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Research Article

Occurrence and characterization of microplastics in herring scad (*Alepes vari*) from Chennai coast of Bengal Bay

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Keywords

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Abstract

The escalating issue of marine plastic pollution is primarily attributed to the extensive utilization of plastics coupled with their improper disposal in oceans. Microplastics (MPs), particles measuring less than 5 mm, result from the degradation processes of larger plastics, exacerbating ocean pollution. In this research, 100 samples of a carnivorous fish, *Alepes vari*, from the Chennai coast of Bengal Bay were collected and analyzed for the presence of MPs, which revealed varied accumulation ratios of MPs within their gastrointestinal tract. Through hydrogen peroxide digestion of 100 fish specimens, it was found that approximately 68% contained MPs, with an average abundance of 9.5 (±0.3) particles per individual. The predominant types of MPs observed were pellets (81.34%) and films (18.66%) with brown pellets comprising the majority (25.97%). Further analysis indicated a higher concentration of MPs in the stomach compared to the intestine. Fourier transform infrared radiation analysis-attenuated total reflection confirmed the presence of Polystyrene polymer in the ingested MPs, suggesting a potential link between the dietary habits of these fish and their ingestion of microplastics.

Article info

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Introduction

Plastics are widely utilized because of their many advantages, including their cost, resilience, and durability. It constitutes significant supplies that have many beneficial implications for society. In 2018, there were about 360 million tons of plastic produced worldwide, with the Middle East and Asia being dominant in the highest production (Leal *et al.*, 2019). In 2015, architecture and construction (13%), agriculture (9%), and product packaging (53%), accounted for the majority of plastic usage in South Africa (Bakir *et al.*, 2020). Between 4.8 and 12.7 million tons of plastic invaded the ocean in 2010 alone; by 2025, this amount is predicted to have increased by an order of magnitude (Jambeck *et al.*, 2015). Macro plastics (>20mm) eventually break down into microplastics (MPs) (less than 5 mm) and mesoplastics (between 5 and 25 mm) (Naidoo and Glassom, 2019). Due to the great mobility and extended residence durations, MPs are either produced (microbeads) or result from the weathering of bigger plastic particles. Primary plastics, which are purposefully utilized as resin pellets or as components of personal care products, can also yield MPs (Carpenter *et al.*, 1972). On the surface of the ocean, the MPs were first discovered in 1972 (Di Beneditto and Awabdi, 2014). However, the possible effects on the marine ecosystem have only lately come to the notice of scientists and the general public.

Once MPs find their way into the ecosystem, the main concerns are related to their possible bioavailability to marine life (Lavers *et al.*, 2014). There have been reports of plastic consumption in several different animal species, including fish, mammals, seabirds, insects, and turtles (Neves *et al.*, 2015; Kershaw and Rochman, 2015; Lusher *et al.*, 2015; Nadal *et al.*, 2016; Peters and Bratton, 2016). Consequently, in recent years, research on plastic pollution has concentrated on the origins, destiny, and ecological impacts of microscopic particles. Numerous research examining fish absorption of MPs have revealed that a wide range of species including pelagic and demersal fishes from different depths and geographical regions are interacting with MPs in the environment (Lusher *et al.*, 2016). According to Kershaw and Rochman, 2015), there are several possible effects of MPs on fish that are not fully known (Cole *et al.*, 2013). Microplastics (MPs) and nanoplastics can undergo several processes once they enter the environment which include ingestion by organisms, exposure to the gills in aquatic species, absorption into various tissues and cells, excretion, and transfer between trophic levels. One significant route of transfer is through the diet, where contaminated prey species can transfer MPs to predatory fish (Jackson *et al.*, 2000). Additionally, microplastics and nanoplastics can be absorbed by cells through various mechanisms, including phagocytosis, macropinocytosis, Clathrin-mediated endocytosis, and Caveolae-mediated endocytosis (Yee *et al.*, 2021). The degree of plastic pollution in the area and fish feeding habits are two major variables influencing fish consumption of plastics. Because of their small size, lower trophic organisms which often exhibit minimal food selection and would take any item of suitable size may find MPs more bioavailable (Rochman *et al.*, 2013). Fish that consume plastic may have internal obstructions and damage to their digestive tracts, according to earlier field research (Besseling *et al.,* 2013). Additionally, it has been demonstrated in experimental settings that fish exposed to plastic suffer detrimental effects (De Sa *et al.,* 2018). In particular, impaired development, hormonal imbalance, reduced eating and/or weight loss, liver disease and toxicity, inflammation, organ transfer, and reduced reproductive output are the adverse effects of MPs in fishes (Browne *et al.,* 2008). Furthermore, data suggests that MPs may serve as transporters for toxic sorbed co-contaminants, such as pathogens, hydrophobic organic compounds, and additives, which may then enter the biota after ingestion (Tanaka *et al.,* 2013). Furthermore, the build-up of chemicals on the outermost layer of MPs could negatively impact fish's health such as the hindered formation of fat deposits, diminished feeding stimuli, enzyme reflex, and failure in reproduction (Derraik *et al.,* 2002). Yet, in comparison to other exposure pathways, the transfer of sorbed co-contaminants from MPs to biota could be minimal (Bakir *et al.,* 2016).

