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Articles

Effect of different types of microplastics on the vital activity of bloodsucking mosquitoes Aedes aegypti L. (Diptera: Culicidae)

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We have experimentally investigated the effect of three different types of microplastics (high-density polyethylene, polypropylene and polystyrene) on body weight, metamorphosis rate and mortality of bloodsucking mosquitoes *Aedes aegypti* (Linnaeus 1762), vectors of protozoal and helminthic diseases of humans and animals. Supplementation of the diet with polypropylene was found to have no effect on mosquito weight at all life stages, while the addition of high-density polyethylene and polystyrene promoted a decrease in larval weight and an increase in adult weight ($p < 0.05$). Ingestion of high-density polyethylene by larvae increased pupal weight and decreased adult weight compared to the control, whereas no such effect was found for polypropylene and polystyrene. High-density polyethylene and polystyrene did not affect mosquito mortality at all stages, but there was a tendency for polypropylene to have an adverse effect on pupal and adult survival. The survival rate of mosquitoes at all life stages in both the control and experimental groups was generally quite high. Supplementation of the diet with different types of plastics did not affect the metamorphosis rate at all stages of mosquito development and was comparable in both the control and experimental groups. The experiments revealed no significant effect of different types of plastics on the vital activity of *Ae. aegypti*. Only high-density polyethylene microparticles were found to significantly affect mosquito body weight, yet this was opposite at the pupal and adult stages.

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Keywords

Aedes aegypti, microplastics, fragments, high-density polyethylene, polypropylene, polystyrene, physiology of mosquitoes

Introduction

Microplastic pollution is becoming an increasingly prevalent challenge. Microplastics (MPs) are ubiquitous in both aquatic and terrestrial ecosystems (Ershova et al. 2022; Surendran et al. 2023; Takahito et al. 2023). Microplastic pollution is undoubtedly an environmental issue of global concern (Bhardwaj et al. 2024).

MPs are man-made polymer particles with a size range between 1 µm to 5 mm at its greatest length (Frias and Nash 2019). MPs are formed as a result of gradual degradation of plastic materials by physicochemical factors (Helmberger et al. 2020), or are released into the environment as initially synthesized small diameter particles (Horton et al. 2017). At present, a large number of different synthetic polymers are being produced worldwide. The most common of these are polyethylene terephthalate (PET), high-density polyethylene (HDPE), polyvinyl chloride (PVC), polystyrene (PS), and polypropylene (PP) (Chamas et al. 2020). Almost all of these compounds can be found in waste streams released into the environment.

Plastic polymers are biochemically inert, but various components such as heat stabilizers, flame retardants, antioxidants, and plasticizers such as bisphenol A (BPA), which are added to plastic products to give them additional marketable properties, pose a greater threat. These components are of low molecular weight and can migrate into the environment or living organisms, causing adverse effects (Romera-Castillo et al. 2018; Liu et al. 2020). Another challenge is the transport of persistent organic compounds, pathogens and other infectious agents in the environment by MPs (Li et al. 2018; Miloloža et al. 2021; Gayalarde et al. 2023).

Due to their small size and low density, MPs are bioavailable to many living organisms in marine, freshwater and terrestrial ecosystems, e.g. fish, crabs, mussels, molluscs, and terrestrial mammals including humans (Watts et al. 2014; Li et al. 2016; Naji et al. 2018; Zhu et al. 2019; Thrift et al. 2022; Leslie et al. 2022). Recent evidence shows that MPs are transferred up the food web via predator-prey interactions, from the copepod *Eurytemora affinis* (Poppe, 1880) to the mysid shrimp *Neomysis integer* (Leach, 1815) (Setälä et al. 2014), and from larvae of *Culex pipiens* mosquito (Linnaeus, 1758) to *Chaoborus flavicans* midge (Meigen, 1830) (Cuthbert et al. 2019). In addition, MPs can be transmitted ontogenetically from larvae to adults by insects that migrate from aquatic to terrestrial habitats during their life cycle. This was reported for mosquitoes of the genera *Culex*and *Aedes*(Al-Jaibachi et al. 2018, 2019; Simakova et al. 2022; Griffin et al. 2023) and for *Chironomus riparius* (Meigen, 1804) (Setyorini et al. 2021).

Bloodsucking mosquitoes of the family Culicidae play a key role in trophic chains. They are an important food source for many animal species. In addition, they participate in filtering organic particles and thus can be used as bioindicators of water quality (Yee and Kaufman 2019). Therefore, the study of mosquitoes in terms of microplastic pollution is important to understand the MP effect on their physiology and ecosystems as a whole.

