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Candidate

Ben Sellers

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Ecotoxicological Effects of Fluoxetine on a Freshwater Bioindicator Snail, Physa acuta

This Senior Project is approved as acceptable

Project Director

Dr. Daniel Elías

Committee Member

Dr. James Bowers

Committee Member

Dr. Emily Weber

Honors Program Co-Director

Dr. Bill Yankosky

Honors Program Co-Director

Dr. Fred Sanborn

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Ecotoxicological Effects of Fluoxetine on a Freshwater Bioindicator Snail, *Physa acuta*

Ben Sellers

Honors Thesis

North Carolina Wesleyan University

Director: Dr. Daniel Elias

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Ecotoxicological Effects of Fluoxetine on a Freshwater Bioindicator Snail, *Physa acuta*

Abstract

Fluoxetine, a widely prescribed antidepressant, is commonly detected in effluent-influenced aquatic systems. To evaluate the impact of fluoxetine on freshwater environments, the bladder snail *Physa acuta* was selected. Egestion, movement, and reproductive outputs (number of egg masses and total number of eggs) were measured after 7 days exposure to environmentally relevant concentrations of fluoxetine (Low: 70 ng/L and High: 350 ng/L). We observed a statistically significant effect of fluoxetine on total number of eggs ($p = 0.005$) with a 1.55× at 70 ng/L (low) and 1.68×at 350 ng/L (high) reduction relative to controls. Egestion, movement, and number of egg masses were not significantly affected by any fluoxetine exposure. Our results suggest that fluoxetine affects reproductive output in *P. acuta*, potentially through energy reallocation toward physiological maintenance and detoxification processes associated with oxidative stress. Reduced egg production and subsequent population declines may, in turn, disrupt ecosystem functions, including predator-prey interactions, nutrient cycling, and overall ecosystem structure.

Keywords: *Physa acuta*, fluoxetine, egestion, movement, reproduction, pharmaceuticals

Statements and Declarations

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Introduction

Pharmaceuticals are detected in aquatic environments due to effluent from wastewater treatment plants, improper disposal of pharmaceuticals, and industrial discharge (Patel et al. 2019). Their abundance is in response to global human population growth and increased demand for pharmaceuticals and limited capacity of wastewater treatment plants (WWTP) to completely remove these compounds (Gao et al. 2012; Khasawneh and Palaniandy 2021). Analgesics and anti-inflammatory drugs (e.g. ibuprofen, acetaminophen), antibiotics (e.g. ciprofloxacin, sulfamethoxazole), antiepileptics (e.g. carbamazepine), and antidepressants (e.g. sertraline, fluoxetine) are among the most frequently detected pharmaceuticals in freshwaters (Patel et al. 2019). In particular, fluoxetine has been detected from 70 ng/L in surface waters to 350 ng/L in WWTP effluent (Martin et al. 2019).

Fluoxetine, the active ingredient in Prozac, is a widely prescribed antidepressant used to treat major depressive disorder, obsessive compulsive disorder, bulimia nervosa, and panic disorder (Sohel et al. 2024). It acts by inhibiting the reuptake of serotonin (Rossi et al. 2004; Sohel et al. 2024). Although fluoxetine is only intended for human use, its detection in aquatic environments and neuroactive properties has the potential to affect non-target organisms, including freshwater invertebrates such as *Physa acuta*.

To evaluate the potential ecotoxicological impacts of fluoxetine, researchers have conducted studies on a wide range of aquatic organisms. For example, exposure to 10-110 ng/L of fluoxetine affected population dynamics of zooplankton (copepods) with a decreased recovery rate after a predator was removed (Michelangeli et al. 2024). The Oregon shore crab (*Hemigrapsus oregonensis*) exposed to 3-30 ng/L exhibited decreased predator avoidance thus increasing predation vulnerability (Peters et al. 2017). Further, Yamindago et al. (2021) observed tentacle contraction and decreased reproductivity of *Hydra magnipapillata* when exposed to 1.5 mg/L of fluoxetine. Lazzara et al. (2012) indicated reproductive effects (reduced estradiol and fewer oocytes) on zebra mussels (*Dreissena polymorpha*) when exposed to 20-200 ng/L of fluoxetine. In vertebrates, fluoxetine (500 ng/L) reduced swimming speed on adults and transgenerational effects on zebrafish (*Danio rerio*) offspring (Al Shuraiqi et al. 2021).

