersal	*Panna microdon* (Bleeker, 1849)	27 (24:3)	13.20±4.34	21.9 - 7.6	28.04±25.98	99.0 – 4.0	0.16±0.10	0.57 – 0.03
	Dendrophysa russelli (Cuvier, 1829)	41 (9:32)	13.56±0.92	15.0 – 11.7	32.90±8.53	52.0 – 18.0	0.16±0.06	0.3 – 0.05

Pelagic	*Johnius borneensis* (Bleeker, 1851)	30 (25:5)	13.86±1.59	16.4 – 10.9	34.33±14.36	65.0 – 14.0	0.27±0.21	0.91 – 0.02
	Johnius weberi (Hardenberg, 1936)	7 (7:0)	16.30±1.56	18.4 – 14.2	42.29±13.24	63.0 – 28.0	0.19±0.08	0.38 – 0.14
Total		105 (65:40)	**Range**	7.6 – 21.9 cm		4 – 99 g		

TL = Trophic Level, n = total number of sample, M = male, F = female, ±SD = ± standard deviation

Table 2 indications the number of discrete fish stomachs observed, the number of individuals from each group with plastic debris, the average anumber of individual pieces of debris per stomach of fishes (including individuals with no debris), the range of single pieces of debris per stomach of fishes in each and every group together with the information on frequency of occurrence.

Table 2. The average number and range of individual pieces of debris per stomach with frequency of occurrence.

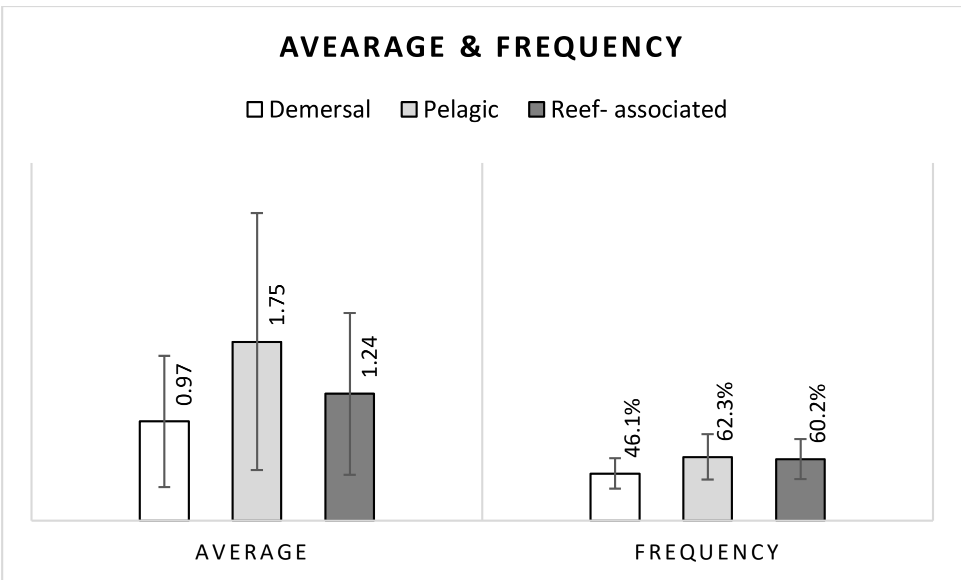
Fish species	Stomach examined	Stomach with debris	Number of pieces of plastic debris/ stomach (average (±SD), Range	Frequency (%)
		Manuscript-1		
Demersal fish species (n= 6)				
Alepes apercna	3	2	2.0 (±2.0), 0-4	67
Dasyatis zugei	3	1	0.3 (±0.6), 0-1	33
Dendrophysa russellii	3	1	0.3 (±0.5), 0-1	33
Leiognathus berbis	8	4	0.9 (±1.0), 0-1	50
Leiognathus fasciatus	3	1	1.3 (±2.3), 0-4	33
Leiognathus splendens	10	6	1.0 (±1.1), 0-3	60
Sub-total	*30*	*15 (50% of total demersal fish)*		
Pelagic fish species (n= 11)				
Alepes melanoptera	8	6	1.3 (±1.0), 0-3	75
Alepes vari	3	2	1.7 (±1.5), 0-3	67
Anodontostoma chacunda	14	8	2.0 (±4.0), 0-15	57
Johnius borneensis	3	2	1.0 (±1.0), 0-2	67
Johnius carouna	20	17	3.8 (±3.2), 0-13	85
Opisthopterus tardoore	3	1	2.0 (±3.5),0-6	33
Rastrelliger brachysoma	3	1	1.0 (±1.7), 0-3	33
Sardinella gibbosa	3	1	0.3 (±0.6), 0-1	33
Sardinella jussieu	8	6	1.3 (±1.3), 0-4	75

Scomberomorus commerson	4	4	4.3 (±1.0), 3-5	100
Scomberomorus guttatus	5	3	0.6 (±0.5), 0-1	60
Sub-total	*74*	*51 (68.92% of total pelagic fish)*		
Reef- associated fish species (n= 7)				
Alepes kleinii	4	2	0.8 (±1.0), 0-2	50
Drepane longimana	3	1	0.3 (±0.6), 0-1	33
Megalaspis cordyla	29	23	1.6 (±1.2), 0-5	79
Sardinella albella	14	12	2.3 (±1.8), 0-5	86
Scomberoides tala	3	2	0.7 (±0.6), 0-2	67
Scomberoides tol	3	2	2.2 (±2.2), 0-4	67
Terapon theraps	5	2	0.8 (±1.3), 0-3	40
Sub-total	*61*	*44 (72.13% of total reef-associated fish)*		
Total	165	110 (66.67% of total fish)		
Manuscript-2				
Demersal fish species (n= 2)				
Panna microdon	27	12	0.85±1.06, 3 – 0	44
Dendrophysa russelli	41	23	0.88±1.12, 5 – 0	56
Sub-total	*68*	*35 (51.47% of total demersal fish)*		
Pelagic fish species (n= 2)				
Johnius borneensis	30	18	0.90±0.88, 3 – 0	60
Johnius weberi	7	8	1.14±1.21, 3 – 0	57
Sub-total	*37*	*26 (70.27% of total pelagic fish)*		
Total	105	61 (58.10% of total fish)		
Grand Total	**270**	**171 (63.33% of total fish)**		

In manuscript-1, out of 165 individual fish stomach examined, 66.67% contained plastic offal. Particularly, these consisted of 15 demersal individual fishes (50%), 51 pelagic individual fishes (68.92%) and 44 reefs- associated individual fishes (72.13%) containing some kinds of plastic matters (Table 2). The average occurrence of plastic particles in the pelagic fish (1.75 particles/ stomach) was higher than that of demarsal fish (0.97 particles/ stomach) by approximately two times (Table -2). On the other hand, in manuscript-2, out of 105 investigated fish stomachs, 57 (54.29%) stomachs contained plastic debris (Table 2). In particular, this involved of 35 individual demersal fishes (51.47%, out of 68 individuals) and 26 individual pelagic fishes (70.27%, out of 37 individuals). The average frequency of occurrence (%) for demersal fish species was 51.47%, which was lower than the average frequency of occurrence of the observed pelagic fishes (70.27%) (Table 2).

Average and frequency (%) of existence of plastic particles in the stomach of different types of fishes have been shown in Fig. 3. In manuscript-1, the average occurrence of plastic debris in the pelagic fish (1.75 particles/ stomach) was higher than that of demarsal fish (0.97 particles/ stomach) by approximately two times (Fig. 3.A). Meanwhile, in manuscript-2, the average number of plastic debris per g of stomach for demersal fishes was 5.41, which was slightly higher than that of the pelagic species (4.67). Contrariwise, pelagic fish species showed higher (1.02) average number of plastic debris per stomach than that of the demersal ones (0.87) (Fig. 3.B).

(A)



(B)

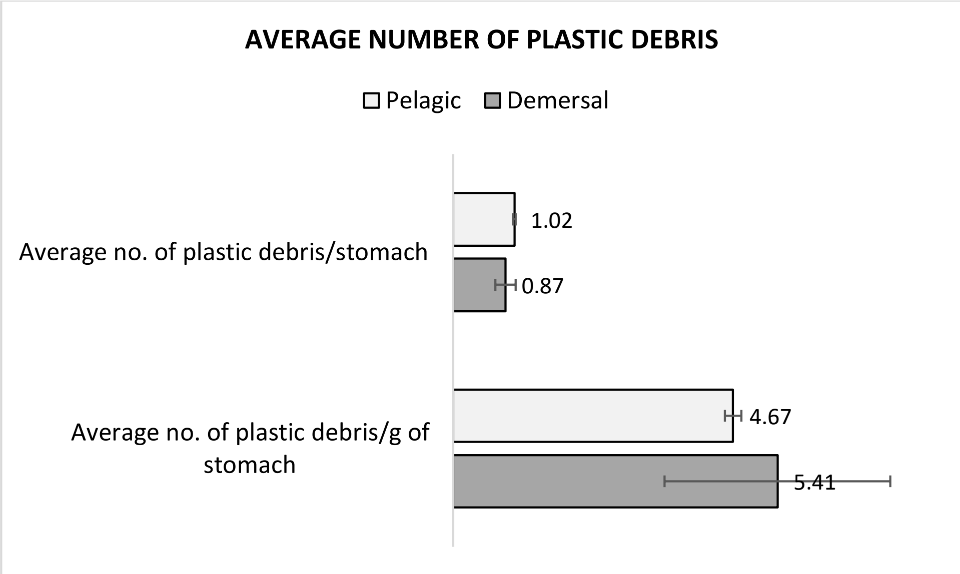


Figure 3. Average number and frequency (%) of occurrence of plastic particles in the stomach of different types of fishes. (A) Manuscript-1; (B) Manuscript-2. Error bars represent standard deviations.

Therefore, it was obvious that the pelagic fish species possessed more plastic debris than the demersal fishes. This may be because of the luxuriant presence of plastic offal in the surface level of marine water bodies. Meanwhile most of the plastic debris tend to levitate on the surface level of the water because of their solidity and structure behavior, pelagic fishes gulp the plastic debris mistakenly as food. According to several studies (WWF, 2018), 80% of plastic offal in our marine environment is introduced from land sources and it can come to our ocean in three leading ways such as dumping plastic offal in the bin when it could be reused, dropping litters and goods that go down the sewer. Once in the ocean, plastic stays in the surface level of the water bodies for certain period of times, breaks down into tiny pieces and then travels to other trophic level of the water bodies such as middle level and finally to the bottom level. This can be one of the vital reasons behind the copiousness of plastic offal in the stomach contents of pelagic fishes.

Comparable investigation was performed by Romeo *et al.* (2015), who worked on the existence of plastic rubbish in the stomach of 3 large pelagic fishes (*Xiphias gladius*, *Thunnus alalunga* and *Thunnus thynnus*) in the Mediterranean Sea and 18.18% of the investigated fish stomach contained certain types of plastic offal, which was inferior than those of the current study.

Plastic debris had diverse forms and colors; transparent, black and pink plastic debris present in all the fish examined species in this exploration. Several circumstantial information on plastic offal found in the stomach content were presented below (Table 3).

Table 3. Colors, types and size of plastic particles found in the stomach contents of different fishes.

