TYPE Original Research
PUBLISHED 06 January 2025
DOI 10.3389/fenvs.2024.1514062

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RECEIVED 19 October 2024
ACCEPTED 13 December 2024
PUBLISHED 06 January 2025

CITATION
Elías D, Ramirez Vieytez J, Funoy Sayar M, Loaisiga J, Harper A and Doll J (2025) Effects of ibuprofen and microplastics on movement, growth and reproduction in the freshwater snail *Physella acuta*.
Front. Environ. Sci. 12:1514062.
doi: 10.3389/fenvs.2024.1514062

Effects of ibuprofen and microplastics on movement, growth and reproduction in the freshwater snail *Physella acuta*

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Emerging contaminants such as microplastics and pharmaceuticals in freshwater ecosystems are a growing concern, seriously affecting aquatic organisms. Ibuprofen, a widely used anti-inflammatory drug, is commonly found in rivers, streams, and water systems where it is discharged. As a result, organisms that inhabit these environments, such as snails, are affected in their natural habitats. This study examines the effects of microplastics (10 μg/L), ibuprofen (500 μg/L), and a combination of ibuprofen (500 μg/L) and microplastics (10 μg/L) on growth, movement, and reproduction in *Physella acuta*, a freshwater snail species. While no significant effects were observed on movement or number of egg clusters, there was a significant decrease in growth when snails were exposed to microplastics or the combination of microplastics and ibuprofen ($p = 0.021$). Additionally, snail egg production decreased when exposed to ibuprofen ($p = 0.001$) but increased when exposed to microplastics ($p = 0.002$). Microplastic exposure produced more eggs than ibuprofen ($p < 0.001$) and ibuprofen and microplastics combined ($p < 0.001$). Our results suggest that ibuprofen affects growth and the total number of eggs, likely due to oxidative stress, neurotoxicity, or disrupted hormonal pathways. In contrast, microplastics may have caused digestive system blockage, thus affecting energy allocation for growth and reproduction. Changes in snail fitness can directly and indirectly affect food webs and nutrient cycles, highlighting the need for research on these pollutants to understand their chronic and long-term effects on ecosystems.

KEYWORDS

ibuprofen, microplastics, *Physella acuta*, ecotoxicology, reproduction, aquatic pollutants

Introduction

The global human population is projected to reach 9.7 billion by 2050, significantly increasing the demand for products and services, including medicines, food, and clothing (Sadigov, 2022; Albahri et al., 2023). A negative consequence of this growing demand is the rise in waste production, including pharmaceuticals and plastics, which contribute to environmental pollution. From 1950 to 2018, plastic production exceeded 6.3 billion tons, with 370 million tons produced in 2019 alone, representing 16% of the previous 68 years (Alabi et al., 2019; Ritchie et al., 2023). Further, it is estimated that 23 million tons of plastic entered aquatic ecosystems in 2016, and it is projected to reach 53 million tons by 2030

(Borrelle et al., 2020). Improper disposal of plastic, with 21% of this plastic being recycled or incinerated, leads to pollution in aquatic and terrestrial ecosystems (Kumar et al., 2021; Shetty et al., 2023). This widespread plastic pollution highlights the critical need to study its long-term ecological impacts.

Microplastics, defined as plastic particles smaller than 5 mm, are an emerging concern due to their potential effects on marine organisms and human health (Prata et al., 2020). Microplastics have multiple applications. For example, polylactic acid (PLA), polyethylene terephthalate (PET) and polyethylene (PE) are found in bottles, polyhydroxyalkanoates (PHA) are used in disposable items, polycaprolactone (PCL) is common in medical devices and food packaging, polypropylene in clothing, polystyrene in food containers, and polyurethane is found in tires, furniture, and insulation (Iwata, 2015; Lambert and Wagner, 2018; González-Pleiter et al., 2019; Krueger et al., 2015; Majewsky et al., 2016; Zhang et al., 2020; Shah et al., 2008).