To date, several published papers mainly address the effect of spherical PS on bloodsucking mosquitoes, and only one paper reports the effects of PE (Al-Jaibachi et al. 2018, 2019; Simakova et al. 2022; Griffin et al. 2023). The investigation of other types of plastics therefore requires further experiments, since no sufficient information is available on the effects of certain types of polymeric materials of different shapes on the physiology of living organisms, including mosquitoes.

The aim of the study was to evaluate the effect of the most common types of plastics, namely PP, PS and HDPE fragments of irregular shape, on physiological parameters including weight, mortality and metamorphosis rate in bloodsucking mosquitoes *Aedes aegypti* (Linnaeus 1762) as model organisms.

Materials and methods

Maintenance of mosquitoes

The *Aedes aegypti* mosquito colony is continuously maintained in the Laboratory of Evolutionary Cytogenetics of Tomsk State University. The larvae are kept in dechlorinated tap water. The larval diet consists of dried bovine liver powder with powdered dried leaves of *Urtica dioica*. The colony is maintained in the laboratory at a temperature of 25 ± 2 °C, relative humidity of 70 ± 5 %, and 16-hour light/8-hour dark photoperiod.

Preparation and characterization of MPs

Plastic particles were obtained by mechanical grinding of pre-frozen (–22 °C) commercial plastics of an appropriate type (PP, PS and HDPE). Micronized particles were obtained in the form of irregularly shaped fragments (Fig. 1). The mean particle size (± SE) along the maximum axis derived from measurement of 500 random HDPE particles was 174 ± 9.96 µm, with 70.4% of particles falling into size categories available for uptake by III instar mosquito larvae (< 200 µm). The mean sizes of PP and PS were 446 ± 23.3 and 63.1 ± 3.24 µm, respectively. For PP, the proportion of particles < 200 µm was the lowest (36.0%), whereas 93.8% of PS fragments showed sizes available for ingestion by mosquito larvae (Fig. 1).

To verify the polymer composition, spectroscopic analysis of the obtained preparations was performed by Raman spectroscopy. Raman spectra were obtained using a continuous semiconductor laser (wavelength 785 nm, power 100 mW) on an InVia Basic confocal dispersive spectrometer (Renishaw, UK). The spectra were measured in the range of 100–1800 cm^{-1}. The spectral resolution was 1 cm^{-1}.

