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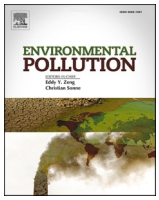

Rapid egestion of microplastics in juvenile barramundi: No evidence of gut retention or tissue translocation☆

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ABSTRACT

Despite many reports of large microplastics being isolated from fish muscle, there are limited exposure studies documenting the transport of microplastics >10 μm from the gastrointestinal tract (GIT) to surrounding tissues. Moreover, egestion rates of microplastics are not commonly studied, especially for carnivorous fish. In this study, experimental data and a literature meta-analysis were combined to understand microplastic translocation to fish tissue and egestion rates. Juvenile barramundi (*Lates calcifer*) were exposed through their diet to polyamide (PA) fibres and polyethylene terephthalate (PET) fibres and fragments (8–547 μm in length) to determine if shape, size, and polymer type influence microplastic translocation and egestion rates. Despite the high concentration (~5000 microplastics g^{-1}) and variable range of PET sizes and shapes used, their translocation from the GIT into other tissues was not observed, thus demonstrating PET fragments and fibres are unlikely to accumulate within barramundi. Moreover, more than 90% of all ingested PET microplastics were egested in less than 24 h, with only one small fragment persisting to 96 h post exposure. Elimination half-lives ranged from 9.2 to 12.2 h, with small PET fragments egested at a faster rate than the larger PET fragments and fibres but with no significant differences. Due to methodological challenges, PA fibres were unable to be quantified amongst the digesta. The meta-analysis of published fish egestion rates revealed that, when considering multiple fish, gut morphology (i.e., presence of a true stomach) rather than microplastic size and shape influenced egestion rates across species. The results presented here demonstrate no concrete evidence for GIT accumulation or translocation into tissue with rapid and efficient egestion of ingested microplastics by fish. These results suggest microplastics are not likely to bioaccumulate in barramundi and/or directly impact their associated food web.

1. Introduction

Ingestion of macro- and microplastics by biota commonly occurs in the marine environment (López-Martínez et al., 2021; Walkinshaw et al., 2020; Wootton et al., 2021). With the abundance of anthropogenic marine litter forecast to increase in the foreseeable future (Isobe et al., 2019), there is an urgent need to evaluate the risks these particles may pose to marine organisms (Koelmans et al., 2022) and elucidate environmental sinks. Ingestion by biota can significantly alter the fate of microplastics in the marine environment. Once ingested, movement and migration of biota can facilitate the transport of plastics to new environments (Bourdages et al., 2021; Grant et al., 2022; Grant et al., 2021; Pérez-Guevara et al., 2021b). Ingestion can also further degrade microplastics (Dawson et al., 2018; Mateos-Cárdenas et al., 2020). After passing through the gut, microplastics are incorporated into faecal material, facilitating the vertical transport of buoyant plastics to the benthos (Cole et al., 2016; Kvale et al., 2020), which is presumed to be a sink for plastics (Abel et al., 2021; de Smit et al., 2021). Finally, predation or scavenging of contaminated prey provide opportunities for the trophic transfer of microplastics to organisms, which, under normal

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circumstances, may not be prone to plastic ingestion (Costa et al., 2020; Hasegawa and Nakaoka, 2021; Nelms et al., 2018).

Ingestion not only influences the fate of microplastics in the environment but also their likely ecological impacts, with bioaccumulation being of particular concern (Parolini et al., 2023). This is especially true for some organisms that are not readily able to egest large plastics (Im et al., 2020; Roman et al., 2020; Wilcox et al., 2018), a phenomenon that has cascaded into a generalised trepidation of plastic and, more specifically, microplastic bioaccumulation across all taxa and ecosystems. Supporting this hypothesis are field studies which have reported microplastics from wild fish muscle tissue, varying from small items <10 μm (Atamanalp et al., 2021; Lopes et al., 2023; Makhdoumi et al., 2021) to large microfibres 1–5 mm in size (Akhbarizadeh et al., 2018; Lu et al., 2024). It is worth noting, however, that many such studies have been criticised for their insufficient use of quality assurance and quality control (Dawson et al., 2021) and require further validation. Furthermore, there is evidence that some marine organisms are adept at expelling nondigestible material through, for example, regurgitation (De Pascalis et al., 2022; Li et al., 2021a; Saborowski et al., 2019), pseudofaeces (Li et al., 2021b; Ward et al., 2019), egestion (Pérez-Guevara et al., 2021a), and moulting (Welden and Cowie, 2016), reducing the likelihood of plastic translocation to tissue and thereby of bioaccumulation. Many fish species sit within this group of organisms, having already been shown to be capable of egesting microplastics after ingestion (Bour et al., 2020; Ory et al., 2018; Xiong et al., 2019), yet, laboratory studies are still needed to validate the translocation of microplastics from the gastrointestinal tract (GIT) into tissues, and specifically those in the larger size range (~500 μm).

The number of controlled studies designed to assess microplastic translocation into tissues, and evaluate risks of bioaccumulation in fish, is still incipient. Very few take into account the diversity of fish feeding habits and mechanisms, or fish life stage—all factors known to influence the bioaccumulation of contaminants (Babut et al., 2017; Barber, 2008; Masset et al., 2019). Consideration of the shape and size of microplastics used in controlled studies analysing bioaccumulation is also important; shape is expected to influence retention within the fish GIT (Grigorakis et al., 2017; Jabeen et al., 2018; Santana et al., 2021), with large irregular microplastics hypothesised to promote gut blockages and smaller plastics hypothesised to translocate readily (Thompson et al., 2024). Microplastic particles >10 μm are unlikely to be capable of translocating from the GIT into other tissues (De Sales-Ribeiro et al., 2020; Kim et al., 2020; Ma et al., 2021a; Ramsperger et al., 2023; Schur et al., 2019), and thus far, laboratory studies have exhibited little, if any, evidence for microplastic bioaccumulation in fish.

