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The interplay of larval age and particle size regulates micro-polystyrene biodegradation and development of *Tenebrio molitor* L.

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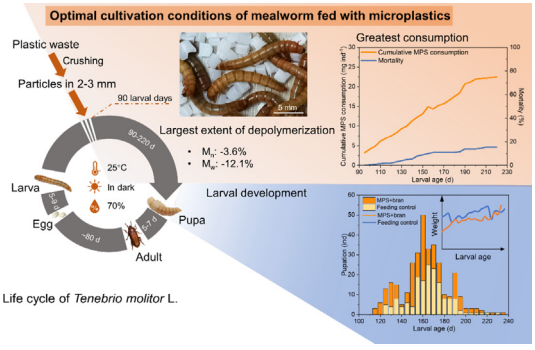
HIGHLIGHTS

- Within limits, the smaller the microplastics, the more plastics mealworms consumed.
- Only mealworms of 3-month-old depolymerized and oxidized MPS.
- Mealworms fed MPS at an older age took in more plastics.
- MPS enhanced mortality, weight loss and delayed/advanced pupation in mealworms.
- Using 3-month-old mealworms with bran performed best in microplastic degradation.

GRAPHICAL ABSTRACT



ABSTRACTARTICLE INFO

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Microplastics, tiny plastic fragments from 1 μm to 5 mm, are widespread globally, even in remote environments. Due to their small sizes, they are easily ingested by organisms and contaminate the food chain. Recently, the biodegradation of some recalcitrant plastics by larva of *Tenebrio molitor* L. (mealworm) has been reported. However, the effects of microplastic feeding on them are limited. In our study, we selected rigid micro-polystyrene (MPS) as the model plastic to investigate the influences of particle size and larval age on plastic consumption and degradation, and the effects of microplastic feeding on the survival and development of mealworms at different larval ages. The smaller the microplastic fragment was, the more plastics the mealworms consumed, though there was a limit on particle size. Mealworms of three-month-old had the highest consumption rate. Both depolymerization and modification on the functional groups were only observed in frass excreted by three-month old mealworms. Additionally, mealworms cofed with wheat bran and MPS of this age had comparable mortality, larval growing curve and pupation distribution as the control group with wheat bran. Our results demonstrated that mealworms in this larval stage had the greatest resistance to high doses of microplastic feeding. We suggested that microplastic waste could be provided to three-month old mealworms as half replacement of bran diet to result in the greatest plastic consumption and degradation.

1. Introduction

Plastic production worldwide in 2020 was 367 million tonnes (PlasticsEurope, 2021). Of this enormous quantity, over 50 % ended up as waste in landfills and only roughly 7 % was recycled (Tiseo, 2021a). Furthermore, 17 % of the recycled plastics were eliminated through

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incineration after repeated processing, and only 2 % of plastics produced were recycled up to 2017 (Tiseo, 2021a). Inevitably, after weathering, the landfilled plastics were further damaged into microplastics (< 5 mm in length), carried away by wind, and entered the environment. Until now, microplastics have been reported to reaching aquatic and remote areas, including deep sea, polar and alpine regions (Van Cauwenberghe et al., 2013; Peeken et al., 2018; Allen et al., 2019; Bergmann et al., 2019). With the increasing exposure to microplastics, there will be a greater chance of ingestion and accumulation along food chains. In addition to gut blockage caused by the direct ingestion of microplastics, the adsorbed persistent organic pollutants (POPs), such as phthalic acid esters (PAEs), dichlorodiphenyltrichloroethane (DDT), polycyclic aromatic hydrocarbon (PAHs) and polychlorinated biphenyls (PCBs) (Pannetier et al., 2019; Černá et al., 2021; Benson and Fred-Ahmadu, 2022), also cause harmful effects on consumers and enrichment along the trophic levels.

Biodegradation of plastics and microplastics attracted broad scientific and public attention due to the extraordinary destructive effects caused by landfilling and incineration. The larvae of insects from the Tenebrionidae (Coleoptera) family (i.e. *Tenebrio molitor*, *T. obscurus*, *Zophobas atratus* and *Plesiophthalmus davidis*) and Pyralidae (Lepidoptera) family (i.e. *Plodia interpunctella*, *Achroia grisella* and *Galleria mellonella*) have been reported to ingest and partly degrade recalcitrant plastics including polystyrene (PS), polyethylene (PE), polypropylene (PP) and polyvinyl chloride (PVC) (Brandon et al., 2018; Kundungal et al., 2019; Peng et al., 2019, 2020a, 2020b; Lou et al., 2020; Woo et al., 2020). Especially, behaviors of mealworm (larva of *Tenebrio molitor*), superworm (larva of *Zophobas atratus*) and greater waxworm (larva of *Galleria mellonella*) in plastic foam degradation have been intensively investigated. Mealworm and superworm from the same family prefer decaying grains and milled cereals, as well as undecayed matter, like meat, flour, bran, crackers, feathers and dead insects. They inhabit dark and damp places, such as grain bins, bird houses, feed processing plants and warehouses (Ghaly and Alkoaik, 2009). In contrast, greater waxworms live on honeycomb in beehives and eat honey, beeswax and the skin of bee pupae (Kong et al., 2019). The different living and feeding habits lead to different plastic degradation patterns between the greater waxworm and larvae from Tenebrionidae.