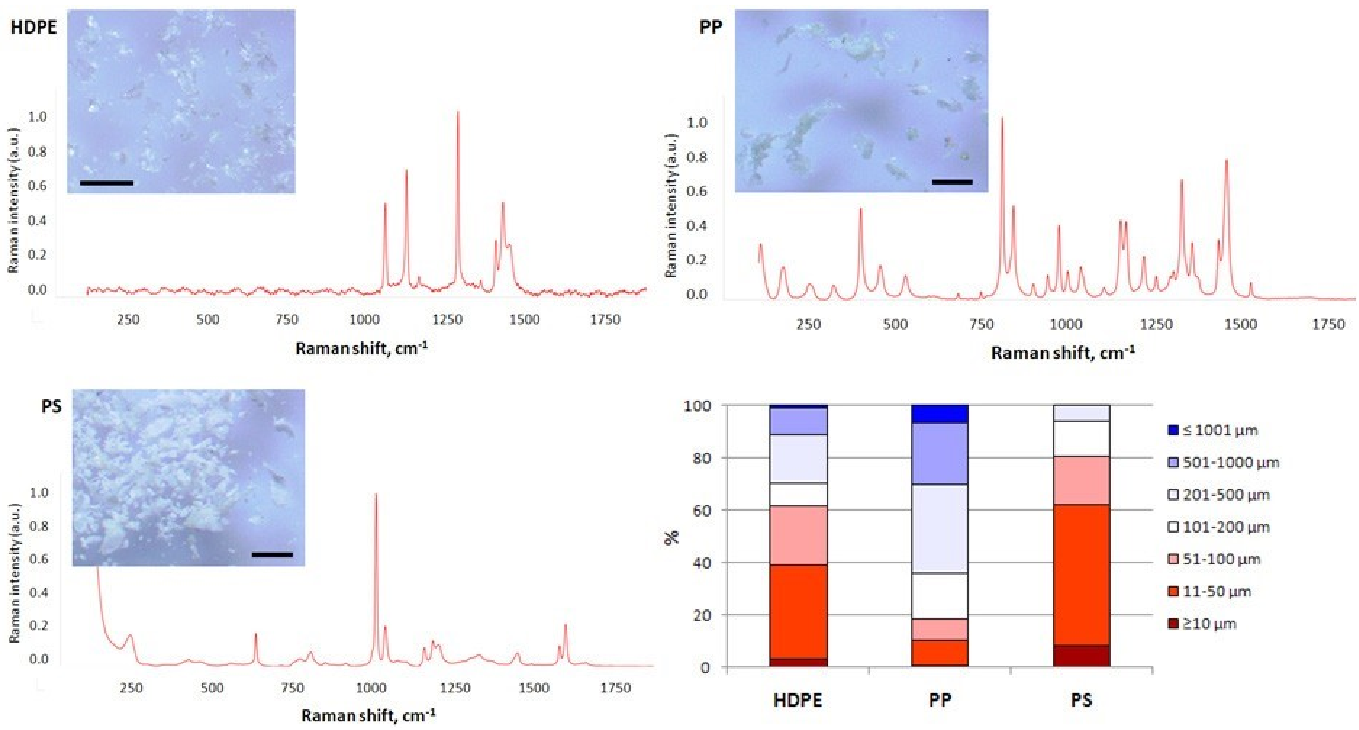

Figure 1. *Microphotographs and Raman spectra of HDPE, PP and PS particles, 0.5 mm scale. Size distribution of each type of plastics, %.*

Experimental

The experiments were performed in 8 replicates in parallel. In each replicate, 15 III instar mosquito larvae were placed in Petri dishes (90 × 90 mm) filled with 75 ml of tap water. Experimental group larvae were reared in the presence of different types of plastics. The experiment included 8 dishes with PP, 8 with PS, 8 with HDPE, and 8 without MPs (control). MPs of each type were weighed on an Explorer Pro EP214C analytical balance with an accuracy of ± 0.0001 g (Ohaus, Switzerland) and then added once to a Petri dish at a concentration of 0.3 mg (0.02 mg per larva). Control group larvae were reared under similar conditions but without MPs supplemented. The larvae of the control and experimental groups were fed with 6 mg of dried bovine liver powder (0.4 mg per larva) every 48 hours. Petri dishes were periodically replenished by water to the 75 ml mark to compensate for water evaporation. The environmental conditions were maintained stable: temperature of 25 ± 2°C, relative humidity of 70 ± 5%, and 16-hour light/8-hour dark photoperiod.

The metamorphosis rate was monitored daily throughout the experiment, and insect mortality was recorded at each life stage. In each replicate, larvae were randomly collected on day 3, pupae on day 6–7, and adults after flight. After that, they were fixed in 1 ml of 70% ethanol. Each individual was placed in a separate 1.5-ml Eppendorf tube. Then, IV instar larvae, pupae and adults selected from the experimental and control groups were washed twice in distilled water and weighed on a Jewelry scale 8068-series microbalance with an accuracy of ± 0.001 g (Smartron, China).

Statistical methods

The data obtained were analyzed using R statistical software (v4.0.5; R Development Core Team 2021). The effect of different types of plastics (PP, PS, HDPE) on mosquito body weight at all life stages was analyzed using ANOVA after log10 transformation to satisfy normality and homogeneity of variance among groups (Shapiro-Wilk test, $p > 0.05$; Levene’s test, $p > 0.05$). We performed post hoc Tukey comparisons when conditions significantly affected response variables at 95% confidence (Fox and Weisberg 2019). We also assessed differences between the control and experimental groups in mosquito body weight using Cohen’s d effect size (Cumming 2012; Sullivan and Feinn 2012) calculated using the R package Durga (Khan and McLean 2023).

Fisher’s exact test (Fisher 1934) was used to assess differences in mortality rates, $p < 0.05$. Pearson’s chi-squared test, p-value = 0.01498, was used to analyze mosquito populations pooled by life stages.

Result

The study employed three types of plastics: HDPE (2), PP (5), and PS (6). The effect of these types of plastics on the vital activity of larvae, pupae and adults of blood-sucking mosquitoes *Ae. aegypti* (body weight, survival rate, metamorphosis rate) was experimentally verified.

Analysis of mosquito body weight at all life stages in the control and three experimental groups revealed some differences. At the larval stage, mosquito body weight in the control group exceeded that in the HDPE- and PS-fed groups, and it did not differ from the PP-fed group. At the pupal stage, no statistically significant differences were revealed between the control and experimental groups. After metamorphosis, adult weight in the control group was lower than that in the experimental groups, and mosquitoes from the HDPE- and PS-fed groups showed statistically significant differences compared with the control (Table 1, Fig. 2).

