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Microplastic Fragments in Stomach Content of Blue Swimming Crab, *Portunus pelagicus* from Wonnapha Coastal Wetland, Chonburi Province, Thailand

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Abstract. The study aim was to search for a quality, quantity, and diversity of microplastics ingestion by the blue swimming crab, *Portunus pelagicus* in Wonnapha coastal wetland, along the Gulf of Thailand located in Chonburi Province. Of 296 crabs from the wetland area were examined in this study, plastic debris ranging from 0.09 µm to 38.6 mm size were detected in 26.35 % of assessed crabs and the average value of 0.73 ±1.4 items/total crabs. Fibers were dominant plastic (77.77%); red and blue were the most common observed plastics (32% and 25 % respectively), but 10.65% were microbeads, similar to those found in commercial facial cleansers, 9.72% were pellets resins, and some of which were fragments. Forty-two of the plastics ranged sizes were smaller than 5 mm. This assessment indicates that microplastics are definitely present in *P. pelagicus*, an important species for human consumption.

Keywords: blue swimming crab, Gulf of Thailand, microplastic, pollution, wetland

1. Introduction

Plastic debris especially microplastics are an emerging pollutant with the potential to affect many aquatic organisms and trophic levels (Farrell & Nelson, 2013; Setälä et al., 2018; Franzellitti et al., 2019). From land and river to the ocean, plastic debris are flowed remotely and randomly (Jambeck et al., 2015, Lebreton et al., 2017; Schmidt et al., 2017). Therefore, coastal wetlands might be an important reservoir of microplastics (Zhang, 2017; Li et al., 2018). Wonnapha coastal wetland located on the coast of Chonburi Province, the Inner Gulf of Thailand, is a study site. It is one of the important natural fishing grounds for small scale fishing for shrimp and blue swimming crab (Eiamsa-Ard and Amornchairojkul, 1997). Green mussel (Perna viridis) farms are also located in this area (Tunkijjanukij, and Intarachart, 2007). However, the areas surrounding mussel rafts have been favored by fishermen for the operation of crab traps and gill nets. Intensive aquaculture and fisheries activities can possibly turn Wonnapha coastal wetland into a hotspot for microplastic pollution. In addition, Chonburi Province has a 160 km-long coastline that is exposed to pollution loads of both domestic and industrial origins. Resulting from the rapid expansion of economic and aquaculture activities, plastic debris problem was reported in Bangsaen and Angsila coastal area, a location site near the Wonnapha coastal wetland (Thushari et al., 2017).

Thus, the issue of plastic waste in this coastal wetland is a critical concern and needs an urgent treatment from community cooperation. According to NOAA (2016) indicated that the accumulation of plastic debris can alter the physical and chemical composition of habitat, and impair critically to nurseries and refuges employed by many different organisms that utilize these habitats and then may reduce the quality of habitat for organisms.

Furthermore, smaller debris items, particularly microplastics (small sized; < 5mm diameter, Lusher et al. (2017), can threaten marine life. In addition, laboratory experiments state that microplastics can play a key role as vectors for heavy metal ions in the marine system and can transfer any toxic organic pollutants that are easily adsorbed on their relatively large surface areas to marine organisms (Brennecke et al., 2016; de Sá et al., 2018). Based on research data, the increasing number of microplastics, both quality, and quantity have been reported in the stomachs of marine life, which can significantly affect life in the sea, causing entanglement, suppression, malnutrition, and frequently death (Baulch and Perry; 2014; Vegter et al., 2014; Kurtela and Antolović, 2019). In addition, Sussarellu et al. (2016) indicated that the ingestion of microplastics during gametogenesis had been shown to cause some adverse effects on feeding and reproduction in oysters, with negative impacts on adult fecundity and offspring quality. These results support the idea of the developing hypothesis that microplastics can reduce reproductive output and suitability in marine species by changing their food consumption and energy allocation. Furthermore, Pitt et al. (2018) also demonstrated that nanoplastic can penetrate the chorion of developing zebrafish, then accumulate in the tissues, and affect physiology and behavior during growth and development. In swimming crabs (Necora puber), microplastics are found in the gill, stomach, testes, and brain, indicating that the reproductive organ and brain of crabs can accumulate microplastics and transfer to other trophic levels (Crooks et al, 2019).