Mealworm, the highly competitive candidate, can degrade microplastics, including PE foam, PP foam, foamy or rigid PS and rigid PVC (Yang et al., 2015a, 2018, 2021b, 2022; Brandon et al., 2018; Wu et al., 2019; Peng et al., 2020b; Zhong et al., 2022). Analysis of microbial community structures, isolation of functional bacteria and transcriptomic analysis were conducted to study its biodegradation mechanism. It is well accepted that microorganisms in gut and the fatty acid degradation pathway play key roles in plastic degradation (Yang et al., 2015b; Zhong et al., 2022). However, research on their persistence in degradation and the effects of microplastics on the survival and, development of insect larvae that degrade plastics are limited. In our previous work (Zhong et al., 2022), we recorded the mortality and relative weight of mealworms fed on microplastics, including low-density PE (LDPE), linear low-density PE, high-impact PS, PS foam and rigid PP until pupation or death. The mortalities of plastic-fed groups were higher than the starvation group, while the relative weight decreased as the starvation group did. The results implied that energy released by plastic digestion could not account for the energy spent on nutrition, which was not in accordance with previous research (Yang et al., 2014, 2015a, 2018, 2021a, 2021b; Brandon et al., 2018; Peng et al., 2019; Lou et al., 2020) because of the longer studied period. Regarding the influence on growth and metamorphosis, mealworms fed with bran and PS were reported to complete all their life stages (Yang et al., 2018), but the effects of plastic on pupation and their life span were not clarified. To fully understand the impacts of plastic feeding on survival and development of mealworms, mortality (or survival rates), larval weight and pupation distribution through the larval stage are essential parameters to record and analyze.

Polystyrene has broad but one-off application and remarkable durability, and occupies a great market share (16 % of the total plastic production in 2018, Tiseo, 2021b). In this study, we selected micro-polystyrene (MPS) as the model plastic and mealworm as the model insect to examine the impact of microplastic feeding on survival, growth and metamorphosis of plastic-degrading larvae at different larval ages. To ensure the ingestion by larvae, foamy plastics were firstly selected for degradation. However, rigid plastic waste remains a huge environmental problem. The global rigid plastic packaging market was valued at $182,100 million in 2020 and is expected to grow by 4.9 % from 2021 to 2028 (Khandelwal and Sumant, 2021). Rigid PP was not degradable by mealworm in our study (Zhong et al., 2022), while contrary result was obtained using foamy PP (Yang et al., 2021a). Therefore, rigid MPS of different particle sizes was selected in our research to extend the application of mealworms in plastic degradation regarding scale and integrity. By minimizing the harmful impact on mealworms, we provided a practical and eco-friendly approach by utilizing mealworms as degradation reactors in recalcitrant plastic waste management. Our research not only enriches the knowledge on the effects of microplastics on plastic-degrading larvae, but also provides a new perspective of their influences on the environment.

2. Materials and methods

2.1. Plastic test materials

Rigid micro-PS (MPS; density: 1.114 g cm^{-3}; shore hardness: 25.6 HD; amorphous) was purchased from Kumho Tire (Korea). MPS in typical sizes (1–2 mm, 2–3 mm and 3–4 mm) was prepared by cutting and passing through mesh sieves. Before experiment, MPS was cleaned and eluted with fresh water twice and distilled water once on the sieve, followed by oven-drying at 60 °C for 12 h until complete dryness.

2.2. Mealworm cultivation and MPS consumption

Stock mealworms (larvae of *T. molitor*) were initially provided by the Urban Protein Engineering Technology Research Center, which obtained the original insect colony from South China Agricultural University, Guangzhou. The experimental mealworms were reared continuously over three generations in our laboratory and all are from the same batch of adults. Before providing the experimental diet, the larvae were reared and fed with sufficient wheat bran (Bob's Red Mill, Milwaukee, USA). Effects of plastic size on consumption were evaluated by feeding mealworms at their young larval stage (YLS, one-month-old, ~3 mg ind^{-1}) with different particle sizes, i.e. 1–2 mm, 2–3 mm and 3–4 mm. To investigate the differences in plastic consumption and degradation of mealworms at different larval ages, mealworms at YLS, middle larval stage (MLS, two-month-old, ~20 mg ind^{-1}) and old larval stage (OLS, three-month-old, ~80 mg ind^{-1}) were fed with MPS alone or cofed with bran. Worms unfed or fed with bran at the same larval age were prepared as starvation and feeding control, respectively. Selected mealworms (50 ind) were subjected to a 48-h starvation period (Yang et al., 2015a). They were then reared on the selected diets in 300 mL (140 mm × 50 mm × 45 mm, L × W × H) glass containers in a controlled-environment chamber (MGC-350HP, Yi Heng, Shanghai, China) under stable conditions (25 °C, 70 % RH, in dark). The initial MPS supplement in each replicate in the plastic solely fed groups and cofed groups were 3.0 g and 1.5 g respectively, while the initial MPS/bran was 1:1 in cofed diet. Half bran based on the consumption in feeding control at each larval age was added to cofed groups every 5 days.

