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Quantifying Polyethylene Microplastics Through Microscopy and Investigating Their Effects on *Daphnia magna* Growth Rate

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Abstract

Polyethylene microplastics are among the most abundant pollutants in freshwater environments (Schwarz et. al. 2019). Their ingestion by aquatic organisms has been linked to physiological stress and potential bioaccumulation across trophic levels (Nelms et. al. 2018). This study investigates whether green-fluorescing, low-density polyethylene microspheres can be reliably quantified within *Daphnia magna* using polarizing light microscopy, and whether exposure affects juvenile growth rates. Two colonies were maintained, with microspheres integrated in algae and water columns as the treatment group. Both live and digested *Daphnia* specimens were analyzed using an Olympus BX51 Polarizing Light Microscope. Microspheres were detected in 47 of 60 digested individuals (average 5.317±5.691 particles), with particles observed in the digestive tract and carapace of all live specimens. Polarizing light microscopy proved effective for microplastic detection, though it was time-intensive. Growth rate effects were tested by placing juveniles into individual vials containing either just algae (control) or algae mixed with the microspheres (treatment) and the lengths were measured every other day for two weeks. Three experimental groups were tested: control-control, control-plastic, and plastic-plastic. Unexpectedly, *Daphnia* exposed in the plastic-plastic group exhibited the highest growth rates (1.150±1.344 mm/day). However, these results were inconclusive due to limited replication and high mortality in the control-control and control-plastic groups (2–4 days) compared to the plastic-plastic group (10–14 days). Future work includes measuring microplastic diameters, exploring alternative analytical methods, and assessing energy trade-offs and potential additive leachates (Labbe et. al. 2020) (Schrank et. al. 2019).

Keywords: *Daphnia, microplastics, microscopy, growth, ecology, environment*

Introduction

Microplastics (1µm–5mm) are defined as plastic fragments present in the environment (Huang et. al. 2022). One of the most prevalent types of plastic is polyethylene ($(C_2H_4)_n$), which is synthesized through the polymerization of ethylene using a metallocene catalyst (ScienceInfo). This results in a highly flexible, durable material with chemical and electric insulation properties (ScienceInfo). Given the estimated 4.8–12.7 million tons of plastic entering the oceanic systems annually (and the steady rise in global plastic production from 1.7 million tons in 1950 to over 300 million metric tons in 2014), polyethylene has emerged as the most abundant plastic in aquatic ecosystems (Schwarz et. al. 2019).

Consequently, growing concern has emerged regarding the impact of microplastics on aquatic ecosystem health (Nelms et. al. 2018). Marine organisms may ingest these particles, potentially leading to adverse effects such as intestinal blockages, chronic inflammation, endocrine disruption, and movement through trophic levels by bioaccumulation and biomagnification. This could result in significant disruption in our ecosystems. For freshwater environments, *Daphnia magna* plays a foundational role in the food web (Yin et. al. 2023). These are filter-feeding platonic crustaceans that primarily consume green algae, reproduce both sexually and through parthenogenesis in the absence of mates, and serve as a key food source for bivalves and various fish species.

Thus, *Daphnia magna* ingesting plastics such as polyethylene may pose significant risks to growth, reproduction, and survival. However, current research presents highly variable findings regarding polyethylene's effects on *Daphnia magna* health. A 2018 study reported no observable impact from polyethylene microspheres, while a 2021 study documented reductions in all three (Canniff et. al. 2018) (An et. al. 2021). However, most studies report a combination of unchanged or diminished outcomes across these metrics (Huang et. al. 2022) (Song et. al. 2026). Proposed explanations for this inconsistency include energy allocation or potential leaching of chemical additives from the microspheres, such as the UV-stabilizer benzophenone-3 ($C_{14}H_{12}O_3$) (Glazier and Callow 1992) (Schrank 2019). Ultimately, this prevents definitive conclusions about the toxicity of polyethylene microplastics in *Daphnia magna*.

In addition to biological concerns, there remains a lack of standardized methods for identifying and quantifying microplastics (Kotar et. al. 2022). While microscopy allows for the manual counting of particles, an interlaboratory study revealed an average recovery rate of 45.7%. The study concluded that while microscopy holds promise, additional methods should also be utilized to improve accuracy and reproducibility. One such method involves using polarizing light microscopy to detect fluorescing microplastics (Labbe 2020). Since plastic polymers exhibit birefringence under cross-polarized light, fluorescent plastic can be more readily distinguished and quantified using this approach.

