Ingestion of polystyrene microparticles impairs survival and defecation in larvae of Polistes satan (Hymenoptera: Vespidae)

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Abstract

Microplastics (MPs) are widespread pollutants of emerging concern, and the risks associated with their ingestion have been reported in many organisms. Terrestrial environments can be contaminated with MPs, and terrestrial organisms, including arthropods, are predisposed to the risk of ingesting MPs. In the current study, the larvae of the paper wasp *Polistes satan* were fed two different doses (6 mg or 16 mg at once) of polystyrene MPs (1.43 mm maximum length), and the effects of these treatments on immature development and survival till adult emergence were studied. Ingestion of the two doses resulted in mortality due to impaired defecation prior to pupation. The survival of larvae that ingested 16 mg of MPs was significantly lower than that of the control. The ingestion of 16 mg of MPs also reduced the adult emergence (11.4%) in comparison to the control (44.4%). MPs were not transferred from the larvae to the adults that survived. These findings demonstrate that MP ingestion can be detrimental to *P. satan*, e.g. larval mortality can decrease colony productivity and thus the worker force, and that MPs can potentially affect natural enemies that occur in crops, such as predatory social wasps.

1- Introduction

Plastic pollution is a global concern and has been detected in marine, freshwater, and terrestrial ecosystems (Lusher, 2015; Lusher et al., 2015; Li et al., 2018; Choi et al., 2021). Agricultural, urban and industrial runoff, landfills, and littering are examples of plastic waste sources (Cozar et al., 2014; Hamid et al., 2018; Machado et al., 2018; Xu et al., 2020). Biotic and abiotic processes cause the fragmentation of environmental plastic waste (GESAMP, 2015; Auta et al., 2018; Hamid et al., 2018). These small fragments are known as microplastics (MPs), defined by Frias and Nash (2019) as "any synthetic solid particle or polymeric matrix, with regular or irregular shapes, and sizes ranging from 1 µm to 5 mm, of either primary or secondary manufacturing origin, which are insoluble in water." Thus, MPs occur in various shapes, such as spheres, fibers, pellets, and beads; they also vary in chemical composition, including polyester, polystyrene, polyethylene, polypropylene, and polyvinyl chloride particles (Ng et al., 2018). Some of these plastics originate from petroleum, including polystyrene, which is often found as expanded polystyrene, popularly known as Styrofoam (Ho et al., 2018). Its lifespan is indefinite, suggesting its potential to accumulate in the environment.

Current data suggest that MP concentrations in freshwater sediments exceed those in marine systems (Obbard et al., 2014; Eerkes-Medrano et al., 2015; Tubau et al., 2015; Yang et al., 2021; Jiang et al., 2022). In these ecosystems, MP contamination has been reported in biota at different trophic levels (Auta et al., 2017; Brookson et al., 2019). Studies on the presence of MPs in the biota of terrestrial ecosystems are limited, despite terrestrial environments being a substantial source of MPs (Jambeck et al., 2015; Machado et al., 2018; D'Souza et al., 2020). Terrestrial ecosystems, for example agricultural soils, can contain more MPs than oceanic basins (Nizzetto et al., 2016; Xu et al., 2020). Therefore, like aquatic organisms, terrestrial organisms can be exposed to and even ingest MPs.

Numerous organisms, including invertebrates, ingest MPs that can pass through the digestive tract without detectable effects (Ugolini et al., 2013; Hamer et al., 2014; Kaposi et al., 2014; Santana et al., 2018; Weber et al., 2018); but damage to organs, tissues, and cells, or changes in behavior, often causing mortality, has also been reported (Blarer and Burkhardt-Holm, 2016; Sussarellu et al., 2016; Alomar et al., 2017; Jin et al., 2018; Lo and Chan, 2018). The ingestion of MPs by earthworms (Lwanga et al., 2016; Jiang et al., 2020; Lahive et al., 2019), nematodes (Lei et al., 2018a, b), fruit flies (Demir et al., 2021), and honeybees (Deng et al., 2021; Wang et al., 2021; Balzani et al., 2022) can be lethal. Instead, ingesting MPs did not appear to cause mortality in snails (Song et al., 2019), nor silkworms (Muhammad et al., 2021). The paucity of data and lack of knowledge concerning the interaction between MPs and terrestrial species is surprising, especially for economically important taxa, including wasps.