Similar responses to fluoxetine have been observed in freshwater gastropods, including *Physa acuta* (Fong and Ford, 2014; Imiuwa et al. 2025) with changes in behavior, reproduction, embryonic development, and effects on multigenerational fitness, highlighting the sensitivity of this species to serotonergic disruption (Sánchez-Argüello et al. 2012; Henry et al. 2022). Thus, *P. acuta* is a relevant organism for assessing pharmaceutical contamination. While these studies have focused on high concentrations (31 µg/L to 250 µg/L), early life stages, or multigenerational effects, our research specifically evaluates acute responses across multiple functional endpoints at environmentally relevant fluoxetine concentrations.

Physa acuta snails are an air-breathing freshwater gastropod widely distributed across the continental United States (Fong and Ford, 2014). As a self-fertilizing hermaphrodite, *P. acuta* is

used for assessment of reproductive endpoints as well as growth and behavioral responses (Imiuwa et al. 2025). Therefore, the objective of this study was to evaluate the effects of environmentally relevant concentrations of fluoxetine on movement, egestion, and reproduction of *Physa acuta*. We hypothesized that fluoxetine exposure would decrease egestion, movement, and reproductive endpoints. Overall, this study aims to improve understanding of the ecological implications of fluoxetine contamination in freshwater ecosystems.

Methods

Physa acuta snails were purchased from Carolina Biological Supply and maintained in a 40 L glass aquarium prior to experimentation. Snails were kept in aerated synthetic spring water (EPA, 2002) under a 16:8 light–dark photoperiod at room temperature (~20°C) and fed fish flakes (Tetramin®) *ad libitum*. A 7-day exposure was conducted to evaluate the effects of fluoxetine on snail egestion, movement, and reproduction. Three snails were randomly assigned to one of three treatments representing environmentally relevant fluoxetine concentrations, control (0 ng/L), low fluoxetine (70 ng/L), and high fluoxetine (350 ng/L).

Snails were placed individually into 250 mL glass jars containing 200 mL of treatment solution. Each treatment included 12 replicates. Water changes, feeding, and treatment renewals were performed twice per week. Fluoxetine (Thermo Fisher Scientific) was used to prepare stock solutions. Nominal treatment concentrations were prepared by dilution using synthetic spring water.

Egestion

Egestion rate was measured at the end of the exposure period. Fecal matter was collected using a pipette and transferred to pre-weighed aluminum weigh boats. Samples were dried for 48 hours at 60°C in a drying oven (Quincy Lab 10GC) and weighed using a precision balance (Ohaus Pioneer®). Snail length or biomass were not recorded; therefore, egestion was not standardized by individual size. Because snails were selected from the same population and size class and randomly assigned to treatments, variation in body size is expected to be similar across treatments and primarily increase within-treatment variability rather than biasing treatment means.

Movement

Snail movement was measured following Bernot et al. (2005) protocol. Individual snails were placed in a 40 L glass aquarium filled to a height of 5 cm with synthetic spring water. A 1 cm^2 grid paper was placed beneath the aquarium. After a 10-second acclimation, snails were observed for 60 seconds, and the number of grid squares crossed was recorded to calculate movement (cm/s).

Reproduction

Reproductive output was measured at day 3, 5, and 7. Egg masses laid on the glass jars were counted under a stereomicroscope (Leica Zoom 2000, Model Z30V), and the number of eggs within each mass was recorded. Total egg masses and total egg number per individual were used as reproductive endpoints.