Thus, this research aims to investigate two key questions: whether green-fluorescing, low-density polyethylene microspheres can be reliably quantified within *Daphnia magna* after exposure using a polarizing light microscope, and whether such exposure influences growth rates compared to a control group. It is hypothesized that microplastics will be found within the exposed *Daphnia* using this method and that these individuals will exhibit a decrease in growth rate compared to the control group.

Materials and Methods

Daphnia colony setup

Live *Daphnia magna* colonies were purchased from CAROLINA™, along with a container of dried algae pellets. Upon arrival, the colonies were transferred into small plastic containers filled with purified drinking water (PRIMO™) to allow for acclimation to the new environment. To prepare the food source, four algae pellets were soaked in purified water for rehydration. Once acclimated, the colony was split into two groups. Half of the colony was transferred into a 34.6 cm x 20.3 cm x 12.7 cm Sterilite® plastic container filled with purified drinking water and approximately half of the rehydrated algae, serving as the control (Figure 1). The other half was placed into an identical container with the same volume of water and algae but with the addition of green fluorescing, low-density polyethylene microspheres ranging 10–63µm (Cospheric) (Figure 1). These microspheres were integrated by gently rubbing them into the algae on weighing paper and dispersing them throughout the water column. Both colonies were maintained for two months to ensure optimal acclimation and

to allow the treated *Daphnia magna* to interact with the plastic. Overhead lighting remained on to promote algae growth, and a heat lamp was positioned above the colonies to maintain a temperature of approximately 21°C. To support oxygenation, the water in each container was briskly stirred every other day.

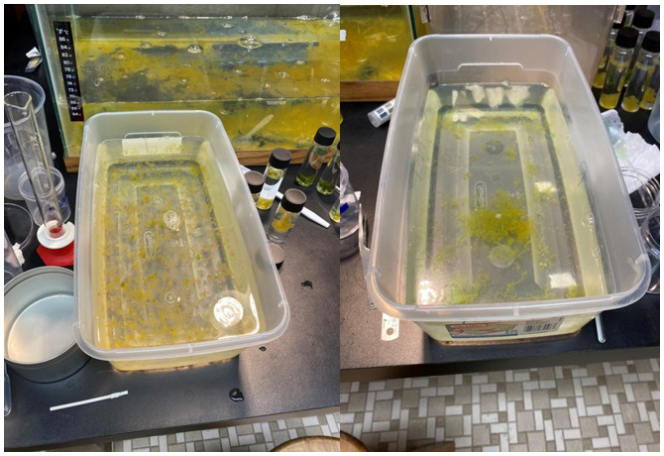

Figure 1: Control (left image) and Treated (right image) *Daphnia* Colony Setup. Each colony was prepared with purified drinking water and algae, and the microspheres were added to the treated colony, giving the algae a more prominent green hue.

Preparation of *Daphnia magna* for analysis

Six *Daphnia magna* were extracted from the treated colony using a plastic pipette and transferred into a rinsed metal container filled with tap water. To remove any externally adhered microspheres, the organisms were allowed to swim briefly before being moved into a second tin of fresh tap water for additional rinsing. After rinsing, the *Daphnia* were placed onto pre-cleaned CAROLINA™ glass microscope slides (three individuals per slide). Excess water was carefully pipetted off the slides, leaving the organisms in place. Two drops of 30% hydrogen peroxide (H_2O_2) were then directly added onto each *Daphnia* to initiate digestion overnight. After this, the remaining carapace structures were flattened using metal tweezers for better analysis. A barrier was drawn around the hydrogen peroxide residue to ensure that only the plastics within the region were counted. Additionally, three *Daphnia* were rinsed and placed on a slide and analyzed while still alive to assess microplastic locations.

Analysis of specimens using a polarizing light microscope

Each prepared slide was examined using an Olympus BX51 Polarizing Light Microscope at 40x magnification. Prior to analysis, the microscope was powered on, the eyepieces and light source were calibrated, and the Infinity3 camera was activated and "white-balanced" to optimize the color gradient of the samples. Live *Daphnia magna* specimens were analyzed first, followed by the digested samples. Each slide was systematically scanned for the presence of microspheres. Upon detection of a plastic particle, or after completing a full scan, the analyzer was inserted to activate the microscope's polarizing function. Fluorescence of the green microspheres was also assessed, and all observed microspheres were counted and documented.