This study combines laboratory-based experiments and literature meta-analysis to assess microplastic translocation to fish tissue and egestion rates after ingestion. Specifically, male juvenile barramundi (*Lates calcarifer*) were dietarily exposed to microplastics to assess and quantify translocation and egestion rates in predatory, carnivorous fish. Barramundi are a commercially valuable aquaculture, and wild-caught species, utilised for human consumption. They are generalist predators, feeding on gastropods, crustaceans, and fish; these feeding habits indicate they may be more susceptible to the ingestion of microplastics through contaminated prey items rather than direct ingestion from the water column. The aims of this experiment were to a) determine if polyamide (PA) fibres and polyethylene terephthalate (PET) fibres and fragments of various sizes (<547 μm) are able to translocate from barramundi GIT to their muscle tissue and liver, b) determine if microplastic size, shape, and polymer type influence barramundi egestion rates, and c) contextualise egestion rates exhibited by barramundi with previously studied fish. To carry this out, a literature review was conducted and data explored to assess environmental, biological, and microplastic characteristics across fishes that may influence microplastic egestion.

2. Methods

2.1. Fish maintenance and husbandry

Fish were acclimated for >2 weeks prior to transfer to their individual tanks for exposure. Details are provided in the Supplementary Material.

2.2. Microplastics Standards and experimental diet

2.2.1. Microplastics standards

Medium royal blue polyamide (PA) flock fibres with a nominal length of 500 μm and 3 decitex (~18 μm) and black polyethylene terephthalate (PET) flock fibres with a nominal length of 500 μm and 3.3 decitex (~17 μm) were purchased from commercial suppliers (Flockit and The Flocking Shop, respectively; Fig. 1). Fluorescent green PET fragments were obtained by grinding a soft drink bottle in a household blender. The bottle was rinsed, then soaked in MilliQ water for approximately 1 week prior to blending. The bottle was cut into smaller sections, snap frozen with liquid N_2, and pulse blended. Fragments were frozen and reground until the whole bottle was fragmented. Fragments were consecutively dry- and wet-sieved through a sieve stack to obtain the desired size fractions; wetting ensured nanoplastic-sized fragments were not attached to the surface of the larger microplastic fragments. Three nominal sizes were obtained: 63–77 μm, 500–547 μm, and ≤547 μm (Fig. 1). Nominal size ranges were validated using the image-analysis software FIJI (Schindelin et al., 2012). All three polymer types were confirmed using a Fourier Transform Infrared Spectrometer (FTIR) (PerkinElmer Spectrum 100) operating in universal attenuated total reflection (ATR) mode (resolution: 16 scans at 4 cm^{-1}, wavenumber: 600–4000 cm^{-1}). Spectra were compared against the NICODOM library and those with >70% similarity were considered a valid identification (Kroon et al., 2018).

2.3. Experimental diet

Preparation of the experimental diets is outlined in detail in the Supplementary Material. Briefly, commercial 6 mm fish food pellets were homogenised in a laboratory blender until a fine powder was formed. The food powder was combined with 5% gelatine and microplastics (0.1%, 0.01%, or 0% concentration), left to set at 4 °C for >3 h, then extruded through a pellet extruder (Multi Granulator Model MG-55) with a 5 mm axial die plate. The extrudate was cut by hand into nominal 4 mm lengths using a modified blade attachment and dried at 50 °C for >3 days. Dried microplastics-dosed pellets were stored in an airtight container at 4 °C until needed.

Four exposure experiments were conducted. The exposure concentrations selected were in line with previous studies modelling microplastics egestion in fish (Liu et al., 2021; Roch et al., 2021) but were intentionally chosen to be above environmentally relevant concentrations to ensure egestion rates, and translocation into muscle and liver (if it occurs), could be quantified. To quantify microplastic uptake (Experiment 1, Table 1), the diet comprised of a 1:1 wt of PET fragments ≤547 μm diameter and PET fibres 500 μm length (each shape represented 50% of the total plastic weight). The final microplastic concentration (MP g^{-1}) within the dosed dietary pellets was 0.1% of the total weight of the pellet and equated to 4467.5 MP g^{-1}.

To assess the influence of size on egestion (Experiment 2, Table 1), the diet comprised of small (63–77 μm) and large PET fragments (500–547 μm). Per gram of microplastic, the number of small microplastics greatly outnumbered the large microplastics, thus the diet was prepared with 5% of small and 95% of large microplastics so that large fragments were not underrepresented. The final microplastic concentration within the dosed dietary pellets was 0.1% of the total weight of the pellet and equated to 624.3 MP g^{-1}.

To assess the influence of shape on egestion (Experiment 3, Table 1),

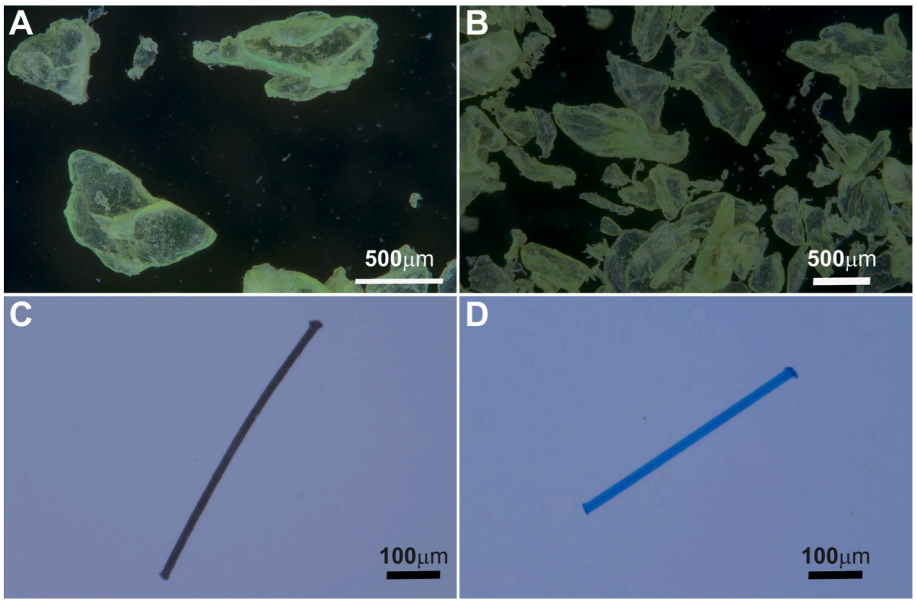

Fig. 1. Microplastics used to spike barramundi food pellets: A) polyethylene terephthalate (PET) fragments 500–547 μm; B) PET fragments ≤547 μm; C) PET fibre; and D) blue polyamide (PA) fibres.

