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# Microplastics Affect Rates of Locomotion and Reproduction via Dietary Uptake in Globally Invasive Snail *Physa acuta*

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**Abstract:** Given the omnipresence and potential of entering the food web, the recently emerged pollutant microplastics (MPs) has become a global threat. The impacts of MPs in marine ecosystems are well documented, but the freshwater environment is relatively understudied. Improper disposal of industrial and commercial waste introduces MPs in the freshwater environment where it is either transported to the ocean or eventually settles down to the bottom. To elicit the impacts on components of fitness, the effect of inert particles on the reproductive and behavioral performance on organisms can only be translated after long-term experiments, but most of the available information on freshwater benthos relies only on short-term experiments. This study investigated the rates of microplastic ingestion, locomotion and reproduction in the globally invasive snail *Physa acuta* (Gastropoda, Pulmonata) at six environmental concentrations (0, 2.5, 5, 10, 20 and 40 mg/200 mL) of polystyrene (PS) MPs particle (size = 32–63µm) for 93 days after maturity. The PS particle ingestion was confirmed by analysis of *P. acuta* excreta and tissue digestion. *P. acuta* displayed Type II functional response to MPs. We measured locomotion speed and reproductive rate for 93 days after maturation at 20, 30 and 40 mg/200 mL concentrations of PS MPs equivalent to $500 \times 10^4$, $750 \times 10^4$ and $1000 \times 10^4$ particles/200 mL, respectively. Average locomotion speed and egg capsule production were significantly lower in the MP applied environment than in the control. The percent reduction in ovisac production and egg hatching success were a direct function of MP concentrations in the medium., although, in a natural setup, plastic debris was observed as a frequent oviposition substrate for *P. acuta* favoring the species in dispersal. The present results point to a higher tolerance of *P. acuta* to MPs and their role as a vehicle of MP transfer from sediment to fish.

**Keywords:** benthos; microplastics; reproduction; locomotion; oviposition substrate



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## 1. Introduction

Microplastics (MPs) (particle size < 5 mm) are increasingly evolving as a global threat [1]. The common occurrence of MPs in higher densities (~$10^3$–$10^4$ particles/L in seawater; 5–34 particles/L in freshwater) has been well documented in marine and freshwater ecosystems [2,3]. The major proportion of MPs in the freshwater environment is generated from improper disposal of industrial, commercial and domestic wastes [4]; additionally, rainfall also contributes to plastic deposition [5–7]. In freshwater ecosystems, MPs are either transported to the ocean or settle down to the bottom, intervening with benthic biota. The majority of benthic organisms are either raptorial predators or particle feeders, feeding on settled debris [8] on the basis of particle size. In light of overlapping dietary niche size with MPs, meso and meiobenthos are more likely to ingest MPs. Data on MPs ingestion by benthos are very limited and mainly confined to marine benthos, and their biological effects have rarely been investigated. Lusher et al. [9] noted only 23 out of 120 studies on MPs, focused on freshwater ecosystem, with only one study focusing on freshwater molluscs. This indicates a dearth of MP research in freshwater ecosystem as well as a scarcity of studies on its impact on freshwater molluscs.

The benthic community serves as a potential bioindicator of MP pollution in lotic ecosystems [10] and plays an important role in structuring the riverine community through bentho–pelagic and aquatic–terrestrial coupling [8,11,12]. The presence of MPs in the environment, and their ingestion, may interfere with feeding, locomotion and reproduction, and hence the inclusive fitness of a population in the community is likely to be affected [13,14]. The inclusive fitness is the number of offspring equivalents that an organism rears, rescues or otherwise supports through its behaviour. The reproductive value is a measure of the combined effects of fecundity and survival. Survival is affected by locomotion patterns/speed. An organism can enhance its fitness, in terms of the search for a mate and escape from predation, with the help of its locomotion ability, optimizing its reproduction output [15]. Their ability to accumulate large quantities of persistent materials, and their contribution to the values of bioaccumulation factor, make snails suitable organisms for the identification of contaminated sites [16,17]. The snail species *Physa acuta* (Draparnaud 1805), is abundantly found in the benthic region throughout a ~2500 km stretch of the river Ganga. It is an invasive species in India and native to North America [18]. Several studies [19–22] have extensively investigated the effects of environmental perturbations and various anthropogenic activities on the feeding, reproduction and invasion of *P. acuta*, yet no quantitative information is available on the dietary and environmental effects of MPs on fitness components (feeding, locomotion and reproduction) of *P. acuta*. MPs are ingested and are likely to interfere with the feeding and food selection in many other related invertebrates [23]. The proliferation and invasion of *P. acuta* along the riverbank at wastewater discharge (higher MPs concentration in sediment) sites have prompted us to study their fitness components in MP contaminated systems. The concentrations of MPs in freshwater have been reported to vary between 5 and 34 particle/L [3] in riparian system, whereas at wastewater discharge sites the concentrations increase more than an order of magnitude, contributed by higher MP particle discharge (up to $6.5 \times 10^7$ MPs/day) through waste water [24]. In functional response experiments, we have used test concentrations ranging from 0 to 40 mg/200 mL, in which the organism showed a saturation level feeding at 20 mg/200 mL. Therefore, for further reproduction and locomotion experiments, MP concentrations $\geq$ 20 mg/200 mL were used to understand the impact of MP concentrations on the eco-physiological attributes of *P. acuta.*

Herein, we aim to investigate the ingestion probability and locomotory and reproductive performance of *P. acuta* exposed to a range of MP concentrations (0 to 40 mg/200 mL) in laboratory controlled conditions.

## 2. Materials and Methods

### *2.1. Preparation and Characterization of MPs*

The MPs used throughout the experiment were without any additives and were obtained from the raw material discards of the plastic processing industry. Freshly collected, virgin MPs were ground and differentially sieved to obtain particles of density 1.055 g/mL and size range 63–32 µm.