Experiment setup for measuring the *Daphnia magna*'s growth rate

To assess growth rates, 50 mL glass vials were prepared by adding 30 mL of purified drinking water (measured with a graduated cylinder) and 0.50±0.02 g of rehydrated algae (weighed on a Denver Instrument XS-410 mass scale) (Figure 2). For the treatment, 0.01 g of polyethylene microspheres were weighed and rubbed into the algae prior to being deposited into the vials. Once the vials were prepared, juvenile *Daphnia magna* were extracted from the respective colonies and placed on a microscope slide. Excess water was removed using a micropipette, and each organism was measured using a SHINWA 15 cm ruler under a BAUSCH & LOMB dissecting microscope (Figure 2). After recording the initial lengths, one juvenile was placed into each vial. Lids were loosely secured to allow air exchange and minimize water evaporation. Three experimental groups were established: *Daphnia* from the control colony placed into control vials without microplastics (control-control), *Daphnia* from the control colony placed into plastic-treated vials (control-plastic), and *Daphnia* from the plastic-treated colony placed into plastic-treated vials (plastic-plastic). Organisms were retrieved every two days for remeasurement under the dissecting microscope. Each trial lasted up to 14 days or until the individual died, defined as no movement following gentle disturbance.

Figure 2: Prepared Experimental Vials. There are five vials for each tested groups (control-control, control-plastic, plastic-plastic) lined vertically.

Date	Daphnia	# MP	Date	Daphnia	# MP	Date	Daphnia	# MP
10/20/2025	A1	8	11/10/2025	A3	3	2/16/2026	B2	16
	A2	7		B1	0		B3	1
	A3	16		B2	0	2/25/2026	P1	10
	B1	0		B3	0		P2	15
	B2	9	1/15/2026	A1	0		P3	7
	B3	12		A2	4		2P1	9
10/27/2025	A1	1		A3	1		2P2	8
	A2	1		B1	1		2P3	23
	A3	1		B2	1			
	B1	8		B3	2			
	B2	0	1/20/2026	A1	2			
	B3	1		A2	6			
10/30/2025	A1	18		A3	4			
	A2	3		B1	1			
	A3	2		B2	2			
	B1	3		B3	5			
	B2	0	2/10/2026	A1	0			
	B3	0		A2	4			
11/5/2025	A1	8		A3	1			
	A2	13		B1	16			
	A3	9		B2	8			
	B1	11		B3	13			
	B2	3	2/16/2026	A1	9			
	B3	0		A2	1			
11/10/2025	A1	0		A3	0			
	A2	0		B1	12			

Table 1: Raw Data Gathered from Microplastic Quantification in an Excel Spreadsheet. The six samples digested at a time were dated and assigned a letter/number based on which of the two slides they rested on (the specimens labeled with P are prepared *Daphnia* from the plastic-plastic growth rate experiments that lived past the allotted experiment time).

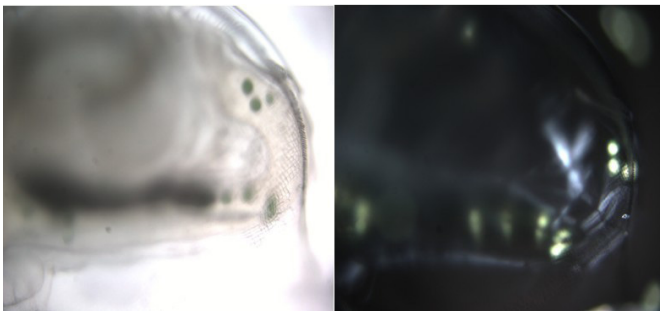

Figure 3: Live *Daphnia* Sample #2 Taken under Regular and Polarizing Light. This showcases microplastics moving through the digestive track under a microscope at 40x magnification without polarized light (left image) versus with polarized light (right image).

Statistics

Averages and standard deviations were calculated for the number of microspheres found per *Daphnia magna* within the prepared quantification samples. To assess growth dynamics, juvenile *Daphnia* were categorized into three initial length ranges: 0.5–0.7 mm, 0.8–1.0 mm, and 1.1–1.3 mm. For each range, the average growth rate (mm/day) and corresponding standard deviation were calculated. Average life expectancy was determined for each size class as well.

