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Low occurrence of microplastic contamination in anchovies, a transboundary species, in Thai waters

Siriporn Pradit[a,*], Prakrit Noppradit[a], Karnda Sengloyluan[b], Thongchai Nitiratsuwan[c], Ong Meng Chuan[d], Prawit Towatana[a]

[a] Coastal Oceanography and Climate Change Research Center, Faculty of Environmental Management, Prince of Songkla University, Songkhla 90110 Thailand
[b] Rubber Engineering and Technology Program, International College, Prince of Songkla University, Songkhla 90110 Thailand
[c] Faculty of Science and Fisheries Technology, Rajamangala University of Technology Srivijaya, Trang 92150 Thailand
[d] Faculty of Science and Marine Environment, Universiti Malaysia Terengganu, Terengganu 21030 Malaysia

*Corresponding author, e-mail: siriporn.pra@psu.ac.th

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ABSTRACT: In this study, size capture determination of the anchovies in the Gulf of Thailand (GoT) and the Andaman Sea showed length ranges of 6.00–9.90 cm and 3.50–12.00 cm and weight ranges of 2.23–8.00 g and 0.34–12.09 g, respectively. The occurrence of microplastics (MPs) in anchovies were investigated by randomly sampling 100 individual anchovies (GoT = 50, Andaman Sea = 50) from 2670 samples. The samples were digested with 10% potassium hydroxide for the analyses of types and amounts of MPs present. Surprisingly, a low level (approximately 8%) of MPs was found, and the size of the MPs was less than 500 μm. All found MPs were of a fragment type with only three types of polymer (polyethylene, polyester, and rayon) identified by FT-IR spectrophotometer. The low occurrence of MP ingestion in the anchovy samples in our study was probably caused by: (1) the dilution effect of the schooling behaviour of anchovies diluting the amount of MPs ingested by a single individual; (2) the dilution effect depending on the seaward distance from the shore (point source of MPs); and (3) the short residence time (6–12 months) of the anchovies living in the ocean gathered by our study. As a result, the low accumulation of MPs was found in the anchovy samples. The appearance of MPs in anchovies is an alarming finding for food security and sustainability in Thailand.

KEYWORDS: Gulf of Thailand, Andaman Sea, anchovy, microplastics, contamination

INTRODUCTION

Marine litter and MPs are currently a serious problem affecting marine and coastal ecosystems and resources, socio-economic aspects, marine tourism as well as seafood security with the increasing amount of plastic waste being dumped into the ocean. The global demand for incremental annual plastic utilisation has been growing constantly due to its convenient properties and the increasing population in recent years [1]. Plastic debris is ubiquitous in the oceans of the world; it is estimated that at least 5.25×10^{12} plastic items weighing 2.7×10^{5} tons are currently floating in the oceans and seas [2]. Thailand is ranked as the 6th largest in the world for marine waste disposal [3, 4]. Despite a number of constantly enforced monitoring and reducing marine litter programmes from many regional and international organizations: the United Nations Environmental Programme (UNEP), the G20, the G7, the European Marine Strategy Framework Directive [5], the Regional Seas Conventions' Action Plans (e.g., OSPAR Regional Action Plan for Marine Litter; UNEP/MAP-Barcelona Convention Regional Plan on Marine Litter Management in the Mediterranean), the harmful effects of MPs in biota are still far from being understood [6]. The impacts of MPs, or plastic litter, on the marine environment has long been the subject of environmental research [7].

