

**ORIGINAL PAPER**

## **Accumulation of microplastics in zooplankton from Chonburi Province, the Upper Gulf of Thailand**

**Darika Buathong<sup>\*a</sup>, Pronsiri Sriwisait<sup>a</sup>, Sittiporn Pnengsakun, Charernmee Chamchoy<sup>a</sup>, Orathep Mue-suae<sup>a</sup>, Supphakarn Phoaduang<sup>a</sup>, Montaphat Thummasan<sup>b</sup>, Duangkamon Sangiamdee<sup>c</sup>, Thamasak Yeemin<sup>a</sup>, Makamas Sutthacheep<sup>a,\*</sup>**

<sup>a</sup>Marine Biodiversity Research Group, Department of Biology, Faculty of Science, Ramkhamhaeng University, Huamark, Bangkok, Thailand

<sup>b</sup>Green Biology Research Division, Research Institute of Green Science and Technology, Shizuoka University, Shizuoka 422-8529, Japan

<sup>c</sup>Department of Chemistry, Faculty of Science, Ramkhamhaeng University, Bangkok, Thailand 10240

\*Corresponding author: *smakamas@hotmail.com*

Received: 18 August 2020 / Revised: 2 September 2020 / Accepted: 9 September 2020

**Abstract :** Microplastics particles cannot be digested by marine organisms, causing physiological problems to different animals worldwide. Zooplankton can ingest microplastic and introduce it into the food web, causing bioaccumulation from basic to top levels, which can later reach humans consumers. We investigated the characteristics and abundance of microplastics ingested by different groups of zooplankton. The samples were collected by using 120  $\mu\text{m}$  mesh plankton net with a mouth diameter 30 cm, by horizontal hauls. Later analyzed under a stereomicroscope and identified by using Fourier transform infrared spectroscopy (FTIR). Microplastics were detected at all dominant zooplankton groups here: chaetognaths, shrimp larvae, cyclopoid copepods, calanoid copepods, and cirripedia nauplius, except for harpacticoid copepods. The highest abundance of microplastics was found in cirripedia nauplius with 1.15 particles per individual, followed by cyclopoid copepods with 0.5 particles per individual. All microplastics found were fibrous, ranging from 0.1 to 0.5 mm in length. The majority (87.7%) were blue. A total of 63.9% of the microplastics come from polyethylene terephthalate, while 27.9% come from polyurethane, and only 8.2% are rayon. We found evidence that zooplankton ingests microplastic in the Upper Gulf of Thailand, potentially introducing it into the local food web. A higher abundance of particles from PET origin evidence a high level of domestic trash and land borne microplastics, possibly carried by the rivers to ocean waters. worldwide, this study indicates its presence in zooplankton of the Upper Gulf of Thailand, and urgent measures are needed to prevent human consumption and related health problems.

**Keywords:** Gulf of Thailand, ingestion, microplastics, zooplankton

### **1. Introduction**

Plastic is considered the wonder product of the last century due to its cheap manufacturing cost, durability, and flexibility. Currently, the annual global production of plastics has increased from 1.5 million tons in the 1950s to >335 million tons in 2016 (PlasticsEurope, 2018). Plastic bags, fishing gear, food containers, and drinks are the most common waste ingredients that are transported to the oceans by rivers, coastal town sewers, floods, and winds and contaminate beaches and resorts (Zhou et al., 2018). The inadequate disposal plan, accidental loss, and fragmentation of larger plastics have contributed to the increasing accumulation of tiny plastic particles and fibers (<5 mm) in the environment (Cole et al., 2011; UNEP, 2016), with plastic now contributing up to 80% of all marine debris (Barnes et al., 2009). Microplastics primarily originate from manufactured items of microscopic size (e.g., exfoliates in cosmetics products) or secondary items derived from the biological and mechanical breakdown of microplastics.

Plastic pollution is a global level environmental issue (Thompson et al., 2004; Cole et al., 2011; Rochman, 2018; Botterell et al., 2019). Plastics debris are widely found in marine environments,