The blue swimming crab, *Portunus pelagicus* is one of the marine crustaceans living in wide geographical habitats such as the coastal waters of the western Indian Sub-continent, Pakistan, Persian Gulf, Red Sea, Mediterranean Sea, East Coast of Africa, and Thai waters (Lai et al., 2010). This species is a benthic carnivore eating mainly on sessile mollusk, invertebrates, and teleost fish (Kunsook et al., 2014). *P. pelagicus* supports substantial commercial and local fisheries in Thailand and is one of the more resilient of the Gulf of Thailand including Wonnapha coastal wetland species, but its fate depends on many factors to survive: hunted extensively in this natural habitat, predators, dissolved oxygen depletion from plankton bloom, vanishing habitat, occupy by *Octolasmis* spp. in their gills, and plastic debris pollution (Jeffries et al., 2005; Buranapratheprat et al., 2008; Khongkhon et al., 2017; Thushari et al., 2017). However, the amount and type of plastics ingested by blue swimming crabs in Wonnapha coastal wetland have not been investigated. The need for assessment of the levels of microplastics in blue swimming crab, one of the wild populations widely distribute throughout the coast of Thailand, is required essentially for the baseline levels of contamination and the risk of microplastics to *P. pelagicus* and coastal wetland ecosystems.

This study, evaluated the ingestion of plastic marine debris in blue swimming crab, *P. pelagicus* in Wonnapha coastal wetland, in terms of size, morphology and color.

2. Materials and Methods

2.1 Crabs Sampling

Blue swimmer crabs were collected randomly from Wonnapha coastal wetland a regular fishing area of local fishermen (13° 16′ 18.49′′ N 100° 55′ 19.42′′ E and 13° 14′ 44.73′′ N 100° 51' 55.04′′ E). Eleven months of samplings were performed from February-December 2017, with the cooperation of local fishermen. A total of 296 crabs were examined. Crabs were placed in ice for euthanasia until no movement. Later, crabs were examined and dissected, respectively. Then the crabs were washed with water to remove the outside debris, mud, algae, and another external skeletal adherent. Their size (carapace width, carapace length), wet weight, and sex were determined. The differentiation between males and females was measured and recorded based on Lai et al. (2010) and Josileen (2011) methods. Each crab was dissected in a metal tray using a scalpel, forceps, and scissors. Then stomachs were removed and inspected in the laboratory. The dissection was carried out under control conditions to prevent airborne contamination (Lusher et al., 2017). All stomach (foreguts) sections were fixed individually in a 45 ml glass bottle containing 70% ethanol solution (Carey, 2011; Nadal et al., 2016) for digestion later.

2.2 Digestion procedure

Briefly, the stomach of *P. pelagicus* was removed from 70% ethanol solution and put into 45 ml clean glass bottles separately for digestion. They were then treated with 1 M KOH solution (Dehaut et al., 2016) for 2-3 days at room temperature. The residual content in each glass bottle was filtered (WhatmanTM cellulose 10 µm; 110 mm Ø). The filter papers were dried at room temperature in a glass Petri dish and kept covered to minimize the exposure risk for one week.