MPs are generally described as small plastic particles of < 5 mm in diameter [7, 8]. MP ingestion may occur directly due to the misidentification and subsequent consumption of MPs as feeding [9]. In a marine environment, MPs are typically found as pellets, fragments, or fibres and consist of diverse polymers [10]. The most commonly used and abundant polymers are high-density polyethylene (HDPE), low-density polyethylene (LDPE), polyvinyl chloride (PVC), polystyrene (PS), polypropylene (PP), and poly(ethylene terephthalate) (PET), which all together account for approximately 90% of the total plastic production worldwide [11]. MPs accumulation in organisms has the potential to cause various adverse effects on health, such as increase in mortality rate, reduction in feeding activity, and inhibition of growth and immunity [1]. Furthermore, MPs have the ability to absorb persistent organic pollutants (POPs) or heavy metals dissolved in seawater. Plastics contaminated by POPs are found globally from the coastal areas to the remote habitats of subtropical gyres [12]. MPs and co-contaminants have the potential to affect the fishery industries and theirs sustainability, increasing pressure on the already threatened fish stocks [13]. Over the time, biofouling occurs on these microplastic pieces, resulting in an increase in their metal absorbing ability. Similarly, the metal absorbing ability of microplastic debris increases with time [4]. Furthermore, a study of biofouling as a source of metal pollution conducted by Bighiu et al [14] showing that the uptake of MPs and absorbed co-contaminants by fish represents a risk to human health since these contaminants may be transferred to fish tissues and eventually humans through consumption.

Fisheries in Thailand comprise both commercial and small-scale ventures, contributing to approximately 2.4 million tons to the world's total fishery production in 2017, of which 1.3 million tons came from marine resources [15]. Marine fisheries are an economically important sector for the livelihood of Thai people, especially the fishermen in the Gulf of Thailand (GoT) and the Andaman Sea [16]. Thailand has a tropical climate with a high diversity of fish and more than 20 types of fishing gear [17]. Thailand's marine fishery resources in the GoT and the Andaman Sea have been categorised into three separate species groups: (1) demersal; (2) pelagic; and (3) anchovies. Anchovies, central to the delicate food chain in marine and ocean ecosystems, spread globally from the islands of Hawaii, Japan, Tahiti, Korea, Australia, and Papua New Guinea to the coastal areas of ASEAN countries including islands in the Indian Ocean, the Arabian Sea, and the Red Sea. Anchovies are small pelagic fish species and commonly range 7–8 cm in length. In 2018, the total production of anchovies in Southeast Asia was about 410 811 metric tons and valued at about 1 billion USD [18]. Indonesia, Thailand, the Republic of Philippines, Myanmar, and Malaysia are the main producers of anchovies in the Southeast Asia Region, using fishing gear such as the anchovy purse seine, anchovy lift net, and anchovy falling net. In Thailand, anchovies can be found all over the coasts and islands in the GoT and the Andaman Sea within the country's territory. For the east coast, its distribution extends along the coast of the GoT, and the west coast to the border with Malaysia. Anchovies are used mainly as raw materials for fish processing, e.g., boiled-dried anchovy and fish sauce, which are meant for both domestic consumption and export. Therefore, the anchovy is economically important in Southeast Asia.

Small and semi-pelagic fish species, such as anchovies, have high economic impact globally and contribute to the bulk of biomass. They are present at the low and middle trophic levels and play a crucial role in pelagic food web in the marine system [19]. Anchovies found in Thai waters belong to family Engraulidae (mostly genus *Encrasicholina* and *Stolephorus*). Little is known about the ingestion of MPs in anchovies. Thus, the objective of this study is to investigate the occurrence of MPs in anchovies from Thai waters.

MATERIALS AND METHODS

Fish sampling and sample preparation

Anchovies in Thai waters are caught by local fishing boats and later sold to fishmongers at the Chumpon and Trang fish markets (personnel communication). A sampling of fish from the two sites was carried out at the beginning of January 2020. A total of 2670 anchovy samples were randomly bought from various fishmongers at the Chumphon and the Trang fish markets (1537 and 1133 fish samples, respectively) without identifying the fish species. The fish samples from the two sites represented the anchovies in the GoT and the Andaman Sea, respectively (Fig. S1). The samples were immediately kept in an icebox, transported from the fish markets to the Prince of Songkla University laboratory, and then frozen until further uses.