Residual MPS was collected and weighed after passing through a 1-mm sieve and blown with air using a rubber suction bulb to remove the frass carefully. MPS consumption was assessed by cumulative consumption (mg ind^{-1}), which computed as the accumulation of the weight of MPS consumed (mg) per surviving larva every 5 days until all studied larvae

died or pupated. Larvae were removed immediately after death. Survived larvae or pupae were counted and weighed. All treatments were carried out with four replicates.

2.3. Evaluation of MPS depolymerization and oxidation by mealworm

To assess plastic degradation, frass of MPS-fed mealworm or the feedstock (50 mg) were freeze-dried for 48 h and stored under −20 °C before analysis. To obtain sufficient frass for analysis, 1000 larvae were raised for each treatment in glass containers (1100 mL, 160 mm × 110 mm × 60 mm, L × W × H). Egested frass or plastic feedstock (20 mg) was freeze-dried for 48 h and stored under −20 °C before analysis. MPS was extracted with 10 mL tetrahydrofuran (THF) at room temperature for 12 h. The extract was further filtered into a glass vial using a 0.45 μm PVDF syringe filter. Number average and weight average molecular weights (M_n and M_w) and molecular weight of the highest peak (M_p) were determined by gel permeation chromatography (GPC) via Agilent 1260 coupled with 1100 refractive index detector, according to methods by Brandon et al. (2018). Modification on functional groups was obtained by Fourier Transform Infrared Spectroscopy (FTIR) on Agilent Cary 630 FTIR. Residual polymers were dissolved in THF and spread in the window until all the solvent was dried. Spectra were recorded in the range of 4000–500 cm^{-1}. All treatments were carried out with two replicates.

2.4. Evaluation of the survival and development of mealworm

The mortality (%) of mealworms was assessed, while the developmental status (growth and metamorphosis) was evaluated according to their larval weight (mg ind^{-1}), pupation rate (%) and pupation distribution. Mortality and pupation rate were calculated as the percentage of dead or pupated mealworms among the initial worms (50 ind). The larval weight (mg ind^{-1}) was the average weight of each surviving mealworm. The pupation distribution consisted of the number and the average weight of pupae recorded on the counting day.

2.5. Statistical analysis

Linear regression was processed by OriginPro (2018) with default parameters. One-way analysis of variance (ANOVA) was used to analyze statistical significance by SPSS (IBM SPSS Statistics 17, $p < 0.05$). Tukey's post hoc test was used if equal variances were assumed, while the Games-Howell test was used if equal variances were not assumed.

3. Results and discussion

3.1. MPS consumption and larval survivorship at different MPS particle sizes

The effects of microplastic particle size were evaluated in terms of cumulative consumption (mg ind^{-1}) and survival of mealworms. The cumulative consumption, which was negatively dependent on the particle size, increased over time in all groups (Fig. 1A). In total, mealworms caused 10.3 mg, 7.2 mg and 1.9 mg reduction of MPS in size range of 1–2 mm, 2–3 mm and 3–4 mm, respectively, until all the experimental larvae were dead. The MPS consumption and mealworm growth pattern of 1–2 mm and 2–3 mm were very similar, especially for the first 45 treated days. However, the consumption in worms fed with 3–4 mm MPS was much lower than the others, which was not significantly different ($p = 0.458$), while those between 3 and 4 mm with either 1–2 mm ($p = 0.001$) or 2–3 mm ($p = 0.001$) were significant. Smaller microplastics enhanced ingestion, but particle size effect declined to a certain extent.

Survivorship of mealworms varied with diets. The surviving periods (counting from commencement of MPS feeding until all the mealworms in a group died) of the three groups (MPS of 1–2 mm, 2–3 mm and 3–4 mm) were 85, 90, and 80 days, respectively (Fig. 1B). Considering the similarities of MPS consumption and survival conditions between the groups fed with MPS of 1–2 mm and 2–3 mm, MPS of 2–3 mm was selected as the experimental particle size in the following study.