The prevalence of MPs in commercially significant fish species in the Bay of Bengal remains poorly understood, highlighting a critical knowledge gap. Therefore, this study aims to address this gap by investigating the accumulation of microplastics (MPs) in the gastrointestinal tract of carnivorous fish, *A. vari*, collected from the Chennai coast of the Bay of Bengal. Through analysis of 100 fish specimens, the research aims to determine the presence and abundance of MPs, identify the predominant types of MPs, assess their distribution within the gastrointestinal tract, and investigate potential links between fish dietary habits and microplastic ingestion, confirmed using Fourier Transform Infrared Radiation analysis- Attenuated Total Reflection (FTIR-ATR).

Materials and methods

In September 2023, 100 fish of Herring scad, *A. vari* of similar size were bought from the fish market at Tiruvanmiyur Beach, Chennai, which is near the Bay of Bengal coastline. The fish, as soon as purchased, was washed with 0.9% saline followed by a sterile water rinse and the morphometric measurements were taken before the experiment such as length and weight (Fig. 1A). The gathered samples were kept in the lab's deep freezer at -20°C. A total of one hundred specimens of fish were subjected to gender determination, resulting in the identification of sixty males and forty females. The average total length of the fish was recorded as 17±1 cm, with a corresponding average fork length of 12.5±1.5 cm. Each fish exhibited a mean weight of approximately 50±20 g. The fish were then grouped, with each group comprising 10 specimens, irrespective of gender. The fish were subsequently dissected ventrally, and the Gastro-Intestinal Tract (GIT) was excised. Its weight was measured using an automated weighing balance (Fig. 1B), showing a total GIT weight of 0.05g, with the stomach accounting for 0.03g and the intestine for 0.02g. The stomach and intestine's dimensions, internal composition, and form were noted. Each fish had their stomach and intestine removed, and to minimize error within groups, three replicas of stomach and intestine were taken, and six individuals in each replicate, were utilized for study. We separated the species into two classes, fish with complicated GIT structures and fish with simpler GIT structures based on the anatomy of the intestinal and stomach tracts. In specifics, the simple class had broad, uncoiled intestines and internally smooth wall stomachs, while the complicated class included internally folding or projecting stomachs together with thin, coiled intestinal structures.

Quality control

To lower the possibility of contamination, equipment such as glassware and dissection instruments was thoroughly cleaned three times using filtered water. Before being used, tap water, saline water, and hydrogen peroxide were passed through a 1 mm filter to remove the contamination. During the studies, gloves and lab coats were used. When the samples were not in use, they were instantly concealed. The tissue-free experimental techniques were carried out as blank experiments. Before being used, the autoclave is used to sterilize the conical flask, Petri dish, and filtration apparatus.

Digestion of sample

To isolate polymers, the entire gastrointestinal tract (GIT) underwent a digestion process. To address plastic accumulation in specific organs, the intestines and stomach were sequentially digested in a 250 mL conical flask (Fig.1C). A digestion protocol (Li *et al.,* 2015; Li *et al.,* 2016) was applied to the GIT to enhance the efficiency of plastic extraction and characterization from the tissue. Organic materials were broken down by adding 200 mL of 30% H_2O_2 and adjusted according to the total sample weight. The sealed conical flask was placed in an oscillating incubator set at 65°C and 80 rpm for 24 to 72 hours, depending on the digestion extent.