These particles can persist in the environment for thousands of years without degrading (Andrady and Koongolla, 2022). Microplastics have been detected in aquatic and terrestrial ecosystems, with polyethylene microplastics accounting for more than 50% of total microplastics floating in the ocean (Issac and Kandasubramanian, 2021), thus representing a risk for multiple species and human health (Li et al., 2023). They have been found in daphnia, salmon, tuna, and shellfish (Guzzetti et al., 2018; Prata et al., 2020; Li et al., 2023) Despite their frequent detection in the aquatic and terrestrial habitats, microplastics effects on ecosystem health remain poorly understood, specifically when combined with other pollutants.

The production of pharmaceuticals has also seen a sharp increase. Similar to plastic, pharmaceuticals contribute significantly to environmental pollution. Ibuprofen (e.g., Advil, Motrin, Midol, Nurofen, Dalsi, etc.) is one of the most used non-steroidal anti-inflammatory drugs (NSAIDs) globally and is considered an essential medicine by the World Health Organization (Miranda et al., 2021; Michalaki and Grintzalis, 2023). The global ibuprofen market is valued at USD 294.4 million in 2020 and is estimated to grow to USD 447.6 million by 2026 (Makuch et al., 2021). In the US, ibuprofen was prescribed 21.3 million times in 2016 (Thammineni et al., 2019). Additionally, 45% of global sales of pharmaceuticals are attributed to NSAIDs and ibuprofen being a major contributor (Schmidt and Redshaw, 2015).

Ibuprofen is frequently detected in water systems due to its widespread use and improper disposal (Austin et al., 2022; Petrie and Camacho-Muñoz, 2021).

Ibuprofen mechanism of action involves the reversible inhibition of cyclooxygenase enzymes (i.e., COX-1 and COX-2). These enzymes are part of the synthesis of prostaglandins, which mediate inflammation and pain (Negres, 2019). Ibuprofen inhibiting these enzymes reduces the levels of prostaglandins and decreases inflammation and pain perception (Negres, 2019).

In aquatic organisms, ibuprofen has been linked to oxidative stress, cytotoxicity, genotoxicity, and neurotoxic effects, including changes in behavior (Sibiya et al., 2023). Changes in enzyme activity, including glutathione S-transferase (GST), catalase, lipid peroxidation, and protein carbonyls, are biomarkers indicating oxidative damage in multiple aquatic organisms such as *Chironomus riparius* (Muñiz-González, 2021), *Danio rerio* (Falfushynska et al., 2022), and *Dreissena polymorpha* (Gonzalez-Rey and Bebianno, 2012). Ibuprofen's effects on acetylcholinesterase (AChE) activity can lead to neurotoxic effects, such as altered behavior, growth, and reproduction. For example, ibuprofen exposure inhibited AChE in *Carassius auratus*, disrupting normal neurotransmission and causing physiological stress (Yang et al., 2019). Similarly, when exposed to ibuprofen, *Daphnia magna* showed altered spontaneous movement, free-swimming distance, duration, and speed under dark conditions (Michalaki and Grintzalis, 2023). In addition, Kovacevic et al. (2016) demonstrated neurotoxic impacts in *D. magna* using metabolomic analysis, revealing disruptions in physiological functions and reductions in organismal fitness.

Both pharmaceuticals and microplastics in freshwater environments present a significant risk to aquatic organisms and water quality. Thus, the co-occurrence of these pollutants in aquatic ecosystems highlights the importance of understanding their combined effects on freshwater species.

Research has shown that microplastics affect marine organisms' health, reproduction, and survival (Pantos, 2022), though the full ecological consequences remain unclear (Santillo et al., 2017; Mishra et al., 2021). For example, European Perch (*Perca fluviatilis*) exposed to polystyrene microplastics displayed inhibited hatching, reduced growth rates, altered feeding preferences, and increased predation risk (Lönnstedt and Eklöv, 2016). In European seabass (*Dicentrarchus labrax*), microplastics inhibited he activity of acetylcholinesterase enzyme, increased lipid oxidation in the brain (Li et al., 2023). Therefore, these studies emphasize the need to further explore the broader ecological impacts of microplastic exposure.

