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Microplastic accumulation in local dominant shellfish from the Khwae Noi Basin in Western Thailand and its environmental factors

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ABSTRACT: Microplastics (MP) have accumulated in environmental components including aquatic organisms. In this study, we investigated MP accumulation in the local dominant shellfish, i.e. 150 individual assassin snails (*Anentome helena*) and 60 individual clams (*Pilsbryoconcha exilis compressa*), sampled from 39 sites in headstream, midstream and downstream areas of the Khwae Noi Basin in western Thailand. MP accumulation was found to be only the filamentous type in approximately 26.0% and 38.3% of the occurrences in assassin snails and clams with averages of 0.5±1.0 and 2.5±3.3 pieces/individual, respectively. MP colors found in assassin snails were blue and black with 54.8% and 45.1% occurrences, and those found in clam were blue and green with 64.5% and 35.4% occurrences, respectively. Principal Correspondence Analysis (PCA) demonstrated that MP accumulation was associated with 9 environmental factors; for example, downstream, land-use area and population size were positively related to MP accumulation, whereas headstream, elevation and pristine area were negatively related to accumulation. Besides, Sorensen Distance of 65.0% similar index was classified into three groups of MP accumulation in the shellfish. Fourier-transform infrared analysis illustrated four types of MP accumulation in the shellfish, comprising polydimethylsiloxane, polyamide, polyester, and polyvinyl chloride. All MP types found in the shellfish were related with those found in water and sediment.

KEYWORDS: microplastic accumulation, freshwater shellfish, FT-IR, water pollution, sediment

INTRODUCTION

Microplastics (MP) are distributed all over the world and have contaminated the environment including water, soil, and organisms with various sizes, colors and types. MP are generally defined as small plastic particles less than 5 mm, and are classified to primary MP and secondary MP [1], and are threatening as micro-pollutants in terrestrial and aquatic environments. A small amount of the MP pollution in the environment occurs through domestic wastewater. These are primary MP, which are ingredients in some products; e.g. face and skin cleanser, toothpastes, and shower gels and cosmetics [2]. But, most are secondary MP fragments or fibers degraded from large plastic debris, aided by external factors such as UV radiation, photodegradation, and plastic expiration as well as plastic discharges from industrial, commercial and agricultural activities [1].

Increased human population and anthropogenic activities inevitably cause MP pollutants in the environment [3]. MP accumulation in aquatic fauna is generally internal as a result of oral ingestion, filter feeding, respiration, and dermal contact with water and sediment [4]. Many studies have revealed that benthic invertebrates, fishes, and aquatic mammals are at risks for MP accumulation, which can cause a blockage of the digestive tract and inflammatory responses [5]. Also, many studies have suggested that MP accumulation in aquatic invertebrates can be used as a bio-indicator for assessing the degradations of habitats in aquatic ecosystem by anthropogenic activities [6].

Recently in Thailand, MP accumulations in aquatic organisms in marine and estuary habitats [7–9], have been discussed in approximately 45 publications (from 2017 to 2022), while those in freshwater habitats are found in only about 6 publications (from 2020 to 2022). MP accumulations have been reported in two rivers in northeastern Thailand, i.e. in eight species of fishes in Chi River [10], and two species of fishes and two species of snails in Nam Pong River [11], as well as in one river in southern Thailand; i.e., two brackish bivalve species in Tapi Phumduang River [12]. Also, MP accumulation has been reported in 14 species of fishes in Ubolratana reservoir [13], *Pantala* sp. (Libellulidae, Odonata) in paddy fields [14], and in *Litopenaeus vanamei* and *Macrobrachium rosenbergii* in culture ponds [15]. However, the number of studies of MP accumulation in aquatic animals is very few when compared to the diversity of Thai freshwater species. To our knowledge, there have been no studies of MP accumulation in any native shellfish in rivers in western Thailand in conservation areas (national parks and wildlife sanctuaries) or in disturbed areas (land-used), or on the correlation of MP accumulation with environmental factors.

The Khwae Noi Basin is an important river system in western Thailand. It provides valuable natural resources, including biological diversity and habitats; e.g. freshwater fishes, aquatic insects, zooplankton, and phytoplankton, all involved in complex food webs [16]. Mammals and other terrestrial organisms utilize aquatic resources of the basin through ecosystem interactions. MP accumulation in aquatic invertebrates can be used as a bio-indicator for assessing the degradations of habitats in aquatic ecosystems by anthropogenic activities. In this study, MP abundance, shapes, sizes, colors and types were investigated in two locally dominant shellfish, the assassin snail, *Anentome helena* (von dem Busch, 1847) and the clam, *Pilsbryoconcha exilis compressa* (Martens, 1860), as well as in water and sediment at headstream, midstream and downstream locations within high to low elevations of the Khwae Noi Basin. MP abundance and its correlation with environmental factors also were studied. These dominant shellfish might be useful as bio-indicators of MP contamination in freshwater ecosystem.