MPs extraction

Random sampling of 100 (GoT = 50, Andaman Sea = 50) individual anchovies from the aforementioned 2670 samples was carried out. Each sample was defrosted and cleaned (Fig. 1a) with distilled water and deionised water (to make sure that no more MPs left in the fish body) before recording the length (cm) and weight (g). Afterward, the entire body of the fish was digested in 10% potassium hydroxide (KOH) and placed in a 250 ml conical flask. Subsequently, 150 ml of 10% KOH solution was carefully added before sealing the flask with aluminium foil and leaving it for 12 h at room temperature for the assimilation process. The conical flask was then heated on a hotplate at 60 °C for 12 h with 1- to 2-min manual shaking at 2-h intervals to remove all organic matter (Fig. 1b). At the end of the 12 h, the obtained solution was immediately filtered (Fig. 1c) through a Whatman GF/F filter (pore size = 0.7 μm). After that, the filter paper was removed and placed into a clean petri dish to completely dry in an oven at 50 °C to be later used for visual identification of MP particles (debris size < 5 mm). Deionised water was used during sample preparation, digestion, and MP identification. Blank was carried out by filtering the distilled water from an 8 cm petri dish filled with distilled water and placed next to the working zone with no observed MP.

Polymer identification

The samples were visually observed under a microscope (Olympus SZ61, Japan; Fig. 1d). The morphotypes of microplastic particles were classified into fibre, fragment [20], and other shapes. To characterise and identify polymer types, the spectra of the suspicious polymer particles less than 1 mm were obtained from micro Fourier transform infrared (μFTIR) spectrometer (Fig. 1e), Frontier model, coupled with a Spotlight 200i FTIR microscope (Perkin Elmer, USA). Meanwhile, suspicious samples with dimensions larger than 1 mm were identified using a Frontier FTIR

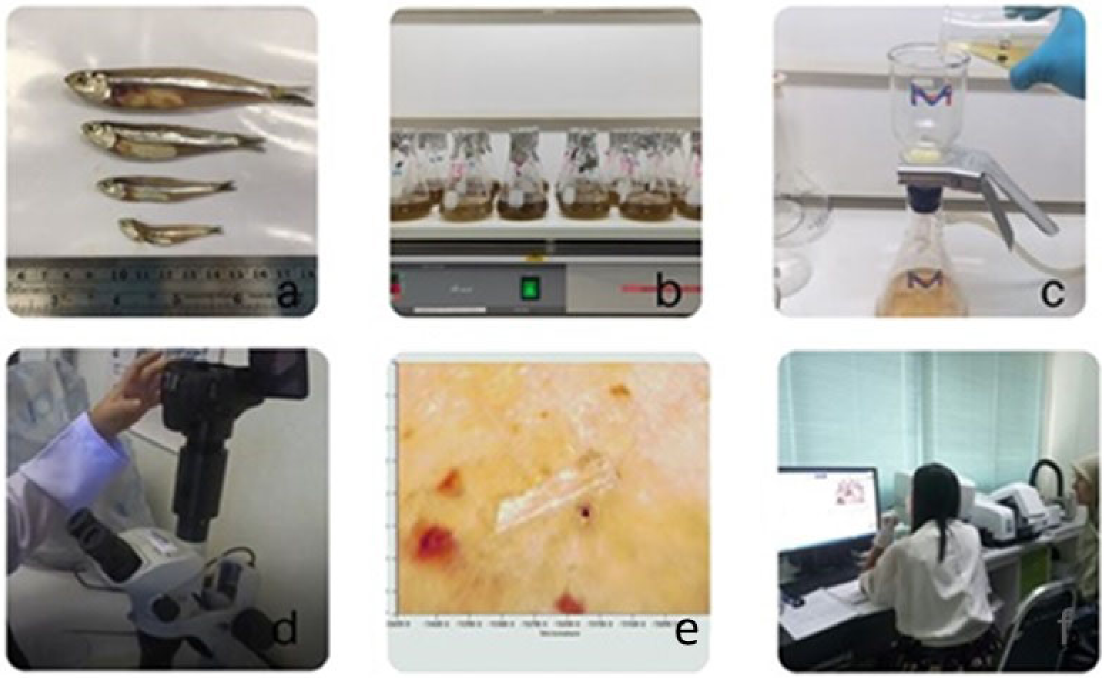

Fig. 1 MP analysis process: (a), anchovy samples; (b), digestion with KOH; (c), filtration; (d), microscopic identification of the MP; (e), MP image; (f), identification by μFTIR analysis.

spectrometer with the attenuated total reflection (ATR) technique. The sample spectra were identified by comparing with the referent polymer spectra in the spectral library obtained from the FTIR programme.