Overusing pharmaceuticals like ibuprofen contributes to high waterway concentrations, negatively impacting aquatic organisms. Ibuprofen exposure has been linked to cytotoxic and genotoxic effects, oxidative stress, and adverse impacts on growth, reproduction, and behavior (Sibiya et al., 2023). Pharmaceuticals and microplastics pose significant risks to water quality and aquatic life due to their bioactivity and toxic metabolites (Chopra and Kumar, 2020).

Ibuprofen is commonly detected in surface waters at concentrations ranging from ng/L to μg/L. However, significantly higher concentrations have been reported in streams influenced by effluents from hospitals and wastewater treatment plants. Specifically, maximum concentrations of 280 μg/L (Taiwan), 414 μg/L (Korea), and 603 μg/L (United Kingdom) have been detected (Almeida et al., 2013; Luo et al., 2014; Jan-Roblero and Cruz-Maya, 2023). These concentrations in effluent-impacted streams highlight the localized contribution of wastewater discharges to pharmaceutical contamination in streams. Understanding the interactions between ibuprofen and microplastics and their combined effects on freshwater ecosystems is crucial for predicting long-term environmental consequences.

Freshwater organisms, including *Physella acuta*, are particularly susceptible to pollutants. *Physella acuta* plays a key role in the ecosystems as a food source for larger species and by contributing to nutrient cycling (McClain et al., 2012; Naldi et al., 2020; Konschak et al., 2021). Due to its rapid population growth and adaptability to various environments, *Physella acuta* has become an important model organism for studying the impact of environmental pollution (Camargo and Alonso, 2017; Prieto-Amador et al., 2021; Nandy et al., 2024). Assessing the effects of ibuprofen and microplastics on *P. acuta* provides valuable information into potential disruptions in nutrient cycling and aquatic food webs.

Our study explores the individual and combined effects of ibuprofen and microplastic exposure on the growth, movement, and reproduction of *Physella acuta*, including the number of egg clusters and total eggs produced. Previous research has focused on the independent effects of microplastics (e.g., Meaza et al., 2021; Mason et al., 2022; Pantos, 2022) and ibuprofen (e.g., De Lange et al., 2006; Muñiz-González, 2021; Jan-Roblero and Cruz-Maya, 2023) on aquatic organisms. However, limited research has addressed their combined effect, particularly on freshwater snails.

We hypothesize that 1) ibuprofen will decrease snail growth, movement, and reproduction by affecting energy allocation due to oxidative stress and neurotoxicity, 2) microplastic exposure will impair metabolic processes (i.e., digestion through physical blockage), reducing growth, movement, and reproduction, and 3) combined exposure will have a greater negative effect on these endpoints than ibuprofen or microplastics alone.

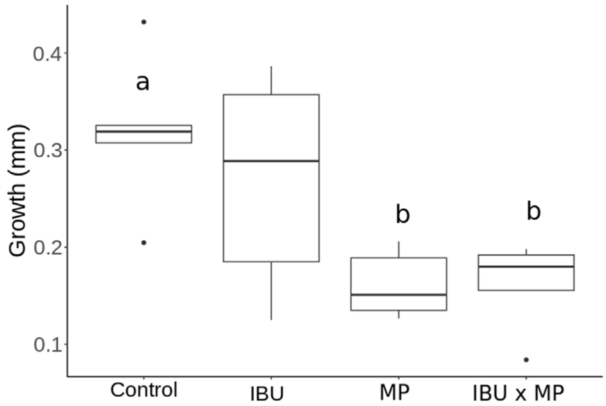

FIGURE 1
Physella acuta growth (mm) as a result of exposure to ibuprofen (500 μg/L; IBU), microplastics (10 μg/L; MP), and combined ibuprofen (500 μg/L) and microplastics (10 μg/L). Different letters represent significant differences between treatments.