3.2. Effect of larval age on microplastic consumption

The consumption of MPS varied with larval stages in mealworms. Cumulative consumption with or without bran increased with the weight of mealworms over time (Figs. 2 & S1). Under the MPS-fed treatment or feeding control (in parentheses), their consumption after 5 days at YLS, MLS and OLS were 0.02 (7.52), 0.47 (41.4) and 2.31 (53.2) mg ind^{-1}, respectively, showing that older larvae had greater MPS consumption. The cumulative total consumption was greater in older larvae fed with MPS with or without bran (Fig. 2A & B). Interestingly, although MPS was provided earlier regarding their development stages in the younger groups, the greatest total MPS consumption was contributed by mealworms in the older group. Differences between the cumulative consumption of MPS fed alone at OLS and other larval stages were significant ($p < 0.001$), but the variation between YLS and MLS was not ($p = 0.110$). The consumption with bran at OLS was significantly greater than that at MLS ($p < 0.001$).

We compared the cumulative consumption at the same larval stage between different groups. The consumption curves overlapped in YLS and MLS groups fed with MPS alone (Fig. S1A) as well as MLS and OLS groups cofed with bran (Fig. S1B). Cumulative consumption produced by older mealworms was greater than the younger ones throughout the

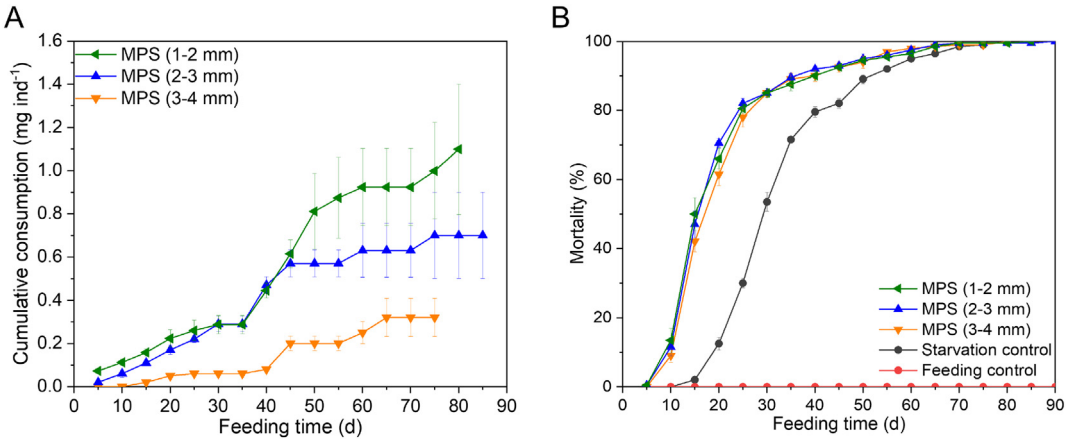

Fig. 1. (A) Cumulative consumption (mg ind^{-1}) and (B) mortality (%) of mealworms at YLS (young larval stage) fed with MPS only. Vertical bars are standard errors of four replicates.

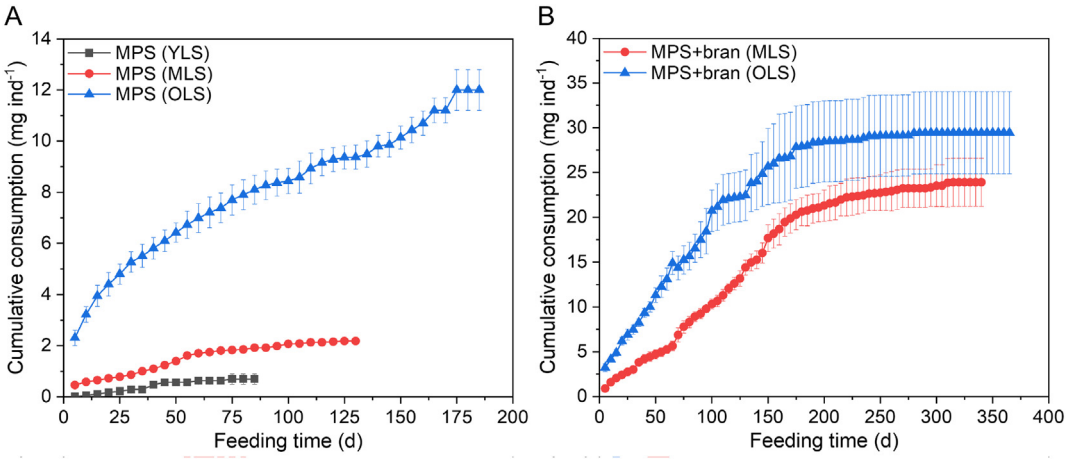

Fig. 2. Cumulative consumption (mg ind^{-1}) of MPS-fed groups (A) and bran cofed groups (B) at different larval stages over MPS feeding time (YLS: young larval stage; MLS: middle larval stage; OLS: old larval stage). Vertical bars are standard errors of four replicates.

experimental period. Besides, the variants in the fitted consumption rate of cumulative consumption over time were extraordinary (Table S1), with a slope of 1.5–2.0 times greater than the younger group fed with or without bran. Our results indicated the ability of plastic consumption increased with larval age when considering both the microplastic consumption rate and the total microplastic consumption.