Fish species	**Details of plastic particles**			
	Length range (mm)	**Width range (mm)**	**Color**	**Type**
Manuscript-1				
Demersal fish species (n= 6)				
Alepes apercna	0.82 – 4.76	0.03 – 0.40	Transparent, Blue	Fibre, Fragment
Dasyatis zugei	16.67	0.16	Green	Fibre
Dendrophysa russellii	1.34	0.02	Blue	Fibre
Leiognathus berbis	0.53 – 6.54	0.02 – 0.23	Transparent, Blue, Black	Fibre
Leiognathus fasciatus	1.60 – 3.29	0.02 – 1.33	Transparent, Red, Blue	Fibre, Fragment
Leiognathus splendens	0.62 – 7.41	0.02 – 0.82	Transparent, Blue, Black	Fibre, Fragment
Pelagic fish species (n= 11)				
Alepes melanoptera	0.52 – 4.83	0.02 – 0.51	Transparent, Green, Black,	Fibre, Fragment

			Red	
Alepes vari	2.75 – 10.55	0.05 – 0.11	Transparent, Yellow, Black, Brown	Fibre,
Anodontostoma chacunda	0.63 – 16.36	0.02 – 2.42	Transparent, Red, Blue, Brown, Black	Fibre, Fragment
Johnius borneensis	2.28 – 10.55	0.03 – 0.04	Blue, Brown	Fibre
Johnius carouna	0.35 – 17.16	0.01 – 1.94	Transparent, Blue, Black, Brown, Green, Red	Fibre, Fragment
Opisthopterus tardoore	1.18 – 8.83	0.01 – 0.04	Black, Blue	Fibre
Rastrelliger brachysoma	2.46 – 5.27	0.04 – 0.40	Transparent	Fibre
Sardinella gibbosa	0.73	0.61	Transparent	Fragment
Sardinella jussieu	0.75 – 5.07	0.04 – 0.96	Transparent, Blue, Black	Fibre, Fragment
Scomberomorus commerson	0.54 – 8.70	0.02 – 0.47	Transparent, Black, Yellow, Red	Fibre, Fragment
Scomberomorus guttatus	1.53 – 2.88	0.03 – 0.04	Transparent, Black	Fibre
Reef- associated fish species (n= 7)				
Alepes kleinii	0.87 – 4.41	0.06 – 2.10	Transparent, Black	Fibre, Fragment
Drepane longimana	2.26	0.05	Black	Fibre
Megalaspis cordyla	0.13 – 15.23	0.01 – 1.74	Transparent, Black, Blue, Brown, Red	Fibre, Fragment
Sardinella albella	1.29 – 8.78	0.01 – 0.81	Transparent, Blue, Black, Red, Brown	Fibre, Fragment
Scomberoides tala	0.83 – 5.94	0.05 – 0.68	Transparent, Black	Fibre, Fragment
Scomberoides tol	0.69 – 6.46	0.02 – 0.09	Transparent, Red, Blue	Fibre
Terapon theraps	1.36 – 3.55	0.02 – 0.30	Transparent,	Fibre,

			Black	Fragment
		Manuscript-2		
Demersal fish species (n= 2)				
Panna microdon	2.08 – 23.48	0.04 – 1.71	Transparent, Blue, Brown, Black, Pink, Violet, Green	Fibre, Fragment
Dendrophysa russelli	1.46 – 20.99	0.04 – 3.85	Transparent, Black, Blue Green, Pink, Violet	Fibre, Fragment
Pelagic fish species (n= 2)				
Johnius borneensis	1.23 – 38.22	0.06 – 2.72	Transparent, Black, Pink, Red, Violet, Blue, Brown	Fibre, Fragment
Johnius weberi	2.12 – 16.75	0.06 – 0.62	Transparent, Black, Brown, Pink	Fibre, Fragment
Sizes of plastic debris found in the stomach of fishes	*0.13- 38.22 mm	**0.01- 3.85 mm		

**Lowest and highest length; **lowest and highest width*

For the two investigations, the plastic items found in the stomach contents of the samples extended from 0.13 mm to 38.22 mm in length and width ranged from 0.01-3.85 mm (Table 3). Though the plastic items obtained were either fibre type or fragment type, most of them were fibre from fish nets (Table 3). These plastic litters had different shapes and different colors. Transparent (38.24%) colored plastic items were the most dominant color of plastics found throughout these investigations (Fig. 4). Since transparent color is nearly illusive to see in the water, aquatic organisms especially fishes erroneously take this sort of plastic litters while gulping, filter-feeding and consuming food. Sometimes, fishes unintentionally ingest plastic litters as a live food as well.

Similar investigation was done by Romeo *et al.* (2015), who reported various color of plastic rubbish such as transparent, white, blue and so on. The plastic debris found in that study ranged from 0.63 to 164.50 mm in length and 0.69 to 60.57 mm in width from the stomach contents of pelagic fishes.

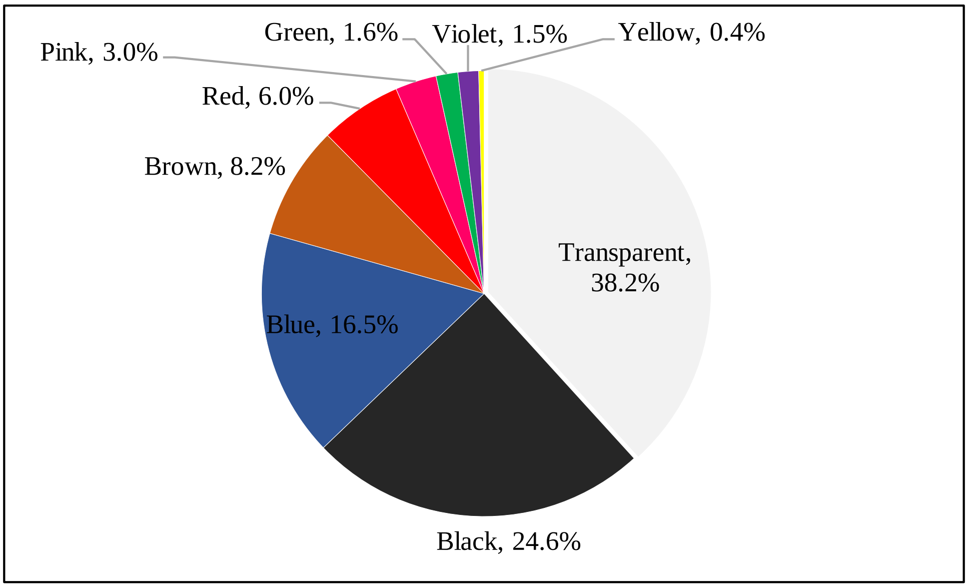
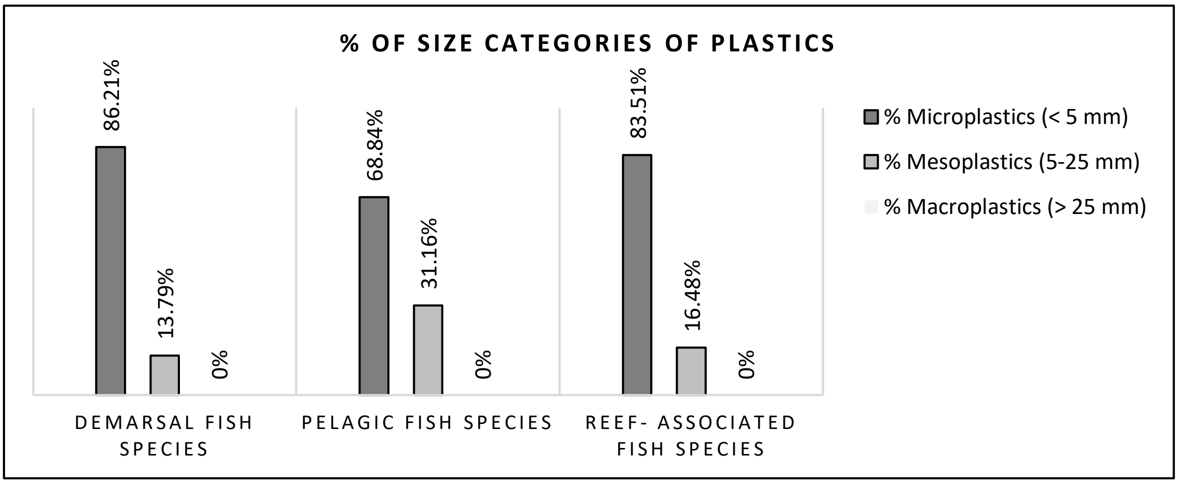


Figure 4. Percentages (%) of color of plastic debris found in the stomach contents of fishes in both investigations.

Plastic debris were different in size in each species of fishes as stated in Figure 5. Mesoplastic were the most plentiful (69.88%) size group found and about 28% of all the plastic debris found were microplastics which were less than 5 mm in diameter (Fig. 5).

Percentages of plastic debris according to their size group were indicated in Figure 5.

(A)



(B)

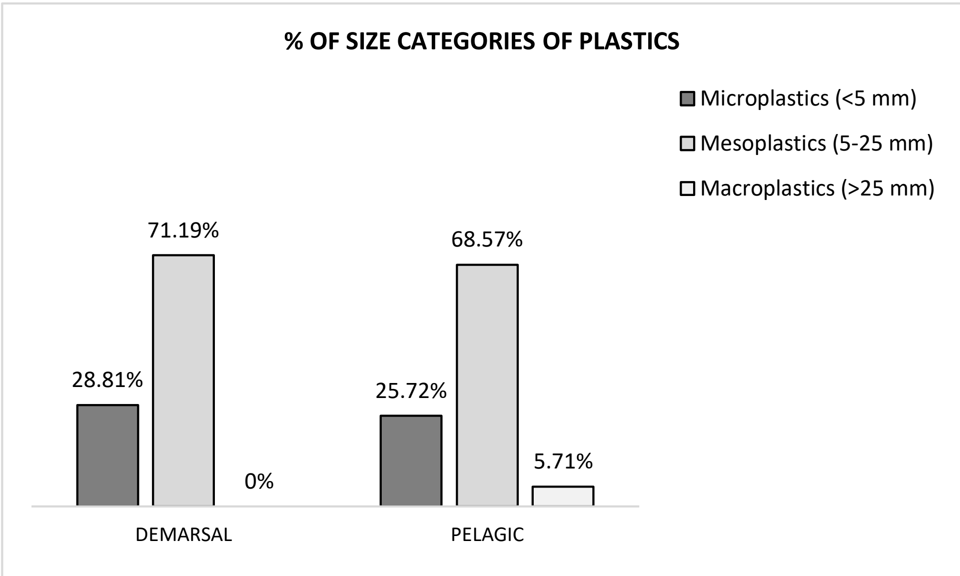


Figure 5. Percentages (%) of plastic debris according to their size group.

Size of the plastic items found in the gut contents were also been categorized. In first investigation, Microplastics (<5mm) were the most abundant (79.52%) size group of plastic items obtained from the stomach content of fishes during this investigation and the rest of them (20.48%) were mesoplastics (5-25 mm) In particular, demersal and reef-associated fish species showed more or less similar amount of microplastics which are 86.21% and 83.51% respectively. Since the sizes of the fish individuals were small, there wasn't any macroplastic (>25 mm) found during the investigation (Fig. 5.A).

Meanwhile, in second investigation, Mesoplastics (69.88%, 5-25mm) were the most abundant size group of plastic debris obtained throughout the investigation, which is higher than the amount of microplastics (27.27%, <5 mm) found by approximately two times. Rest of the plastic debris found were macroplastics (2.85%, >25 mm). In particular, 25.72% and 28.81% of microplastic debris were obtained from pelagic and demersal fish species, respectively. Contrariwise, pelagic and demersal species showed 68.57% and 71.19% mesoplastics respectively in their stomach contents (Fig. 5.B). Only 5.71% macroplastic was found from the pelagic fish throughout the investigation, while no macroplastics were obtained from demersal fishes.

Furthermore, there was a number of more scientists who also obtained microplastic debris in fish gastrointestinal tract (Lusher et al., 2013; Murphy et al., 2017; Phillips and Bonner, 2015; Tanaka and Takada, 2016).

Different colors (transparent, black, blue and green) and forms (fibre and fragment) of plastic debris found throughout the research were presented in Figure 6.

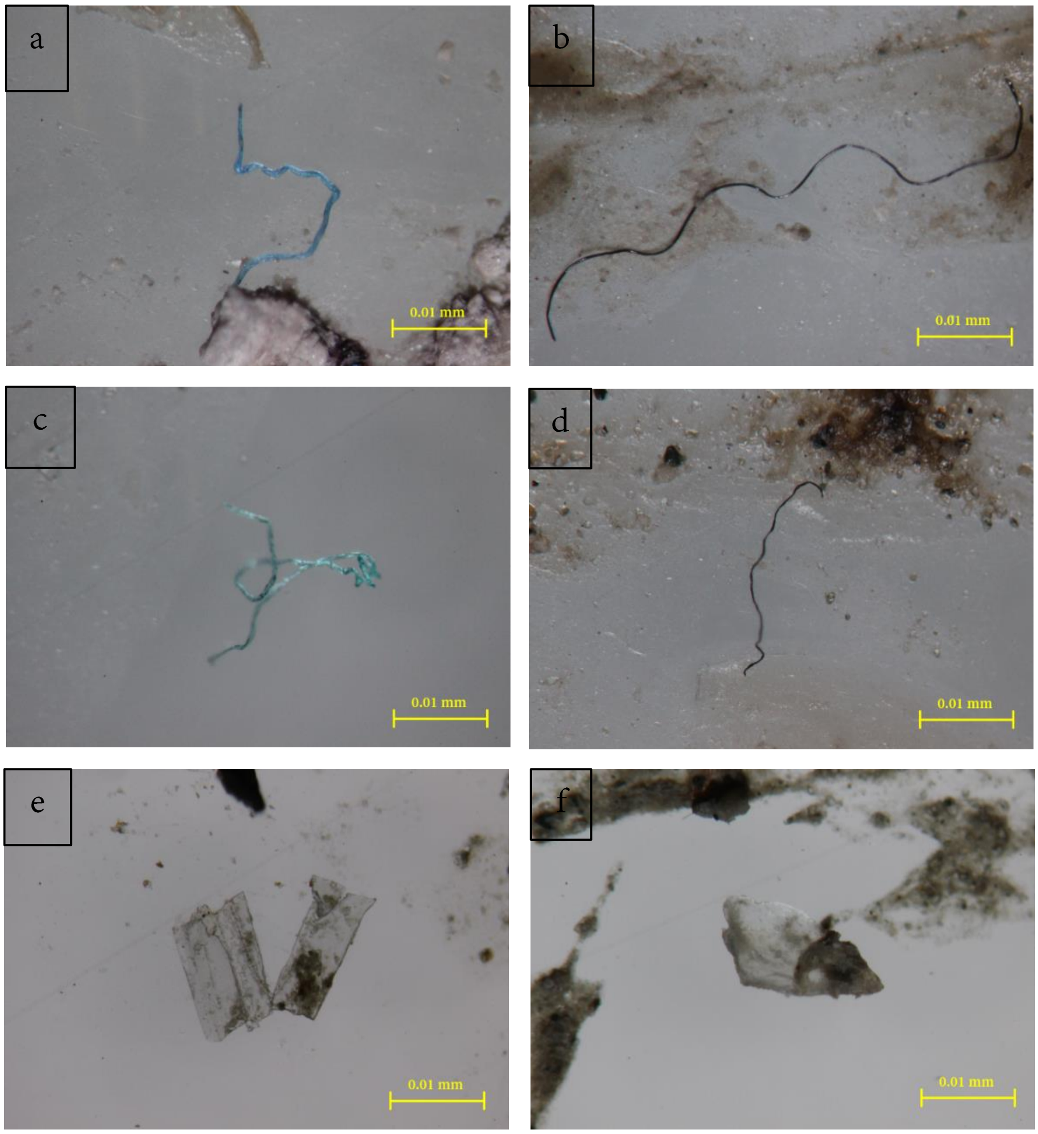


Figure 6. Photographs of fibre (a-d) and fragment (e-f) forms of plastic debris found in the stomach contents of fishes under stereo zoom microscope.