Methods

Snails (*Physella acuta*) were purchased from Carolina Biological Supply. Snails were kept in a 40 L glass aquarium with aerated synthetic spring water under a 16:8 photoperiod, room temperature (~20°C), and fed fish flakes (Tetramin®). *Physella acuta* snails were estimated to be juveniles and adults based on their shell lengths (Núñez, 2010) ranging from 3.94 mm to 8.47 mm, with a mean of 6.68 mm (SD 1.045 mm). Three snails were placed in 200 mL glass jars. Each treatment included five replications (n = 20 experimental units; 60 snails). Water changes, treatment renewals, and feedings were performed biweekly. Similar to Bartolini et al. (2017) and Rivi et al., 2021, snails were marked using a permanent marker to differentiate between each other. No detrimental effects were observed as in Garlick-Ott and Wright (2022).

Clear polyethylene microspheres (density: 0.96 g/cm^3, size range: 10–90 μm) were purchased from Cospheric LLC (United States) to study the effects of microplastic exposure. Ibuprofen stock solutions (≥98% purity, Sigma Aldrich, United States) were prepared, and aliquots were added to each glass jar to achieve the target treatment concentration of 500 μg/L.

Snails were exposed for 14 days to four treatments: control, ibuprofen (IBU, 500 μg/L), microplastics (MP, 10 μg/L), and ibuprofen and microplastics mixture (IBU × MP, 500 μg/L and 10 μg/L). Environmentally relevant concentrations of ibuprofen (500 μg/L) and microplastics (10 μg/L) were chosen to represent concentrations commonly detected in contaminated aquatic ecosystems (ibuprofen: Pharms UBA Umwelt Bundesamt, 2024; Jan-Roblero and Cruz-Maya, 2023, and microplastics: Goldstein et al., 2012; Oliveira et al., 2013; Rochman et al., 2014).

Experimental design

Snail shell length was measured using ImageJ (version 1.53) software to determine snail growth. Individual snails were placed in a 40 L aquarium filled with synthetic spring water (5 cm height) for quantifying movement. A one cm^2 grid paper was placed underneath the aquarium. Distance traveled was not recorded for the first 10 s to allow snails to acclimatize but was recorded for the next one minute. Snail reproduction was measured using a stereo microscope by counting the number of egg clusters and the total number of eggs.

Statistical analysis

Four treatment groups (IBU alone, MP alone, IBU and MP, and control) were tested for their effects on growth, movement, number of egg clusters, and total number of eggs. Growth and movement are continuous variables modeled using ANOVA for normally distributed responses or the Kruskal-Wallis test for non-normally distributed responses. If statistically significant differences were found among treatments, a Tukey's Honest Significant difference test was used for multiple comparisons across groups for normally distributed response variables. Dunn's test was used for multiple comparisons across groups for non-normally distributed response variables. Normality was checked using the Shapiro-Wilkes test. The number of egg clusters and the total number of eggs are count data, and the effects of the treatment groups were assessed using a generalized linear model with a Poisson error distribution. Multiple comparisons across groups were made using the expected marginal means estimated with the emmeans () function and the contrast () function from the emmeans package version 1.10.4 (Lenth, 2024). Alpha was set at 0.05 for all analyses. All analysis was conducted using R version 4.4.1 (R Core Team, 2024). Figures were constructed in R using the ggplot2 () package version 3.5.1 (Wickham, 2016).

Results

Growth

Snail growth ranged from 0.16 mm to 0.32 mm after 14 days of treatment exposure (Figure 1; Table 1). Snails exposed to microplastics alone and ibuprofen and microplastics combined

TABLE 1 Changes in *Physella acuta* growth, movement, number of egg clusters, and total number of eggs after 14 days of exposure to control, ibuprofen (IBU; 500 μg/L), microplastics (MP; 10 μg/L), and ibuprofen and microplastics (IBU × MP; 500 μg/L × 10 μg/L). Numbers in parentheses represent the standard deviation.