2.3 Identification of Microplastic

2.3.1 Visual Identification

The filters were observed under a stereomicroscope (Olympus; sz3060), and images were taken with the stereomicroscope SZX7 with digital camera/DP22 image processing software. A visual assessment was applied to identify stomach content separately as natural or plastic particles first (Hidalgo-Ruz et al., 2012). The morphology characteristic identification criteria were used (Norén, 2007; Löder & Gerdts, 2015). Particles were also compared to a number of photos depicting the known microplastics from various prior studies and also two sources of plastic ingredients in commercial products were used as references (UNEP, 2016; Viršek et al., 2016; Tanaka and Takada, 2016). However, the particles which presented the characteristic feature of synthetic polymer were identified as microplastics. In case of doubt, a hot metal tip was applied on the object following a hot needle test (Karlsson et al., 2017). The quantification of plastic debris ingestion was grouped by color, size (length, width) and product type categories or shape: fibres, fragments, bead and pellets. The size of plastic particles found in the stomach of blue swimming was determined using image processing software v.6. then categorized as microplastics (<5 mm), mesoplastics (5–25 mm) and macroplastics (>25 mm) following the method of Pham et al. (2017). The frequency of plastic debris occurrence in these crabs was estimated by the proportion of the individuals examined where plastics were present in the crab stomach contents.

2.4 Statistic analysis

Data were analysed using Minitab software v.18, chi-square test was used to compare the proportion of the plastic occurrence in the stomach between sexes, with significant difference attributed where $p \leq 0.05$. The wet weight and carapace width were expressed as a mean with a standard deviation (mean ±SD).

3. Results

3.1 Shape, composition and size of microplastics

For microplastic ingestion investigation, 296 individuals of *P. pelagicus* were analyzed. The range of total carapace width (CW) of blue swimming crab is from 56.88 to 135.40 mm (95.00±11.43 mm), and the fresh weight is from 20.87 to 164.34 g (64.68±25.34 g). Plastic debris were found in both sexes of crab stomach. A total of 216 plastic particles were found in 26.35% of all sampled individuals (78 individuals) (Table 1). With the averaging number of plastic items ingested by crabs, 2.84±1.52 particles per individual were recorded. The average number of particles ingested by the total number of crabs sampled (n = 296) was 0.73±1.4 (range 0-9) particles per individual. The majority of crabs containing plastic debris in stomachs were females. However, there were no significant differences in microplastic ingestion between female and male crabs ($p>0.05$).

3.2 Microplastic characterization

Among all 216 particles found, plastic particles were identified as 168 fiber (77.77%; Figs.2-3), 23 microbead (10.65%; Fig. 2 C1-C2), and 4 fragments (1.85%; Fig. 4 (C4)) 21 pellets (9.72%; Fig. 4 (C3). Fibers were the most commonly found type of microplastics in the stomach of blue swimming, among ingested plastic fibers. The variation of size and number of fishing gear fragments were mainly found in male crabs (Fig. 3). The bead composed of 2 yellowish color granules and irregular green shapes were found in male crabs (Fig. 4 (C1, C2). The rest as shown in Fig. 2 C2-C4, were found in both male and female crabs.

From 296 crab specimens, plastic particle sizes ranging from 0.09 µm up to 38.6 mm were detected in the stomach of 78 crabs. In the size of plastic particle investigation, the plastic particle size distribution of microplastics (< 5 mm), mesoplastics (5-25 mm), and macroplastics (> 25 mm) were 42%, 36.5%, and 20.83%, respectively. The macroplastics were abundantly found in male crab stomachs. Furthermore, microplastic particles were found in a variety of colors. The most common colors were red (32.4%) and blue (25%). Meanwhile, black (18.51%), green (11.11%), yellowish (11.11%), clear or transparent (1.39%) and pink (0.5%) were least commonly found (Fig.1)

Table 1. Summary of morphological measurements of *P. pelagicus* collected from Wonnapha coastal wetland

Specimens	No. of Crabs	Carapace width (mm)		Wet weight (g)		Individuals with plastic found in their stomachs
		Range	Average (mm±SD)	Range	Average (g±SD)	
Total	296	56.88-135.40	95.00 ± 11.43	20.87-164.34	64.68± 25.34	78
Females	147	56.88-135.40	94.30 ± 13.98	20.87-164.34	62.92 ± 31.03	42
Males	149	65.70-115.70	96.06 ± 8.65	23.56-124.12	67.23 ± 49.66	36

