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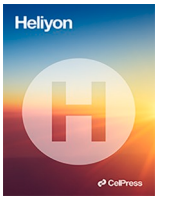

Research article

# Assessing the ecological consequences of biodegradable plastics: Acute, chronic and multigenerational impacts of poly-3-hydroxybutyrate microplastics on freshwater invertebrate *Daphnia magna*

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ABSTRACT

Microplastics, pervasive contaminants in freshwater ecosystems, have raised ecological concerns. Efforts are underway to substitute conventional plastics with biodegradable alternatives that should be more easily decomposed in the environment. However, the biodegradation of these alternatives depends on specific conditions such as temperature, humidity, pH, and microorganisms, which are not always met. Consequently, these biodegradable alternatives can also fragment and generate microplastics, which can be ingested and affect biota. In this study, we investigated the acute, chronic, and multigenerational effects of two fractions (particles <63 μm and particles <125 μm) of biodegradable poly-3-hydroxybutyrate (P3HB) at varying concentrations on the inhibition, mortality, reproduction activity, and growth of the freshwater invertebrate *Daphnia magna*. No acute effects were observed for either size fraction. However, during chronic and multigenerational experiments, an increase in the concentration of P3HB microplastics corresponded with increased mortality, reduced reproductive activity, and slower growth among the mother organisms. Given the important role of *D. magna* in the food chain, these findings suggest that biodegradable microplastics may indeed negatively affect freshwater ecosystems.

## 1. Introduction

Microplastics (MPs) are defined as small plastic particles ranging from 1 to 1000 μm in size [1], and have become ubiquitous, found in diverse locations worldwide, spanning from urban centers to remote regions, both on land and in the ocean [2]. Currently, there is a growing scientific and societal concern about their impact on freshwater and marine organisms [3]. Nevertheless, research on microplastic contamination in freshwater ecosystems remains comparatively limited when compared to the marine realm [4]. This

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discrepancy may be attributed to the conventional perception of freshwater environment solely serving as conduits for transporting MPs into the oceans. However, the widespread presence of MPs in freshwater environments around the world has prompted a swift evolution in studies exploring their potential impacts [5].

The risks associated with MPs are related to their physical and chemical characteristics, encompassing factors such as composition, shape, and even color [6]. Their size renders them accessible as a potential food source for a wide range of organisms [7]. Subsequently, this can result in the obstruction of feed appendages, leading to reduced food intake, aggregation causing blockages in the digestive tract of organisms, or potential translocation into the circulatory system [8,9]. Furthermore, MPs have the capacity to introduce toxic substances into organisms. This occurs through two mechanisms: firstly, the gradual leaching of additives added during production to enhance plastic properties, and secondly, their large surface area and hydrophobic properties enable MPs to accumulate hydrophobic organic pollutants from the environment, which may subsequently be released within the bodies of organisms after ingestion [10,11].

With the growing effort to replace conventional plastics with eco-friendly alternatives, degradable materials are coming to the fore [12,13]. Biodegradable polymers (BDPs), emerging as viable substitutes for conventional plastics across various industrial sectors, are polymeric materials capable of undergoing decomposition by microorganisms into carbon dioxide, water, and biomass under aerobic conditions, or into methane and carbon dioxide under anaerobic conditions [14–16]. Comparable as conventional plastics, BDPs can also naturally break down into small particles, generating a substantial quantity of MPs [17,18]. However, despite their biodegradability, these MPs can persist for extended periods due to the influence of various environmental factors (biotic and abiotic) environmental factors such as temperature, humidity, pH, biologically active substances, and the presence and activity of microorganisms [19,20]. These conditions are rarely met in aquatic environments.

Poly-3-hydroxybutyrate (P3HB) is a notable representative of biodegradable polymers. This polymer belongs to the group of biodegradable linear polyesters known as polyhydroxyalkanoates (PHAs). PHAs are synthesized as an intracellular reserve for carbon and energy sources by various bacteria (gram-negative, and gram-positive), thriving under aerobic and anaerobic conditions [21–23]. P3HB is a relatively hydrophobic material characterized by an approximate crystallinity of 50 %, a high melting point (180 °C), and low elasticity. Its density (1.23 g/cm$^3$) suggests a predisposition to settle down in aquatic environments, although factors such as turbulence, water flow, and thunderstorms can lead to its resuspension within the water column [24–26].

The commonly used model organism for studying the influence of MPs on freshwater biota is the aquatic invertebrate *Daphnia magna*. This filter-feeding species is capable to non-selectively ingest particles varying from <1 μm to approximately 70 μm in size [27,28]. According to Scherer et al. [29], the probability of MPs ingestion by freshwater invertebrates depends on factors such as the properties and concentration of MPs. Daphnids are also preferred for these studies because of their global abundance in aquatic environments and their significant role in bridging primary producers and higher trophic level consumers [30].

The majority of research in this domain has focused on the impact of conventional plastics on zooplankton including polystyrene (PS), polyethylene (PE), polypropylene (PP), polyvinyl chloride (PVC), polyethylene terephthalate (PET), and others [31]. While PE, PET, and PS MPs usually did not cause immobilization and mortality of *D. magna* during the standard acute exposure period (48 h), adverse effects became evident with longer exposures (96–120 h) [30,32–36]. In addition, PS MPs can cause significant reduction of the filtering capacity and a reduction of the swimming velocity of *D. magna* [37,38]. In chronic tests, PE and PET MPs exhibited detrimental effects on the growth and reproductive activity of *D. magna* [39,40], while PS MPs were found to increase mortality and affect growth and reproduction, depending on the initial age of the test organisms [30,41]. Additionally, Bosker et al. [42] observed a negative impact of MPs on the population size and biomass of these organisms highlighting potential repercussions for the freshwater ecosystem due to the importance of *D. magna* in the food chain.