Social wasps (Hymenoptera: Vespidae) are effective biological control agents (Southon et al., 2019), and prey on a variety of agricultural and urban pests (Prezoto et al., 2019). During foraging, adults interact with the air, water basins, soil, plants, and other animals (Richards, 2000). Contamination by MPs has been demonstrated in many of these locations and resources (Al-Jaibachi et al., 2018; Wen et al. 2018; Azeem et al., 2021), which are used by wasps for nest construction or feeding (Richards, 2000), such as mood, water, nectar, and insect prey. The detection of pollutants in the larval fecal masses suggests an oral exposure route, in which adult wasps would prey on contaminated food, then offer it to larvae (Urbini et al., 2006). Moreover, agricultural areas where social wasps are particularly common (Barbosa et al., 2016), are among the most MP-contaminated terrestrial environments (Nget al., 2018). Therefore, social wasps may be exposed to MPs.

Oral exposure to MPs may pose consequences to wasps. The lumens of the midgut and hindgut are not connected in the larvae of most Apocritan Hymenoptera (many wasps, bees, and ants) (Peters, 2012). Consequently, during larval development, the midgut accumulates waste, such as indigested particles from preys. Prior to metamorphosis, the lumens of the midgut and hindgut become connected, and this accumulated material or meconium (enclosed by the peritrophic matrix) is expelled from the body (Spradbery, 1973; Kojima, 1996). This defecation helps the larvae eliminate the pollutants ingested. Alternatively, it could represent a critical period during which these insects are more vulnerable to detrimental effects associated with the ingestion of plastics (Murray and Cowie, 2011; Wright et al., 2013; Puskik et al., 2020).

In this work, the effects of ingesting polystyrene MPs on the development of the social wasp *Polistes satan* were investigated. The following hypotheses were tested: (1) larvae ingest food with polystyrene MPs; (2) wasp mortality at the immature stage increases with MP ingestion; and (3) larval defecation is impaired by MP ingestion. To this end, a laboratory bioassay was performed to compare food ingestion, survival, and defecation between larvae that ingested food contaminated with high doses of polystyrene MPs (acute oral exposure) and larvae that ingested food without MPs (control).

2- Methods

2.1- Microplastics

Polystyrene granules (Sigma-Aldrich, Saint Louis, MO, USA) were ground in an ordinary blender. The granules were crushed in order to obtain the smallest possible particles in the form of powder stuck to the wall of the blender jar. This powder was removed with the help of a brush and stored. For characterization, microparticles were stuck to double-sided adhesive tape previously mounted on aluminum blocks (stubs), metallized with gold in a sputtering device, and observed under a scanning electron microscope (SEM VP1430 LEO, LEO Electron Microscopy Ltd., Cambridge, UK Kingdom) at the Centro de Microscopia e Microanálise da Universidade Federal de Viçosa (NMM-UFV).

2.2- Wasps

In November 2020, combs from 18 colonies of *P. satan* were collected from the roof of a house on a coffee farm in the municipality of Alpinópolis, Minas Gerais state, southeast Brazil (-20° 51' 49.00" S, -46° 23' 17.02" W). In this species, a single colony can contain multiple combs, each of which is independently attached to the substrate by a peduncle (Supplementary Material S1), thus allowing the collection of immature wasps while keeping most of the colony intact. Forceps were used to collect one comb full of late-instar larvae from each colony (Supplementary material S1). These combs were individually kept in plastic cages of 1 L and transported in the dark within a thermal bag to the Laboratório de Abelhas e Vespas (LAV) at the Universidade Federal de Viçosa. The day after, last-instar larvae (5th instar) were carefully removed from the nest cell with forceps, sized by estimating the maximum head width (Eickworth, 1969), then individualized in a well of a 96-well-plate (Fig. 1), which was then kept in a plastic tray within an incubator at 28 ± 1^{O}C, RH 70 ± 5% in the dark. Our sample comprised 99 larvae (3–12 individuals per colony; 18 colonies).

