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RESEARCH

Microplastic exposure reduces multi-trophic biomass yield in a freshwater integrated aquaculture system

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Abstract

Freshwater integrated multi-trophic aquaculture (IMTA) systems are increasingly used to enhance nutrient utilization efficiency; however, their performance under microplastic (MP) exposure remains poorly characterized. This study evaluated the effects of water-borne and feed-borne polyethylene (PE) MP (10–20 µm) on biomass production and filtration performance in a three-compartment freshwater IMTA system comprising rainbow trout (*Oncorhynchus mykiss*), freshwater mussels (Unionidae), and duckweed (*Lemna minor*). Fluorescent spherical MPs were applied in four treatments ($n = 3$ tanks per treatment): control (no MPs), feed-borne MPs (5 particles per 100 g feed), water-borne MPs (35 particles L^{-1} at fish-tank inflow), and combined exposure. The exposure phase lasted 45 days with 15-day sampling intervals, followed by a 45-day depuration phase. Fish growth responses differed modestly among treatments during exposure, with transient reductions observed in MP-exposed groups relative to the control at intermediate sampling points. Mussel filtration capacity showed limited variation among treatments, with no consistent treatment-specific suppression across time. Duckweed biomass accumulation was lower under combined exposure compared with the control, whereas variability was higher in MP treatments during early growth stages. An integrated multi-trophic performance index calculated from terminal measurements indicated moderate reductions in overall system productivity under combined exposure relative to the control. During depuration, growth trajectories across treatments converged. These results indicate that elevated microplastic exposure can influence biomass distribution and productivity within freshwater IMTA systems, particularly under combined exposure scenarios.

Keywords Microplastic pollution · Bioremediation · Multi-trophic interactions · Aquaculture

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Introduction

Aquaculture has become the fastest-growing food production sector globally and now plays a central role in meeting the rising demand for high-quality aquatic protein. According to recent estimates, global aquaculture production reached approximately 136 million tonnes in 2023, comprising both aquatic animals and algae (FAO, 2025). As production intensifies, aquaculture systems increasingly face constraints related to water quality, feed efficiency, waste management, and emerging contaminants. Among these, microplastic (MP) pollution has recently been recognized as a novel operational challenge with potential consequences for animal performance, production efficiency, and product quality. Unlike classical chemical pollutants, MPs are physically persistent particles that can enter culture systems through multiple pathways and directly interact with cultured organisms (Lusher et al. 2017). Experimental evidence indicates that MP exposure can affect feeding-related processes and physiological performance, including altered energy metabolism and impaired growth, depending on dose and polymer characteristics (Bhat et al. 2024).

Integrated multi-trophic aquaculture (IMTA) has emerged as a promising concept to address these challenges by co-culturing species from different trophic levels, allowing waste from one compartment to become a resource for another (Buck et al. 2018). In freshwater IMTA systems, finfish provide dissolved and particulate nutrients for filter-feeding bivalves and primary producers, while aquatic plants or algae contribute to water purification and additional biomass production (Boyd 2017). When appropriately designed, IMTA systems can improve nutrient recovery, enhance productivity per unit input, and buffer environmental fluctuations compared with monoculture systems. As managed agroecosystems, IMTA systems rely on ecological processes such as nutrient recycling, trophic coupling, and biological filtration to maintain performance under variable environmental conditions (Khanjani et al. 2022). In parallel with the drive toward intensification and circularity, however, MP pollution has emerged as a growing concern for both capture fisheries and aquaculture (Vázquez-Rowe et al. 2021).

MPs are commonly defined as plastic particles ranging from approximately 1 µm to 5 mm in size, originating either as primary MPs manufactured at small sizes or as secondary particles generated through the fragmentation of larger plastic debris (Lusher et al. 2017). MPs are now ubiquitous in aquatic environments and have been documented in surface waters (Shafi et al. 2024), sediments (He et al. 2021), and the water column (Beheshtimaal et al. 2025). They have also been detected in a wide range of aquatic organisms, including commercially important finfish (Bhattacharya et al. 2025) and bivalves (Cho et al. 2021). The rapid growth in global plastic production, combined with inadequate waste management, exacerbates this problem, with several million tonnes of plastic estimated to enter aquatic environments annually, where they are progressively converted into micro- and nanoplastics through physical, chemical, and biological processes (Manon et al. 2026). These particles can sorb and transport other contaminants and are sufficiently small to be ingested by organisms at multiple trophic levels (Lusher et al. 2017). Laboratory and mesocosm studies have demonstrated that MP exposure can induce a range of sub-lethal effects in fish, including oxidative stress, inflammation, altered energy metabolism, and impaired growth and reproductive performance, often in a dose- and polymer-dependent manner (Atamanalp et al. 2023; Bhat et al. 2024). Recent syntheses further suggest that chronic exposure may compromise physiological resilience, particularly when MPs interact with co-occurring stressors such as pathogens or elevated temperature.

Evidence from aquaculture environments confirms that MPs are not limited to marine or open-water systems but represent an operational challenge for inland aquaculture as well. MPs have been detected in culture ponds (Huang et al. 2024), cages (Vazirzadeh et al. 2025), and raceways (Adeel et al. 2025), as well as in commercial fish feeds (Mohsen et al. 2024) and farmed finfish (Matias et al. 2024) and shellfish (Can Tuncelli & Erkan 2024). Recent studies on tilapia (*Oreochromis niloticus*) and panga (*Pangasius hypophthalmus*) cultured in ponds in Bangladesh revealed widespread MP contamination of gastrointestinal and muscle tissues, dominated by fibers and polyethylene and polyester polymers, raising concerns about trophic transfer to consumers (Manon et al. 2026). Parallel surveys of fish ponds and processed fish products further indicate that MPs are embedded along aquaculture value chains from primary production to processing. Although current risk assessments suggest that human exposure via seafood consumption remains relatively low in most cases, the progressive accumulation of MPs in farmed stocks warrants attention, particularly for species consumed whole, such as bivalves and small fish (Lusher et al. 2017).

Despite growing awareness of MP contamination in aquaculture, understanding its ecological and production-level consequences remains challenging due to the limited realism of most experimental designs. Many laboratory studies rely on single-species approaches and elevated MP concentrations that poorly reflect farming conditions (Lusher et al. 2017; Ockenden et al. 2021; Thompson et al. 2024). While such studies have been instrumental in identifying mechanistic toxicity pathways (Bhat et al. 2024), they offer limited insight into whole-system responses. In real-world aquaculture systems, MPs can reach organisms via multiple exposure routes. Waterborne exposure may arise from contaminated intake water, urban effluents, surface runoff, or the degradation of on-farm plastic materials (Hossain et al. 2023; Nabi et al. 2024; Su et al. 2024a). Feed-borne exposure represents an additional pathway, as MPs may be present in feed ingredients or introduced during feed processing, storage, and handling (Su et al. 2024b; Siddique et al. 2025). In IMTA systems, MPs may be transferred across trophic levels and compartments, potentially influencing organismal performance and system functions such as nutrient cycling and biomass production (Alomar et al. 2022; Fraissinet et al. 2024a, b).

To date, no experimental study has evaluated how low, environmentally realistic MP loads delivered via different exposure routes affect overall biomass production in a freshwater IMTA system integrating finfish, filter-feeding bivalves, and aquatic plants. This knowledge gap is critical because IMTA systems operate as interconnected networks in which functional impairment at one trophic level may propagate to others, ultimately altering system-scale outputs (Rusco et al. 2024; Azhar & Memiş, 2024). Moreover, extractive compartments within IMTA systems may act as sinks for particulate pollutants such as MPs, concentrating them into bivalve or plant biomass rather than allowing uncontrolled downstream transport (Fraissinet et al. 2024a, b). Whether this process provides functional buffering or merely redistributes MPs internally remains unresolved.

The present study addresses this gap using a controlled freshwater IMTA system comprising rainbow trout (*Oncorhynchus mykiss*), freshwater mussels (Unionidae), and duckweed (*Lemna minor*). The system was exposed to low concentrations of PE MPs via three exposure routes: water-borne (W-MP), feed-borne (F-MP), and combined water- and feed-borne exposure (FW-MP), alongside an MP-free control. Growth performance, feed utilization, and biomass yield were quantified at both the compartment and whole-system levels. We hypothesized that water-borne and feed-borne MPs would exert distinct effects on trout growth, mussel filtration capacity, and duckweed biomass production, with combined exposure producing the greatest functional impairment. At the system scale, we further

hypothesized that IMTA would confer partial resilience against MP-induced productivity losses through compensatory responses among trophic compartments.

