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Reply to the 'Comment on "Expanded polystyrene is not chemically degraded by mealworms"' by W.-M. Wu and C. S. Criddle, *RSC Sustainability*, 2026, 4, DOI: 10.1039/D5SU00247H

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We thank Wu and Criddle for their commentary and welcome this scientific dialogue. Our approach was designed to rigorously assess the potential for insect-mediated expanded polystyrene (EPS) degradation by comparing pure and commercial EPS under controlled conditions that eliminated cannibalism artifacts. Our results demonstrate that mealworms mechanically fragment EPS but achieve no genuine biochemical degradation. Pure EPS remained chemically unaffected after gut passage, while commercial EPS showed only modest additive-mediated oxidative changes, and not enzymatic polymer backbone cleavage. Additional studies on superworms (*Zophobas morio*) with both EPS and polyvinyl chloride (PVC) yielded corresponding results, confirming that the absence of plastic metabolism spans multiple insect species and polymer types. Here we address Wu and Criddle's concerns regarding mass balance, isotopic interpretation, and analytical methods while demonstrating how experimental artifacts in previous studies generate false evidence for biodegradation. Simple scalability calculations reveal the fundamental impracticality of any insect-based approach: treating one ton of polystyrene would require over sixty million mealworms, producing more than four tons of dead biomass while generating vast quantities of microplastics and achieving zero meaningful degradation. Our controlled methodology establishes that insect-mediated plastic treatment is neither chemically viable nor economically feasible.

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Sustainability spotlight

This study provides definitive evidence that mealworms and superworms cannot biodegrade polystyrene or PVC, instead fragmenting these polymers into extensive amounts of particulates while achieving zero chemical degradation. Our cross-species validation and rigorous analytical methods resolve conflicting claims in the literature and demonstrate that insect-mediated plastic treatment faces insurmountable biological and economic barriers. Processing one ton of polystyrene would require over 60 million mealworms, producing more than 4 tons of dead biomass and generating 4–8 tons of CO_2-equivalent emissions – exceeding the environmental impact of conventional waste management. By eliminating experimental artifacts like cannibalism, this work prevents misdirection toward economically unviable solutions. These findings support SDG 12 (Responsible Consumption and Production) and SDG 13 (Climate Action) by redirecting innovation toward scalable technologies that genuinely reduce plastic waste burdens.

Introduction

Biodegradation of expanded polystyrene (EPS) and other synthetic polymers by insect larvae has been investigated extensively since Yang *et al.* (2015) first reported polystyrene degradation by yellow mealworms.[1] Over the past decade, numerous studies have claimed that darkling beetle larvae (*Tenebrio molitor*, *Tenebrio obscurus*, and *Zophobas atratus*) can degrade various plastics, with reported plastic mass reductions ranging from 30–70%.[2–8] However, the field faces significant methodological inconsistencies and contradictory findings regarding the mechanisms underlying these reported degradation capabilities.

Examination of published studies reveals several recurring limitations that complicate interpretation of results. Many studies employ group housing densities of 100–250 larvae per container without individual monitoring[2–4,7,9] which confounds survival and growth data through cannibalism. Yang *et al.*[9] explicitly mentioned that "both the unfed mealworms and mealworms fed PS alone engaged in cannibalism," with survivors consuming dead mealworms to maintain biomass. Additionally, most studies rely primarily on plastic mass loss measurements without comprehensive molecular analysis[10,11] potentially conflating mechanical processing with biochemical

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degradation. The use of commercial plastic products containing variable additive compositions without comparing to additive-free polymers further complicates interpretation, making it impossible to distinguish additive-mediated oxidation from genuine backbone depolymerization.[1–6] Finally, incomplete mass balance analyses fail to account for physical fragmentation into microplastics that become unrecoverable, leading to systematic overestimation of degradation.[12,13]

These limitations have led to conflicting claims about insect-mediated plastic degradation capabilities. While some researchers report substantial biodegradation with maintained larval health,[2–4] others observe toxicity and mortality when insects are fed plastic-only diets,[11,14] with survival rates comparable to or worse than complete starvation. The inconsistent application of analytical protocols for distinguishing between mechanical consumption and biochemical utilization has perpetuated these contradictions in the literature. For example, early claims of polyethylene biodegradation by wax moth larvae were refuted when high-resolution Raman microscopy revealed only mechanical hole-making without digestion,[15] demonstrating how rigorous analytical methods are essential to distinguish mechanical fragmentation and additive-mediated oxidation from true enzymatic depolymerization.

In our EPS study,[16] we investigated whether mealworms can degrade expanded polystyrene by feeding individually housed larvae pure EPS (without additives) and commercial EPS (containing additives), monitoring growth, survival, and chemical changes over 125 days. This approach eliminated cannibalism artifacts and distinguished additive effects from polymer backbone degradation. Results showed both formulations failed to sustain growth and proved toxic, with survival below starvation controls and no larvae completing metamorphosis. GPC analysis revealed molecular weight reductions in commercial EPS (12.7% decrease in M_w) characterized by selective reduction of higher molecular weight fractions, while pure EPS showed no statistically significant changes after gut passage. These findings indicate that additives facilitated limited oxidative degradation rather than enzymatic depolymerization of the polymer backbone. We concluded that mealworms cannot degrade polystyrene nor derive nutrition from EPS.