2.3- Bioassay

Two days after transference to the well-plates, the larvae were provided with food containing MPs (1.43 mm maximum length) (supplementary material S1) over a single feeding event at 8 A.M. To the best of our knowledge, there were no publications on the ingestion of MPs by wasps that we could refer to. Each larva was allowed to ingest for five minutes a mashed *T. molitor* pupa mixed with 6 mg of MPs (lowest dose) or 16 mg of MPs (highest dose), weighed on a precision scale. The particles were added to mashed prey body, simulating ingestion of MPs via the food web, therefore allowing us to test potential oral exposure risk. Attempting to add the particles into liquid food (sucrose solution or water) would be difficult because the particles are not soluble in water. In the control group, mashed *T. molitor* pupae without MPs were added to larvae. Afterwards, the larvae (treated or control) received food without MPs until they started cocoon weaving.

When the larvae started cocoon weaving, they were transferred individually to 2 mL-microcentrifuge tubes (Supplementary material S1); the tube opening was plugged with cotton to allow them to develop (Supplementary material S1). The survival of individuals was checked every day for up to 40 days, from the beginning of cocoon weaving until adult emergence. The larvae that died before the start of cocoon weaving (N = 2) were not counted. Thus, our final sample for the bioassay comprised 97 individuals, distributed as follows: 34 and 27 individuals fed 6 mg and 16 mg of MPs respectively, and 36 individuals not fed MPs (control). It was registered whether or not each larvae ate the *T. molitor* prey (with or without MPs) at the beginning of the treatment. The larvae were inspected daily to check for the presence of regurgitated MPs. Survival was assessed once per day, from the beginning of larval cocoon weaving until adult emergence or immature death. Individuals were considered dead if they did not move in response to the mechanical stimulation. Deaths were recorded before, during, and after defecation (Fig. 2). The number of adults that emerged was recorded. Dead, immature or emerged adults were individually stored in ethanol 70%, and then dissected to track the location of MPs inside the gut or in the abdominal caveat.

2.4- Statistical Analysis

To test if larvae ingest food with MPs, the proportion of wasps that ate all the *T. molitor* prey (with or without MPs) in each treatment (0, 6 and 16mg MPs) at the beginning of the treatment was counted.

To test whether MP ingestion causes mortality at the immature stage, survival curves were obtained with Kaplan-Meier estimators and were first analyzed using the log-rank test. Subsequently, pairwise comparisons were performed using the Bonferroni's adjustment.

To further investigate whether MP ingestion causes mortality at the immature stage, it was examined whether MP ingestion by wasp larvae affected the probability of reaching adulthood by fitting binomial generalized linear models. Whether an individual reached adulthood was considered the response variable, diet treatment a fixed effect, the wasp nest origin a random effect, and the maximum head width of the larvae as a covariate. The random effect resulted in a singular fit with forced us to discard it from the model, and then the covariate was tested by the chi-squared test in a reduced model (without the random effect). Finally, since the covariate was not significant ($P > 0.05$), estimates were obtained from the simplest model (without both random and covariate effects) and compared using Tukey's test.

To test whether impaired defecation at the immature stage was a consequence of ingesting MPs, the effect of diet treatments on the probability of immature wasps dying before, during, and after defecation was examined. To this end, binomial generalized linear models were fitted. Firstly, the data from (only) dead larvae were binarized into "before" or "not before," "during" or "not during," and "after" or "not after" the defecation stage to create three different binomial variables. In each model, one of these three variables was entered as the response variable, and diet treatment was entered as a fixed effect. In these studies, random effect (nest origin) was not tested since it also showed singularity fits and covariate effect (maximum head width of the larvae) was discarded because it did not show any improvement of the models ($P > 0.05$).

