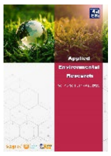
App. Envi. Res. 46(1) (2024): 007

Applied Environmental Research

- RESEARCH FOR SUSTAINABLE PLANET -



Research Article

Exploring Microplastics in Seawater and Zooplankton on the Eastern Coast of Thailand: A Case Study in Phuket Province

Pensiri Akkajit[1,*], Sirima Yuankerd[1], Arsanchai Sukkuea[2]

[1] Faculty of Technology and Environment, Prince of Songkla University, Phuket Campus, Phuket 83120, Thailand
[2] School of Engineering and Technology, Walailak University, 222 Thaiburi, Thasala, Nakhon Si Thammarat 80160, Thailand

*Correspondence Email: pensiri.a@phuket.psu.ac.th

Abstract

Microplastics can accumulate in the environment and be transferred to different trophic levels, making them a concern for the aquatic food chain. As far as the researcher is aware, this study is the first to analyze microplastics in seawater and zooplankton along the eastern coastline of Phuket, Thailand, emphasizing their ecological significance. Analysis of the seawater samples identified 256 microplastic particles, with an average concentration of 52.6±21.4 and 32.6±30.3 particles L^{-1} for 20–300 µm and >300 µm classes, respectively. Four zooplankton species—copepod, fish larvae, bivalvia larvae, and shrimp larvae—were chosen for examination. The findings indicated that microplastics can move to higher trophic levels, and their interaction with zooplankton resulted in 26 microplastic particles among the 120 zooplankton individuals observed. This yielded an average ingestion rate of 0.22±0.57 particles per individual zooplankton, with the highest rate observed among fish larvae. The micro-Fourier Transform Interferometer (µFT-IR) analysis verified the presence of polyethylene terephthalate (PET), polyethylene (PE), polyester, urea-formaldehyde (UF), and polyamide (PA). This underscores the prevalence of these polymers in daily and human-made plastic sources, emphasizing the need to implement effective measures to address microplastic pollution in ecologically vital areas to safeguard marine environments.

ARTICLE HISTORY

Received: 7 Sep. 2023
Accepted: 29 Jan. 2024
Published: 24 Feb. 2024

KEYWORDS

Bivalvia larvae;
Copepod;
Fish larvae;
Microplastics;
Phuket;
Shrimp larvae;
Zooplankton

Introduction

Phuket, a popular island destination in Thailand's southern region, attracts a large number of tourists each year. However, such a high level of tourism has the potential to negatively affect marine ecosystems and the environment. The production of a substantial amount of waste is one notable consequence, especially in coastal areas. Research has shown that microplastics (MPs), plastics with diameters less than 5 mm, are found in beach sediments in Phuket [1–2]. Due to their small particle sizes, MPs are of great concern since they can travel long distances, be consumed directly and indirectly, and ultimately transferred through the food chain [3]. Organisms living in surface waters are likely to encounter MPs with a density lower than that of seawater, such as polystyrene (PS), polypropylene (PP), and polyethylene (PE). On the other hand, benthic organisms residing at the lowest level of a water body, such as a lake or ocean, are more prone to encountering denser types of plastic, including polyethylene terephthalate (PET) and polyvinyl chloride (PVC) [4]. The harmful effects of MPs on aquatic organisms have been reported [5–6], and the ingestion of MPs by organisms is dependent on the abundance and size of MPs, availability of natural prey, and physiological and behavioral characteristics of the organism [7]. Consequently, this represents a route whereby MPs could enter the food web and transfer up the trophic levels.

Zooplankton encompasses a diverse species of marine organisms, both vertebrates and invertebrates [8]. Their

https://doi.org/10.35762/AER.2024007

significance extends to a vital role within marine ecosystems and the aquatic food web. As primary consumers, they feed on suspended algae while simultaneously serving as secondary consumers by preying upon other zooplankton. The digestion of MPs in zooplankton is due to several factors. MPs may also be directly ingested by aquatic organisms through accidental consumption or the misidentification of microplastics as food [9].