including coastal zones, estuaries, open ocean, the deep sea, and polar marine environments (Van Cauwenbergh et al., 2013; Cozar et al., 2014; Turra et al., 2014; Lusher et al., 2015; Tang et al., 2018). Being similar in size to natural food items and suspended organic particles, microplastics can be ingested by a variety of marine organisms such as zooplankton, sea cucumber, decapods, mussels, lugworms (Graham and Thompson, 2009; Murray and Cowie, 2011; Cole et al., 2013; Farrell and Nelson, 2013; Setälä et al., 2014; Van Cauwenbergh et al., 2015; Sun et al., 2017, 2018a, 2018b;) and fishes (Lusher et al., 2013; Rochman et al., 2013). In addition, Gall and Thompson (2015) reported encounters between organisms and marine debris for a total of 693 species; of this debris, 92% were plastics. Marine organisms can unintentionally ingest microplastics, whether capturing them while filter- or deposit-feeding, mistaking them for preys when foraging, or even by ingesting organisms of lower trophic levels contaminated with these particles, i.e., trophic transfer (GESAMP, 2015). As a consequence, plastics are now considered as the most common and persistent pollutants, which ultimately end up in the coastal and oceanic environment through numerous pathways, including riverine and atmospheric transport, beach littering, via aquaculture, shipping, and fishing activities (Lebreton et al., 2017; Villarrubia-Gomez et al., 2017). Given the exponentially increasing demand and insufficient waste management, coupled with the resistance of synthetic polymers to environmental degradation, it is expected that the marine plastic inventory will continue to increase for a few more decades (Jambeck et al., 2015). However, few studies reported ingestion of microplastics by zooplankton in Thailand and the presence of microplastics in coral reefs adjacencies. This study aims to investigate the characteristics and abundance of microplastics ingested by different groups of zooplankton on a beach in the Upper Gulf of Thailand.

## 2. Materials and Methods

### 2.1 Study site and samples collection

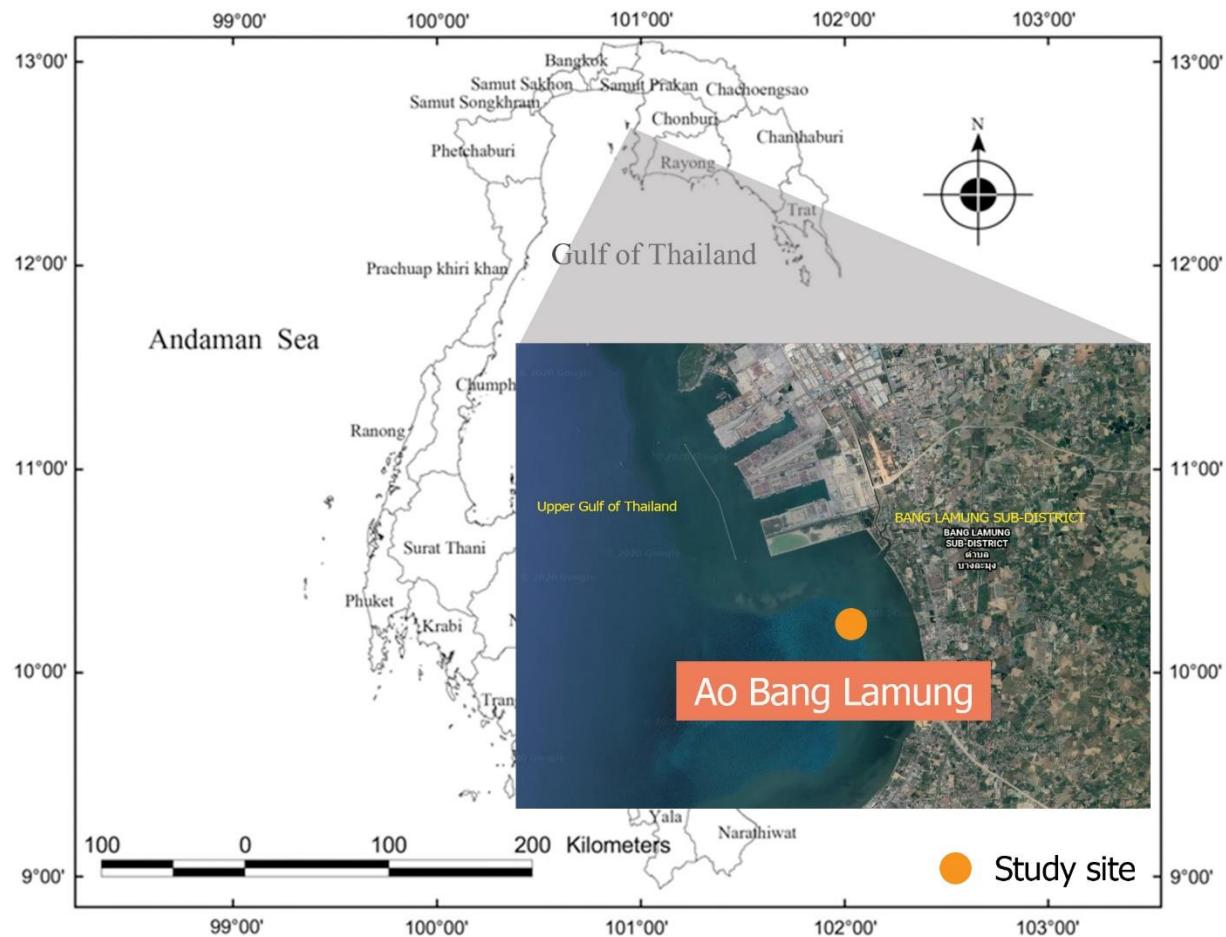
This study was carried out at Ao Bang Lamung, a beach near an industrial complex on Chonburi Province, the Upper Gulf of Thailand, in 2019 (Figure 1). The zooplankton samples were collected by horizontal tows using a standard 120  $\mu\text{m}$  mesh plankton net with a mouth diameter of 30 cm with 5 meters depth. Zooplankton samples were preserved in formaldehyde for cell counts. Zooplanktons were identified group and counted the number of cells by using a stereomicroscope. The form and density of microplastics were assessed in at least 20 individuals of each zooplankton group.