All data were analyzed using R software (R Core Team, 2020) with a significance level of $P < 0.05$.

3- Results

P. satan larvae ingested food containing MPs. All larvae (N = 97) were fed *T. molitor*, regardless of the presence or concentration of MPs. After MP ingestion, *T. molitor* were not detected on the head of the larvae.

The ingestion of MPs killed the immature larvae. The overall log-rank test indicated a significant survival difference among the treatments (χ^2 = 9.8, df = 2, *P* = 0.007, Fig. 3A). The survival curve of larvae that ingested the highest dose (16 mg) of MPs differed from that of the control (χ^2 = 8.14, df = 1, *P* = 0.013). The survival curve of the larvae treated with the lowest dose (6 mg) of MPs did not differ from that of the larvae treated with the highest dose of MPs (χ^2 = 2.75, df = 1, *P* = 0.291) or the control (χ^2 = 3.54, df = 1, *P* = 0.18).

Ingestion of MPs by larvae also affected the probability of reaching adulthood. In the binomial generalized linear models, the inclusion of wasp nest origin as a random effect was discarded due to a singular fit, as was the inclusion of head size as a covariate (larval head width: mean = 2.99 mm; standard deviation = 0.18; range = 2.64–3.5 mm) due to its non-significance (χ^2= 0.1034, df = 1, *P* = 0.7478). Therefore, a pairwise comparison between treatments was carried out with estimates obtained from the most parsimonious and simple model, that is, that with only the fixed effect (treatments and control), as shown in Fig. 3B. Reaching adulthood was more frequent in the control (44.4%), followed by larvae treated with the lowest (20.6%) and highest concentration of MPs (11.1%), respectively. The only statistically significant difference in the probability of reaching adulthood occurred between the control and larvae treated with the highest concentration of MP (z = -2.659, P = 0.021).

Ingestion of MPs impaired defecation at the larval/pupal transition. As is shown in Fig. 4, most deaths (> 60%) occurred after defecation and only a few deaths (< 5%) occurred before defecation. Deaths during the defecation only occurred in the larvae treated with MPs, corresponding to 29.60% and 33.33% of deaths in larvae treated with the lowest and highest doses of MPs, respectively. The treatments did not affect the probability of dying before defecation (χ^2= 1.3532, df = 2, *P* = 0.5083), but did affect the probability of dying during defecation (χ^2= 12.403, df = 2, *P* = 0.002). The treatments also increased the probability of death after defecation (χ^2= 14.269, df = 2, *P* = 0.0008), which was likely a consequence of the many deaths of treated larvae during defecation.

The ingested MPs were only found in the endoperitrophic space or food bolus (Supplementary Material S1). In larvae that died before defecation, MPs were homogeneously distributed along the lumen of the midgut. Instead, the peritrophic matrix was partially egested in the larvae that died during defecation. In such cases, the MPs were concentrated at the end of the larval body, near the anus, thereby preventing the luminal content or meconium from being completely egested, resulting in anomalous defecation. MPs can always be recovered from the meconium in individuals that defecate successfully. MPs were not detected in adults derived from larvae that had ingested these particles.

4- Discussion

In this study, it was demonstrated that *P. satan* larvae ingested food containing high doses of MPs. Therefore, ingestion is a likely exposure route for MPs in the social wasp larvae. Some animals can recognize and avoid ingesting MPs (Ryan et al., 2019; Xu et al., 2022), while others regurgitate MPs after ingesting them (Saborowski et al., 2019). None of these behaviors were detected in *P. satan* larvae, because all the larvae consumed *T. molitor* prey regardless of the presence or dose of MPs, and MPs were never found over the larval head or around the larval body after MP ingestion. In our experiment, the contaminated prey was offered to the larvae for five minutes, so they had the opportunity to ingest it or not. The exposed larvae fully ingested the contaminated prey just as they ingested prey without MPs before and after exposure, and just as control larvae ingested prey without MPs. Other insect larvae, including mealworms (Wu et al. 2019), silkworms (Muhammad et al., 2021), and fruit flies (Demir et al. 2022), are capable of ingesting MPs and they have been successfully used in experiments with these pollutants.