Zooplankton frequently ingest MPs since they are similar in size to their natural prey. The small size of MPs makes them potentially accessible to a wide range of organisms that consume particles of similar dimensions [10–11]. Additionally, many zooplankton species exhibit filter-feeding behavior, during which they inadvertently capture and ingest MPs. Furthermore, MPs can be ingested indirectly by zooplankton through trophic transfer [12]. Microplastics detected in zooplankton mainly range in size from 20–300 μm [8, 13]. Although MPs between 20–300 μm are considered to be small, this size range is important due to their potential for adverse effects if present in higher concentrations [3], while MPs above 300 μm are considered to be normal in size [14]. Zooplankton can indiscriminately ingest MPs via filter-feeding in the range of 1.4–30.6 μm in diameter, depending on the species, life stage, and MP size [15]. Van Raamsdonk et al. [16] reported that particles smaller than 25 μm are considered to have the highest probability of passing the intestine and entering the mammal. Previous studies have demonstrated the effects of MPs on zooplankton. Once ingested, MPs can interrupt food ingestion and impact marine organisms such as causing a reduction in feeding capacity, detrimental alterations to intestinal function, tumor formation, immune response, and feeding disruption [17–18].

Few studies have examined the occurrence of MPs in seawater, sediment, and organisms on Mai Khao Beach in Phuket, Thailand [2]. However, none have reported specifically on the ingestion of MPs by zooplankton. In another region of Thailand, MPs were detected in the seawater at Bandon Bay on the east coast of Surat Thani Province in Southern Thailand, with a mean abundance of 0.33 particles m^{-3} [19]. Prarat and Hongsawat [20] detected MPs in seawater at an average concentration of 1,781.48±1,598.36 particles m^{-3} along the coastal area of Rayong Province on the eastern coast of the Gulf of Thailand.

The present study investigates the presence of MPs in both seawater and zooplankton on Mai Khao Beach, along Phuket's eastern coastline in Southern Thailand. The study focuses on assessing MPs at this critical sea turtle nesting site, underlining its importance for sea turtle survival and the urgent need for preservation. This study targets MPs measuring 20–300 μm and >300 μm, resembling organisms like mesoplankton and microplankton that are vital to the aquatic food chain. The ingestion of MPs is examined in four zooplankton species, including copepod, fish larvae, bivalvia larvae, and shrimp larvae. The copepods are important prey species for higher trophic levels, such as amphipods, fish, or seabirds. Additionally, fish larvae play a significant role as indicators of fish populations and recruitment patterns. Bivalvia larvae, encompassing the larval stages of mollusks such as clams and mussels, are crucial for understanding the reproductive cycles and dispersal mechanisms of these economically and ecologically important organisms. Shrimp larvae, on the other hand, are essential components of the zooplankton community and serve as valuable food sources for numerous organisms. These organisms are key constituents of the zooplankton community, fulfilling crucial roles in aquatic ecosystems by establishing links in the food web, which represents the flow of energy and nutrient transfer among diverse organisms in a specific habitat, showcasing complex relationships between different species. The four types of zooplankton examined in this study are interconnected with the specific characteristics of the study area through their ecological roles and contributions to the intricate web of energy and nutrient transfer within the aquatic ecosystem. The relationships between these organisms highlight the complex and dynamic nature of the interactions in a specific habitat. These findings contribute to the understanding of interactions between MPs, zooplankton, and the aquatic food chain, emphasizing the importance of studying and managing the pollution capability of MPs in marine ecosystems.

Methodology

1) Study area and sample collection

Seawater and zooplankton sampling were conducted on Mai Khao Beach, along the eastern coastline of Phuket (Figure 1). Mai Khao Beach is one of the few beaches in Phuket, serving as a vital breeding ground for nesting sea turtles, further emphasizing its ecological significance. Mai Khao Beach stretches over 11 km in length, making it the longest beach in Phuket. The beach is in proximity to Phuket International Airport, encompassed by the Sirinat National Park, known for its high productivity and abundant biodiversity, including mole crabs and seagrasses. Three sampling stations (S1–S3) were selected for this study. S1 represents the northernmost point of Phuket, S2 is located behind Phuket International Airport, known as a plane-spotting beach, and S3 is situated within the Sirinat National Park.