Table 1
Microplastic (MP) treatment (polyethylene terephthalate, PET; and polyamide, PA) and measured concentration (MP g^{-1}) with standard deviation (SD) of each of the five diets.

Experimental Diet	Polymer, Shape and Size	MP Size	Nominal MP Concentration % of total MPs	Final MP Concentration % of total pellet mass	Measured Concentration[a] Mean MP g^{-1} (SD)
Control	–	–	0	0	0
1 – Uptake	PET fragments; mixed	≤547 μm	50%	0.1%	2043.7 (331.3)
	PET fibres	500 μm	50%		2423.8 (32.0)
2 – Egestion (size)	PET fragments; small	63–77 μm	5%	0.1%	607.1 (11.6)
	PET fragments; large	500–547 μm	95%		17.2 (1.4)
3 – Egestion (shape)	PET fragments; large	500–547 μm	90%	0.1%	24.5 (3.5)
	PET Fibres	500 μm	10%		523.4 (28.2)
4 – Egestion (polymer)	PET Fibres	500 μm	50%	0.01%	264.9 (8.7)
	PA Fibres	500 μm	50%		N/A

[a] Fish were fed 2.4 g of food per day.

the diet was comprised of PET fibres (500 μm) and large PET fragments (500–547 μm). Although both types of microplastics had the same nominal length, the fibres were considerably thinner; thus, the number of fibres greatly outnumbered the number of large microplastics per gram. To ensure large fragments were not underrepresented, the diet was prepared with 10% fibres and 90% large fragments, with a final microplastics concentration of 0.1% of the total weight of the pellet and equated to 547.9 MP g^{-1}.

To assess the influence of polymer type on egestion (Experiment 4, Table 1), the diet comprised of 1:1 PA fibres (500 μm) and PET fibres (500 μm), each polymer type representing 50% of the total plastic weight. The final microplastic concentration within the dosed dietary pellets was 0.01% of the total weight of the pellet, equating to approximately 500 MP g^{-1}. A lower concentration of microplastics was used in this experiment to ensure the number of microplastics was relatively consistent across the Egestion Experiments 2–4.

2.4. Fish feeding Protocol

Barramundi are a predatory ambush feeding fish. Thus, pellets were offered one-by-one to each fish individually and occurrence of consumption was recorded, including whether they consumed the pellet as it floated on the surface, as it sank through the water column, or after it had settled on the bottom. The total number of pellets not consumed for each fish was also recorded. If a fish did not consume any of the dosed pellets on the day of the exposure, it was excluded from the study.

2.4.1. Experimental design

Prior to exposure, fish were acclimated within their individual tanks until the entire cohort commenced feeding on the control diet food pellets. Fish were exposed to one of five experimental diets with two variables per experiment. The exact composition of microplastics differed between each treatment to ensure relative consistency between the abundance of particles in each diet and across experiments (Table 1).

2.5. Uptake (experiment 1)

Fish were exposed to fish pellets dosed with PET fibres (500 μm) and fragments (≤547 μm) for 48 h. Fish were offered 2.4 g of dosed pellets once daily. After 30 min, uneaten food pellets were removed from the tanks. Control fish were fed non-dosed food pellets. Five exposed fish were sampled at 0, 6, (dosed once), 24 (dosed twice), and 48 h (dosed thrice), along with three control fish at 0 and 48 h. Fish were humanely euthanised using an ice slurry and stored frozen (−20 °C) in low density polyethylene (LDPE) Ziploc bags until analysed.

2.6. Egestion (experiments 2–4)

Three egestion experiments were conducted over 96 h, all with the same experimental design but each examining one of three microplastic physicochemical characteristics: shape (500 μm PET fibres and 547 μm irregular PET fragments), size (547 μm and 77 μm irregular PET fragments), or polymer type (blue 500 μm PA fibres and 500 μm black PET fibres).

Fish were pulse-exposed to dosed fish pellets. Fish were offered 2.4 g of dosed pellets for 30 min, after which, uneaten food pellets were removed from the tanks. Control fish were fed non-dosed food pellets. From 24 h, all remaining fish were offered the control diet daily. Five exposed fish were sampled at 0, 2, 6, 12, 24, 48, and 96 h along with 3 control fish at 0 and 96 h. Fish were humanely euthanised using an ice slurry and stored frozen (−20 °C) in LDPE Ziploc bags until analysed.

2.6.1. Microplastics isolation from fish tissues and enumeration

Fish were defrosted at room temperature within their individual LDPE Ziploc bags. Once defrosted, they were measured (TL) and weighed (w.w.) (Table 1). After flushing the outer surface of the fish with MilliQ water, they were placed in a stainless-steel dissection tray and dissected according to their exposure experiment. Uptake experiment 1 fish were separated into ~50 g of skinless muscle tissue, liver, and GIT from the oesophagus to the anus. Swim bladders were discarded. For egestion experiments 2–4, only the GIT was dissected from the carcass, with the liver and swim bladder removed. All dissected samples were flushed liberally with MilliQ water, placed into Schott bottles, weighed, capped, and frozen at −20 °C.

