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Assessing the impact of weathered polystyrene collected from the marine environment on oxidative stress responses in *Zophobas morio* larvae: A preliminary study

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ABSTRACT

Foamed polystyrene (PS) is a prevalent material in consumer products and thus represents one of the largest constituents of marine litter. PS may interact with and be ingested by various organisms, including insects, which are starting to be used in biodegradation of plastic wastes. This study examines the physiological performances and the potential oxidative stress related to dietary environmental PS exposure of *Z. morio* larvae. Experimental groups were fed different diets: bran and oatmeal (control), weathered PS collected from the marine environment, and virgin PS. Over a 30-day feeding period, larvae growth, survival, and PS consumption were measured, together with antioxidant enzymatic activities and gene expression profiles. Results showed that PS ingestion supports larval growth similarly to bran, but the two PS-fed groups consumed a different amount of plastic, suggesting that weathered PS might affect the patterns of PS consumption by larvae. We denoted that PS consumption was associated with a decline in total antioxidant capacity, which showed the highest decrease in the environmental PS group. Marked increases in oxidative stress biomarkers such as superoxide dismutase (SOD) activity/mRNA levels and malondialdehyde content were also observed in the environmental PS-fed group, indicating an elevated oxidative stress response, probably due to pollutants adsorbed/absorbed on PS. Differently to SOD, catalase at both mRNA and enzyme activity levels was found to be significantly reduced by PS digestion, regardless of PS type. The mRNA levels of glutathione s-transferase (GST) were significantly higher in the environmental PS-fed larvae compared to the virgin PS-fed group. In addition, Principal Component Analysis clearly distinguished between larvae fed different PS types, highlighting the enhanced oxidative stress caused by the ingestion of PS collected from marine environments. These results support the potential of insects in plastic biodegradation processes but also demonstrate the health hazards of using environmental PS as principal dietary component in *Z. morio* larvae, thus questioning their use in downstream productions.

1. Introduction

Foamed polystyrene (PS), a ubiquitous material used in a variety of consumer products (*e.g.* protective and food packaging, appliances and construction material), has emerged as a prominent component of marine litter and source of environmental microplastics (MPs). In this regard, recent data show that only construction and packaging generate about 530 000 tonnes of foamed PS waste produced in Europe (Turner, 2020). With its widespread distribution and persistence, foamed polystyrene particles are increasingly encountered in diverse ecosystems, including soils, sediments, and aquatic habitats (Siddiqui et al., 2023). The main routes of marine environment contamination by foamed plastic debris are represented by both internal waters and direct littering, especially in the littoral zone (Turner, 2020; Chelomin et al., 2023). In this latter, foamed waste is widely and rapidly dispersed due to its lightness and low-density properties and it is particularly difficult to retrieve (Turner, 2020). Floating and fragmented foamed PS is exposed to weathering that may produce significant quantities of MPs (Ho et al., 2020; Moyal et al., 2023). Foamed PS MPs may then interact with and be ingested by a range of organisms resulting in adverse health effects.

The potential consequences of plastic/microplastic ingestion on insects are of particular interest because some species have been recently

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used as food ingredients for farm animals and humans (Dobermann et al., 2017). In addition, insects play a key role in the actual strategy (*i.e.* biodegradation of plastics) suggested for reducing the burden of global plastic pollution (Siddiqui et al., 2024). The ability to digest plastic, including PS, by insect larvae has in fact been suggested as a promising sustainable approach for treatment of plastic waste. Plastic degradation by insects proved to be highly efficient due to a variety of gut microbiome-related mechanisms which allow to depolymerize and mineralize the ingested plastics (An et al., 2023; Siddiqui et al., 2024). Up to now different lepidopteran and coleopteran insect larvae have been employed in biodegradation of plastic products (Siddiqui et al., 2024). Among these, larvae of *Zophobas morio* have shown the capacity of eating foams of expanded polystyrene (EPS) in a fully gut microbe dependent way (Peng et al., 2020; Yang et al., 2020a; Tay et al., 2023). However, this species seems to have some limitations in the PS depolymerization process as demonstrated by the consistent quantities of undigested polymers with high molecular weights found in egested PS frass (Peng et al., 2020; Jung et al., 2023).

These plastic fragments could bioaccumulate in the gut before being excreted causing oxidative stress, a condition arising from an imbalance between reactive oxygen species (ROS) production and antioxidant defence mechanisms that leads to cellular damage and dysbiosis. Biodegradation of PS has been found to induce oxidative stress responses in mealworms resulting in negative physiological consequences (Peng et al., 2023). These findings were supported by a significant increase in the main oxidative stress biomarkers such as the activity of antioxidant enzymes (*i.e.,* superoxide dismutase, SOD), and lipid peroxidation (*i.e.*, malondialdehyde, MDA, content). SOD, as first-line antioxidant defense, was found to be up-regulated in mealworms that ingest MPs (Peng et al., 2023). These results clearly indicate an involvement of antioxidant systems in scavenging activity of free radicals produced by digestion and biodegradation of MPs. Similarly, increases in the MDA content, indicating oxidative damage, were reported in both plastic-fed mealworms (Peng et al., 2023) and MP-exposed earthworms (Cheng et al., 2020; Wang et al., 2022).