METHODOLOGY

Study area

This study was carried out on the Khwae Noi Basin, located between 500000 to 1658326 and 391554 to 1437349 (Universal Transverse Mercator Co-ordinate System, UTM), which is one of the largest river systems in Thailand, comprising 1st–5th order streams and approximately 353 km^2 (Fig. 1). Many tributaries provide a diversity of micro-habitats for many species of shellfish. Dominant shellfish species include the assassin snail, a famous ornamental water snail, and clams, an importantly local human food in Thailand and elsewhere in Southeast Asia. These species have high abundance, co-exist in the same habitat, and are distributed throughout from headstream to downstream.

Microplastics samplings and preparations

Shellfish

Assassin snails and clams were selected for this study based on their different feeding behaviors and habitats. Assassin snails are a benthic aquatic predator and scavenger on live snails and decaying organic material. It is the top carnivore on water snails and inhabits various substrates in small riffle areas where it feeds on very small organisms and scrapes or brushes particles from surfaces of small rocks and substrate sediments in the river. It is a commercially valuable ornamental water snail. In contrast, the clam represents a filter feeder living in pool habitats with muddy clay bottoms and is embedded in sediment as sand to silt < 0.5 mm. Its whole body is normally eaten as traditional Thai and other Southeast Asian dishes.

Shellfish sampling

Shellfish were collected from 39 sampling sites at tributaries of the Kwhae Noi Basin (Fig. 1). Each site was classified by stream order using 1:50000 maps and recorded using GPS (SD±5.0 m). Shellfish were sampled from three zones as follows. (1) Headstream (13 sites) areas have minimal disturbance and include national parks and wildlife sanctuaries no industry, small- scale agriculture, and local villages with small populations. (2) Midstream (13 sites) areas have medium-size agricultural areas; e.g. with cassava, corn, pine apple, and sugar cane, with higher numbers of local villages with higher populations. (3) Downstream (13 sites) areas have more and larger agricultural areas compared with headstream and midstream, and there are many anthropogenic activities, e.g., small industries and municipal discharge. ArcGIS, a geographical information systems (GISs) was used to derive land use all sampling sites.

Shellfish were randomly sampled within an area of 35.0 to 50.0 m^2 at each site. Fifty assassin snails were collected from benthic substrates of littoral/sublittoral streams for each headstream, midstream and downstream area. Twenty clams were collected in sandy, clayey, and muddy habitats in each headstream, midstream and downstream area. Headstreams ($n = 13$ sampled sites) were located from 392488 to 1658569, to 392004 to 1547954 (UTM), comprising mostly 1st order streams. Midstreams ($n = 13$ sample sites) were located from 392004 to 154795 and 500000 to 1547726, and 3922004 to 1547726, and downstreams ($n = 13$ sampled sites) were located from 500000 to 1547726 and 391554 to 1437349, and were composed of 2nd–3rd stream orders and 3rd–4th order streams, consecutively. Shellfish samples were collected by dip net, 0.03 cm^2 mesh, with 50.0 cm diameter, and by diving where water was deep, from September 2021 to March 2022. These periods included wet and dry seasons during which MP can be found in terrestrial urban floodplains and sediment in the study areas. After they were collected, samples were transported in a 4.0 °C insulated box to the freshwater laboratory of Mahidol University, Kanchanaburi Campus and stored at −80 °C until analysis.

Soft tissue preparation

After thawing, assassin snails were heated in a 60–70 °C water bath for approximately 4–5 min, and then the whole soft body was carefully separated by forceps; clam soft bodies were separated by scalpel.

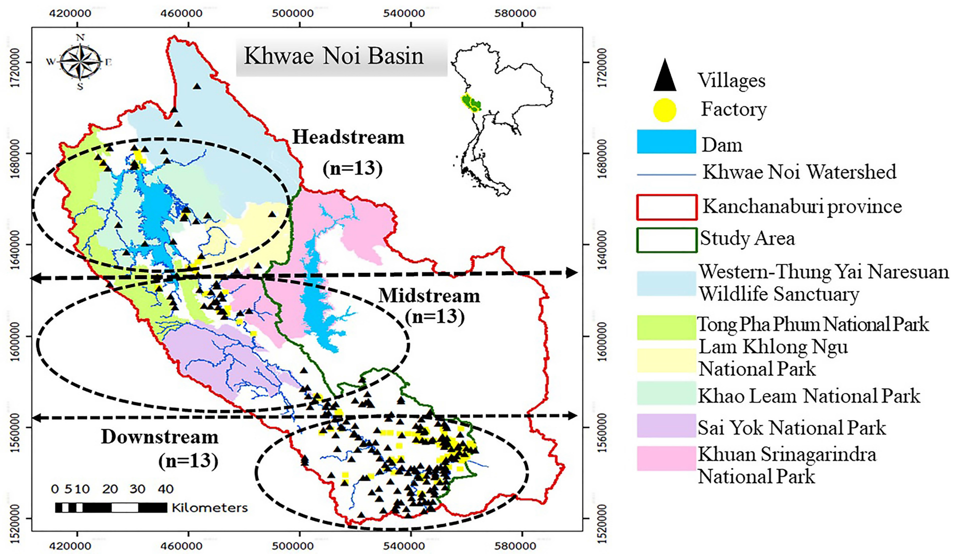

Fig. 1 The Khwae Noi Basin, outlined in green, illustrating headstream, midstream, and downstream areas associated with conservation areas and human activities.