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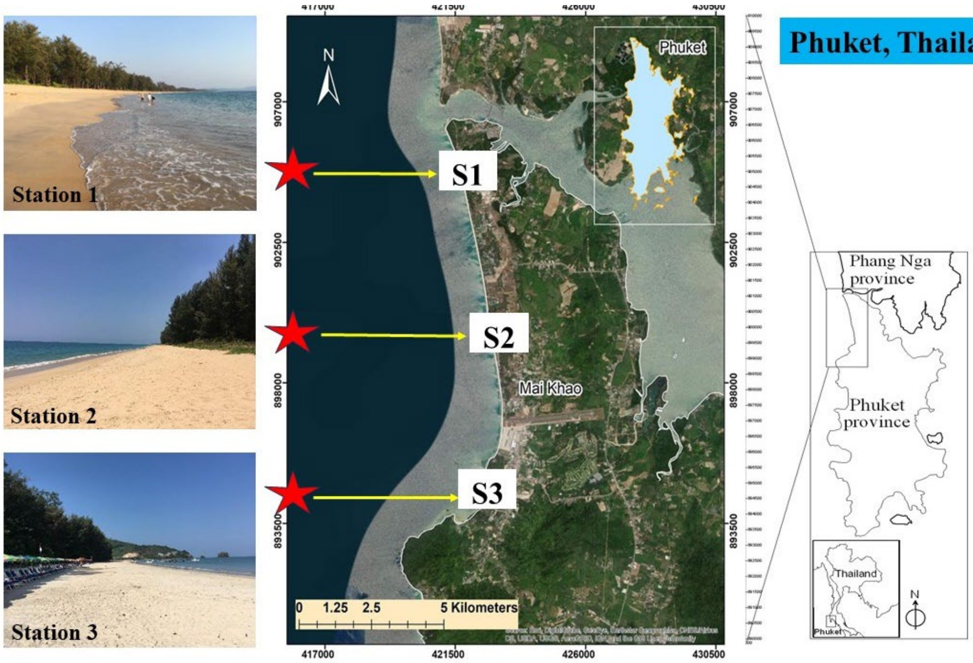


Figure 1 Sampling stations on Mai Khao Beach Phuket.

At each of the sampling stations (S1–S3), seawater and zooplankton samples were collected 3 km away from the shoreline at a depth of 0–2 m below the surface. Sampling activities were conducted during daylight hours (8 am–4 pm) in August 2020, corresponding to the low season. The collection process involved pumping the water through a zooplankton filter bag equipped with 20 µm and 300 µm mesh and a flowmeter (HYDRO-BIOS, Germany). The tows were conducted for approximately 180 min using a Rocker 801 pump, resulting in a total collection of 8,000 liters of seawater. The net was then carefully transferred into sampling bottles, and the samples were immediately fixed by adding a 10% formaldehyde solution with a volume of 25 mm into the bottle to maintain the condition of the zooplankton before further analysis. The preserved zooplankton samples were returned to the laboratory and processed immediately.

2) Zooplankton analysis

The collected samples of zooplankton were then subjected to separation and analysis. Individuals of the four species were carefully picked out using forceps (Feather Type), visually examined, and divided into the following groups: copepod, fish larvae, bivalvia larvae, and shrimp larvae. One milliliter of the sample was placed into counting trays designed for zooplankton, known as the Bogorov counting chamber. This facilitated examination and sorting under a stereomicroscope (Olympus SZ40) to classify the zooplankton into four main groups at each sampling station prior to the microplastic digestion process.

The identification of zooplankton followed the process described in several taxonomic guidebooks [21], and the volume of filtered seawater obtained from flow meter readings was used to estimate zooplankton density, expressed as the number of individuals per liter (ind./liter), using Eq. 1 as given by Ramarn et al. [22].

$$C = NV2/V1 \qquad \text{(Eq. 1)}$$

Where, C is the density of zooplankton (ind./L), N is the number of zooplankton in one mL, V2 is the volume of concentrated sample (250 mL), and V1 is the volume of water filtered by the plankton net (8,000 L).

3) Extraction of MPs from zooplankton and seawater

Four dominant taxonomic groups were determined for MP analysis based on the zooplankton composition. To ensure a comprehensive and statistically significant dataset for each zooplankton species, MP ingestion was assessed by randomly choosing 30 individuals per species (15 individuals per mesh size), totaling 120 zooplankton individuals across the four species. This approach accounts for potential variations in MP ingestion within a species and the impact of different mesh sizes on sampling results. The digestion procedure was modified from Prata et al. [23]. All the zooplankton were digested using 30% hydrogen peroxide (H_2O_2) with 1% potassium hydroxide (KOH) at 50 °C for 24 h or until all organic tissue had been removed without damaging microplastic samples. The samples were then cooled down and transferred using vacuum filtration through a Whatman glass microfiber filter (GF/F, 0.7 µm, 110 mm diameter, Whatman, UK). After digestion, samples were directly examined under a stereomicroscope for the presence of MPs with a Samsung S22 Ultra used to photograph each particle and categorized according to shape (fragment, fiber, pellet, and film). Size classes were considered as those corresponding to the mesh size (20–300 μm and >300 μm).