Results of the current study emphasize the pervasive existence of plastics in the lower Gulf of Thailand. Moreover, the high frequencies of micro and mesoplastics in the marine fishes from the lower Gulf of Thailand represent a further warning signal for marine conservation as well as for the soundness of human health. However, more detailed information and investigations are needed to determine this sort of relevance.

5. CONCLUSIONS AND RECOMMENDATIONS

Findings of the present investigation highlight prevalent presence of plastic rubbish and high frequencies micro and meso-plastics in the marine fishes from the lower Gulf of Thailand and signify a further cautionary sign for marine conservation as well as for the consciousness of human well-being. These fundamental findings signify a vital preliminary stage in exploring some eco-toxicological facets such as the possible effects associated to the transmission of impurities on human well-being and the valuation of the existence and effect of plastic pollution on other types of marine organisms. The accession of the microplastics as well as other sized plastic litters in commercially important marine fishes suggestively reveals the anthropogenic stress on the fishery and marine food security in the lower Gulf of Thailand. Health perils are feasible when people consume these defiled marine organisms. Moreover, effective management programs in the study area and contiguous areas for the plastic pollution are immediately required. Wherefore, related to the high ingesting of these species in the Gulf of Thailand, this issue necessitates profounder enquiry in the futurity. Moreover, definitely recommended that microplastic contamination in marine organisms and their food chain in adjacent Provinces must be explored in order to make sure the wellbeing circumstances for environment as well as human safety. Moreover, this is an elementary research work which need further in detail investigations.

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APPENDIX A

PUBLISHED MANUSCRIPT - 1

Title	First evidence of existence of microplastics in stomach of some commercial fishes in the lower Gulf of Thailand.
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Volume (Issue)	16 (6)
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Year	2018

FIRST EVIDENCE OF EXISTENCE OF MICROPLASTICS IN STOMACH OF SOME COMMERCIAL FISHES IN THE LOWER GULF OF THAILAND

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Abstract. Microplastics have turned into a key global environmental issue in the current decade because of their marine ubiquity, bioavailability and capability of carrying toxic chemicals. The study focuses, for the first time, on the existence of plastic debris in the stomach contents of some commercially important marine fishes caught from the lower Gulf of Thailand during August to November of 2017. Size and weight range of the samples were 8.5 to 37.1 cm and 8 to 133 g. Results highlighted the ingestion of plastics in the 66.67% samples (110 out of 165 samples). The plastics ingested were microplastics (79.52%) (<5 mm), mesoplastics (20.48%) (5-25 mm). No macroplastic was found during this study since the study dealt with small fishes only. Transparent color plastics were the most dominant colors found in the stomach of fishes examined. Net fibres were the major types of plastics found during this study. There was no relationship found between size of plastics and different biological features of the investigated fishes. These initial findings signify an imperative phase in exploring ecotoxicological perspectives such as the existence and impact of plastic debris on the food chain; the probable effects related to the transmission of contaminants on human health etc.
Keywords: *marine litter, microplastic, plastic ingestion, gut content, plastic pollution, anthropogenic debris, Gulf of Thailand*

Introduction

Plastics are synthetic organic polymers, which are obtained from the poly-merisation of monomers extracted from oil or gas (Derraik, 2002; Rios et al., 2010; Thompson et al., 2009). In modern society, plastic has attained a crucial status, with widespread industrial, medicinal, municipal and commercial applications. Since mass production began in the 1940s, the annual plastic production has amplified from 1.5 million tonnes in the 1950s to 322 million tonnes in 2015 (PlasticsEurope, 2016). At present, plastic has been the fastest-growing urban waste and accounted for 60-80% of marine debris (Moore, 2008). Plastic waste has been assembled in the environment at a turbulent rate through inadvertent release and indiscriminate abandonment. Plastic has become a pervasive and dominant component of marine debris due to its lightweight, cumulative global production, durable nature and continuing inappropriate dumping (Derraik, 2002; Gregory, 2009; Moore, 2008; Thompson et al., 2009). Plastic is responsible for around 92% of all negative encounters between organisms and marine litter. The impacts of large plastic objects (i.e., macroplastic) on marine life were widely reported. It can cause various problems for fish and wildlife, such as ingestion, entanglement and death (Gall and Thompson, 2015). Above 660 marine species were known to be affected globally by plastic litter directly or indirectly (Dias and Lovejoy, 2012).

According to various studies, plastic litter is divided into three categories such as macroplastics, mesoplastics and microplastics (Browne, 2010; Fendall and Sewell, 2009). Plastic items break down into gradually smaller fragments due to oxidation, ultraviolet (UV) radiation and mechanical forces, which is below 5 mm in diameter and called microplastic (Barnes et al., 2009; Cole et al., 2011; Lippiatt et al., 2013). According to Lippiatt et al. (2013), size range of microplastics are <5 mm to 0.1 μm (Lippiatt et al., 2013). Microplastics are widely distributed, in deep sea sediments and surface water (Song et al., 2015; Woodall et al., 2014), from lakes to open sea water (Eriksen et al., 2014; Imhof et al., 2013), and in various marine organisms through the trophic levels (Boerger et al., 2010; De Witte et al., 2014; Murray and Cowie, 2011; Van Cauwenberghe and Janssen, 2014; Van Franeker et al., 2011). Numerous studies focused on the introduction of plastic and other anthropogenic debris in marine habitats and food web through digestion by diverse marine organisms, ranging from zooplankton to vertex predators (Fossi et al., 2014, 2012; Ivar Do Sul and Costa, 2014). In the stomach of Mediterranean organisms such as elasmobranches, turtles, teleosts and some invertibrates, plastic debris was also recorded (Deudero and Alomar, 2014; Lazar and Gračan, 2011). The effect of debris ingestion by marine wildlife was more explicit in those areas categorized by convergence currents, where anthropogenic debris was amassed (Moore et al., 2001).

From earlier studies, noteworthy amounts of plastics amassed into the marine environment and coastal ecosystems were primarily from Asian countries including Thailand which had moderately high economic growth rates (Jambeck et al., 2015). Plastic has also been acknowledged as one of crucial component in Municipal Solid Waste (MSW) composition of Thailand (Chiemchaisri et al., 2007; Kaosol, 2009). Therefore, land based plastic can be the core source of plastic pollution in coastal waters (Jambeck et al., 2015). However, at present, there are very few studies conducted on plastic pollution in Thailand. Particularly, no study was done on microplastic contamination in marine fish in lower southern Gulf of Thailand.

This study investigated, for the first time, the occurrence of plastic debris in the stomach content of various commercially important marine fishes together with the relationship between total plastic length and different biological features of fishes and details on plastic debris found in the stomach content of fishes.

Materials and methods

Study site

The study location (Sathing Phra District; 7°28’24”N, 100°26’18”E) was selected at Songkhla Province, the lower Gulf of Thailand (*Fig. 1)* to symbolize the coast with different anthropogenic activities. Neighboring districts of Sathing Phra are

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Singhanakhon of Songkhla Province, Pak Phayun of Phatthalung Province, Lrasae Sin and Ranot of Songkhla Province. To the east of Sathing Phra is the Gulf of Thailand. Sathing Phra is a coastal fishery community with commercial fishing and culture performance. However, the area is presently affected by adjacent fishing settlement and some tourism activities.

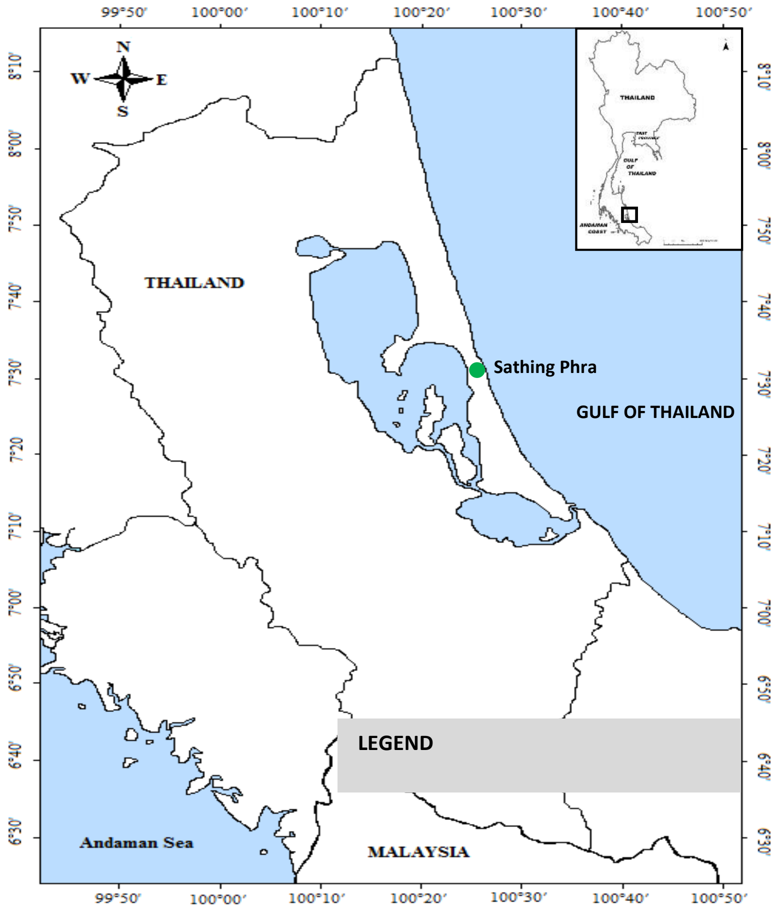

Figure 1. *Map of the study site in Songkhla Province, the lower Gulf of Thailand*

Sampling and identification of fishes

Fish samples were randomly collected from August to December of 2017 from the lower Gulf of Thailand. Fishes were caught by using different types of nets such as Shrimp net, Mackerel net and small traditional fishing boats were used to catch the fishes. Mackerel nets were hung vertically from a boat in the water with its bottom edge held down by weights and its top edge buoyed by floats. Particular information of the location and fishes were recorded. Then the fish samples were kept in an icebox with adequate ice and transported to the laboratory where they were immediately frozen and kept at -20 °C until further analysis.

Species of fish samples were primarily identified by the local fishermen and then their habitat, trophic level, sex and details about the species were assigned and verified according to the standard taxonomic keys of Talwar and Jhingran (1991); Froese and Pauly (2017); SEAFDEC (2014). Fishes were identified to species where possible and pictures were taken of individual fish for subsequent identification.

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Analytical methods

In the laboratory, each fish was defrosted subsequently and sampled fishes were thoroughly rinsed by filtered distilled water to remove sediments and impurities from the external veil. After thoroughly rinsing the samples, each specimen sample was measured (Total length, TL; Fork length, FL and mouth size – *Fig. 2*) and Weighed (Total weight, W).