Characterization of the polymeric material of the MPs was carried out by Fourier Transform Infrared Spectroscopy (FTIR), a standard analytical technique used to identify unknown polymer types. MP samples produced a resultant spectrum in the Far-IR range of 689/cm to 4000/cm, at a resolution of 0.7/cm using a JASCO FT/IR-4600 FT-IR spectrometer. The type of polymer was identified by matching the obtained spectra against the inbuilt library database (Sadtler database NDUPP-X).

Conversion of MP weight from mg/mL to particles/mL was carried out using the following mathematical equation [25].

$$\text{MPs (particles/L)} = \frac{\text{TMP}_{\text{stock}}\ \text{(particles/L)} \times \text{C}_{\text{x}}\ \text{(mg/L)}}{\text{C}_{\text{stock}}\ \text{(mg/L)}} \quad (1)$$

where $\text{TMP}_{\text{stock}}$ is the total number of MPs in the stock solution, $\text{C}_{\text{x}}$ is the concentration (x) of MPs in the experiment and $\text{C}_{\text{stock}}$ is the concentration of MPs in the stock solution.

By using equation 1, we converted the MP particles mg/mL to particles/mL, and we obtained low (20 mg/200 mL or $2.5 \times 10^4$ particles/mL), medium (30 mg/200 mL or $3.75 \times 10^4$ particles/mL) and high plastic concentrations (40 mg/200 mL or $5 \times 10^4$ particles/mL).

### *2.2. Test Organism*

The freshwater snail *P. acuta* (Gastropoda, Pulmonata, Physidae) is a hermaphrodite mollusc, depicting both cross and self-fertilization [26]. Snails were sampled from the southern bank of the River Ganga (Patna, Bihar) and transported to the Ecosystem Ecology laboratory, Central University of South Bihar (Gaya, Bihar, India) within seven hours of sampling. Ten individuals of *P. acuta* were used as a starter culture and incubated at $20 \pm 1.5$ °C temperature in an enamel tray containing 1 L filtered Ganga River water (GRW) for laboratory acclimatization. After one-week of acclimation, culture was expanded from the enamel tray to an aquarium for mass culture (Volume: 25 L), while progressively increasing the ratio of standard snail water (SSW) to GRW, completely replacing GRW in 15 days. SSW (Table S1) was prepared following Kawata and Ishigami [27]. The SSW medium was changed, and crushed fish food was provided, every alternate day. The dissolved oxygen level of the culture medium was maintained >6 mg/by continuous aeration [28]. Freshly matured individuals were used for the experiment. Individuals were considered as mature when the first batch of egg capsules was laid.

### *2.3. Experimental Setup*

In order to obtain convincing information on MP effects on the components of inclusive fitness, we conducted short-term confirmatory tests on dietary intake and long-term age-specific effects of MPs on locomotion speed and reproduction rates in *P. acuta*. Two long-term (93 days after maturity) studies on locomotion speed and reproduction rate were conducted in SSW and in SSW applied with three concentrations of PS particles.

#### 2.3.1. Short Duration Experiment

Two short-duration tests, (I) confirmatory test of MPs ingestion and (II) functional responses of *P. acuta*, were conducted in sequence.

Experiment I: Confirmatory test of MPs particle ingestion

Ingestion of MP particles were confirmed through post feeding excrement analyses. The PS particles used for the feeding trials were stained with Nile Red (NR) (Stock-1 mg NR:1 mL acetone). An amount of 100 µL NR stock solution dissolved in one mL of acetone was used for the staining. The experimental design for the confirmatory test consisted of 20 transparent plastic containers (15 × 10 cm) (experimental container) ((1 treatment + 1 control) × 10 replicate) (one individual per container). Mature individuals (20 in number) of *P. acuta* were taken from the stock culture and transferred to the experimental container, with SSW 200 mL and crushed fish food. In the treatment, 40 mg stained PS (plastic particles $1000 \times 10^4$/200 mL) particles were added on the SSW surface and mixed with a glass rod. Snails were allowed to feed for 24 h duration. In control containers, snails were given only fish food. After 24 h of incubation, the excrements were collected in a glass petri dish from both control and treatment and observed under a stereo zoom microscope (Leica-M205-FA) to record the digested and undigested components in faeces.

Experiment II: Effect of MP particle concentration on ingestion

A total of 60 freshly mature *P. acuta* individuals, starved for 24 h, were use in the experiment. The experimental design for the effect of MP concentration on the particle ingestion test consisted of 60 transparent plastic containers (15 × 10 cm) ((5 treatment + 1 control) × 10 replicates). The plastic containers (50 in number) were filled with 200 mL SSW, to which five concentrations (2.5 mg, 5 mg, 10 mg, 20 mg and 40 mg) of MPs were added; no natural food was given. Control containers were filled with 200 mL SSW with fish food. Mature *P. acuta*, starved for 24 h, were introduced to each of the experimental containers and incubated for 60 min. After 60 min of, snails were gently washed with distilled water and transferred to another pre-labelled beaker with SSW and set aside for

another 24 h for collection of faecal pellets. After 24 h, the faecal pellets egested were collected. The snails were washed with distilled water, and their shell was removed. The inner soft tissue was isolated and digested in 10% KOH solution at 50 °C for 5 h. The faecal pellets were stained with Nile Red and dried in the dark for 24 h. The dry faecal pellets were then crushed, suspended in distilled water, and mixed with a vortex mixer. The digested inner tissue and the faecal pellet solution were then observed under a microscope (Olympus CX21LED, 100× magnification) on a Sedgwick-Rafter counting cell, and the number of MPs were counted carefully. The MP ingestion data obtained were transformed using the Michaelis–Menten equation [29]:

$$V_0 = V_{max}\, S/K_m + S \quad (2)$$

where $V_0$ is the rate of ingestion, $V_{max}$ is the saturation value of the ingestion rate, S is the concentration of the MPs and $K_m$ is the MP concentration at which $V_{max}/2$ is reached.

2.3.2. Long Duration Experimental Set up

To elucidate the impact of PS MPs on locomotion speed, egg capsule production, hatching success and embryonic development, we conducted five experiments in sequence.