However, the majority of data on MP toxicity in plastivores are referred to laboratory studies focused on using commercial plastics. In this regard, laboratory experiments have exposed organisms to pre-production MPs, usually referred to as “virgin” MPs. These MPs are not representative of those found in the environment because they lack the complex chemical cocktail and biofilm that are associated with environmental MPs (Amariei et al., 2022; Bucci et al., 2022; Herrera et al., 2022). In fact, preliminary data have demonstrated that persistent organic pollutants (POPs), such as polycyclic aromatic hydrocarbon (PAHs), polychlorinated biphenyl (PCBs) and pesticides represent the most common categories of contaminants found on the surface of weathered MPs (Capriotti et al., 2021; Cormier et al., 2022; Wang et al., 2022; Bao et al., 2024). MP-associated POPs are at levels giving rise to both potential toxicity and transfer along the trophic web. It has been shown that the interaction between MPs and POPs can result in synergistic or antagonistic effects on organisms. A key factor influencing the adsorption of POPs to MPs is the presence of biofilm, a consortium of algae, bacteria, and other microorganisms that can increase the uptake of adsorbed contaminants and exacerbate their potential ecological impacts, creating a complex matrix of environmental contamination (Scott et al., 2021; Arienzo et al., 2021).

To date, little is known about the potential negative effects of weathered PS on insect larvae. Thus, the aim of the current study was to identify the adverse effects related to ingestion of environmental PS litter in *Z. morio* larvae by measuring oxidative stress biomarkers and physiological performances. Our study begins with a preliminary observation that superworms willingly eat weathered (*i.e.* environmental) PS, without adverse consequences neither on survival nor on growth. We then assessed the oxidative stress responses induced by a PS diet through the analyses of the total antioxidant capacity (TAC) as well as SOD, glutathione s-transferase (GST), and catalase (CAT) activities/transcripts and the concentration of MDA.

2. Materials and methods

2.1. Larvae and rearing condition

A total of 210 adult *Zophobas morio* larvae (initial average body weight: 773.7 ± 25.4 mg) were purchased from a commercial farm (Factory serpens lepidoctera; F.S.L.; Frosinone, Italy) and cultured indoors with their common food bran (Yang et al., 2020b; Zielinska et al., 2021; Fasce et al., 2022). Individuals were left to acclimate before the experiment set up and then placed in a 60 × 60 × 30 cm Vision Cage. Each Vision cage housed two boxes, each containing 35 larvae, assigned to one of the following experimental groups: control, environmental polystyrene (Env PS), and clean PS (PS). The cages were heated with a 28 W heating mat on the bottom using a Habistat pulse thermostat for temperature stabilization and the environmental conditions within the cages were kept constant (27.0 ± 0.5 °C temperature and 70 ± 10 % humidity), as monitored by a digital thermo-hygrometer (Yang et al., 2020a; Wang et al., 2022).

2.2. PS-feeding test

Individuals within each group were fed *ad libitum* a different experimental treatment diet over a 30-d exposure period, specifically: the control group was provided with bran, oatmeal, and carrot slices for hydration; the Env PS group was given a 10.00 g block of environmental PS, along with carrot slices; the PS group received 10.00 g of clean, synthetic PS together with carrot slices. The supplementation of bran and oatmeal diet with fresh carrots improve access to certain essential nutrients, positively affecting larvae growth in different darkling beetles (Tenebrionidae).

Environmental PS was collected from the shoreline at one site along a coastal area of the Marche region close to the Tronto river mouth (Italy; 42°53′43.4"N 13°54′57.6"E), which is known to be characterized by agricultural runoff and urban discharges (Palermo et al., 2008; Annibaldi et al., 2015; Cocci et al., 2017). Fourier-transform infrared spectroscopic analysis was used for the rapid identification and characterization of the PS. The clean PS was recovered from uncontaminated newly synthesised PS seed trays. Foods for the control group were periodically replaced in order to prevent mould growth. In the PS and Env PS groups, the block of PS was neither modified nor replaced during the entire experimental period.

2.3. Superworms growth conditions and measurements

The larval monitoring period lasted specifically from June 26, 2023 to July 25, 2023. During this time, measurements were taken twice a week to acquire data on the increasing weight of the larvae, the weight loss of the growth substrate (i.e. PS), mortality, and the general health status of the larvae involved in the experiment. A continuous visual inspection was adopted in order to prevent the proliferation of mould. Weight measuring of both PS and larvae were made with a digital electronic high-accuracy scale (>0.001) laboratory balance. The uneaten PS block and small undigested PS particles resulting from the superworms eating were subjected to a drying process at 40 °C for 2 h before each weighing. This method was applied because the moisture absorbed by the PS, due to environmental conditions, can increase its weight, leading to not reliable weight measurements. The amount of PS consumed was calculated as the initial weight of PS minus the combined weight of residual PS block and undigested PS fragments found in the boxes (Peng et al., 2022; Mapfumo et al., 2024; Lopes et al., 2024).

2.4. Molecular analysis of antioxidant-related genes

Total RNA was extracted from larval whole bodies using Trizol

Table 1
List of primers used in this study.

Gene	Primer Sequence (5'→3')	Size (bp)	GenBank	Efficiency (%)
CAT	CCTCAACCGCAATCCCAAAA			
	CCCCATAGTTGAAAAGCCGG	128	XM_965010.4	91.3
SOD	CAGGACAAAATGCCGACCAA			
	AGTCACATGAACCGGAGCTT	102	XM_064067465.1	94.9
GST	GCGAAGGTTCTGCTTACGTC			
	AGACTGAAGACTCCCGCAAA	125	XM_064060641.1	95.2
GAPDH	TCGTGCTTCTTTGGAACGTG			
	CAACCAAGAATCCACCTTCTGC	146	LC496554.1	98.8

Reagent (Thermo Scientific), according to the manufacturer's instructions (Invitrogen). The concentration and quality of RNA was achieved using a Qubit™ 4 Fluorometer (Thermo Fisher Scientific) with the Qubit RNA HS Assay Kit and the Qubit RNA IQ Assay Kit. cDNA was produced using the All-In-One 5X RT MasterMix according to manufacturer's instructions (G592; abm). For the genomic DNA (gDNA) removal the mixture was incubated at 37 °C for 15 min and cDNA synthesis was obtained at 60 °C for 20 min. The reaction was stopped by heating at 95 °C for 2 min.