Then, samples were weighed. After rinsing with ultrapure water to eradicate surface residues, each whole soft body was put into a glass bottle. Individual samples of shellfish were digested by adding 30–35 ml of 10% KOH and 25–30 ml of H_2O_2 (30% v/v) into soft tissues. They were placed in an incubator at 60 °C for 24 h. A saturated NaCl solution, 300 g/l concentration, was filtered and added into the flasks to separate MP by flotation. The saline solution was kept for 12 h at 20–25 °C room temperature, and the supernatant was then pipetted and filtered through a glass microfiber filter (Whatman GF/C 1.2 µm pore size).

Quality assurance and control

In this study, we controlled the quality in the laboratory with quality laboratory standards, including special care for protecting from contamination all essential equipment, including metal scissors, tweezers, scalpel and glassware. All equipment was cleaned with a non-ionic detergent, cleaned with 70% alcohol, and rinsed five times with filtered distilled water (0.70 µm pore). Then, high quality aluminum foil was used to wrap all equipment, which was then oven-dried at 40 °C. The workstation was carefully cleaned before and after the analysis of all samples. In addition, soft tissues of shellfish were rinsed with filtered distilled water to clean any possible MP on their exoskeletons from various environments. Whole soft tissues were rinsed under laminar flow, placed in small glass Petri dishes, then covered with aluminum foil.

Water

Diversity of MP in water was investigated to correlate with their accumulation in the shellfish. Plankton nets, 20 µmwith 30 cm diameter and mouthed with a 150 ml glass bottle container, was used to collect MP in the surface water in 39 sampled sites. First, the plankton net was cleaned with bottled drinking water and rinsed 5 times using river water at the sampling site. MP in water were randomly collected with a 10 m line from the center to the margin of the river in each site for 5 replicates and pooled together for each site, for predicting MP diversity in the river. Then, water samples were preserved in 4 °C insulated boxes [17]. From 750 ml of each sample, 300 ml were taken for duplicate measurements. Before identification of MP types in a sample, 300 ml of water were filtered using Whatman GF/C (Glass microfiber filter 1.2 µm) paper.

Sediment

MP in sediments were studied to predict MP diversity relating to their accumulation in the shellfish that lived in different habitats in the basin. Sediment samples from the 39 sites sampled for shellfish were collected by shovels in littoral and sublittoral zones of the rivers and by Ekman dredge sampler (T.Science GS-600 model, 15×15×15 cm dimension) in deep areas of the river. The collected sediment was then placed into a 0.5 l glass container. For each site, sediment was collected for 3 replications and analyzed. The sediment samples were preserved in 4 °C insulated boxes. All sediment samples were processed according to Wang et al [18] and Peng et al [19]. One kg of each sample in wet weight was dried in the oven at 100 °C for 48 h. The dried sediment was thoroughly mixed and then $\leqslant 100$ g was dissolved in 400 ml of NaCl 30% solution. Next, the mixture was stirred for 2 min using a stirring spoon. After being stirred, it was allowed to settle until no more visible material floated on to the surface of supernatant. The floating material in the supernatant was filtered using a vacuum pump on Whatman GF/C (Glass microfiber filter 1.2 µm) filter paper, and then used to identify the MP types in the sediment.

Microplastic analysis

Soft tissues of assassin snails ($n = 150$ individuals) and clams (60 individuals) were used to analyze MP types. The supernatant samples from the shellfish were used to identify the abundance of MP types and colors using filter paper placed on small Petri-dishes 60 mm × 15 mm after they were incubated at 60 °C, 48 h. Their physical characteristics were visually examined and divided into categories, filaments (fibers), rods, fragments, and pellets, under a stereomicroscope. MP found were photographed with a camera (Dino-Lite Pro AM423) attached to the stereomicroscope. MP sizes were measured for their longest dimension under the camera program (DinoCapture 2.0, 1.5.43 version). Size classes were defined in six categories: ⩽ 50 µm (very small MP), ⩾ 51–100 µm (small MP), ⩾ 101–200 µm (relatively small MP), ⩾ 201–300 µm (medium size MP), ⩾ 301–400 µm (relatively large MP), and ⩾ 401–500 µm (large MP).

Fourier-transform infrared (FT-IR) spectrometer (Shimadzu, FTIR-8900) with a NaCl cell measurement technique was used to identify chemical compositions and IR bands (1/cm) for classifying MP diversities and types in the assassin snail, clam, water and sediment supernatant samples. For FT-IR analysis, 82 pieces from snails, 155 pieces from clams, 180 pieces from water, and 58 pieces from sediment were analyzed. Each source was pooled as three replications to analyze their compositions of functional chemical compositions with bond assignments and IR bands (1/cm) for classifying MP types.