3.3. Effect of larval age on microplastic degradation

Microplastic consumption does not directly imply the ability of degradation. To evaluate depolymerization and oxidation levels, frass of mealworms containing the incomplete digested polymer was collected. Changes in molecular weight varied at different larval stages. Since MPS consumption was too little to be measured at YLS, comparisons were only carried out between mealworms at MLS and OLS. Compared with the feedstock, M_n and M_w of the residual polymer egested by mealworms at OLS were reduced by 3.6 % and 12.1 %, respectively, and M_p which reflects the molecular weight distribution pattern decreased from 125 to 85 kDa. These results indicated that mealworms at OLS broke down the long-chain structure of MPS and produced fragments of smaller molecular weight. In contrast, M_n and M_w in mealworms at MLS increased by 28.1 % and 2.2 %, respectively, and M_p (125 kDa) remained unchanged. Depolymerization of PS was not observed after being treated by mealworms at MLS.

In contrast, molecular modifications were similar at MLS and OLS. According to the spectra of FTIR (Fig. 3), broadened peaks of hydroxyl groups (3700–3100 cm^{-1}) and shifted peaks caused by methylene from 1000 to 750 cm^{-1} to 1100–800 cm^{-1} provided additional evidences of oxidation and cleavage of the polymer after ingestion by either group. However, different from our previous work (Zhong et al., 2022), the peak caused by carbonyl groups in 1900–1600 cm^{-1} was too small to be noticed, while the appearance of a new peak in 1500–1250 cm^{-1} produced by C—N stretch vibration was apparent in collected residual from both groups. The stretch vibration caused by C—N usually appeared in 1230–1030 cm^{-1} in the FTIR spectra. Only when the N connects with the benzene ring directly will it cause the shift to the higher frequency area (1360–1250 cm^{-1}) due to conjugation. Thus, the oxidation of MPS by mealworm was generated by insertion or replacement of N between the backbone and the side (aryl) group. The different chemical compositions in rigid MPS (with rubber as additives) and expanded PS may lead to varying priorities in modifying functional groups. In short, depolymerization and oxidation of MPS after ingestion by mealworms at OLS were proven by the reduction of molecular weight and modification of functional groups. In contrast, only oxidation was observed at MLS.

3.4. Effect of MPS intake on survival and development of mealworm at different larval ages

MPS feeding was harmful to the survival, growth and metamorphosis of mealworms, and older worms were more resistant to the negative effects. Regarding survival, mortalities of mealworms fed with MPS alone (Fig. 4A) at YLS and MLS finally reached 100 %, while that at OLS was 95.2 %, even higher than the starvation control of the same stage (90.0 %). Besides, the fitted death rate (Table S1) declined, and the surviving periods (i.e. YLS: 90 days, MLS: 135 days, OLS: 185 days) expanded with the postponement of the start of MPS feeding. Differences of mortalities between YLS and the OLS were significant ($p = 0.004$), while not between MLS with either YLS ($p = 0.101$) or OLS ($p = 0.426$). Although the growth trends between starvation and MPS-fed groups of each larval stage were similar, the time to reach plateau (data range of mortality and larval age in Table S1) and the fitted death rate varied (Table S1). The fitted death rates of MPS-fed alone and starvation control (in parentheses) at YLS, MLS and OLS were 3.47 (3.46), 1.71 (1.79) and 1.02 (0.95), while

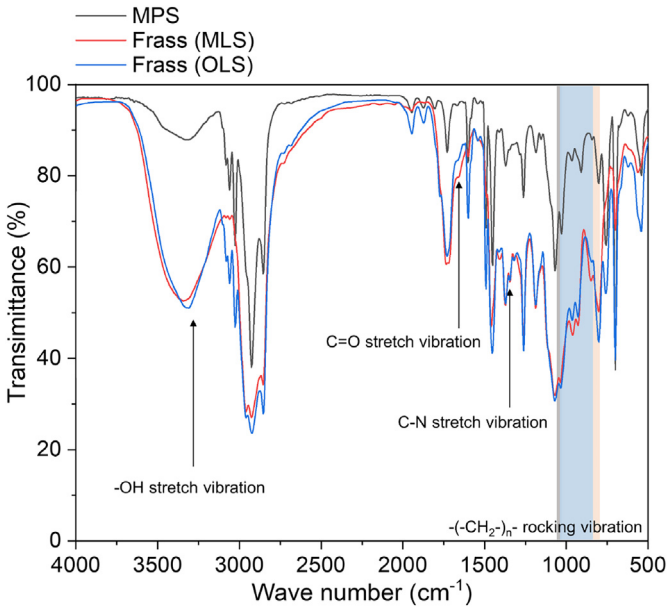

Fig. 3. FTIR spectra of MPS feedstock and frass from mealworm at MLS and OLS. Orange and blue shading represented the methylene rocking vibration region of feedstock, while gray and blue shading represented those of collected frass.