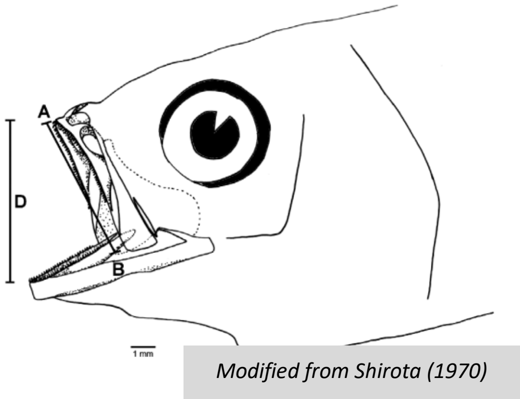

Figure 2. *Measurement of mouth gape size of fish*

In order to measure the mouth size of fish, the method proposed by Shirota (1970) was applied, which is as follows:

Mouth gap size of fish:

$$D = \sqrt{2AB} \qquad \text{(Eq.1)}$$

where, D = Mouth gape size, AB= Measurement of upper-maxilla length

Equation 1 was performed based on the recommendations found in Shirota (1970). Considering that the conceptual size of the maximum width of food (in this case, plastic particles) corresponds to 50% of D (Shirota, 1970). Then subsequently, each fish was dissected from the upper part of the oesophagus to remove the stomach according to the methods published elsewhere (Claessens et al., 2013; Lusher et al., 2013; Rocha-Santos and Duarte, 2015). Stomach contents were then separately placed inside a petri dish. In laboratory, stomach contents were examined in order to identify plastic debris, which were then counted, grouped by color and measured (length) by the Stereo Zoom Microscope (OLYMPUS SZ2-ILST). To determine the length of each particle of debris, all photographed pieces were digitally measured using the software package ImageJ 1.4.3.6 (Public domain). The ingested plastics were categorized as microplastics (<5 mm), mesoplastics (5-25 mm) and macroplastics (>25 mm) following Galgani et al. (2013).

According to *Equation 2*, to figure the total magnification of an image, the power of the objective (1X, 2X, 3X, 4X) which were set according to the precision of the plastic debris were multiplied by the power of the eyepiece (10X).

Total magnification:

$$\text{Total magnification} = \text{Power of objective} \times \text{Power of eyepiece} \qquad \text{(Eq.2)}$$

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Preclusion of contamination

Prominent maintenance was taken to preclude sample adulteration during dissection, extraction, sorting and visual identification. Our method includes numerous steps such as (i) Implement personal hygiene program, (ii) Use seperate equipments, (iii) Clean and sanitize all work surfaces etc. to avoid procedural contamination, cross-contamination and/or misidentification of natural debris (e.g., shells, algae, and coral) as anthropogenic debris. To obviate cross- contamination, all utensils and glassware were rinsed three times with distilled water between samples.

Statistical analysis

All data analysis was performed in Microsoft excel for mean, minimum and maximum. The frequency of plastic debris occurrence (*F%*) in these fish was estimated by the quantity of the individuals observed where plastics were present in the stomach contents. The R 3.2.0 (R Core Team, 2015) statistical software was used to analyze the data.

Results

Overall, 258 plastic fragments were recognized from the stomach content of 110 fishes (66.67%) and in particular 15 demersal individual fishes (50%), 51 pelagic individual fishes (68.92%) and 44 reef- associated individual fishes (72.13%) (*Table 2*). *Table 1* reports mean values and range of fork length, total length and weight of fishes together with the information on sex ratio and habitat of examined fishes.

Table 1. *Mean values and range of fish lengths and weight for each fish species with their habitat*

Trophic level	Fish species	Sample n (m+f)	Fork length (cm)		Total length (cm)		Weight (g)	
			Mean±SD	Range	Mean±SD	Range	Mean±SD	Range
Demersal	Alepes apercna (Grant, 1987)	3 (2+1)	12.5±0.4	13.0-12.2	14.5±0.6	15.2-14.0	38.3±3.2	42.0-36.0
	Dasyatis zugei (Müller & Henle, 1841)	3 (2+1)	-	-	36.6±0.5	37.1-36.2	86.0±3.6	89.0-82.0
	Dendrophysa russellii (Cuvier, 1829)	3 (1+2)	-	-	12.9±1.7	14.0-11.0	32.7±4.2	36.0-28.0
	Leiognathus berbis (Valenciennes, 1835)	8 (2+6)	8.5±0.9	9.8-7.5	9.6±1.0	10.9-8.5	13.3±4.3	20.0-8.0
	Leiognathus fasciatus (Lacepède, 1803)	3 (2+1)	8.0±0.2	8.1-7.8	9.5±0.1	9.6-9.5	13.3±1.2	14.0-12.0
	Leiognathus splendens (Cuvier, 1829)	10 (3+7)	8.7±0.3	9.3-8.2	9.9±0.5	11.0-9.3	13.2±3.2	19.0-8.0
Pelagic	*Alepes melanoptera* (Swainson, 1839)	8 (3+5)	15.4±0.8	17.1-14.4	17.2±0.8	18.2-16.0	57.1±8.5	73.0-49.0
	Alepes vari (Cuvier, 1833)	3 (0+3)	17.2±0.3	17.4-16.9	19.0±0.8	19.6-18.1	83.7±4.0	88.0-80.0
	Anodontostoma chacunda (Hamilton, 1822)	14 (8+6)	11.0±1.4	13.2-9.3	13.1±1.6	16.0-11.1	32.6±13.6	59.0-17.0
	Johnius borneensis (Bleeker, 1851)	3 (3+0)	-	-	12.7±0.3	12.9-12.4	24.7±1.5	26.0-23.0
	Johnius carouna (Cuvier, 1830)	20 (9+11)	-	-	16.1±3.1	22.7-12.0	53.9±29.4	129.0-15.0
	Opisthopterus tardoore (Cuvier, 1829)	3 (0+3)	10.3±0.4	10.7-10.0	11.7±0.4	12.1-11.4	11.0±2.6	14.0-9.0
	Rastrelliger brachysoma (Bleeker, 1851)	3 (0+3)	15.2±0.3	15.5-14.9	17.3±0.4	17.6-16.9	43.3±2.1	45.0-41.0
	Sardinella gibbosa (Bleeker, 1849)	3 (3+0)	13.9±1.1	14.6-12.6	15.6±1.1	16.3-14.3	32.7±7.5	37.0-24.0
	Sardinella jussieu (Lacepède, 1803)	8 (4+4)	10.8±0.6	11.5-10.0	12.7±0.7	13.7-12.1	22.5±4.3	32.0-19.0
	Scomberomorus commerson (Lacepède, 1800)	4 (1+3)	21.1±3.7	26.4-18.6	23.5±4.3	29.8-20.5	98.3±56.4	182.0-61.0
	Scomberomorus guttatus (Bloch & Schneider, 1801)	5 (5+0)	17.0±0.6	17.9-16.3	19.9±0.8	21.1-19.0	52.4±6.1	62.0-47.0
Reef-associated	*Alepes kleinii* (Bloch, 1793)	4 (1+3)	12.4±0.6	12.8-11.5	14.0±0.5	14.3-13.3	29.5±3.1	32.0-25.0
	Drepane longimana (Bloch & Schneider, 1801)	3 (1+2)	-	-	15.6±0.8	16.3-14.7	116.7±14.7	133.0-105.0
	Megalaspis cordyla (Linnaeus, 1758)	29 (25+4)	16.3±1.1	18.3-13.8	18.0±1.4	20.7-15.3	53.7±12.3	77.0-36.0
	Sardinella albella (Valenciennes, 1847)	14 (6+8)	12.5±1.2,	13.8-10.2	14.2±1.3	15.5-11.6	30.9±7.4	40.0-19.0
	Scomberoides tala (Cuvier, 1832)	3 (1+2)	16.2±1.5	17.1-14.4	18.0±2.4	19.4-15.2	51.0±17.3	62.0-31.0
	Scomberoides tol (Cuvier, 1832)	3 (0+3)	15.2±0.3	15.5-14.9	16.9±0.7	17.6-16.2	47.3±3.1	50.0-44.0
	Terapon theraps (Cuvier, 1829)	5 (4+1)	13.4±0.9	14.8-12.4	14.1±0.9	15.4-13.2	49.6±13.5	73.0-40.0
	Total	165 (86+79)						

n = total number of sample, m = male, f = female, FL = fork length, TL = total length, Wt. = weight, ±SD = ± standard deviation

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Table 2 shows the number of individual fish stomachs examined, the number of individuals from each group with anthropogenic debris, the average number of individual pieces of debris per stomach of fishes (including individuals with no debris), the range of individual pieces of debris per stomach of fishes in each group together with the information on frequency of occurrence. Average and frequency (%) of occurrence of plastic particles in the stomachs of different types of fishes have been shown in *Figure 3.*

Table 2. *The average number and range of individual pieces of debris per stomach with frequency of occurrence*

Fish species	Stomach examined	Stomach with debris	Number of pieces of debris/stomach (average (±SD), Range	Frequency (%)
Demersal fish species (n = 6)				
Alepes apercna	3	2	2.0 (±2.0), 0-4	67
Dasyatis zugei	3	1	0.3 (±0.6), 0-1	33
Dendrophysa russellii	3	1	0.3 (±0.5), 0-1	33
Leiognathus berbis	8	4	0.9 (±1.0), 0-1	50
Leiognathus fasciatus	3	1	1.3 (±2.3), 0-4	33
Leiognathus splendens	10	6	1.0 (±1.1), 0-3	60
Pelagic fish species (n = 11)				
Alepes melanoptera	8	6	1.3 (±1.0), 0-3	75
Alepes vari	3	2	1.7 (±1.5), 0-3	67
Anodontostoma chacunda	14	8	2.0 (±4.0), 0-15	57
Johnius borneensis	3	2	1.0 (±1.0), 0-2	67
Johnius carouna	20	17	3.8 (±3.2), 0-13	85
Opisthopterus tardoore	3	1	2.0 (±3.5),0-6	33
Rastrelliger brachysoma	3	1	1.0 (±1.7), 0-3	33
Sardinella gibbosa	3	1	0.3 (±0.6), 0-1	33
Sardinella jussieu	8	6	1.3 (±1.3), 0-4	75
Scomberomorus commerson	4	4	4.3 (±1.0), 3-5	100
Scomberomorus guttatus	5	3	0.6 (±0.5), 0-1	60
Reef-associated fish species (n = 7)				
Alepes kleinii	4	2	0.8 (±1.0), 0-2	50
Drepane longimana	3	1	0.3 (±0.6), 0-1	33
Megalaspis cordyla	29	23	1.6 (±1.2), 0-5	79
Sardinella albella	14	12	2.3 (±1.8), 0-5	86
Scomberoides tala	3	2	0.7 (±0.6), 0-2	67
Scomberoides tol	3	2	2.2 (±2.2), 0-4	67
Terapon theraps	5	2	0.8 (±1.3), 0-3	40
Total	165	110 (66.67% of total fish)		