After being collected on board, the MPs in seawater were then transferred to 500-mL glass beakers. The sodium chloride (NaCl) solution (density 1.20 g cm^{-3}) was added, and the supernatant was then removed and filtered for density separation. Subsequently, 20 mL of aqueous 0.05 M Fe(II) solution ($FeSO_4 \cdot 7H_2O$) was added to the beaker, followed by 20 mL of 30% H_2O_2 for organic digestion. The solution was left at room temperature for 5 min and then placed on a hot plate at 75 °C for 30 min while covered with a watch glass. When natural organic material was detected, an additional 20 mL aliquot of H_2O_2 was introduced, and the process was repeated until no natural organic material remained visible. Density separation was performed once more by adding 6 g of NaCl per 20 mL of the sample to increase the density of the aqueous solution and heated to 75 °C until the salt had been dissolved. After digestion, these solutions were filtered with Whatman GF/F (0.7 μm, 110 mm diameter) using a vacuum pump and dried at 60 °C for 24 h for further analysis. The particles retained on the filter were then subjected to color and shape analysis, and the MP abundance in seawater samples was reported as particles of MP per liter of seawater (particles L^{-1}). MP particles were additionally characterized using a Micro-Fourier Transform Infrared Spectrometer (μFT-IR) (Jasco FT/IR-6600) for the qualitative identification of various polymer types using spectra in the range of 4000–600 cm^{-1} and analyzed using OPUS 7.5 spectral software. The plastic composition was determined using Bio-Rad's Informatics Spectroscopy Software and Spectral Database within the spectra library. Only spectra that demonstrated more than a 90% match with the standard database were considered acceptable.

4) Statistical analysis and quality control

Descriptive statistics (mean, standard deviation, and range) were employed to interpret the results. The analysis of variance (ANOVA) revealed significant differences between the means of independent groups ($p<0.05$). For quality control, blank samples were used throughout this study to monitor and account for potential contamination during analysis. Three replicates of seawater and zooplankton samples were used to assess the precision and repeatability of the analysis.

Results and discussion

1) Zooplankton abundance

The zooplankton density of the four collected zooplankton species across the two mesh sizes (20–300 μm and >300 μm) is presented in Table 1. Figure 2 illustrates examples of the zooplankton species identified in this study across different size ranges (20–300 μm and > 300 μm). The results indicated that among the zooplankton, copepods were the most prevalent, followed by bivalvia and fish larvae. Copepods are among the most abundant and diverse zooplankton groups in marine environments, distributed across the entire water column, particularly abundant in surface water [24]. The average densities of copepods using the 20–300 μm and >300 μm mesh net were 24.46±4.47 and 53.41±20.2 ind./L, respectively.

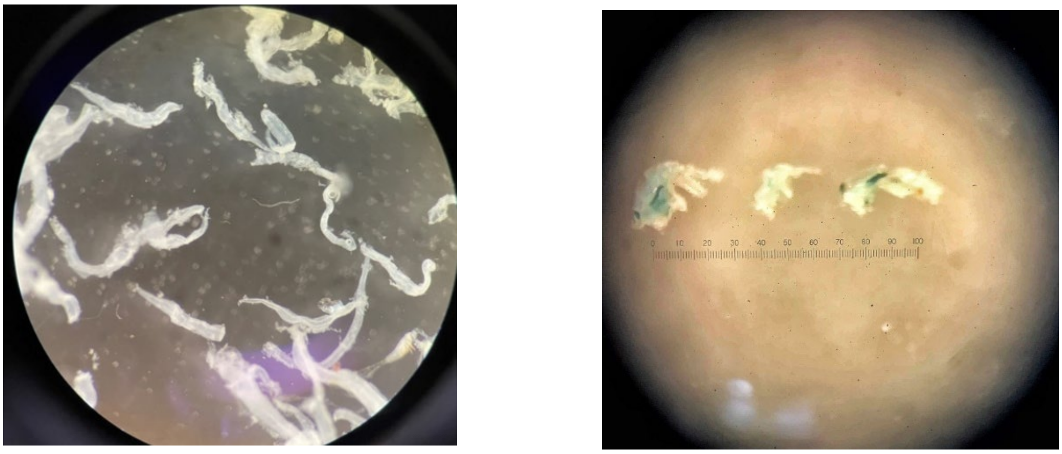
Table 1 Average density (ind./L) of four zooplankton species from the two mesh sizes (20–300 μm and >300 μm)

Zooplankton species	Density (ind./L)	
	20–300 μm	> 300-μm
Copepod	24.46±4.47	53.41±20.2
Fish larvae	0.78±0.53	9.85±4.72
Bivalvia larvae	15.81±3.42	16.28±8.97
Shrimp larvae	0.65±0.81	1.17±0.41

The results of this study align with those of another investigation where a positive relationship was observed between the abundance of fish larvae and copepods. In the previous research, 122 copepod species and a total of 96 fish species were identified in the northern Taiwan Strait associated with the hydrography [25]. In this study, the average densities using the 20–300 μm and >300 μm mesh nets of the bivalvia and fish larvae were 15.81 ±3.42 and 16.281±8.97 ind./L, and 0.78±0.53 and 9.854±4.72 ind./L, respectively.