RESULTS AND DISCUSSION

Determination of length-weight relationship

The length of the anchovy samples obtained from the Andaman Sea and GoT showed a positive relation with weight with a correlation coefficient (R^2) of the Andaman Sea samples ($R^2 = 0.78$) higher than that of the GoT ($R^2 = 0.37$) (Fig. 2a,b). The possible explanation for this characteristic is that anchovies from the Andaman Sea have less species diversity than those from the GoT, and therefore the former with less species diversity would likely result in higher length-weight relation than the later.

For the relationship between length and weight with the frequency of occurrence, a normal distribution (bell curves) was observed around their mean values, and the bell curves of the GoT had more narrow characteristics than those of the Andaman Sea. If assuming that the species diversity of anchovies in both the GoT and the Andaman Sea were low, the more narrow bell shaped curves of the GoT were probably caused by the smaller age difference among individual anchovies in the schools than those of the Andaman Sea.

Anchovies grow rapidly after hatching and reach 3 cm in length approximately 56 days later. During the first 1–6 months, anchovies increase in length by an average of 8 mm per month. The length increasing rate decreases at an average of 3.8 mm per month during the subsequent 6–12 months. The average age of anchovies is 1–1.2 years. Therefore, they are suitable for fishery purposes at the age of approximately 7–9 months. Hence, the average length of anchovies obtained from our study was assumed based on the ages of 6–12 months.

Occurrence of MPs in anchovies

Surprisingly, pieces of plastic debris were only found in 8 out of the 100 investigated samples (8%), and all were MPs (Table 1). Although all the sizes of MP particles (debris size < 5 mm) were investigated from the 100 samples, only MPs with a size less than 500 μm were found, which was consistent with the study of Tanaka and Takada [21]. They found that over 80% of the MPs size was < 1000 μm, and more than half was < 500 μm. Nine MPs were found in eight of the ten analysed livers of *E. encrasicolus*. Their sizes ranged from 124 μm to 438 μm with an average of 323 μm (± 101 μm). The unexpectedly tiny MP fragments found in the anchovy samples in this study were probably caused by the feeding behaviour of the fish, which fed mostly on phytoplankton and zooplankton; and, therefore, accidentally engulfed the very tiny MPs suspended among the plankton. This is