Results

Polyethylene microspheres were identified in all three live *Daphnia magna* specimens analyzed, with particles observed in both the digestive tract and the carapace. One individual also exhibited microspheres lodged within the reproductive canal. Among the digested samples, 47 out of 60 contained at least one microsphere, with particle counts ranging from 0–23 per specimen. The average ± standard deviation was 5.317±5.691 microspheres found (Table 1). While the Infinity3 camera provided sufficient resolution to detect microspheres near the surface of the carapace and in the hydrogen peroxide residue, the use of the polarizer brightly illuminated the green fluorescence of the microplastics and was essential for identifying particles embedded deeper within the specimens (Figure 3).

With five vials per trial, three trials were completed for the control-control and control-plastic groups, and two trials for the plastic-plastic group. In the control-control, *Daphnia* in the 0.5–0.7 mm and 0.8–1.0 mm initial size exhibited average growth rates of 0.288±0.064 mm/day (8 individuals) and 0.250±0.122 mm/day (6 individuals), respectively (Table 2). The 1.1–1.3 mm range included only one individual, which grew 0.1 mm total. Thus, no statistical analysis was conducted for this range. The control-plastic group showed similar growth rates for the 0.5–0.7 mm and 0.8–1.0 mm ranges, both averaging 0.250±0.058 mm/day (4 individuals) (Table 3). However, the 1.1–1.3 mm range demonstrated a significant increase, with an average growth rate of

0.400±0.321 mm/day (7 individuals). In what appears to be a contrast to the previously mentioned literature, the plastic-plastic group exhibited the highest growth rates across all ranges. *Daphnia* in the 0.5–0.7 mm range grew at 1.150±1.344 mm/day (2 individuals), while those in the 0.8–1.0 mm range grew 0.750±0.071mm/day (2 individuals) (Table 4). The 1.1–1.3 mm group averaged 0.483±0.232 mm/day (6 individuals). Overall, the lowest growth rate was observed in the control-plastic group within the 0.5–1.0 mm range, while the highest was in the plastic-plastic group in the 0.5–0.7 mm range.

Initial Length (mm)	Sample Size	Range of Growth (mm)	Growth Rate Avg±SD (mm/day)
0.5 - 0.7	8	0.2 - 0.3	0.288±0.064
0.8 - 1.0	6	0.2 - 0.5	0.250±0.122
1.1 - 1.3	1	0.1	N/A

Table 2: Growth Rate Results for Control-Control. Initial length range of *Daphnia* juveniles, total sample size, seen range of growth, and the average and standard deviation for the growth ranges displayed for the control-control group.

Initial Length (mm)	Sample Size	Range of Growth (mm)	Growth Rate Avg±SD (mm/day)
0.5 - 0.7	4	0.2 - 0.3	0.250±0.058
0.8 - 1.0	4	0.2 - 0.3	0.250±0.058
1.1 - 1.3	7	0.2 - 1.0	0.400±0.321

Table 3: Growth Rate Results for Control-Plastic. Initial length range of *Daphnia* juveniles, total sample size, seen range of growth, and the average and standard deviation for the growth ranges displayed for control-plastic group.

Initial Length (mm)	Sample Size	Range of Growth (mm)	Growth Rate Avg±SD (mm/day)
0.5 - 0.7	2	0.2 - 2.1	1.150±1.344
0.8 - 1.0	2	0.7 - 0.8	0.750±0.071
1.1 - 1.3	6	0.2 - 0.7	0.483±0.232

Table 4: Growth Rate Results for Plastic-Plastic. Initial length range of *Daphnia* juveniles, total sample size, seen range of growth, and the average and standard deviation for the growth ranges displayed for plastic-plastic group.

The life expectancy of the *Daphnia magna* varied notably across the experimental groups. The control-control group exhibited the shortest survival times, averaging 2.750, 3.1657, and 2.00 days for the 0.5–0.7 mm, 0.8–1.0 mm, and 1.1–1.3 mm ranges, respectively (Figure 4). The control-plastic group showed similar results, with average lifespans of 2.5 and 2.75 days for the 0.5–0.7 mm and 0.8–1.1 mm ranges, respectively (Figure 5). However, the 1.1–1.3 mm range in this group demonstrated a marked increase in longevity, averaging 5.286 days. In contrast, the plastic-plastic group exhibited the longest survival times across all size ranges. *Daphnia* in the 0.5–0.7 mm range lived an average of 8 days, those in the 0.8–1.0 mm range survived 11.5 days, and individuals in the 1.1–1.3 mm range lived for an average of 10 days (Figure 6). Notably, six *Daphnia* from the plastic-plastic group exceeded the 14 allotted days. These individuals were subsequently rinsed and digested for microplastic quantification, revealing particle counts ranging from 7–23 microspheres.