Several studies have ventured into investigating the effects of biodegradable MPs. Savva et al. [43] compared the sublethal effects and food intake of *D. magna* of polyhydroxybutyrate (PHB, not specified), PLA MPs, and conventional ones revealing that biodegradable MPs had a more pronounced toxic impact. The chronic effect of PLA, PVC, and polyurethane (PUR) was investigated by Zimmermann et al. [44] who observed that all 3 types of MPs negatively affected the test organisms, with PVC showing the most significant impact on reproduction and PLA causing the highest mortality. While the authors did not draw specific conclusions regarding the potential toxicity of biodegradable MPs, recent research suggests that their presence might lead to nutrient depletion in the medium due to biofilm formation and biosorption [45].

Hence, it is evident that the anticipated rise in the usage of biodegradable plastics will lead to a corresponding surge in environmental contamination by MPs. While conventional MPs pose various risks to aquatic organisms, it remains uncertain whether biodegradable MPs have similar adverse effects. Therefore, the aim of this study was to evaluate the impact of P3HB MPs on the freshwater organism *D. magna.* We examined both the short-term and long-term consequences, including the effects on mortality and reproductive activity of these organisms, with a focus on multigenerational outcomes.

## 2. Material and methods

### 2.1. Preparation of microplastics

P3HB MPs (ENMAT Y3000, spherical shape) from TianAn Biologic Materials Co., Ltd., Ningbo City, China were used. To prepare size fractions, a suspension of P3HB MPs in MilliQ water was sieved through stainless-steel mesh sieves with openings of 63 and 125 μm. Subsequently, both size fractions (<63 μm and <125 μm) were dried in glass beakers in a fume hood at room temperature. The particle sizes do not precisely match both size fractions (for details see Procházková et al. [45]) because ultrasonication was not applied during the process. However, for clarity in the text, we refer to the mesh size used for particle preparation i.e. <63 μm and

<125 μm.

### 2.2. Daphnia magna culture

The *D. magna* test organism obtained from laboratory culture at the Institute of Chemistry and Technology of Environmental Protection (Faculty of Chemistry, Brno University of Technology, Czech Republic) was cultured in ASTM reconstituted hard water [46]. This water was enriched with selenium, vitamins (biotin, thiamine, cyanocobalamine, according to the OECD guideline No. 202 [47]), and 20 μl/L seaweed extract (Marinure, Glenside, Scotland) according to Rosenfeldt et al. [48]. The medium was regularly replaced three times a week and the organisms were nourished with green algae *Desmodesmus subspicatus* providing an equivalent to 200 μg carbon per organism daily. The culture was maintained under controlled conditions with a temperature of 20 ± 1 °C and a light-dark cycle of 16:8 (800–1000 lux).

Twenty-four hours prior to initiating the experiments, all offsprings were removed from mother cultures. After an additional 24 h period, all newborn neonates were collected with a plastic pipette and quantified. These age-synchronized test organisms were randomly distributed among various treatment (see Section 2.3, 2.4, and 2.5). and control groups.

### 2.3. Acute experiments

In the acute experiments, both size fractions of P3HB MPs (<63 and < 125 μm) were examined using two exposure methods: testing in P3HB suspension and testing of P3HB leachate. The suspension was prepared by weighing the desired amount of P3HB and then transferred to a volumetric flask, which was subsequently filled with culture medium (as described in Section 2.2). This suspension underwent an ultrasonification for 10 min to disperse the particles. For the leachate, a suspension of P3HB in a culture medium was prepared and then incubated for 96 h under the same conditions as the acute test with *D. magna*. Following incubation, the suspensions was filtered through a 0.8 μm pore size filter.

The acute experiments were conducted in 150 mL glass beakers, with each beaker containing 50 mL of either test suspension, leachate, or medium. The concentration of P3HB microparticles was 0, 6.25, 12.5, 25, 50, and 100 mg/L 1 mg of fraction <63 μm corresponded to $3.20 \cdot 10^{10}$ particles, while for fraction <125 μm it was $2.39 \cdot 10^{10}$ particles [45]. This concentration range was selected based on previous studies on the toxicity of MPs to *D. magna* [32,33,35] as well as the concentration limit stipulated for acute toxicity testing of chemicals according to OECD guideline No. 202 [47].

For each treatment, two acute experiments were conducted: 1) newborn neonates of *D. magna* (<24 h old, and 2) 7-day-old *D. magna* individuals (age-synchronized neonates were fed with green algae for 7 days) were exposed to prepared suspensions, leachates, or media. The observation period was prolonged from 48 h to 96 h, during which immobilization of *D. magna* individuals was visually assessed at 24, 48, 72, and 96 h. According to OECD guideline No. 202 [47] the organisms were not provided with food, and the suspension, leachate, or medium was not changed throughout the experiments. Each experiment was repeated three times and consisted of four replicates of the same treatment.

### 2.4. Chronic experiments

Chronic experiments were conducted for both size fractions of P3HB MPs (<63 and < 125 μm). We investigated the effects of chronic exposure on fundamental life-history parameters in *D. magna*, specifically focusing on survival, growth, age at first reproduction, and total offspring number after exposure to various concentrations of P3HB MPs. The experiments were designed according to the standard OECD No. 211: 21-d Daphnia reproduction test [49]. The organisms were exposed as newborn neonates (<24 h old).

Initially, we utilized the same concentrations of P3HB MPs as in the acute test (6.25, 12.5, 25, 50, and 100 mg/L) along with a control group. However, due to the high mortality observed in the test organisms at high concentrations, we subsequently reduced the P3HB MPs concentrations to 1.56, 3.13, 6.25, 12.5, and 25 mg/L.

The neonates were individually incubated in 150 mL glass beakers filled with 100 mL of test suspension or control medium. These vessels were kept at 21 °C under a 16:8 light:dark cycle, and the organisms were provided with a daily diet of green algae *D. subspicatus* (200 μg carbon per organism daily). Test suspensions and control medium were changed three times per week. Each test vessel held one neonate, and there were ten replicates within each concentration. Each experiment was repeated three times and lasted 21 days.