SYBR green-based real time PCR with cross-species primer pairs (Table 1) was used for evaluating transcription profiles of CAT, SOD, GST target genes. Data were normalized to GAPDH levels as the most stable reference gene for each assay run (Tables S1, 2). The optimized reaction included: 5.0 µl yourSIAL® Green Mix 2X, 0.5 µl each of forward and reverse primers (10 µM) and 2 µl template cDNA, 2 µl DEPC-H_2O. Thermal-cycling temperatures were at 95 °C for 2 min, followed by 40 cycles at 95 °C for 5 s and 60 °C for 30 s. Dissociation-curve analysis indicated the production of a single amplification product. The products were confirmed by gel electrophoresis in 2.0 % agarose gels and visualized with a non-toxic dye (SafeView Classic, abm).

2.5. Antioxidant enzyme analysis and oxidative stress indices in the whole body

A portion of whole bodies from individual larva was homogenized on ice with a phosphate buffer solution (0.1 M; pH 7.5). Then, the homogenate was centrifuged at 10,000 rpm for 15 min at 4 °C, and the resulting supernatant was stored at -80 °C for biochemical assays. Total protein concentrations in crude enzyme extract were determined according to the Bradford Protein Assay (Biorad). Commercial kits were used for the determination of the activities of superoxide dismutase (Total Superoxide Dismutase Activity Assay Kit; MyBioSource MBS2540402), catalase (Catalase Assay Kit; MyBioSource MBS2540413) and glutathione S transferase (Glutathione S-transferase Microplate Assay Kit MBS8243172). The cumulative effect of all antioxidants was analysed by using a colorimetric assay kit (Total Antioxidant Capacity,T-AOC, Colorimetric Assay Kit; MyBioSource, MBS2540515) by measuring the absorbance at 520 nm. Quantitative measurement of MDA was achieved with a competitive inhibition enzyme immunoassay technique (General Malondialdehyde, MDA ELISA Kit; MyBioSource MBS2700234).

All procedures and sample dilutions were performed following the protocol described in the respective operating manuals.

2.6. Statistical analyses

To examine the normality and homoscedasticity of the data, the Shapiro-Wilk W-test and Levene's test were applied. One-way analysis of variance (ANOVA) followed by Tukey's post hoc test was used to detect significant differences between the treatments. Values of $p < 0.05$ were considered significant. Principal component analysis (PCA) was performed to assess the differences in composition profiles of all the oxidative stress markers in *Zophobas morio* larvae grouped by treatments.

2.7. Results and Discussion

As in previous studies (Yang et al., 2020a; Sun et al., 2022; An et al., 2023), exclusively PS-fed superworms easily approach the styrofoam blocks producing hollows as result of intense chewing and burrowing activities. The two PS-fed groups consumed a different amount of plastic, suggesting that weathered PS might affect larvae consumption patterns (Fig. 1). Indeed, the mass loss of PS was 54.9 ± 1.9 %, and 64.9 ± 7.4 % for the PS-fed and Env PS-fed, respectively, by the end of the experiment. However, the observed differences were not significantly different. Overall, our findings are slightly different to existing literature suggesting increased levels of Env PS foam mass loss in our study. The accumulated loss of the Env PS foam mass was close to that observed in previous studies with *Z. atratus* larvae fed PS plus bran over 33 days (Peng et al., 2020). The latter study has shown that co-feeding of both *Z. atratus* and *T. molitor* larvae with bran and PS significantly increases their PS consumption (Brandon et al., 2018; Yang et al., 2018; Peng et al., 2020). In this regard, our findings suggest the potential of PS, particularly the Env PS in stimulating the feeding behaviour of superworms. Survival rate and average body weight of superworms were monitored during the experiment and are reported in Fig. 1.

The survival rates of groups fed with PS appeared both slightly lower than those of the control group during the feeding trial but were not significantly different neither from each other nor from control (Fig. 1A). Overall, these results seem in contrast to a previously reported

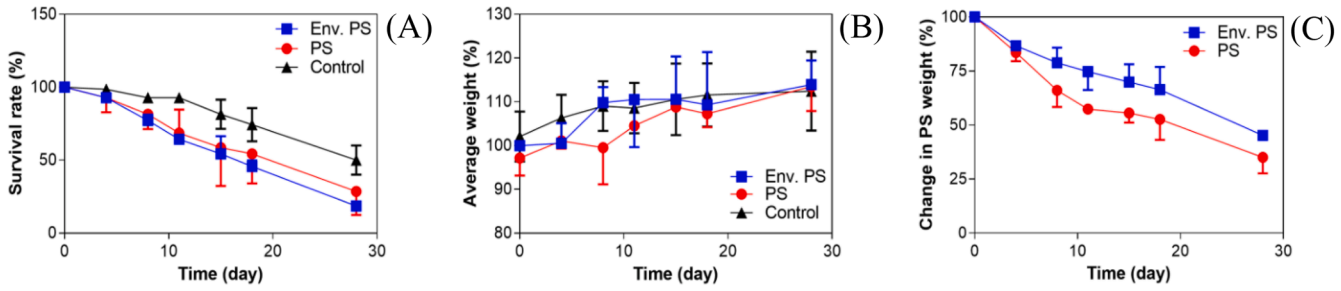

Fig. 1. Survival indices of *Zophobas morio* larvae and polystyrene (PS) consumption rate over the test period. (A) Survival rate (%), (B) weight change (%) and (C) PS consumption (%) by superworms *Zophobas morio* fed virgin PS or environmental PS (Env PS).