Treatment	Growth (mm)	Movement (cm/s)	# Of egg clusters	Total # of eggs
Ctrl	0.32 (0.08)	0.04 (0.03)	0.60 (0.89)	16.40 (23.77)
IBU	0.27 (0.11)	0.06 (0.04)	0.80 (0.84)	8.00 (7.62)
MP	0.16 (0.03)	0.07 (0.07)	2.80 (1.30)	29.20 (11.03)
IBU x MP	0.16 (0.05)	0.02 (0.02)	1.80 (2.49)	12.80 (17.54)

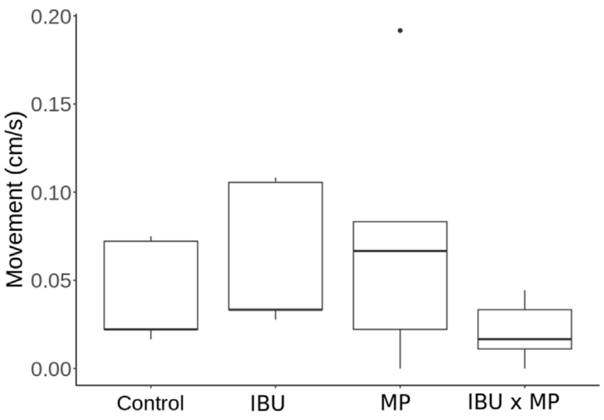

FIGURE 2
Physella acuta movement (cm/s) as a result of exposure to ibuprofen (500 μg/L; IBU), microplastics (10 μg/L; MP), and combined ibuprofen (500 μg/L) and microplastics (10 μg/L). There were no significant differences between treatments.

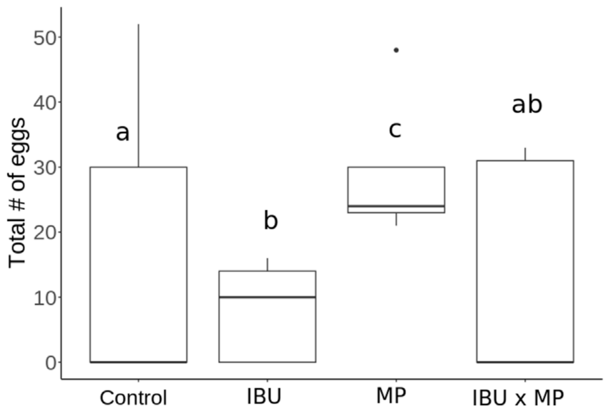

FIGURE 3
Physella acuta total number of eggs as a result of exposure to ibuprofen (500 μg/L; IBU), microplastics (10 μg/L; MP), and combined ibuprofen (500 μg/L) and microplastics (10 μg/L). Different letters represent significant differences between treatments.

showed a 50% significant decrease in mean growth compared to the control group (p = 0.021). Snails exposed to ibuprofen alone exhibited a 15.6% decrease in mean growth.

Movement

Snail movement ranged from 0.02 cm/s to 0.07 cm/s after 14 days of treatment exposure (Figure 2; Table 1). Mean snail movement decreased by 50% in the combined ibuprofen and microplastics treatment (0.02 cm/min) compared to the control (0.04 cm/min). However, snails exposed to microplastics showed a 75% increase in the mean movement (0.07 cm/min), and those exposed to ibuprofen alone showed a 50% increase (0.06 cm/min) compared to the control. There was no significant difference between treatments.

Egg clusters

The number of egg clusters ranged from 0.6 to 2.8 after 14 days of treatment exposure (Figure 3; Table 1). Compared to the control, mean snail production of egg clusters increased 1.33× (33%) when exposed to ibuprofen, 4.67× (367%) when exposed to microplastics, and 3× (200%) when exposed to ibuprofen and microplastics combined. There was no significant difference between treatments.