### 2.2 Extraction of MPs from zooplankton

The zooplankton samples were cleaned with distilled water two to three times to ensure no plastic was attached to their body surface. Then, rinsed zooplankton of each group was transferred to a 20-ml scintillation vial for storage. The zooplankton samples were treated with 30% hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) and heated up to 55-65 °C until they were completely digested. Microplastics particles were separated from the digested samples by flotation in saturated sodium chloride solution (250 g/ml) (Mathalon and Hill, 2014). After 24h of floatation at room temperature, the overlying water was vacuum filtered through a 20  $\mu\text{m}$  pore size filter. Several blanks containing only  $\text{H}_2\text{O}_2$  in an empty vial were run to correct for potential air-borne particle deposition in the laboratory. No contamination of blanks was observed during the experiments. Each filter was placed into a clean glass petri dish for observation under a stereoscopic microscope and photographed with a digital camera.

### 2.3 Microplastics Identification and Qualification

The microplastics samples on the membrane were observed under a stereomicroscope.



**Figure 1** Map of Ao Bang Lamung, the Upper Gulf of Thailand showing the locations of the study

All plastic particles were used to record the microplastic's quantity, color, shape, and size. Microplastic particles were further identified by using Fourier transform infrared spectroscopy (FTIR). The polymer types were identified by comparing the sample spectra with FTIR spectral libraries.

### 2.3 Data Analysis

Due to the high amount of zeros in samples, differences of microplastics abundance among zooplankton groups were examined by Kruskal-Wallis Rank Sum test in R, package "stats" version 3.6.1, and the post-hoc Dunn's Multiple Comparisons test, package "FSA"

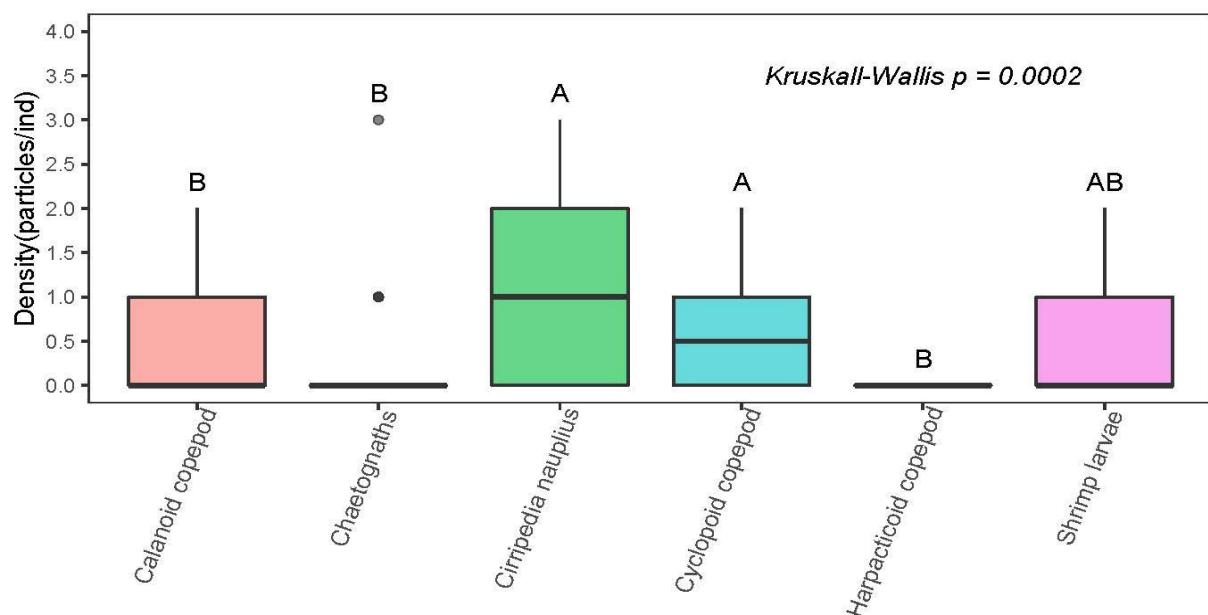
version 0.8.30, was used to analyze differences between groups.