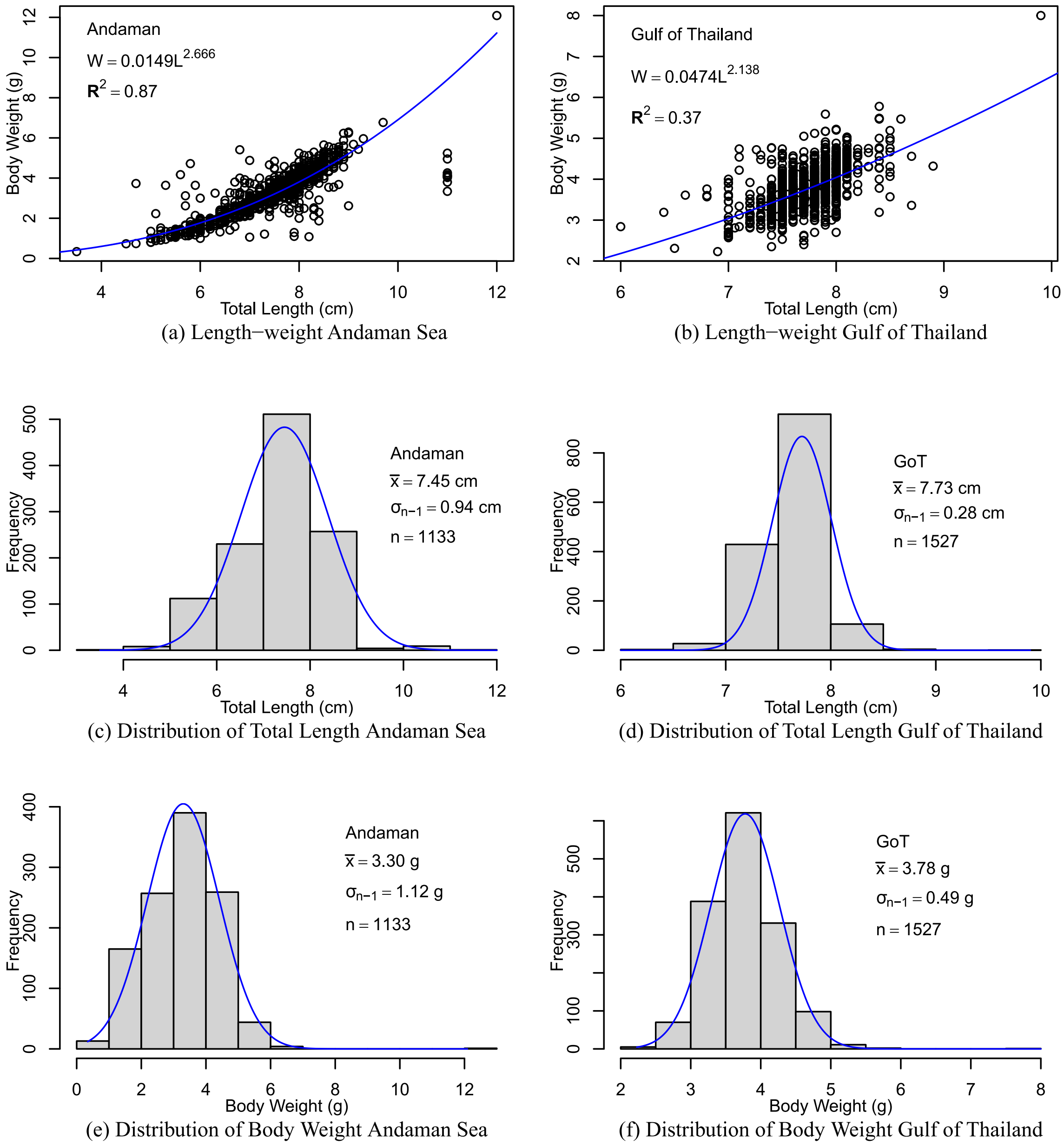

Fig. 2 Length-weight relationship of the anchovy samples obtained from the Andaman Sea and from the Gulf of Thailand.

Table 1 MPs found in anchovies ($n = 100$) from 2 sites in Thai waters (the Gulf of Thailand and the Andaman Sea).

Area	Batch no.	Fish examined	Fish with MPs	Number of MPs (pieces)	Remarks
GoT	1	12	3	3	Polyethylene (2), Rayon (1)
	2	14	1	1	Rayon (1),
	3	12	1	1	Polyester (1)
	4	12	1	1	Rayon (1)
Andaman	1	12	2	2	Polyethylene (1), Rayon (1)
	2	14	0	0	
	3	12	0	0	
	4	12	0	0	
Total		100	8	8	

agreed well with the characteristics of anchovies since they are small-sized fish and thus can only feed on small-sized prey and food corresponding to the small size of their mouths [22]. Furthermore, this is the first evidence of the MP debris found in the anchovies in Thai waters since there has been no available published data concerning the MPs ingested by anchovies in Thai waters. Thus, further studies should be carried out to investigate this matter. Planktivorous fish are particularly susceptible to accumulating microplastic with similar size, shape, and colour to their prey [23, 24].

The amount of ingested MPs (6 pieces) in anchovies from the GoT was more than that (2 pieces) from the Andaman Sea (Table 1). This was probably caused by the topographic factor regarding the different slope characteristics of the coastal areas between the GoT and the Andaman Sea. The coastal area of the Andaman Sea comprises a steep slope (submerged shoreline), whereas the GoT has a gentle slope (emerging shoreline). Hence, at the same distance from the shore, the Andaman Sea has higher seawater volume than that of the GoT to dilute the MP concentration in the seawater, resulting in a lower amount of MPs found in anchovies from the Andaman Sea. Another possible explanation is the different current characteristics between the GoT and the Andaman Sea. The current of the GoT in a shallow semi-enclosed bay on the continental shelf of Asia certainly has lower dilution capacity of MPs contamination than that of the Andaman Sea with the current of open sea on the steep continental shelf. The two aforementioned factors probably reduce the chance of up-taking MPs in the contaminated seawater by anchovies from the Andaman Sea compared with that from the GoT.