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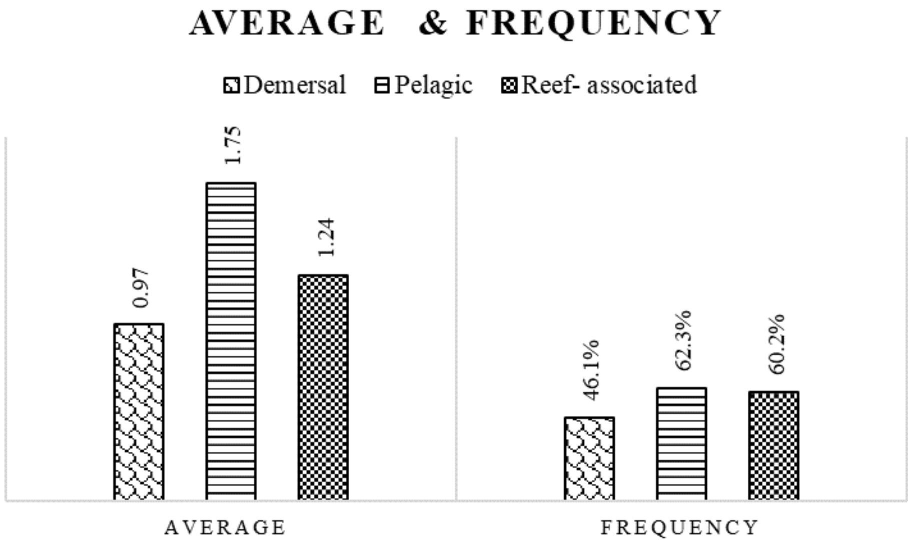
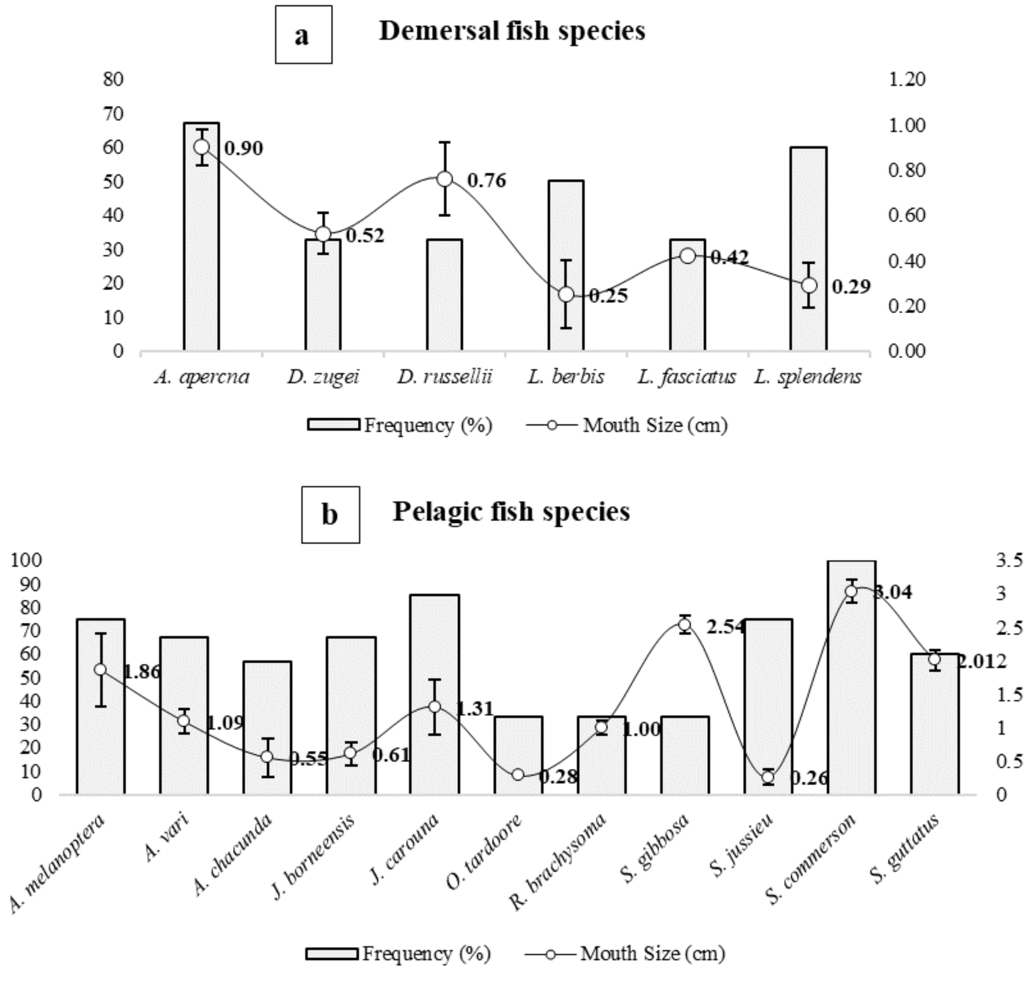


Figure 3. *Average (number of particles/ stomach) and frequency (%) of occurrence of plastic particles in stomach of different types of fishes*

In *Figure 4a-c*, comparison between mouth size of individual fish species and frequency of occurrence of plastic particles in stomach was shown.



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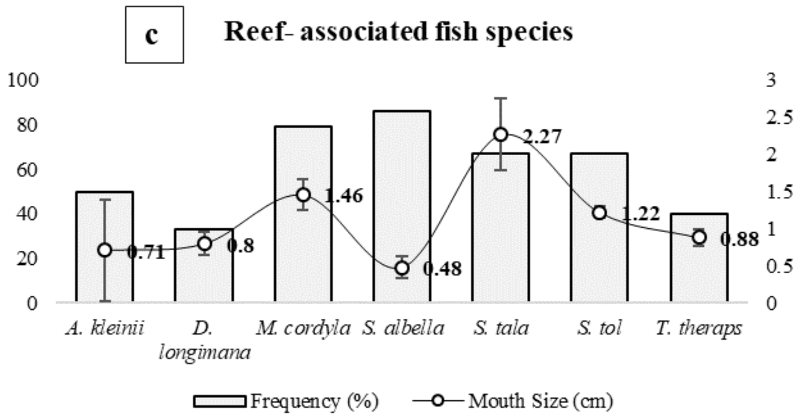

Figure 4. *Relevance between frequency (%) of occurrence of plastic particle in stomach and mouth size (cm) of individual fish species. Error bars represent standard deviations*

Plastic particles found in fish stomach had different shapes and colors; Transparent colored plastics were the most dominant color present in the stomachs of examined fishes. Colors, types and size of plastic items found in the stomach contents of fishes were briefly described below (*Table 3*). Color ranking of plastic particles found in the stomach content of fishes have also been shown in *Figure 5*.

Table 3. *Colors, types and size of plastic particles found in the stomach contents of different fishes*

Fish species	**Details of plastic particles**			
	Length range (mm)	**Width range (mm)**	**Color**	**Type**
Demersal fish species (n = 6)				
Alepes apercna	0.82 – 4.76	0.03 – 0.40	Transparent, Blue	Fibre, Fragment
Dasyatis zugei	16.67	0.16	Green	Fibre
Dendrophysa russellii	1.34	0.02	Blue	Fibre
Leiognathus berbis	0.53 – 6.54	0.02 – 0.23	Transparent, Blue, Black	Fibre
Leiognathus fasciatus	1.60 – 3.29	0.02 – 1.33	Transparent, Red, Blue	Fibre, Fragment
Leiognathus splendens	0.62 – 7.41	0.02 – 0.82	Transparent, Blue, Black	Fibre, Fragment
Pelagic fish species (n = 11)				
Alepes melanoptera	0.52 – 4.83	0.02 – 0.51	Transparent, Green, Black, Red	Fibre, Fragment
Alepes vari	2.75 – 10.55	0.05 – 0.11	Transparent, Yellow, Black, Brown	Fibre,
Anodontostoma chacunda	0.63 – 16.36	0.02 – 2.42	Transparent, Blue, Red, Black, Brown	Fibre, Fragment
Johnius borneensis	2.28 – 10.55	0.03 – 0.04	Brown, Blue	Fibre
Johnius carouna	0.35 – 17.16	0.01 – 1.94	Transparent, Blue, Brown, Black, Red, Green	Fibre, Fragment
Opisthopterus tardoore	1.18 – 8.83	0.01 – 0.04	Blue, Black	Fibre
Rastrelliger brachysoma	2.46 – 5.27	0.04 – 0.40	Transparent	Fibre
Sardinella gibbosa	0.73	0.61	Transparent	Fragment

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Sardinella jussieu	0.75 – 5.07	0.04 – 0.96	Transparent, Blue, Black	Fibre, Fragment
Scomberomorus commerson	0.54 – 8.70	0.02 – 0.47	Transparent, Black, Yellow, Red	Fibre, Fragment
Scomberomorus guttatus	1.53 – 2.88	0.03 – 0.04	Transparent, Black	Fibre
Reef-associated fish species (n = 7)				
Alepes kleinii	0.87 – 4.41	0.06 – 2.10	Transparent, Black	Fibre, Fragment
Drepane longimana	2.26	0.05	Black	Fibre
Megalaspis cordyla	0.13 – 15.23	0.01 – 1.74	Transparent, Blue, Red, Black, Brown	Fibre, Fragment
Sardinella albella	1.29 – 8.78	0.01 – 0.81	Transparent, Blue, Red, Black, Brown	Fibre, Fragment
Scomberoides tala	0.83 – 5.94	0.05 – 0.68	Transparent, Black	Fibre, Fragment
Scomberoides tol	0.69 – 6.46	0.02 – 0.09	Transparent, Blue, Red	Fibre
Terapon theraps	1.36 – 3.55	0.02 – 0.30	Transparent, Black	Fibre, Fragment

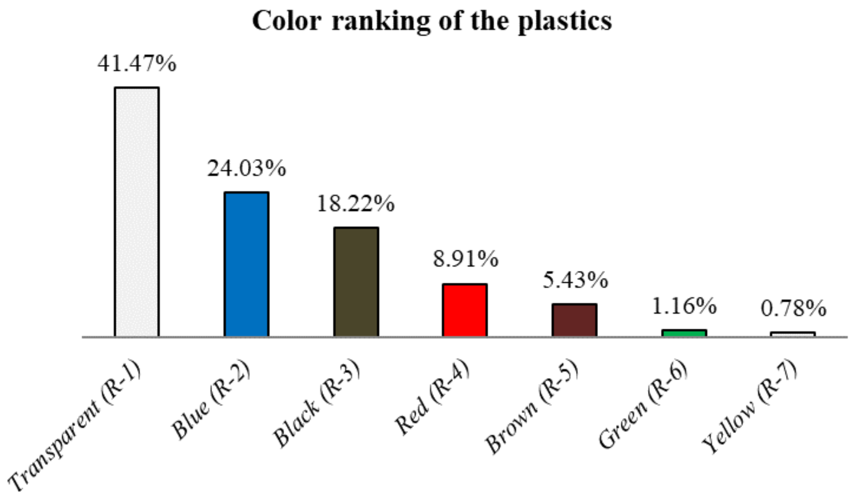
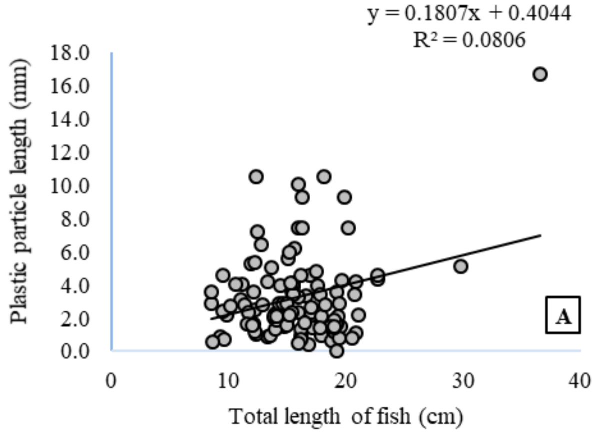


Figure 5. *Color ranking of plastic particles found in the stomach contents of fishes (R-1 = Rank-1)*

Relationship between size of plastic litters and different biological features of fishes are given in *Figure 6A-C*.



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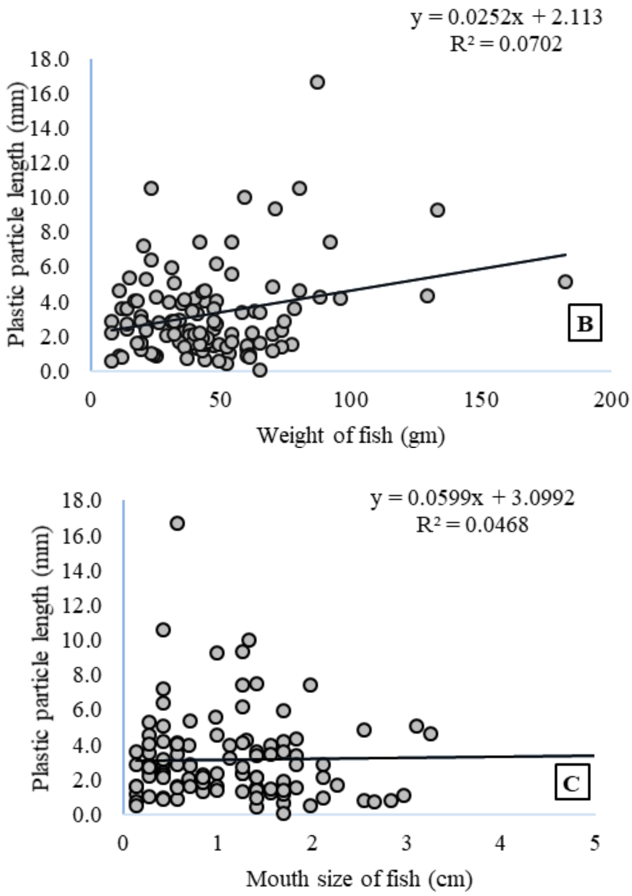

Figure 6. *Relationship between size of plastic fragments found in stomach contents and different biological features of fishes. (A) Plastic fragments length VS Total length of fish, (B) Plastic fragments length VS Weight of fish and (C) Plastic fragments length VS Mouth size of fish*

Plastic debris found in stomach contents of fishes differed in size in each group of fishes as reported in *Figure 7*. Microplastics which are less than 5 mm in size were the most abundant (79.52%) size group found during the research work (*Fig. 7*).