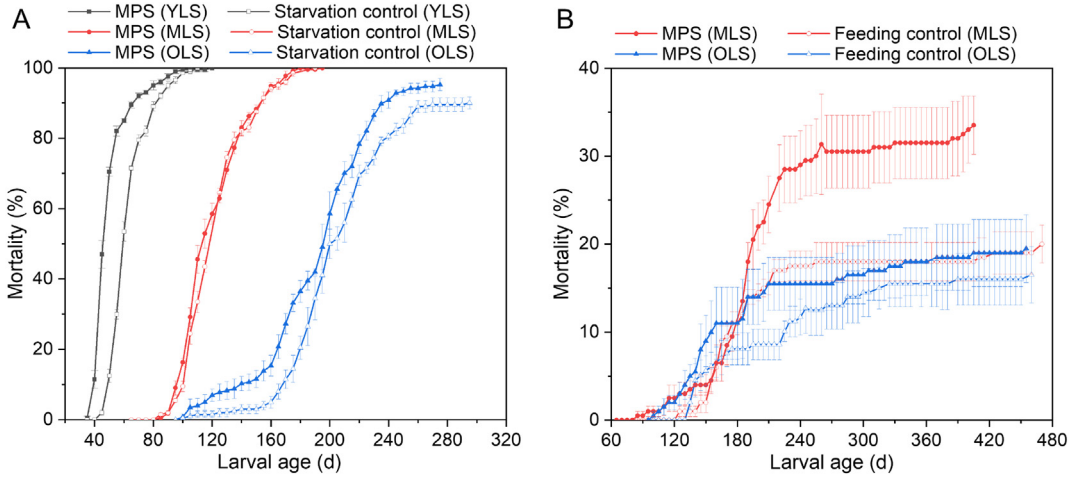

Fig. 4. Mortality (%) of MPS-fed groups (A) and bran cofed groups (B) at different larval ages over the whole larval stage. Vertical bars are standard errors of four replicates.

the time taken to reach plateau (d) was 30 (40), 50 (50) and 70 (90), respectively. At MLS, the higher fitted death rate in MLS starvation control and the same fitted data range (90–140 larval days) indicated that ingestion of MPS at MLS had similar negative effects to starvation on mealworms. Contrastingly, higher fitted death rates and longer time needed to reach plateau were observed at YLS and OLS. The greater side-effects on mealworms at YLS and OLS are probably caused by the incomplete development at YLS and the greatest ingestion of MPS at OLS. With the growth of mealworms, the resistance to the harmful impacts caused by MPS ingestion increased but was eliminated after ingesting excessive MPS.

Regarding the bran cofed groups, mortality of mealworms (Fig. 4B) at OLS was 19.5 %, which was significantly lower than that at MLS ($p < 0.05$), but slightly higher than its feeding control. In contrast, mortality of MLS cofed group was significantly higher than its feeding control ($p \leq 0.05$). The surviving periods of OLS cofed group (365 days) and its feeding control (370 days) were similar, while a considerable variation was observed at MLS (MPS cofed group: 345 days; feeding control: 410 days). Additionally, the fitted death rate of MLS cofed group was nearly two times greater than that of OLS (Table S1). Half replacement of bran diet with MPS at OLS had little influence on the survival of mealworms.

Metamorphosis was evaluated by pupation rate and pupation distribution over time. Compared with the feeding control, mealworms fed with MPS and bran at MLS not only reduced their pupation rate (i.e. feeding control: 80 %; MPS cofed with bran: 67 %) but also postponed their pupation (Fig. 5A). Besides, no pupation was observed at MLS with MPS as the sole diet. In contrast, mealworms fed with MPS at OLS had comparable pupation rate (i.e. feeding control: 85 %; MPS cofed with bran: 84 %; MPS: 2 %) and pupation time to its feeding control (Fig. 5B).