Materials and methods

IMTA system and treatments

The experiment was conducted at the Istanbul University Aquatic Vertebrate Experimental Unit (IUSUCAN), Sakarya, Türkiye, in a freshwater IMTA system comprising three trophic levels: rainbow trout (*Oncorhynchus mykiss,* Walbaum 1792), freshwater mussels (*Unionidae,* Rafinesque 1820), and duckweed (*Lemna minor,* Linnaeus 1753). Each trophic level was housed in dedicated fiberglass units: circular tanks (Ø 90 cm; ~ 600 L) for trout, circular tanks (Ø 80 cm; ~ 400 L) for mussels, and shallow rectangular units (200 × 50 × 10 cm) for duckweed. Groundwater was pre-treated by mechanical sand filtration (Nozbart, Türkiye; 620-mm diameter, 80-cm height, 0.302 m^2 filtration area, 15 $m^3\ h^{-1}$ flow capacity, 50 $m^3\ h^{-1}\ m^{-2}$ filtration velocity, 1.5-bar working pressure, 4-bar test pressure, 90-kg sand load), followed by UV sterilization (UVTEKNIK UV-40 system, Türkiye; equipped with a 320-W high-output UV lamp, 19-mm diameter, 1554-mm length) and polypropylene cartridge filtration (WATECH, Türkiye; 10-µm melt-blown, FDA-compliant material, max. pressure 125 psi). Water movement in the system was maintained using a circulation pump (Nortek HCP 300 T, Türkiye; 2200 W/3 HP, 400 V, 50 Hz, 2850 rpm, 20-m head, 420 L min^{-1} maximum flow). After treatment, water was collected in a reservoir before entering the culture tanks. During the study, water flowed unidirectionally from the reservoir, through the fish tank, the mussel tanks, and into the duckweed tanks. This flow was maintained at ~ 10 L min^{-1} (~ 24 complete exchanges day^{-1} in fish tanks). Water entered each fish tank tangentially to promote circular flow and internal mixing, minimizing dead zones. Aquarium air stones were used to provide continuous aeration, additional turbulence, and vertical mixing.

Prior to stocking experimental organisms, the system was operated under steady-state flow conditions without fish, mussels, or duckweed to verify MP dosing accuracy and spatial homogeneity. The peristaltic pump was calibrated to deliver the target nominal concentration of 35 MPs L^{-1} at the fish-tank inflow. During calibration, 10-L water samples were collected from each tank at five independent time points under operational flow ($n = 5$ per tank). Samples were filtered and fluorescent MPs were enumerated under UV illumination.

The measured concentration across tanks was 34.5 ± 3.2 MPs L^{-1} (mean ± SD), confirming agreement with the nominal target concentration and demonstrating low temporal and spatial variability. No evidence of stratification, surface aggregation, or sediment accumulation was observed during validation runs. Based on these results, particle distribution was considered near-homogeneous under steady-state conditions prior to the introduction of experimental organisms.

Prior to incorporation into the MP-containing diets, spherical fluorescent PE MPs, which are hydrophobic in their pristine state, were initially dispersed in a 0.1% Tween-80 (Merck, Germany) solution to facilitate suspension, and were subsequently rinsed with filtered deionized water in accordance with the manufacturer's technical recommendations. For feed-borne exposure treatments (F-MP and FW-MP), a commercial trout grower diet (Alabalık Büyütme Tam Yemi, MoyFeed®, Özpekler, Türkiye) was used. The proximate composition of the diet is provided in the "Fish stocking and growth assessment" section.

To incorporate MPs into the feed matrix, commercial pellets were mechanically ground into a uniform meal. Fluorescent PE MPs were added directly to the dry feed mixture to achieve a nominal concentration of 5 MPs 100 g^{-1} feed. The mixture was thoroughly homogenized prior to re-pelleting. Controlled amounts of water were added to achieve suitable plasticity, and the mixture was re-pelleted to obtain a uniform 4.0-mm pellet diameter suitable for the experimental fish size.

This matrix incorporation approach ensured internal embedding of MPs within the pellet structure rather than surface coating, thereby minimizing potential particle loss through leaching. To control for potential processing effects associated with grinding and re-pelleting, the control diet underwent identical processing without MP addition (process-matched control). Subsamples of MP-containing pellets were inspected under UV illumination to confirm uniform particle distribution.

For water-borne exposure treatments, spherical fluorescent PE MPs (nominal size 10–20 µm; Cospheric LLC, USA) were dosed using a programmable peristaltic pump (EIN'S, Türkiye) to achieve 35 MPs L^{-1} at the fish-tank inflow, with the suspension injected directly into the inflow line upstream of the fish tank inlet. The injection point was located approximately 30 cm before tank entry to allow initial mixing within the pressurized inflow pipe. Downstream modules (mussel and duckweed units) received MPs solely via advective transport from the fish tanks, reflecting realistic trophic transfer rather than direct dosing. Four treatments were established in triplicate ($n = 3$ tanks per treatment): Control (no MPs), feed-borne MPs (F-MP; 5 MPs 100 g^{-1} feed), water-borne MPs (W-MP; 35 MPs L^{-1}), and combined exposure (FW-MP; 5 MPs 100 g^{-1} + 35 MPs L^{-1}).

The selected MP concentrations were designed to represent an experimentally elevated contamination scenario relative to reported freshwater levels in Turkey, while remaining within the range applied in controlled exposure studies. For water-borne MPs, the nominal concentration of 35 MPs L^{-1} was informed by reported surface-water concentrations of 33 MPs L^{-1} in Küçükçekmece Lagoon (Çullu et al. 2021) and riverine densities ranging from 4 to 293 MPs m^{-3} in Turkish freshwater systems (Erdoğan 2020; Özgüler et al. 2022). Given that most riverine values are reported in volumetric units substantially lower than 35 MPs L^{-1}, the applied concentration represents a high-end experimental scenario rather than typical background conditions.

The selected feed-borne MP dose (5 MPs 100 g^{-1} feed) was based on documented contamination levels in fishmeal and commercial aquafeeds. Reported concentrations include approximately 3.37 MPs 100 g^{-1} in fishmeal (Gündoğdu et al. 2021) and up to 5.4 MPs 100 g^{-1} in formulated feeds (Yao et al. 2021), indicating that the applied level corresponds to the upper range of currently observed contamination.

The exposure phase lasted 45 days with sampling at 15-day intervals, followed by a 45-day depuration phase. Tanks were randomly assigned to treatments at trial start. A schematic of the system and flow path is provided in Fig. 1.

Fish stocking and growth assessment

Rainbow trout (86.49 ± 3.12 g, 20.29 ± 0.24 cm) were stocked at 50 fish per 600 L tank, corresponding to an initial biomass of approximately 7 kg m^{-3}. Fish were acclimated for 4 weeks using the same commercial trout grower diet employed during the experimental phase (Alabalık Büyütme Tam Yemi, MoyFeed–Özpekler, Türkiye; 4 mm pellets). According to the manufacturer's declaration, proximate composition (as-fed basis) was: 44% crude protein, 18% crude lipid, 1.69% crude fiber, 8.07% crude ash, 1.02% calcium,

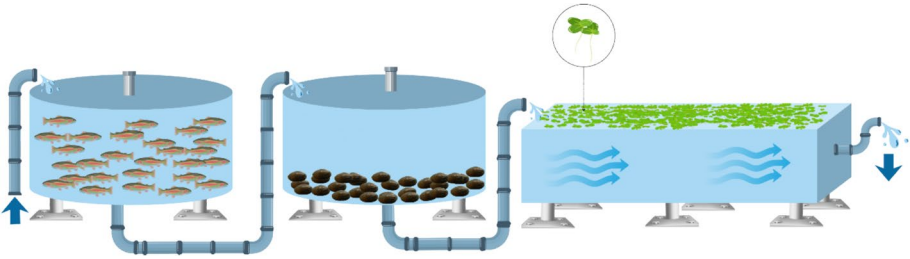


Fig. 1 Experimental design of the freshwater IMTA system comprising three trophic levels: rainbow trout (*Oncorhynchus mykiss*, Walbaum 1792), freshwater mussels (*Unionidae,* Rafinesque 1820), and duckweed (*Lemna minor,* Linnaeus 1753)

0.75% phosphorus, and 0.65% sodium. The ingredient formulation included fish meal, poultry meal, wheat flour, soybean meal, sunflower meal, guar meal, fish oil, soybean oil, blood meal, wheat gluten, corn gluten, and a vitamin–mineral premix.

During exposure, fish were fed twice daily (09:00, 16:00) at 2% body weight per day, adjusted at each 15-day sampling interval based on measured biomass. For the F-MP and FW-MP treatments, fish received the re-pelleted MP-containing diet prepared as described in the "IMTA system and treatments" section.