Data Analysis

The overall effect of fluoxetine treatments on snail egestion, movement, total egg mass, and total number of eggs was evaluated using Analysis of Variance when data were normally distributed or Kruskal-Wallis test when data were not normally distributed. Normality was assessed using Shapiro-Wilks test. Post-hoc analysis was completed if there was a statistically significant effect. Pair-wise comparisons for normally distributed data were analyzed with a Tukey test while non normally distributed data were analyzed with Dunn's test. Statistical analysis was conducted in R 4.2.2 (R Core Team 2022). Alpha was set at 0.05 for all analysis.

Results

Egestion was not normally distributed (all Shapiro-Wilks p-values < 0.05), while movement, total egg mass, and total number of eggs were normally distributed (all Shapiro-Wilks p-values > 0.05). Egestion (Kruskal-Wallis $\chi^2 = 3.826$, df = 2, p = 0.148), movement ($F_{2,33} = 1.653$, p = 0.207) and total egg mass ($F_{2,33} = 2.584$, p = 0.091) were not significantly different across treatments (Table 1). Although there was no statistically significant effect because of the high variability, mean snail egestion decreased 2× when exposed to 70 ng/L and 6.7× when exposed to 350 ng/L of fluoxetine. There was a significant difference in the total number of eggs across treatments ($F_{2,33} = 6.186$, p = 0.005; Figure 1). The control group was significantly different from the low treatment (Tukey p = 0.020) and the high treatment (Tukey p = 0.008). There was no significant difference between the low and high treatments (Tukey p = 0.924). Relative to the control, total egg production decreased 1.55× at 70 ng/L and 1.68× at 350 ng/L.

Table 1: Mean (Standard deviation) for egestion, movement, and reproduction of *Physa acuta* after a 7-day exposure to control (0 ng/L), low (70 ng/L), or high (350 ng/L) fluoxetine treatments. Reproduction includes total egg masses and the total number of eggs produced.

Treatment	Egestion (mg/snail/day)	Movement (cm/s)	Total Egg Mass	Total Number of Eggs
Control	0.142 (0.149)	0.032 (0.021)	3.67 (1.15)	68.4 (11.2)
Low	0.067 (0.085)	0.044 (0.018)	2.50 (1.68)	44.0 (30.1)
High	0.021 (0.019)	0.034 (0.016)	2.83 (0.94)	40.8 (17.3)