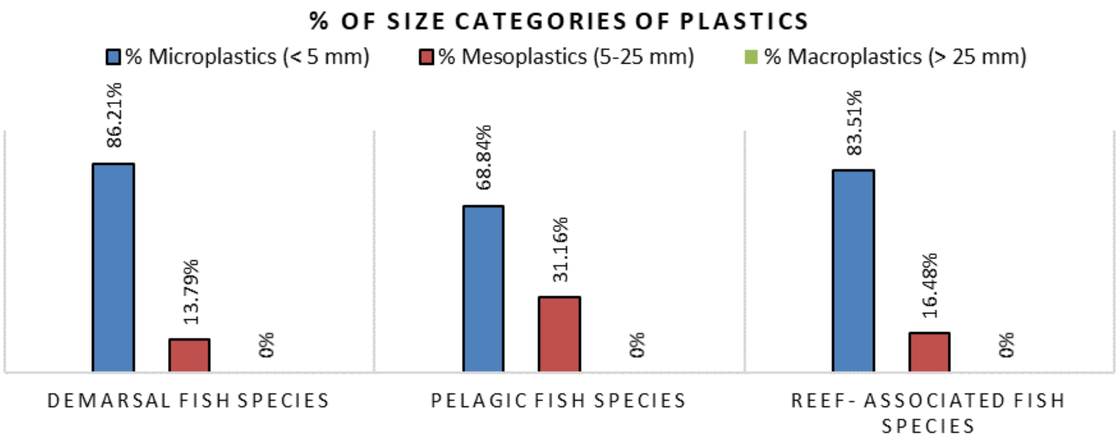

Figure 7. *Percentage of size categories of plastic in the stomach contents of each type of fish*

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In *Figure 8*, some illustrations of plastic debris found in the stomach contents of fishes are shown.

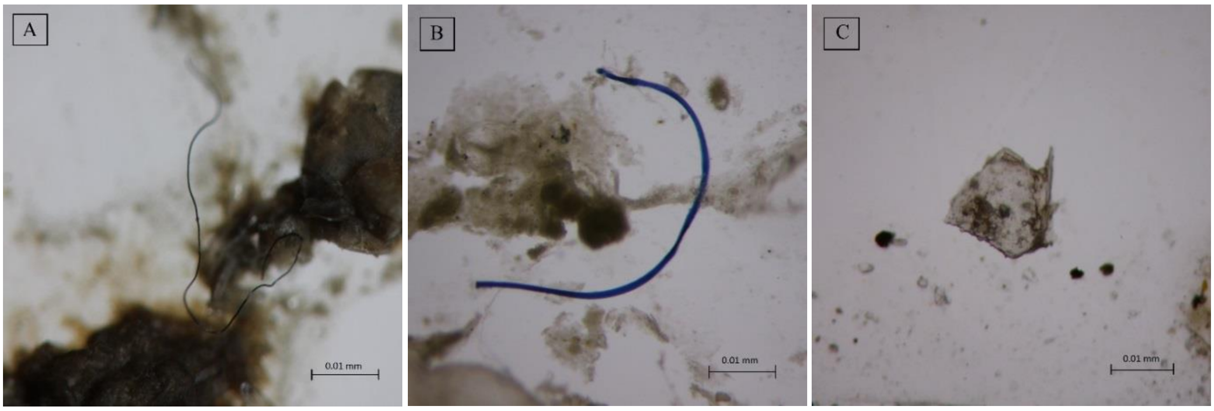

Figure 8. *Photographs of plastic debris found in the stomach content of fishes. Net fibre (A, B) and plastic fragment (C)*

Discussion

Recent investigations (Fossi et al., 2012) revealed data on the effect of microplastics on massive filter-feeding creatures such as baleen whales and sharks in the Mediterranean sea, which can possibly devour microplastic offal. No data has been reported on microplastic ingestion by fishes from lower Southern Thailand. This study provided the first published record of plastic polymers in the stomach contents of some commercially important fishes from the lower Gulf of Thailand (*Fig. 1*). Size and weight of the examined fishes ranges from 8.5 to 37.1 cm and 8 to 133 g. respectively (*Table 1*). Of the 165 fish stomachs examined, 66.67% contained plastic items. Particularly, this consisted of 15 demersal individual fishes (50%), 51 pelagic individual fishes (68.92%) and 44 reefs- associated individual fishes (72.13%) containing some kinds of plastic matters (*Table 2*). The average occurrence of plastic particles in the pelagic fish (1.75 particles/ stomach) was higher than that of demarsal fish (0.97 particles/ stomach) by approximately two times (*Fig. 3*). This probably implied that the plastic contamination in the study area was possibly related to the density of the plastic particles. HDPE (high density polyethylene), LDPE (low density polyethylene), and PP (polypropylene) which make up containers and plastic bags for example do float, as their density is less than that water. Higher density plastics such as PET (polyethylene terephthalate), PVC (polyvinyl chloride), and PS (polystyrene solid), do sink. The second probable explanation was that the pelagic fishes in the study area had more herbivorous (plankton feeding) fishes than carnivorous fishes as compared to the demarsal fishes and therefore was responsible for the higher occurrence of plastic pieces in the pelagic fishes than that of the demersal ones.

Since this is the first study on plastic ingestion by fishes from the lower Gulf of Thailand, there is no other study in Thailand to compare with. However, there were some other findings from different places around the world. Possatto et al. (2011) who perceptibly recorded ingested plastic from fishing nets in 23% of the three species of catfish (*C. spixii, C. agassizii and S. herzbergii)* found in the estuary of River Goiás in Northeast Brazil, but lower in comparison with a study by Boerger et al. (2010), who visually acknowledged plastic in 35% of 670 individuals of five mesopelagic and one

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epipelagic fish species caught with a manta trawl (0.33 µm mesh size) in the North Pacific Gyre. Furthermore, Lusher et al. (2013), who informed 36.5% of the 504 gastrointestinal tracts from 10 species of fish from the English Channel to have plastic contents. Since Thailand was one of the five countries who dumped more plastic (60%) into the oceans than the rest of the world combined (GlobalPost, 2016), the result from the current study showed alarmingly high percentage (66.67%) of plastic ingestion by fishes.

This current investigation indicated that the pelagic fish species had more plastic substances in their gut content in average, which was 1.75 plastic litter/stomach (*Fig. 3*). Contrarily, demersal fish species had less plastic items (0.97 plastic litter/ stomach) than other classes of fishes (*Fig. 3*). Besides, *Figure 2* represents the frequency (%) of occurrence of plastic particles in the stomach contents of different classes of fishes. Pelagic fish species showed the highest (62.3%) frequency of occurrence of plastic items, whereas, demersal fishes showed the lowest (46.1%) frequency of occurrence of plastic matters in the stomach contents of fishes (*Fig. 3*). Similar study was done by Romeo et al. (2015), who worked on large pelagic fishes (*Xiphias gladius, Thunnus thynnus* and *Thunnus alalunga*) in the Mediterranean Sea and found 18.18% of the fish stomach contained some kinds of plastic matters, which was lower than those of the current study. The ingestion of microplastics by mesopelagic fish was also reported in the Pacific Ocean (Boerger et al., 2010; Davison and Asch, 2011). Since most of plastic particles tend to float on the surface level of the water because of their density and structural behavior, pelagic fishes ingest the plastic particles erroneously as food. Sometimes filter-feeding fish species ingest plastic matters while filtering the water by their gill and gulping during lack of oxygen situation. On the other hand, reef-associated fish species showed around 60% frequency of occurrence (*Fig. 3*). In addition, reef-associate species are also threatened because of plastic pollution. Some plastic particles with comparatively high density settle down on the reef bed and are erroneously taken by reef-associated fishes. Plastic litters (macro to microplastics) can also be ingested through predation action, in particular, when predatory fish catch their small prey aggregated in schools. This kind of feeding comportment may enhance the feasibility of ingesting plastic debris together with the prey (Romeo et al., 2015).

In this current study, relevance between mouth size (cm) of the individual fish and frequency (%) of occurrence of plastic particle in stomach content was also been investigated (*Fig. 4*). Nevertheless, comprehensively there was no relativity found between frequency of occurrence of plastic matters and mouth size of individual fishes excluding some species such as *A. apercna, D. zugei, R. brachysoma, S. commerson, S. guttatus, D. longimana* and *S. tala.* These species indicated moderately positive relevance between frequency of occurrence and mouth size of fish (*Fig. 4*). Differently, *L. berbis, L. splendens, A. chacunda, J. borneensis, S. jussieu* and *S. albella* revealed comparatively negative relativity (*Fig. 4*). Since this is the inceptive search on this sort of relativity, there is no convenient investigation to correlate with.

The plastic items found in the stomach contents of the samples ranged from 0.13 mm to 17.16 mm in length and width ranged from 0.01-2.42 mm (*Table 3*). Though the plastic items obtained were either fibre type or fragment type, most of them were fibre from fish nets (*Table 3*). These plastic litters had different shapes and different colors. Transparent (41.47%) colored plastic items were the most dominant color of plastics found during this investigation (*Fig. 5*). Differently, Yellow (0.78%) colored plastics were the smallest group of plastic litters obtained in the current study (*Fig. 5*). Since

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transparent color is nearly illusive to see in the water, aquatic organisms especially fishes erroneously take this sort of plastic litters while gulping, filter-feeding and consuming food. Sometimes, fishes unintentionally ingest plastic litters as a live food as well. Size of the plastic items found in the gut contents were also been categorized. Microplastics (<5 mm) were the most abundant (79.52%) size group of plastic items obtained from the stomach content of fishes during this investigation and the rest of them (20.48%) were mesoplastics (5-25 mm) In particular, demersal and reef-associated fish species showed more or less similar amount of microplastics which are 86.21% and 83.51% respectively. Since the sizes of the fish individuals were small, there was no macroplastic (>25 mm) found during the investigation (*Fig. 6*). One of the recent study also showed the dominancy of transparent colored plastic items in the stomach contents of fishes done by Romeo et al. (2015), who also found microplastic as the most abundant size group of plastics in fish gut of albacore and bluefin tuna. Furthermore, there are a number of more scientists who also found microplastic particles in fish gastrointestinal tract (Lusher et al., 2013; Murphy et al., 2017; Phillips and Bonner, 2015; Tanaka and Takada, 2016). In the current investigation, for the first time, relevance between size of plastic litters and different biological features of fishes such as total length, weight and mouth size were investigated (*Fig. 6*). The scatterplots (*Fig. 6*) shows the points fall randomly on the plot, which indicates that there is no linear relationship between the variables ($p < 0.05$). It means that there has no discernable increasing or decreasing linear pattern in those graphs (*Fig. 6*). As expected, the sizes of microplastic particles (<5 mm) were so small until all sizes of fishes even the small ones with small mouths could swallow them. Big carnivorous fish could contaminate with microplastic by eating the microplastic contaminated herbivorous fish, whereas the big plankton feeding fish could intake microplastic particles by eating plankton mingled with microplastic debris. However, more detailed information and investigations are needed to determine this sort of relevance. Results of the current study emphasize the pervasive existence of plastics in the lower Gulf of Thailand. Moreover, the high frequencies of micro and mesoplastics in the marine fishes from the lower Gulf of Thailand represent a further warning signal for marine conservation as well as for the soundness of human health.

Conclusions and recommendations

The accession of the microplastics as well as other sized plastic litters in commercially important marine fishes suggestively reveals the anthropogenic stress on the fishery and marine food security in the lower Gulf of Thailand. Health perils are feasible when people consume these defiled marine organisms. These fundamental findings signify a vital initial phase in exploring some ecotoxicological aspects such as the possible effects associated to the transmission of contaminants on human health and the assessment of the existence and effect of plastic debris on other types of marine organisms. Moreover, effective management programs in the study area and contiguous areas for the plastic pollution are immediately required. Wherefore, linked to the high consumption of these species in the Gulf of Thailand, this topic necessitates profounder investigation in the futurity. In addition, it is definitely recommended that microplastic contamination in marine organisms and their food chain in other adjacent provinces should be explored to make sure the safety circumstances of environment and human health. Moreover, this is an elementary research work which need further in detail investigations.

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APPENDIX B

PUBLISHED MANUSCRIPT - 2

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Ingestion of microplastics by some commercial fishes in the lower Gulf of Thailand: a preliminary approach to ocean conservation

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Abstract Microplastics have been acknowledged as evolving marine contaminants of noteworthy apprehension, due to their ubiquity, persistence and toxic potentiality. It is very urgent and important to study about microplastic pollution not only in Thailand but also for the world because of its harmful effects on marine biota as well as for human health. The study focused on the presence of plastic debris in the stomach contents of some economically important fish caught in the lower Gulf of Thailand between January to April 2018. Size and weight range of the samples were 7.6 to 21.9 cm and 4 to 99 gm. Results highlighted the ingestion of plastics in the 54.29% samples. The ingested plastics were microplastics (27.27%; <5 mm), mesoplastics (69.88%; 5-25 mm) and macroplastic, (2.85%; >25 mm). Fibres were the major forms of plastics found during this study. These preliminary findings underlined the ubiquitous presence of microplastics in the lower Gulf of Thailand marine biota, as well as the water column where pelagic fish live, and feed and it also representd an urgency to reduce the use of plastics or to ensure the proper recycling it.