### 3. Results

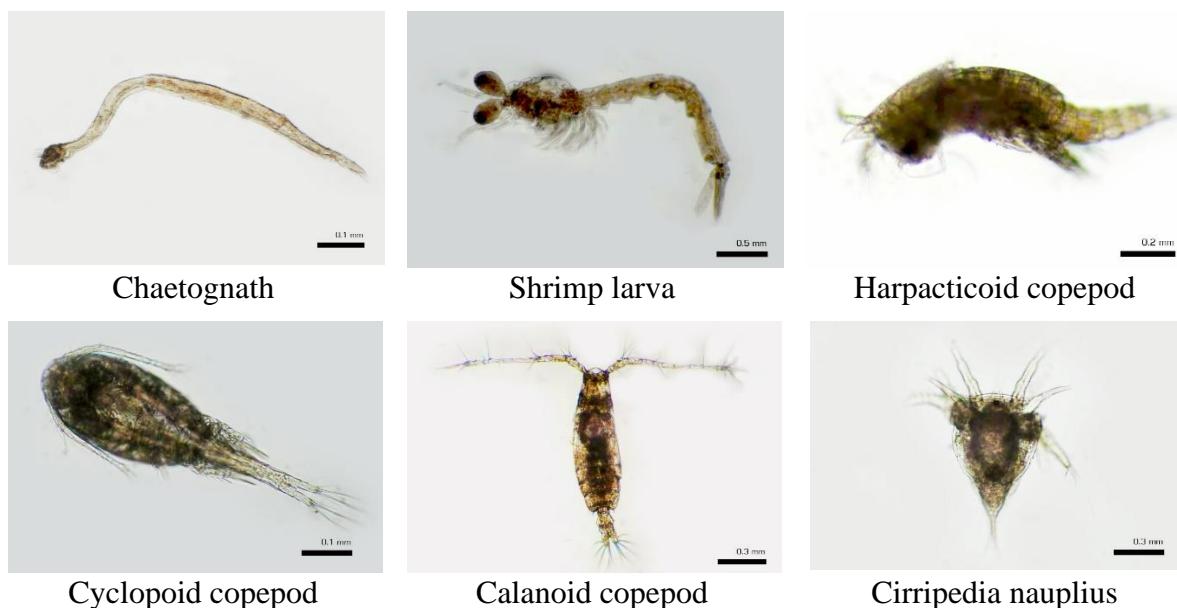
The density of zooplankton ranged between 42 and 6,166 individual  $m^{-3}$ , while size of zooplankton were ranged 0.8 to 5.2 mm (Table 1) Six dominant zooplankton groups were selected for microplastics analysis under a stereomicroscope, i.e. chaetognaths, shrimp larvae, harpacticoid copepods, cyclopoid copepods, calanoid copepods and cirripedia nauplius. These six major groups accounted for over 84% of total zooplankton abundance. Microplastics were detected in all zooplankton groups examined except for harpacticoid copepods. The abundance of microplastics

significantly varied across zooplankton groups (Figure 2) and the highest abundance of microplastics were found in cirripedia nauplius ( $1.15 \pm 0.05$  particles/ind.) followed by cyclopoid copepods ( $0.50 \pm 0.03$  particles/ind.), calanoid copepods ( $0.45 \pm 0.04$  particles/ind.), shrimp larvae ( $0.45 \pm 0.03$  particles/ind.) and chaetognaths ( $0.30 \pm 0.02$  particles/ind.) while no microplastic was detected in harpacticoid copepod. (Figure .2).