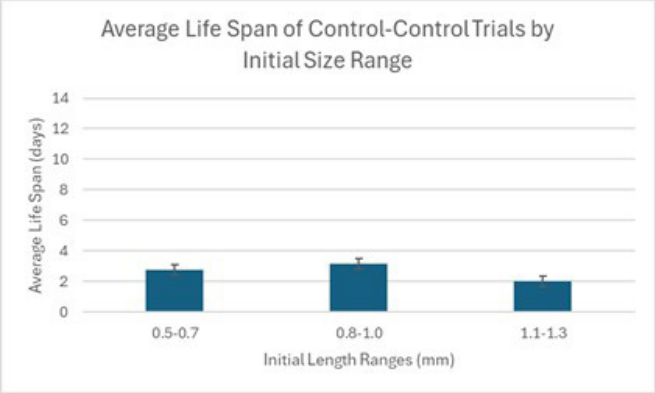

Figure 4: Average Life Spans for Each Range of Initial *Daphnia* Lengths for Control-Control. They averaged approximately 3 days for the 0.5–0.7 and 0.8–1.0 mm initial lengths, decreased to 2 days for the 1.1–1.3 mm range.

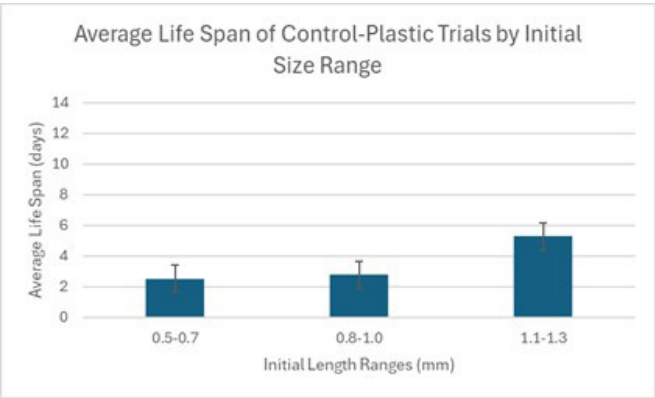

Figure 5: Average Life Spans for Each Range of Initial *Daphnia* Lengths for Control-Plastic. The 0.5–0.7 and 0.8–1.0 mm initial lengths both average around 3 days and then increased to 5 days for the 1.1–1.3 mm range.

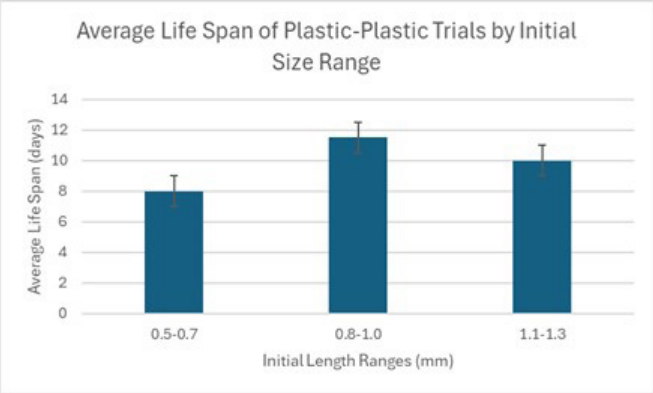

Figure 6: Average Life Spans for Each Range of Initial *Daphnia* Lengths for Plastic-Plastic. The 0.5–0.7 mm range average at 8 days, the 1.1–1.3 was 10 days, and then the highest lifespan was the 0.8–1.0 mm range at almost 11 days.