Experiment I (Locomotion speed)

Thirty snails were kept in a 2 L aquarium containing MPs, and 10 snails were kept in a 1 L aquarium (without MPs) for a week to acclimatize them to experimental conditions. The experiment consisted of control (without MPs) and three treatment levels, low ($2.5 \times 10^4$ particles/mL), medium ($3.75 \times 10^4$ particles/mL) and high ($5 \times 10^4$ particles/mL). The experiment concentrations were selected according to the results obtained from short-duration experiment II. Locomotion was observed by placing individual snails in 10 transparent plastic containers (15 × 10 cm), already marked with a grid of 1 cm squares. The container was filled with 200 mL fresh SSW, and known concentration of MPs were sprinkled on the water surface and mixed thoroughly. The PS MPs were initially afloat; however, they eventually remained in the water column for the observation duration after thorough mixing. No MPs were added to the control container. The number of squares travelled by each snail over a 15-min duration was counted, and the total distance travelled by each snail was determined. To elucidate the effect of PS particles on locomotion rate, the experiment was monitored for 93 consecutive days. The snails were provided with crushed fish food, daily, and the test medium was changed on every alternate day. The experiment was conducted in 10 replicates for each concentration, exposing a total of 30 snails to MPs and an additional 10 snails as the control.

Experiment II (Fecundity)

To understand the overall effects of PS MPs on fecundity, we investigated (a) egg capsule production and (b) egg hatching success and embryonic development.

(a) Egg capsule production

The present work estimates the number of egg capsules produced/female ($n$ = 10) for 93 days in control and in PS particle-applied medium (particle concentration: $500 \times 10^4$, $750 \times 10^4$ and $1000 \times 10^4$/200 mL). A total of 40 individuals of freshly matured snails were used in this experiment. Egg capsules produced by snails in the control and the treatments were counted daily and transferred to the predefined petri plates (d-90 mm) containing 15 mL of SSW. The same conditions were provided for the egg capsules as for the adult snails. After 10 days of hatching, juveniles were transferred to a 250 mL glass beaker, keeping minimum variation from that of the adult environment.

(b) Egg hatching success

The egg capsules produced/female by *P. acuta* in the control and the treatments were collected for 20 consecutive days and placed in separate petri plates. The eggs inside the egg capsules were observed daily at 12 h intervals under a stereoscopic microscope (Leica-M205-FA). Freshly hatched juveniles were counted for the estimation of egg hatching success

in the control and in the medium applied with three concentrations of MPs ($2.5 \times 10^4$, $3.75 \times 10^4$ and $5 \times 10^4$ particles/mL). Hatching success was estimated by the following mathematical equation.

$$\text{Hatching success} = \frac{\text{Total number of juveniles hatched}}{\text{Total number of eggs in an egg capsule}} \times 100 \quad (3)$$

Observations were continued until the last egg of the egg capsule hatched to juvenile.

### *2.4. Statistical Analysis*

The data for locomotion rate were subjected to one-way ANOVA for significant difference between locomotion rate of snails across different concentrations followed by post-hoc comparisons using Dunnett's T3 test. To compare the effects of concentrations, age and their interactions on the locomotion rate, a factorial two-way ANOVA was conducted. For egg capsules, we determined the mean and standard error of the total egg capsule produced in the treatment and the control. The hatching success data generated in percentage were arch-sine transformed prior to use for the one-way ANOVA test. One-way ANOVA, followed by Dunnett's T3 test was carried out for hatching success. All analyses were carried out using SPSS software (version 25.0) (IBM, Armonk, NY, USA).

## 3. Results

### *3.1. Short Term Experiment*

#### 3.1.1. Experiment I

After 24 h of feeding on a natural diet (fish food), the excrement collected from the control appeared small and twisted (Figure 1a), and those collected from the treatment appeared rope-like and C shaped (Figure 1b). The undigested, stained component of the snail's excrement collected from treatment indicated the presence of plastic particles in the faeces (Figure 1b).

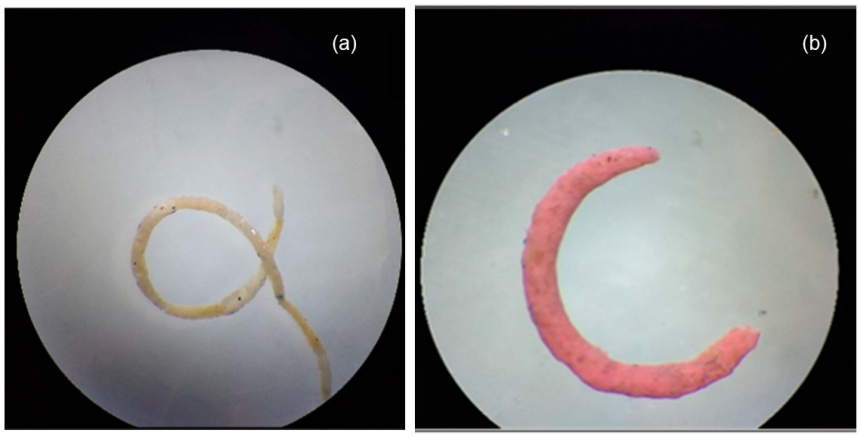


**Figure 1.** Microphotograph (40x magnification) of excrement of *P. acuta* in (**a**) Twisted rope shape, (**b**) C-shape.

The functional group region (4000/cm to 1450/cm) of the obtained FTIR spectra confirmed that the polymers used in the experiment were PS particles (Figure 2).

#### 3.1.2. Experiment II

There was no mortality observed in *P. acuta* during their exposure to MPs. The functional response curve was plotted using the Michaelis–Menten transformation, and the $V_{max}$ and $K_m$ were different according to treatments. *P. acuta* displayed Type II functional response as the rate of MP ingestion increased with the concentration of MPs in the treatment. MP ingestion rate was highest (4819.277 MPs particles) at $1000 \times 10^4$/200mL particle concentration (Figure 3).