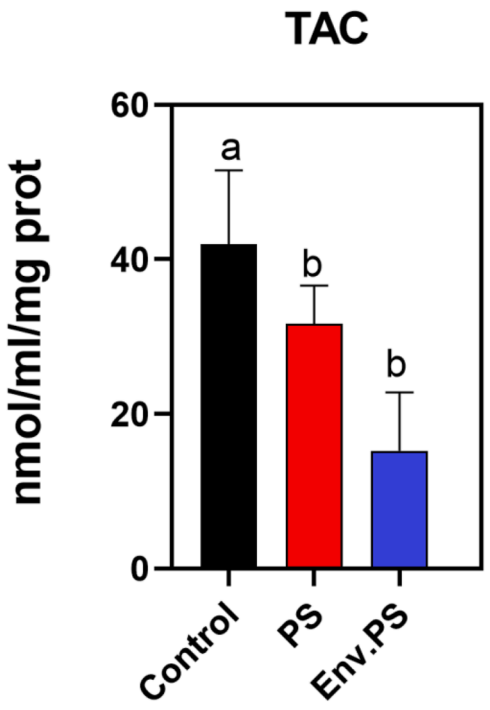

Fig. 2. Changes in the total antioxidant capacity (TAC) of superworms (*Zophobas morio*) after dietary exposure to virgin PS (PS) or environmental PS (Env PS). Different letters denote a significant differences (*p < 0.05).

survival rate of ~90 % after 35 days, observed at similar experimental settings but with significantly lower larvae densities (Peng et al., 2020). Our recorded survival rates are quite close to that (~70 %) reported by Yang et al. (2020b) which used a similar population density and that (>80 %) of Zhong et al. (2023) which however used a younger larval stage of *T. molitor*. The average weights of PS fed and control superworms were not statistically different at the end of the feeding trial (Fig. 1B). These findings suggest that ingesting PS as an exogenous food source could sustain growth and life activities of superworms as much as bran. However, data on the survival rates could indicate a potential influence of larval age on the safety of plastic biodegradation process in *Z. morio*. Basically, our results are in line with those reported in previous studies with different species of plastivores (Brandon et al., 2018; Yang et al., 2018; Peng et al., 2020).

PS consumption was previously associated with increased endogenous oxidative damage to superworms (Zielinska et al., 2021; Chen et al., 2023). Interestingly, herein we observed that the increase in oxidative stress levels was paralleled by a decrease in TAC activity. TAC displayed a significant reduction in both PS feeding trial groups, showing the highest decrease in the Env PS group (Fig. 2).

After 30 days of continuous plastic feeding, both the activities and mRNA levels of antioxidant and detoxifying enzymes (*i.e.* SOD/CAT/GST) were found differently and significantly modulated in PS-fed superworms (Fig. 3). Notably, both gene expression and activity of SOD were all significantly higher in Env PS-fed than the bran-fed control or (see the mRNA levels) the PS-fed group (Fig. 3A). This data points out very clearly that the oxidative stress response is mainly driven by what accumulates and potentially leaches from Env PS (*e.g.* POPs) producing higher levels of H_2O_2. Differently to SOD, CAT was found to be significantly modulated by PS digestion, regardless of PS type, at both mRNA and enzyme activity levels (Fig. 3B). Muhammad et al. (2021) reported a strong size-dependent induction of SOD, but not CAT, gene expression in response to PS in silkworms, highlighting a defence mechanism that prevents oxidative stress. Changes in CAT activity following microplastic exposures were reported in earthworms suggesting the increase of H_2O_2 through disproportionation reaction (Chen et al., 2020).