Statistical analysis

The association among abundance of MP pieces and environmental factors in the shellfish were analyzed by Principal Correspondence Analysis (PCA), PCoA-Plot technique and similarity index by Sorensen Distance for identification using the PC-ORD program, version 3.5. The distribution of MP pieces in the environment analyzed by PCA analysis was subjected to cluster analysis with group average linkage and Sorensen distance values to identify groups. MP abundance was $\log(x + 1)$ transformed and normalized for the distribution. In preparation for ordination analysis, some adjustments were made to the data. No MP were found in some individuals of shellfish which required some adjustments to be made to the data in preparation for ordination analysis. The statistical package available for PCA analysis required that a dummy variable of 0.00001 MP piece/individual [20] be added to each of the shellfish individuals that lacked MP for statistical analysis.

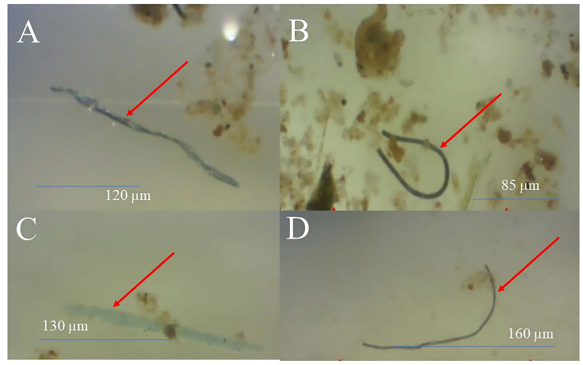

Fig. 2 Filamentous MP accumulations in assassin snails: (A) blue and (B) black, and in clams: (C) blue and (D) green.

RESULTS AND DISCUSSION

Microplastics abundance in shellfish

MP accumulation in 150 assassin snails with body lengths from 0.5 to 5.5 cm and body weights from 0.02 to 3.2 mg with averages 2.4±0.8 cm and 1.5±0.7 mg, respectively. Body length and weight of assassin snails were positively correlated with MP accumulation at $p \leqslant 0.05$, $r^2 = 0.57$. MP accumulation increased with increasing body lengths from 0.5–5.5 cm. Filamentous MP was the only type found, totally 82 pieces, occurring at 0–4 pieces/individual, with an average of 0.5±1.0 pieces/individual, at about 26.0% occurrence.

In this study, we found filamentous MP accumulation in assassin snails in the range of 80 µm to 250 µm lengths, with an average of 150±30 µm (Fig. 2A and 2B). MP lengths ranged ⩾ 50–100 µm, ⩾ 101–200 µm, ⩾ 201–300 µm, and ⩾ 301–400 µm, with approximately 10.0% (0.3±1.0 pieces/individual), 8.0% (0.1±0.5 pieces/individual), 4.6% (0.04±0.2 pieces/individual) and 3.3% (0.03±0.1 pieces/individual) occurrences, respectively (Fig. 3B and 3C). Lengths ⩽ 50 µm and ⩾ 400–500 µm were not present. Two colors of filamentous MP were identified with 54.9% and 45.1% occurrences in blue and black, respectively. The filamentous MP accumulation in assassin snails from headstream, midstream and downstream were significantly different at $p \leqslant 0.05$, with filamentous MP absent in headstream assassin snails ($n = 50$), while midstream ($n = 50$) and downstream ($n = 50$) had occurrences of approximately 14.0% (average MP per individual: ⩾ 50–100 µm; 0.5±1.2 and ⩾ 301–400 µm; 0.1±0.3) and 64.0% (average MP per individual: ⩾ 50–100 µm; 0.5±1.2, ⩾ 101–200 µm; 0.4±0.8, and ⩾ 201–300 µm; 0.1±0.4), respectively (Figs. 3A and 5A).