Each tank initially contained 50 rainbow trout, corresponding to a total biomass of approximately 4.3 kg. At this biomass, feeding at 2% body weight per day corresponded to approximately 86 g feed $tank^{-1}$ day^{-1}. With a crude protein content of 44%, this equates to approximately 38 g protein $tank^{-1}$ day^{-1}. Assuming protein contains 16% nitrogen (protein/6.25), the estimated initial daily nitrogen input was approximately 6.1 g N $tank^{-1}$ day^{-1}, decreasing proportionally as tank biomass declined over successive sampling intervals. At each 15-day sampling interval, 15 fish per tank were removed for growth measurements and not returned to the system. No fish were transferred between tanks or treatments. Consequently, tank biomass gradually decreased over time; however, feeding rates were adjusted proportionally based on the remaining biomass to maintain consistent relative feeding intensity (2% body weight per day). On each sampling day (every 15 days), fish were fasted for 24 h, then 15 fish per tank were randomly netted and lightly anesthetised with 2-phenoxyethanol (0.1–0.3 mL L^{-1}), within the effective range reported for rainbow trout (Ucar & Atamanalp 2010). Anesthetised fish were blot-dried and weighed (wet mass, g) using a wet-weighing, tare-corrected procedure; total length (cm) was recorded on a moist board.

Growth outcomes such as specific growth rate (SGR, % day^{-1}), average daily gain (ADG, g day^{-1}), and feed conversion ratio (FCR) were computed at the tank level using standard formulas:

$$\mathrm{SGR} = \left[\ln\left(W_t\right) - \ln(W_0)\right] \times 100/t \tag{1}$$

$$\mathrm{ADG} = \left[W_t - W_0\right]/t \tag{2}$$

$$\text{Feed conversion ratio (FCR)} = \frac{\text{total feed intake (g)}}{\text{wet weight gain (g) over each interval}} \tag{3}$$

Here, W_0 and W_t are mean body masses at interval start and end; t is days. "Total feed intake" was approximated by feed offered per tank because uneaten pellets were not quantitatively recovered; reported FCRs are therefore upper-bound estimates of true biological efficiency. As secondary indicators, Fulton's condition factor (K) and the coefficient of variation of body mass (CV) were computed per tank per date (Tunçelli & Pirhonen 2021).

$$K = 100 \times W/L^3 \tag{4}$$

$$\text{CV}\ (\%) = 100 \times\ \text{SD}/\text{mean} \tag{5}$$

Here, W was fish weight (g) and L total length (cm), mean represented the average body mass within a tank, and SD corresponded to the standard deviation of body mass measurements.

Freshwater mussel growth measurement

Wild Unionidae (comprising *Unio crassus* and *Anodonta cygnea* species) were collected from Lake Sapanca tributaries and acclimated for 2 weeks. In each of the 12 parallel IMTA units (four treatments × three replicates), 20 adult mussels were stocked per tank (10 *U. crassus* and 10 *A. cygnea*), corresponding to approximately 1.0 kg total wet biomass and a density of ~ 30 ind m^{-2}. Individuals were arranged in three vertical layers to minimize self-shading and promote even flow. A *Spirulina* suspension (prepared fresh daily) was provided once per day as a standardized food source (15 mg/ml/individual/day). Every 15 days, 10 mussels per tank were randomly measured for shell dimensions (length, width, height; mm) and wet weight (g) per tank before being returned to their tanks. In the morphometric table (Table 1), endpoint somatic and shell metrics were recorded separately for *Unio crassus* and *Anodonta cygnea*.

Per-capita filtration rate and particle retention were estimated at each sampling by clearance-rate assays (Coughlan 1969; Fraissinet et al. 2023) on *Unionidae* ($n = 3$ per treatment). Mussels were incubated for $t = 1$ h in vessels with volume v (L) containing a suspended algal surrogate (*Spirulina*). Initial and final algal concentrations (C_0, C_t) were counted with a Thoma haemocytometer. Filtration rate (FR) and percentage particle retention (%PT) were computed as follows:

$$\text{FR} = [v/(DW \times t)] \times ln(C_0/C_t) \tag{6}$$

$$\%\text{PT} = [(C_o - C_t)/C_o] \times 100 \tag{7}$$

where DW is mussel dry weight (g). Thus, FR expresses cleared water volume per unit mussel biomass and time, while %PT represents the proportion of particles removed during the assay. Filtration-rate values were averaged at the tank level prior to statistical analysis to maintain the experimental unit as tank ($n = 3$ per treatment).

For dimensioning purposes, nitrogen input was estimated based on initial biomass (4.3 kg $tank^{-1}$) and a feeding rate of 2% body weight day^{-1}. This corresponded to approximately 86 g feed $tank^{-1}$ day^{-1}, yielding ~ 38 g protein $tank^{-1}$ day^{-1} (44% crude protein). Assuming 16% nitrogen in protein, the initial nitrogen input was ~ 6.1 g N $tank^{-1}$ day^{-1}. Nitrogen input declined proportionally over time as tank biomass decreased.

For the purpose of dimensioning the mussel biofilter, an average nitrogen removal capacity of 0.3 mg N day^{-1} g^{-1} mussel (wet weight) was assumed (Kim et al. 2011). With

Table 1 Mean (±SD) total length (cm), body mass (g), Fulton's condition factor (K), and within-tank coefficient of variation of body mass (CVweight, %) of rainbow trout by sampling day and treatment

Day	Treatment	Length (cm)	Weight (g)	K	CVweight (%)
Day 0	CTRL	20.22 ± 0.13	86.47 ± 0.54	1.04 ± 0.02	6.17 ± 0.26 [a]
	F-MP	20.39 ± 0.20	85.47 ± 2.68	1.01 ± 0.00	6.12 ± 0.12 [a]
	W-MP	20.25 ± 0.26	86.39 ± 3.43	1.04 ± 0.01	5.94 ± 0.38 [a]
	FW-MP	20.29 ± 0.37	87.65 ± 4.99	1.05 ± 0.01	6.16 ± 0.56 [a]
Day 15	CTRL	20.97 ± 0.03	101.11 ± 1.26	1.10 ± 0.02	6.70 ± 0.14 [a]
	F-MP	21.05 ± 0.26	99.76 ± 2.36	1.07 ± 0.03	5.89 ± 0.52 [a]
	W-MP	21.19 ± 0.38	101.22 ± 4.46	1.06 ± 0.03	5.93 ± 0.14 [a]
	FW-MP	21.09 ± 0.22	98.90 ± 1.88	1.04 ± 0.01	5.70 ± 0.52 [a]
Day 30	CTRL	21.96 ± 0.11	113.06 ± 0.71	1.07 ± 0.02	6.27 ± 0.69 [a]
	F-MP	21.76 ± 0.51	110.96 ± 8.02	1.07 ± 0.02	5.65 ± 0.96 [a]
	W-MP	21.66 ± 0.32	108.86 ± 4.94	1.06 ± 0.02	5.66 ± 0.55 [a]
	FW-MP	21.72 ± 0.34	107.46 ± 5.62	1.04 ± 0.03	6.24 ± 0.18 [a]
Day 45	CTRL	22.75 ± 0.28	127.79 ± 2.20	1.08 ± 0.02	5.76 ± 0.60 [a]
	F-MP	22.78 ± 0.47	127.65 ± 5.90	1.08 ± 0.04	5.42 ± 0.93 [a]
	W-MP	22.44 ± 0.20	123.28 ± 2.02	1.09 ± 0.01	5.38 ± 0.74 [a]
	FW-MP	22.45 ± 0.23	124.26 ± 2.99	1.10 ± 0.01	4.79 ± 0.80 [a]
Day 60	CTRL	23.64 ± 0.38	145.06 ± 4.08[ab]	1.10 ± 0.03	5.45 ± 0.52 [a]
	F-MP	23.83 ± 0.24	148.98 ± 2.62 [a]	1.10 ± 0.01	3.85 ± 0.89 [a]
	W-MP	23.22 ± 0.30	139.35 ± 2.79 [b]	1.11 ± 0.03	4.91 ± 0.88 [a]
	FW-MP	23.26 ± 0.19	139.20 ± 4.57 [b]	1.10 ± 0.01	4.71 ± 0.76 [a]
Day 75	CTRL	24.67 ± 0.24	168.90 ± 3.43	1.13 ± 0.01 [ab]	4.02 ± 0.43 [a]
	F-MP	24.64 ± 0.26	172.51 ± 4.94	1.15 ± 0.01 [a]	4.69 ± 0.28 [a]
	W-MP	24.42 ± 0.13	166.62 ± 2.03	1.13 ± 0.01 [b]	4.19 ± 0.24 [a]
	FW-MP	24.41 ± 0.07	164.34 ± 0.67	1.13 ± 0.01 [b]	4.27 ± 0.46 [a]
Day 90	CTRL	25.92 ± 0.41	207.04 ± 4.73	1.19 ± 0.02	3.85 ± 0.43 [a]
	F-MP	25.79 ± 0.25	208.56 ± 2.89	1.21 ± 0.02	4.18 ± 0.81 [a]
	W-MP	25.55 ± 0.06	203.64 ± 2.08	1.22 ± 0.01	3.80 ± 0.26 [a]
	FW-MP	25.48 ± 0.18	202.73 ± 2.62	1.23 ± 0.02	3.93 ± 0.33 [a]

roughly 1000 g wet mussel biomass per tank (20 adults), this yields a theoretical removal capacity of about 300 mg N $tank^{-1}$ day^{-1} (0.3 g N day^{-1}). When compared with the 6.1 g N day^{-1} introduced via feed, this indicates that the mussel assemblage can remove a non-trivial, but limited, fraction of the total nitrogen load. The estimated removal capacity indicates that mussels contribute meaningfully to particulate nitrogen polishing but do not function as the sole nitrogen sink within the IMTA unit.