A higher density of zooplankton was observed in the larger mesh sieve (>300 μm) in comparison to the smaller one, particularly with respect to copepods and fish larvae (Table 1). Zooplankton within a size range >300 μm was more likely to be retained by the larger mesh sieve during the sampling process due to the sieve's greater pore size, which effectively captured larger organisms while allowing smaller ones to pass through. However, the results of the present study contrast with those of Figueiredo and Vianna [26], who found higher MP abundance using a fine mesh net (64 μm net) compared to the 200 μm net. Wu et al. [27] found that the abundance of copepods collected by the 100 μm mesh plankton net was about two orders of magnitude higher than that collected in the East China Sea by the 330 μm mesh plankton net. Additionally, Garcia et al. [28] found that the average abundance of copepods in Brazil, using the 120 μm mesh plankton net, was five times higher than that of the 300 μm net.

a) Examples of zooplankton



b) % distribution of zooplankton composition

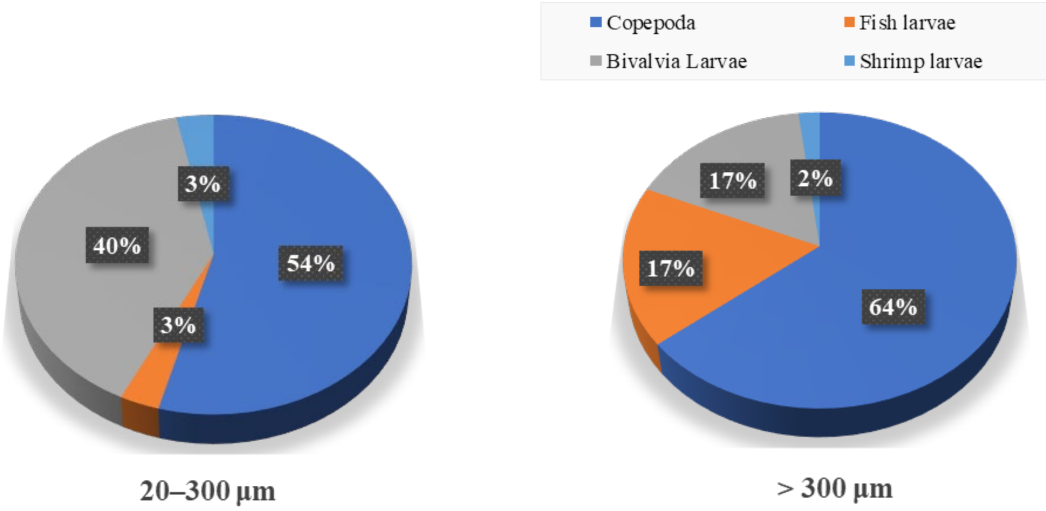


Figure 2 Zooplankton species identified in this study within different size ranges (20–300 µm and > 300 µm).

2) Microplastics in seawater

The seawater samples collected at the same stations as the zooplankton samples revealed the characteristics of MPs, including shape categories and color composition, as illustrated in Figure 3. In total, 256 particles of MPs were identified from the seawater samples at three stations (S1–S3) using the 20–300 µm and >300 µm mesh sieves, ranging from 37.8–77.1 particles L^{-1} (20–300 µm) and 12–67.8 particles L^{-1} (>300 µm) with an average concentration of 52.6±21.4 particles L^{-1} and 32.6±30.6 particles L^{-1}, for 20–300 and >300-µm sizes, respectively.

The dominant shape of MPs was found to be fiber, contributing >53% of the total MPs abundance in seawater. The other most abundant types were fragments and films, contributing 22% and 23% of the total MPs abundance, respectively. Fibers are a diverse category of synthetic plastic polymers commonly used in the production of clothing, curtains, personal hygiene products, and sanitary items. Another potential source of these synthetic fibers is wastewater from sanitary facilities [1–2].