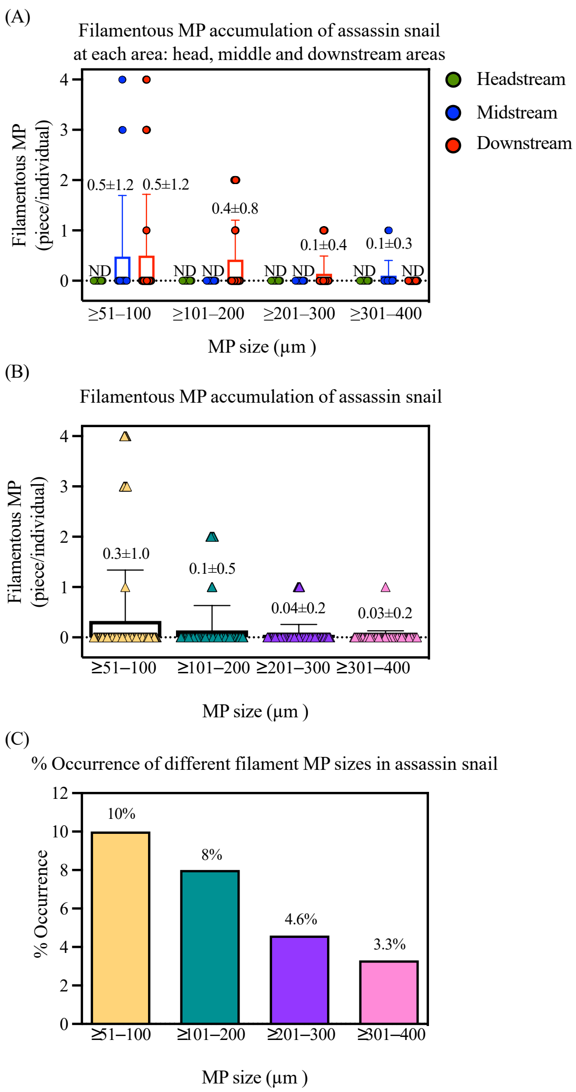

Fig. 3 Accumulation of filamentous MP by size in assassin snails at each area (A) and overall areas (B) of this study. The percentage of occurrences of filamentous MP classified by four size classes in assassin snails (C) was demonstrated for overall areas of this study. ND; not detected or not found.

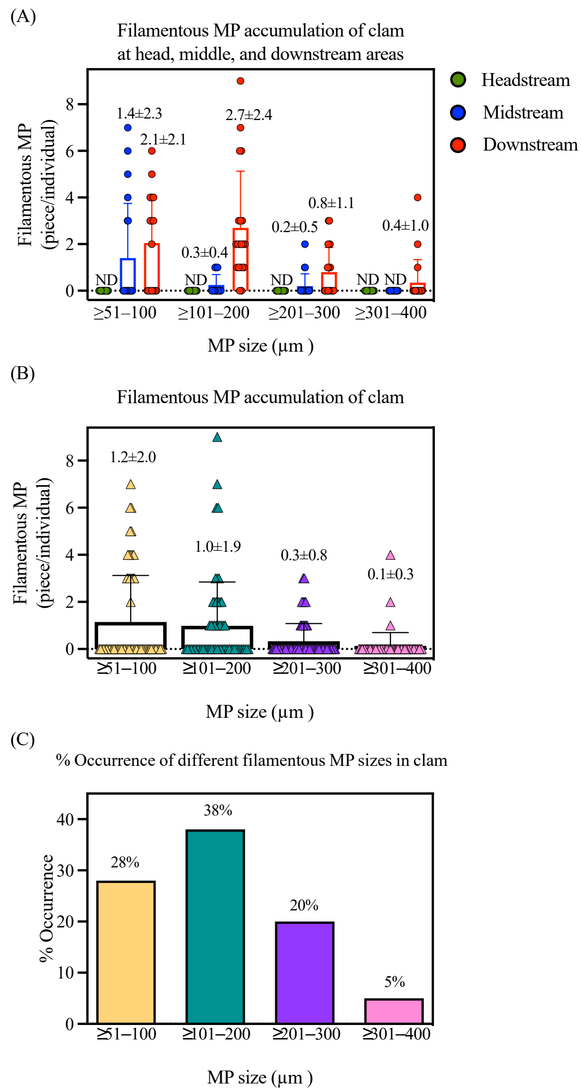

Fig. 4 Accumulation of filamentous MP by size in clams at each area (A) and overall areas (B) of this study. The percentage of occurrences of filamentous MP classified by four size classes in clams (C) was demonstrated for overall areas of this study. ND; not detected or not found.

Only filamentous MP was found in clams ranging from 2.8–8.2 cm in body length and 4.5–350 mg body weight with averages of 3.2±1.2 cm and 200±120 mg, respectively. Body weight was significantly positively correlated to MP accumulation ($p \leqslant 0.05$, $r^2 = 0.6$). Similarly, other studies found positive relationships among the numbers of MP accumulation and body weights (sizes) in some aquatic organisms, and their habitats; e.g., *Mytilus edulis* and *Perna viridis* [21], *Corbicula fluminea* [22], *Modiolus modiolus* and *Mytilus* spp [23].