Discussion

Microplastics were detected within the digestive tract and carapace of *Daphnia magna* following over two months of exposure to polyethylene spheres. Since the microspheres were suspended in the algae, this supports the idea that *Daphnia* may ingest microplastics if the particles are embedded in their food (Yin et. al. 2023). An explanation for the presence of microplastics in the carapace may lie in the organism's shell structure. A 2024 study demonstrated that microplastics can be swept beneath the shell (Funke et. al. 2024). However, this study also noted that this phenomenon could be size-dependent. To explore this further, future studies should quantify the diameters of these retained microplastics using a polarizing light microscope (either through an eyepiece-mounted measuring scope or through digital analysis with the Infinity3 camera system) to recognize a pattern in size ranges found. These findings underscore the vulnerability of *Daphnia magna* to microplastic pollution and raise concerns regarding chronic toxicity and trophic transfer of these particles through aquatic food webs (Nelms et. al. 2018).

Polarizing light microscopy successfully revealed fluorescent microspheres that were otherwise undetectable using standard brightfield microscopy. However, there is still a risk of overlooking particles during analysis (Kotar et. al. 2022) (Labbe et. al. 2020). Additionally, this process is notably time-intensive; each *Daphnia* specimen was carefully scanned to ensure precision and accuracy, which limited the study to 60 samples over a five-month period. To improve efficiency and reduce observer bias, alternative methods, such as Flow Cytometry, may be considered.

Contrary to the proposed hypothesis, the growth rate results did not align with expectations. The growth rate began to increase in the control-plastic group and continued to increase in the plastic-plastic group. Previous studies have not reported increased growth rates following long-term exposure to polyethylene microplastics (Canniff et. al. 2018) (An et. al. 2021) (Huang et. al. 2022) (Song et. al. 2026). However, the reliability of these is limited due to the lack of completed trials, largely due to the low survivability in the experimental groups. Several factors may have contributed to the high mortality rate observed in the control colony. One plausible explanation is inadequate acclimation to the vials. *Daphnia* are highly sensitive to environmental stressors, and disruptions can significantly increase mortality (Nelms et. al. 2018). At the time of this study, a new colony was introduced to the existing control population, which may not have fully adjusted at the time of the study. To improve reliability in future experiments, it is recommended that multiple *Daphnia* generations be raised in a simulated aquatic environment to ensure proper acclimation before testing.

However, the survival duration appears to possibly correlate with observed growth rates; as average lifespan increased, so did growth rate in both the control-plastic and plastic-plastic groups. This trend raises the possibility that fluorescent polyethylene microspheres may not be negatively impacting *Daphnia* growth rates. Given that the previous literature reports variation in growth, reproduction, and survival, further investigation should be done to assess potential energy allocation trade-offs. Plastic additives, such as fluorescent dyes or UV-stabilizers, may leach into the water or bodies of the *Daphnia* (Schrank 2019). However, while Cospheric reports using less than 30% additives in their microspheres, the specific chemicals remain protected as a "trade secret" according to the Safety Data Sheet (Cospheric). Therefore, to accurately assess additive leaching, future studies should consider sourcing microspheres from manufacturers that transparently disclose chemical constituents.

Conclusion

This study demonstrates that green-fluorescing polyethylene microspheres can be successfully detected and

quantified within *Daphnia magna* using polarizing light microscopy. These microplastics were observed in both live and digested specimens, primarily within the digestive tract and carapace. This supports the concern that *Daphnia* are incorporating microplastics found in their environment, mainly through possible ingestion (Yin et. al. 2023). Future studies should quantify the diameters of plastic found to determine if there is a preferred size range being integrated within the *Daphnia*.

While polarizing light microscopy improved visualization of these particles compared to a standard microscope, this method remains highly time-insensitive and susceptible to potential undercounting (Kotar et. al. 2022) (Labbe et. al. 2020). As a result, an alternative method should be investigated to improve efficiency on microplastic analysis.

Conflicting with the hypothesis and literature, the *Daphnia* that were exposed to polyethylene microspheres did not exhibit reduced growth rates (Canniff et. al. 2018) (An et. al. 2021) (Huang et. al. 2022) (Song et. al. 2026). Instead, the plastic-plastic group showed the highest average growth rate. However, these findings remain inconclusive due to limited replication caused by the high mortality in the control groups (possibly resulting from acclimation stress associated with the experimental setup) (Nelms et. al. 2018). Therefore, definitive conclusions regarding the biological effects of polyethylene microplastics on *Daphnia magna* cannot be made and future replications should investigate long-term energetic trade-offs and additive leaching from the fluorescent plastics (Glazier and Callow 1992) (Schrank 2019).

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