### 2.5. Multigenerational experiments

For multigenerational experiments, we subjected four consecutive generations (F0 – F3) of *D. magna* to exposure to P3HB MPs. The exposure of each generation followed the OECD guideline No. 211 [49] and the setup and conditions were consistent with those employed in the previous chronic experiments (see Section 2.4). The exposure concentration was set at 1.56 mg/L and both size fractions of P3HB microplastics were evaluated. In addition, for each experiment, a control group (consisting of only the medium) was included. The suspensions were changed three times per week and Daphnia were provided with a daily diet of green algae *D. subcapitatus* (equivalent to 200 μg of carbon per organism).

To initiate the next generation, the offsprings originating from the third brood, sourced from at least three different parental animals, were pooled and exposed as previously described. Each generation was exposed for a period of 21 d, during which survival, growth, age at first reproduction, and total offspring number were determined.

### 2.6. Determination of the growth rate of D. magna

All *D. magna* organisms, both before and after the chronic and multigenerational experiment (survivors) were captured using a Nikon D3100 digital camera equipped with an AF-S Micro NIKKOR 40 mm 1:2.8 G lens (Nikon, Japan) on laminated graph paper. The body lengths were subsequently measured utilizing the ImageJ software, extending from the apex of the helmet to the base of the apical spine [50].

### 2.7. Data analysis

The average growth rate after 21 days of exposure was calculated as follows:

$$\mu = \frac{L_e - L_b}{t}$$

where $\mu$ (mm/d) is the average growth rate, $L_e$ (mm) is the body length at the end of the experiment, $L_b$ (mm) is the body length at the beginning of the experiment, and $t$ (d) is the exposure time (21 days).

The statistical significance of the differences between the control and individual concentrations was assessed using Dunnett's test in the R program, with differences being deemed significant when $p < 0.05$.

## 3. Results

### 3.1. Acute experiments

In this experiment involving P3HB MPs (whether suspension or leachate and whether with neonates or 7-day-old organisms), no instance resulted in an increase in daphnid mortality, even after 96 h of exposure.

### 3.2. Chronic experiments

The preliminary experiments involving higher concentrations (6.25, 12.5, 25, 50, and 100 mg/L) of both size fractions of P3HB MPs in suspension revealed a diminishing reproductive activity of maternal organisms with increasing concentration (Fig. S1A). Notably,

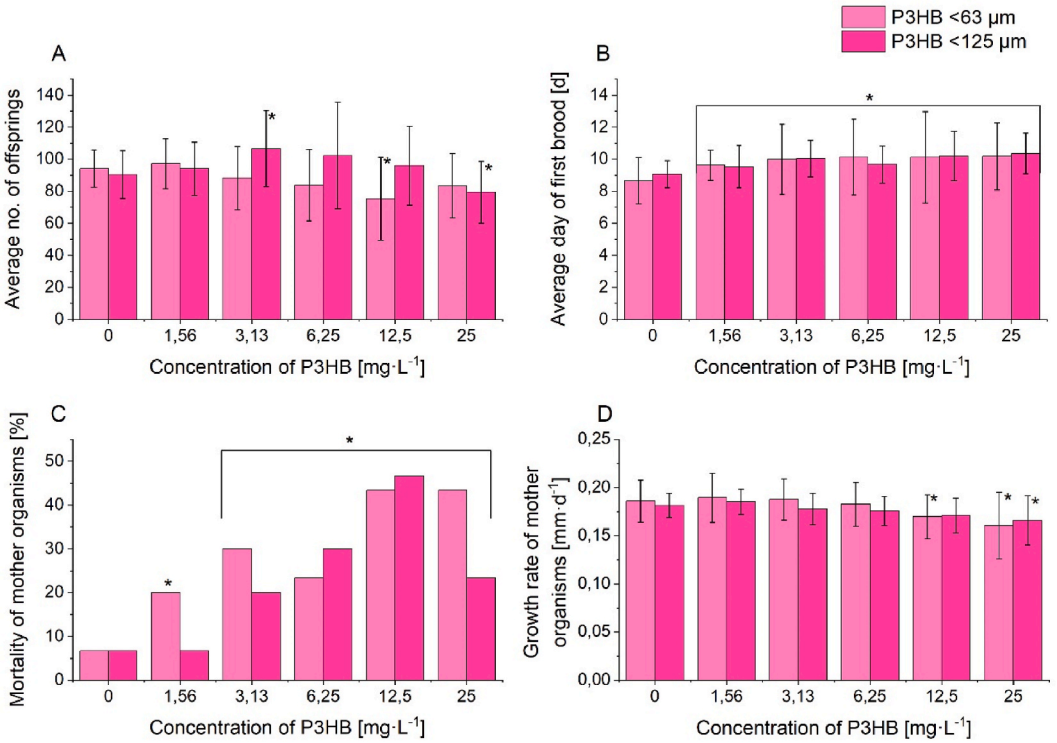


**Fig. 1.** Results of chronic experiments involving both size fractions of P3HB MPs (<63 µm and <125 µm; n = 30): A – average number of offsprings per mother organism, B – average day of first brood, C – mortality of mother organisms, D – specific growth rate of mother organisms from the beginning to the end of the 21-day experiment. Asterisks indicate statistically significant deviations from the control ($p < 0.05$).

for the larger size fraction (MPs <125 μm), a marginal stimulation of reproductive activity, compared to the control group, was initially observed up to a concentration of 25 mg/L. In addition, for MPs <63 μm, there was a delay in the onset of the first brood at high concentrations (50 and 100 mg/L), while for MPs <125 μm, this phenomenon was only observed at the highest concentration, i.e. 100 mg/L (Fig. S1B). A substantial mortality rate among maternal organisms was recorded for both size fractions, specifically reaching 53.3 % for MPs <63 μm and 46.7 % for MPs <125 μm at a concentration of 100 mg/L (Fig. S1C). Due to high mortality, we subsequently reduced the concentration of P3HB MPs in suspension to 1.56, 3.13, 6.25, 12.5, and 25 mg/L.