2.6.2. Digestion

Fish liver and GIT were digested following the method outlined in the Supplementary Material. Briefly, tissues were defrosted, and the tissue submerged in a defatting solution of 9:1 hexane:ethanol (1:10 *w/v* sample solvent ratio) (Ido et al., 2019). Samples were sonicated for 20 min then left at room temperature (RT) for approximately 9–18 h, after which the solvent was decanted into a Schott bottle and retained for filtration. A second aliquot of solvent was added to the sample, sonicated again for 20 min, and left to stand at RT for a period to bring the total extraction time to 24 h. The second volume of solvent was decanted and retained. The tissue was lyophilised for 15 min to remove any remaining solvent, digested overnight (~18 h) in 200 ml 10% KOH at 40 °C, then diluted with 200 ml MilliQ water. The defatting and KOH digestion solutions were then both filtered over 11 μm nylon filters (GIT) or 8 μm Polycarbonate filters (liver) using a glass Millipore vacuum filtration system. As the food pellets were composed of nondigestible material (i.e., bones, vegetable material, feather, and chitin), GIT filters were clarified with sodium hypochlorite solution (commercial bleach) for 30 s, and then flushed with MilliQ water followed by 70% ethanol. As controls, 1 g of dosed pellets from each diet was also digested using the method outline above.

Fish muscle tissue was defatted and digested as outlined above. However, samples were filtered onto 8 μm PC filters, without the sodium hypochlorite clarification step. Filters were stored in polystyrene Petri dishes with lids and sealed with parafilm until quantification.

2.6.3. Quality assurance and quality control

Laboratory coats were verified by ATR-FTIR to be comprised of 100% cotton with polyester stitching. Laboratory coats and clothing and were brushed with a sticky lint roller to remove loose fibres before commencing work. Plastic laboratory consumables were avoided in preference for glass or metal equivalents. Dissection and filtration were conducted on an open-air laboratory bench, which was wiped clean with MilliQ water and a bamboo cloth. Before use, KOH, ethanol, bleach, and hexane were prefiltered using 0.45 μm Whatman PTFE filters. Items were cleaned prior to use. Cleaning involved washing with tap water and detergent, reverse osmosis (RO) water, and MilliQ water in triplicate. All cleaned items were wrapped in aluminium foil until use. Utensils were not set down on any surface after commencing work. The filtration system was washed between each sample and was rinsed with MilliQ water during assembly. Wash bottles and Schott bottle lids were comprised of Teflon.

Fish were rinsed with RO water before dissection. Furthermore, the dissected muscle, GIT, and liver were flushed with MilliQ water before digestion. Non-exposed fish were used as experimental controls and processed alongside exposed fish. Further, three negative controls per extraction batch were prepared using a subsample of the digestion solution, comprising of potassium hydroxide, hexane:ethanol, and sodium hypochlorite, and processed alongside samples, following the same procedure. Three positive controls per extraction batch were prepared using three individual non-dosed food pellets to which 5 pieces of each plastic used in the experiments (i.e., PA fibres, PET fibres, PET fragments) were added. These were processed, using the same procedure, alongside samples. Putative microplastics identified in the negative controls were analysed using ATR-FTIR.

2.6.4. Microscopic quantification and ATR-FTIR analysis of putative microplastics

Filters were examined using a Leica M205C microscope under brightfield (PET and nylon fibres) or fluorescence light (PET fragments) (NIGHTSEA OSFA-UV 360–380 nm). Filters were visually scanned in a grid search pattern at 25x magnification for 500 μm particles and 60x for 77 μm particles. Microplastics within the GIT contents from the uptake experiment were not enumerated, but classified as presence/absence, representing a positive control confirming pellet ingestion. Microplastics were enumerated in dosed pellets, muscle, liver, and GIT samples taken from egestion experiments, with each filter counted twice. Every item isolated from the fish liver, muscle, and controls were also analysed by ATR-FTIR, as outlined above. For the GIT samples, items were confirmed by visual assessment, and where putative particles were ambiguous, those were also analysed by ATR-FTIR. If particles did not return a strong spectral match to the dosed plastics (i.e., PA or PET), they were excluded.

2.6.5. Meta-analysis of depuration data

For the meta-analysis of published work, a data extraction workflow was followed. Firstly, Web of Science was searched for published studies quantifying microplastics egestion (≥1 μm) in fish. Each publication and supplementary material were searched for raw data of microplastic concentration in the GIT over time. Where raw data was unavailable publicly, the primary author was contacted directly for access to the raw data. If this proved unsuccessful, the free software GetData Graph Digitizer, version 2.26.0.20, was used to extract data from graphs within the publication. Microplastic concentration was converted to MP g^{-1} before calculating the egestion rate. Studies that failed to provide weight data for exposed fish were excluded. Fish trophic level was obtained from FishBase, version August 2022. Microplastic egestion rates and elimination half-life were calculated using GraphPad, version 10.3.0.

Microplastic characteristics explored for influence on egestion were polymer type, shape, size, exposure concentration (total number), exposure method (dietary or aqueous; repeated or single), and fish feeding regimes (prior to exposure, throughout exposure, during depuration period). Biological factors explored for influence on egestion were fish trophic level, fish species, gut anatomy, and water temperature.

2.7. Data analysis

One-way and two-way ANOVAs were used to compare fish length and weight across experiments and over time points. Tissue-specific uptake was calculated as the concentration of microplastics (MP g^{-1}) in the liver and muscle. Concentration of microplastics in the GIT was used to estimate the egestion rates and elimination half-life of the ingested microplastics, using the following equation:

$$C_t = C_0 e^{-k_e t}$$

Where C_t = the amount of microplastics in the fish GIT at a particular time, C_0 = the amount of microplastics in the fish GIT at time 0, k_e = rate constant as number of microplastics per hour, t = measurement time (C_t). Elimination half-life was calculated as the following:

$$t_{1/2} = \ln(2) k_e$$

Where $t_{1/2}$ = microplastic elimination half-life, and k_e = egestion rate constant.