In their recent comment on our study,[17] Wu and Criddle raise important questions about our experimental approach and data interpretation. While we appreciate their attention to population variability and analytical methods, we believe our methodology helps clarify contradictions in the biodegradation literature.[2–4,11,14] Establishing rigorous experimental standards is crucial for realistic assessment of biological plastic treatment approaches.[18–20] Current waste management frameworks, including the EU's Packaging and Packaging Waste Regulation,[21] US EPA's National Strategy,[22] and EU Waste Framework Directive,[22] allow "biodegradable" designations without mandating the controlled experiments, pure polymer testing, cannibalism elimination, isotopic validation,[23] that our and others' work demonstrates are essential for distinguishing genuine enzymatic depolymerization from additive oxidation and physical fragmentation.

Results and discussion

Understanding evidence of PS biodegradation: addressing confounding factors

Wu and Criddle raise several concerns about our experimental design and conclusions, particularly questioning whether our findings from Australian mealworms can be generalized to all populations. We appreciate their acknowledgment that our approach of comparing pure *versus* commercial polymers is "technically sound" for characterizing biodegradation. We agree that many studies in the literature have documented apparent polystyrene degradation by mealworms from various geographical sources. However, we believe our investigation helps clarify the mechanisms underlying these observations.

Our study was specifically designed to address two critical confounding factors that have influenced previous interpretations. First, we examined the role of EPS additives by comparing commercial EPS, which contains additives, fillers, and residual processing agents, to laboratory-made pure EPS without additives. This allowed us to distinguish apparent degradation signals arising from non-polymer components from those attributable to the polymer backbone itself.

Second, we eliminated larval cannibalism during experiments, preventing the confounding introduction of biogenic carbon from insect tissue into frass, gut content, or biomass measurements. This confound is substantial: Yang *et al.*[9] reported PS-fed mealworms (120 larvae at ~2 larvae per cm^2) achieved 86.7 ± 3.3% survival over 32 days, exceeding unfed controls (54.2 ± 2.5%), yet both groups scavenged nutrients by consuming shed exoskeletons and dead mealworms. Similarly, Bożek *et al.*[11] acknowledged cannibalism "can skew survival data" but used group housing (20 larvae per container), where protein, lipid, and carbohydrate measurements from homogenized larvae could incorporate cannibalized individuals, artificially inflating apparent nutrient retention. Both studies used commercial plastics without pure polymer controls, preventing definitive conclusions about polymer backbone degradation *versus* additive modification. Our individual housing eliminates these artifacts, demonstrating that apparent survival advantages in group-housed studies reflect cannibalistic supplementation rather than PS metabolism.

These methodological differences directly explain the apparent discrepancies between our findings and previous studies. Rather than viewing this as a limitation, we believe it demonstrates the critical need for standardized experimental protocols that can distinguish genuine biodegradation from experimental artifacts.

Mass balance claims and their pitfalls: artifacts in CO_2 recovery, isotopic shifts, and missing mass?

Assessing mass balance in insect–plastic feeding studies requires caution, as technical limitations make accurate accounting difficult. In our experiments, we consistently observed substantial accumulation of small plastic particles in and around the rearing containers during EPS feeding (Fig. 1 and Video S1 in the SI), demonstrating that physical

fragmentation—not biodegradation—represents the major fate pathway for ingested EPS. As documented in Video S1, these particles are extremely light and difficult to collect quantitatively, with even smaller fragments generated during mastication being undetectable visually while potentially representing significant mass.

These methodological limitations expose a fundamental flaw in how biodegradation is often assessed. Apparent "mass loss" of expanded polystyrene after feeding trials usually reflects physical fragmentation rather than enzymatic depolymerization. Gravimetric recovery of macroscopic EPS particles underestimates the production of micro- and nanoplastics during mastication, creating an illusion of degradation when the polymer remains chemically intact. Accurately recovering and characterizing these small fragments from biological matrices remains technically challenging, with validated protocols still under development. Although Peng *et al.*[24] demonstrated nanoplastic extraction from frass, comprehensive quantitative recovery is not yet established.

As a result, unrecovered microparticles are easily misattributed to degradation. Many studies calculate biodegradation by subtracting the mass of recovered plastic and solvent-extracted frass from the initial input, without validated methods to detect small particles in frass, gut contents, or the environment.[1,2,9,25] As Jendrossek (2024)[23] critically notes, apparent "missing mass" in many published studies more likely reflects unrecovered particulate matter rather than genuine biodegradation.

Distinguishing CO_2 from metabolism *versus* artifacts

The study of Yang *et al.*[1] represents the sole attempt in mealworms to demonstrate polystyrene mineralization through gas-phase analysis, reporting up to 47.7% carbon recovery as CO_2 over 16 days, which they interpreted as metabolic activity. However, their experimental design contains critical methodological limitations that preclude definitive interpretation.

Fig. 1 Snapshot from Video S1 showing accumulation of small commercial EPS particles in mealworm rearing containers, illustrating physical fragmentation during mastication. This video was recorded over 3 days of commercial EPS feeding specifically to demonstrate the physical breakdown of EPS into small fragments, and this is not footage from our main experimental trials.

Most importantly, the absence of aerosol filtration in their continuous-flow system means that micro- and nanoplastic particles released during mastication could have been entrained in the CO_2 collection apparatus. Elemental analysis combusts all carbon-containing materials, including plastic particles – generating false positives for biological CO_2 production. Without orthogonal gas-phase measurements or chemical validation of collected carbonates, plastic-derived particles cannot be distinguished from true respiratory CO_2. Furthermore, the study lacks essential controls: starved larvae to establish baseline metabolic CO_2 release, and microplastic particle-only incubations to quantify abiotic contributions from particulate contamination. These omissions render the gas-phase analysis ambiguous regarding the source of recovered carbon. These considerations informed our decision to prioritize direct chemical analysis over gas-phase measurements.