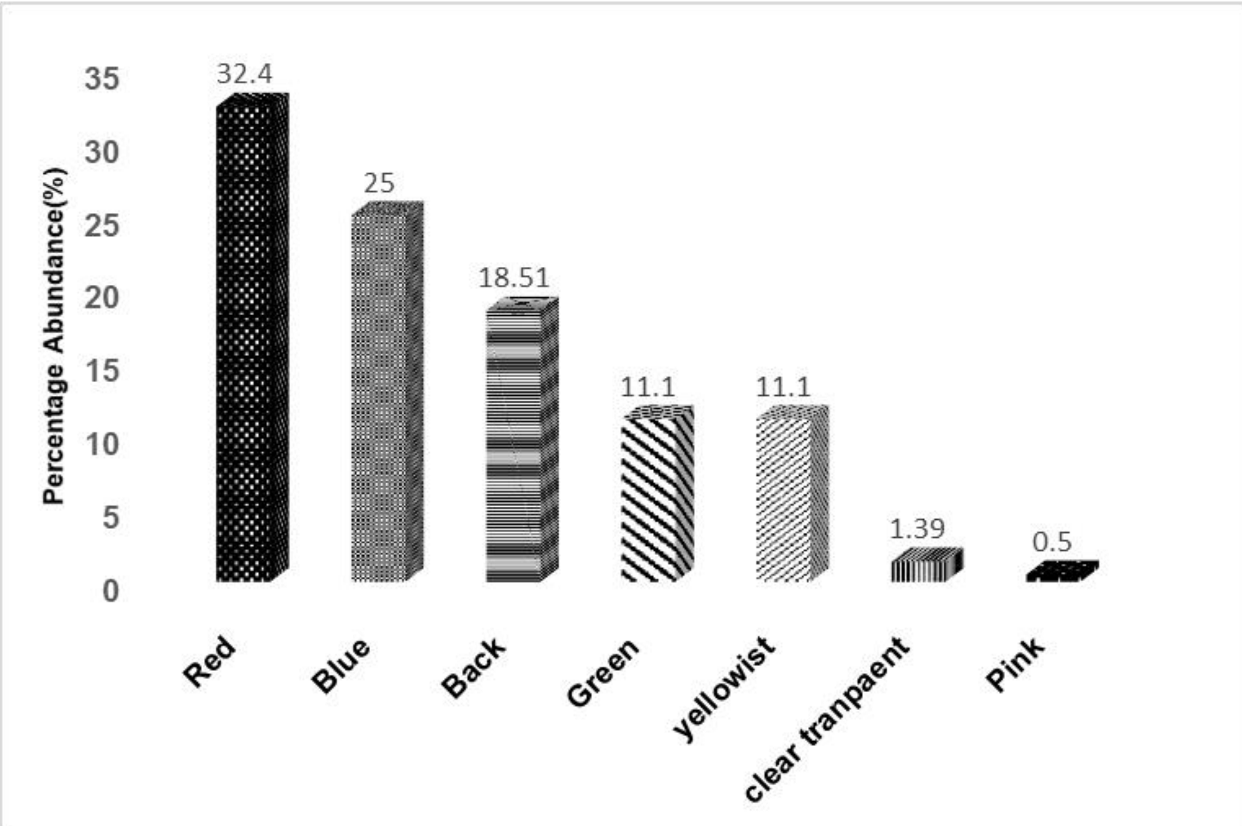


Figure 1. Percentage abundance (%) of microplastic based on colors

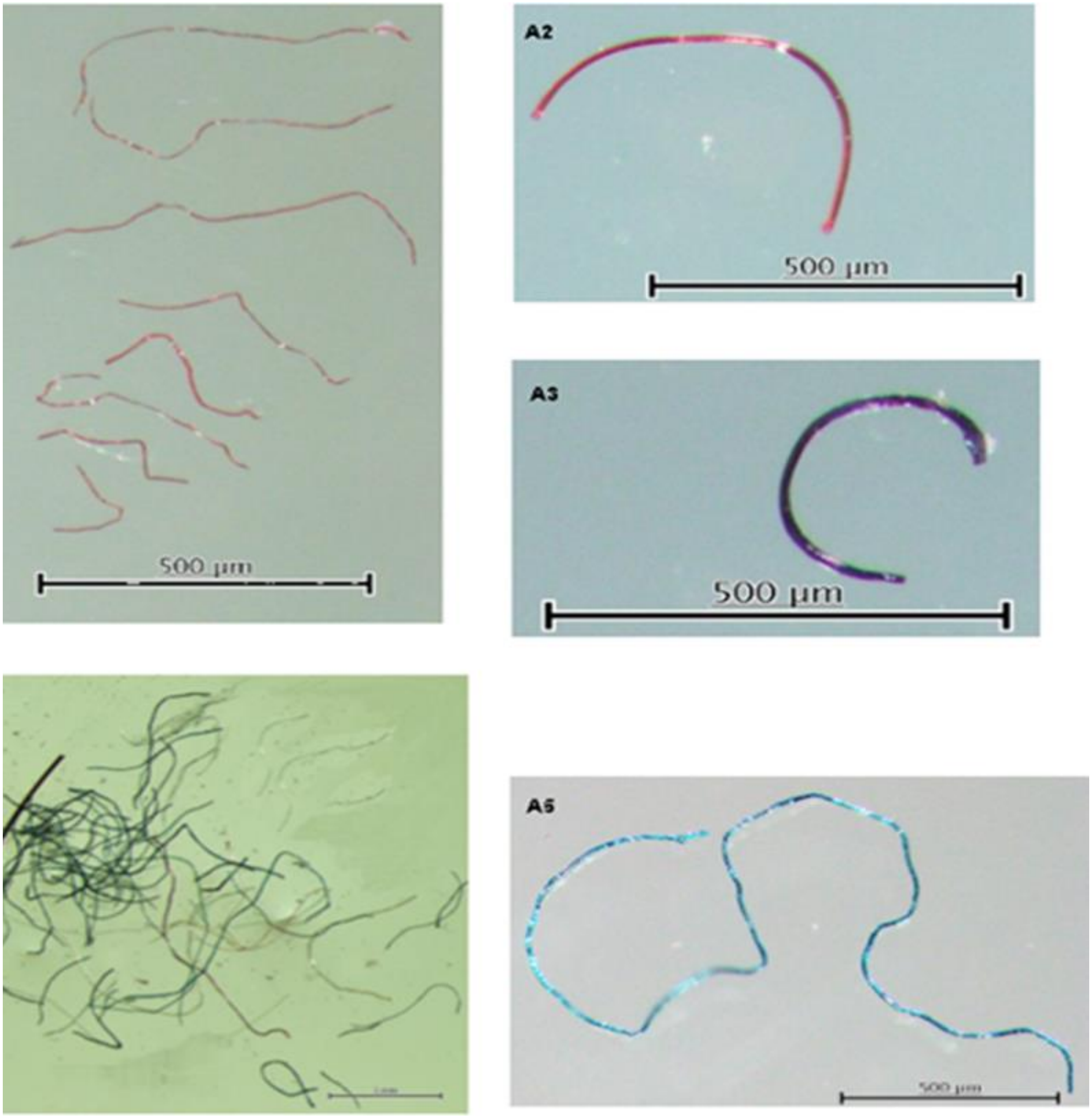


Figure 2. Examples of microplastic particles in *P. pelagicus* stomach: A1-A5; Fibres, Scale bar increasing magnification with image J software