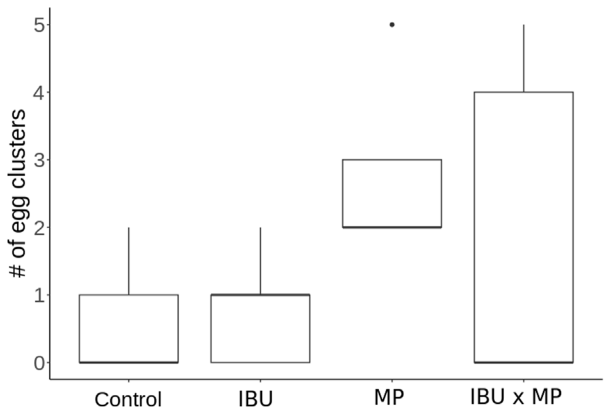

FIGURE 4
Physella acuta number of egg clusters as a result of exposure to ibuprofen (500 μg/L; IBU), microplastics (10 μg/L; MP), and combined ibuprofen (500 μg/L) and microplastics (10 μg/L). There were no significant differences between treatments.

Total egg count

Total egg count ranged from 8 to 16.4 after 14 days of exposure to treatments.(Figure 4; Table 1) Mean snail total egg count decreased by 51% when exposed to ibuprofen ($p = 0.001$), by 22% when exposed to combined ibuprofen and microplastics, and increased by 78% when exposed to microplastics ($p = 0.002$). Additionally, snails exposed to microplastics produced 265% × (3.65) more eggs on average than snails exposed to ibuprofen ($p < 0.001$) and 56% × (2.3) more eggs on average than snails exposed to ibuprofen and microplastics combined ($p < 0.001$).

Discussion

This study assessed the individual and combined effects of ibuprofen and microplastic exposure on *Physella acuta* growth, movement, and reproduction. Snail growth significantly decreased when exposed to microplastics alone and in combination with ibuprofen ($p = 0.021$; Figure 1). These findings support our hypotheses, implying that the reduction in growth likely results from a trade-off between growth and detoxification. Specifically, nutrient absorption impairment caused by microplastic physical blockage of the digestive system likely contributed to these effects (Imhof et al., 2013; Jeyavani and Vaseeharan, 2023). Previous studies on *Physa fontinalis*, amphipods, and rotifers also indicated reduced growth when exposed to microplastics (Au et al., 2015; Jeong et al., 2016; Michler-Kozma et al., 2022). This highlights the susceptibility of freshwater biota to short-term pollutant exposure, which could lead to effects on ecosystem functions.

Although we did not directly assess chemical interactions (i.e., synergistic, additive, antagonistic), the reduced growth observed with ibuprofen and microplastics combined may suggest that in addition to an impaired digestive system, oxidative stress from ibuprofen likely contributed to the effects of microplastics alone (Srain et al., 2021; Jan-Roblero and Cruz-Maya, 2023). In response to ibuprofen, aquatic organisms exhibit detoxification responses (Batucan et al., 2022). For example, glutathione S-transferase (GST) activity increased to prevent oxidative damage in *Chironomus riparius* after ibuprofen exposure (Muñiz-González, 2021). In *Danio rerio* (zebrafish), ibuprofen increased oxidative stress markers, including catalase, glutathione S-transferase, and protein carbonyls (Falfushynska et al., 2022). For *Dreissena polymorpha* (zebra mussel), ibuprofen elicited lipid peroxidation and disrupted enzymatic responses (Gonzalez-Rey and Bebianno, 2012). These findings suggest that oxidative stress and impaired detoxification processes are key mechanisms reducing snail fitness when exposed to ibuprofen and microplastics.

Reduced growth in *P. acuta* can disrupt ecosystem dynamics. For example, decreased feeding is associated with smaller snails (e.g., Silva et al., 2020). Thus, lower grazing may promote algal growth, affecting food available to primary consumers (Lowe and Hunter, 1988; Konschak et al., 2021). A reduction in snail populations and a corresponding decrease in egestion may also affect nutrient cycling (Mulholland et al., 1991; Perrotta et al., 2020), altering nitrogen and phosphorus availability (Elias and Bernot, 2017). Additionally, smaller snail populations can affect predator-prey dynamics by reducing food sources for predators (Justice and Bernot, 2014; Krupsi et al., 2018). These changes can affect biodiversity and community structure (Swamikannu and Hoagland, 1989; Konschak et al., 2021; Kumari et al., 2023).