Interpreting $\delta^{13}C$ enrichment—biomass incorporation or kinetic fractionation?

Yang *et al.* also reported small but statistically significant $\delta^{13}C$ enrichment in fatty acids extracted from ^{13}C-labelled PS-fed mealworms relative to bran-fed controls, interpreting these isotopic shifts as direct evidence for plastic carbon incorporation into biomass.[1] This interpretation fundamentally misinterprets well-established principles of isotopic fractionation during metabolic stress. Dietary stress induces isotopic repositioning through preferential metabolism of isotopically lighter compounds. During starvation, enzymatic processes exhibit kinetic isotope effects wherein ^{12}C-containing substrates undergo catabolism at marginally higher rates than their ^{13}C counterparts. As metabolic demands deplete ^{12}C-enriched lipid reserves, residual tissue pools become progressively enriched in ^{13}C, generating isotopic signatures indistinguishable from those Yang *et al.* attribute to exogenous carbon assimilation. This phenomenon exhibits taxonomic universality. Chironomid larvae demonstrate significant bulk $\delta^{13}C$ increases over 9–12 day fasting periods, with tissue ^{13}C enrichment resulting from preferential depletion of isotopically light lipid fractions.[26] Meta-analyses across diverse taxa confirm that $\delta^{13}C$ responses during nutritional stress exhibit complex, heterogeneous patterns modulated by lipid extraction protocols and tissue-specific metabolic rates.[27]

Yang *et al.*'s isotopic analysis[1] lacked the critical controls needed for mechanistic interpretation. Although they applied compound-specific isotope analysis of fatty acids, the absence of parallel starvation controls prevents discrimination between plastic-derived carbon assimilation and generic stress-induced fractionation of endogenous lipids. Without this baseline, their $\delta^{13}C$ enrichments remain ambiguous and cannot be uniquely attributed to assimilation of PS-derived carbon. In addition, Jendrossek's (2024)[23] theoretical assessment demonstrates that the $\delta^{13}C$-CO_2 enrichment reported by Yang *et al.* is roughly four orders of magnitude lower than would be expected

if the claimed amount of ^{13}C-labelled EPS had actually been metabolised, further suggesting that the reported shifts are unlikely to reflect true metabolic incorporation.

Taken together, these considerations demonstrate that published mass balance approaches, whether based on apparent "missing mass," CO_2 recovery, or isotopic enrichment in biomass, are prone to artifacts unless supported by direct chemical evidence of backbone cleavage and assimilation. Insect feeding on EPS consistently produces vast amounts of micro- and nanoplastic particles, but no measurable biochemical transformation. Without rigorous controls and validated analytical methods, signals attributed to biodegradation are more parsimoniously explained by physical fragmentation, particulate contamination, or starvation physiology.

Given our primary objective to establish chemical evidence for biodegradation and bioassimilation, we focused our analysis on direct indicators of polymer backbone modification and metabolic incorporation. Chemical characterization of frass-derived material revealed only minimal alteration modification of the polymer backbone, providing definitive evidence that consumption occurred without corresponding biodegradation. The lack of measurable growth benefits in larvae, together with statistically insignificant molecular weight changes in pure EPS, provides clear evidence that consumption does not translate into genuine biochemical transformation. This approach directly addresses the fundamental question of whether mealworms possess enzymatic capabilities for EPS depolymerization, independent of the technical complexities associated with microplastic quantification in biological systems.

Population specificity *versus* biological limitations

To evaluate whether our findings reflect population-specific effects or broader biological limits, we conducted validation experiments using superworms (*Zophobas morio*). Superworms have displayed superior plastic-degrading abilities compared to mealworms.[28] We fed the superworms pure and commercial EPS diets using the same individual housing and mechanical stimulation protocols detailed in the Materials and methods section based on our recent PVC study.[29] Complete experimental procedures are described in the Materials and methods section.

Individual housing triggers natural pupation in superworms, requiring mechanical stimulation to maintain larval development (detailed in our concurrent PVC study).[29] Control experiments confirmed effective growth maintenance under stimulation, while unstimulated larvae exhibited complete mortality by day 100 due to failed pupation (Fig. 2a). When superworms were transitioned from bran to EPS-exclusive diets at different developmental stages (T_0, T_{70}, T_{105}), results replicated our mealworm findings precisely (Fig. 2b–d). Both pure and commercial EPS consumption produced progressive weight loss and survival outcomes indistinguishable from starvation controls, regardless of developmental timing or species.

We also conducted a separate experiment collecting frass from superworms on both EPS diets and starvation diets. After polystyrene extraction, we analyzed the polymer using GPC and FTIR to study molecular weight and polymer backbone changes. The molecular weight analysis revealed no significant changes across all GPC parameters (M_n, M_w, M_z) for either EPS formulation after digestive passage ($p > 0.05$) (Fig. 3). FTIR analysis showed absence of carbonyl formation, contrasting with prominent biological signatures in starvation controls (Fig. 4).

The replication of negative results across phylogenetically related species from independent sources demonstrates that EPS consumption limitations reflect biochemical constraints rather than population-specific enzymatic deficiencies. These findings, combined with our parallel PVC degradation studies showing identical consumption-without-degradation patterns, establish that the absence of genuine biodegradation capability transcends species.

Independent validation from geographically distinct sources strongly supports these conclusions. Urbanek *et al.*[10] and Bożek *et al.*[11] using Polish mealworms reported weight losses of 10–20% in PS-fed groups with no nutritional benefit over starvation, with biochemical analyses demonstrating severe depletion of lipids, carbohydrates, and proteins consistent with starvation-induced catabolism. Réjasse *et al.* (2022)[30] used deuterated PE and sensitive microspectroscopy with wax moth larvae but "could not obtain evidence for wax worm-dependent biodegradation and/or bioassimilation of products derived from PE", results obtained regardless of whether conventional or axenic (sterile) larvae were used, demonstrating that gut microbiome presence does not enable polymer degradation.