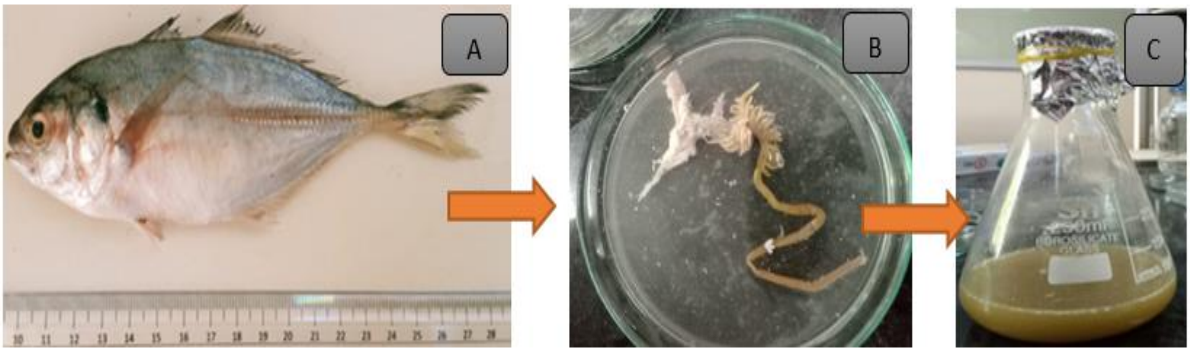

Figure 1: A) *Alepes vari*, B) Dissected GIT showing stomach and intestine, and C) digestion by KOH.

A filtered saturated saline solution with a density of 1.2 g/ml was prepared. Approximately 800 mL of filtered NaCl solution was added to the container to utilize flotation for separating plastics from the dissolved solution in the GIT. After mixing and allowing it to settle overnight, the clearing level was observed. The mixture was then filtered using a 47 mm, 5µm pore-size cellulose nitrate filter (Whatman AE98) through a vacuum pump. Subsequently, the filters were retained for microscopic examination of plastic particles in sterilized petri dishes with covers (Li *et al.*, 2015; Li *et al.*, 2016). Initially, the particles within the filter paper were visually inspected using a binocular microscope (Hidalgo *et al.*, 2012). Plastic materials were categorized into four types according to their physical properties: sheets (irregularly flat, flexible), pellets (spherical, ovoid), films (thin, soft, transparent), and fibers (elongated) (Choy and Drazen, 2013). The longest or widest dimensions of each particle were then measured to the nearest millimeter. Based on their size, all plastic items were classified into three main groups: tiny microplastics (<2 mm), large microplastics (2-5 mm), and mesoplastics (5-25 mm); those exceeding 25 mm were designated as macroplastics (Collignonn *et al.*, 2014; Romeo *et al.*, 2016). The isolated polymers were identified with the help of Fourier Transformed Infrared Spectroscope-Attenuated Total Reflectance (FTIR-ATR).

Results

Abundance of MPs

MPs have accumulated in 68 of the 100 fish studied. A total of 670 MPs were recovered from the Gut of fish accounting for 9.5±0.3 particles per fish. Two different morphologies of MPs namely pellets (81.34%) and films (18.66%) were isolated from the entire Gut (Fig. 2). Three different colors of pellets such as brown pellets (25.97%), black pellets (31.79%), and white pellets (23.58%) were identified along with the orange film (18.66%) (Fig. 3). the degree of aggregation of these various colored and shaped MPs in the stomach and intestine is recorded (Fig. 4). The represented data shows that the stomach had 380 MPs whereas the intestine had 290 MPs, indicating that the stomach had more MPs than the intestine.