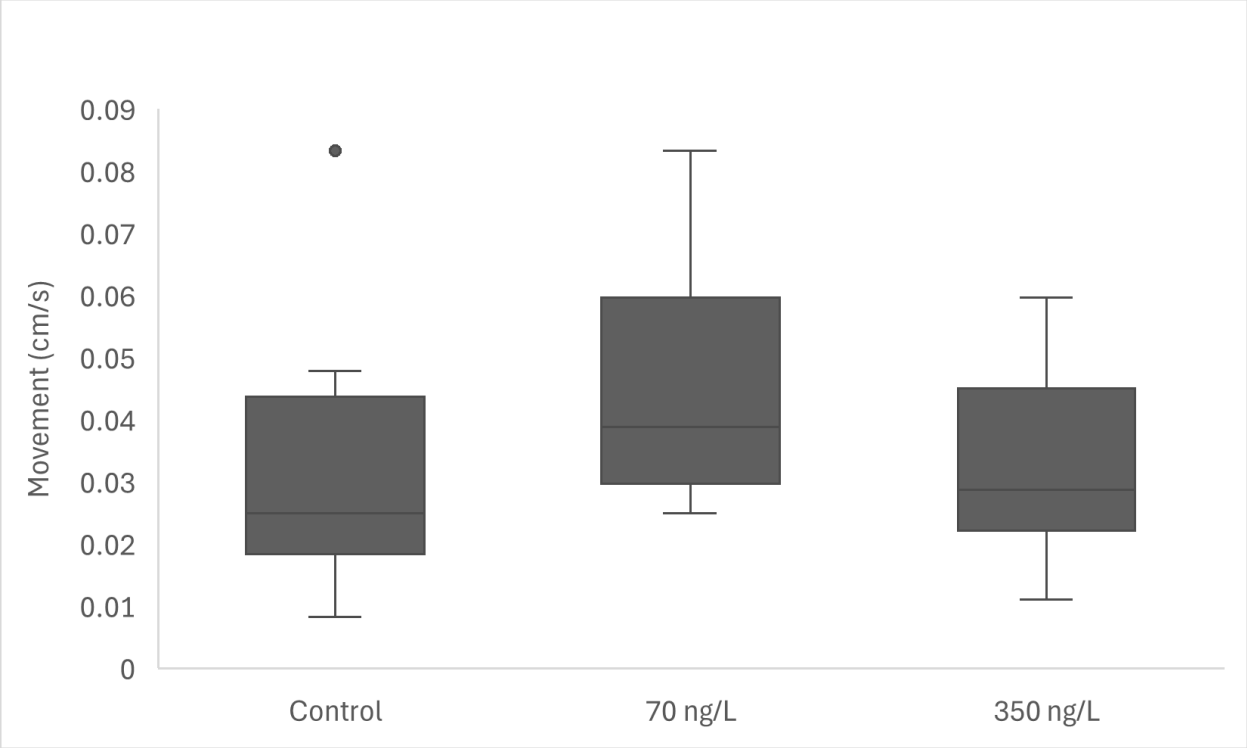


Fig. 1 Movement (cm/s) of *Physa acuta* snails across fluoxetine treatments (control: 0 ng/L, low: 70 ng/L, and high: 350 ng/L). No significant difference was observed between groups. Outliers are displayed as points.