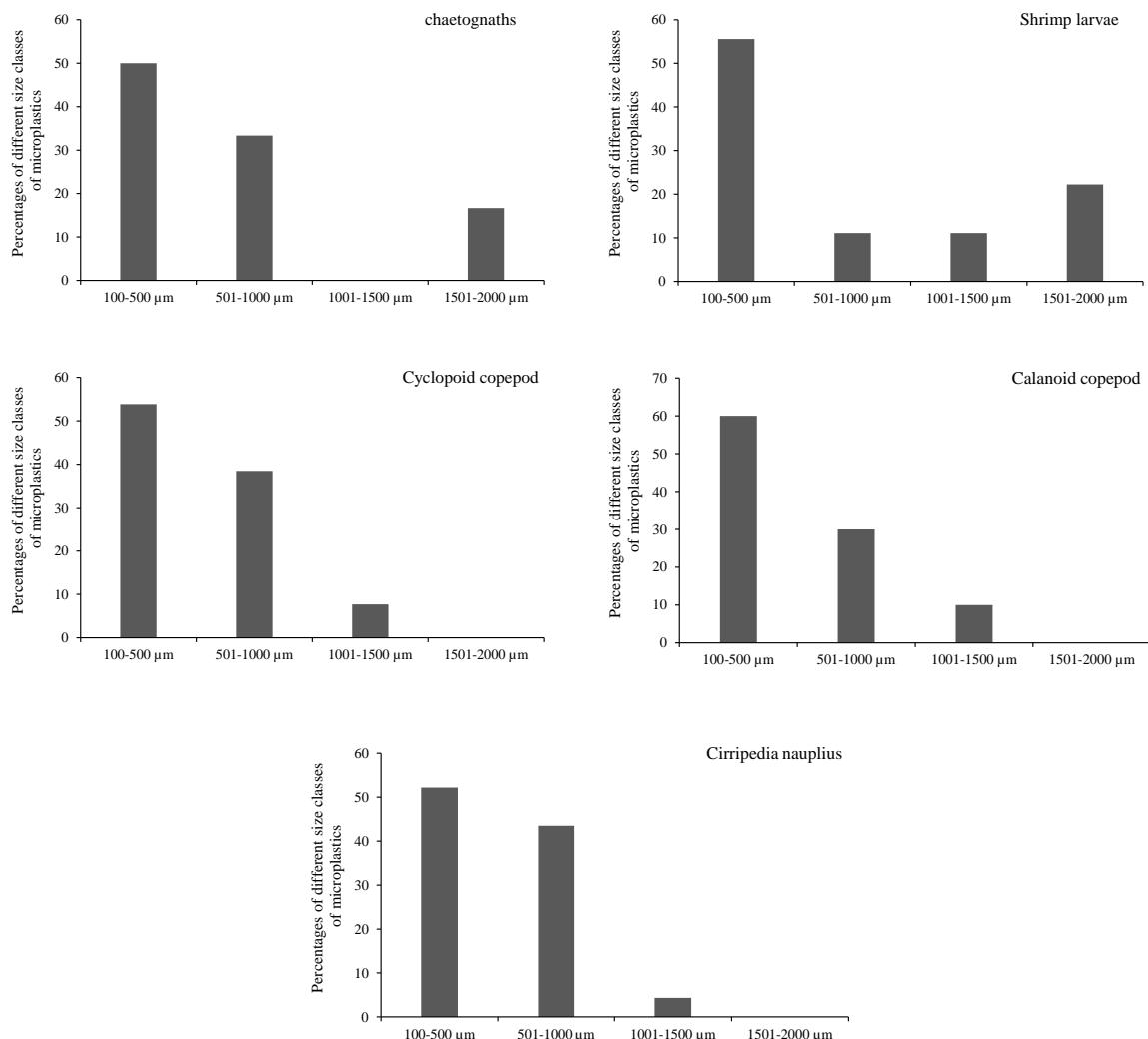
The shape of all microplastics in zooplankton consisted of fibers. Mostly, the length of the microplastic particles ranged from  $100 \mu\text{m}$  to  $500 \mu\text{m}$ . Across all zooplankton groups, approximately 87.7% of the microplastics were blue, followed by red (8.7%), brown and red (1.8%) plastics also well represented (Figure .4 and 5).