Welsch's ANOVA was used to compare rate constants between the different microplastics. Data analysis was performed in Graphpad 10.2.3.

3. Results

Across the four experiments, non-exposed control fish and procedural analysis controls were completely free of microplastics, confirming nil cross-contamination. Across all treatments, ten fish were excluded due to non-ingestion of the dosed or control food pellets.

Fish weight ranged from 109.1 to 318.6 g and length ranged from 18 to 27.6 cm across the four experiments. There was no significant difference between fish length ($F_{(3, 144)} = 27.07$, $p > 0.05$) and weight ($F_{(3, 117)} = 23.65$, $p > 0.05$) over the three egestion experiments or between sampling time points ($F_{(6, 75)} = 1.116$, $p > 0.05$). However, fish exposed to microplastics for 48 h in the uptake experiment were significantly smaller (length 22.6 ± 1.6 cm, weight 164.1 ± 31.02 g) than those used in the egestion experiments ($F_{(3, 117)} = 23.65$, $p < 0.001$, Fig. 2A–C).

The measured concentration of microplastics in each diet is presented in Table 1. Across the egestion experiments, the total combined exposure of both test microplastics was ~550 MP g^{-1}.

3.1. Uptake

All fish observed consuming food pellets dosed with PET fibres (~500 μm) and PET fragments (<547 μm) were found to have microplastics within their stomach and/or intestine. Controversially, all liver and muscle tissue samples from exposed fish were completely free of dosed microplastics at all time points (0, 6, 24, and 48 h). Due to the viscosity of the digested liver and muscle samples, 8 μm filters were used. Therefore, the presence of nanoparticles and small microplastics (<8 μm) within these tissues cannot be completely discounted.

3.2. Egestion

Large and small PET fragments, originating from the same source material and consistent in terms of polymer composition and shape, were almost completely eliminated within 24 h (Fig. 2E). Interestingly, the two individual fish which still had large PET fragments within their GIT also contained the highest concentration of small PET fragments at 24 h (381 and 72 small fragments, respectively). Beyond 24 h, large fragments were no longer present in the GIT content, however, five individual small fragments, isolated across three fish, were present in GIT samples from the 48- and 96-h sampling points. Together, these results suggest that microplastic egestion may be overarchingly dependent on inter-fish variability or possibly exposure concentration, with fragment size acting as a secondary variable, facilitating the persistence of a limited number of very small microplastic fragments beyond 24 h post ingestion.

There was also little difference in egestion between the large PET fragments and fibres (Fig. 2F). Large fragments were all egested in <24 h. One PET fibre was isolated from a fish at 24 h, and another fish sampled at 48 h also contained two fibres (Table 3, Table S6).

The blue PA fibres, used to assess the egestion of different polymer types with the same size and shape (Experiment 4), could not be quantified in the fish GIT due to the blue dye being leached from the fibres during the extraction procedure. The transparent fibres were impossible to distinguish amongst the recalcitrant material remaining on the filters after digestion (Fig. S3). Thus, only the PET fibres were enumerated. PET fibres co-exposed with PA persisted longer in the GIT of the barramundi than PET fibres co-exposed with PET fragments (Fig. 2G). Numerous (>100) PET fibres co-exposed to PA fibres were still present in fish GIT at 24 h. However, almost all fibres were completely