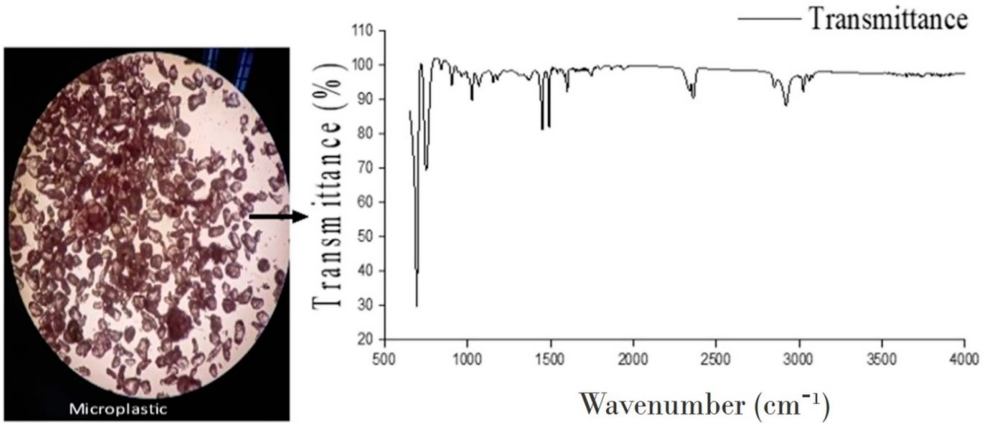


**Figure 2.** Photograph of MPs and the FT-IR analysis spectrum of MPs used in the experiments, corresponding with the reference spectra of polystyrene (PS).

### *3.2. Long Duration Experiment*

#### 3.2.1. Experiment I

The frequency of linear locomotion was maximum, followed by crawling and circular motion. The snails protruded their neck from the hard shell, twisting their whole-body, and then proceeded to swim on the surface of the water in an inverted position. Specific differences in locomotion speed were highly significant in different MP concentrations in comparison to the control (df = 3, $p < 0.001$, one-way ANOVA followed by Dunnett's T3 test) (Figure 4).

In our investigation, locomotion speed in all treatment was not uniform compared to the control group (two-way ANOVA). The differences between minimum and maximum locomotion speed were significantly higher at higher MP concentrations. Concentrations yielded an effect size of 0.148, indicating 14.8% of the variance (df = 3; $p < 0.001$), and age yielded an effect size of 0.205, indicating 20.5% of the variance in the locomotion rate of snail (df = 92; $p < 0.001$) (two-way ANOVA) (Figure 5).

In the control group, the locomotion speed was slower after day 74, whereas in the treatment group (40 mg/200 mL MPs concentration), the reduced locomotory speed was observed very early after day 32, followed by 30 mg/200 mL MPs concentration after 63 days and 20 mg/200 mL after 72 days (Figure 5a–d).

#### 3.2.2. Experiment II

(a) Egg capsule production

In the present study, the cumulative total of egg capsules produced during 93 days of experimental duration was significantly lower in the treatments than in the control (Figure 6; $p < 0.001$; one-way ANOVA). Total number of egg capsule produced during 93 days of experimental duration in the control was 100, and the experimental setup at three concentrations for all replicates used were 90 (20 mg/200 mL), 70 (30 mg/200 mL) and 58 (40 mg/200 mL). Variations in daily egg capsule production were significantly affected by the age (df = 92; $p < 0.001$), concentrations (df = 3; $p < 0.001$) and their interactions (df = 276; $p < 0.001$; two-way ANOVA). Age yielded an effect size of 0.229, indicating 22.9% variance; concentrations yielded an effect size of 0.052, indicating 5.2% variance, whereas their interaction (age vs. concentrations) yielded an effect size of 0.414, indicating 41% variance in egg capsule production.

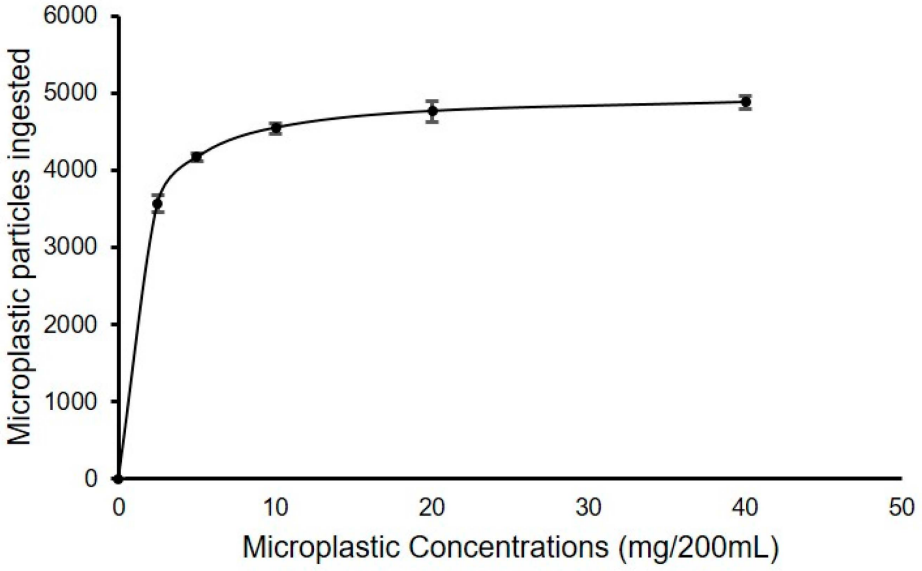


**Figure 3.** MP particle ingestion rate by *P. acuta* at six concentrations of MP (each data point represents mean and standard errors of 10 replicates).