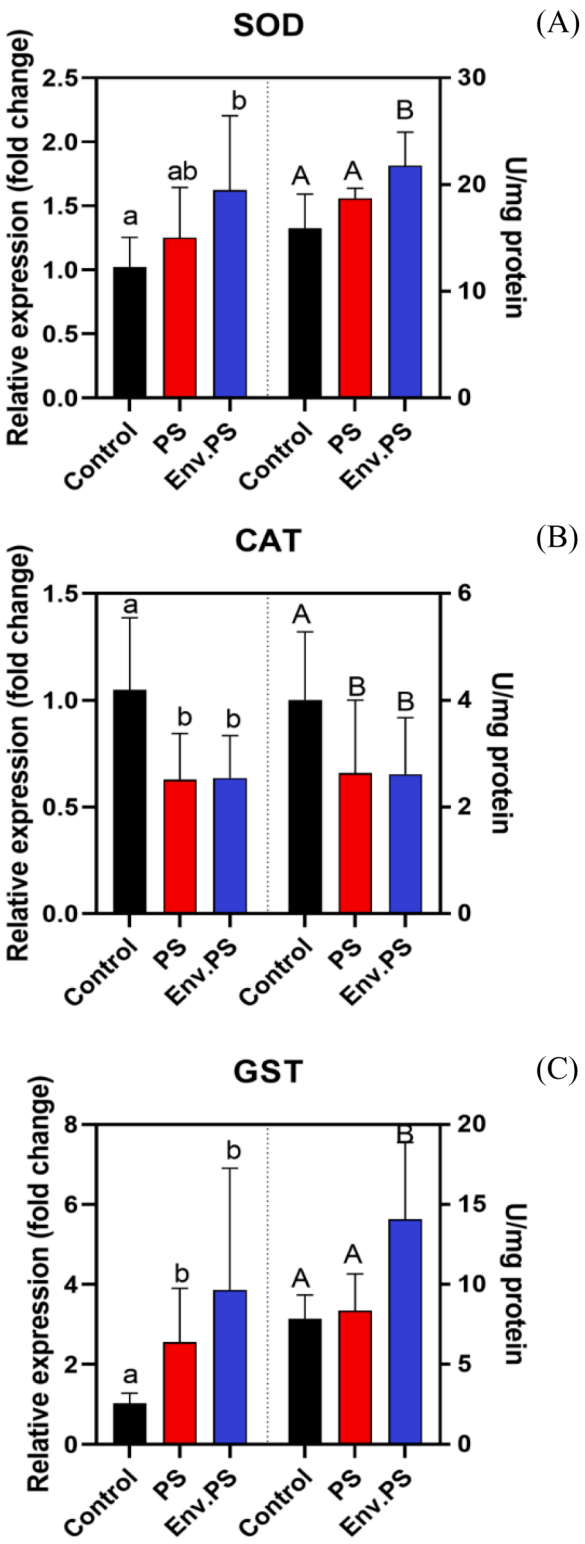

Fig. 3. Gene expression (left panel) and activity (right panel) of the enzyme superoxide dismutase (A; SOD) catalase (B; CAT) and glutathione S transferase (C; GST) in *Zophobas morio* larvae fed different diets: bran and oatmeal (control), weathered PS collected from the marine environment, and virgin PS. Lowercase letters indicate a statistical significant ($p<0.05$) difference in mRNA levels and uppercase letters denote a statistical significant ($p<0.05$) difference in the enzymatic activity among groups.

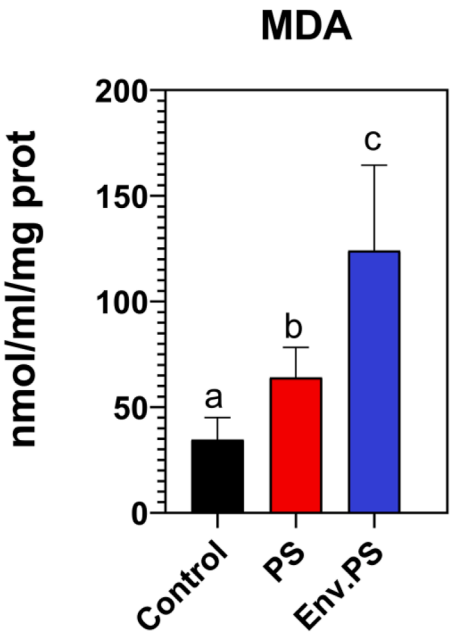

Fig. 4. The effect of PS feeding on malondialdehyde (MDA) content in *Zophobas morio* larvae after 30-d dietary exposure to virgin PS (PS) or environmental PS (Env PS). Lowercase letters indicate a statistical significant ($p<0.05$) difference in MDA levels among groups.

In particular, a modulation of CAT, specifically attributable to PS fragments, has been observed in different animals including crustaceans and fish (Lei et al., 2018; Liu et al., 2019). The combination of increased SOD and decreased CAT in the gut of superworms thus suggests an imbalance in antioxidant defence, a condition that could cause DNA damage. The antioxidant response is completed by the detoxification action of GST that is one further biomarker of MPs-induced oxidative stress (Fig. 3C). Similarly to SOD, feeding of PS induced a significant increase in GST gene expression with respect to bran dieting. Notably, the mRNA levels of GST were significantly higher in the Env PS-fed larvae compared to the virgin PS-fed group. The GST expression profiles were consistent with the activity patterns only in larvae exposed to Env PS. This outcome suggests that the well-known effects of PS digestion on ROS production in mealworms (Peng et al., 2023) could be amplified by the presence of chemical pollutants absorbed/adsorbed on PS. In this regard, significantly elevated levels of GST activity were found in adult beetles, *Leptinotarsa decemlineata,* exposed to pesticides (Clements et al., 2018). Transcriptomic analysis of mealworms (*Tenebrio molitor*) after long-term exposure to plastic feeding revealed an upregulation of GSTs in the plastic-fed groups suggesting that intermediates of PS degradation could act as xenobiotics (Zhong et al., 2022). As the role of GST is pivotal for detoxifying lipid peroxidation products, we evaluated the oxidative damage in lipids by assessing MDA content (Fig. 4).

Feeding of PS significantly increased MDA levels within superworms when compared to control individuals. These results are consistent with the accumulation of MDA content found in both mealworms and earthworms under exposure to plastics (Cheng et al., 2020; Peng et al., 2023). However, the observed effect is again significantly higher in larvae from the Env PS group compared to "virgin" PS specimens. These observations corroborated that digestion and biodegradation of polluted-PS would overwhelm the ability of the GST system to cope with oxidative stress induced by lipid peroxidation even more than virgin PS.

Finally, we carried out a Principal Component (PCA) analysis with vectors using values of all the oxidative stress markers assessed to test whether the major groups could be distinguished, and which variables would affect such distinction (Fig. 5). Control larvae were mostly grouped on the right part of the biplot, and all of the Env PS-fed specimens were spread in the left part of the biplot. Interestingly, Fig. 5 shows that PS-fed and control superworms could not be differentiated with the adopted biomarkers. The vector analysis suggested that increased levels of MDA, SOD (both transcripts and activity) and GST expression were more associated with Env PS-fed larvae, whereas the values of TAC and CAT expression were negatively associated with the PS-fed group. This finding highlights the induction of oxidative stress following PS consumption, with a higher expression of oxidative markers (*i.e.* MDA and SOD activity) being able to distinguish Env PS (*i.e.* polluted PS) from virgin PS.