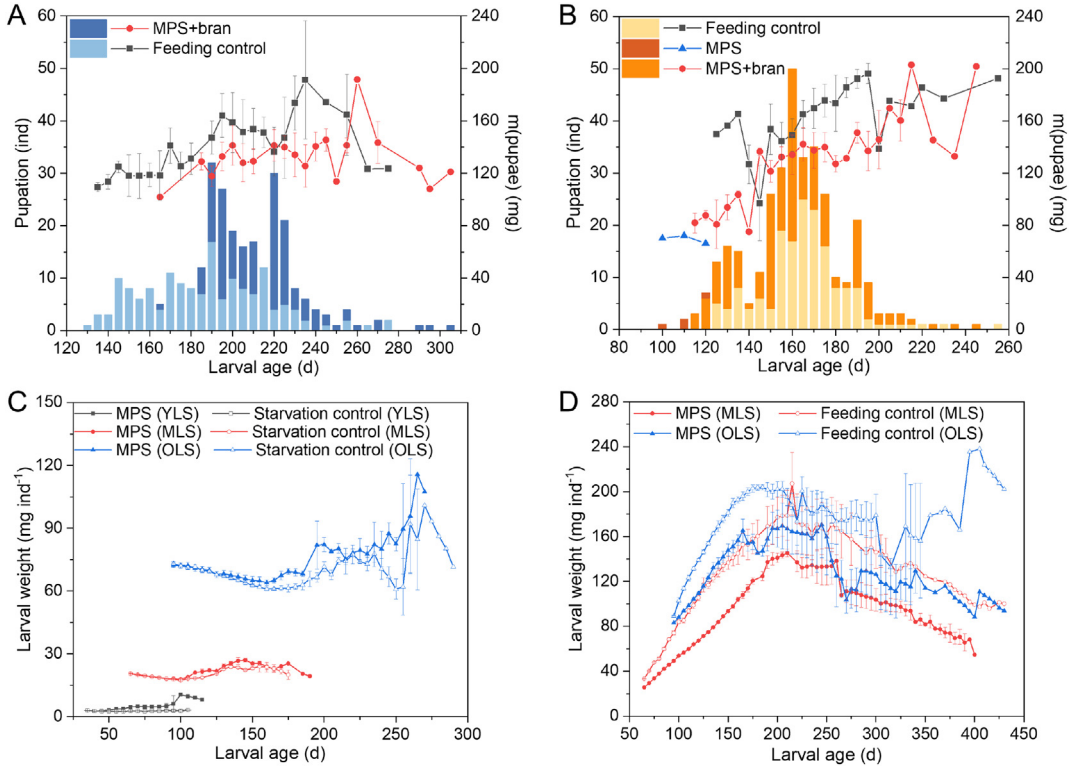

Fig. 5. Pupation distribution of mealworms at MLS (A) and OLS (B). Larval weight (mg ind^{-1}) of MPS-fed groups (C) and bran cofed groups (D). Vertical bars are standard errors of four replicates.

The growth status of mealworms as reflected by larval weight was not constant over time. Larval weight of the three groups fed with MPS alone and the starvation control had similar development pattern (Fig. 5C), declining slowly followed by an increase and then a rapid decline till the end. Our previous study demonstrated that it was caused by the death of weaker larvae (Zhong et al., 2022). Regarding the cofed groups, the growth pattern of MPS co-diet and their feeding control (Fig. 5D) were similar, rising steadily to a peak at 200–225 days after hatching, followed by a steady decrease towards the end of the experiment. The steady increase implied that the larvae fed with half bran grew continuously. The larval age corresponding to the highest peak in Fig. 5B was identical to when the mealworms finished their pupation (Fig. 5A & B, 250 larval days). After failure to pupation, mealworms slowed down their movement, reduced their food intake (Fig. 2D, even the ingestion of bran in the feeding control decreased from 8.95 mg ind^{-1} d^{-1} after 130 larval days to 1.09 mg ind^{-1} d^{-1} after 470 larval days) and became senescent for death as larvae, which led to the reduction of larval weight. Moreover, the gaps in the fitted growth rate within the linear range between cofed groups and their feeding control were similar (MLS with bran: 0.78, adj. R^2 = 0.9974, 65–210 larval days; MLS feeding control: 1.29, adj. R^2 = 0.9928, 65–210 larval days; OLS with bran: 1.12, adj. R^2 = 0.9957, 95–165 larval days; OLS feeding control: 1.65, adj. R^2 = 0.9841, 95–165 larval days), which demonstrated that the half replacement of bran by MPS could not supply sufficient energy for growth as bran. However, the overlap between OLS and the feeding control of MLS inferred the greater resistance to MPS in older mealworms.

Similarities in larval weight increase and pupation pattern between the feeding control and mealworm cofed with bran at OLS implied that feeding mealworm with MPS and bran at this age eliminated the most harmful effects on development. Considering the great reduction of MPS uptakes and the high probability of pupation failure, we suggested stop feeding mealworms microplastic diet after 220 larval days.

3.5. Discussion

Mealworm was able to take in rigid microplastics in smaller sizes and the harmful impacts on survival, growth and metamorphosis would be effectively eliminated when exposed to microplastics in the presence of wheat bran at their OLS. On one hand, our research provided a practical and eco-friendly approach to utilizing mealworms as degradation reactors in recalcitrant microplastic waste management. On the other hand, we enriched the knowledge on the effects of microplastic feeding on plastic-degrading larvae. The direct harmful effects of MPS feeding on mealworms were weight loss and higher mortality. MPS feeding without bran produced higher mortalities at OLS than the starvation control, while YLS and MLS have the same highest mortalities (100 %). The highest average larval weight of mealworm fed with MPS and bran at OLS was 17 % higher than at MLS. The resistance to the harmful impacts caused by MPS ingestion increased with larval age, but such positive effect can be eliminated when ingesting excessive MPS.