Part	Stage	Mean, mg	SD, mg	N, ind	P adj
Control	Larval	1.56	0.33	25	–
HDPE	Larval	1.13	0.11	38	0.000
PP	Larval	1.56	0.27	25	1.000
PS	Larval	1.33	0.38	36	0.008
Control	Pupal	1.23	0.08	26	–
HDPE	Pupal	1.23	0.11	31	1.00
PP	Pupal	1.15	0.26	26	0.987
PS	Pupal	1.06	0.27	34	0.142
Control	Adult	0.76	0.1	29	–
HDPE	Adult	0.97	0.23	31	0.021
PP	Adult	0.83	0.2	30	0.983
PS	Adult	1.03	0.13	29	0.000

Table 1. *Mean body weight of Aedes aegypti mosquitoes in the control and experimental groups*

Note: SD – standard deviation, N – number of mosquitoes, P adj – p-value (F, ANOVA).

The study of body weight variations during metamorphosis revealed the following. In the experiments, body weight at three stages of mosquito metamorphosis showed different dynamics. In the control group, there was a decrease in body weight from larvae to adults. Similar changes were observed in the PP-fed group. In the PS-fed group, body weight was observed to decrease in pupae and adults, whereas adult weight was similar to that of pupae. A slightly different dynamics was observed in the experiment with HDPE; pupal weight was found to increase compared to larval weight, and adult weight was observed to decrease, which on average was less than that of larvae and that of pupae (Fig. 3).

Thus, PP had no effect on mosquito body weight at all life stages, whereas HDPE and PS supplemented to the diet decreased larval weight and increased adult weight. HDPE exhibited the most pronounced effect on mosquito body weight during metamorphosis.