In the subsequent experiment involving a lower concentration of P3HB MPs, a minor stimulation (3.3 %) of reproductive activity was noted for MPs <63 μm when compared to the control group. However, the reproductive activity exhibited a decline with increasing microplastic concentration up to 19.9 %, and 11.2 % at concentrations 12.5 and 25 mg/L, respectively (Fig. 1A). Conversely, for MPs <125 μm, an inhibition of reproductive activity (12.2 %) was monitored only at a concentration of 25 mg/L of P3HB MPs in suspension (Fig. 1A). Additionally, a slight delay in the average day of the first brood of juveniles (up to 17.6 % for MPs <63 μm, and 14.2 % for MPs <125 μm) was observed for both size fractions at all concentrations of P3HB MPs in suspension (Fig. 1B). Moreover, an increase in the concentration of P3HB MPs in suspension led to elevated mortality rates among mother organisms for both size fractions (up to 43.3 % for MPs <63 μm, and 46.7 % for MPs <125 μm, Fig. 1C). The last parameter monitored was the growth rate of the mother organisms. For both size fractions of P3HB MPs, following a minor growth stimulation at the lowest microplastic concentrations in suspension (1.9 % for MPs <63 μm, and 2.1 % for MPs <125 μm), there was a subsequent slight inhibition of growth (13.6 % for MPs <63 μm, and 8.6 % for MPs <125 μm, Fig. 1D).

### 3.3. Multigenerational experiments

The same effects as in the chronic experiment were also observed in the multigenerational experiment. The impact of the lowest concentration (1.56 mg/L) of both size fractions of P3HB MPs on the organism *D. magna* was assessed, specifically focusing on reproductive activity, the day of the first brood, mortality of mother organisms, and growth rate.

In the first generation (F0) of maternal organisms, a stimulation of reproductive activity was documented for both size fractions of P3HB (3.2 % for MPs <63 μm, and 4.0 % for MPs <125 μm). However, in the subsequent generations (F1–F3), a slight inhibition of reproductive activity was observed in all instances (Fig. 2A). In the last generation of mother organisms (F3) it reached 23.6 % for MPs <63 μm, and 19.2 % for MPs <125 μm. Furthermore, a delay in the first day of reproduction was observed in all cases (Fig. 2B) along

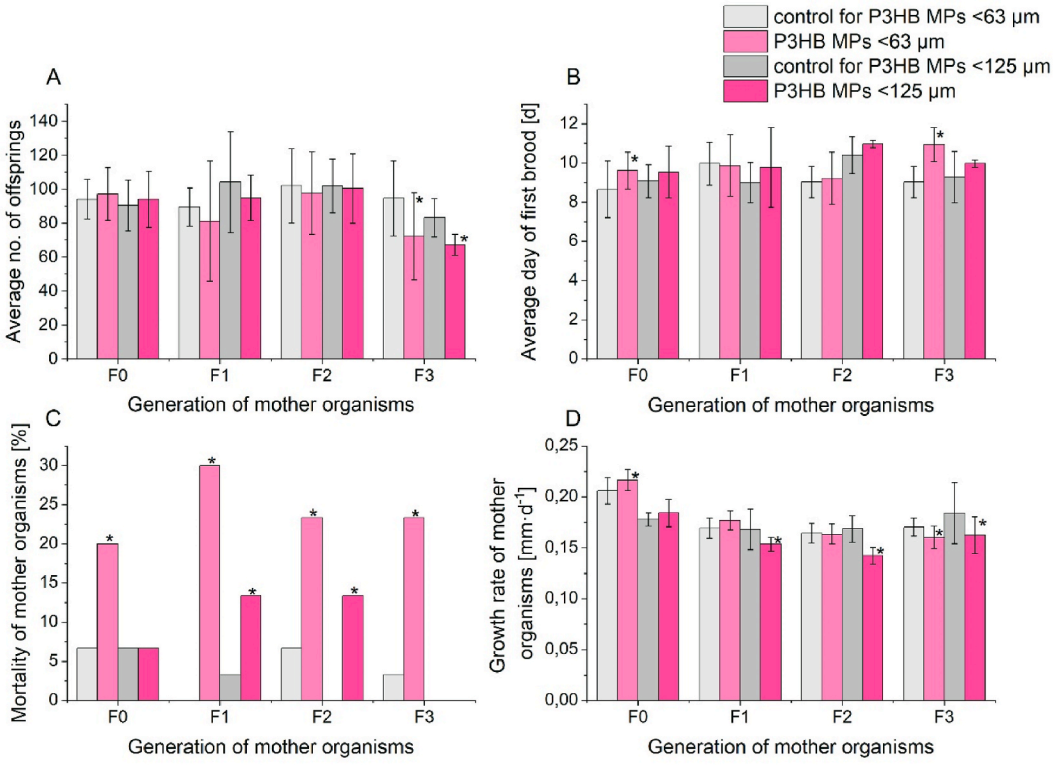


**Fig. 2.** Results of multigenerational experiments involving both size fractions of P3HB MPs (<63 μm and <125 μm, concentration 1.56 mg/L; n = 30): A – average number of offsprings per mother organism, B – average day of first brood, C – mortality of mother organisms, D – specific growth rate of mother organisms from the beginning to the end of the 21- day experiment. Asterisks indicate the statistically significant deviations from the control ($p < 0.05$).

with a slight increase in the mortality of mother organisms, consistent with the results of the chronic experiment (Fig. 2C).

For MPs <63 μm, a decline in the growth rate of the mother organisms compared to the control was observed in the F2 and F3 generations (0.4 % and 5.9 %). Conversely, for MPs <125 μm, this decrease in growth rate was evident as early as the F1 generation and reached 8.6 % (Fig. 2D).