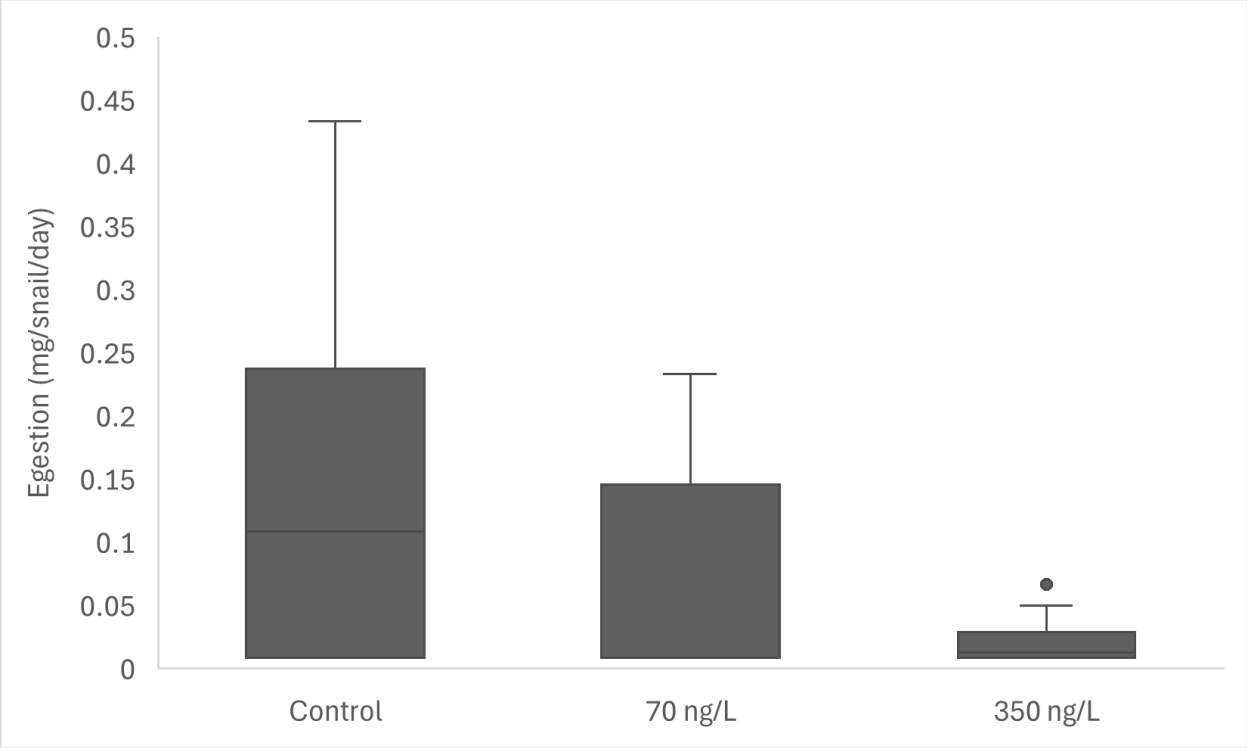


Fig. 2 Egestion (mg/snail/day) produced by Physa acuta across fluoxetine treatments (control: 0 ng/L, low: 70 ng/L, and high: 350 ng/L). Boxplots show that egestion decreased as fluoxetine concentration increased, but no significant difference was observed. Outliers are displayed as points.