Identification of MPs

FTIR identification revealed 4 types of polymers including polyethylene (3 pieces), rayon (4 pieces), and polyester (1 piece) (Table 1, Fig. 3). All of the 8 MPs found were in fragment form. Non-MPs, such as natural fragments and palm tree, were also found. All samples were observed to have a strong vibration peak at 1030 cm^{-1} of Si—O—Si bond that might have been formed from other contaminants or the glass filter used in the sample preparation. Absorption peaks of $-CH_2$ symmetric stretching and asymmetric stretching were found in all the samples. Polyethylene (PE) showed an additional peak of about 1470 cm^{-1} of —CH bending, a characteristic peak of PE [25]. Additional peaks might come from other contaminations. Rayon is a cellulose fibre generated from natural sources of cellulose and synthetic cellulose fibres. Rayon fibre is commonly used to produce artificial silk and other textiles. In the study, characteristic peaks of rayon fibre were found at 1738 cm^{-1} and 1420 cm^{-1}, referred to as C=O stretching and $-CH_2$ and —O—CH— bending [26]. Polyester was found in one sample showing a strong vibration peak of C=O bond in a polyester structure at 1710 cm^{-1} [27]. Other vibration peaks were dependent on the chemical structures of polyester that could not be specifically identified by the FTIR technique. Rayon particles may originate from clothing lint in laundry. Pradit et al [4] found 5 polymer types including polyester and rayon in the stomachs of fish (*Arius maculatus*) from Songkhla Lake, a lagoon in the coastal region of Thailand.

Possible explanation of low occurrence of MPs

The MPs found in this study had a low level of occurrence compared with previous research in other areas (Table 2). In our study, the amounts of MPs present in anchovies were much lower than those in the Tokyo Bay [21], the Mediterranean Sea [28], and the Lombok Island [29]. However, our results were consistent with the low-level occurrence of MPs in anchovies (*Engraulis ringens*) in Chile reported by Ory et al [30]. High incidences of MPs present in fish are often found in waters near populated areas where MPs are abundant [30]. A low concentration of MPs was found in this study, and all of the MPs were the fragment type. Three possible explanations responsible for the low occurrence of MP ingestion in the anchovy samples in our study are as follows:

(1) Dilution effect is caused by the lifestyle behaviour of anchovies since it is well known that open-water fish like anchovies usually travel in large schools for protection. When threatened, a school of thousands of anchovies spreading out over several hundred meters will contract into a writhing sphere with only a few meters across, thereby obstructing the attempt of a natural predator to hunt a single individual. This schooling behaviour can definitely dilute the number of MPs able to be ingested by individual anchovies.

(2) The dilution effect is influenced by seaward distance from the shore. Almost all plastic wastes and their fragments, including MPs, originate from human activities in the terrestrial environment. Eventually, they are commonly transported and reach their destination at sea. Hence, activities around highly populated coastal areas can be a significant source of plastic debris including MPs. This means that the amounts of plastic wastes and MPs are high near the shore and gradually decrease seaward depending on the distance away from the coast. The habitants of anchovies in both the GoT and the Andaman Sea in this study are far away from shore (3 km). Consequently, low amounts of MPs suspended with anchovy food in sea water are available for ingestion by schools of anchovies in accordance to the dilution effect of seaward distance from the shore.

(3) The average age of the anchovy in this study, based on the average length of the anchovy samples, was 6–12 months old. Thus, the 6–12 month residence time of the anchovy samples from the two local fish