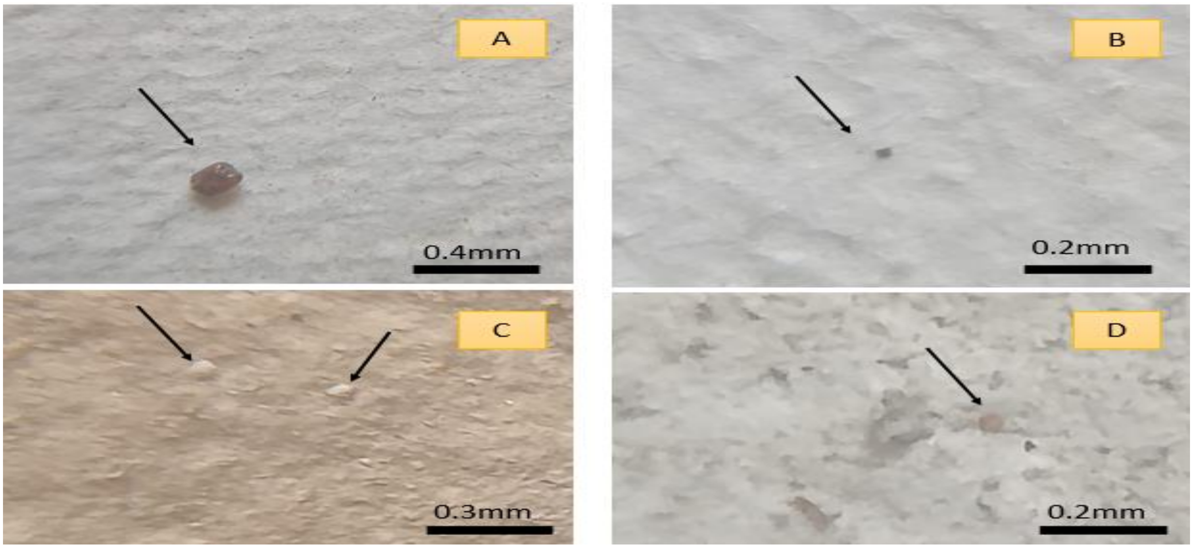

Figure 2: Different morphology of microplastics isolated from *Alepes vari* (A-brown pellet, B-black pellet, C-white pellet, D-orange film).

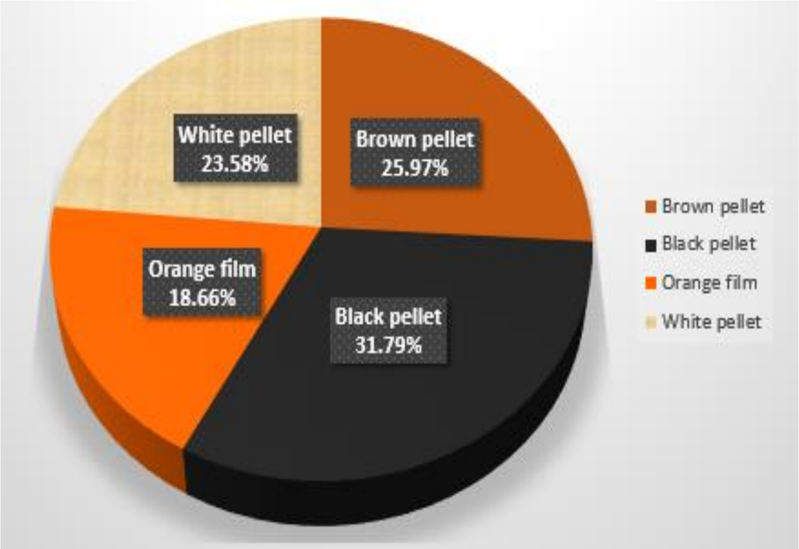

Figure 3: Various kinds of pellets isolated from *Alepes vari*.

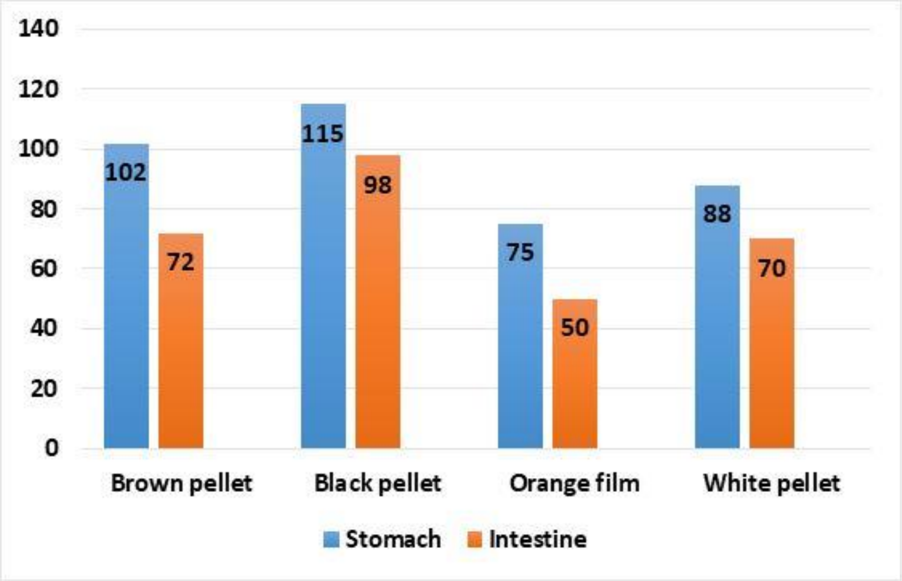

Figure 4: Differences in the number of microplastics isolated from stomach and intestine.