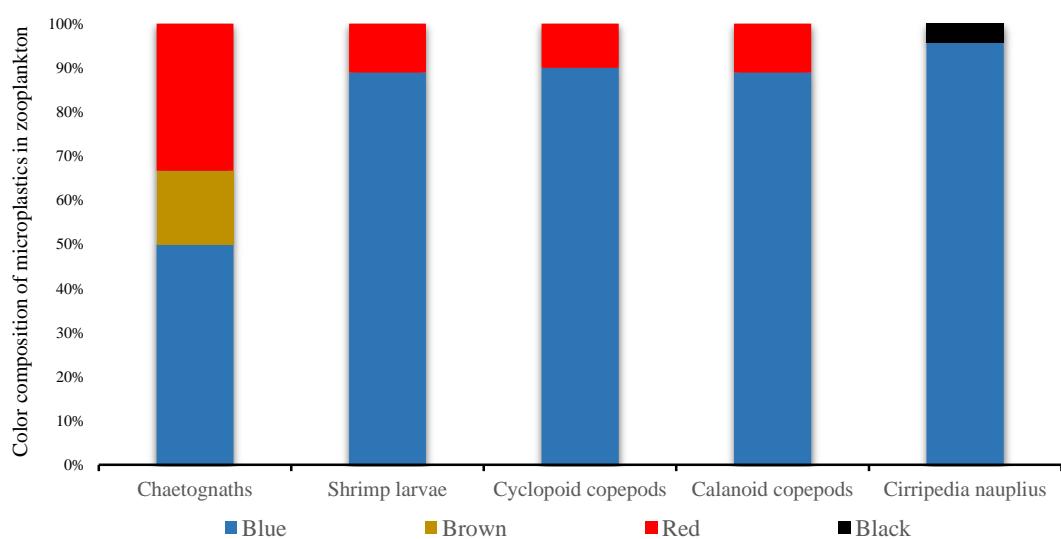
**Figure .2** The number of microplastics in different zooplankton groups



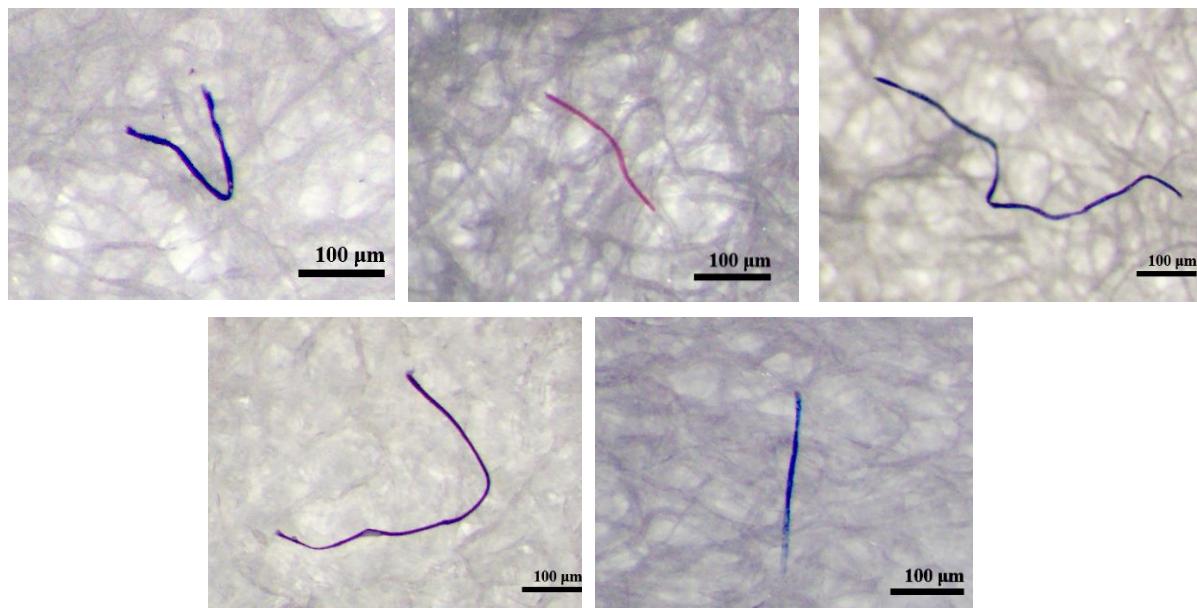
**Figure .3** Dominant zooplankton in the study site