The toxicities and impacts of microplastics on insects have been extensively studied in the past few years because of the widespread of microplastics in every environment. A positive correlation was observed between microplastic concentration in the sediment and both uptake of MPS and weight loss by aquatic lugworms (*Arenicola marina*) (Besseling et al., 2013). Significantly higher mortality and weight reduction were found when exposed to the high dose of microplastics to earthworms and *Folsomia candida*, but affected differently on their reproduction (Lwanga et al., 2016; Zhu et al., 2018a). Moreover, bacterial diversity and microbiota in the gut were altered under exposure. The response mechanism against microplastics in gut may be the same for terrestrial, aquatic and plastic degrading worms (Lwanga et al., 2016). The increased microplastics uptake dilutes and limits their nutrient bioavailability, which further causes weight loss and lower growth rates. The more adaptable to diverse living conditions and a broader range of food, such as grains, cereals, meal, flour, bran, crackers, feathers and dead insects (Ghaly and Alkoaik, 2009), may increase resistant strength under high doses microplastic feeding in mealworm.

Moreover, addition of bran in MPS-fed diet would improve the resistance to the negative effects caused by MPS. Larvae at MLS without bran all died while few larvae at OLS pupated in advance. In contrast, with half bran, larvae at MLS postponed their pupation, while those at OLS performed almost the same as the feeding control. Studies on the effects of microplastics or macroplastics on pupation of mealworms are lacking. Sole polylactic acid (PLA) diet accelerated mealworm pupation, which failed to eclose (Peng et al., 2021), but this was not in accordance with our research. Nutrient deficiencies may disturb the expression patterns of the hormonal system by failure in the production of prothoracicotrophic, molting or juvenile hormone. However, the mechanism and critical factors to regulate pupation under microplastic pressure are entirely lacking. It was stated that the high proportion of carbohydrates (73 %) and most of the B vitamins such as thiamin, riboflavin and pantothenic acid in bran significantly impacted the growth and survival of mealworms (Ho, 2018). According to the dynamic energy budget model (Matyja et al., 2020), energy was assimilated from food and accumulated as a reserve throughout the larval stage of mealworm, where energy was mobilized and released to maintain their lives. Pupation only occurs when the density of the reproduction buffer achieves a certain threshold (Llandres et al., 2015). Therefore, after reserving enough energy and essential nutrients from bran feeding at OLS, mealworm could pupate with little influence. In contrast, insufficient essential nutrients led to the postponement of pupation for worms at MLS.

The trophic transfer of microplastics in food chain also raised concerns. Mealworm is the traditional high-protein feed for poultry and fish. The ingested PS was mineralized to biomass in mealworms (Yang et al., 2015a), and plastic residuals were detected in their frass. Lwanga et al. (2017) confirmed that high concentration of microplastics was collected from chicken feces after ingestion of microplastics-fed earthworms. The food chain model for predator-prey relationship (Zhu et al., 2018b) confirmed that the co-occurrence of predators and preys enhances the transportation of microplastics in soil. It is clear that microplastics might be accumulated through the food chain in the natural environment. Besides, it is widely accepted that POPs would accumulate along with the transport of microplastics in the environment, which can be another hazard in trophic transfer. Thereby, the safety of mealworms fed with microplastics or the hazard of microplastics with POPs to their predators or the entire environment, and the corresponding posttreatment must be carefully considered in future plastic management.

4. Conclusion

Mealworms preferred ingesting MPS in smaller sizes, but the increment narrowed down as the particle size reduced to a certain extent. In our study, mealworms fed MPS at an older age took in more plastics, which caused higher mortality, weight loss and postponed/advanced pupation depending on the developmental stages as larvae. Depolymerization of the long-chain structure of MPS and oxidation of the functional groups were observed in frass excreted by mealworms only at old larval stage. Additionally, the harmful effects of MPS feeding on growth and survival of mealworms would largely be eliminated when supplied MPS with bran. Considering the ability of MPS consumption and depolymerization as well as the effects on development, mealworms at old larval stage with bran supplement are highly recommended in MPS degradation.

CRediT authorship contribution statement

Zheng Zhong: Conceptualization, Investigation, Methodology, Writing – original draft preparation, Validation, Software; Xi Zhou: Investigation; Yichun Xie: Writing – review & editing; Lee Man CHU*: Supervision, Methodology, Writing – review & editing, Funding acquisition, Project administration.

Data availability

Data will be made available on request.

Declaration of competing interest

The authors declare no competing financial interest.

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Appendix A. Supplementary data

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