Keywords: Microplastics, marine litter, plastic ingestion, plastic debris, marine pollution, ocean conservancy

Introduction

Plastic pollution is the gathering of plastic substances in the environments which have several hostile effects on wildlife, wildlife habitat as well as on human beings (Moore, 2017; Parker, 2018). Plastic production by human are high because of its durability, light weight, attractive appearance and low cost (Hester and Harrison, 2011; Thompson *et al.*, 2004; Jambeck *et al.*,

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2015). As of 2018, approximately 380 million tonnes of plastic are manufactured worldwide every year. From the 1950s up to 2018, an estimated 6.3 billion tonnes of plastic has been produced globally, of which an estimated 12% has been ignited and another 9% has been recycled. The rest has been abandoned in landfills or the natural environment (The Economist, 2018).

Plastic contamination can distress land, waterways and ocean. Several living organisms, mostly marine organisms, can be affected either through disclosure to chemical toxicants within plastics that intervene with their physiology or by mechanical effects, for example, harms related to ingestion of plastic rubbish or entanglement in plastic substances. Above 660 marine species were known to be oppressed worldwide by plastic debris directly or indirectly (Dias and Lovejoy, 2012). Nearly 92% of all adverse encounters between marine litter and organisms occurs because of plastic waste (Gall and Thompson, 2015).

According to several studies, plastics that act as pollutants are categorized into micro-, meso-, or macro-plastics, based on size (Hammer *et al.*, 2012; Browne, 2010; Fendall and Sewell, 2009). Following to a recent investigation, size range of microplastics are <5 mm to 0.1 µm (Lippiatt *et al.*, 2013). Plastic matters gradually breakdown into minor trashes due to oxidation, ultraviolet (UV) radiation and mechanical forces, which is lower than 5 mm in diameter, termed microplastic (Barnes *et al.*, 2009; Cole *et al.*, 2011; Lippiatt *et al.*, 2013). Microplastics are extensively distributed, in deep sea sediments and surface water (Song *et al.*, 2015; Woodall *et al.*, 2014), from lakes to open sea water (Eriksen *et al.*, 2014; Imhof *et al.*, 2013), and in numerous marine organisms through the trophic levels (Boerger *et al.*, 2010; De Witte *et al.*, 2014; Murray and Cowie, 2011; Van Cauwenberghe and Janssen, 2014; Van Franeker *et al.*, 2011). Several studies focused on the induction of plastic and other anthropogenic rubbish in marine domiciles and food web through ingestion by varied marine organisms, ranging from zooplankton to vertex predators (Fossi *et al.*, 2012, 2014; Ivar Do Sul and Costa, 2014). The zones with convergence currents and where anthropogenic debris was accumulated, the consequence of debris assimilation by marine wildlife was more obvious (Moore *et al.*, 2001).

The influence of microplastic assimilation on diverse marine entities with miscellaneous feeding mechanisms has been studied in different parts of the world (Thompson *et al.*, 2004; Leslie *et al.*, 2013; De Witte *et al.*, 2014). Furthermore, these earlier investigations indicated that the eco-toxicological situations of certain species were associated to the environmental stress levels in their territories (Nayar *et al.*, 2004). Nevertheless, there was no investigation performed in Southern Thailand.

According to prior explorations, striking extents of plastics accumulated into the marine environment and coastline ecosystems were predominantly from Asian countries including Thailand which had soundly extraordinary economic progress rates (Jambeck *et al.*, 2015). Moreover, Plastics have also been acknowledged as one of vital constituent in Municipal Solid Waste (MSW) composition of Thailand (Kaosol, 2009; Chiemchaisri *et al.*, 2006). In this manner, land based plastics can be the primary source of plastic pollution in coastal water (Jambeck *et al.*, 2015). Furthermore, Thailand is one of the five countries which dump more plastic (60%) into the oceans than the rest of the world combined and the other countries are China, Indonesia, the Philippines and Vietnam (GlobalPost, 2016). Even so, there was no investigation done on microplastic pollution in marine fish, especially, in the lower Gulf of Thailand.

Sathing Phra District, located in the northern part of Songkhla Province in the lower Gulf of Thailand, is one of the most rapid industrialized development areas in Songkhla Province. Thus, these quick expansions of man-made activities pose a possible threat of plastic pollution in this and neighboring areas. That's why this investigation have certain noteworthy significance in terms of knowing the extent of plastic pollution in these areas and resolving it.

The objective of the study was to investigate occurrence, frequency, amount, and forms of plastics ingested by some commercial and abundant fishes in the lower Gulf of Thailand: Panna croaker *Panna microdon* (Bleeker, 1849), Goatee croaker *Dendrophysa russelli* (Cuvier, 1829), Sharpnose hammer croaker *Johnius borneensis* (Bleeker, 1851) and Weber's croaker *Johnius weberi* (Hardenberg, 1936). Considering the hazard associated to the plastic pollution, this study provides an imperative involvement to the knowledge and understanding of plastic occurrence in these commercial fishes.

Materials and methods

Species selection and sampling site

A total of 27 Panna croaker (*Panna microdon*), 41 Goatee croaker (*Dendrophysa russelli*), 30 Sharpnose hammer croaker (*Johnius borneensis*) and 7 Weber's croaker (*Johnius weberi*) were collected during January to May, 2018 from Sathing Phra District, Songkhla Province in the Lower Gulf of Thailand (Figure 1). These four diverse species (2 demersal and 2 pelagic) belonging to the family Sciaenidae were preferred because of their abundance and commercial significance and the study site represents the coast with different anthropogenic activities.

Figure 1. Geographical location of the study site in Songkhla Province, the Lower Gulf of Thailand

Sampling and species identification

Immediately after collection of fishes, certain details of the samples and the sampling site were noted. Then the samples were taken to the laboratory in an icebox with sufficient ice in it and preserved at -20 ℃ for further analysis purpose.

For species identification, particular information (trophic level and sex) were assigned with the help of fishermen and then verified according to the standard taxonomic keys of Talwar and Jhingran (1991); Froese and pauly (2018), SEAFDEC (2014).

Investigative techniques and avoidance of adulteration

In the laboratory, at first, each fish was thawed gradually and cleaned by filtered water to dispel sediments and impurities from the extraneous veil. Then, specimens were measured (total length) and weighed (total body weight). Each fish was dissected from the upper part to the oesophagus to remove the stomach according to the methods published elsewhere (Claessens *et al*., 2013; Lusher *et* *al.*, 2013; Rocha-Santos and Duarte, 2015). Gut contents were then distinctly placed inside petri dishes and examined in order to distinguish plastic debris, which were counted, assembled by color, measured by the Stereo Zoom Microscope (OLYMPUS SZ2-ILST). The ingested plastics were categorized as microplastics (<5 mm), mesoplastics (5-25 mm) and macroplastics (>25 mm) following the method of Galgani *et al.* (2013). To determine the length of each particle of debris, all photographed were digitally measured using the software package ImageJ 1.4.3.6 (public domain).

Conspicuous protection was taken to prevent sample contamination throughout the whole investigation such as during dissection, extraction, sorting and visual identification. This technique includes several stages to avoid technical contamination, cross- contamination and/or misidentification of natural debris (e.g., shells, algae, and coral) as plastic debris.

Statistical analysis

The frequency of plastic debris occurrence (*F%*) in these fish samples was estimated by the proportion of the examined individuals where plastic debris were present in the stomach contents. All data analysis was accomplished by Microsoft excel for mean, minimum and maximum. One-way ANOVA was performed to compare results among the groups. Differences at $p<0.05$ were considered statistically significant.

Results

Entirely, 105 discrete fishes (68 demersal and 37 pelagic individuals) were perused for the presence of plastic debris throughout this investigation. Among them, 57 individuals (54.29%) have plastic debris in their stomach in different size and shape (Table 1). In detail, some sorts of ingested plastic debris present in the 35 individual demersal fishes (51.47%) and 22 individual pelagic fishes (59.46%) were found (Table 2).

The mean values and range of total length, body weight and stomach weight together with the information on trophic level and sex ratio of examined fishes were exhibited in Table 1.

The average number of plastic debris per stomach of fishes including the range together with the information on stomach containing different amounts of plastic debris was found. In particular, *Johnius borneensis* shows the highest (60.00%) and *Panna microdon* shows the lowest (44.44%) frequency of occurrence of plastic debris in the stomach content of fishes

(Table 2). Average number of plastic debris per stomach and per g of stomach were briefly presented in Figure 2.

Plastic debris had different shapes and colors; transparent, black and pink plastic debris present in all the fish examined species in this exploration. Several circumstantial information on plastic debris found in the stomach content were presented in Table 3. Entirely, pelagic fish species shows longer (9.93 mm) plastic debris than that of the demersal (8.59 mm) ones (Figure 3).

Color and form of plastic debris found in the stomach content of the examined fishes were presented in Figure 4. Transparent debris (35%) were found the most common whereas the green (2%) were the least common plastic debris found in the stomach content of the fishes (Figure 4). Furthermore, fibre type plastics (84%) were the most dominant form of plastic debris found throughout this investigation (Figure 4).

The plastic debris were different in size in each species of fishes as reported in Figure 5. Mesoplastics were the most abundant (69.88%) size group found and about 28% of all the plastic debris found were microplastics which were less than 5 mm in diameter (Figure 5). Percentages of plastic debris according to their size group were indicated in Figure 5.

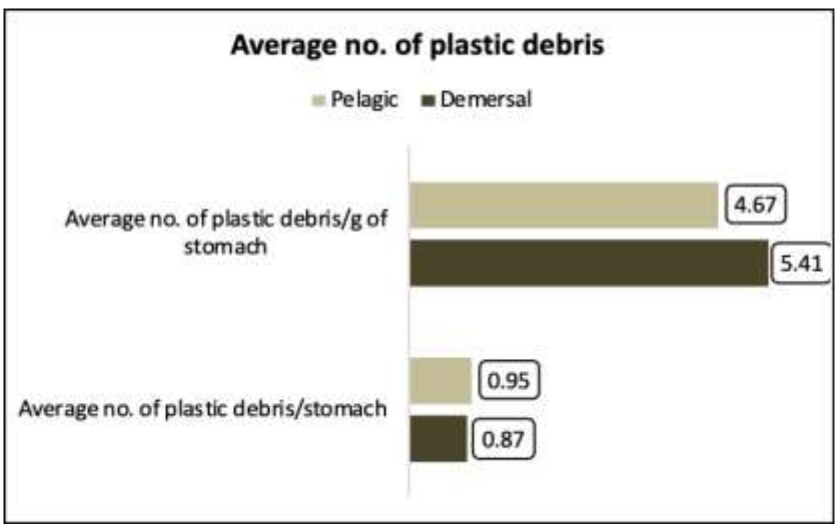

Figure 2. Average number of plastic debris per stomach and per g of stomach of different group of fishes

Table 1. Mean and range of total length, body weight and stomach weight for each fish species with their trophic level

Fish species	Trophic level	Sample (M:F)	Total length (cm)		Body weight (g)		Stomach weight (g)	
			Mean ±SD	Range	Mean ±SD	Range	Mean ±SD	Range
Panna microdon (Bleeker, 1849)	Demersal	27 (24:3)	13.20 ±4.34	21.9 - 7.6	28.04 ±25.98	99.0 – 4.0	0.16 ±0.10	0.57 – 0.03
Dendrophysa russelli (Cuvier, 1829)		41 (9:32)	13.56 ±0.92	15.0 – 11.7	32.90 ±8.53	52.0 – 18.0	0.16 ±0.06	0.3 – 0.05
Johnius borneensis (Bleeker, 1851)	Pelagic	30 (25:5)	13.86 ±1.59	16.4 – 10.9	34.33 ±14.36	65.0 – 14.0	0.27 ±0.21	0.91 – 0.02
Johnius weberi (Hardenberg, 1936)		7 (7:0)	16.30 ±1.56	18.4 – 14.2	42.29 ±13.24	63.0 – 28.0	0.19 ±0.08	0.38 – 0.14
Total		105 (65:40)						

M= Male, F= Female, SD= Standard deviation

Table 2. Average No. of plastic debris found in the stomach of the examined fishes

Trophic level	Fish species	No. of stomach examined	No. of the stomach with plastic debris	No. of pieces of plastic debris/stomach avearge ±SD, range	Frequency of occurrence (%)	
Demersal	*Panna microdon* (Bleeker, 1849)	27	12	0.85 ±1.06, 3 – 0	44.44	51.47
	Dendrophysa russelli (Cuvier, 1829)	41	23	0.88 ±1.12, 5 – 0	56.10	
Pelagic	*Johnius borneensis* (Bleeker, 1851)	30	18	0.90 ±0.88, 3 – 0	60.00	59.46
	Johnius weberi (Hardenberg, 1936)	7	4	1.14 ±1.21, 3 – 0	57.14	