**Figure 4** Size composition of microplastics in zooplankton



**Figure 5** Color composition of microplastics in zooplankton at Ao Bang Lamung



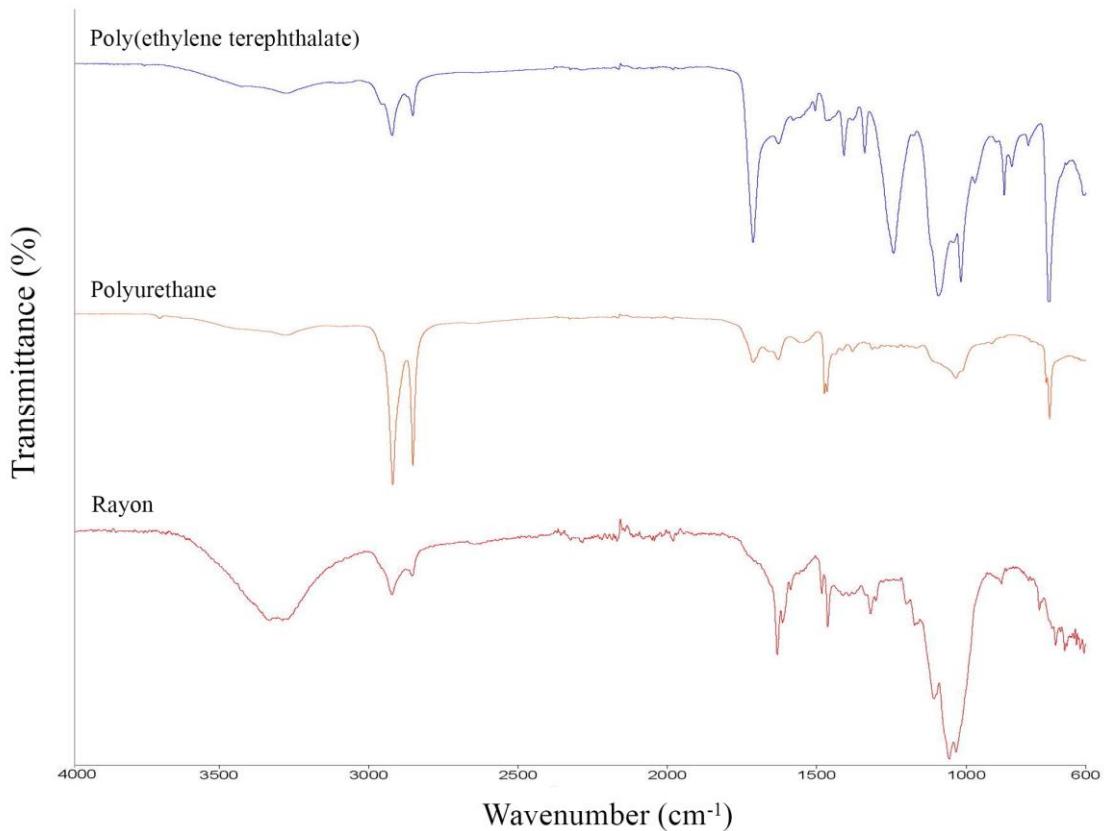
**Figure .6** Type and size of microplastics found in zooplankton at Ao Bang Lamung

**Table 1** Characteristic of plastic ingested by zooplankton collected from all zooplankton groups.

Zooplankton group	Density of zooplankton (individual m <sup>-3</sup> )	Average size of zooplankton (mm)	Average size of microplastics (μm)	Frequency of occurrence (%)
Chaetognaths	42.7±5.1	3.3±0.5	656.7±617.9	20
Shrimp larvae	153.3±18.3	2.6±0.5	327.7±319.4	35
Harpacticoid copepod	625.6±74.9	0.9±0.1	Not found	Not found
Cyclopoid copepod	2163.7±257.5	0.8±0.5	517.9±319.9	30
Calanoid copepod	6166.7±740.8	1.1 ± 0.1	458.6±311.3	50
Cirripedia nauplius	4300.7±516.6	0.8 ± 0.2	526.3±360.49	45

Characteristic and size particles of microplastics in zooplankton were shown in Figure .6. The FTIR microscope analysis of the 61 representative microplastics (6 particles from chaetognaths, 9 particles from shrimp larvae, 13 particles from cyclopoid copepod, 10 particles from calanoid copepod, and 23 particles from cirripedia nauplius) was

identified and classified into three polymer categories: polyethylene terephthalate, polypropylene, and rayon. The dominant polymers in the zooplankton group were polyethylene terephthalate (63.9%), followed by polyurethane (27.9%) and rayon (8.2%) (Figure .7).



**Figure .7** FTIR spectra of the selected microplastic particles

#### 4. Discussion

Detection of microplastics in most zooplankton groups depicts its bioavailability within this community. Given that zooplanktons are the primary consumers in the marine food chain, they are susceptible to the hazardous effects of microplastics litters due to accidental ingestion (Botterell et al., 2019). In this study, microplastics were detected in zooplankton around Ao Bang Lamung, Chonburi Province, the Upper Gulf of Thailand, indicating microplastic contamination in these areas. The microplastic abundance in zooplankton are in accordance with previous studies reported in the Yellow Sea (0.07-1.17 particles/ind; Sun et al., 2018a), but slightly higher than that in the Northeast Pacific Ocean (0.03-0.06 particles/ind; Desforges et al., 2015), Portuguese coastal waters (0.04-0.14; Frias et al., 2014), Kenya's