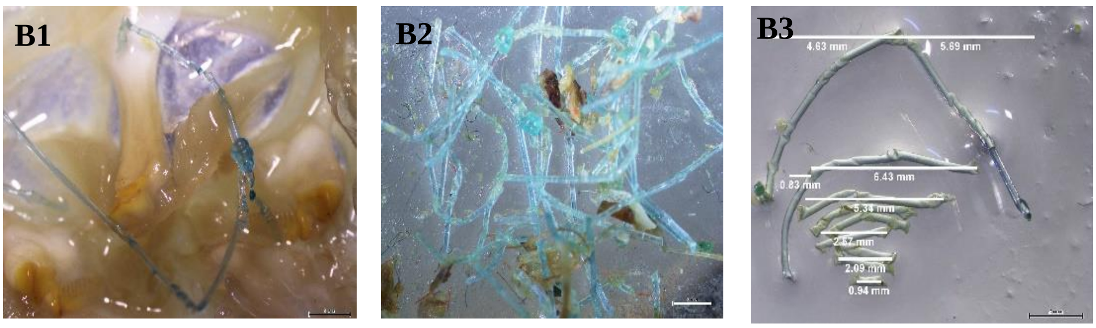
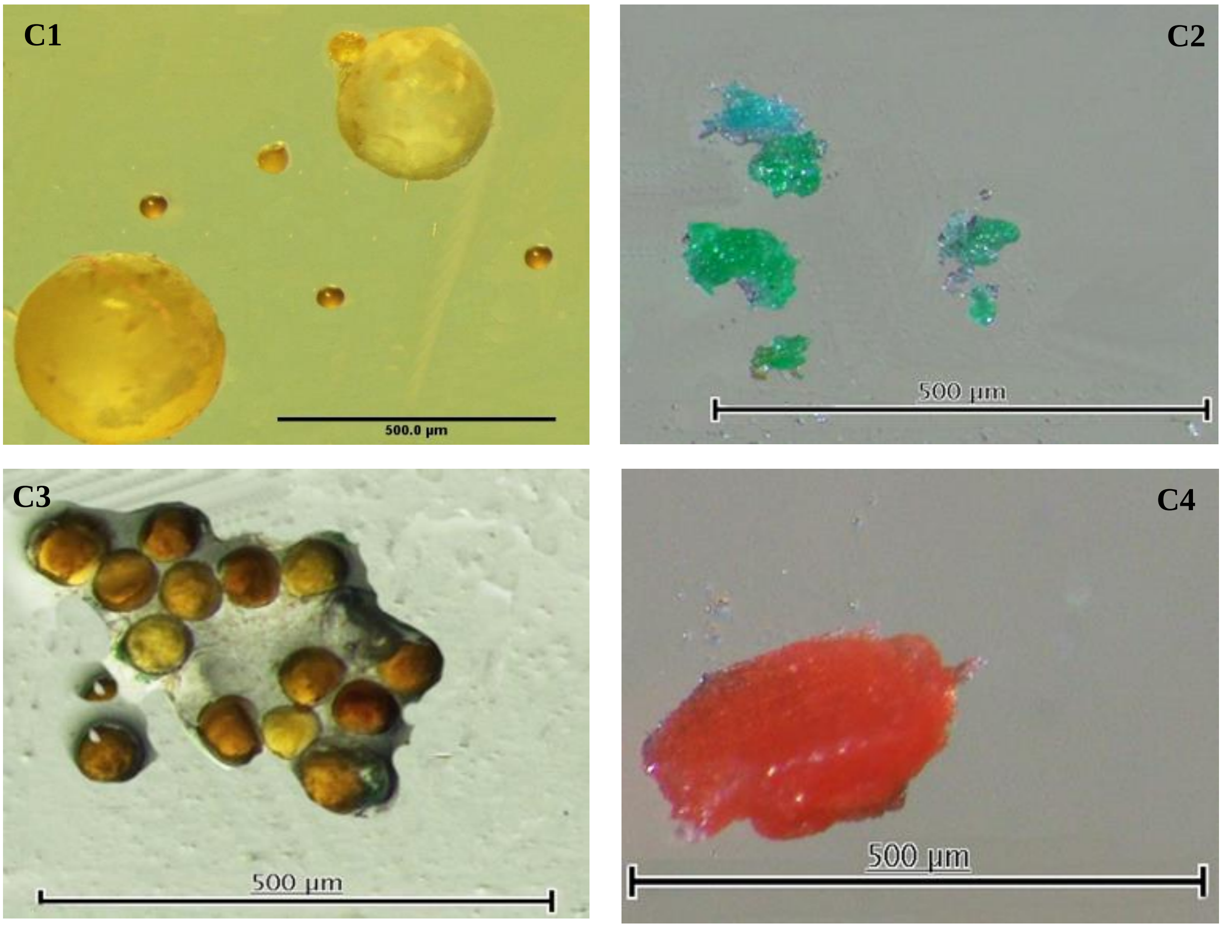