MPs appeared in eight colors: black, transparent/white, red, yellow, blue, brown, green, and purple. Among these, transparent/white accounted for 62% and 25% of the total abundance for the 20–300 µm and >300 µm size categories, respectively. Black constituted 17% and 36%, and blue represented 8.4% and 12.6% for the 20–300 µm and >300 µm size categories, respectively. The results of this study are consistent with a prior investigation that examined the distribution of MPs in beach sediment on Mai Khao Beach. The previous study revealed a predominance of white (44.94%) and blue (23.60%) MPs, with a prevailing fiber shape (94.5%) [2].

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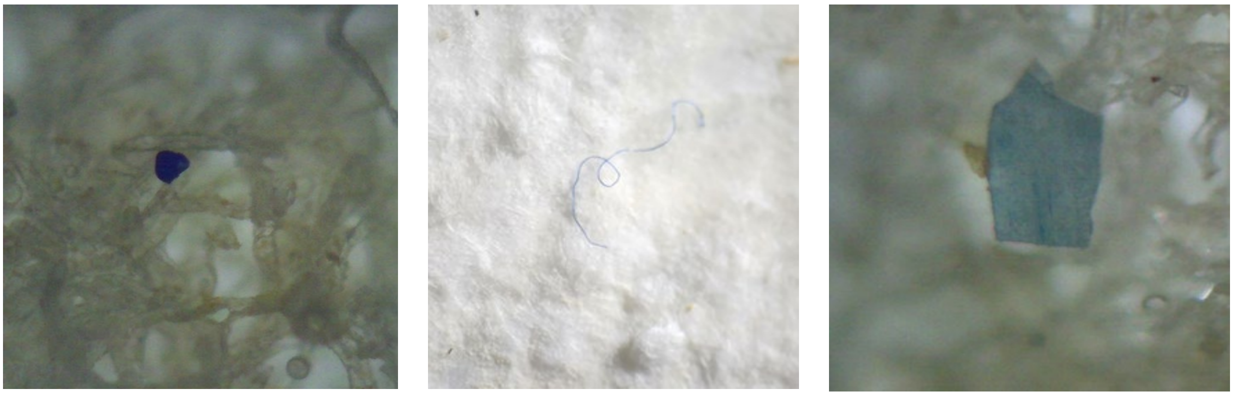
a) Images of different shapes of MPs (film, fiber and fragment)



b) % distribution of color composition

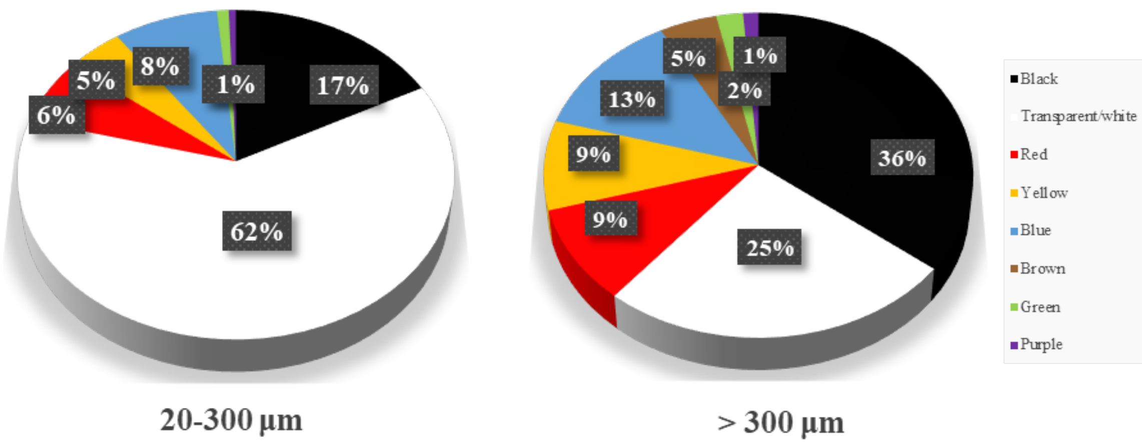


Figure 3 Characteristics of MPs found in the seawater samples a) shape categories of MPs and b) color composition of MPs within different size ranges (20–300 µm and > 300 µm).

Regarding polymer identification, from the samples subjected to µFTIR analysis, 80% were identified as PET, while the remaining samples consisted of PP, polyamide (PA), and polyether urethane (PU). PET and PP are widely used synthetic plastic polymers commonly found in disposable plastic containers, protective packaging, bottles, and trays. Additionally, PU is frequently utilized as a cushioning material in various consumer and commercial products. Since these polymers constitute the most prominent synthetic fibers in the world of fiber production, their existence is significant [29]. According to the results, the white/transparency, black, and blue fibers of MPs originating from PET and PP, as well as the presence of PA and PU identified in the seawater under study, can be attributed to anthropogenic origins since these materials were identified as polymers commonly used in consumer products [30].