The convergence of negative findings across geographically distinct populations (Australia, Poland, France), multiple insect species (*T. molitor*, *Z. morio*, *G. mellonella*), polymer types (EPS, PVC, PE), and diverse analytical approaches (GPC, FTIR, isotope labeling, microspectroscopy) establishes fundamental biochemical constraints rather than population-specific variation.

GPC analysis: statistical interpretation and cross-species validation

Statistical analysis of molecular weight distributions requires evaluation of measurement precision relative to observed variations. For pure EPS processed by mealworms, apparent changes of +20.88% (M_n), +1.34% (M_w), and +3.52% (M_z) exhibited no statistical significance ($p > 0.05$), indicating these variations represent analytical uncertainty rather than chemical modifications. The substantial coefficient of variation (25.95%) within pure EPS control groups reflects inherent measurement challenges associated with laboratory-synthesized foam matrices rather than polymer degradation.

Commercial EPS demonstrated statistically significant reductions in M_w (−12.70%) and M_z (−17.40%) with constant M_n values, indicating selective modification of higher molecular weight fractions consistent with additive-mediated oxidative processes rather than backbone depolymerization. These changes, while statistically detectable, represent modest physical degradation processes insufficient to support claims of meaningful biochemical transformation.

To conduct cross-species validation, we collected frass from superworms fed EPS diets, extracted the polystyrene, and

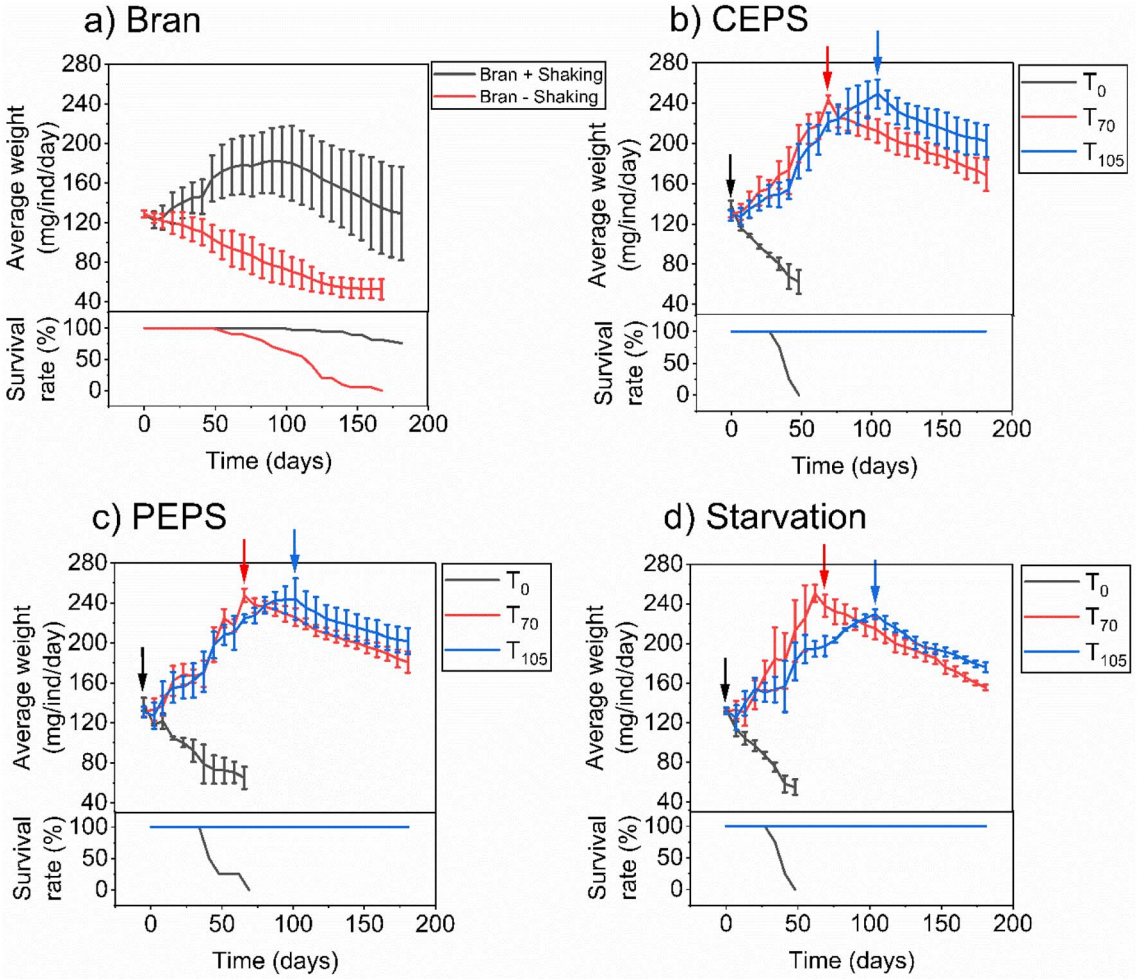

Fig. 2 Growth curves and survival rates of superworms fed with (a) bran with and without mechanical stimulation, (b) bran followed by commercial EPS (CEPS), (c) bran followed by pure EPS (PEPS), and (d) bran followed by starvation. New data generated here, using the methods described in the Materials and methods section (based on ref. 29). Arrows indicate the time at which superworms were switched from bran diet to the respective treatment. T_0, T_{70}, T_{105} indicate diet switching after 0, 70, and 105 days. T_0 curves (black) terminate early due to complete mortality, after which growth measurements become undefined. In panels (b)–(d), survival curves for T_{70} (red lines) and T_{105} (blue lines) overlap almost completely due to identical survival patterns; the blue lines (T_{105}) remain visible while T_{70} curves are obscured by this overlap. Error bars represent standard deviation among individual specimens ($n = 8$ per treatment group).