The survival rate of mosquitoes at all life stages was high in both the control and experimental groups. In the control group, the highest mortality (11%) was recorded at the larval and pupal stages. In the PP-fed group, the highest mortality (20.2%) was observed at the pupal stage, while in the HDPE-fed group, the highest mortality (10.8%) was reported for the larval stage; in other groups, mortality did not exceed 10% (Table 2). Analysis of mosquito populations at different stages (Pearson's Chi-squared test, p-value = 0.01498) revealed a relationship between the survival rate and the experiment. Mortality was slightly higher in the PP-fed group (12.0%), and in other experimental groups, it did not exceed 10%: 8.1% in the control (plastic-free), 5.7% in the HDPE-fed group, and 6.3% in the PS-fed group.

In general, the reported types of plastics did not significantly affect the mortality of *Ae. aegypti* mosquitoes compared to the control group, yet PP had an adverse effect on the survival rate of mosquitoes (Table 2, Fig. 4).

The observations on the metamorphosis of *Ae. aegypti* mosquitoes revealed no significant differences in the rate of transition from one stage to another (Table 3, Fig. 5). In both the control and experimental groups, transition to the fourth larval stage occurred on day 3–4, that to the pupal stage on day 4–11 with a maximum on day 7, and that to the adult stage on day 7–13 with a maximum on day 10.

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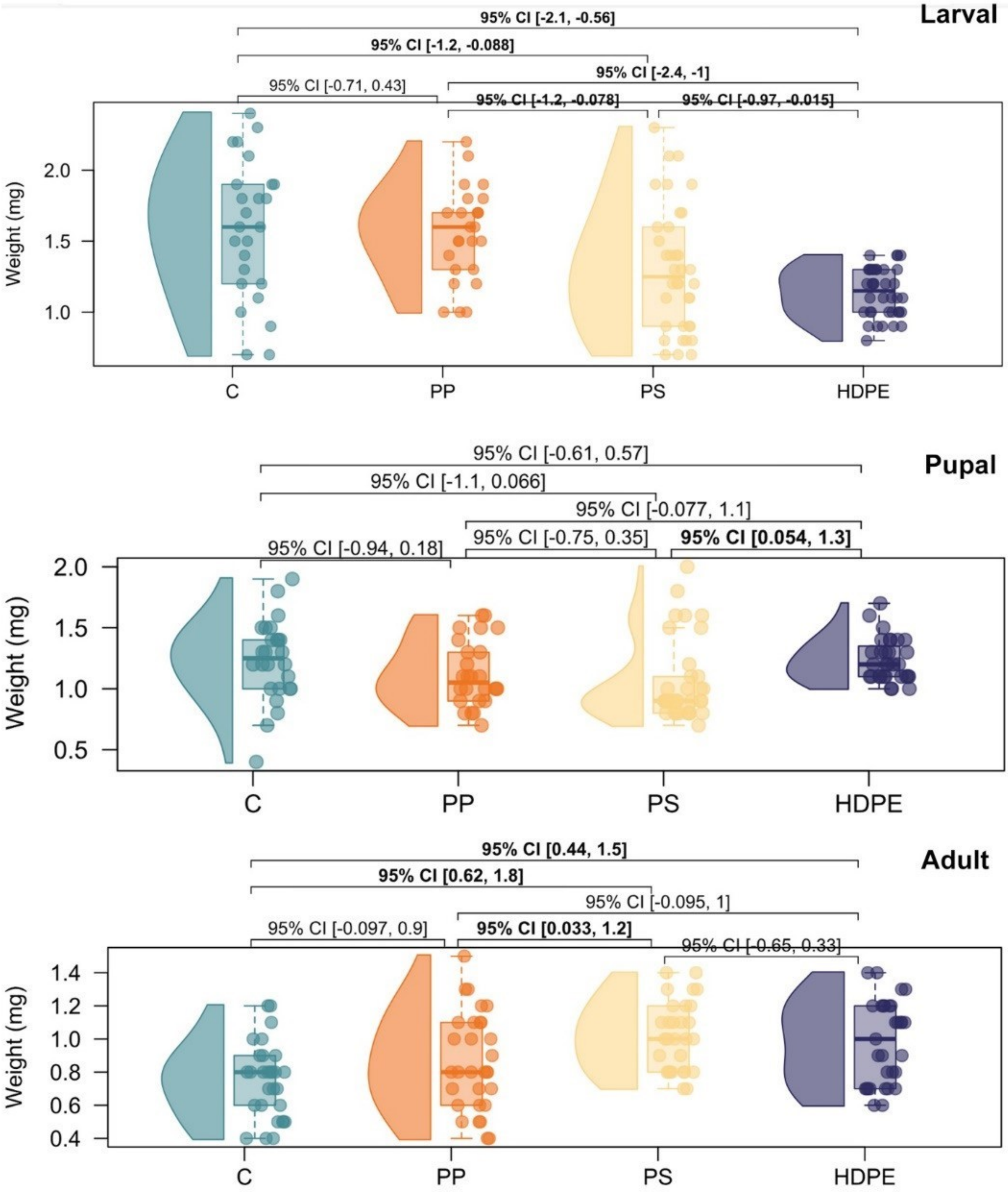