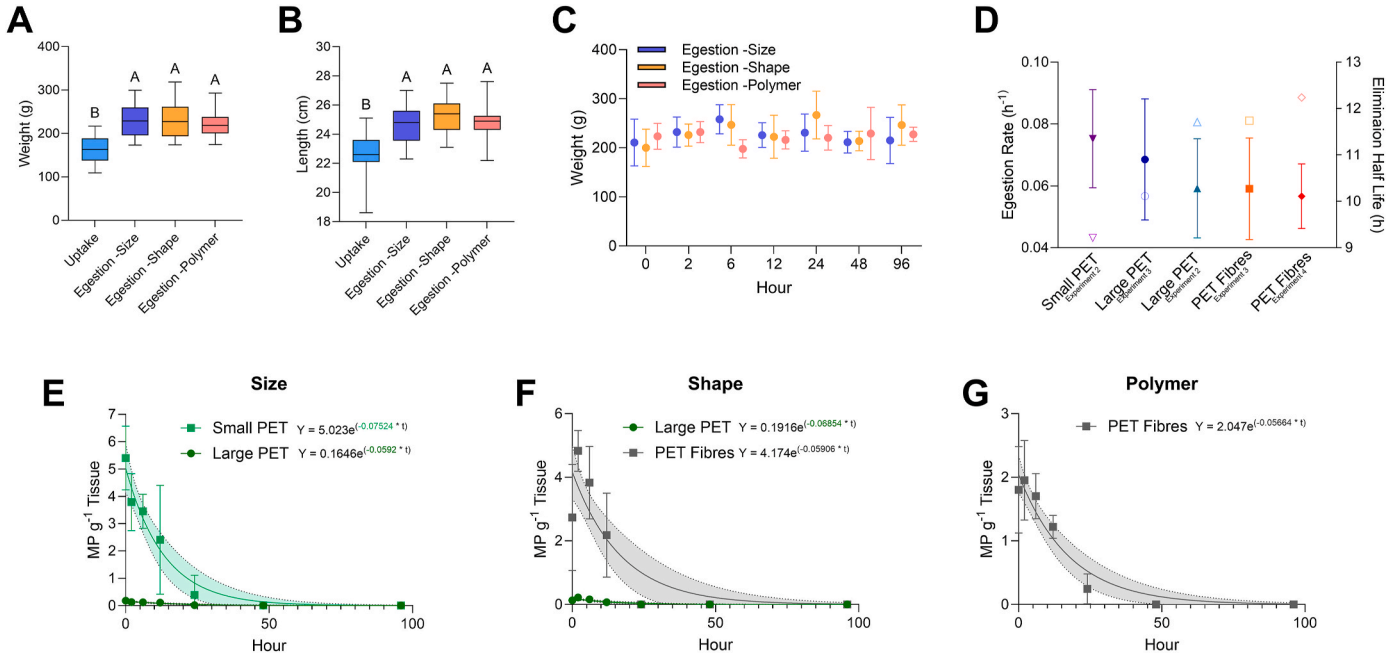

Fig. 2. A) Fish weight and B) length across the four experiments (letters denote significant differences), C) fish weight across egestion time points, D) microplastic egestion rates (solid) and elimination half-life (outline), and E-G) mean microplastic concentration (MP g^{-1} ± SD) of polyethylene terephthalate (PET) fibres and fragments over time after dietary exposure in juvenile barramundi (*Lates calcarifer*) (symbols) and modelled egestion rate (solid lines) with 95% confidence intervals (shading).

egested by 48 h, with only 1 fibre present at this time point. Across the three egestion experiments, more than 90% of all microplastics present in the fish diet were egested within <24 h (Table 3).

Across the three egestion experiments, small PET fragments had the fastest egestion rate at 0.075 h^{-1}, with an elimination half-life of 9.2 h. The elimination half-life of the large fragments and fibres ranged from 10.1 to 12.2 h, with depuration rates between 0.057 and 0.069 h^{-1} (Table 2). There was no significant difference between the depuration rates for any of the microplastics used in the egestion experiments ($F_{(4, 139.7)} = 0.2336$, $p > 0.05$).

A literature search identified 13 studies quantifying microplastic egestion across 11 fish species and which were compatible for meta-analysis (Fig. 3C). Two studies exposed fish to microplastics on consecutive days before analysing egestion (Fig. 3A). These data were excluded when exploring concentration as a factor to remove possible confounding effects (Fig. 3B). Of all the factors analysed, only gut morphology appeared to consistently impact egestion rate (Fig. 2D). The absence of a true stomach in stomachless fish seemed to increase microplastic egestion rates (Fig. 3D). No discernible influence was found across trophic level, or fish species (Fig. 3E), fish weight, water temperature, microplastic polymer (data not shown), shape, and size (Fig. 3A). A slight trend of slower egestion rates was observed for fish exposed to microplastics through diet and possibly elevated exposure concentration (Fig. 3B).

Table 3
Total polyethylene terephthalate (PET) microplastic egestion (% ± standard deviation) across the three egestion experiments.

Microplastic	Microplastics egested <24 h (%) (SD)	Number of Fish with microplastics >24 h
Small PET Fragments	93.71 (10.17)	3
Large PET Fragments	95.16 (11.67)	0
PET fibres	93.65 (6.19)	2

4. Discussion

Despite the extremely high dose of microplastics ingested by juvenile barramundi, no plastic items were isolated from the liver or muscle tissue, confirming that fragments and fibres >8 μm do not translocate from dietary content within the gut into these other tissues. This exposure study also corroborates a recent survey of wild barramundi from the Great Barrier Reef, which did not find any microplastic particles in the muscle tissue (Dawson et al., 2022). Fish studied in the uptake experiment ingested approximately 10,722 individual microplastic items per day. The exposure dose used in this study is four orders of magnitude higher than environmental levels (Santana et al., 2021). Hence, the absence of microplastics in the liver and muscle reveals that if dietary exposure did indeed contribute to microplastic translocation, this phenomenon would be extremely rare in the environment. Few laboratory exposure studies using small microplastic beads (<5 μm) had previously supported the low likelihood of microplastic translocation into fish tissue (Santana et al., 2017). In fact (Zeytin et al., 2020), calculated that for every 1.87×10^7 microplastics ingested, only 1 microplastic translocated into fish muscle, further contributing to the lack of strong evidence of microplastic translocation to fish tissues. This contrasts with previous published environmental studies where large microplastics have been isolated from the nondigestive tissue of several different fish species, including the liver (Guilhermino et al., 2021; Raza et al., 2022) and muscle (Atamanalp et al., 2021; Barboza et al., 2020; Guilhermino et al., 2021; Lu et al., 2024; Makhdoumi et al., 2021; Raza et al., 2022; Selvam et al., 2021). However, in laboratory exposure studies under controlled conditions (Batel et al., 2020; De Sales-Ribeiro et al., 2020; Elizalde-Velázquez et al., 2020; Kim et al., 2020), and environmental studies where particular attention is paid to minimising sample contamination (Dawson et al., 2021) and thorough use and analysis of negative controls (Dawson et al., 2022; Hosseinpour et al., 2021; Huang et al., 2020; Rasta et al., 2021; Su et al., 2019), large microplastics are not isolated from nondigestive tissue. Rigorous quality assurance and quality control is essential for reliable data, especially when testing such an ecologically relevant hypothesis, like tissue translocation and bioaccumulation, which has particular importance for food safety and ecological impacts.