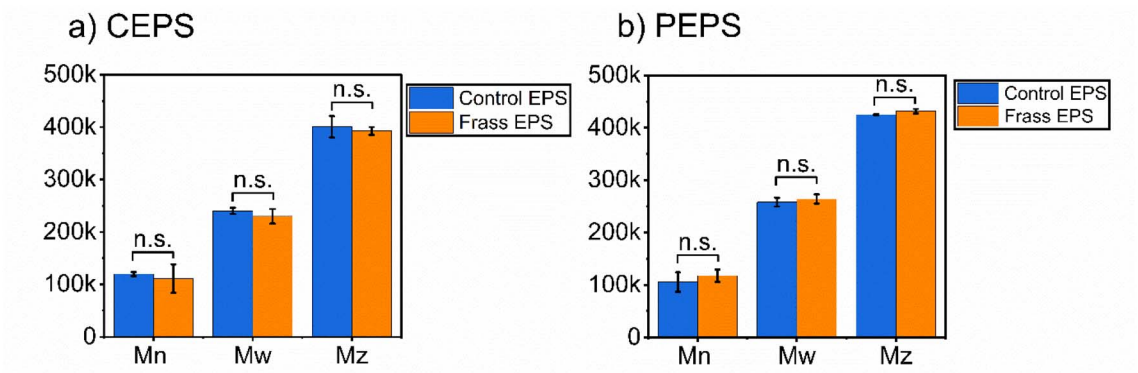

Fig. 3 Molecular weight distributions (M_n, M_w, M_z) of (a) commercial EPS (CEPS) and (b) pure EPS (PEPS) before and after consumption by superworms. New data generated here, using the methods described in the Materials and methods section (based on ref. 29). Control EPS (blue) and frass EPS (orange) show no statistical differences across all molecular weight parameters. n.s. indicates no significant difference ($p > 0.05$). Error bars represent standard deviation from triplicate measurements.

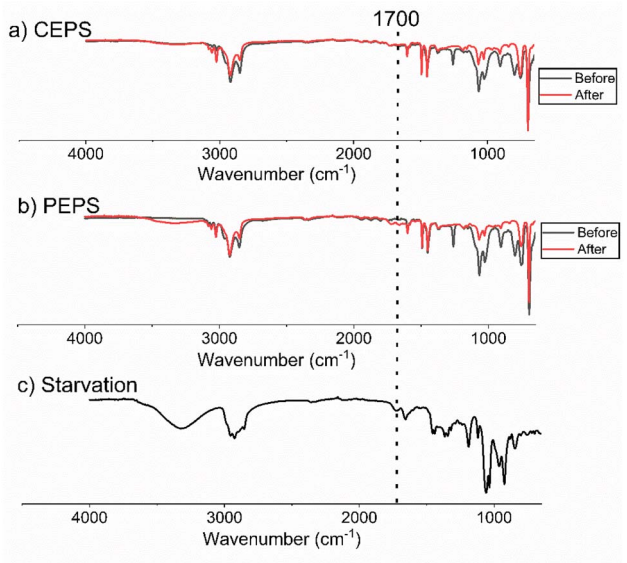

Fig. 4 FTIR spectra of (a) commercial EPS (CEPS), (b) pure EPS (PEPS) before (black) and after (red) consumption by superworms, and (c) starvation control frass. New data generated here, using the methods described in the Materials and methods section (based on ref. 29). Dotted line indicates carbonyl region (1700 cm^{-1}). Carbonyl peaks appeared only in starvation controls, demonstrating biological interference.

examined the molecular weight of the recovered material. Under identical analytical conditions as for mealworms, superworms exhibited no statistically significant molecular weight changes for either commercial or pure EPS formulations across all parameters (M_n, M_w, M_z; $p > 0.05$, Fig. 3). This demonstrates that the modest commercial EPS changes observed in mealworms represent experimental variability or species-specific physical processing differences rather than genuine enzymatic depolymerization capabilities.

The replication of statistically insignificant molecular weight variations across phylogenetically related species supports our "chemically unaffected" characterization. If commercial EPS possessed inherent susceptibility to insect-mediated depolymerization, consistent degradation patterns would appear across species with similar consumption rates. The absence of such consistency confirms that observed molecular weight modifications reflect limited, non-enzymatic processes rather than biodegradation mechanisms.

FTIR analysis: addressing biological interference

As noted in authoritative critiques by Gu[12] and Jendrossek,[23] FTIR analyses in plastic biodegradation studies "suffer from interference by extracellular polymeric substances and microbial metabolites that adhere to plastic specimens, producing false positive signals for material changes." Biological metabolites generate carbonyl signatures mimicking polymer oxidation. Similarly, Weber *et al.*[31] demonstrated that protein and lipid contamination from biological sources produces FTIR peaks at 1744, 1651, and 1545 cm^{-1} that are easily misattributed to polymer degradation products.

Our new superworm data provides empirical validation of this interference (Fig. 4). We collected frass from superworms fed EPS diets or under starvation, extracted the polystyrene, and examined FTIR spectra of the recovered material. We found no carbonyl peak formation at 1700 cm^{-1} for commercial or pure EPS samples, contrasting with mealworm samples fed with commercial EPS exhibiting measurable carbonyl formation. This species-specific divergence indicates gut chemistry variations rather than consistent EPS degradation mechanisms.