Figure 2. *Body weight of Aedes aegypti mosquitoes at the larval, pupal and adult stages in the control (C) and experimental (PP, PS, HDPE) groups. Violins show distribution of the data in each group. Boxplots display median and the 75th and 25th percentiles. Whiskers extend to the minimum and maximum values, but exclude outliers that are beyond 1.5 times the interquartile range. Circles indicate individual values. Brackets show 95% confidence intervals of bootstrapped Cohen's d for pairwise comparison.*

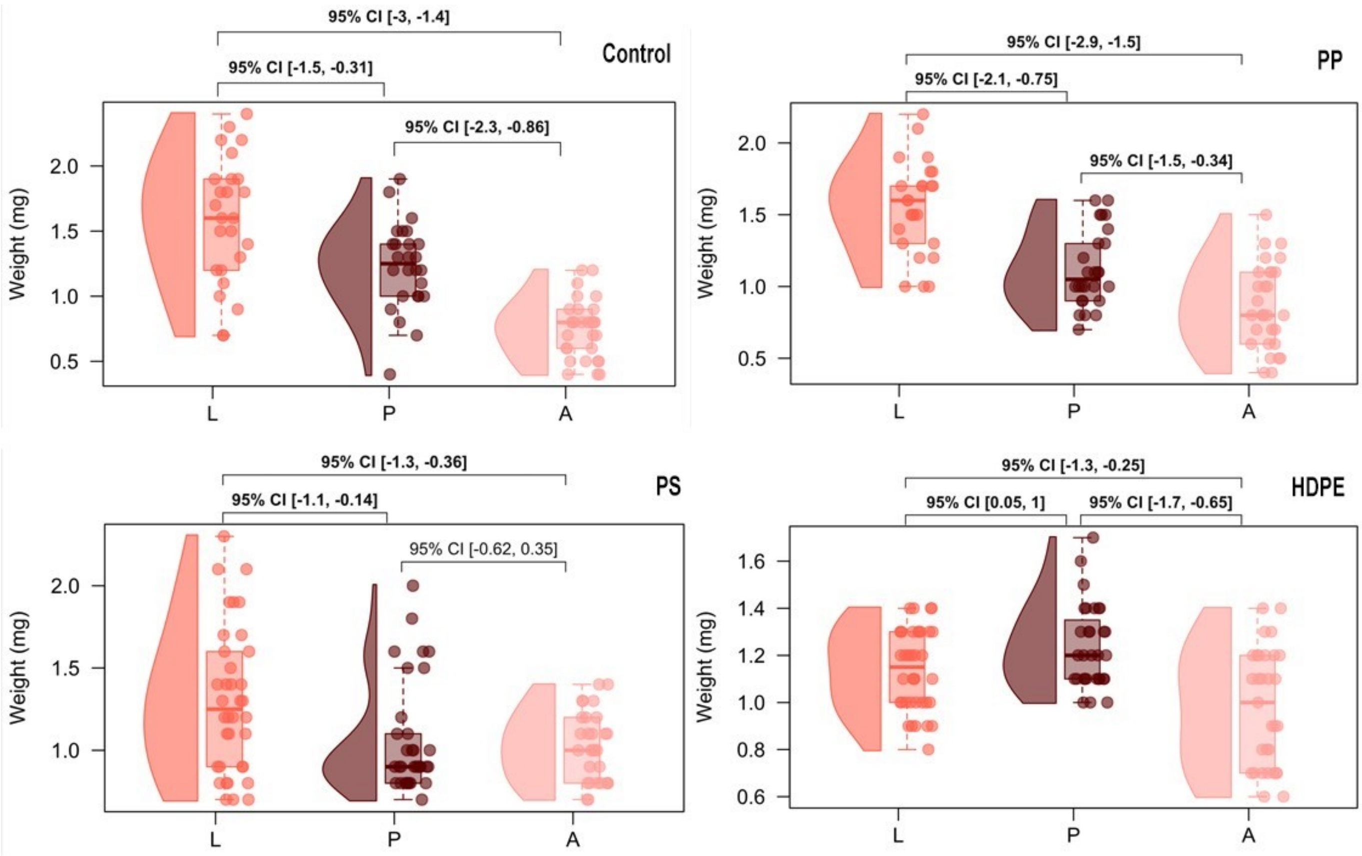

Figure 3. *Comparison of Aedes aegypti mosquito weight at different life stages in the control (C) and experimental (PP, PS, HDPE) groups. Violins show distribution of the data within each group. Boxplots display the median and the 75th and 25th percentiles. Whiskers extend to the minimum and maximum values, but exclude outliers that are beyond 1.5 times the interquartile range. Circles indicate individual values. Brackets show 95% confidence intervals of bootstrapped Cohen's d for pairwise comparison.*

Part	Percentage, %						Count, ind.		
	Larval	Pupal	Adult	Larval	Pupal	Adult	Larval	Pupal	Adult
C	10.9	11.2	1.1	89.1	88.8	98.9	110	98	87
HDPE	10.8	3.7	1.9	89.2	96.3	98.1	120	107	103
PP	9.2	20.2	5.7	90.8	79.8	94.3	120	109	87
PS	8.3	7.3	2.9	91.7	92.7	97.1	120	110	102

Table 2. *Survival rate of Aedes aegypti mosquitoes in the control and experimental groups*

Stage	Part	Observation days										
		d3	d4	d5	d6	d7	d8	d9	d10	d11	d12	d13
Larval	C	94.9	5.1	–	–	–	–	–	–	–	–	–
	HDPE	86.6	13.4	–	–	–	–	–	–	–	–	–
	PP	92.9	7.1	–	–	–	–	–	–	–	–	–
	PS	90.4	9.6	–	–	–	–	–	–	–	–	–
Pupal	C	–	2.7	4.1	23.3	42.5	5.5	–	19.2	2.7	–	–
	HDPE	–	–	2.9	21.7	49.3	1.5	–	21.7	2.9	–	–
	PP	–	1.2	4.8	19.3	41.0	4.8	–	16.9	12.0	–	–
	PS	–	1.4	8.0	20.3	47.3	–	–	23.0	–	–	–
Adult	C	–	–	–	–	5.6	–	11.1	72.2	8.3	–	2.8
	HDPE	–	–	–	–	–	–	2.9	94.2	–	–	2.9
	PP	–	–	–	–	2.9	–	–	68.5	5.7	–	22.9
	PS	–	–	–	–	3.1	0.0	3.1	87.5	–	6.3	–

Table 3. *Metamorphosis rate of Aedes aegypti mosquitoes in the control and experimental groups*