Over the three egestion experiments, the juvenile barramundi egested microplastics quickly alongside food waste. As reported in previous studies, microplastic size and shape seemed to influence egestion rates (Roch et al., 2021; Santana et al., 2021), where larger microplastics were depurated slower than smaller microplastics, with the fibres depurating the slowest. Small particles that persisted within the fish GIT beyond 24 h were also the most abundant within the dosed pellets. It is possible that increased concentration also increases the likelihood of a small number of particles persisting in the GIT. However, the lack of significant differences between the egestion rates of different microplastic shapes and sizes suggests that egestion of particles 8–547 μm can also be overarchingly driven by external factors, such as fish size, gut complexity, or temperature (Bermudes et al., 2010; Gilannejad et al., 2019). Food storage within the barramundi stomach occurs within the pyloric section, which promotes digestion of protein-rich foods and regulates the release of digestate into the intestine (Purushothaman et al., 2016). In this study, microplastics mirrored the gut residence time of food, with typical food passage time ranging from 10 to 16 h (N. Bourne, Pers. Comm). In barramundi, gut residence time and digestibility are linked to water temperature. The fish in this study were exposed in winter, when water temperatures are mild and energy loss is increased. Under summer conditions, elevated water temperatures (~30 °C) are expected to increase the food egestion rate (Bermudes et al., 2010), thus microplastic egestion would also be expected to increase. Overall, although studies modelling microplastics egestion in fish are rare, the few studies which are available also demonstrate a rapid egestion of microplastic particles (Bour et al., 2020; Grigorakis et al., 2017; Jovanović et al., 2018; Ma et al., 2021b; Ohkubo et al., 2020; Ohkubo et al., 2022; Roch et al., 2021).

Based on the performed literature review, the microplastic egestion rates expressed by barramundi were also comparable to other true stomach fish exposed via diet, with a half-life ranging between 9 and 12 h, slightly slower than stomachless fish. For most fish, microplastics were egested at similar rates as food (Grigorakis et al., 2017; Marnane and Bellwood, 1997; Ohkubo et al., 2020; Roch et al., 2021), adding weight to the hypothesis that gut morphology is the dominant factor

Table 2
Egestion rate constant k_e (h^{-1}) and elimination half-life (h) with 95% confidence intervals (CI) of polyethylene terephthalate (PET) fragments and fibres in juvenile barramundi (*Lates calcarifer*).

	Experiment 2		Experiment 3		Experiment 4
	Small PET Fragments	Large PET Fragments	Large PET Fragments	PET fibres	PET fibres
Egestion Rate k_e (h^{-1})	0.075	0.059	0.069	0.059	0.057
95% CI	0.027–0.037	0.025–0.037	0.028–0.040	0.023–0.032	0.016–0.021
Elimination Half-life (h)	9.212	11.709	10.113	11.737	12.237
95% CI	3.017–5.025	4.486–8.718	3.706–6.950	4.162–7.636	3.355–5.026