Duckweed growth monitoring

Lemna minor was collected from local freshwater streams and pre-adapted for 7 days in shallow tanks under controlled laboratory conditions. Nutrients were supplied by adding compost (0.5 g L^{-1}) and approximately 1% (v/v) sludge to the bottom of each tank at the start of the experiment. Compost and sludge inputs were applied identically across

all treatments in terms of timing, mass/volume, and replenishment schedule to ensure standardized organic loading and comparable background conditions among experimental groups. No treatment-specific modifications were made during either the exposure or depuration phases.

LED lighting was maintained under a 10:14 h light:dark photoperiod. At 15-day intervals, biomass yield (fresh weight, g) and frond number were recorded from five randomly selected 10 × 10 cm quadrats per tank. Root length was measured from fronds within the same quadrats at each sampling interval.

Duckweed biomass and root length were selected as indicators of primary-producer performance and as functional proxies for potential in-system particle interception and water clarification within the IMTA loop. This approach is consistent with previous applications of *L. Minor*- Unionidae biofilters in trout aquaculture and freshwater IMTA configurations (Azhar & Memiş, 2023, 2024).

Water quality monitoring

Water temperature, dissolved oxygen, pH, conductivity, salinity, and total dissolved solids (TDS) were measured daily at 09:00, including on sampling days, using a portable multiparameter instrument (SevenGo Duo SG23, Mettler Toledo, Switzerland). The system was maintained under stable conditions, with setpoints as follows: temperature at 13 ± 0.3 °C, pH between 6.9 and 7.0, and conductivity ranging from 420 to 440 µS cm^{-1}. Dissolved oxygen concentrations in the flow-through system averaged around 9 mg L^{-1} and did not fall below 8 mg L^{-1} in any tank. Measurements were conducted at both the inflow and outlet of each trophic unit. These conditions, including cold, well-oxygenated water and near-neutral pH, fall within the optimal ranges recommended for rainbow trout grow-out farming systems (Woynarovich et al. 2011).

Integrated multi-trophic performance index

To summarize treatment effects at the system level, an integrated multi-trophic performance index (IMPI) was calculated based on terminal measurements from all three trophic compartments. The index is deliberately simple and empirical, but conceptually follows the logic of multi-species productivity indicators used in IMTA ecosystem models, where the performance of co-cultured modules is evaluated jointly rather than in isolation (Ren et al. 2012).

For each treatment k and module j (fish, mussels, duckweed), the percentage deviation from the corresponding control mean of a module-specific performance metric was first calculated:

$$\Delta_{k,j}(\%) = 100 \times \frac{\mu_{k,j} - \mu_{CTRL,j}}{\mu_{CTRL,j}} \tag{8}$$

where $\mu_{k,j}$ is the mean value of the selected performance variable in treatment k (fish: ADG or SGR; mussels: per-capita filtration capacity; duckweed: specific growth rate or pre-harvest biomass) and $\mu_{CTRL,j}$ is the corresponding mean in the control group (CTRL).

An integrated multi-trophic performance index was then obtained for each treatment by averaging across modules:

$$\mathrm{IMPI}_{k,j} = \frac{1}{J}\sum_{j=1}^{J}\Delta_{k,j} \tag{9}$$

with $J = 3$ trophic levels (fish, mussels, duckweed). Negative IMPI values indicate an overall reduction in system productivity relative to CTRL, whereas positive values indicate an improvement. In line with its exploratory intent, the IMPI was used descriptively in the "Integrated signal across trophic levels" section to summarize cross-module productivity patterns; no additional formal hypothesis testing was performed on this composite index. Fish ADG, mussel filtration rate, and duckweed biomass were chosen because they represent the primary functional outputs (growth, clearance, plant production) of each trophic level in this IMTA system.

Statistical analysis

All continuous variables were inspected for normality (Shapiro–Wilk test) and variance homogeneity (Levene's test). Statistical analyses were carried out using R and IBM SPSS Statistics (R Core Team 2026; IBM Corp., Armonk, NY, USA). Primary analyses utilized linear mixed-effects models (LMMs), which are appropriate for repeated-measures designs (Bates et al. 2015; Kuznetsova et al. 2017). The LMMs included Treatment, Time, and their interaction as fixed effects, with Tank as a random intercept term, and baseline values as covariates when applicable. Variables were log- or Box–Cox-transformed when model assumptions required it. Post hoc pairwise contrasts and multiplicity-adjusted p-values were obtained as estimated marginal means using the emmeans package (Lenth 2023). Effect sizes (partial η^2 and Cohen's d) with 95% confidence intervals were reported (Cohen 1988). As a sensitivity analysis aligned with common aquaculture reporting practices, repeated-measures ANOVA was additionally performed (Treatment as the between-subjects factor; Time as the within-subjects factor; with Greenhouse–Geisser correction where sphericity assumptions were violated; Greenhouse & Geisser 1959). For all LMMs and repeated-measures ANOVA models, residual diagnostics (Q–Q plots and residuals versus fitted values) indicated that model assumptions were adequately met after transformation where applied.

All statistical inferences were conducted at the tank level. Tanks ($n = 3$ per treatment) were considered the true experimental replicates throughout the study. For fish growth analyses, a linear mixed-effects model was used with TankID specified as a random intercept to account for clustering of repeated measurements within tanks.

For mussel and duckweed endpoints, individual organisms or quadrat measurements were first averaged within each tank, and tank-level means were used as the unit of statistical inference. No statistical tests were performed using individual organisms within the same tank as independent replicates, thereby avoiding pseudo-replication.

Results

Fish growth performance

A Linear Mixed-Effects Model was employed to analyze the effects of treatment and time on trout body weight, specifying Day and Group as fixed factors and TankID as a random

intercept term. The model indicated highly significant main effects for time, treatment, and their interaction: Day ($F(6, 47.7) = 3592.15$, $p < 0.001$), Group ($F(3, 10.9) = 9.12$, $p = 0.003$), and Day × Group ($F(18, 47.7) = 2.23$, $p = 0.014$). The high marginal ($R^2 = 0.986$) and conditional ($R^2 = 0.996$) values suggest that fixed effects, along with the random tank effect, accounted for the majority of variance in fish body weight. The Intraclass Correlation Coefficient (*ICC*) was 0.73, justifying the inclusion of TankID as a random factor. Although the Day × Group interaction was significant ($p = 0.014$), post hoc comparisons indicated that treatment differences were restricted to specific intervals during exposure, whereas final body weight (Day 90) did not differ among treatments ($p > 0.05$).

Growth responses showed modest, time-dependent differences among treatments during the exposure phase (Table 1; Fig. 2). Over the 45-day exposure, mean body mass increased from approximately 85–90 g to 120–140 g across all groups. By Day 30, all MP-exposed groups exhibited slightly lower mean body mass relative to CTRL (113.06 ± 0.71 g), with means of 110.96 ± 8.02 g (F-MP), 108.86 ± 4.94 g (W-MP), and 107.46 ± 5.62 g (FW-MP). Post hoc estimated marginal means comparisons did not support a feed-borne-specific suppression at this time point, and the lowest means were observed in the combined and waterborne treatments rather than exclusively in F-MP.

This pattern was reflected in interval Average Daily Gain (ADG) during days 15–30: CTRL 0.80 g day^{-1}, F-MP 0.75 g day^{-1}, W-MP 0.51 g day^{-1}, and FW-MP 0.57 g day^{-1} (Fig. 2B). Thus, the transient mid-exposure reduction in growth should be interpreted as a modest treatment-associated effect without clear dominance of a single exposure route. During the exposure phase, body mass differed modestly among treatments at some intermediate time points (Table 1; Fig. 2A). At day 30, mean body mass was 113.06 ± 0.71 g (CTRL) versus 110.96 ± 8.02 g (F-MP), 108.86 ± 4.94 g (W-MP), and 107.46 ± 5.62 g (FW-MP), indicating small, non-exclusive deviations among exposed groups. This pattern was reflected in average daily gain (ADG) during days 15–30: CTRL 0.80 g day^{-1}, F-MP 0.75 g day^{-1}, W-MP 0.51 g day^{-1}, and FW-MP 0.57 g day^{-1} (Fig. 2B). CVweight remained low and similar across treatments, suggesting that treatment effects were reflected primarily in mean growth rather than size dispersion. SGR varied less dramatically, remaining in a narrow band (approximately 0.38–0.41% day^{-1}) during exposure and rising to approximately 0.44% day^{-1} by day 90. FCR coupled predictably to these dynamics (Fig. 2D). During exposure, FCR generally remained between 1.0 and 1.6 in CTRL, W-MP, and FW-MP, but briefly exceeded 2.0 in the F-MP group between days 15 and 30, consistent with the transient depression in ADG. In the later depuration phase (75–90 days), FCR rose above 3.0 in all treatments, reflecting size-related declines in feed efficiency rather than persistent treatment differences; this should be interpreted as an upper-bound estimate as it was based on feed offered rather than measured feed intake.