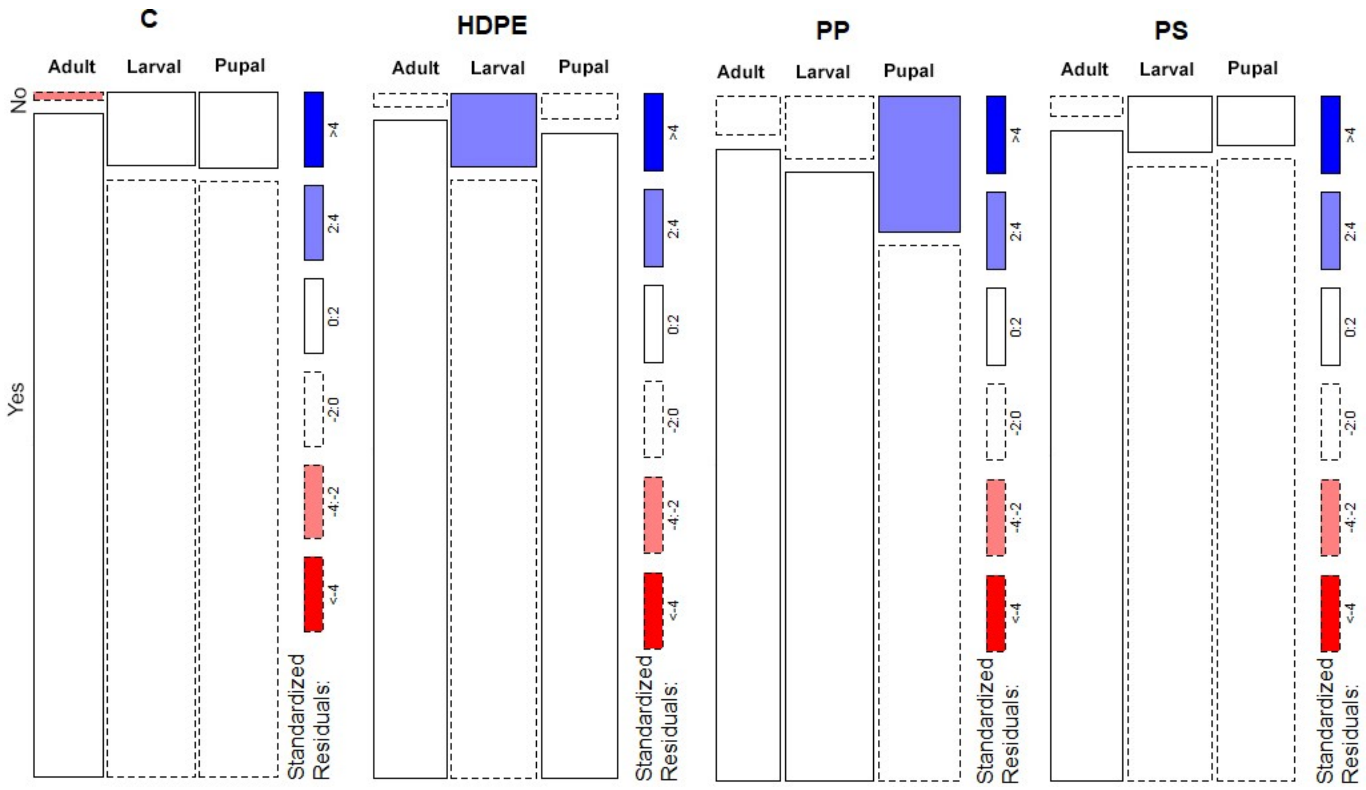

Figure 4. *Survival rate of Aedes aegypti mosquitoes in the control and experimental groups.*

Discussion

In recent years, a number of papers have been published on the effects of MPs on mosquitoes, yet the results of these studies vary considerably. For instance, Al-Jaibachi et al. (2019) reported that 2- and 15-µm PS microspheres at concentrations up to 200 psc/ml did not significantly affect the mortality rate of *Culex pipiens* mosquitoes during their transition from the aquatic larval to terrestrial adult stage nor did they affect adult weight. A similar study was conducted on *Aedes aegypti* mosquitoes (Linnaeus 1762) (Simakova et al. 2022), where 2-µm PS microspheres were used at a higher concentration (8.0×10^6 pcs/ml), which, however, had no significant effect on mosquito survival. The analysis of the effect of MPs on mosquito body weight showed that the mean weight in the experimental groups exceeded that in the control group. In a recent experiment, Griffin et al. (2023) exposed *Aedes albopictus* (Skuse 1895) and *Culex quinquefasciatus* (Say 1823) mosquitoes to 1–53-µm PE microspheres at concentrations of 60–6000 pcs/ml. The results showed that some PE concentrations caused the death of both *Aedes* and *Culex* larvae. The lowest concentration did not affect the development or survival rate of *Cx. quinquefasciatus*, in contrast to *Ae. albopictus*. However, PE concentration of 6000 pcs/ml led to 100% larval mortality in both genera (Griffin et al. 2023).

Apparently, a different type of plastics (PE as opposed to earlier experiments with PS) used in the latter study was one of the contributing factors in the observed differences in mosquitoes.