## 4. Discussion

The extensive utilization of plastics resulted in a pressing environmental concern such as the pollution caused by MPs. Numerous studies have highlighted the impact of conventional MPs on aquatic organisms, particularly *D. magna*, a keystone species in freshwater ecosystems. This study introduces P3HB, a possible alternative to conventional plastics, and seeks to shed light on its potential ecological implications.

Recent studies [30,32–36] showed that while conventional MPs such as PE, PET, or PS do not induce an increased immobilization and mortality of *D. magna* in acute tests (48 h), an extended exposure period (96–120 h) gradually escalates their negative effect. Interestingly, the results of this study diverge from this pattern, as we did not observe an elevation in *D. magna* immobilization even after 96 h in any of the tested scenarios (suspension/leachate, juveniles/7-day-old organisms). However, during chronic experiments, an increase in mortality alongside increasing concentration of both size fractions of P3HB in suspension was observed. Additionally, there was a progressive reduction in the growth of daphnia and an inhibition of their reproductive activity. In accordance to our results, PE and PET MPs did exert a negative effect on growth and reproductive activity during chronic experiments [39,40]. Also, PS particles have been found to elevate mortality levels and disrupt the growth and reproduction of organisms [30,41]. In addition, Savva et al. [43] have suggested that the sublethal effects and their impact on food intake in *D. magna* may be more pronounced in the presence of biodegradable MPs such as PHB and PLA compared to conventional MPs.

In the multigenerational experiment, a slight inhibition of reproductive activity and a deceleration in the growth of organisms were observed in the subsequent generations F1 to F3. Schür et al. [27] reported that PS MPs induced a multigenerational effect in *D. magna*. Exposure to MPs resulted in increased mortality in *D. magna*, as well as reduced reproduction and growth over the course of four generations. To make a comparison with the effects of natural particles, they used kaolin, which did not yield such an effect.

The observed adverse effects on growth, reproduction, and mortality during chronic experiments highlight the pressing need to address MPs pollution. These effects can be attributed to multifaceted mechanisms, which may involve mechanical damage to organisms through adsorption to their appendices and body surface [51], nutrient depletion in test medium [45], and the ingestion of MPs [7]. Savva et al. [43] reported that biodegradable MPs (PHB and PLA) showed a greater post-exposure inhibition effect on the feeding activity of *D. magna* than high-density polyethylene (HDPE). As recently reported in our work [52], the ingestion of P3HB MPs depended on both their size and concentration in the surrounding environment. For example, at a concentration of 25 mg/L, *D. magna* ingested P3HB MPs up to 10.3 wt% of MPs <63 μm and 6.3 wt% of MPs <125 μm, respectively. Furthermore, as the concentration of P3HB MPs in suspension increased, so did the content of P3HB found in *D. magna* [52]. Therefore, the escalating mortality and growth inhibition of *D. magna* with increasing P3HB MPs concentration in suspension (as depicted in Fig. 1C and D) is probably related to a higher ingestion of P3HB MPs from the suspension. The fact, that a more pronounced effect was observed with smaller particles (<63 μm) confirms our assumption that the impact of P3HB MPs on *D. magna* is particle size-dependent. This observation aligns with the research conducted by Ref. [53], who reported that secondary P3HB nanoplastics induced up to 85 % immobilization of *D. magna* after 48 h of exposure.

The potential effects of biodegradable plastics on biota are poorly understood, but even less is known about whether these effects are influenced by their biodegradability. Biodegradation is associated with the development of a biofilm on the surface of the plastics [54], which applies also to P3HB MPs [45]. Biofilm formation on MPs can modify their ingestion by organisms, for example, some MPs coated with biofilms can release into the water noxious signals, known as infochemicals, prompting nearby organisms to ingest them [55]. The development of biofilm on the surface of P3HB MPs could possibly be influenced by the time lag in the mortality of *D. magna* when compared to conventional ones. When plastic particles or products are released into the aquatic environment, a coating layer of inorganic and organic pollutants rapidly forms on their surface. Subsequently, the formation of biofilm on the plastic surface, lasting minutes to hours, is mostly likely the initial interaction with the surrounding biota [54]. During the acute test (96 h) the organisms might ingest not only P3HB MPs but also the biofilm presents on their surface, using it as a source of nutrients. However, after a longer period (during chronic experiments), mechanical blockage of the digestive tract due to accumulated P3HB MPs probably led to increased mortality.

In the natural environment, biodegradable plastics may pose some additional, secondary problems. They are expected to degrade in the natural environment, even if their complete degradation is not always achieved due to unfavorable conditions (e.g. low temperature, unsuitable pH, microorganisms), they generally degrade faster than conventional plastics [56]. Therefore, their biodegradation can lead to oxygen depletion and affect nutrient levels, as shown in our previous study [45]. This in turn can affect organisms that are sensitive to lower oxygen level or plants that require certain nutrients for their growth. Another issue associated with biofilm formation on MPs is its potential to transport pathogens [57,58]. Therefore, further research is essential to elucidate the precise mechanisms responsible for the observed differences and to assess the broader ecological consequences of adopting P3HB particles as a more environmentally friendly alternative.

## 5. Conclusion

This research has demonstrated that the presence of P3HB MPs did not induce acute effects on *D. magna*. However, in chronic and

multigenerational experiments, we observed an increase in mortality, a decrease in reproductive activity, and a slower growth rate of mother organisms, all of which were dependent on the concentration of P3HB MPs in suspension. Furthermore, smaller MPs appeared to elicit more pronounced effects. Both of these phenomena can be attributed to the ingestion of MPs, which increases with higher concentrations of MPs in suspension and smaller particle sizes. The biofilm formation on the MPs surface also facilitates their ingestion. Subsequently, the blockage of the digestive tract in *D. magna* leads to adverse consequences. It is evident that biodegradable MPs can be comparable or even more hazardous to *D. magna* than conventional MPs, which requires careful consideration of their use and management to mitigate their potential environmental impacts. Balancing the benefits of biodegradability with the risks of increased toxicity is therefore a crucial challenge for policymakers, researchers, and industries.