Figure 3. Examples of microplastic particles in *P. pelagicus* stomach: B1-B3; Crab gill nets. Scale bar increasing magnification with image J software



Figrue 4. Examples of microplastic particles in *P. pelagicus* stomach: C1-C2; Microbeads; Pellets and C4; Fragment. Scale bar increasing magnification with image J software

4. Discussion

There are reports on microplastic contamination in a variety groups of marine organisms such as zooplankton (Amin et al., 2020), sessile invertebrates (Thushari et al., 2017), oyster and crabs (Waite et al., 2018), brown shrimp (Devriese et al., 2015), ghost crab (Costa et al., 2018), cetaceans (Baulch and Perry, 2014), and commercial fishes (Azad et al., 2018). Our study has shown a similar trend in microplastic contamination of blue swimming crab, *P. pelagicus* from Wonnapha coastal wetland. The amount of plastic contamination is 0.73-9 particles/individual. At least one microplastic item is found within each crab. That means microplastics are widespread and abundant in this area. Likewise, Thushari (2017) reported that Bangsaen beach had the highest average number of plastic debris both macro (> 2 cm) and meso debris (2 mm-2 cm). It is possible that plastic debris in Bangsaen can flow through the tidal change to a connected area, Wonnapha coastal wetland. The microplastic particles found in the stomach content of *P. pelagicus* prominently are microplastic fiber with different colors and sizes. This implies that this habitat is a sink for fibers, especially fishing ropes that may come from fishery activities in the area. However, the shape and color of these fibres may play a part in the toxicity of ingested microplastics depending on type, location and the presence of organic pollutants at the surface of microplastics (Pannetier et al., 2019). In this study, microbeads were found in crab stomachs. According to Ertel (2018) demonstrated that 300 μm commercial microbeads were ingested by sand fiddler crab (Uca pugilator), which microbeads alone can be toxic and can serve as vectors introducing additional toxins due to bioaccumulation of plastics, including toxins that adhere to plastics, throughout the food web. Similarly, the incidence of plastic pellets was found in the stomachs of blue swimming crabs in Wonnapha coastal wetland. It is possible that this crab may have a chance to expose to various chemical contaminants absorbed from surrounding seawater (Teuten et al., 2009; Ertel, 2018; Pannetier et al., 2019) and then interfere with biological and physiological functions to an extent (Franzellitti et al., 2019). According to Ogata et al. (2009), global aquatic contamination by plastic pellets and pollutants were well documented. Later, persistent organic pollutant concentrations in plastic resin pellets from remote islands were reported similar to the concentration in plastic fragments collected in the open ocean based on the explanation of sorption/desorption equilibrium in seawater (Heskett et al., 2012). Plastic resin pellets may play a role as pollutant transport media and toxic chemicals in marine environments (Mato et al., 2001). Therefore, microplastics in blue swimming crab stomachs indicated that the crabs act as a pollutant accumulator and mediator to other organisms in trophic levels, including humans.

4. Conclusion

Our findings firstly indicate that plastic pollution in the Wonnapha coastal wetland are directly impacted on blue swimming crabs. Some of these plastic polymers likely originate from lost and discarded fishing gears (gill nets), whereas other plastics are microbeads commonly used in commercial facial cleansers and pellets resins, an industrial raw material used in the plastic industry. However, to the best of our knowledge, the result reported in this study is the first evidence of microplastic contamination in the blue swimming crab, a coastal wetland species. Therefore, we recommend that the study of plastic types and their potential hazard associated with the plastic debris ingestion of crabs, the impacts to population, health, growth, and development in wild habitats, and human food safety are needed for further investigation.

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