The 155 pieces of filamentous MP (Fig. 2C and 2D) found in clams ranged from 65 µm to 350 µm in length with an average of 115.0±45.0 µm, and 0–9 pieces/individual were found with an average of 2.5±3.3 pieces, with 38.0% occurrence. MP lengths ranged ⩾ 50–100 µm, ⩾ 101–200 µm, ⩾ 201–300 µm, and ⩾ 301–400 µm, with 28.3% (1.1±1.9 pieces/individual), 38.3% (0.9±1.8 pieces/individual), 20.0% (0.3±0.7 pieces/individual) and 5.0% (0.1±0.5 pieces/individual) occurrences, respectively (Fig. 4B and 4C). Lengths ⩽ 50 µm and ⩾ 401–500 µm were not found. The colors of filamentous MP were blue and green with 64.5% and 35.4% occurrences, respectively. The MP accumulations in clams living in headstream, midstream, and downstream were significantly different

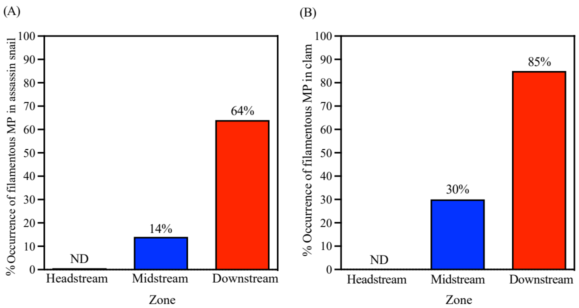

Fig. 5 Percent occurrence of MP in headstream, midstream and downstream environments: assassin snails, 64.0% downstream ($n = 50$), 14.0% midstream ($n = 50$) (A); clam, 85.0% downstream ($n = 20$), 30.0% midstream ($n = 20$) (B). ND; not detected or not found.

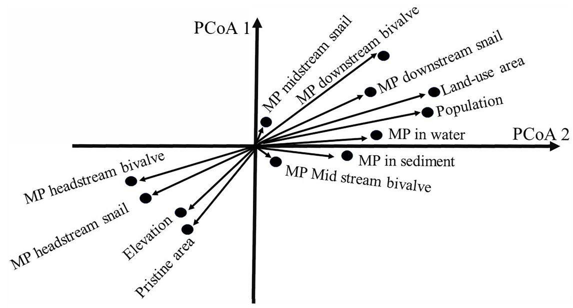

Fig. 6 Principal Correspondence Analysis of MP accumulation in assassin snails and clams, associated with 9 environmental factors, explaining > 75.4% of the total variance with a 60.0% eigenvalue.

at $p \leqslant 0.05$. MP were absent in headstream clams ($n = 20$), while occurrences at midstream ($n = 20$) and downstream ($n = 20$) were approximately 30.0% (average MP per individual: ⩾ 50–100 µm; 1.4±2.3, ⩾ 101–200 µm; 0.3±0.4, and ⩾ 201–300 µm; 0.2±0.5, and ⩾ 301–400 µm) and 85.0% (average MP per individual: ⩾ 50–100 µm; 2.1±2.1, ⩾ 101–200 µm; 2.7±2.4, ⩾ 201–300 µm; 0.8±1.1, and ⩾ 301–400 µm; 0.4±1.0), respectively (Figs. 4A and 5B).

The studies of MP accumulation in oysters, *Saccostrea cucullate*, from the coastal waters of China [24] and in eight commercial mussels, *M. galloprovincialis*, *P. viridis*, *Ruditapes philippinarum*, *Crassostrea gigas*, *Sinonovacula constricta*, *Scapharca subcrenata*, *Meretrix lusoria*, and *Busycon canaliculatu*, from Qingdao and Xiamen [25] found that the most dominant MP type was filamentous, with most less than 100 µm [24] and 100–200 µm [25], and filamentous MP were generally accumulated in the shellfish, *Placopecten magellanicus* [26]. Weber et al [27] and Scherer et al [28] reported that the snails, *Lymnaea stagnalis* and *Physella acuta*, accumulated the most MP in the range of 10–90 µm, which is in accordance with our study.

MP accumulations in clams was higher than in assassin snails by shellfish weight; i.e. pieces/individual, g. However, the average amount of MP accumulation in the two species was not different when based on the number of pieces of MP per individual. The number of MP was calculated based on the body weight of soft tissues (MP pieces/individual), with MP accumulation in assassin snails higher than in clams owing to the average number of MP piece per individual in both species, but assassin snails' weight is less than clams'. Hoellein et al [29] reported that MP accumulation was higher in smaller (by body weight) than larger freshwater mussels, *Dreissena* sp. Similarly, de la Torre et al [30] found that small body weight of *Semimytilus algosus* had more MP accumulated than large body weight of *Tegula atra*. In contrast, species of *S. algosus* and *Chiton granosus* were similar in soft tissue weight, but were not different in MP accumulation [30].

We found that MP in both species were filamentous with blue, black and green colors and lengths of 50–500 µm, which was similar to the phytoplankton eaten by adult and veliger stages of shellfish [31], and diatoms (*Fragilaria* sp., *Navicula* sp., *Pinularia* sp., *Tabelaria* sp.), green algae (*Scenedesmus* sp., *Coelastrum* sp., *Eudorina* sp., *Pediastrum simplex*, *P. duplex*, *Volvox* sp.), Zygnematophyceae (*Closterium* sp., *Zygnema* sp., *Cosmarium* sp.). Filamentous algae were found in approximately 60.0% of the stomachs of freshwater shellfish in rivers [32]. As small MP particles are < 500 µm, they can combine with mucus of phytoplankton forming aggregations [33] and with dissolved solid organic particles formed by hydrophobicity, Van Der Waals force, Pi–Pi ($\pi - \pi$) bond and electrostatic interaction [34]. For feeding, assassin snails use a radula, a tongue-like structure supporting rows of teeth like a rasp, which is protruded from the mouth and used to gather food including items as small as MP. For clams, the small MP particles are filtered through the inhalant aperture into their digestive system. However, the MP accumulation in clams was higher than that in assassin snails presumably because clams feed all the time while assassin snails move their body and feed intermittently. We have observed assassin snails to move about 30 cm per day.