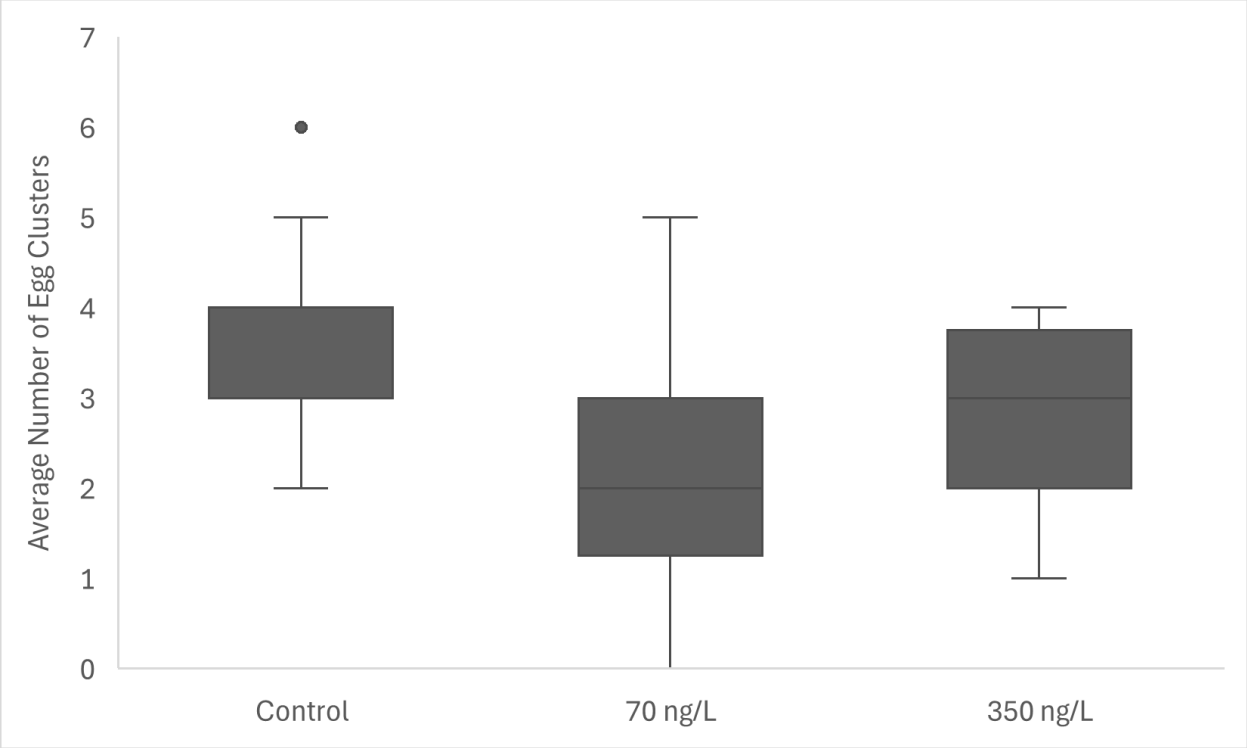


Fig. 3 Number of egg clusters produced by *Physa acuta* across fluoxetine treatments (control: 0 ng/L, low: 70 ng/L, and high: 350 ng/L). Boxplots show that snails in the control laid more egg masses than the snails exposed to fluoxetine, but no significant difference was recorded. Outliers are displayed as points.

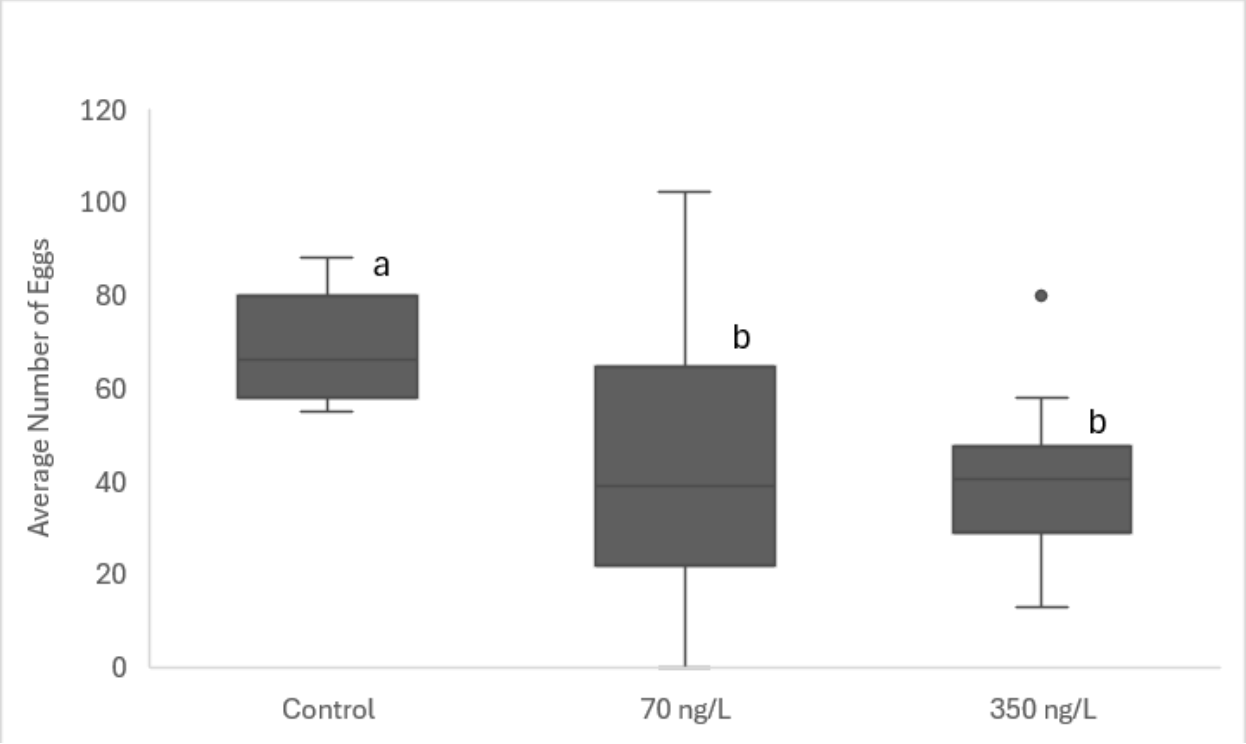


Fig. 4 Total number of eggs produced by *Physa acuta* across fluoxetine treatments (control: 0 ng/L, low: 70 ng/L, and high: 350 ng/L). Boxplots show that control snails produced more eggs than those exposed to low or high concentrations. Outliers are displayed as points. Different letters indicate significant differences.