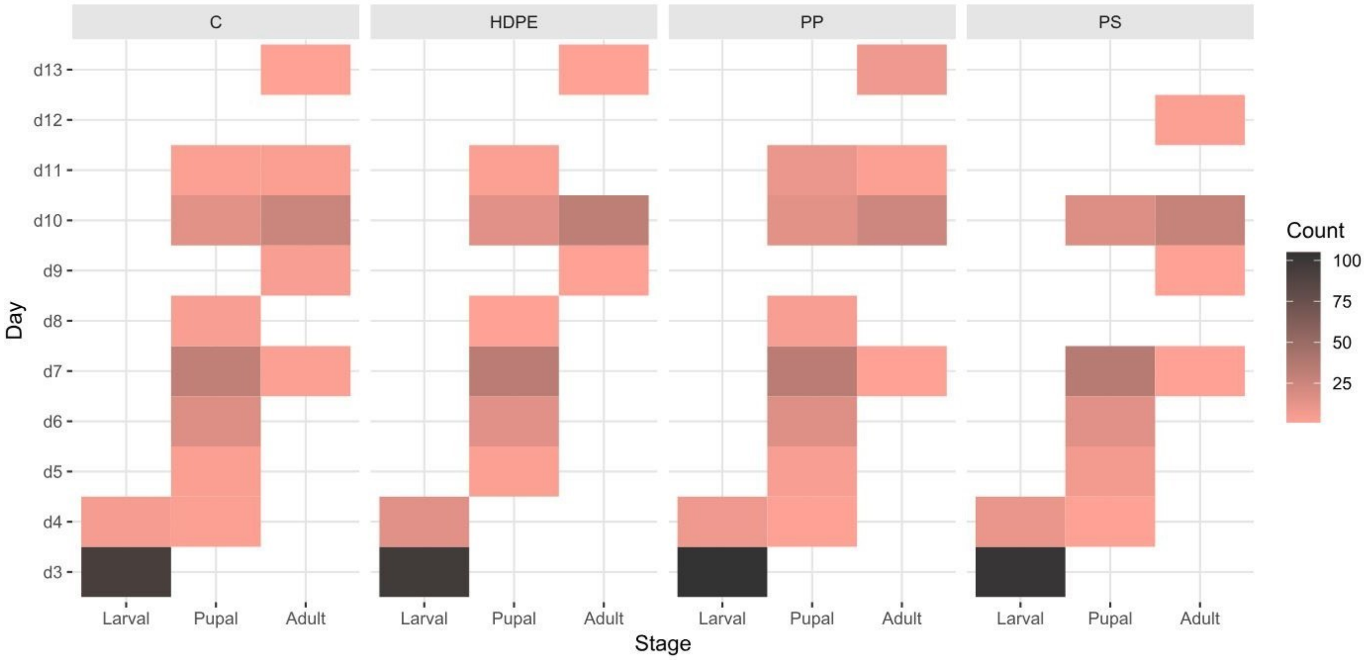

Figure 5. *Metamorphosis rate of Aedes aegypti mosquitoes in the control (plastic-free) and experimental (fed with different types of plastics) groups.*

Meanwhile, spherical plastic particles are not prevalent in freshwater ecosystems, whereas fibres and irregularly shaped fragments are the most frequently reported shapes. Plastic particles with sizes ranging from a few nanometres to several millimetres are typically found in natural environment (Li et al. 2020; Szymańska and Obolewski 2020; Frank et al. 2022; Bhardwaj et al. 2024). HDPE, PP and PS particles represented by fragments of irregular shape and size were used to simulate microplastic pollution in a way that was closer to natural conditions (Fig. 1). The average sizes of plastic particles (along the maximum axis) were 174, 446 and 63.1 µm for HDPE, PP and PS, respectively. Among HDPE particles, 70.4% of the particles were < 200 µm in size and could therefore be ingested by III instar mosquito larvae. Among PP and PS particles, 36.0 and 93.8% of the particles were available for ingestion by larvae. Different particle size and availability for ingestion by mosquito larvae, along with the polymer composition, may have resulted in the effects detected. Thus, in the experimental groups, the largest-sized PP particles had no effect on mosquito body weight at all life stages, whereas HDPE and PS added to the diet decreased larval weight and increased adult weight. Of the three types of plastics, contrary to expectations, HDPE with an average particle size exhibited the most significant effect on mosquito body weight during metamorphosis.

Conclusion

It has been experimentally proved that different types of plastics do not significantly affect the vital activity of bloodsucking mosquitoes *Aedes aegypti*. PP had no effect on mosquito body weight at any stage, while HDPE and PS decreased larval weight and increased adult weight ($p < 0.05$). The results showed that HDPE affected ($p < 0.05$) pupal and adult weight during metamorphosis, whereas PP and PS had no such effect. HDPE and PS did not affect mosquito mortality at any stage compared to the control group, but PP tended to have an adverse effect on the survival rate of pupae and adults. The metamorphosis rate was not affected by any of the three types of plastics. Consequently, none of the three types of plastics had a significant effect on the vital activity of bloodsucking mosquitoes.

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