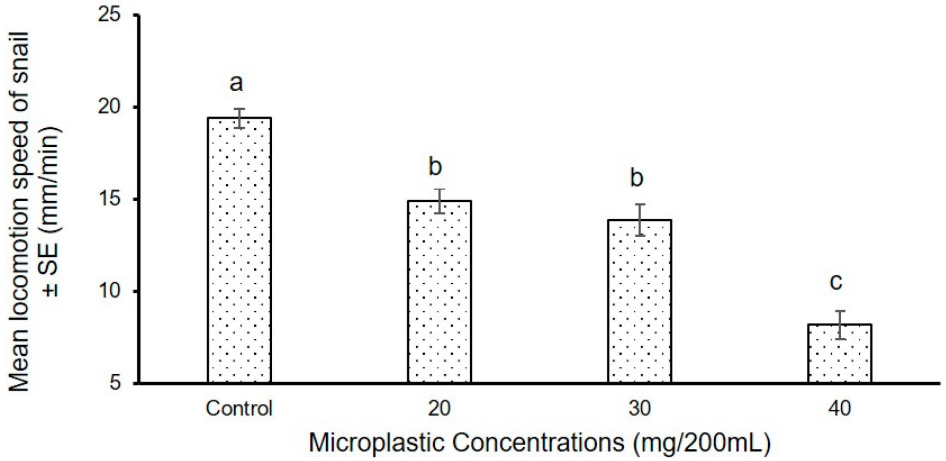


**Figure 4.** Weighted average of 93 days' locomotion speed of *P. acuta* in control (without MPs) and in the medium applied with three concentrations of MPs (20, 30 and 40 mg/200 mL) ($n$ = 93). Data points with differences superscribed with different alphabets are statistically significant ($p < 0.05$).

The effect of age for average experimental duration on the control was not significant. The effect of age on daily egg capsule production was significant ($p < 0.001$) in the treatment cohorts (Figure 7a–d). Number of egg capsule produced daily was uniform during 93 days of experimental duration (Figure 7a), whereas in the treatments, no egg capsule production occurred after 78, 84 and 88 days, respectively, at high, medium and low concentrations (Figure 7b–d). Among the three different concentrations, the highest concentration (40 mg/200 mL) used in the experiment had a highly significant effect on egg capsule production (Figure 6). Although there was a ~10% reduction in total egg capsule production at low MP level, the differences between the control and lower concentration were not significant (Figure 6).

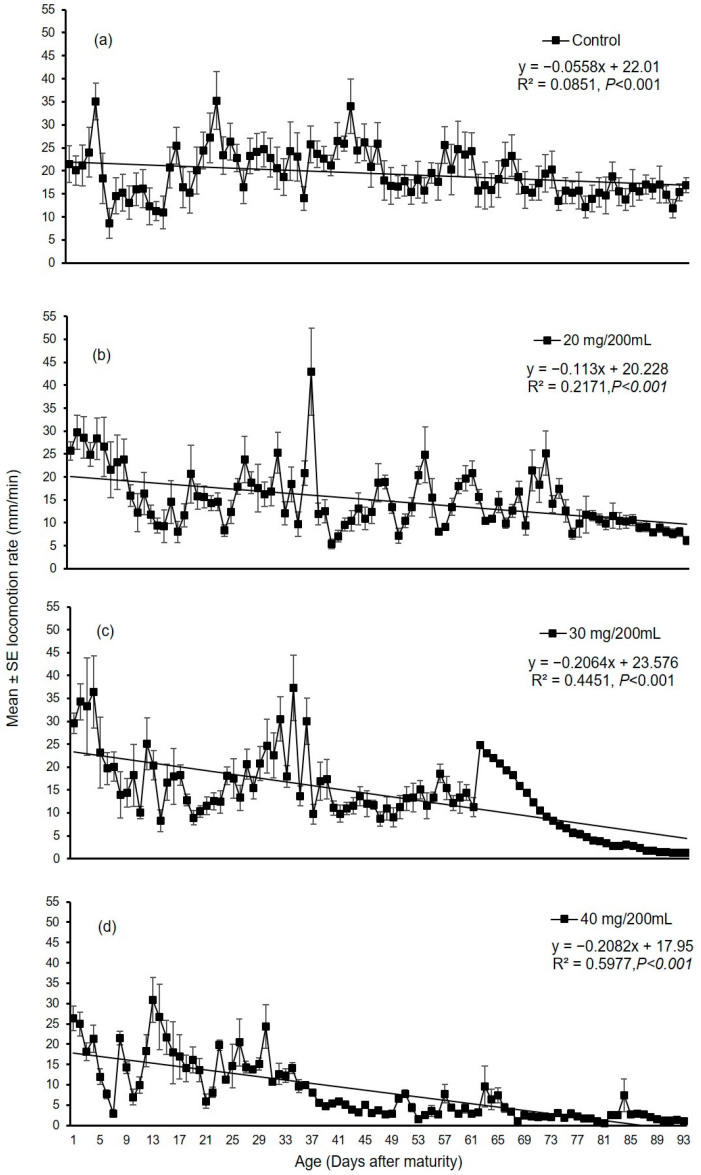


**Figure 5.** Age (post maturity) specific locomotion speed (mm/min) of *P. acuta*, in (**a**) control, and in MP particles applied medium at concentrations of (**b**) 20, (**c**) 30 and (**d**) 40 mg/200 mL ($n$ = 10).

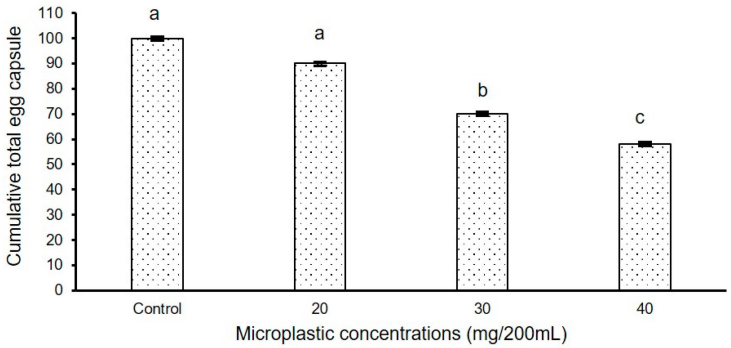


**Figure 6.** Cumulative total (Mean ±SE) of egg capsule production/individual by *P. acuta* for 93 days of experimental duration in control, and in the medium with three concentrations (20, 30 and 40 mg/200 mL) ($n$ = 10) of polystyrene microplastics. Data points with differences superscribed with different letters are statistically significant ($p < 0.05$).