Our bioassay simulated a likely contamination scenario. Under field conditions, social wasp larvae are fed by foragers and cannot choose the quality or quantity of food they will receive, but they can choose whether or not to ingest. The absence of food avoidance and regurgitation related to MP ingestion indicated that the risk of exposure of social wasp larvae to MPs in realistic scenarios cannot be neglected.

The use of high dosages (6 or 16 mg of MPs) in our assay is in line with previous studies with aquatic organisms (Au et al. 2015; Ogonowski et al. 2016; Watts et al. 2016; Yin et al. 2018) and may be useful to determine a potential tipping point for harmful effects of MPs in *P. satan* (Cunningham and Sigwart 2019). The ingestion of 6 mg or 16 mg of MPs by wasp larvae simulate a heavily polluted environment. This worst-case scenario, however, can be supported by recent studies showing that other hymenopterans, like solitary bees, can build nests partially (MacIvor and Moore, 2013; Wilson et al., 2020; Quintos-Andrades et al., 2021) or even fully (Allasino et al., 2019) using plastic collected in landscapes. Finally, the ingestion of high doses of MPs by *P. satan* emphasizes the ability of wasp larvae to ingest MPs and the negative effects associated with their ingestion, thereby constituting an appropriate approach for the first screening of the potential effect of this contaminant in social wasps.

The ingestion of polystyrene MPs caused mortality at the immature stage and also decreased the probability of reaching adulthood, at least in wasps that were fed the highest dose of MPs, thus confirming the detrimental effect of this contaminant. Ingestion of MPs by other terrestrial (silkworms: Muhammad et al., 2021) and marine (sea urchins: Kaposi et al., 2014; slipper snails: Lo and Chan, 2018; decapod crustaceans: Cole et al., 2013) larvae did not appear to be lethal. The type, size, shape, and dose of ingested MPs could explain the different results regarding the mortality of invertebrate larvae that ingested MPs. Future studies with different taxa are important to better understand which species are more or less sensitive to MPs.

In this study, mortality during defecation only occurred in larvae that ingested MPs, confirming that impaired defecation is a consequence of ingesting MPs. The time of larval defecation seems to represent a critical period in which social wasp larvae could be more vulnerable to detrimental effects associated with MP ingestion. Impaired defecation after plastic ingestion has been demonstrated in lobsters (Murray and Cowie, 2011), marine worms (Wright et al., 2013), and vertebrates (Puskik et al., 2020). Therefore, this seems to be a common effect across animal taxa.

Here, a successful larval-rearing protocol for social wasps was described. To our knowledge, the only previous test was performed almost a century ago by Gaul (1940), who reported the successful rearing of *P. fuscatus* from eggs to adults. Our protocol can be used or adapted to test the lethal and sublethal effects of ingestion of different pollutants across wasp development.

5- Conclusions

The ingestion of polystyrene MPs during post-embryonic development is detrimental to paper wasps, at least under high experimental dosages. Furthermore, a protocol for the *in vitro* larval rearing of these predators was described and successfully used to test the effects of MP ingestion on wasps. The risk of ingesting MPs by *P. satan* larvae cannot be neglected, as they did not avoid or regurgitate food containing MPs, which caused mortality due to impaired defecation. Thus, during the larval period, individuals are vulnerable to particulate anthropogenic pollutants. Massive defecation by larvae is an evolutionarily conserved trait among most Apocritan Hymenoptera, and potentially, ingesting MPs can also be detrimental in ants and bees.

Declarations

Ethical Approval

Not applicable.

Consent to Participate

Not applicable.

Consent to Publish

All authors consent to publish this manuscript.

Availability of data and materials

All data will be made available on request.