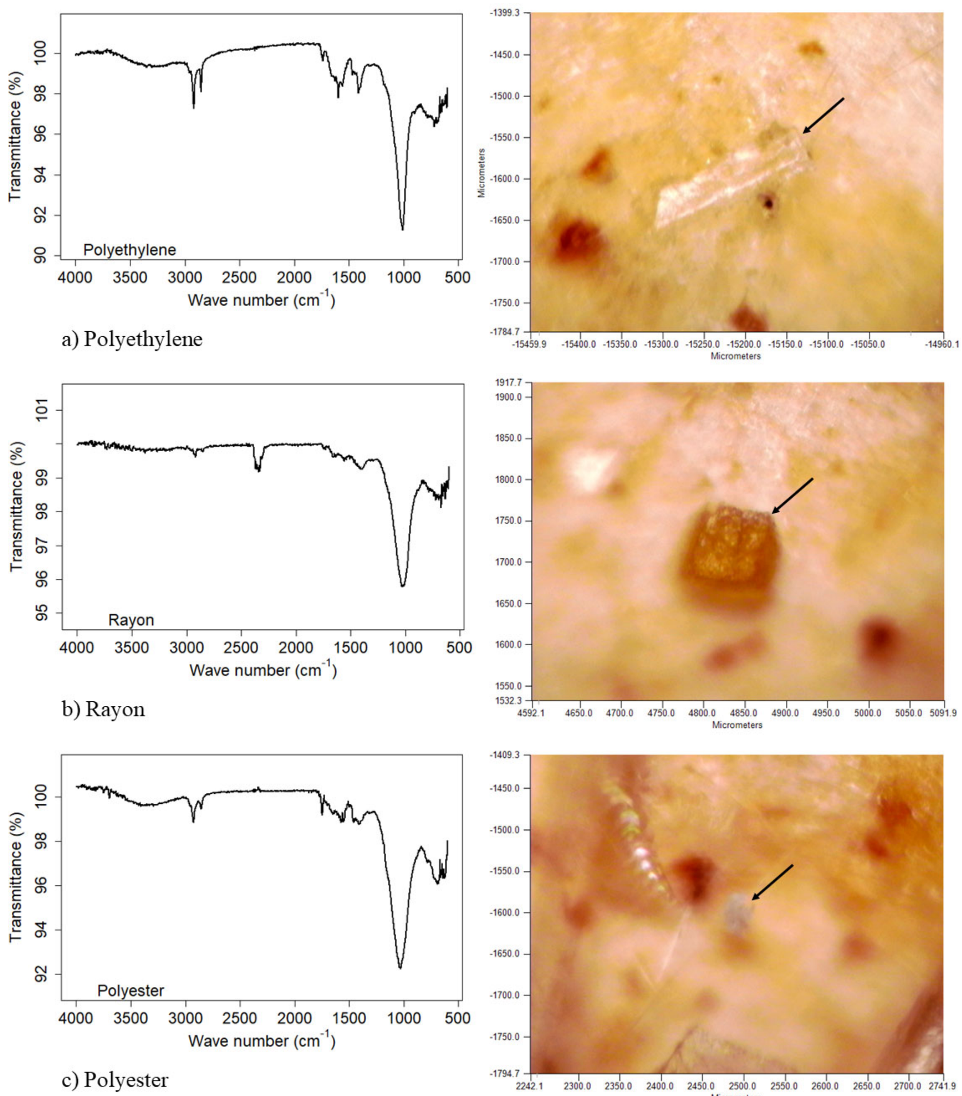

Fig. 3 FTIR spectra and photographs of microplastic fragments: (a), Polyethylene; (b), Rayon; and (c), Polyester.

Table 2 Microplastic ingestion of anchovies and other pelagic fish in Thai waters (the Gulf of Thailand and the Andamen Sea) and other areas.

Species of Anchovy	Location	Type	Polymer type	Length of fish (cm)	MPs (%)	Reference
Engraulis japonicas	Tokyo Bay	Fragment (86%) Bead (7.3%) Filament (5.3%)	PE (52%), PP (43.3%), Polystyrene		77	[21]
Engraulis encrasicolus	Mediterranean Sea		PE, a copolymer of styrene and acrylonitrile	12.4 ± 1.0	80	[28]
Engraulis ringens	Southeast Pacific Ocean: Chile	Fragment	PE, PP	15.0 ± 0.2	7.7	[30]
Stolephorus spp	Lombok Island, Indonesia	Fibre, Fragment, Film, Foam	PE, PP, Polystyrene, polyester	7.59 ± 1.68	88	[29]
Family Engraulidae	Thai waters (the Gulf of Thailand and the Andaman Sea)	Fragment	PE, rayon, polyester	7.45 ± 0.94 (Andaman) 7.73 ± 0.28 (GoT)	8	This study

markets was fairly short compared with the commercial fish, which might have a few years of residence time before being caught. It is recognised that the longer residence time of the fish living in the sea results in the higher accumulation of MPs ingested by fish. Thus, the low amount of MPs present in the anchovy samples was attributed to the short residence time in the sea of the fish, resulting in low MP accumulation.