Environmental factors

PCA as Pearson Correlation demonstrated abundance of filamentous MP correlated with 9 environmental factors: headstream, midstream, downstream, elevation, pristine area, land-used area, population, MP in water and MP in sediment as illustrated in Fig. 6, described by explaining > 75.4% of the total variance with an eigenvalue of 60.0%. PCA indicated that area of land use had the highest influence on contamination of MP in the basin, with high contaminations in water and sediment, resulting in high MP accumulations in the two species of shellfish. In contrast, elevation and pristine area factors were correlated with low contaminations of MP in water and sediment, and low MP accumulations in shellfish. The MP accumulations of shellfish were classified by 65.0% similarity index by Sorensen Distance, being classified into 3 groups. The first group contained 50 assassin snails and 20 clams in headstreams with high elevation at ⩾ 300 m, pristine areas (conservation areas with lowest number of villages and smallest human population), and no MP accumulation in shellfish. The second group comprised 7 assassin snails and 6 clams in midstream with medium elevation at 150–200 m, illustrating MP accumulations at 0.02±1.2 and 0.1±2.0 piece/individual for assassin snail and clam, respectively, having medium disturbance areas with increasing land-used area. The third group composed of 32 assassin snails and 17 clams that was defined as aquatic habitat degradation, which clearly comprised land-used areas, having high abundance of MP in water and sediments related to high MP accumulation in downstream assassin snail and clam at 0.3±1.4 and 2.1±1.6 piece/individual with low elevation at ⩽ 150 m.

Both species are in midstream and downstream, excepted headstream, having the higher percentage of MP accumulation according to Berglund et al [35] that found the highest MP accumulation in freshwater duck mussel (*Anodonta anatina*) at the downstream of the Swedish River where are disturbed from wastewater treatment plant, rural farmland and city location area. The studies by Corcoran [36] discussed the impact of urban streams found high MP accumulation, which was directly related to high MP accumulation in the Asian clam (*C. fluminea*). The amount of MP accumulation in the freshwater snail, *Bellamya aeruginosa*, varies with the distance from a city where human activities; e.g. industry and tourism, contribute to the dispersion of MP in the Taihu Lake environment in China [37]. According to Limbago et al [38] the occurrence of MP accumulation in water is positively correlated to areas downstream of urbanization, including high MP density in water body of upstream region where is on urbanization [39]. Hence, MP accumulations in water and sediment of streams, lakes, and coastal regions cause accumulation in aquatic organisms from various point and non-point sources of anthropogenic activities [40].

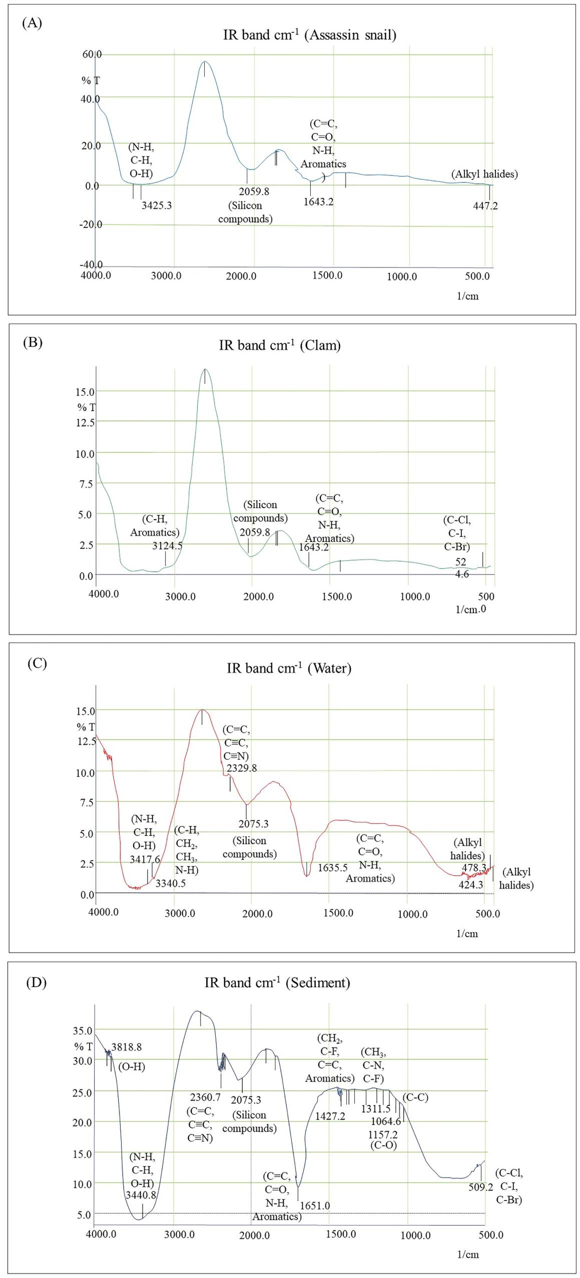

Fig. 7 FT-IR spectrum of microplastic types found in assassin snail (A), clam (B), water (C) and sediment (D) from the Khwae Noi Basin.