Reproductive output was measured by counting the number of egg clusters and the total number of eggs (Table 1). Treatments did not affect number of egg clusters (Figure 3) but significantly decreased (ibuprofen) or increased (microplastics) the total number of eggs (Figure 4). Contrary to previous research (e.g., Michler-Kozma et al., 2022; Kumari et al., 2023; Merbt et al., 2024) that reported reduced reproductive outputs under microplastic exposure, we found a significant increase in egg production with microplastic exposure ($p < 0.001$). Several factors could explain this difference: 1) microplastic type and size influence their bioavailability and toxicity (Alak et al., 2022). The size and characteristics of polyethylene (PE) microspheres used in this study (10–90 μm) likely influenced their bioavailability and interaction with *Physella acuta*. Particles in this size range are small enough to be ingested but large enough to cause physical blockages in the digestive tract, impairing nutrient absorption. Additionally, these microplastics may adhere to mucus membranes, causing stress responses (Alak et al., 2022; Imhof et al., 2013). In addition to physical effects, PE microplastics often contain chemical additives, such as plasticizers, stabilizers, and flame retardants, which can act as endocrine-disrupting chemicals (Chen et al., 2019; Lin et al., 2023; Bucci et al., 2024). Furthermore, the hydrophobic nature of PE microplastics allows them to adsorb other contaminants which can worsen their toxicity. 2) exposure period and concentration can influence reproductive outcomes. Pedersen et al. (2020) found no changes in quagga mussel reproduction within 24 h, even at elevated microplastic concentrations of 0.8 g/L, however reduced sac production and egg hatching was observed on *Physella acuta* after 93 days exposure of 150 mg/L of polystyrene (Kumari et al., 2023); 3) reproductive stage and experimental conditions contribute to egg production. Saha et al. (2019) found that *P. acuta* produces more eggs with age. Feeding schedules, water quality, and organism density per experimental unit may also influence energy allocation toward reproduction, contributing to variability across studies. These findings highlight the need for standardized protocols to assess the complexity of ibuprofen and microplastics effects on reproduction accurately (de Ruijter et al., 2020).

Ibuprofen exposure decreased the total egg count. Although research on ibuprofen's impact on *P. acuta* is limited, similar effects on reproduction have been reported in other aquatic organisms and pollutants. For example, *P. acuta* egg production decreased when exposed to fluoxetine (Sánchez-Argüello et al., 2009; Henry et al., 2022) and reclaimed water (Aquilino et al., 2018). When exposed to ibuprofen, impaired reproduction was reported for *Planorbis carinatus*, *Moina macrocopa*, and *Oryzias latipes* (Das et al., 2019). When exposed to ibuprofen and microplastics combined, there was no significant effect on the total number of eggs, thus complicating predictions of multiple-pollutant exposure (Fent et al., 2006; Di Poi et al., 2018; Zhang et al., 2022). Reduced reproduction in *P. acuta* could alter community structure and function. Specifically, declining snail populations and their corresponding roles as grazers and detritivores (Wikström and Hillebrand, 2012; Tchakonte et al., 2023) and as a food source for predators (Gilioli et al., 2017) may reduce biodiversity. Similarly, smaller populations can impact nutrient cycling in freshwater ecosystems (Auld, 2018).

Movement increased when exposed to combined ibuprofen and microplastics (Figure 2). Kumari et al. (2023) reported decreased movement when exposed to microplastics alone. Even though this increase was not statistically significant, it may have biological relevance as it can reflect behavioral changes or stress responses (Calow and Forbes, 1998; Almeida and Nunes, 2019), including feeding behaviors and predator avoidance (Justice and Bernot, 2014; Elias and Bernot, 2017), and changes in food web dynamics and nutrient cycling (Newman et al., 1996; Hall et al., 2003; Vannatta, 2021).