Within each sampling day, values sharing the same superscript letter are not significantly different (LMM with Tukey-adjusted pairwise comparisons, $p > 0.05$). Values are calculated from 10 mussels per tank ($n = 3$ tanks per treatment).

Water quality

Environmental conditions remained tightly controlled and showed no meaningful treatment-related differences. Throughout the trial, water temperature ranged from 13.1 to 13.7 °C, salinity from 0.19 to 0.21‰, dissolved oxygen from ~ 8.8 to 9.1 mg L^{-1}, pH from 6.9 to 7.4 (stabilizing at ~ 6.9–7.0 after day 30), conductivity from ~ 422 to 436 µS cm^{-1}, and Total Dissolved Solids (TDS) from 211 to 218 mg L^{-1}. These values fall within the

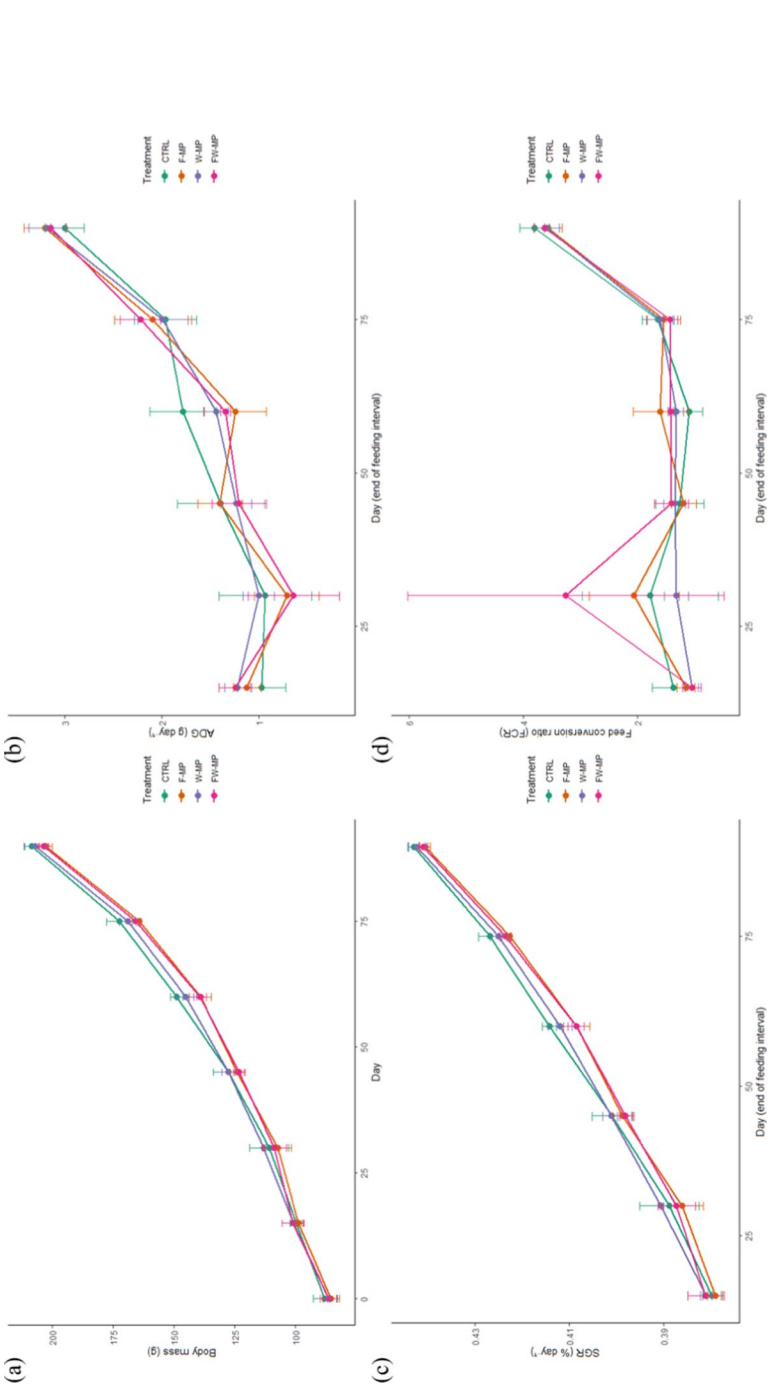


Fig. 2 Growth performance of rainbow trout under microplastic exposure. **a** Body mass (g); **b** average daily gain (ADG, g day^{-1}); **c** specific growth rate (SGR, % day^{-1}); **d** feed conversion ratio (FCR). Treatments are CTRL (no MPs), F-MP (feed-borne MPs, 5 MPs 100 g^{-1} feed), W-MP (water-borne MPs, 35 MPs L^{-1}), and FW-MP (combined exposure, 5 MPs 100 g^{-1} feed + 35 MPs L^{-1} water). Points represent tank means ± SD ($n = 3$). ADG, SGR, and FCR are plotted at the end of each 15-day feeding interval (days 15, 30, 45, 60, 75, and 90). Error bars represent SD from three independent tanks ($n = 3$)

recommended ranges for rainbow trout and remained stable across treatments, supporting the interpretation that observed differences in growth and trophic-level performance primarily reflect MP exposure rather than water-quality drift.

Freshwater mussel performance

Per-capita filtration capacity (L ind^{-1} h^{-1}) differed consistently among treatments and increased over time (as shown in Fig. 3). The ranking was consistent across analyzed time points (CTRL > W-MP > F-MP > FW-MP), with gradual improvements during exposure and further gains after depuration. For illustration, *Unionidae* means rose from baseline values of ~ 0.66–0.82 L ind^{-1} h^{-1} to ~ 0.75–0.95 L ind^{-1} h^{-1} by the end of depuration, corresponding to a relative increase of approximately 15%, while the combined-exposure group (FW-MP) remained clearly below CTRL at study end. A two-way ANOVA with treatment and time as fixed factors (tank-level means) revealed significant main effects of both treatment ($F_{(3,24)} = 5.65$, $p = 0.0045$) and time ($F_{(2,24)} = 5.34$, $p = 0.0121$), but no treatment × time interaction ($F_{(6,24)} = 0.29$, $p = 0.94$). Post hoc comparisons on the treatment main effect showed that CTRL mussels had higher filtration rates than FW-MP based on the treatment main effect (averaged across time) (Tukey, $p = 0.0038$), whereas F-MP and W-MP occupied an intermediate position (shared "ab" group). For the time main effect,

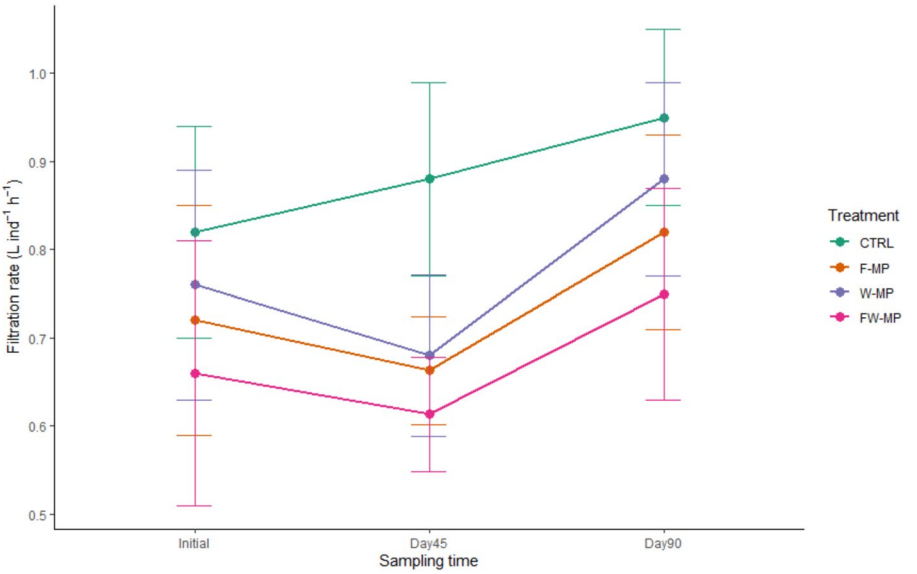


Fig. 3 Freshwater mussel filtration rate under microplastic exposure. Time course of per-capita filtration capacity of freshwater mussels (Unionidae) in the freshwater IMTA system under different microplastic (MP) exposure regimes. Treatments are: CTRL (no MPs), F-MP (feed-borne MPs, 5 MPs 100 g^{-1} feed), W-MP (water-borne MPs, 35 MPs L^{-1}), and FW-MP (combined exposure). Filtration rate (L ind^{-1} h$^{-.1}$) was analyzed using three functional time levels: baseline (day 0), end of the 45-day exposure phase (day 45), and end of the 45-day depuration phase (day 90). Although intermediate exposure measurements were recorded, statistical comparisons were performed using these three time levels. Symbols represent treatment means ± SD ($n = 3$ tanks per treatment)

filtration at day 90 was significantly higher than at day 45 (Tukey, $p = 0.026$), with initial values statistically intermediate between these two sampling points.