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Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

Author Contributions

Conceptualization: Gustavo Ferreira Martins; writing the original draft: all authors; data curation: André Rodrigues de Souza; funding acquisition: André Rodrigues de Souza, Maria Augusta P. Lima, Fábio Santos do nascimento and Gustavo Ferreira Martins; investigation: André Rodrigues de Souza, Thaís Andrade Viana and Gustavo Ferreira Martins; statistical analyses: Rodrigo Cupertino Bernardes and Wagner Faria Barbosa

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Figures

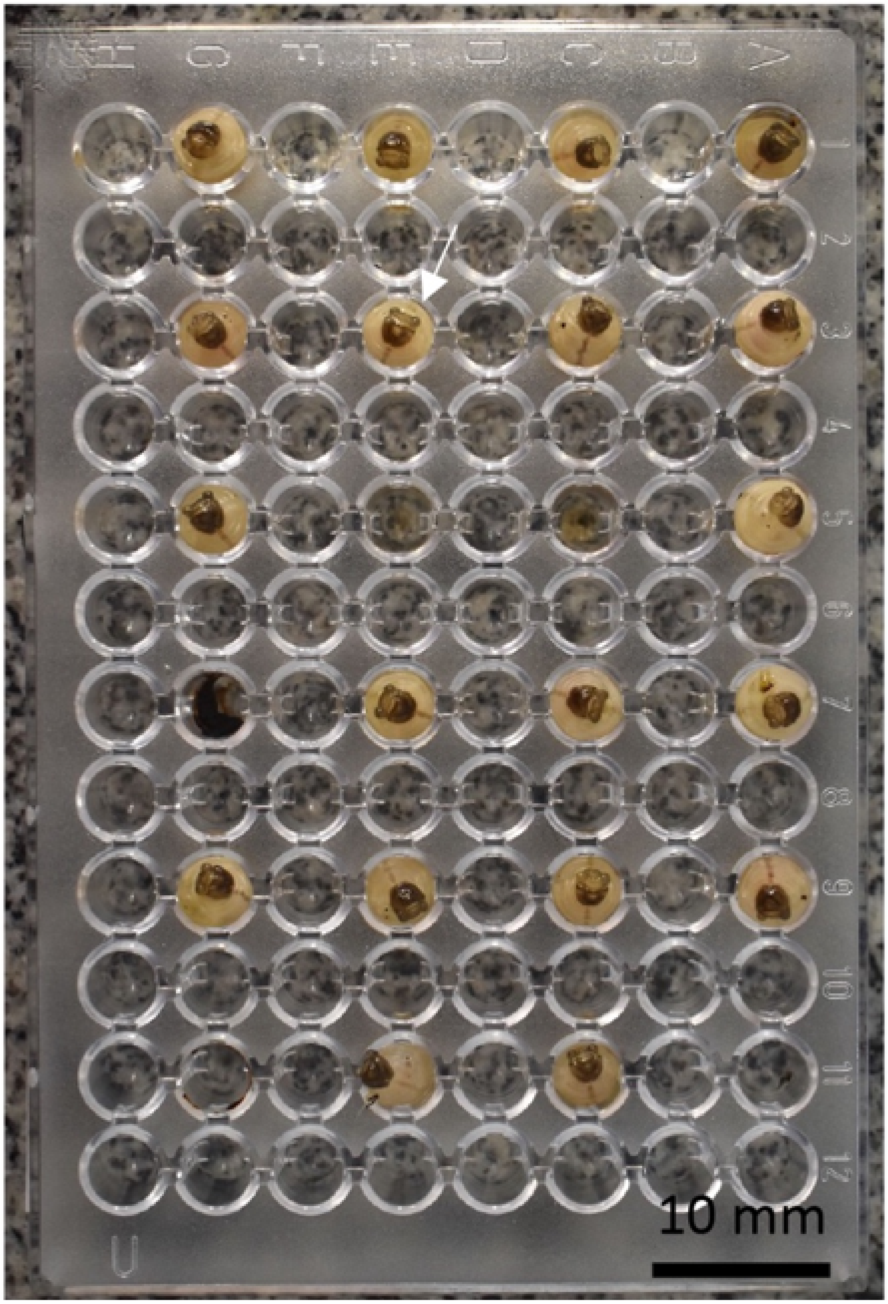

Figure 1

Polistes satan larvae in the wells of a 96-well-plate. Right after transference to the well-plates, the larvae were hand-fed (Supplementary Material S1) from 8 A.M to 6 P.M with different food sources alternately every two hours, six times per day. This procedure simulates the progressive provisioning of larvae by adult wasps under field conditions. In each feeding event, it was offered an 8 μL drop of sugar:water solution (1V:1V), or a 2 μL drop of water (both offered with a micropipette), or a mashed *Tenebrio molitor* (Coleoptera:Tenebrionidae) pupae (offered with forceps) from a laboratory population reared on wheat bran and pieces of sugarcane.

Figure 2

A *Polistes satan* larva that successfully defecated. Note the relatively large and dark meconium (M).