## Data availability statement

Data will be available on request, for more information contact corresponding author.

## CRediT authorship contribution statement

**Petra Procházková:** Writing – original draft, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Gabriela Kalčíková:** Writing – review & editing. **Eliška Maršálková:** Writing – review & editing, Conceptualization. **Martin Brtnický:** Writing – review & editing. **Helena Zlámalová Gargošová:** Writing – review & editing, Resources. **Jiří Kučerík:** Writing – original draft, Visualization, Supervision, Project administration, Funding acquisition, Conceptualization.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e36302.

## References

[1] N.B. Hartmann, T. Hüffer, R.C. Thompson, M. Hassellöv, A. Verschoor, A.E. Daugaard, et al., Are we speaking the same language? Recommendations for a definition and categorization framework for plastic debris, Environ. Sci. Technol. 53 (2019) 1039–1047.
[2] S. Du, R. Zhu, Y. Cai, N. Xu, P.-S. Yap, Y. Zhang, et al., Environmental fate and impacts of microplastics in aquatic ecosystems: a review, RSC Adv [Internet] 11 (2021) 15762–15784 [cited 2023 Nov 29], http://xlink.rsc.org/?DOI=D1RA00880C.
[3] L.C. de Sá, M. Oliveira, F. Ribeiro, T.L. Rocha, M.N. Futter, Studies of the effects of microplastics on aquatic organisms: what do we know and where should we focus our efforts in the future? Sci. Total Environ. 645 (2018) 1029–1039.
[4] C. Pradinaud, S. Northey, B. Amor, J. Bare, L. Benini, M. Berger, et al., Defining freshwater as a natural resource: a framework linking water use to the area of protection natural resources, Int J Life Cycle Assess [Internet] 24 (2019) 960–974 [cited 2023 Dec 4], http://link.springer.com/10.1007/s11367-018-1543-8.
[5] A.A. Horton, A. Walton, D.J. Spurgeon, E. Lahive, C. Svendsen, Microplastics in freshwater and terrestrial environments: evaluating the current understanding to identify the knowledge gaps and future research priorities, Science of The Total Environment [Internet] 586 (2017) 127–141 [cited 2023 Dec 4], https://linkinghub.elsevier.com/retrieve/pii/S0048969717302073.
[6] J. Yin, Y. Long, W. Xiao, D. Liu, Q. Tian, Y. Li, et al., Ecotoxicology of microplastics in Daphnia: a review focusing on microplastic properties and multiscale attributes of Daphnia, Ecotoxicol Environ Saf [Internet] 249 (2023) 114433, https://doi.org/10.1016/j.ecoenv.2022.114433 [cited 2023 Dec 4].
[7] M. Cole, P. Lindeque, E. Fileman, C. Halsband, R. Goodhead, J. Moger, et al., Microplastic ingestion by zooplankton, Environ. Sci. Technol. 47 (2013) 6646–6655.
[8] F. Murray, P.R. Cowie, Plastic contamination in the decapod crustacean Nephrops norvegicus (Linnaeus, 1758), Mar Pollut Bull [Internet] 62 (2011) 1207–1217 [cited 2023 Dec 4], https://pubmed.ncbi.nlm.nih.gov/21497854/.
[9] M.A. Browne, A. Dissanayake, T.S. Galloway, D.M. Lowe, R.C. Thompson, Ingested microscopic plastic translocates to the circulatory system of the mussel, Mytilus edulis (L.), Environ Sci Technol [Internet] 42 (2008) 5026–5031 [cited 2023 Dec 4], https://pubs.acs.org/doi/10.1021/es800249a.
[10] S. Anbumani, P. Kakkar, Ecotoxicological effects of microplastics on biota: a review, Environ. Sci. Pollut. Res. Int. 25 (2018) 14373–14396 [cited 2021 Apr 20], http://link.springer.com/10.1007/s11356-018-1999-x.
[11] J. Oehlmann, U. Schulte-Oehlmann, W. Kloas, O. Jagnytsch, I. Lutz, K.O. Kusk, et al., A critical analysis of the biological impacts of plasticizers on wildlife, Philosophical Transactions of the Royal Society B: Biological Sciences [Internet] 364 (2009) 2047–2062 [cited 2021 Apr 20], https://royalsocietypublishing.org/doi/10.1098/rstb.2008.0242.
[12] E. Amasawa, T. Yamanishi, J. Nakatani, M. Hirao, S. Sato, Climate change implications of bio-based and marine-biodegradable plastic: evidence from poly(3-hydroxybutyrate-co-3-hydroxyhexanoate), Environ. Sci. Technol. 55 (2021) 3380–3388.

[13] M.A.S. Patwary, S.M. Surid, M.A. Gafur, Properties and applications of biodegradable polymers, J. Res. Updates Polym. Sci. 9 (2020) 32–41 [Internet], https://lifescienceglobal.com/pms/index.php/jrups/article/view/7780.

[14] J.H. Song, R.J. Murphy, R. Narayan, G.B.H. Davies, Biodegradable and compostable alternatives to conventional plastics, Phil. Trans. Biol. Sci. 364 (2009) 2127–2139.

[15] G.E. Luckachan, C.K.S. Pillai, Biodegradable polymers- A review on recent trends and emerging perspectives, J. Polym. Environ. 19 (2011) 637–676.

[16] X. Zhao, K. Cornish, Y. Vodovotz, Narrowing the gap for bioplastic use in food packaging: an update, Environ Sci Technol [Internet] 54 (2020) 4712–4732. https://pubs.acs.org/doi/10.1021/acs.est.9b03755.