Morphometric data further supported modest structural growth in CTRL and relative suppression under F-MP and FW-MP, especially in *Anodonta cygnea* (Table 2). In both *Unio crassus* and *A. cygnea*, shell length, geometric volume, and whole wet mass increased from baseline to day 90. Crucially, the end-point whole wet mass of *A. cygnea* was significantly lower in the F-MP (37.01 ± 0.70 g) and FW-MP (34.49 ± 2.70 g) groups compared to CTRL (43.00 ± 2.50 g, Duncan's $p < 0.05$), confirming that both feed-borne and combined exposure regimes led to structural growth inhibition in this species. W-MP generally occupied an intermediate position.

Condition index (CI) and meat yield (%) were broadly stable across treatments, suggesting that MP exposure primarily influenced growth trajectories and filtration performance rather than gross condition factors.

Different letters indicate significant differences among treatments (Duncan's multiple range test, $p < 0.05$). Values are based on tank-level means calculated from 10 mussels per tank ($n = 3$ tanks per treatment). Statistical comparisons were performed using tank means as the unit of replication.

Lemna growth performance

Early SGR was clearly treatment-sensitive during the first interval (0–15 days), ranking CTRL > W-MP > F-MP > FW-MP 0–15 (Table 4), with the strongest initial suppression observed under combined exposure. Linear mixed-effects modelling (tank as random intercept) confirmed a significant Treatment effect during this interval, with reduced SGR under F-MP ($p = 0.020$) and FW-MP ($p = 0.004$) relative to CTRL. These results indicate that early growth kinetics were particularly vulnerable to trophic MP exposure, especially when feed- and water-borne pathways acted simultaneously.

Thereafter, group differences in SGR diminished. Growth rates increased substantially across all treatments during 15–30 days and 30–45 days (Interval effect: $p < 0.001$), reflecting compensatory biomass accumulation following early suppression. Significant Interval × Treatment interactions for FW-MP ($p = 0.025$–0.030) indicated partial recovery of SGR in later intervals. By the depuration phase (45–90 days), daily SGR values converged among treatments (CTRL ~ F-MP ~ W-MP ~ FW-MP), demonstrating that the initial suppression under combined exposure was largely transient once external particle pressure was reduced (Table 3).

Root length remained largely stable at ~ 13 °C, with only small net changes from baseline to study end (CTRL + 3.2%, F-MP + 2.4%, W-MP + 2.4%, FW-MP + 0.8%). End-point means were similar among treatments; however, the standard deviation (SD) for root length was consistently higher in the FW-MP group (~ 9 mm) across all time points (Table 4) compared to the other treatments (SD ~ 3.5–4.1 mm). This pronounced increase in between-replicate variance suggests greater heterogeneity in colony-level responses under combined trophic exposure, despite the absence of strong mean separation. Values were averaged at the tank level prior to statistical comparison.

Pre-harvest biomass rose markedly from the approximately 600 g baseline in all treatments (Fig. 4). CTRL maintained the highest means throughout (e.g., day 15: 1217.63 ± 158.95 g; day 90: 1406.39 ± 127.90 g), with W-MP tracking closely at several time points (day 30: 1342.94 ± 137.28 g; day 90: 1389.58 ± 198.17 g). FW-MP consistently yielded the lowest totals (day 15: 663.81 ± 174.17 g; day 90: 872.32 ± 279.65 g).

Table 2 Somatic and shell metrics (mean ± SD) of *Unio crassus* and *Anodonta cygnea* at baseline (initial) and day 90. Mussel and shell mass (g), linear shell dimensions (length, width, height; mm), geometric volume (cm^3), condition index (CI, %), and meat yield (%)

Species	Treatment	Mussel (g)	Shell (g)	Length (mm)	Width (mm)	Height (mm)	Volume (cm^3)	CI (%)	Meat yield (%)
Unio crassus	Initial	30.08 ± 4.70	17.80 ± 2.70	64.25 ± 4.50	34.83 ± 1.10	23.58 ± 2.00	52.96 ± 7.30	57.02 ± 7.10	62.81 ± 1.60
	CTRL	33.31 ± 2.40	20.30 ± 0.40	66.88 ± 1.20	36.25 ± 0.50	25.38 ± 0.50	61.53 ± 2.50	54.30 ± 6.30	62.08 ± 2.10
	F-MP	27.43 ± 5.40	17.62 ± 3.10	63.96 ± 4.30	33.76 ± 1.40	24.67 ± 2.20	53.70 ± 10.00	51.00 ± 0.70	60.81 ± 1.00
	W-MP	29.17 ± 1.10	18.11 ± 1.40	64.70 ± 1.30	35.14 ± 0.60	24.26 ± 0.60	55.21 ± 3.30	52.88 ± 1.10	61.73 ± 0.90
	FW-MP	27.34 ± 3.30	17.21 ± 1.80	63.38 ± 3.30	34.43 ± 1.30	23.90 ± 1.30	52.39 ± 7.20	52.30 ± 2.10	61.33 ± 0.50
Anodonta cygnea	Initial	39.79 ± 5.30	13.92 ± 1.30	89.67 ± 4.40	47.42 ± 3.10	26.97 ± 2.70	114.70 ± 21.00	34.70 ± 4.30	74.10 ± 1.50
	CTRL	43.00 ± 2.50[a]	14.00 ± 0.90	96.33 ± 3.50	51.72 ± 1.50	27.25 ± 0.40	136.41 ± 7.20 [a]	31.64 ± 3.40	75.40 ± 2.30
	F-MP	37.01 ± 0.70[b]	14.99 ± 4.60	91.06 ± 1.70	50.08 ± 1.50	27.00 ± 1.00	124.23 ± 4.40 [ab]	29.82 ± 1.30	71.51 ± 6.40
	W-MP	39.18 ± 3.30[ab]	14.52 ± 1.30	94.92 ± 1.50	47.57 ± 6.60	27.83 ± 1.00	128.45 ± 15.20 [ab]	31.00 ± 6.40	72.95 ± 1.70
	FW-MP	34.49 ± 2.70[b]	12.06 ± 0.60	88.90 ± 9.40	46.69 ± 5.90	26.49 ± 0.50	113.91 ± 27.40 [b]	31.78 ± 9.60	74.07 ± 0.70

Table 3 Interval-specific SGR (% day^{-1}) of *Lemna minor*, calculated following the 80% harvest rule. Each value is based on tank-level means ($n = 3$ per treatment)

Interval (days)	Control	Feed-borne MPs	Water-borne MPs	Combined
0–15	8.31	6.26	6.77	4.27
15–30	11.28	11.52	12.93	10.83
30–45	10.63	10.66	10.03	11.35
45–90*	3.75	3.83	3.89	3.94

*The 45–90-day span covers 45 days in a single step (two intermediate 15-day harvests are aggregated); daily rates are therefore not directly comparable to the 15-day intervals but still illustrate convergence among treatments during depuration

Dispersion was generally tight in CTRL and W-MP (typical CV 6–15%), whereas F-MP and particularly FW-MP exhibited markedly higher variability during the exposure phase, indicating reduced production stability under trophic MP pressure.

When SGR was computed per interval accounting for the 80% harvest rule (Table 4), early growth suppression under FW-MP (0–15 d: 4.27% day^{-1} vs. CTRL 8.31% day^{-1}) was followed by convergence during subsequent intervals, including depuration (45–90 d: ~ 3.75–3.94% day^{-1}). Together, these results demonstrate that MP exposure primarily altered early growth dynamics and increased inter-tank variability, while longer-term relative growth rates partially recovered once exposure intensity declined.

Integrated signal across trophic levels

Across the IMTA modules, MP exposure imposed measurable short-term functional costs, including reduced trout ADG and feed efficiency, lower duckweed biomass accumulation, and decreased mussel filtration activity. Although several parameters partially recovered during the depuration phase, the combined exposure treatment (FW-MP) consistently exhibited the weakest performance across modules and remained numerically below CTRL in the mussel filtration endpoint at study termination.