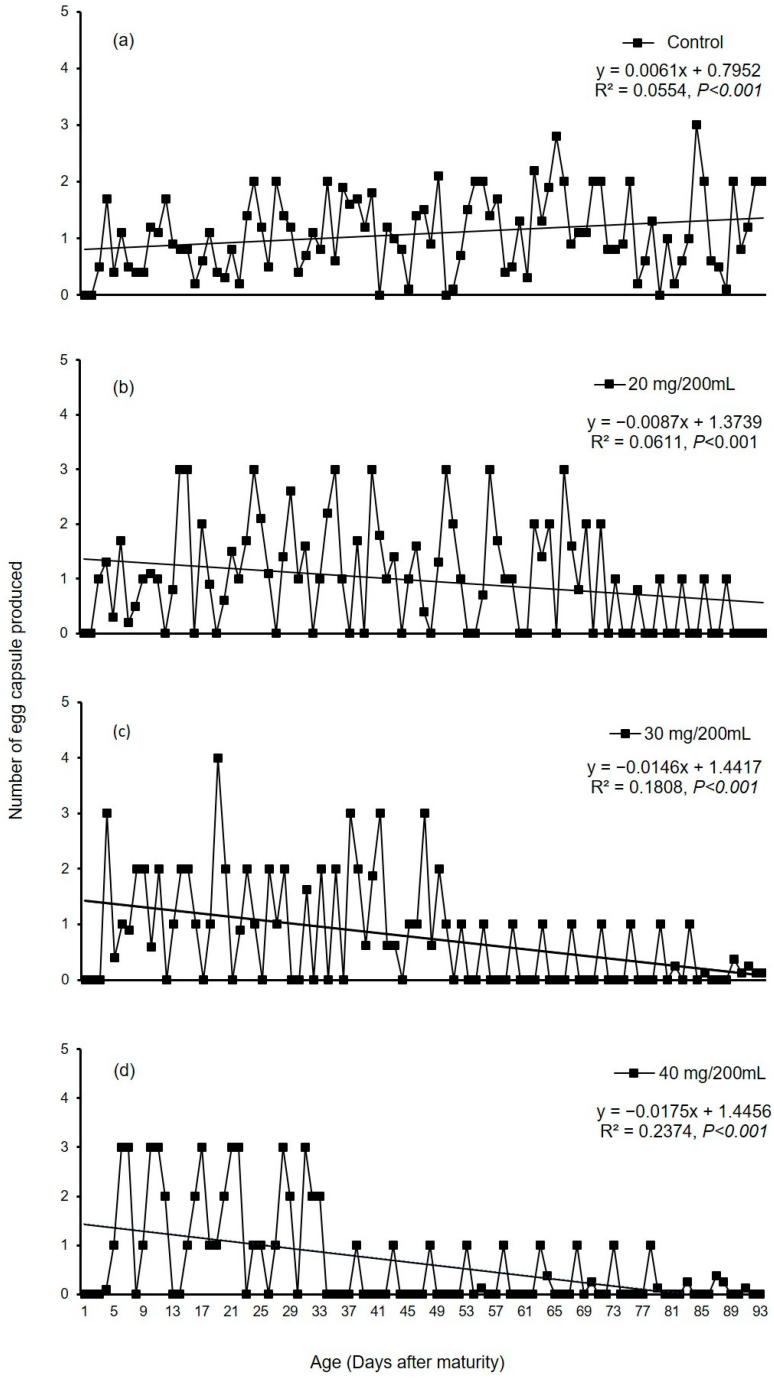


**Figure 7.** Age-specific egg capsule production in *P. acuta* ($n$ = 10), in (**a**) control (without Ms), and in the medium applied with three concentrations of (**b**) 20, (**c**) 30 and (**d**) 40 mg/200 mL of Ms.

(b) Egg hatching success and Embryonic development

The detailed analyses of the number of eggs in each egg capsule and juveniles hatched in the control and the treatments showed differential results. The cumulative number of hatched juveniles produced from all the eggs recorded during experimental duration were significantly lower in the treatments than in the control ($p < 0.01$; one-way ANOVA, followed by Dunnett's T3 test) (Figure 8a).