3. Conclusion

The present work supports previous studies showing that

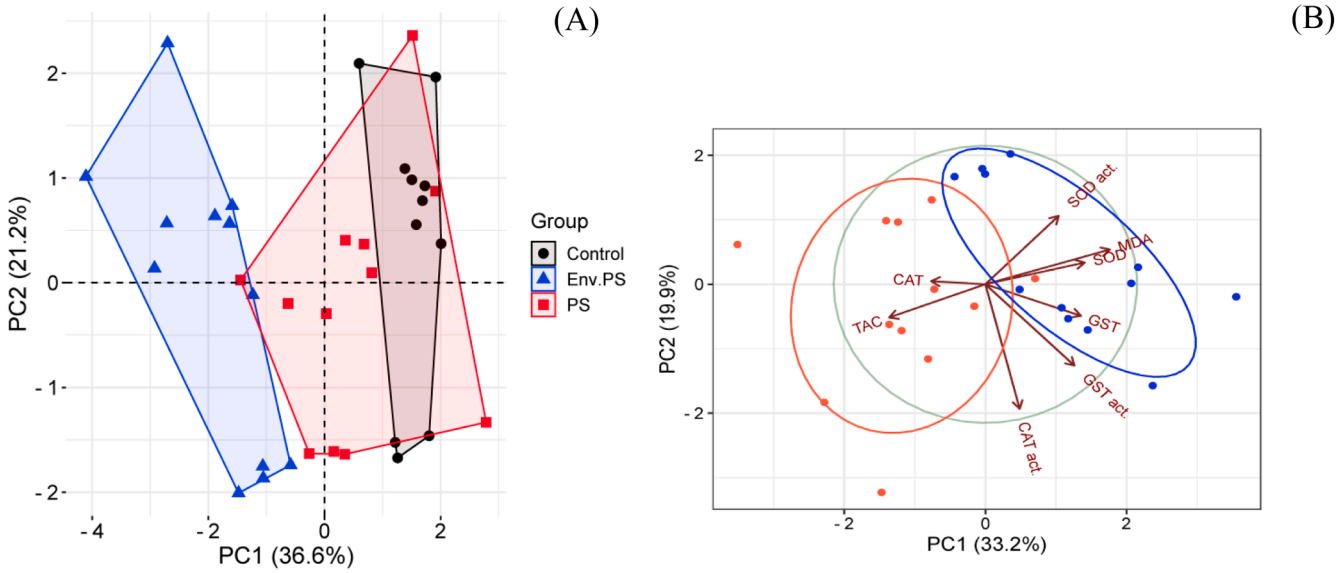

Fig. 5. Principal component analysis (PCA) results for all the oxidative stress markers in *Zophobas morio* larvae grouped by treatment (Black: Control; Red: PS-fed; Blue: Environmental PS-fed. (A) PCA with polygons demarcating the treatment and control groups. (B) PCA correlation biplot between the two PS treatments. Dots represent samples and arrows represent variables. Ellipses correspond to the confidence interval in each case.

superworms can be profitably used in reducing PS waste (Gan et al., 2021; Jung et al., 2023; Quan et al., 2023). However, our results illustrate that Env PS may have adverse biochemical effects on *Z. morio*, which provides a reference for studying the effect of marine litter on the health status of insects. In particular, downstream applications such as animal or human feed production using plastic-eating superworms might not be achievable due to the toxicity of PS to insect organisms. The study that has been carried out also confirms that feeding of "virgin" PS may be much less impactful for insects. In contrast, PS waste may adsorb and concentrate other toxic pollutants (*e.g.* metals, PAHs, pesticides, pharmaceuticals) from the surrounding environment. These sorbed pollutants can be released from Env PS once they are ingested by superworms, thus triggering a synergistic effect with plastic ingestion. Understanding toxicity of Env PS on plastivores is therefore critical for developing sustainable plastic waste biodegradation and downstream processing.

CRediT authorship contribution statement

Paolo Cocci: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Valentina Mazzocchi:** Investigation, Formal analysis. **Mario Marconi:** Writing – original draft, Methodology, Investigation, Conceptualization. **Gilberto Mosconi:** Investigation, Formal analysis. **Francesco Alessandro Palermo:** Writing – review & editing, Writing – original draft, Supervision, Resources, Investigation, Formal analysis, 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.

Data availability

Data will be made available on request.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.envadv.2024.100593.

References

Amariei, G., Rosal, R., Fernandez-Pinas, F., Koelmans, A.A., 2022. Negative food dilution and positive biofilm carrier effects of microplastic ingestion by D. magna cause tipping points at the population level. Environ. Pollut. 294, 118622.

An, R., Liu, C., Wang, J., Jia, P., 2023. Recent advances in degradation of polymer plastics by insects inhabiting microorganisms. Polymers 15. . (Basel).

Annibaldi, A., Illuminati, S., Truzzi, C., Libani, G., Scarponi, G., 2015. Pb, Cu and Cd distribution in five estuary systems of Marche, central Italy. Mar. Pollut. Bull. 96, 441–449.

Arienzo, M., Ferrara, L., Trifuoggi, M., 2021. Research progress in transfer, accumulation and effects of microplastics in the oceans. J. Mar. Sci. Eng. 9, 433.

Bao, X., Gu, Y.T., Chen, L., Wang, Z.J., Pan, H., Huang, S.R., Meng, Z.Y., Chen, X.J., 2024. Microplastics derived from plastic mulch films and their carrier function effect on the environmental risk of pesticides. Sci. Total Environ. 924.

Brandon, A.M., Gao, S.H., Tian, R.M., Ning, D.L., Yang, S.S., Zhou, J.Z., Wu, W.M., Criddle, C.S., 2018. Biodegradation of polyethylene and plastic mixtures in mealworms (Larvae of *Tenebrio molitor*) and effects on the gut microbiome. Environ. Sci. Technol. 52, 6526–6533.

Bucci, K., Bikker, J., Stevack, K., Watson-Leung, T., Rochman, C., 2022. Impacts to larval fathead minnows vary between preconsumer and environmental microplastics. Environ. Toxicol. Chem. 41, 858–868.