Microplastics types

For FT-IR analysis, bond assignments and IR bands (1/cm) of functional chemical compositions were identified in 10 types of MP in samples of tributaries in the Khwae Noi Basin where the shellfish were investigated (Fig. 7, Table S1). A total of 180 pieces of MP were found in water and 58 pieces in sediment with an average of 60.0±19.7 pieces/300 ml and 19.3±4.0 pieces/100 g. MP types did not significantly differ between water and sediment (t-test, $p < 0.05$). Four chemical types of MP were found to accumulate in the two species of shellfish: polydimethylsiloxane (PDMS), polyamide (PA), polyester (PES), and polyvinyl chloride (PVC). Assassin snails accumulated PDMS, PA and PES, and clams accumulated PVC, PDMS, and PA.

Sathish et al [41] reported that polyethylene terephthalate (PET), polypropylene (PP), and polyethylene (PE) were found in clams from the southern coast of India. PP and PE were most commonly found in clams from the Middle-Lower Yangtze River Basin [22], while PE was dominant in clams from the Tuticorin coast of the Gulf of Mannar [41] and in green-lipped mussels from the New Zealand coast [42]. PET and PE were recorded in clams from the Oregon coast, USA [43] and mussels from Chinese coastal waters [21]. The MP types accumulated in shellfish in our study were different from those in other studies; however, PDMS was expected in assassin snails and clams, which were similarly found in mantis shrimp, *Oratosquilla oratoria* from Liaohe Estuary, China [44]. Presumably, downstream areas are close to urban communities, which commonly use PDMS, then causing its accumulation in water, sediment, clams, and assassin snails sampled from these areas.

CONCLUSION

This study provides evidence of microplastic (MP) accumulation in freshwater shellfish, the assassin snail (*C. helena*) and a clam (*P. exilis compressa*), the dominant species in the Khwae Noi Basin in western Thailand. They were sampled from headstream, midstream and downstream areas of the basin. The results showed that the MP accumulations in the two species differed based on their feeding behaviors and habitats. MP accumulation in both species was correlated with 9 environmental factors: headstream, midstream, downstream, elevation, pristine area, land-used area, population, MP in water, and MP in sediment. Headstream, together with elevation and pristine area, did not positively correlate with MP accumulation in the shellfish. On the other hand, MP accumulation increased with greater land-use causing the increment of MP in water and in sediment of the midstream and downstream areas. Hence, it seems clear that anthropogenic activities cause MP accumulation in shellfish.

Appendix A. Supplementary data

Supplementary data associated with this article can be found at http://dx.doi.org/10.2306/scienceasia1513-1874.2023.030.

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Appendix A. Supplementary data

Table S1 Microplastic types found in water, sediment, and shellfish classified by chemical compositions of bond assignment and IR band by FT-IR.

Environment	IR band (1/cm)	Bond assignment	Microplastic type
Water	3417.6	N—H, C—H, O—H	PP, PE, PET, PVC, PDMS, PA, PES, PU, PS, PMMA
	3340.5	C—H, CH_2, CH_3, N—H	
	2329.8	C=C, C≡C, C≡N	
	2075.3	Silicon compounds	
	1635.5	C=C, C=O, N—H, Aromatics	
	478.3	Alkyl halides	
	424.3	Alkyl halides	
Sediment	3818.8	O—H	PP, PE, PET, PVC, PDMS, PA, PES, PU, PS, PMMA
	3440.8	N—H, C—H, O—H	
	2360.7	C=C, C≡C, C≡N	
	2075.3	Silicon compounds	
	1651.0	C=C, C=O, N—H, Aromatics	
	1427.2	CH_2, C—F, C=C, Aromatics	
	1311.5	CH_3, C—N, C—F	
	1157.2	C—O	
	1064.6	C—C	
	509.2	C—Cl, C—I, C—Br	
Assassin snail	3425.3	N—H, C—H, O—H	PDMS, PA, PES
	2059.8	Silicon compounds	
	1643.2	C=C, C=O, N—H, Aromatics	
	447.5	Alkyl halides	
Clam	3124.5	C—H, Aromatics	PVC, PDMS, PA
	2059.8	Silicon compounds	
	1643.2	C=C, C=O, N—H, Aromatics	
	524.6	C—Cl, C—I, C—Br	