Discussion

This study found that exposure to environmentally relevant concentrations of fluoxetine significantly reduced total number of eggs in *Physa acuta*. Although egestion was not significantly affected, we observed a decrease in snail egestion when exposed to 70 ng/L and 350 ng/L of fluoxetine. These results suggest that fluoxetine is likely to impact reproductive processes in *P. acuta*, potentially through physiological stress mechanisms (e.g. oxidative stress) that alter energy allocation rather than directly affecting feeding or movement.

Fluoxetine in snails is metabolized by the cytochrome P450 enzyme which can lead to the production of free-radical compounds such as superoxide, hydrogen peroxide, and the hydroxyl radical (Orozco-Hernandez et al. 2023). ATP production is susceptible to oxidative damage by these radicals (Xu et al. 2025). For freshwater snails, reduced ATP availability under induced oxidative stress commonly results in decreased reproductive investment as energy is reallocated toward antioxidant defenses and cellular repair (Nowakowska et al. 2011; Basopo and Ngabaza 2015).

Similar to our results, Henry et al. (2022) noted a decreased number of *P. acuta* egg masses when exposed to 32.7 ng/L of fluoxetine, as well as no significant effect on movement. In contrast, Sanchez-Arguello et al. (2009) observed increased reproduction of *P. acuta* when exposed to fluoxetine concentrations (31.3 µg/L and 62.5 µg/L), while at 250 µg/L, they indicated a marked reduction in reproductive outputs (i.e. egg masses). However, the concentrations used by Sanchez-Arguello et al. (2009) are ~1000× greater than the ones used in our research and Henry et al. (2022).

Thus, in freshwater ecosystems influenced by fluoxetine pollution, a reduction of egg masses and corresponding population reduction can have effects on ecosystem functions and structure including nutrient cycles, prey and predator interactions, and population dynamics. Specifically, *P. acuta* is an important algae grazer. Reduced grazing can increase algal biomass, primary productivity, and alter nutrient cycle dynamics (Preston et al. 2022; Le Roux et al. 2025). In addition, as a common prey, declines in *P. acuta* could potentially increase interspecific and intraspecific competition and alter energy allocation across trophic levels (Preston et al. 2022; Le Roux et al. 2025). Also, *P. acuta* has a role as an intermediate host for parasites (Pathak et al. 2023). A reduction in available hosts could disrupt parasite life cycles, leading to broader food web alterations, reduced biodiversity, and shifts in species interactions and community structure (Sures et al. 2017).

Overall, there is a need to investigate the full ecosystem effects of fluoxetine on freshwater organisms such as snails, insects, crayfish, isopods, amphipods, fish, and amphibians. Further, antidepressants (e.g. sertraline, citalopram), painkillers (e.g. ibuprofen, acetaminophen), antibiotics (e.g. sulfamethoxazole, erythromycin), hormones (e.g. estradiol), cardiovascular

drugs (e.g. propranolol) and neuroactive drugs (e.g. carbamazepine) are commonly detected in surface waters (Samal et al. 2022; Almazrouei et al. 2023; Paíga et al. 2024; Savíc et al. 2022) highlighting the importance of chemical mixtures research.

These results indicate that reproduction is a particularly sensitive endpoint for detecting fluoxetine effects in freshwater gastropods and suggest potential population-level consequences. Given the ecological role of *P. acuta* as an algal grazer, prey, and parasite host, reduced reproductive output could contribute to broader ecosystem effects.

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