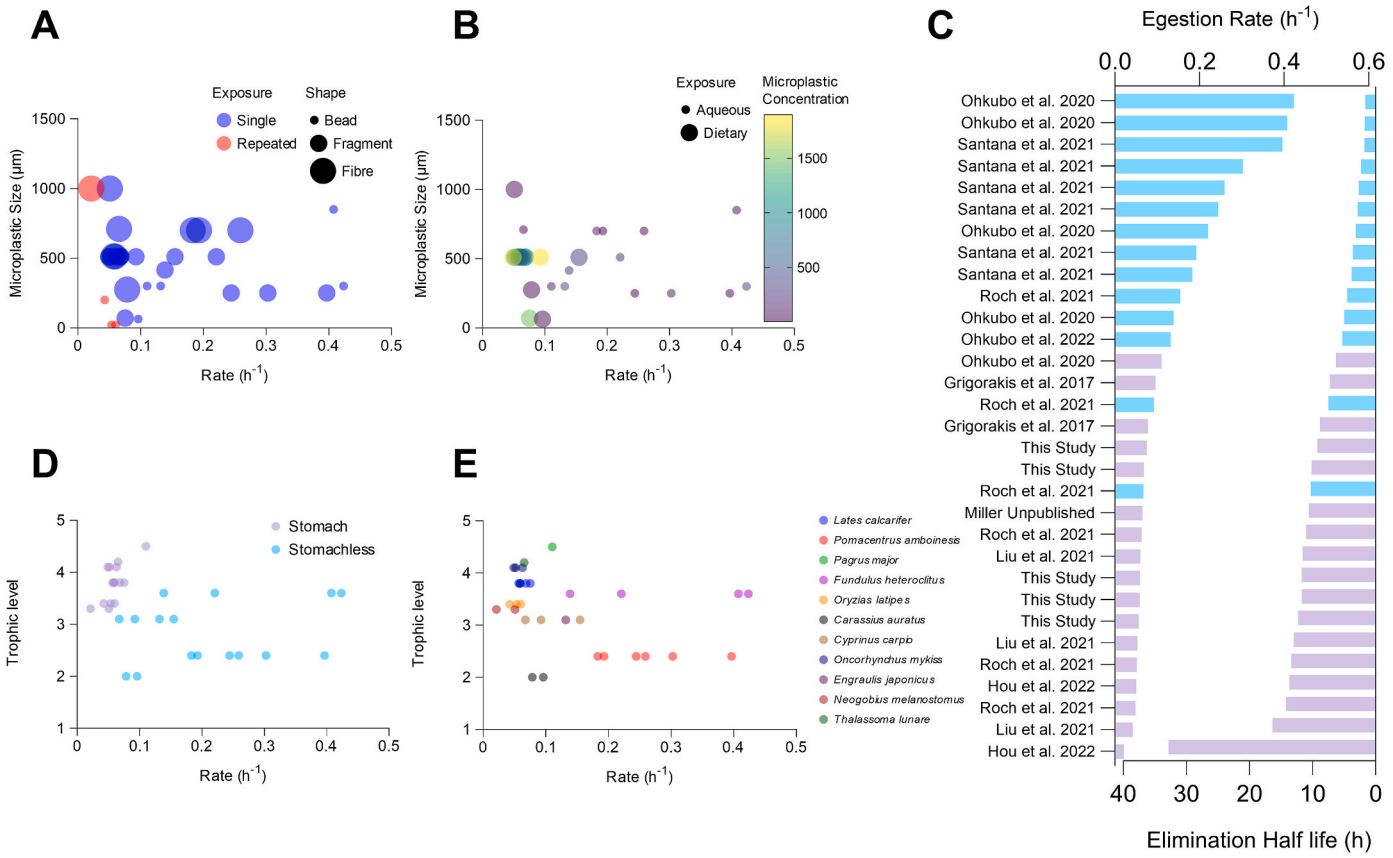

Fig. 3. Meta-analysis of published microplastic egestion from exposed fish. A) Relationship between egestion rate of all fish and microplastic size, shape, and exposure. Red bubbles represent fish that were exposed to microplastics over multiple days. Blue bubbles represent single exposed fish. Bubble size represents microplastics shape, small: bead, medium: fragment, large: fibre. B) Egestion rate of all single exposed fish (blue panel A) compared to exposure concentration. Large bubbles indicate dietary exposure, small bubbles indicate aqueous exposure. C) Calculated egestion rates and elimination half-life used in the meta-analysis. Purple bars indicate fish with a true stomach, blue bars indicate stomachless fish. D) Relationship between egestion rate of all fish (sorted by gut morphology, purple dots indicate true stomach and blue dots stomachless) and trophic level. E) Relationship between egestion rate of all fish species and trophic level.

influencing egestion rates. In fact, considering multiple marine fish species, the meta-analysis revealed that only gut morphology influenced microplastic egestion rates. The similarity amongst egestion rates also suggests that microplastics are likely not translocated or bioaccumulated in any fish. If this were the case, then egestion might be expected to be independent of gut morphology. In contrast with contaminants that are translocated to fish tissues, such as methyl mercury (377 days) (Amlund et al., 2007), PFAS (3.9–28 days) (Martin et al., 2003), and titanium dioxide nanoparticles (1.9–5.7 days) (Mona et al., 2023), the microplastic elimination half-lives of all fish in this meta-analysis could be considered quite rapid, contributing to the evidence of low likelihood of bioaccumulation. The possible exception was Hou et al. (2022), in which a 7-day exposure to 1 mm fibres resulted in a 32 h elimination half-life for stomachless gobies.

Overall, the lack of assimilation of microplastics into nondigestive tissues, along with the rapid egestion rate, confirms that the microplastics tested in this study do not bioaccumulate within barramundi. Furthermore, there was no evidence of gut blockage or difficultly egesting the microplastics, suggesting that microplastics between 8 and 547 µm do not accumulate in the barramundi GIT. This, and the evidence from the meta-analysis, adds to the growing body of work finding that microplastic particles do not bioaccumulate in fish (Elizalde-Velázquez et al., 2020; Hou et al., 2022; Ohkubo et al., 2020; Santana et al., 2021; Santana et al., 2017). In fact, several studies have proposed that previous studies which have isolated microplastics from nondigestive tissues are likely a result of cross-contamination (De Sales-Ribeiro et al., 2020; Jovanović et al., 2018) or artefacts from fluorescent labelling (Catarino et al., 2019; Schur et al., 2019).

Fish, including barramundi, frequently ingest non-food or non-digestible items (Kapoor et al., 1976), and gut morphology is well adapted to suit trophic level and diet composition (Duque-Correa et al., 2024). Wild barramundi prey on prawns, molluscs, and small fish, and their GIT content reflects this composition (Dawson et al., 2020; Purushothaman et al., 2016). They are therefore adapted to egest non-digestible items within their faecal material and could be expected to competently egest microplastics alongside other recalcitrant items. The release of microplastics from faecal material is expected to play a role in the fate and transport of microplastics through the marine environment (Cole et al., 2016; Porter et al., 2018). Microplastics ingested by fish are exposed to chemical digestive processes as they transit through the GIT, altering their surface chemistry (Wheeler et al., 2021). After swallowing, microplastics mix with particulate food and mucus secretions in the oesophagus, then move into the stomach and intestine, where they mix with hydrochloric acid, digestive proteins, and enzymes (Purushothaman et al., 2016). Such biomolecules are known to coat the surface of microplastics forming an ecocorona, which is permanently fixed to the surface. Micro- and nanoplastics covered with an ecocorona have been demonstrated to have significantly altered zeta potential, hydrophobicity, and settling velocity (Schvartz et al., 2023; Zhang et al., 2022), and it has been proposed that egested microplastics, laden with ecocorona, may significantly impact biogeochemical cycling (Cole et al., 2016). However, as wild barramundi have not demonstrated significant microplastic ingestion, their contribution to the geochemical cycling of microplastics remains to be seen.

5. Conclusions

Despite extensive research over the past decade, there is minimal evidence presented thus far for the translocation of microplastics (>10 μm) from the fish GIT into muscle or other nondigestive organs. This laboratory-based study used high exposure concentrations, i.e., >5000 MP g^{-1}, and not a single fibre or fragment was isolated from tissues outside of the GIT. The gut passage of microplastics was considered normal and similar to what has been recorded for natural food, with rapid egestion observed for all microplastic sizes and shapes ingested. Across fish species, the presence of a true stomach resulted in slightly slower egestion rates than stomachless fish. Regardless of this morphological delineation, microplastics do not reside within the fish GIT for significant periods of time.

CRediT authorship contribution statement

Amanda L. Dawson: Writing – review & editing, Writing – original draft, Visualization, Validation, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Marina F.M. Santana:** Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Formal analysis, Data curation, Conceptualization. **Michelle Perez:** Writing – review & editing, Formal analysis, Data curation. **Kelley Meehan:** Writing – review & editing, Formal analysis, Data curation. **Hannah McCarthy:** Writing – review & editing, Formal analysis, Data curation. **Keegan Vickers:** Writing – review & editing, Formal analysis. **Cherie A. Motti:** Writing – review & editing, Writing – original draft, Validation, Project administration, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Animal Ethics

The animal study was reviewed and approved by the James Cook University Animal Ethics Committee (Approval Number A2690).

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Declaration of competing interest

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2025.125884.

Data availability

Data is available at https://apps.aims.gov.au/metadata/view/3c15896d-cbd7-4afe-870f-1945f2bcef4b.

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