Most significantly, in our new superworm data carbonyl peaks appeared prominently in starvation control frass (Fig. 4c) where no plastic was present, demonstrating that carbonyl signatures in biological systems can originate from natural metabolites and organic compounds in frass rather than polymer oxidation. This directly contradicts Wu and Criddle's claim that "the strong C=O peak in the commercial EPS suggests significant oxidative degradation".

Additional analytical tools for residual PS: methodological priorities and evidence standards

Additional analytical techniques such as ^{1}H NMR, XPS, and Py-GC/MS have been suggested to further characterize plastic processing. Our combination of GPC and ATR-FTIR already targets the two primary indicators of polymer degradation – molecular weight reduction and backbone modification – providing a robust mechanistic assessment.

Our comparative approach between pure and commercial EPS allows us to isolate specific variables more effectively than exhaustive analytical surveys of individual systems. The clear distinction we observed between pure EPS (no significant molecular weight changes) and commercial EPS (modest but statistically detectable changes in higher molecular weight fractions) provides compelling evidence for our conclusions about additive-mediated *versus* backbone degradation processes.

We believe that additional techniques, while potentially providing confirmatory information, would not fundamentally alter our primary conclusions given the clarity of our core analytical results and the replication of findings across multiple species and polymer types.

Density of EPS foam diets: physical properties and degradation accessibility

Pure EPS (0.034 ± 0.004 g cm^{-3}) and commercial EPS (0.021 ± 0.003 g cm^{-3}) differed in density by ~1.6-fold, a factor that could influence mechanical processing efficiency. However, we believe that this physical parameter, while potentially affecting consumption rates, does not explain the absence of chemical degradation observed in our experiments. Our direct comparison of pure and commercial EPS under identical rearing conditions was designed to isolate chemical effects from physical ones. GPC analyses showed minimal molecular weight reduction in either foam type, indicating limited backbone cleavage regardless of density. While density may affect how efficiently larvae fragment and ingest EPS, the enzymatic cleavage of aromatic C–C bonds remains fundamentally limited

by high activation energies and stringent catalytic requirements that are unlikely to depend on bulk surface area.

Impact of incubation method: balancing experimental control and biological relevance

Individual housing protocols might negatively affect mealworm physiology, a potential confounding factor in controlled feeding experiments. However, in our experiments, individually reared bran-fed mealworms exhibited average growth rates of 0.9 mg per individual per day over 100 days, compared with 1.1 mg per individual per day for group-reared specimens reported by Zhong *et al.*[32] This 18.2% differential reflects nutritional supplementation through cannibalism in group housing rather than physiological stress from isolation protocols.

Individual housing therefore removed cannibalism as a confounding factor while still maintaining normal physiological status. These data demonstrate that our experimental conditions preserved larval health while providing the experimental control necessary for unambiguous interpretation.

PS as sole diet and toxicity: distinguishing degradation from nutritional adequacy

Wu and Criddle's acknowledgment that EPS diets are "toxic to mealworms" supports our findings yet directly contradicts Yang *et al.* (2020),[8] who claimed superworms survived 28 days on only Styrofoam diet with higher survival rates than unfed controls, highlighting the importance of rigorous methodology.

Our individual housing design over 125+ days was designed to distinguish nutritional inadequacy from active toxicity while eliminating cannibalism artifacts. If polystyrene were truly bioavailable through chemical depolymerization and assimilation, even without other nutrients, it should confer a measurable survival advantage over complete starvation. For example, pure sugar diets have been shown to slow mobilization of endogenous lipid reserves, reduce weight loss, and delay mortality in the two-spotted ladybird *Adalia bipunctata* L. (Coleoptera, Coccinellidae),[33] likely by providing ATP through glycolysis or other metabolic pathways. In contrast, both pure and commercial EPS diets in our experiments led to greater weight loss and higher mortality than starvation alone, indicating net physiological costs of ingestion rather than any nutritional benefit.

Multiple independent studies demonstrate that PS consumption provides no survival advantage and often causes accelerated mortality, indicating active toxicity rather than mere nutritional inadequacy. Using bioenergetic modeling, Matyja *et al.*[14] showed PS-fed larvae exhibited survival indistinguishable from complete starvation with zero pupation and no structural growth, concluding that "PS and/or possible products of its degradation are none or insufficient source of mass and energy for larvae." Zhong *et al.*[32] found PS-fed larvae not only failed to pupate but exhibited mortality exceeding starvation controls; critically, even mixed diets with half nutritious bran showed reduced pupation, proving plastic imposed physiological costs uncompensated by concurrent nutrients. Biochemical analyses by Bożek *et al.*[11] and Leluk *et al.*[34] revealed PS-fed mealworms lost more mass than starved controls with severe depletion of lipids and carbohydrates, representing classic starvation metabolism rather than successful utilization.

Cannibalism in group-housed experiments masks this toxicity. Yang *et al.*[9] used group housing and initially observed PS-fed mealworms with higher survival than unfed controls at one month, but by three months both groups collapsed to identical starvation-level survival. Post-mortem examination revealed "both the unfed mealworms and mealworms fed PS alone engaged in cannibalism," with survivors consuming dead mealworms to maintain biomass. This demonstrates that short-term survival advantages in group-housed studies reflect cannibalistic supplementation rather than PS metabolism. The consistency of negative findings across studies provides definitive evidence that polystyrene cannot serve as a carbon or energy source for mealworm metabolism.