To integrate cross-module responses, an Integrated Multi-Trophic Performance Index (IMPI) was calculated based on the relative percentage deviation from CTRL at the end of the study for three functional endpoints: trout ADG, mussel filtration rate, and duckweed pre-harvest biomass. End-of-study values for each treatment were normalized to CTRL (set at 0%), and the arithmetic mean of percentage deviations across the three modules was used to obtain a composite system-level indicator. This index is presented descriptively to summarize cross-trophic performance patterns rather than for formal hypothesis testing.

The IMPI indicated a marginal system-level change under W-MP (− 1%), a moderate productivity reduction under F-MP (− 9%), and a pronounced decline under combined exposure (FW-MP; − 18%), reinforcing the consistent ranking CTRL > W-MP > F-MP > FW-MP (Table 5). These integrated results suggest that while single-route exposure induced limited system-wide disruption, simultaneous feed- and water-borne exposure produced additive or synergistic productivity costs at the multi-trophic scale.

Negative values indicate an overall reduction in system productivity relative to the MP-free control; values are used descriptively of cross-module performance rather than for formal hypothesis testing.

Table 4 Root length (mm; mean ± SD) of *Lemna minor* under different MP exposure regimes across sampling days. Measurements represent five 10 × 10 cm quadrats per tank, $n = 15$ per treatment per sampling day

Treatment	Initial	Day 15	Day 30	Day 45 (start of the depuration)	Day 90 (end of depuration)
CTRL	10.40 ± 3.60	10.55 ± 3.52	10.70 ± 3.45	10.50 ± 3.60	10.73 ± 3.54
F-MP (Feed MP; 5 MP 100 g^{-1})	12.50 ± 3.60	12.70 ± 3.55	12.90 ± 3.50	12.60 ± 3.56	12.80 ± 3.51
W-MP (Water MP; 35 MP L^{-1})	11.20 ± 4.10	11.40 ± 4.05	11.60 ± 4.10	11.35 ± 4.00	11.47 ± 4.06
FW-MP (Feed + Water MP)	12.00 ± 8.80	12.20 ± 8.90	12.30 ± 9.30	11.90 ± 9.00	12.10 ± 9.10

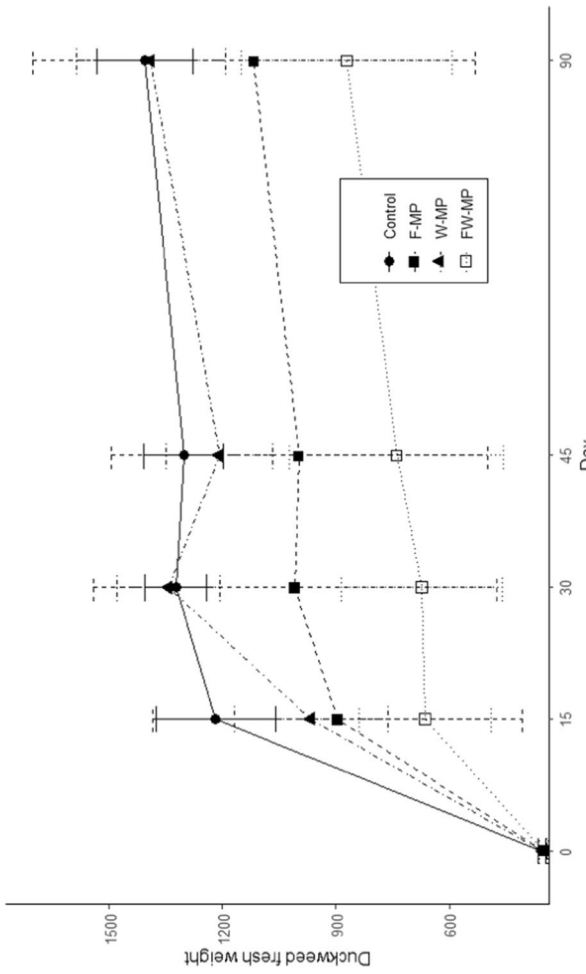


Fig. 4 Duckweed biomass under microplastic exposure. Time course of duckweed (*Lemna minor*) biomass (fresh weight, g) in the freshwater IMTA system under different microplastic (MP) exposure regimes. Treatments are: CTRL (no MPs), F-MP (feed-borne MPs, 5 MPs 100 g^{-1} feed), W-MP (water-borne MPs, 35 MPs L^{-1}), and FW-MP (combined exposure, 5 MPs 100 g^{-1} feed + 35 MPs L^{-1} water). Biomass was measured immediately before harvest at days 0, 15, 30, 45, and 90. At each sampling, ~ 80% of the canopy was harvested and 20% was left to regrow until the next interval; the final interval (45–90 days) thus spans 45 days. Symbols show treatment means ± SD ($n = 3$ plant beds per treatment; ~ 1.0 $m.^{2}$ each). Error bars represent SD from three independent tanks ($n = 3$)

Table 5 Integrated multi-trophic performance index (IMPI, % deviation from CTRL) combining end-of-study trout growth (ADG), mussel filtration rate, and duckweed pre-harvest biomass

Treatment	IMPI (% deviation from CTRL)
CTRL	0.0
W-MP	− 1.0
F-MP	− 9.0
FW-MP	− 18.0

Discussion

This study indicates that exposure route structured performance patterns within the freshwater IMTA system under the tested conditions. While final trout body mass did not differ significantly among treatments at Day 90, interval-specific growth metrics (ADG and FCR) revealed transient differences during the exposure phase. At the multi-trophic level, combined exposure generated the most consistent reductions across functional endpoints, whereas single-route exposures were associated with comparatively moderate system-level changes. These findings should be interpreted strictly as performance-level responses within the experimental framework applied here.

Trout growth responses under different exposure routes

Rainbow trout growth performance was most strongly affected by feed-derived MP exposure. Feed-borne MPs caused a transient reduction in growth and feed-use efficiency, whereas water-borne MPs produced only minor effects on fish performance. This pattern suggests that co-ingestion of MPs with nutrients may represent a comparatively more impactful exposure context under the present experimental conditions, although direct quantification of internal MP burdens was not performed. A recent systematic synthesis of micro- and nanoplastic exposure in aquaculture species similarly highlights exposure route as a critical determinant of biological outcome severity and variability across taxa (Mohsen et al. 2025). Comparable growth rates and feed conversion values reported for trout reared in Lemna–Unionidae biofiltered ponds under MP-free conditions (Azhar & Memiş, 2024) support the interpretation that the observed growth depression was attributable to MP exposure rather than limitations of the IMTA configuration or water quality.

Mechanistically, it is plausible, based on previous experimental work in salmonids, that co-ingested MPs may interfere with gut mixing, nutrient–epithelium contact, or induce localized inflammatory responses. However, the present study was designed to quantify system-level performance endpoints (growth, filtration, and biomass production) rather than physiological or histopathological responses. Consequently, no histological, enzymatic, oxidative stress, or tissue-burden markers were assessed within this experimental framework, and the above mechanisms should therefore be interpreted as literature-supported hypotheses rather than directly demonstrated processes in this dataset. If such processes occur, they could increase maintenance energy demands and potentially divert resources away from somatic growth. In contrast, water-borne MPs at the concentrations used here are encountered more diffusely and intermittently, which may result in a comparatively lower effective gastrointestinal exposure. Similar mechanisms have been proposed

for salmonids exposed to PE-rich MPs, where inflammation and oxidative stress were linked to impaired nutrient assimilation (Bhat et al. 2024).

Importantly, trout exhibited strong compensatory growth once MP exposure ceased. During the depuration phase, growth rates converged across treatments, and final body masses were nearly indistinguishable. This recovery aligns with evidence that rainbow trout retain only a small fraction of ingested MPs and rapidly eliminate the remainder (Kim et al. 2020). Together, these findings indicate that low, environmentally realistic MP loads impose reversible performance costs rather than permanent growth suppression in trout, underscoring the value of depuration windows in aquaculture systems facing episodic MP exposure.

Freshwater mussels under combined exposure

Freshwater mussels (Unionidae) exhibited the most consistent functional deviation across trophic modules, particularly under combined exposure. Filtration capacity consistently ranked CTRL > W-MP > F-MP > FW-MP across analyzed time levels, and the combined-exposure group did not fully converge with CTRL by the end of the depuration phase. Structural growth in *Anodonta cygnea* was lower under feed-borne and combined exposure relative to CTRL, whereas condition index and meat yield remained broadly stable. These patterns indicate that MP exposure modulated feeding-related performance and growth trajectories without clear evidence of systemic deterioration in gross condition metrics. These findings are consistent with previous reports of MP-associated suppression of clearance rates in bivalves exposed to contaminated water (Harris et al. 2020; von Moos et al. 2012). the exposure concentrations applied here were comparatively moderate, the directionality of response aligns with those studies. This suggests that functional feeding parameters in suspension feeders may respond to particle exposure even at non-extreme concentrations, though direct particle burdens in tissues were not quantified in the present study.