Capriotti, M., Cocci, P., Bracchetti, L., Cottone, E., Scandiffio, R., Caprioli, G., Sagratini, G., Mosconi, G., Bovolin, P., Palermo, F.A., 2021. Microplastics and their associated organic pollutants from the coastal waters of the central Adriatic Sea (Italy): investigation of adipogenic effects *in vitro*. Chemosphere 263, 128090.

Chelomin, V.P., Dovzhenko, N.V., Slobodskova, V.V., Mazur, A.A., Kukla, S.P., Zhukovskaya, A.F., 2023. Expanded polystyrene-debris-induced genotoxic effect in littoral organisms. Toxics 11.

Chen, Y., Liu, X., Leng, Y., Wang, J., 2020. Defense responses in earthworms (Eisenia fetida) exposed to low-density polyethylene microplastics in soils. Ecotoxicol. Environ. Saf. 187, 109788.

Chen, Z., Zhang, Y., Xing, R., Rensing, C., Lu, J., Chen, M., Zhong, S., Zhou, S., 2023. Reactive oxygen species triggered oxidative degradation of polystyrene in the gut of superworms (Zophobas atratus Larvae). Environ. Sci. Technol. 57, 7867–7874.

Cheng, Y., Zhu, L., Song, W., Jiang, C., Li, B., Du, Z., Wang, J., Wang, J., Li, D., Zhang, K., 2020. Combined effects of mulch film-derived microplastics and atrazine on oxidative stress and gene expression in earthworm (Eisenia fetida). Sci. Total. Environ. 746, 141280.

Clements J., Schoville S., Clements A., Amezian D., Davis T., Sanchez-Sedillo B., Bradfield C., Huseth A.S., Groves R.L., 2018. Agricultural fungicides inadvertently influence the fitness of Colorado potato beetles, *Leptinotarsa decemlineata*, and their susceptibility to insecticides. Sci Rep-Uk 8.

Cocci, P., Capriotti, M., Mosconi, G., Palermo, F.A., 2017. Transcriptional variations in biomarkers of *Mytilus galloprovincialis* sampled from Central Adriatic coastal waters (Marche region, Italy). Biomarkers 22, 537–547.

Cormier, B., Cachot, J., Blanc, M., Cabar, M., Clerandeau, C., Dubocq, F., Le Bihanic, F., Morin, B., Zapata, S., Begout, M.L., Cousin, X., 2022. Environmental microplastics disrupt swimming activity in acute exposure in Danio rerio larvae and reduce growth and reproduction success in chronic exposure in D. rerio and Oryzias melastigma. Environ. Pollut. 308, 119721.

Dobermann, D., Swift, J.A., Field, L.M., 2017. Opportunities and hurdles of edible insects for food and feed. Nutr. Bull. 42, 293–308.

Fasce, B., Ródenas, L., López, M.C., Moya, V.J., Pascual, J.J., Cambra-López, M., 2022. Nutritive value of wheat bran diets supplemented with fresh carrots and wet Brewers' grains in yellow mealworm. J. Insect Sci. 22, 3.

Gan, S.K., Phua, S.X., Yeo, J.Y., Heng, Z.S., Xing, Z., 2021. Method for zero-waste circular economy using worms for plastic agriculture: augmenting polystyrene consumption and plant growth. Methods Protoc. 4.

Herrera, A., Acosta-Dacal, A., Perez Luzardo, O., Martinez, I., Rapp, J., Reinold, S., Montesdeoca-Esponda, S., Montero, D., Gomez, M., 2022. Bioaccumulation of additives and chemical contaminants from environmental microplastics in European seabass (*Dicentrarchus labrax*). Sci. Total. Environ. 822, 153396.

Ho, W.K., Law, J.C., Zhang, T., Leung, K.S., 2020. Effects of weathering on the sorption behavior and toxicity of polystyrene microplastics in multi-solute systems. Water Res. 187, 116419.

Jung, H.Y.N., Shin, G., Park, S.B., Jegal, J., Park, S.A., Park, J., Oh, D.X., Kim, H.J., 2023. Circular waste management: superworms as a sustainable solution for biodegradable plastic degradation and resource recovery. Waste Manag. 171, 568–579.

Lei, L., Wu, S., Lu, S., Liu, M., Song, Y., Fu, Z., Shi, H., Raley-Susman, K.M., He, D., 2018. Microplastic particles cause intestinal damage and other adverse effects in zebrafish *Danio rerio* and nematode Caenorhabditis elegans. Sci. Total. Environ. 619-620, 1–8.

Liu, Z., Yu, P., Cai, M., Wu, D., Zhang, M., Huang, Y., Zhao, Y., 2019. Polystyrene nanoplastic exposure induces immobilization, reproduction, and stress defense in the freshwater cladoceran Daphnia pulex. Chemosphere 215, 74–81.

Lopes, I.G., Wiklicky, V., Vinnerås, B., Yong, J.W.H., Lalander, C., 2024. Recirculating frass from food waste bioconversion using black soldier fly larvae: Impacts on process efficiency and product quality. J. Environ. Manage. 366, 121869.

Mapfumo, E., Hemmerling, D., Bukutu, C., Acharya, S., Paterson, E., Nobert, S., MacElheren, M., Golizeh, M., 2024. Superworm (Coleoptera: Tenebrionidae, *Zophobas morio*) degradation of UV-pretreated expanded polystyrene. FACETS 9, 1–15.

Moyal, J., Dave, P.H., Wu, M.J., Karimpour, S., Brar, S.K., Zhong, H., Kwong, R.W.M., 2023. Impacts of biofilm formation on the physicochemical properties and toxicity of microplastics: a concise review. Rev. Environ. Contam. Toxicol. 261.