Characterization of MPs

In total, 17 distinct microplastic samples were analyzed using Fourier-transform infrared spectroscopy (FTIR). All samples exhibited characteristic peaks corresponding to the polystyrene polymer, with variations only in their quantities of availability in the gut isolates. This consistent presence of polystyrene can be attributed to the shoaling behavior of the fish species *A. vari*. Due to their similar feeding habits, habitat, and shoaling behaviour, these fish are likely to encounter and ingest similar types of microplastic particles.

Further, the FTIR analysis revealed the predominant presence of polystyrene polymer in the sample, with other polymeric peaks being insignificantly detected. Additionally, the other minor peak values did not meet the threshold for detecting characteristic spectral peaks of any other polymer. The peaks observed in the spectrum (Fig. 5), spanning wavenumbers between 600 cm^{-1} to 3000 cm^{-1}, corresponding to specific functional groups within the polymer structure. Notably, peaks observed at wavenumbers such as 2346.3 cm^{-1}, 2122.7 cm^{-1}, and 2040.7 cm^{-1} indicate C=C stretching, while the peak at 872.19 cm^{-1} signifies C-H bending, consistent with the structural features of polystyrene polymer.

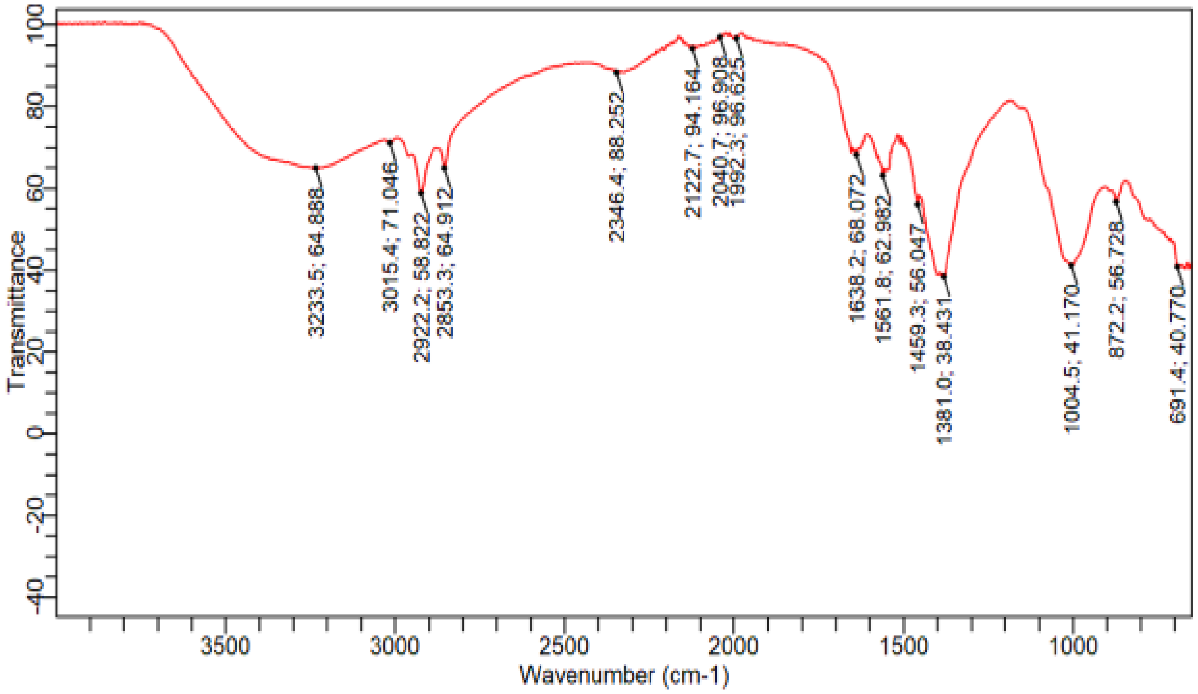

Figure 5: Peaks obtained in the FTIR-ATR Spectrum, confirms the presence of polystyrene polymer.