[17] J. Fojt, J. David, R. Přikryl, V. Řezáčová, J. Kučerík, A critical review of the overlooked challenge of determining micro-bioplastics in soil, Sci. Total Environ. 745 (2020) 140975 [Internet], https://linkinghub.elsevier.com/retrieve/pii/S0048969720345046.

[18] H.Y. Sintim, A.I. Bary, D.G. Hayes, M.E. English, S.M. Schaeffer, C.A. Miles, et al., Release of micro- and nanoparticles from biodegradable plastic during in situ composting, Sci. Total Environ. 675 (2019) 686–693.

[19] S. Agarwal, Biodegradable polymers: present opportunities and challenges in providing a microplastic-free environment, Macromol. Chem. Phys. 221 (2020) 2000017 [Internet], https://onlinelibrary.wiley.com/doi/10.1002/macp.202000017.

[20] X.-F. Wei, M. Bohlén, C. Lindblad, M. Hedenqvist, A. Hakonen, Microplastics generated from a biodegradable plastic in freshwater and seawater, Water Res [Internet] 198 (2021) 117123. https://linkinghub.elsevier.com/retrieve/pii/S0043135421003213.

[21] K. Leja, G. Lewandowicz, Polymer biodegradation and biodegradable polymers – a review, Pol. J. Environ. Stud. 19 (2010) 255–266.

[22] S. Philip, T. Keshavarz, I. Roy, Polyhydroxyalkanoates: biodegradable polymers with a range of applications, J. Chem. Technol. Biotechnol. 82 (2007) 233–247 [Internet], https://onlinelibrary.wiley.com/doi/10.1002/jctb.1667.

[23] A. Pospisilova, I. Novackova, R. Prikryl, Isolation of poly(3-hydroxybutyrate) from bacterial biomass using soap made of waste cooking oil, Bioresour. Technol. 326 (2021) 124683.

[24] L. Savenkova, Z. Gercberga, V. Nikolaeva, A. Dzene, I. Bibers, M. Kalnin, Mechanical properties and biodegradation characteristics of PHB-based films, Process Biochem. 35 (2000) 573–579.

[25] R. Turco, G. Santagata, I. Corrado, C. Pezzella, M. Di Serio, In vivo and post-synthesis strategies to enhance the properties of PHB-based materials: a review, Front Bioeng Biotechnol [Internet] 8 (2021). https://www.frontiersin.org/articles/10.3389/fbioe.2020.619266/full.

[26] J.C. Anderson, B.J. Park, V.P. Palace, Microplastics in aquatic environments: implications for Canadian ecosystems, Environ. Pollut. 218 (2016) 269–280.

[27] C. Schür, S. Zipp, T. Thalau, M. Wagner, Microplastics but not natural particles induce multigenerational effects in Daphnia magna, Environ. Pollut. 260 (2020) 113904.

[28] C.K. Frydkjær, N. Iversen, P. Roslev, Ingestion and egestion of microplastics by the cladoceran Daphnia magna: effects of regular and irregular shaped plastic and sorbed phenanthrene, Bull. Environ. Contam. Toxicol. 99 (2017) 655–661.

[29] C. Scherer, N. Brennholt, G. Reifferscheid, M. Wagner, Feeding type and development drive the ingestion of microplastics by freshwater invertebrates, Sci Rep [Internet] 7 (2017) 17006. Available from: https://www.nature.com/articles/s41598-017-17191-7.

[30] Y.S. Eltemsah, T. Bøhn, Acute and chronic effects of polystyrene microplastics on juvenile and adult Daphnia magna, Environ. Pollut. 254 (2019) 112919.

[31] O. Pikuda, E.R. Dumont, Q. Chen, J.-R. Macairan, S.A. Robinson, D. Berk, et al., Toxicity of microplastics and nanoplastics to Daphnia magna: current status, knowledge gaps and future directions, Trends in Analytical Chemistry [Internet] 167 (2023) 117208, https://doi.org/10.1016/j.trac.2023.117208 [cited 2023 Oct 9].

[32] A.J. Kokalj, U. Kunej, T. Skalar, Screening study of four environmentally relevant microplastic pollutants: uptake and effects on Daphnia magna and Artemia franciscana, Chemosphere 208 (2018) 522–529.

[33] A. Jemec, P. Horvat, U. Kunej, M. Bele, A. Kržan, Uptake and effects of microplastic textile fibers on freshwater crustacean Daphnia magna, Environ. Pollut. 219 (2016) 201–209.

[34] S. Rehse, W. Kloas, C. Zarfl, Short-term exposure with high concentrations of pristine microplastic particles leads to immobilisation of Daphnia magna, Chemosphere 153 (2016) 91–99.

[35] P.M. Canniff, T.C. Hoang, Microplastic ingestion by Daphnia magna and its enhancement on algal growth, Sci. Total Environ. 633 (2018) 500–507.

[36] G. Jaikumar, J. Baas, N.R. Brun, M.G. Vijver, T. Bosker, Acute sensitivity of three Cladoceran species to different types of microplastics in combination with thermal stress, Environ. Pollut. 239 (2018) 733–740.

[37] J. Colomer, M.F. Müller, A. Barcelona, T. Serra, Mediated food and hydrodynamics on the ingestion of microplastics by Daphnia magna, Environ. Pollut. 251 (2019) 434–441.

[38] S. Magester, A. Barcelona, J. Colomer, T. Serra, Vertical distribution of microplastics in water bodies causes sublethal effects and changes in Daphnia magna swimming behaviour, Ecotoxicol. Environ. Saf. 228 (2021) 113001.

[39] D.G. de Castro, A.L.F. Destro, E.C.L. Coimbra, A.L.L. da Silva, A.H. Mounteer, Effects of PET microplastics on the freshwater crustacean Daphnia similis Claus, Acta Limnologica Brasiliensia [Internet] 2023 (35) (1976). Available from: http://www.scielo.br/scielo.php?script=sci_arttext&pid=S2179-975X2023000100706&tlng=en.