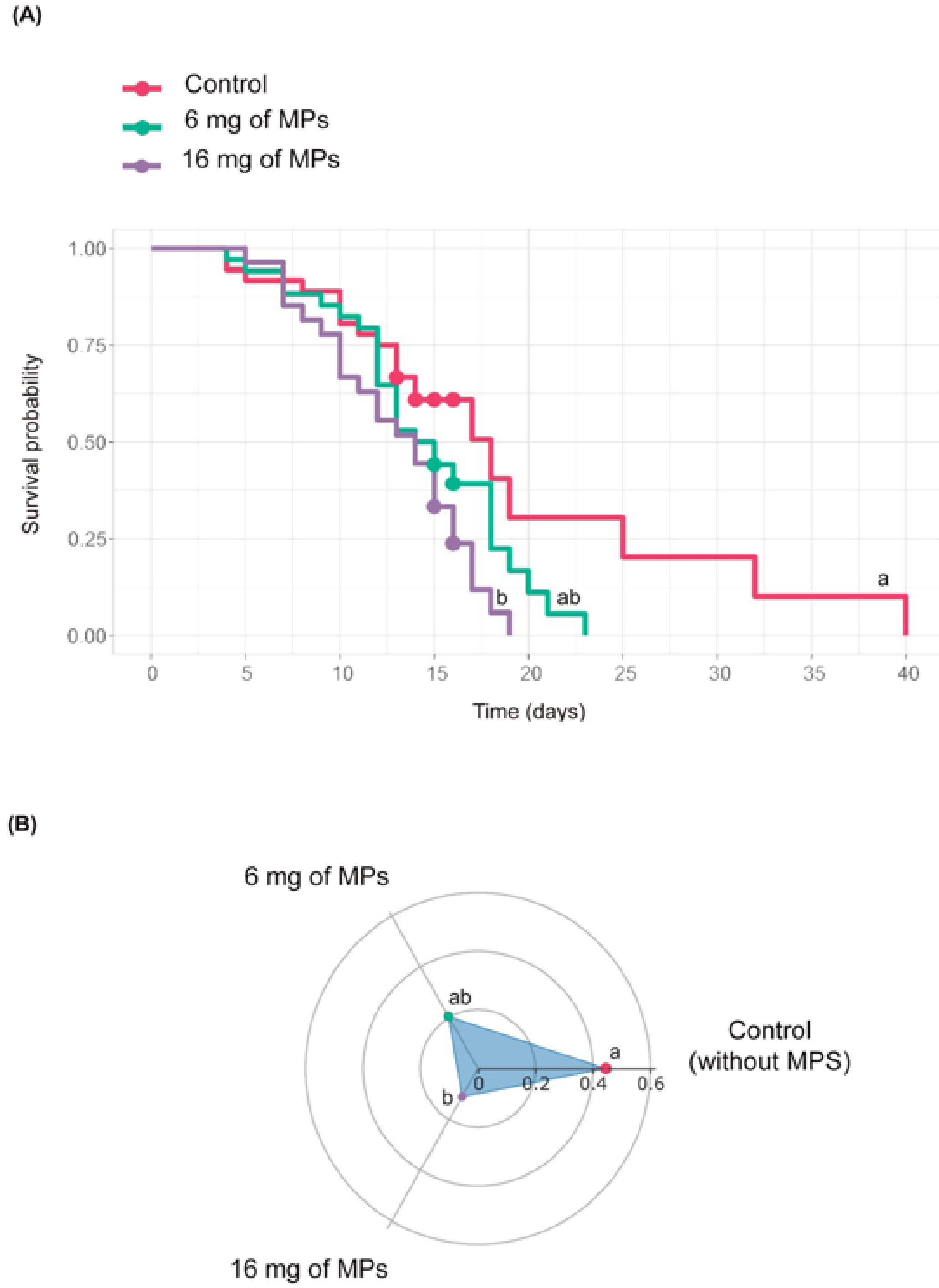

Figure 3

Survival curves (A) and probability to reach adulthood (B) in *Polistes satan* immature that ingested food with two different doses of MPs and the control (without MPs). In (A), the individuals that reached the adulthood were treated as censored data and were indicated by closed circles in the lines. In both (A) and (B), different letters (a,b) indicate significant differences ($P < 0.05$) among treatments.

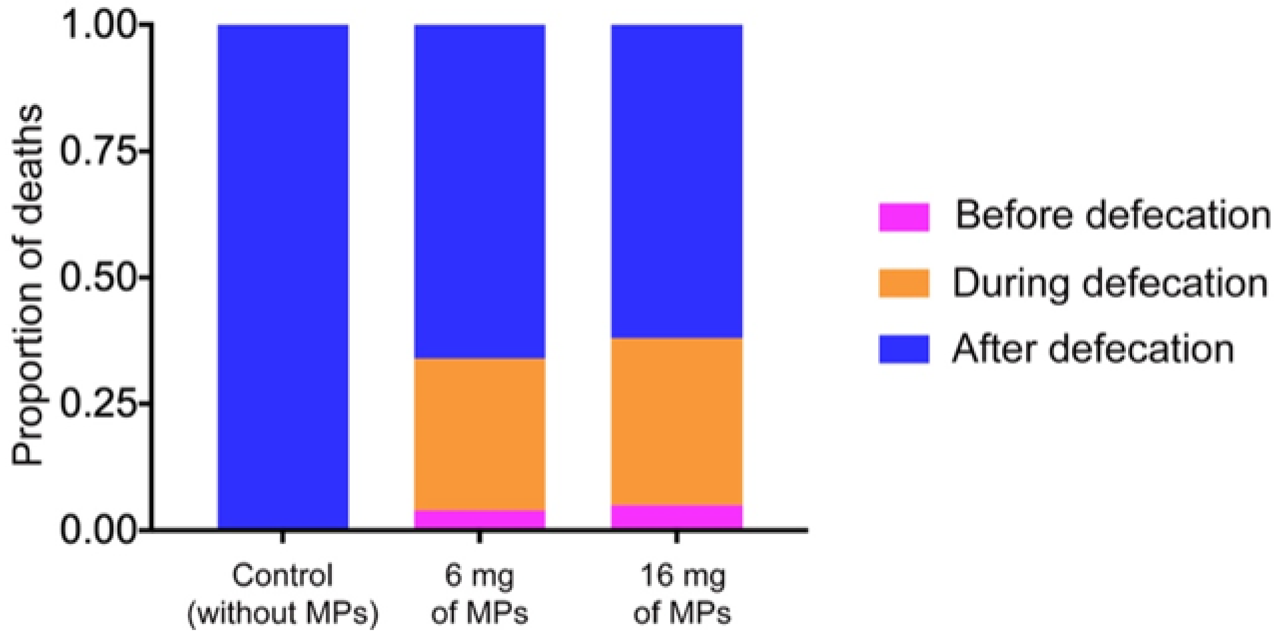

Figure 4

Proportion of deaths during the development of *Polistes satan* immature that ingested food with two different doses of MPs and the control (without MPs).

Supplementary Files

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