In marine IMTA systems, filter-feeding assemblages have been shown to remove substantial proportions of suspended MPs from the water column, with concurrent particle accumulation in tissues (Fraissinet et al. 2024a, b). The present freshwater data demonstrate comparable functional performance modulation in unionid mussels; however, no direct measurements of particle retention, clearance kinetics, or internal load were conducted here. Therefore, parallels with marine retention dynamics should be interpreted cautiously.

The persistent filtration reduction observed under combined exposure may reflect cumulative interaction between suspended particles and filtration structures. As continuous suspension feeders, mussels experience repeated particle contact across gill and digestive epithelia. Under dual exposure pathways, both water-derived and feed-associated particles may increase the effective particle encounter rate at the filtration interface. Nevertheless, because neither tissue-level MP concentrations nor histopathological endpoints were assessed in this dataset, these explanations remain mechanistic hypotheses rather than demonstrated processes.

From a systems perspective, reduced filtration performance has implications for IMTA functionality, as mussel filtration contributes to particulate removal and water quality regulation. However, the present study quantifies functional performance outcomes only and does not directly assess particle attenuation efficiency along the hydraulic flow path.

Similar trade-offs between MP removal potential and biological costs to filter feeders have been discussed in the context of mussels as nature-based solutions for MP mitigation

(Cole 2023). In this framework, unionid mussels may represent a biologically active interface between suspended particles and system productivity, with exposure-route-dependent performance consequences under controlled experimental conditions.

Duckweed buffers MP exposure but exhibits increased variability

Duckweed (*Lemna minor*) was the least affected component in terms of mean biomass production. Although combined exposure caused a short-term reduction in specific growth rate during early exposure, growth rates converged across treatments during depuration, and endpoint biomass differed only modestly among groups. These results are consistent with previous IMTA and aquaponic studies showing that *Lemna* spp. can maintain high productivity under nutrient-driven conditions (Paolacci et al. 2022a,b; Stejskal et al. 2022).

A key and novel finding, however, was the pronounced increase in between-replicate variability under combined MP exposure. While mean biomass and root length remained comparable, standard deviation and coefficient of variation were consistently higher in FW-MP tanks. This indicates that MP stress in duckweed manifested primarily as heterogeneous colony-level responses rather than uniform suppression of average productivity.

Potential mechanisms may include localized particle aggregation at the root surface, uneven spatial distribution of MPs within plant beds, and microscale shading or physical interference effects. These mechanisms were not directly quantified in the present study and should therefore be considered as plausible but unverified explanations. Experimental studies on duckweed exposed to MPs similarly report increased growth variability and physiological heterogeneity rather than consistent biomass decline (Ceschin et al. 2023). Even biodegradable polymers have been shown to alter root architecture and growth dynamics (Procházková et al. 2023), suggesting that particle–plant interactions extend beyond conventional plastics.

From a production perspective, increased variability may be as relevant as changes in mean yield. In IMTA and aquaponic operations, predictability of plant biomass influences harvest scheduling, downstream processing, and integration as feed or biofertilizer inputs. Thus, even when average productivity remains stable, elevated variance under MP exposure could reduce operational reliability.

Integrated system responses suggest partial buffering but functional redistribution

When responses across all trophic levels were integrated using the multi-trophic performance index (IMPI), a coherent system-level signal emerged. This route-dependent modulation aligns with recent systematic evaluations of micro- and nanoplastic impacts in aquaculture organisms, which emphasize exposure pathway as a key structuring variable for growth, physiological, and behavioral endpoints (Mohsen et al. 2025). Relative to the MP-free control, overall productivity declined by approximately 1% under W-MP, 9% under F-MP, and 18% under FW-MP. The negligible reduction under water-borne exposure indicates that the IMTA configuration maintained functional performance under the tested water-column MP concentration.

In contrast, feed-borne MPs ere associated with larger productivity penalties across modules, consistent with more direct trophic transfer. Although the combined treatment produced the strongest reduction, overall system output remained above 80% of control levels, indicating retained functional integrity.

Importantly, the apparent buffering of water-borne MPs at the system level should not be interpreted as particle removal. Rather, it likely reflects redistribution of stress across trophic compartments. Because MP burdens in water and tissues were not quantified in this study, conclusions regarding interception, accumulation, or elimination remain inferential. Existing literature indicates that filter feeders and aquatic plants can retain MPs within biomass (Li et al. 2019; Fraissinet et al. 2024a, b), suggesting that IMTA-mediated buffering may involve internal partitioning rather than degradation or true removal.

Accordingly, IMTA buffering should be interpreted as functional redistribution of stress within the system, not elimination of MP particles. This distinction has implications for co-product management and food-safety assessment in integrated production systems.

Implications, limitations, and outlook

Several practical implications arise from these findings. First, reducing feed-borne MP contamination should be prioritized, as diet-derived particles imposed the greatest productivity costs. Improved raw-material screening, cleaner feed-processing lines, and reduced plastic abrasion during storage and handling represent tangible mitigation strategies. Second, monitoring and filtration of intake water remain important, particularly in systems drawing from MP-contaminated surface waters. Third, IMTA designs can be optimized for MP management by adjusting the density and placement of mussels and macrophytes to maximize capture while ensuring that accumulated MPs are removed via planned depuration and biomass handling.

This study has limitations. Exposure was limited to one MP type (spherical PE, 10–20 µm) and a 90-day timeframe under controlled conditions. Responses to other particle shapes, polymers, or longer-term exposure may differ, particularly under interacting stressors such as pathogens or fluctuating water quality. Nonetheless, by applying environmentally realistic MP loads within a multi-trophic freshwater IMTA configuration, this study offers an integrated performance-based assessment of productivity responses under controlled aquaculture conditions. While mechanistic pathways were not directly investigated, the results provide quantitative evidence on how exposure route influences growth and filtration dynamics at the system level.

Overall, the results indicate that IMTA can enhance resilience to MP pollution, but not without cost. Preventing MP entry, especially via feed, combined with targeted buffering, depuration, and monitoring, offers a realistic pathway toward MP-aware, production-secure aquaculture systems.

Conclusion

Within a controlled freshwater IMTA system combining trout, mussels, and duckweed, both exposure route and trophic identity modulated functional responses to the tested MP loads. Feed-borne MPs were associated with comparatively larger short-term deviations in trout growth metrics and feed conversion during the exposure phase, whereas water-borne MPs at 35 MPs L^{-1} produced relatively minor changes in fish performance endpoints. Mussels exhibited partial functional recovery during depuration but remained numerically below control filtration rates under combined exposure at study end. Duckweed maintained overall biomass production, with MP-related effects expressed primarily as increased between-replicate variability rather than sustained reductions in mean growth. At the integrated system level,

descriptive productivity deviations relative to the MP-free control were small under water-borne exposure (−1%), moderate under feed-borne exposure (−9%), and more pronounced under combined exposure (−18%). These results indicate that, under the tested concentration regime, system-level productivity metrics remained comparatively stable under single-route exposure, while simultaneous feed- and water-borne exposure was associated with greater cross-module performance modulation.

Importantly, these findings reflect functional performance outcomes rather than confirmed particle removal, attenuation, or redistribution dynamics. MP concentrations along the hydraulic flow path and tissue-level burdens were not quantified; therefore, no direct conclusions can be drawn regarding interception efficiency, internal accumulation, or clearance processes within the system. The present study documents performance-level responses under controlled exposure conditions, not fate-level resolution of MPs within the IMTA configuration.

From an applied perspective, reducing MP entry, particularly through feed quality control, may represent a higher priority intervention than low-level water-column exposure control under comparable contamination scenarios. Intake management, on-site filtration, optimized extractive-module stocking, and planned depuration windows may contribute to maintaining production stability under mixed exposure contexts. Future research should integrate particle tracking, tissue burden quantification, histopathological and physiological endpoints, and broader dose–response frameworks across particle types and morphologies to mechanistically link exposure route with system-level productivity outcomes.

Author contribution Gokhan Tuncelli: Writing – original draft, Writing – review & editing, Validation, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization, Resources. Muhammad Hanif Azhar: Writing – review & editing, Methodology, Investigation, Data curation. Devrim Memiş: Investigation, Writing – review & editing, Project administration, Supervision, Resources, Conceptualization. Nuray Erkan: Project administration, Funding acquisition, Conceptualization, Writing – review & editing, Supervision, Resources. Idil Can-Tuncelli: Writing – review & editing, Project administration, Conceptualization. Özkan Özden: Investigation, Project administration. Eda Dagsuyu: Investigation, Project administration. Ismet Burcu Turkyilmaz Mutlu: Investigation, Project administration. Refiye Yanardag: Writing – review & editing, Investigation, Project administration.

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Data availability Data will be made available on request from the corresponding author upon reasonable request.

Declarations

Ethics approval Ethical approval for this study/case/case series was obtained from the Istanbul University Local Ethics Committee for Animal Experiments (Approval Number: 2023/28).

Competing interests The authors declare no competing interests.

Clinical trial number Not applicable.

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