[40] G. Jaikumar, N.R. Brun, M.G. Vijver, T. Bosker, Reproductive toxicity of primary and secondary microplastics to three cladocerans during chronic exposure, Environ. Pollut. 249 (2019) 638–646.

[41] B. De Felice, V. Sabatini, S. Antenucci, G. Gattoni, N. Santo, R. Bacchetta, et al., Polystyrene microplastics ingestion induced behavioral effects to the cladoceran Daphnia magna, Chemosphere 231 (2019) 423–431.

[42] T. Bosker, G. Olthof, M.G. Vijver, J. Baas, S.H. Barmentlo, Significant decline of Daphnia magna population biomass due to microplastic exposure, Environ. Pollut. 250 (2019) 669–675.

[43] K. Savva, M. Farré, C. Barata, Sublethal effects of bio-plastic microparticles and their components on the behaviour of Daphnia magna, Environ Res [Internet] 236 (2023) 116775. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0013935123015797.

[44] L. Zimmermann, S. Göttlich, J. Oehlmann, M. Wagner, C. Völker, What are the drivers of microplastic toxicity? Comparing the toxicity of plastic chemicals and particles to Daphnia magna, Environ. Pollut. 267 (2020) 115392.

[45] P. Procházková, S. Mácová, S. Aydın, H. Zlámalová Gargošová, G. Kalčíková, J. Kučerík, Effects of biodegradable P3HB on the specific growth rate, root length and chlorophyll content of duckweed, Lemna minor, Heliyon [Internet] 9 (2023) e23128. Available from: https://linkinghub.elsevier.com/retrieve/pii/S2405844023103367.

[46] ASTM, E729-96 Standard Guide for Conducting Acute Toxicity Tests on Test Materials with Fishes, Macroinvertebrates, and Amphibians, 2007.

[47] OECD, Test No. 202: Daphnia Sp. Acute Immobilisation Test [Internet], OECD, 2004. Available from: https://www.oecd-ilibrary.org/environment/test-no-202-daphnia-sp-acute-immobilisation-test_9789264069947-en.

[48] R.R. Rosenfeldt, F. Seitz, R. Schulz, M. Bundschuh, Heavy metal uptake and toxicity in the presence of titanium dioxide nanoparticles: a factorial approach using Daphnia magna, Environ. Sci. Technol. 48 (2014) 6965–6972.

[49] OECD, Test No. 211: Daphnia Magna Reproduction Test [Internet], OECD, 2012. Available from: https://www.oecd-ilibrary.org/environment/test-no-211-daphnia-magna-reproduction-test_9789264185203-en.

[50] P. Karatzas, G. Melagraki, L.A. Ellis, I. Lynch, D. Varsou, A. Afantitis, et al., Development of deep learning models for predicting the effects of exposure to engineered nanomaterials on Daphnia magna, Small [Internet] 16 (2020) [cited 2024 Jan 2], https://onlinelibrary.wiley.com/doi/10.1002/smll.202001080.

[51] L. Guilhermino, A. Martins, S. Cunha, J.O. Fernandes, Long-term adverse effects of microplastics on Daphnia magna reproduction and population growth rate at increased water temperature and light intensity: combined effects of stressors and interactions, Sci. Total Environ. [Internet] 784 (2021) 147082 [cited 2023 Dec 26], https://linkinghub.elsevier.com/retrieve/pii/S0048969721021525.

[52] P. Procházková, G. Kalčíková, E. Maršálková, H. Zlámalová Gargošová, J. Kučerík, Innovative approach for quantitative determination of ingested microplastics by Daphnia magna: use of differential scanning calorimetry and thermogravimetry, J. Therm. Anal. Calorim. (2024). https://doi.org/10.1007/s10973-024-12985-0.

[53] M. González-Pleiter, M. Tamayo-Belda, G. Pulido-Reyes, G. Amariei, F. Leganés, R. Rosal, et al., Secondary nanoplastics released from a biodegradable microplastic severely impact freshwater environments, Environ. Sci.: Nano 6 (2019) 1382–1392.
[54] E.S. Okeke, T.P.C. Ezeorba, Y. Chen, G. Mao, W. Feng, X. Wu, Ecotoxicological and health implications of microplastic-associated biofilms: a recent review and prospect for turning the hazards into benefits, Environ. Sci. Pollut. Res. Int. 29 (2022) 70611–70634 [cited 2024 Jan 2], https://link.springer.com/10.1007/s11356-022-22612-w.
[55] Z.L.R. Botterell, N. Beaumont, M. Cole, F.E. Hopkins, M. Steinke, R.C. Thompson, et al., Bioavailability of microplastics to marine zooplankton: effect of shape and infochemicals, Environ Sci Technol [Internet] 54 (2020) 12024–12033 [cited 2023 Dec 28], https://pubs.acs.org/doi/10.1021/acs.est.0c02715.
[56] P. Fan, H. Yu, B. Xi, W. Tan, A review on the occurrence and influence of biodegradable microplastics in soil ecosystems: are biodegradable plastics substitute or threat? Environ Int [Internet] 163 (2022) 107244 [cited 2023 Sep 26], https://linkinghub.elsevier.com/retrieve/pii/S0160412022001702.
[57] C.D. Rummel, A. Jahnke, E. Gorokhova, D. Kühnel, M. Schmitt-Jansen, Impacts of biofilm formation on the fate and potential effects of microplastic in the aquatic environment, Environ Sci Technol Lett [Internet] 4 (2017) 258–267 [cited 2023 Dec 28], https://pubs.acs.org/doi/10.1021/acs.estlett.7b00164.
[58] M. Trojan, M. Koutný, M. Brtnický, J. Holátko, H. Zlámalová Gargošová, J. Fojt, et al., The interaction of microplastics and microbioplastics with soil and a comparison of their potential to spread pathogens, Appl. Sci. 14 (2024) 4643.