In addition to changes in ecosystem structure and function, long term effects of reduced *Physella* snails' populations can be significant. Smaller populations are more vulnerable to genetic drift, inbreeding, and disrupted adaptive responses which eventually can affect survival and reproduction success. Genetic diversity is critical for an effective population size and adaptive responses (Grueber et al., 2019). For example, for colonizing populations, especially since *Physella acuta* is considered an invasive species, smaller populations could lead to reduced fecundity, earlier maturation (Fruh et al., 2017; Chapuis et al., 2024), reduced population density, and persistence of this conditions over generations (Szucs et al., 2017). Further, smaller populations exposed to chronic pollution are more susceptible to environmental pressures affecting birth and death rates. Thus, leading to impaired recovery from perturbations (Lopez et al., 2009).

Our study partially supports our hypothesis: microplastic and ibuprofen exposure negatively affect *P. acuta*. Specifically, microplastics alone and combined with ibuprofen significantly reduced snail growth, likely because of nutrient absorption impairment and energy trade-offs with detoxification processes. Ibuprofen decreased the total egg count because of impaired reproductive function. In contrast, microplastic exposure increased egg production, indicating variability in reproductive responses potentially influenced microplastic type, exposure duration, and reproductive stage. Ibuprofen and microplastics combined did not significantly affect egg count. Although increased movement with combined exposure was not statistically significant, it may still indicate biological stress responses, which could impact feeding behaviors and food web dynamics. Overall, based on our findings, we suggest that oxidative stress, neurotoxicity, and impaired digestive system affect snail fitness. The effects of these pollutants on *P. acuta* identified in this study: reduced growth and reduced reproductive incomes can lead to ecological impacts, including disrupted nutrient cycling, altered prey-predator dynamics, and decreased biodiversity in freshwater ecosystems.

Further, these findings contribute to the larger literature, by exploring the combined effects of ibuprofen and microplastics on *Physella acuta*, by addressing the knowledge gap of these pollutants and understanding multiple stressor exposure which is more relevant of real environmental conditions. The 14-day exposure period can serve as a starting point for other research to address long term impacts of ibuprofen and microplastics alone and combined. Specifically, exploring multi generation effects of these pollutants.

Future research should explore interactions between pollutants (i.e., synergistic, additive, antagonistic), wider range of pollutant concentrations, exposure times, and additional endpoints. For example, multi-generational studies would be able to address chronic impacts on *P. acuta* populations and their adaptive responses. Additionally, studies need to confirm the different mechanisms we suggested as key factors affecting snail fitness, therefore integrating biochemical assays in addition to ecotoxicological dose-response experiments. Further, to better understand the effects of these pollutants, other ecological relevant endpoints should be studied including egg sizes, ingestion volume, and snail speed. Furthermore, standardization of protocols to reduce variability, including microplastic types, sizes, and concentrations, is critical for reliable risk assessment and management strategies. We also propose field studies that explore the larger ecological impacts of reduced snail fitness on community structure and function. Therefore, continuing research like this contributes to and strengthens conservation strategies and mitigation efforts to protect freshwater environments from pharmaceuticals and microplastics.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.

Author contributions

DE: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing–original draft, Writing–review and editing. JR: Writing–original draft. MF: Writing–original draft. JL: Writing–original draft. AH: Writing–original draft. JD: Formal Analysis, Methodology, Validation, Visualization, Writing–review and editing.

Funding

The author(s) declare that financial support was received for the research, authorship, and/or publication of this article. This research was supported by internal grants for professional development and the Evan and Marcy Duff Professional Development Grant, both awarded by North Carolina Wesleyan University.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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The author(s) declare that Generative AI was used in the creation of this manuscript. Grammar and typos check.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fenvs.2024.1514062/full#supplementary-material

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