Moreover, the results of the current study were compared with those of Sun et al. [11], who reported an average concentration of MPs in seawater equating to 0.13±0.20 pieces m^{-3}. The majority of these were in the form of fragments (42%), with the primary polymer types being PE and PP, accounting for 88.13%. The average concentrations of microplastics collected from the surface water of Lake Kallavesi, Finland, equated to 1.8±2.3 pieces m^{-3} (>300 µm), 12±17 pieces m^{-3} (100–300 µm), and 155±73 pieces m^{-3} (20–100 µm). These findings indicate that the majority of the identified microplastics were in the form of fibers (64%) and the remainder fragments [30]. In another study, Min et al. [31] reported an average microplastic abundance in the surface seawater from the south of Jeju Island, Korea, of 0.46±0.27 particles L^{-1}, with the majority consisting of fragments and fibers (69% and 31%, respectively).

3) Microplastics in zooplankton

Thirty individuals per zooplankton species (15 individuals per mesh size), comprising copepods, fish larvae, bivalvia larvae, and shrimp larvae, were selected randomly for MP analysis. Table 2 shows the average number of MP ingested per individual zooplankton. The findings indicate that every species examined had ingested MPs, with a total of 26 MP particles detected among the 120 digested zooplankton specimens. On average, each individual zooplankton had ingested 0.22±0.57 particles/ind. (mean ± standard deviation) of MPs, mainly present as fibers and fragments. The degree of MP ingestion displayed variation across species, with fish larvae demonstrating a comparatively elevated ingestion rate of 1.2±0.35 particles/ind. for sizes ranging from 20–300 µm and 1.8±0.62 particles/ind. for sizes exceeding 300 µm. Based on the average MP ingestion (particles/ind.), the ranking among species in the present study is as follows: fish larvae > shrimp larvae > copepods > bivalve larvae.

There is a similarity in size between MPs and phytoplankton, enabling their ingestion by zooplankton, transferring them from trophic level to trophic level [32]. A positive correlation between the size of MP ingested and the size of zooplankton has been reported [33]. When compared to the MPs in seawater samples, those ingested by zooplankton were considerably smaller. This emphasizes the zooplankton's preference for smaller-sized microplastics [34]. Notably, in the present study, despite the ingestion of larger MPs (>300 µm) by zooplankton being higher than that of smaller MPs (20–300 µm), the difference was not statistically significant ($p<0.05$).

Table 2 The identified microplastics and polymer types ingested by zooplankton species

Zooplankton species	**Average no. of MP ingested per zooplankton individual (particles/ind.±SD)**	
	20-300 µm	**>300 µm**
Copepod	0.6±0.56	1.3±0.41
Fish larvae	1.2±0.35	1.8±0.62
Bivalvia larvae	0.6±0.35	1.4±0.35
Shrimp larvae	0.9±0.59	2.0±0.41

In comparison to studies examining MP ingestion in multiple zooplankton species, Niyomthai et al. [35] investigated the ingestion of MPs by three distinct groups of zooplankton—calanoid copepods, chaetognaths, and shrimp larvae—on Pak Meng Beach in Trang Province, Thailand. The study found that shrimp larvae had the highest abundance of ingested MPs, with an average of 0.70±0.10 particles/ind. In a study by Zavala-Alarcyn et al. in 2023 [36], the ingestion rate of MPs by zooplankton collected from Manzanillo Bay and Navidad Bay in the central Mexican Pacific was observed to be approximately 0.02 particles/ind. for copepods and 0.005 particles/ind. for fish larvae, with the average length of the ingested particles measured at 468.1±113.8 µm. Among the ingested MPs, fragments constituted the highest proportion at 54.2%, followed by fibers at 34.2%. A study carried out by Aytan et al. [37] focused on copepods in the Black Sea, investigating their ingestion and egestion of MPs. The analysis of 351 fecal pellets revealed the existence of four microplastic particles. Among the MPs ingested, fragments were the most identified type, followed by films and fibers, while the colors of the ingested particles included black, blue, and red. In Sepanggar Bay, Sabah, Malaysia, MP ingestion was more pronounced among higher trophic zooplankton, while fibrous and small MPs were highly ingested by zooplankton [38].