Wu and Criddle's proposal to monitor reactive oxygen species (ROS) is also problematic. While oxidative stress represents an interesting biochemical endpoint, ROS generation can arise from multiple mechanisms unrelated to polymer depolymerization: mechanical abrasion of gut epithelia by plastic fragments,[35] translocation of nano-sized particles across cell membranes,[36] leaching of toxic additives,[37] or general starvation-induced oxidative imbalance.[38] Thus, ROS generation occurs through multiple mechanisms unrelated to biodegradation, making it unsuitable as evidence for enzymatic depolymerization.

Need for comparative transcriptome and microbiome analysis?

Several prior studies have applied transcriptomic and microbiome profiling to insects before and after EPS diets in an effort to assess biodegradation.[39–41] Such approaches can provide phenotypic information – for example, shifts in gut microbial composition, activation of stress–response pathways, and alterations in host metabolism – that help describe how organisms respond to plastic exposure. However, important limitations constrain their application to biodegradation assessment.

Gut microbial communities and host gene expression patterns shift rapidly under starvation and dietary stress, including exposure to non-nutritive plastics and their leachates. In fish models, for example, polystyrene microplastics disrupt gut microbiota, trigger inflammation, and alter immune and epithelial gene expression despite the plastic itself remaining chemically intact.[42] Similar responses are documented across multiple species, but these changes typically reflect general toxicological stress, such as gut abrasion, nanoparticle uptake, or additive leaching, rather than enzymatic polymer degradation.[43–45]

Such stress-induced shifts in gene expression and microbiome composition therefore represent non-specific physiological responses to foreign material or starvation. Without complementary chemical evidence of depolymerization and carbon assimilation into biomolecules, transcriptomic and microbiome data remain ambiguous and cannot be taken as proof of biodegradation.

Table 1 Initial mealworm mass, EPS consumption, and requirements: mealworms needed ($\times 10^6$) and corresponding live biomass (t) per ton of commercial EPS across studies

Initial mealworm mass (mg)	EPS consumption rate (mg per individual per day)	Feeding duration (days)	Total EPS consumed per individual (mg)	Mealworms ($\times 10^6$)/biomass required (t) per ton EPS	Reference
22 ± 1	0.10	25	2.5	400/8.8	16
38.1 ± 2.1	0.11	76	8.36	120/4.5	
47.3 ± 3.6	0.09	82	7.38	135/6.4	
62.2 ± 1.7	0.11	140	15.4	64/4	
71.7 ± 1.6	0.04	180	7.2	138/9.9	
87.9 ± 1.3	0.03	180	5.4	185/16.2	
79.2 ± 1.4	0.11	32	3.52	284/22.5	2
76.5 ± 1.5	0.18	32	5.76	173/13.2	9
73.9 ± 0.8	0.2	32	6.4	156/11.5	
91.1 ± 0.7	0.14	32	4.48	223/20.3	
46.3 ± 0.8	0.08	32	2.56	390/18	
84 ± 1.2	0.16	32	5.12	195/16	

Reassessing high-purity polymer biodegradation claims

Wu and Criddle cite reports of mealworm-mediated biodegradation of several high-purity polymers, including polyethylene, polypropylene, PVC, and PET, as evidence that insect plastic degradation is a general phenomenon.[46–49] However, these studies are plagued by the same confounding factors discussed above, including cannibalism, reliance on gravimetric "missing mass" calculations, and insufficient chemical validation of polymer backbone cleavage.

To test this directly, in a separate study we examined pure PVC consumption in both mealworms and superworms under the same controlled conditions as our EPS experiments.[29] Despite active feeding, neither species exhibited growth on this diet, nor did molecular weight analysis reveal any significant polymer changes after gut passage. The replication of negative results across two polymers (EPS and PVC) and two insect species demonstrates that the absence of degradation is not restricted to polystyrene alone. These findings provide direct empirical validation of our methodology and highlight broader biochemical limits to insect-mediated plastic processing.

Practical constraints

Mealworm-mediated EPS treatment faces insurmountable practical barriers. Based on our measured consumption rates (~0.11 mg EPS per individual per day over 140 days), processing a single ton of EPS would require ~64 million mealworms and generate more than 4 tons of dead larval biomass (Table 1). This generous estimate assumes complete consumption and biodegradation, which is not observed. These figures closely match Billen *et al.*'s techno-economic analysis,[50] which calculated 4–10 tons of larvae per ton of plastic waste, treatment costs above €300 per ton, and energy inputs driving costs beyond €1000 per ton. Their study also showed that processing one ton of EPS would emit 4–8 tons of CO_2-equivalents, exceeding the footprint of conventional waste management. Thus, even if some degree of biodegradation were to occur, the enormous biomass requirements, energy demands, and carbon emissions would still render insect-based EPS treatment economically prohibitive and environmentally counterproductive.

Beyond economic and carbon emission concerns, insect-mediated mechanical fragmentation poses serious environmental and health risks. Recent investigations confirm that insect processing achieves only mechanical degradation rather than enzymatic digestion,[15] converting macroplastic waste into micro- and nanoparticles that readily bioaccumulate through food chains and contaminate water supplies. Given that microplastic contamination is already pervasive, with nanoparticles detected even in commercially bottled water,[51,52] deliberately processing plastics through insect fragmentation would exacerbate an already critical environmental and public health crisis. Rather than solving the EPS waste problem, insect-based processing would accelerate the production of these hazardous mobile fragments, transforming manageable macroplastic waste into more bioavailable microplastic pollution that threatens ecosystems and human health through pathways far more difficult to control or remediate than conventional waste management.