Muhammad, A., Zhou, X., He, J., Zhang, N., Shen, X., Sun, C., Yan, B., Shao, Y., 2021. Toxic effects of acute exposure to polystyrene microplastics and nanoplastics on the model insect, silkworm *Bombyx mori*. Environ. Pollut. 285, 117255.

Palermo, F.A., Mosconi, G., Angeletti, M., Polzonetti-Magni, A.M., 2008. Assessment of water pollution in the Tronto River (Italy) by applying useful biomarkers in the fish model *Carassius auratus*. Arch. Environ. Contam. Toxicol. 55, 295–304.

Peng, B.Y., Li, Y., Fan, R., Chen, Z., Chen, J., Brandon, A.M., Criddle, C.S., Zhang, Y., Wu, W.M., 2020. Biodegradation of low-density polyethylene and polystyrene in superworms, larvae of Zophobas atratus (Coleoptera: Tenebrionidae): Broad and limited extent depolymerization. Environ. Pollut. 266, 115206.

Peng, B.Y., Sun, Y., Wu, Z., Chen, J., Shen, Z., Zhou, X., Wu, W.-M., Zhang, Y., 2022. Biodegradation of polystyrene and low-density polyethylene by *Zophobas atratus* larvae: Fragmentation into microplastics, gut microbiota shift, and microbial functional enzymes. J. Clean. Prod. 367, 132987.

Peng, B.Y., Sun, Y., Zhang, X., Sun, J., Xu, Y., Xiao, S., Chen, J., Zhou, X., Zhang, Y., 2023. Unveiling the residual plastics and produced toxicity during biodegradation of polyethylene (PE), polystyrene (PS), and polyvinyl chloride (PVC) microplastics by mealworms (Larvae of Tenebrio molitor). J. Hazard. Mater. 452, 131326.

Quan, Z., Zhao, Z., Liu, Z., Wang, W., Yao, S., Liu, H., Lin, X., Li, Q.X., Yan, H., Liu, X., 2023. Biodegradation of polystyrene microplastics by superworms (larve of *Zophobas atratus*): Gut microbiota transition, and putative metabolic ways. Chemosphere 343, 140246.

Scott, J.W., Gunderson, K.G., Green, L.A., Rediske, R.R., Steinman, A.D., 2021. Perfluoroalkylated substances (PFAS) associated with microplastics in a lake environment. Toxics 5, 106.

Siddiqui, S.A., Manap, A.S.A., Kolobe, S.D., Monnye, M., Yudhistira, B., Fernando, I., 2024. Insects for plastic biodegradation - A review. Process Saf. Environ. Prot. 186, 833–849.

Siddiqui, S.A., Singh, S., Bahmid, N.A., Shyu, D.J.H., Dominguez, R., Lorenzo, J.M., Pereira, J.A.M., Camara, J.S., 2023. Polystyrene microplastic particles in the food chain: characteristics and toxicity. A review. Sci. Total. Environ. 892.

Sun, J.R., Prabhu, A., Aroney, S.T.N., Rinke, C., 2022. Insights into plastic biodegradation: community composition and functional capabilities of the superworm (*Zophobas morio*) microbiome in styrofoam feeding trials. Microb Genom. 8.

Tay, J.H., Asib, N., Abd Aziz, N.A., Tan, G.H., 2023. Biodegradation of expanded and extruded polystyrene with different diets by using larvae (Coleoptera: Tenebrionidae). Pertanika J. Trop. Agric. 46, 459–483.

Turner, A., 2020. Foamed polystyrene in the marine environment: sources, additives, transport, behavior, and impacts. Environ. Sci. Technol. 54, 10411–10420.

Wang, Q.L., Adams, C.A., Wang, F.Y., Sun, Y.H., Zhang, S.W., 2022. Interactions between microplastics and soil fauna: A critical review. Crit. Rev. Environ. Sci. Technol. 52, 3211–3243.

Yang L., Liu Y., Gao J., Peng X.W., Bai Z.H., Zhuang X.L., 2020a. Biodegradation of expanded polystyrene foams in zophobas morio: effects of gut microbiota. Huan. Jing. Ke Xue 41, 5609-5616.

Yang, S.S., Brandon, A.M., Andrew Flanagan, J.C., Yang, J., Ning, D., Cai, S.Y., Fan, H.Q., Wang, Z.Y., Ren, J., Benbow, E., Ren, N.Q., Waymouth, R.M., Zhou, J., Criddle, C.S., Wu, W.M., 2018. Biodegradation of polystyrene wastes in yellow mealworms (larvae of *Tenebrio molitor* Linnaeus): factors affecting biodegradation rates and the ability of polystyrene-fed larvae to complete their life cycle. Chemosphere 191, 979–989.

Yang, Y., Wang, J., Xia, M., 2020b. Biodegradation and mineralization of polystyrene by plastic-eating superworms Zophobas atratus. Sci. Total Environ. 708, 135233.

Zhong, Z., Nong, W., Xie, Y., Hui, J.H.L., Chu, L.M., 2022. Long-term effect of plastic feeding on growth and transcriptomic response of mealworms (*Tenebrio molitor* L.). Chemosphere 287, 132063.

Zhong, Z., Zhou, X., Xie, Y., Chu, L.M., 2023. The interplay of larval age and particle size regulates micro-polystyrene biodegradation and development of Tenebrio molitor L. Sci. Total Environ. 857, 159335.

Zielinska, E., Zielinski, D., Jakubczyk, A., Karas, M., Pankiewicz, U., Flasz, B., Dziewiecka, M., Lewicki, S., 2021. The impact of polystyrene consumption by edible insects and on their nutritional value, cytotoxicity, and oxidative stress parameters. Food Chem. 345.