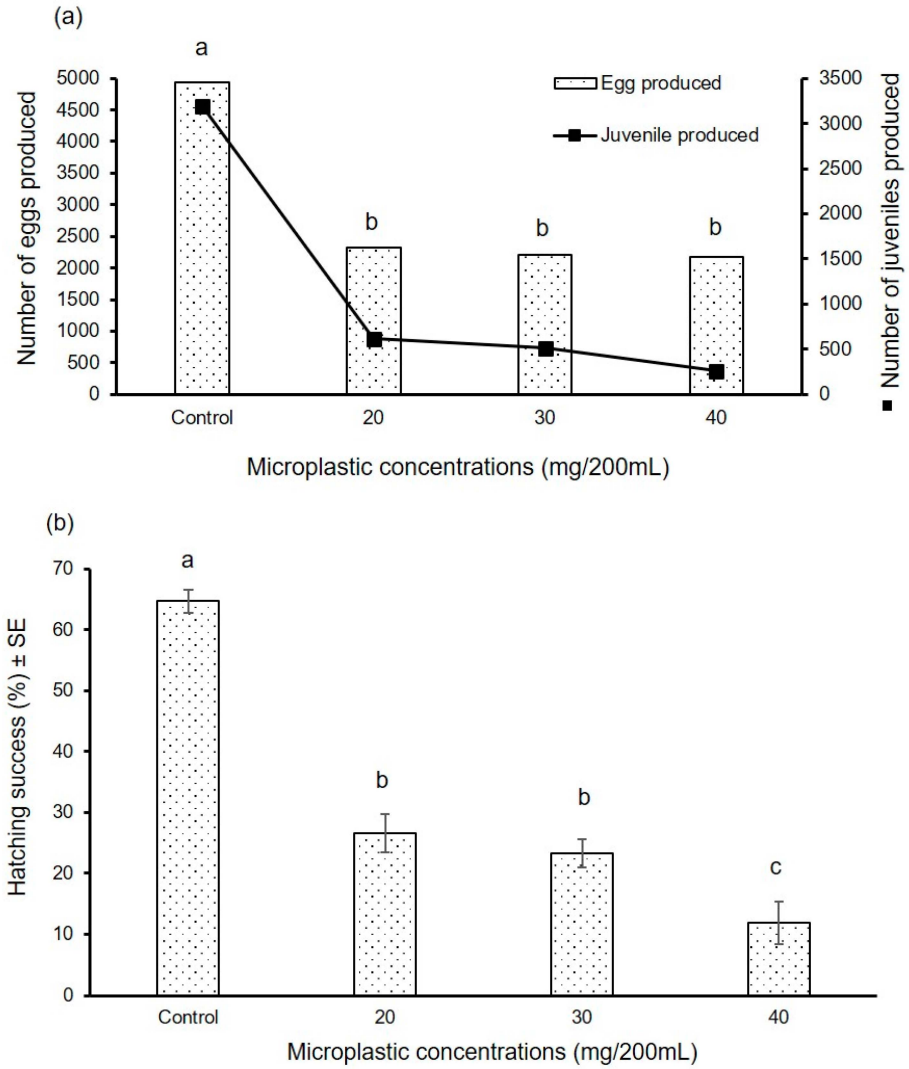


**Figure 8.** Reproductive output of *P. acuta* ($n$ = 10) in terms of (**a**) total number of eggs produced and juveniles hatched for 93 days after maturity and (**b**) egg hatching success (±SE) in control and in the medium applied with three concentrations (20, 30 and 40 mg/200 mL) of microplastics. Data points with differences superscribed with different letters are statistically significant ($p < 0.05$).

Egg hatching success was significantly ($p < 0.001$; one-way ANOVA, Welch test) affected by the presence of MPs in the environment (Figure 8b). The number of hatched juveniles in the control were maximum (3191), followed by 20 mg/200 mL MPs concentration (618), 30 mg/200 mL MPs concentration (511) and 40 mg/200 mL MPs concentration (260). In this study, we observed accumulated MPs on the surfaces of egg capsules and delayed hatching of eggs in the treatments with all three MP concentrations. Underdeveloped eggs were visible over a three-week period, and eggs without nuclei appeared from exposure to three MPs without any further development post three weeks, in comparison to the control (Figure 9a–c).

The effect of MPs was clearly visible on embryonic development. We observed abnormalities in the embryogenesis of MP particle-treated eggs, whereas no abnormality was observed in the case of the control (Figure 9a–d).

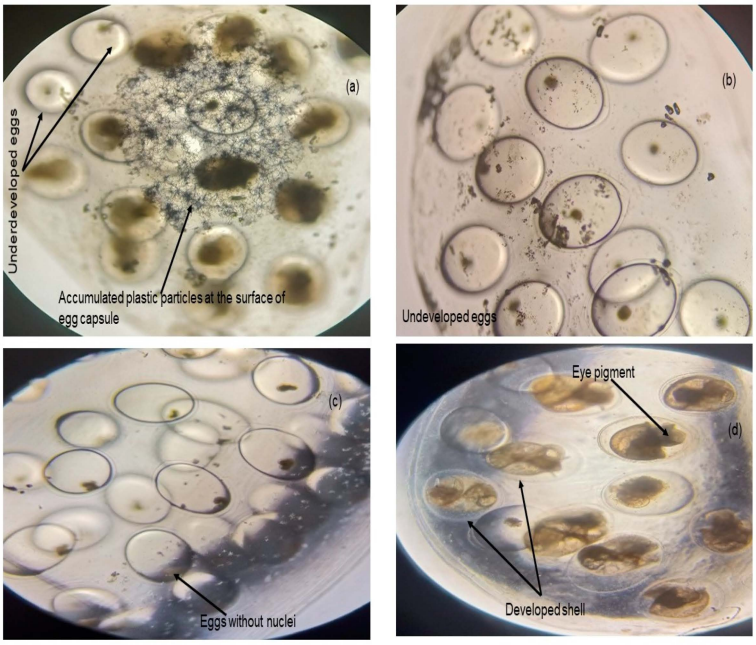


**Figure 9.** Embryonic developmental stages of *P. acuta* in medium applied with three concentrations (20, 30 and 40 mg/200 mL) of microplastics: (**a**) accumulation of microplastics on the outer surface of the egg capsules, (**b**) undeveloped eggs in the egg capsule, over a period of three weeks, (**c**) non-nucleated eggs in microplastic-applied medium and (**d**) egg capsules with hatchlings in control over a period of 3 weeks.

## 4. Discussion

Our results persuasively confirm that dietary intake of PS particles by *P. acuta* interfere with locomotion and have negative impacts on egg preproduction, which points to the effect of PS particles on the inclusive fitness of this species. *P. acuta* is an exotic invasive species and has proved its dominancy over other native species of molluscs. Here we attempt to quantify the number of MPs ingested by *P. acuta*, with varying MPs concentrations. Our data indicate that MP consumption in *P. acuta* is largely driven by the MP concentration in the surrounding environment, as in the Type II functional response using the Michaelis–Menten equation. Studies conducted on other invertebrates, such as *Carcinus maenas* [30] and *Brachionus calyciflorus* [31], also revealed a typical Type-II functional response with MPs. The effects of MPs on banded tilapia (*Tilapia sparrmanii*) were similar to our findings, where tilapia exhibited a saturating type II functional response curve to polyethylene (PE) MPs [32]. Our study consisted of MPs floating in the water column, while the residual MPs obtained from the soft tissue of *P. acuta* sunk to the bottom of the experimental container, suggesting potential adsorption while being inside the organism. In the present study, the size of MP particles (63–32 µm) was equivalent to the commercial food (crushed fish food) that is readily ingested by *P. acuta*. Dietary intake of MPs is most prevalent among various organisms, e.g., in mussels [33,34], oysters [35], *Mytilus galloprovincialis* [36] and other deposit feeders, such as sea cucumbers [37] and crabs [38]. Several adverse effects of MPs have been identified, such as the reduction of emergence rate in *Chironomus sancticaroli* [39,40], reproductive success in *Emerita analoga* [41] and obstruction of the gut passage in *Panorbella campanulata* [42] due to ingestion. A previous study reported that *Physella acuta* can ingest MPs (PS) of particle size 1, 10 and 90 µm, with an ingestion rate of 118 particles/hr. The presence of PS particles affects the ingestion of natural food [43–45]. A short duration study (28 days) on *Gammarus fossarum* revealed its possibility of ingesting polyamide (PA) and PS particles and observed that these plastic particles decrease the food assimilation efficiency, but no obvious impact on locomotion and reproduction was observed [46,47]. *P. acuta* is a prey to organisms at higher trophic levels, such as various freshwater fishes such as *Oreochromis niloticus*, *Mormyrus kannume*, *Mylopharyngodon piceus*, *Procambarus clarkia*,

*Lepomis macrochirus and Lepomis gibbosus* [16,48–52], while the faecal material of snail is eaten by detritivorous zooplankton [53], thus transporting MPs throughout the trophic level.