Table 3. Length, width, color and form of plastic debris found in the stomach content of fishes

Trophic level	Fish species	Detail info of plastic debris found in the stomach of fishes			
		Length range (mm) (Mean ±SD)	**Width range (mm) (Mean ±SD)**	**Color**	**Form of plastic debris**
Demersal	*Panna microdon* (Bleeker, 1849)	2.08 – 23.48 (8.50 ±5.56)	0.04 – 1.71 (0.27 ±0.46)	Transparent, Blue, Brown, Black, Pink, Violet, Green	Fibre, Fragment
	Dendrophysa russelli (Cuvier, 1829)	1.46 – 20.99 (8.64 ±5.00)	0.04 – 3.85 (0.54 ±1.02)	Transparent, Black, Blue Green, Pink, Violet	Fibre, Fragment
Pelagic	*Johnius borneensis* (Bleeker, 1851)	1.23 – 38.22 (10.02 ±8.86)	0.06 – 2.72 (.36 ±0.64)	Transparent, Black, Pink, Red, Violet, Blue, Brown	Fibre, Fragment
	Johnius weberi (Hardenberg, 1936)	2.12 – 16.75 (9.60 ±4.99)	0.06 – 0.62 (0.18 ±0.18)	Transparent, Black, Brown, Pink	Fibre, Fragment

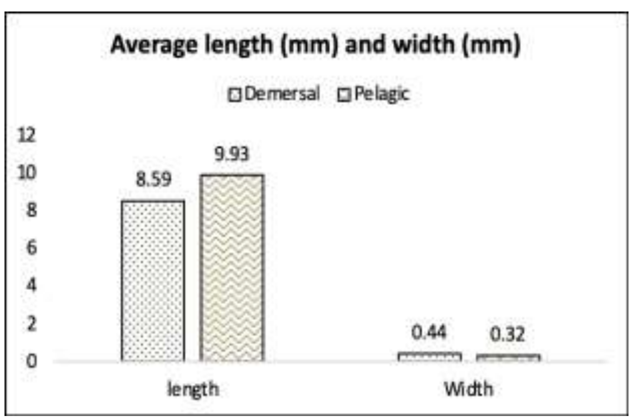

Figure 3. Average length (mm) and width (mm) of plastic debris in the stomach content of different groups of fishes

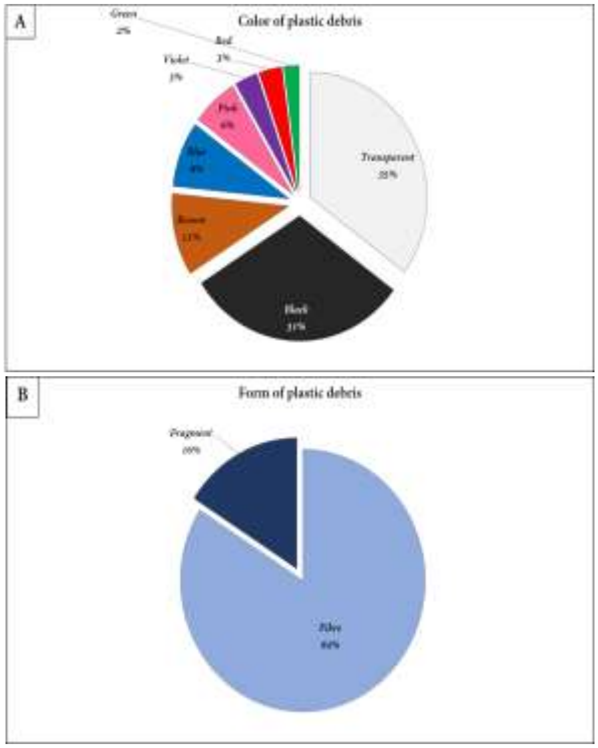

Figure 4. Percentages (%) of color and form of plastic debris found in the stomach of fishes. (A) % of color groups, (B) % of form of plastic debris

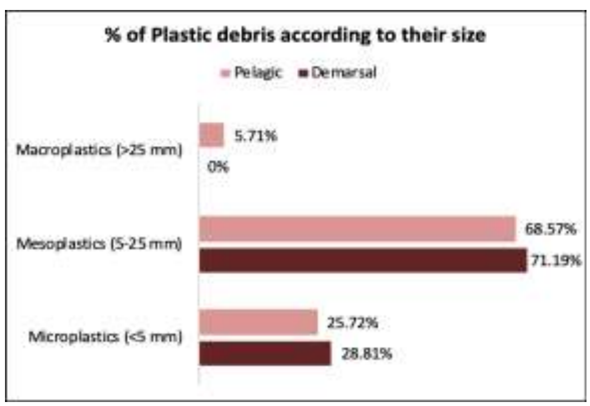

Figure 5. Percentages (%) of plastic debris according to their size group

Different colors (transparent, black, blue and green) and forms (fibre and fragment) of plastic debris found throughout the research were presented in Figure 6.

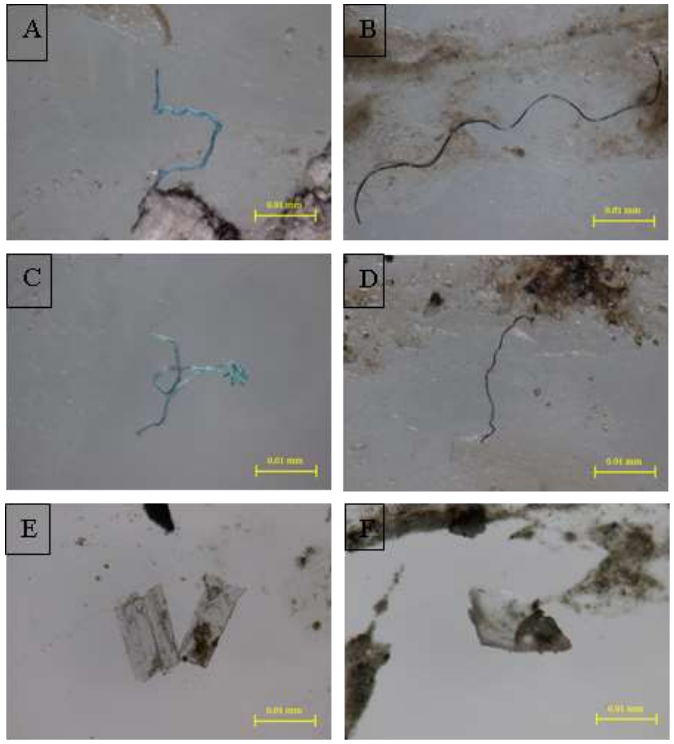

Figure 6. Photographs of fibre (A-D) and fragment (E-F) types of plastic debris found in the stomach contents of fishes under stereo zoom microscope

Discussion

This present enquiry publicized various vital evidence on plastic debris together with the data on frequency of occurrence, amount, forms of plastic debris and specific brief info on the plastic debris found in the gastrointestinal contents of some commercial marine fishes from the lower Gulf of Thailand (Figure 1). Previously, there were very few studies on plastic pollution in Thailand (Thushari *et al.*, 2017), especially there wasn't any investigation done on fishes in lower Gulf of Thailand. Recent studies (Fossi *et al.*, 2012) shown data on the effects of microplastic on huge filter-feeding individuals such as baleen whales and sharks in the Mediterranean Sea, which could probably gulp microplastic punk. Total length and body weight of the examined fishes range from 7.6 to 21.9 cm and 4 to 99 g. Stomach weight of the samples range from 0.03 to 91 g (Table 1). Out of 105 investigated fish stomachs, 57 (54.29%) stomachs contained plastic debris (Table 2). In particular, this involved of 35 individual demersal fishes (51.47%, out of 68 individuals) and 22 individual pelagic fishes (59.46%, out of 37 individuals). The average frequency of occurrence (%) for demersal fish species was 51.47%, which was lower than the average frequency of occurrence of the observed pelagic fishes (59.46%)(Table 2). As stated in Figure 2, the average number of plastic debris per g of stomach for demersal fishes was 5.41, which was slightly higher than that of the pelagic species (4.67). Contrariwise, pelagic fish species showed higher (0.95) average number of plastic debris per stomach than that of the demersal ones (0.87). Therefore, it was obvious that the pelagic fish species possessed more plastic debris than the demersal fishes. This may be because of the luxuriant presence of plastic debris in the surface level of marine water bodies. Meanwhile most of the plastic debris tend to levitate on the surface level of the water because of their solidity and structure behavior, pelagic fishes gulp the plastic debris mistakenly as food. According to several studies (WWF, 2018), 80% of plastic in our ocean is from land sources and it can come to our ocean in three main ways such as throwing plastic in the bin when it could be recycled, littering and products that go down the drain. Once in the ocean, plastic stays in the surface level of the water bodies for certain period of times, breaks down into tiny pieces and then travels to other trophic level of the water bodies such as middle level and finally to the bottom level. This can be one of the vital reasons behind the copiousness of plastic debris in the stomach contents of pelagic fishes. Since the present study is one of the preliminary studies on plastic ingestion by fishes from the lower Gulf of Thailand, there isn't any other study in Thailand to associate with. Comparable investigation was performed by Romeo *et al.* (2015), who worked on the existence of plastic rubbish in the stomach of 3 large pelagic fishes (*Xiphias gladius, Thunnus thynnus and Thunnus alalunga*) in the Mediterranean Sea and 18.18% of the investigated fish stomach contained certain types of plastic offal, which was inferior than those of the current study.

The length of the plastic offal found in the stomach contents of the examined fishes ranged from 1.46 to 23.48 mm and 1.23 to 38.22 mm in terms of demersal and pelagic fishes, respectively. Moreover, for demersal ones, width ranged from 0.04 to 3.85 mm and 0.06 to 2.72 mm for pelagic species (Table 3). On average, highest length (9.93 mm) of plastic debris was reported in pelagic species (Figure 3). In particular, *Johnius borneensis* showed the longest plastic debris in their stomach content, which was 38.22 mm in length (Table 3).

Though the plastic debris obtained from the stomach contents were either fibre or fragment type, most of them (84%) were fibre type (Figure 4). Meanwhile, Plastic debris found in the stomach of the examined fishes had different shapes and color. Transparent, black and pink colored plastic debris were found in all the groups of fishes. Among all the plastic debris found, transparent (35%) colored plastic debris was the most abundant one and black color (31%) was the neighboring one (Figure 4). Oppositely, Green colored (2%) plastic debris was the least common one found in the stomach content of the examined fishes (Figure 4). Since transparent color is almost impracticable to ascertain in the water, aquatic organisms mainly fishes erroneously consume this sort of plastic offal while filter-feeding ones, gulp and consume it as their food. Even sometimes, fishes inadvertently ingest plastic debris as a live foodstuff as well. Similar investigation was done by Romeo *et al.* (2015), who reported various color of plastic rubbish such as transparent, white, blue and so on. The plastic debris found in that study ranged from 0.63 to 164.50 mm in length and 0.69 to 60.57 mm in width from the stomach contents of pelagic fishes.

Size of the plastic debris found in the stomach contents of the fishes were also been characterized. Mesoplastics (69.88%, 5-25mm) were the most abundant size group of plastic debris obtained throughout the investigation, which is higher than the amount of microplastics (27.27%, <5 mm) found by approximately two times (Figure 5). Rest of the plastic debris found were macroplastics (2.85%, >25 mm). In particular, 25.72% and 28.81% of microplastic debris were obtained from pelagic and demersal fish species, respectively. Contrariwise, pelagic and demersal species showed 68.57% and 71.19% mesoplastics respectively in their stomach contents (Figure 5). Only 5.71% macroplastic was found from the pelagic fish throughout the investigation, while no macroplastics were obtained from demersal fishes. Furthermore, there was a number of more scientists who also obtained microplastic debris in fish gastrointestinal tract (Lusher *et al.*, 2013; Murphy *et al.*, 2017; Phillips and Bonner, 2015; Tanaka and Takada, 2016).

Findings of the present investigation highlight prevalent presence of plastic rubbish and high frequencies of meso and micro-plastics in the marine fishes from the lower Gulf of the Thailand and signify a further cautionary sign for marine conservation as well as for the consciousness of human well-being. It is absolutely suggested that microplastic pollution in marine organisms and their food chain in other neighboring provinces must be discovered to make sure the safety situations of environment and human health. These ultimate conclusions denote a vital preliminary point in discovering certain eco-toxicological aspects such as the probable effects related to the transmission of pollutants on human health and the valuation of the presence and effect of plastic debris on other types of marine entities. Additionally, operative management plans in the study area and adjoining areas for the plastic contamination are instantly mandatory.

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