Conclusions

Our investigation, though yielding results that differ from some earlier reports, was designed to rigorously assess the potential for mealworm-mediated EPS degradation as a practical waste management strategy. Across two insect species (*T. molitor* and *Z. morio*) and two polymer types (EPS and PVC), our results consistently show no biochemical modification of polymer backbones despite mechanical consumption. The limited oxidative changes observed in commercial EPS appear to stem from additives rather than enzymatic depolymerization, while pure polymers remain chemically unaffected by gut passage.

By implementing controlled additive analysis, eliminating cannibalism artifacts, using individual monitoring protocols, and applying chemical characterization, we establish methodological standards that help distinguish between mechanical processing and genuine biochemical degradation. Such rigor is essential for a realistic appraisal of biological approaches to plastic waste management.

Our findings establish that rigorous experimental controls reveal the absence of genuine biodegradation, while scale analysis

demonstrates the fundamental impracticality of insect-based plastic treatment. In addition to the absence of true biodegradation and the risks of micro- and nanoplastic generation, these factors render insect-based treatment logistically prohibitive, economically unviable, and environmentally counterproductive.

Materials and methods

Superworm cultivation and experimental design

Superworms (*Zophobas morio* larvae, Biosupplier, NSW, Australia) were maintained using a three-generation breeding protocol identical to that established for *T. molitor*, with the addition of fresh vegetables to the wheat bran diet. Mature larvae were individually separated to induce pupation after reaching maturity, as *Z. morio* larvae require isolation to trigger metamorphosis.[53]

For the experimental phase, 48 superworms with an average initial weight of 125 ± 10 mg were individually housed in 120 mL glass containers to prevent cannibalism. Since isolation can trigger premature pupation in *Z. morio* larvae, mechanical stimulation was implemented immediately upon separation from the colony. A mechanical shaker (Model LSK-0330M, Laboao company) operating at 120 strokes per minute was used, with each container undergoing five daily shaking sessions lasting 5 minutes each, following established protocols.[29] This mechanical stimulation has been demonstrated to extend the larval stage and prevent premature metamorphosis compared to non-agitated controls.

Before introducing the experimental diets, all superworm larvae underwent a 48 hour starvation period to ensure complete gut clearance. Following this preparation, superworms were divided into three experimental groups ($n = 8$ per group): pure EPS (PEPS), commercial EPS (CEPS), and starvation controls. EPS samples were prepared by cutting thin sections (10 ± 5 mg) that were provided to individual superworms. Environmental conditions were maintained at 25 ± 2 °C in darkness throughout the experimental period.

The experimental design followed the same age-stratified approach used for mealworms, with superworms transitioned from bran to EPS-exclusive diets at different developmental stages: T_0 (immediately after isolation), T_{70} (after 70 days on bran), and T_{105} (after 105 days on bran). This stratified approach allowed evaluation of EPS effects across different developmental stages.

Survival rate and growth curve

Individual superworm weights were recorded every five days throughout the experimental period. Survival rates were estimated using Kaplan–Meier analysis following the same equation as established for *T. molitor*:[16]

$$S_t(\%) = (S_{t-1} \times (1 - d/N)) \times 100$$

where S_t is the estimated survival probability at time t, S_{t-1} is the estimated survival probability at the previous time point, d is the number of deaths at time t, and N is the number of individuals at risk at the beginning of time t.

Chemical analysis and statistical methods

For molecular weight analysis, frass was collected from superworms on both EPS diets and starvation diets over a 35 day period. Frass samples (50 mg) were extracted with tetrahydrofuran (THF, 10 mL) in 30 mL glass vials for 2 hours at room temperature. The extracts were filtered through 0.22 μm PTFE sterile syringe filters (Sigma-Aldrich) into clean glass vials. After complete THF evaporation under a stream of nitrogen gas, the residue (approximately 20 mg) was re-suspended in THF to a final concentration of 1 mg mL^{-1}.

Gel permeation chromatography (GPC) was performed using an Agilent Technologies 1260 Infinity II system equipped with a refractive index detector (RID) to determine number-average molecular weight (M_n), weight-average molecular weight (M_w), and Z-average molecular weight (M_z). The system was calibrated using polystyrene standards (Sigma-Aldrich). Sample aliquots (20 μL) were analyzed using THF as the mobile phase at 40 °C with a flow rate of 1.0 mL min^{-1} over a 30 minute runtime. The chromatographic setup consisted of a Polargel-M guard column (50 × 7.5 mm) and a Polargel-M main column (300 × 7.5 mm, Agilent Technologies), specifically selected for separating polymers within the range of 1 to 500 kDa. All analyses were performed in triplicate to ensure statistical significance and reproducibility.

Attenuated total reflectance-Fourier transform infrared (ATR-FTIR) spectroscopy was performed on an Agilent Cary 630 FTIR instrument to analyze functional group modifications. Residual polymers were dissolved in THF and spread on the ATR window, allowing complete solvent evaporation. Spectra were recorded in the range of 650–4000 cm^{-1}. Each experimental condition was replicated twice.

Statistical methods. Statistical analyses employed two-sample t-tests for weight comparisons and GPC molecular weight distributions. All analyses were performed in triplicate with statistical significance set at $\alpha = 0.05$. Complete methodological details, including breeding protocols and mechanical stimulation parameters, are described in our peer-reviewed PVC degradation study.[29]

Conflicts of interest

There are no conflicts to declare.

Data availability

Data for this article, including figures, are available at Zenodo at **https://doi.org/10.5281/zenodo.17292309**.

Supplementary information (SI) is available. See DOI: **https://doi.org/10.1039/d5su00725a**.

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