In our study, the presence of MPs reduced the locomotion speed of *P. acuta*. Similar to our findings, *Crassostrea gigas* [54] and *Daphnia magna* showed a considerable decrease in swimming speed after ingesting high-density PE [55]. A decreased speed with increasing age could be one explanation, depending on how age affects locomotory speed. A study of the impact of PS MPs on mud snails, *Potamopyrgus antipodarum*, exhibits behavioural alterations that significantly increase reaction times as compared to the controls [56,57]. A decrease in the foraging speed of *Achatina fulicam* was observed after the consumption of plants grown on plastic-contaminated soil, negatively affecting its growth rate [58]. In *Pomacea paludosa*, polypropylene MP ingestion had an impact on energy acquisition, and serious damage to digestive gland cells was reported by Jeyavani et al. [59]. Locomotion speed is one of the important aspects that determine success or failure in an organism's pursuit of food, the search for a mate, and escape from predation [15]. In the present case, the snail is a hermaphrodite, so locomotion speed has a larger impact on the search for food and escape from fish predation [16,49]. In our study, MP ingestion decreased the reproduction in *P. acuta*. Similar to our finding, in *H. azteca* (Table S2) decreased reproduction was observed in both acute (10 days) and chronic (42 days) bioassay tests after treatment with PE and PP [60]. Ziajahromi et al. [23], in their study, observed plastic concentration affecting the reproduction of zooplankton *Ceriodaphnia dubia* against MP polyester fibres (size 25.7 ± 10 to 1150 ± 160 µm) and PE beads (size 1–4 µm). The delayed egg hatching and lower hatching success in MP-contaminated environments can be attributed to the following: (i) the accumulation of MP particles on the egg surface (Figure 6a), (ii) higher ingestion of PS particles causing lesser nutrient assimilation, early ageing effects and longer post-reproductive period and (iii) production of the higher number of un-nucleated eggs destined to be nutrient source for viable eggs [61]. A study conducted on *Danio rerio* exposed to MPs revealed that MP accumulation inside the body of larvae did not impact the survival of developing fish, but a delay in hatching was observed [62]. A study on *P. acuta* (without MPs treatment) found that, normally, egg production increases with age, but the rate of oviposition was low in the first and last week of the reproductive cycle compared to the middle week [63]. The early age effect on locomotion and reproduction in MP-contaminated environments further points to the depletion of energy reserve, resulting in a smaller number of fertile eggs in the egg capsule [64]. A plausible explanation is the micro plastic-induced changes in digestive enzyme functioning that may affect the snail's ability to acquire energy from their natural diet, which reduces their energy reserves [43]. The energetic consequences of MP ingestion could contribute to slower locomotion speed, poor reproductive output and reduced egg-hatching success. Plastic concentration is found to be very high at sewage discharge points in the freshwater ecosystem [24]. However, in the natural setup, plastic debris was observed as a frequent oviposition substrate (Figure S3) for *P. acuta*, favouring the species in dispersal and invasion despite affecting locomotion and reproduction.

## 5. Conclusions

This study confirms the ingestion of PS MPs (size 63–32 µm), and it showed a type-II functional response curve. A decrease in locomotion rate, reducing reproduction ability, was observed; the effect was seen at maximum in higher concentrations. Despite the high concentration of MPs, *P. acuta* was able to reproduce; this suggests the tolerance of *P. acuta* to high concentrations of MPs, which leads to proliferation of its population, even in an MP-contaminated environment. It is capable of transferring the MPs to higher trophic level and to lower organism such as zooplankton via its faecal pellets. In nature, microplastics provide a base for oviposition (Figure S3) and act as vehicles of dispersion, which may directly impact the fitness component of this species. There is a high probability of the trophic transfer of MPs through *P. acuta* to higher trophic levels (as prey) and to lower trophic level through its faecal matter. Global plastic production is increasing every year; it rose from

1.5 million tonnes in 1950 to 311 million tonnes in 2014 [65], rising to 367 million tonnes in 2020 [66], with 5–10% of this waste ending in the aquatic ecosystem every year as litter [67]. There will be a continuous increase in plastic production in the coming years, along with increased plastic debris. The annual increase of plastic production is a great matter of concern. Plastic polymers are usually perceived as biochemically inert, but the presence of toxic residual monomers (e.g., vinyl chloride) cannot be ruled out [68]. Additionally, recent investigations suggest that MPs may act as carriers of contaminants [68]; resultantly, MP plastic additives—particularly plasticizers, flame retardants, dyes and fillers—may exert chronic effect [7,22,69] with a potential to alter the community structure through the invasive snail *P. acuta* and other deposit and particle feeders.

To understand the impacts of plastics on the invasion of *P. acuta*, further comparative studies are needed focusing on the responses of native and invasive benthos to MPs in natural setups and on the fate of ingested MPs in the aquatic food web.

**Supplementary Materials:** The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/w15050928/s1, Figure S1: Microplastic film with egg capsules of *P. acuta*, collected from the limnetic zone of the river Ganga, at Danapur (25°38′29.41″ N, 85°02′42.17″ E); Table S1: Composition of Standard Snail Water (SSW) used as culture medium for *P. acuta*. Table S2: Different studies conducted on the effects microplastics on various eco-physiological attributes of freshwater invertebrates.

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