marine environment (0.16-0.46; Kosore et al., 2018) and Terengganu coastal waters (0.003-0.14; Amin et al., 2020). Significant differences were observed in the number of microplastic among the different zooplankton groups. Zooplankton lifestyle/feeding habits can influence microplastic ingestion. It has been reported that omnivorous and carnivorous zooplankton are more susceptible to accidental microplastic ingestion or accumulation via contaminated prey species than herbivores (Sun et al., 2017, 2018b). In a planktonic food web, fish larvae are positioned above copepod and chaetognath, and they predominantly feed on smaller zooplankton, thereby increasing their chances of microplastic ingestion.

Microplastic ingestion by marine organisms occurs accidentally and mainly depends on

the abundance and debris size (Rodríguez-Seijo and Pereira, 2017). The present results showed microplastics ingested have a similar size range from those reported in the Northeast Pacific; 555.5-816.1 (Desforges et al., 2015); the Northern South China Sea; 125-167  $\mu\text{m}$  (Sun et al., 2017) and Terengganu coastal waters 49.5-1135  $\mu\text{m}$  (Amin et al., 2020). Further, many planktons lack the prey selectivity and feed on anything of its palatable size (Moore, 2008). Sun et al. (2017) recorded a 30 fold higher microplastic ingestion by smaller-sized zooplankton (collected with the net of 160  $\mu\text{m}$  mesh) than the larger-sized group (collected with the net of 505  $\mu\text{m}$  mesh size). On the other hand, Christaki et al. (1998) found that the size of microplastic fibers played a crucial role in the clearance rate in ciliate *Strombidium* and plastic microsphere (0.75  $\mu\text{m}$ ) were indistinguishable from fluorescently labeled algae cells. Some zooplankton like calanoids, shrimps, and fish larvae can feed on prey items that can reach lengths of up to 1.54 mm (Baier and Purcell, 1997).

The composition of microplastic particles in zooplankton at Ao Bang Lamung indicated that the zooplankton tended to ingest relatively small fibers. These results showed a similar report in coastal areas of China (Sun et al., 2017, 2018a, 2018b) and the Bohai Sea (Zheng et al., 2020). The microplastics particles were mainly composed of blue color, which was consistent with the color composition of microplastics in the seawater. Blue has also been reported as the most dominant fiber color in marine areas such as the Swedish west coast, the Northeast Atlantic Ocean, and the South African coast (Norén, 2007; Lusher et al., 2014; Nel and Froneman, 2015). The color and shape of microplastics may affect the ingestion choices of marine organisms (Boerger et al., 2010;

Wright et al., 2013). According to the review of Gago et al. (2018), blue is the most common microplastics color in seawater and sediments because blue plastic products are very common and are often used in fishing nets, ropes, and other fishing gear.

Three different types of microplastics were detected in the present study, of which polyethylene terephthalate (PET) showed the highest ingestion in the zooplankton. PET is one of the most widely used and commonly identified plastic polymers in various marine habitats globally, and our results are consistent with earlier reports (Murray and Cowie, 2011; Patterson et al., 2019). For instance, polyethylene terephthalate is widely used in fibers for clothing, containers for liquids and foods, thermoforming for manufacturing, combination with glass fiber for engineering resins, and most of the nets used in marine fisheries.

Knowledge on the mechanism of microplastic transfer across the food web and implications on their health remains unclear. Setälä et al. (2014) reported that microplastics could be transferred via planktonic organisms to higher trophic levels; in addition, the transfer of microplastics between trophic levels has also been demonstrated among adult marine invertebrates (Spear et al., 1995; Graham and Thompson, 2009; Murray and Cowie, 2011; Rochman et al., 2013; Sun et al., 2018b). This indicates the bioaccumulation potential of these contaminants, as predatory fish have also been found to contain microplastic (Avio et al., 2015; Zhang et al., 2016; Murphy et al., 2017). This study further justifies the importance of following studies on microplastic ingestion by plankton, extending the sample collection both spatially and temporally, as zooplankton abundance and distribution may vary based on locality and season. To conclude,

the results from this study have successfully shown that zooplankton sampled at Ao Bang Lamung in the Upper Gulf of Thailand was able to ingest microplastics.

### Acknowledgements

We are most grateful to the staff of Marine Biodiversity Research Group, Faculty of Science, Ramkhamhaeng University, for their fieldwork assistance. This research was funded by a budget for research promotion from the Thai Government to Ramkhamhaeng University and partly supported by the Coral & Coastal Conservation Foundation.

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