The ingestion of MPs and the corresponding polymer types by these zooplankton species are detailed in Figure 4. The µFT-IR analysis confirmed that ingested particles consisted of either PET, PE, polyester, Urea-formaldehyde (UF), PA, and other types. These plastic types are extensively used in various applications. For example, polyester serves as a prevalent textile fiber, UF is a thermosetting resin in laminates and coatings, and PA is used in textiles, carpets, ropes, and automotive components [39]. The majority of identified polymers in zooplankton were found to be PET, in similarity to those present in seawater. Figure 4 shows the polymer composition of MPs found in zooplankton samples. PET, a crucial material used in the production of food containers, plastic bottles, plastic bags, ropes, fishing gear, and the textile industry, was among the detected microplastic types. Insufficient waste management and the discharge of wastewater into the sea can lead to the introduction of these types of plastic into the environment. It has been reported that the polymer types identified in zooplankton samples are consistent with those found in Thai waters [40]. Fish have been reported to ingest PET and rayon, commonly used in clothing, beverage containers, and food packaging [41]. Furthermore, PET and PP have been mostly detected in cyclopoid copepods and calanoid copepods at Ko Sichang, Chonburi Province [40]. Hence, anthropogenic activities along Mai Khao Beach, such as those exhibited by restaurants at S1, wastewater discharge, and the tourist hotspot at S2, could result in plastic discharges, leading to microplastic contamination in seawater and subsequent transfer to higher trophic levels, including zooplankton. Overall, these findings suggest a potential transfer of microplastics from seawater to zooplankton, with fish larvae showing a higher propensity for ingestion. The identification of specific polymers through advanced analysis provides insight into the sources of microplastics, enabling targeted intervention strategies for pollution mitigation.

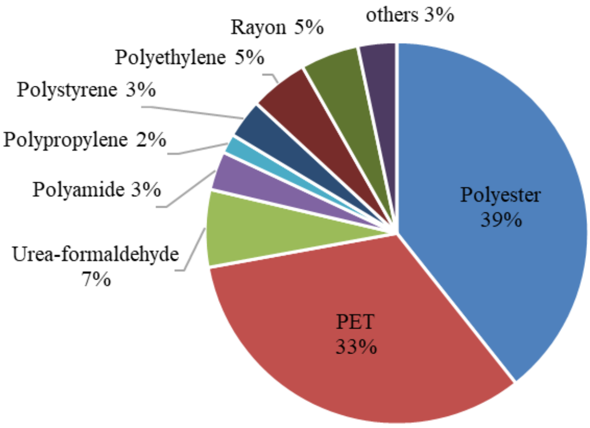


Figure 4 Identified MP polymeric proportions in zooplankton species.

The results of this study have been compared with those of other research identifying the predominant polymeric types of MPs in both seawater and zooplankton from the Yellow Sea. These studies have reported that PP and PE constitute the major polymer types, accounting for 88.13%. Fiber is the dominant shape of MPs found in zooplankton (46%), with the average size being 154.62±152.90 µm [13]. In addition, six different types of polymers—polycarbonate (PC), polycarbonate and polypropylene (PC-PP), PET, PP, low-density polyethylene (LDPE), and polypropylene-polystyrene (PS)—were the most abundant MPs ingested (42.8%) from five taxonomic groups of zooplankton in two bays of the Mexican Central Pacific [36]. Overall, these findings suggest a potential transfer of MPs from seawater to zooplankton, with fish larvae showing a greater propensity for ingestion. The identification of specific polymers through advanced analysis provides insight into the sources of MPs, enabling targeted intervention strategies for pollution mitigation.

Conclusion

This study highlights the presence of MPs in seawater and their ingestion by zooplankton on Mai Khao Beach, Phuket, Thailand. The analysis of MPs in seawater revealed the presence of 256 MP particles, notably with higher concentrations observed in the 20–300 µm class. The size of microplastics ingested had a positive correlation with that of zooplankton. Fish larvae showed the highest MP ingestion, likely due to the similarity in size between MPs and zooplankton. The findings demonstrate the transfer of MPs to the zooplankton, underscoring the critical need to address microplastic pollution in marine ecosystems, particularly in ecologically significant areas like Mai Khao Beach. To understand the long-term effects of MP ingestion on zooplankton and its impact on the marine food web, further research is essential. Such research should focus on both the spatial and temporal distribution of MPs, enabling informed decision-making for conservation and strategies to support the sustainable management of marine ecosystems.

Acknowledgment

The authors would like to express their sincere thanks to the National Science and Technology Development Agency (NSTDA) for its financial support. We would also like to extend our appreciation to the Faculty of Technology and Environment, Prince of Songkla University, Phuket Campus, for providing access to facilities and scientific equipment and Personal Research Funding under Walailak University (Grant No. WU 66249).

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https://doi.org/10.35762/AER.2024007