Discussion

With the escalation of marine plastic pollution driven by extensive plastic usage and improper disposal, MP particles measuring less than 5mm, have become a significant contributor to oceanic contamination. This study aims to investigate the presence of MPs in the GI tract of carnivorous fish *A. vari*, collected from the Chennai coast of the Bay of Bengal. Analysis of 100 fish samples revealed varying accumulation ratios of MPs within their gastrointestinal tract. Hydrogen peroxide digestion of the fish specimens showed that approximately 68% contained MPs, with an average abundance of 9.5±0.3 particles per individual. The primary forms of microplastics (MPs) identified were pellets (81.34%) and films (18.66%), with brown pellets constituting the majority (25.97%), which revealed a greater concentration of MPs in the stomach compared to the intestine. FTIR-ATR analysis of the isolated ingested microplastic samples confirmed the presence of polystyrene polymer predominantly, suggesting a potential link between the dietary habits of these fish and their ingestion of microplastics.

Much research highlighted the pervasive presence of microplastics in the gastrointestinal tracts of various fish species, shedding light on the extent of plastic pollution in aquatic ecosystems. Studies conducted by Lusher *et al.* (2013) and Rochman *et al.* (2015) demonstrated the ingestion of MPs by marine organisms, including fish, due to the prevalence of plastic debris in marine environments. Similarly, findings by Carbery *et al.* (2018) and Li *et al.* (2019) corroborated these observations, revealing the widespread occurrence of microplastic ingestion across different fish species and geographical locations. Moreover, the research conducted by Catarino *et al.* (2018) reported the potential adverse effects of microplastic ingestion on fish health and ecosystem dynamics. Their findings underscore the urgent need for effective mitigation strategies to address plastic pollution in aquatic environments. This highlights the critical importance of developing and implementing measures to reduce the prevalence of microplastics, thereby safeguarding marine life and maintaining the health of aquatic ecosystems.

Furthermore, Prusty *et al.* (2023) found microplastic contamination in *Harpadon nehereus* from Indian fishing harbours. Morphometric analysis showed all specimens were contaminated (6.98±6.73 MPs/g), with Jaffrabad exhibiting the highest levels. Predominant MPs were black and blue threads (1–2 mm), mainly composed of polyethylene, polystyrene, and polyurethane. This poses significant risks to seafood safety via trophic transfer, posing hazards to human health. By fostering a greater understanding of the risks associated with MP contamination and implementing effective mitigation strategies, we can strive towards safeguarding both marine environments and human health (Abbasi *et al.,* 2018). Hao *et al.* (2023) reported that high concentrations of polystyrene MPs accumulation led to significant intestinal damage and reduced weight gain in grass carp fish. Additionally, they found that smaller and more concentrated MP particles caused greater liver congestion and intensified oxidative stress.These add a notable concern regarding the potential bioaccumulation of MPs in humans via the consumption of contaminated fish. Further investigation into the translocation and deposition of MPs in edible tissues is crucial, as is the examination of their impacts on human health. To address the influx of waste into aquatic ecosystems, public awareness campaigns on ocean plastic pollution should be intensified, along with improvements in waste management practices, especially concerning plastic disposal.

Hence, the pervasive presence of microplastics in marine fish underscores the alarming reality of plastic pollution in our oceans. As these fish are integral components of the food chain, their contamination with microplastics poses a direct risk to human health through the consumption of seafood. Urgent action is imperative to address this issue, as unchecked plastic contamination threatens not only marine ecosystems but also the safety of the global food supply chain and the health of human populations.

Conclusions

In this study on *A. vari*, microplastics were detected in 68 out of 100 fish, with 670 MPs recovered, averaging 9.5 ± 0.3 particles per fish. The MPs were primarily pellets (81.34%) and films (18.66%), with pellets in brown (25.97%), black (31.79%), and white (23.58%), and orange films (18.66%). MPs were more concentrated in the stomach (380 MPs) than in the intestine (290 MPs). FTIR results of 17 samples identified polystyrene as the predominant polymer. This consistent presence of polystyrene is likely due to the shoaling behavior and similar feeding habits of *Alepes vari*. The ingestion of polystyrene microplastics in edible fish poses significant health risks to humans, necessitating urgent measures to mitigate marine plastic pollution.

Conflicts of interest

The authors declare no conflict of interest and each author had an equal contribution to this study.

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