CONCLUSION

Concerning the occurrence of MP contamination in the anchovies in this study, it was shown to be a relatively low concentration. However, further studies should be conducted to monitor the amounts of MPs in other aquatic animals as well since they could pose potentially harmful effects on the health of aquatic organisms, ecosystems, and humans. It is well known that anchovies are important to the bottom-middle trophic level of the food web. Therefore, not addressing the MP contamination of anchovies will certainly affect our food security.

Appendix A. Supplementary data

Supplementary data associated with this article can be found at http://dx.doi.org/10.2306/scienceasia1513-1874.2022.069.

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Appendix A. Supplementary data

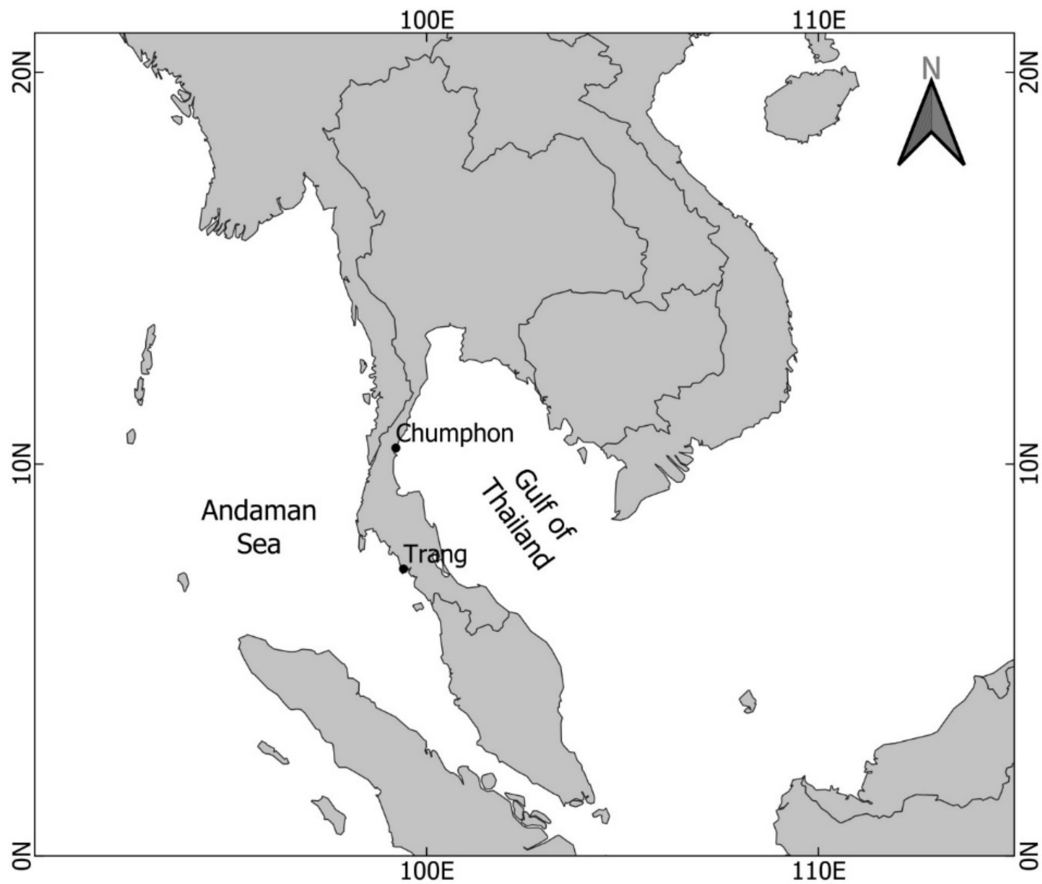

Fig. S1 Sampling locations at the Chumphon fish market (GoT site) and Trang fish market (Andaman Sea site).