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Lessons in Rearing Mealworms for Plastics Degradation

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PURPOSE: The primary objective of this research is to determine if plastics-degrading gut bacterial communities from a nonoptimal insect host can be successfully transplanted into the gut of the optimal mealworm host for large scale composting. To achieve this goal, foundational questions about basic mealworm husbandry needed to be addressed, including proper housing and feeding regimes, expected plastics degradation rates, and survivability on plastics as a food source. This technical note serves as a mealworm husbandry protocol and a guide for lessons learned in the early stages of experimentation dealing with establishment of plastics-degrading mealworm colonies.

BACKGROUND: The need for waste reduction and novel sources of manufacturing feedstocks are common themes on DoD/Army installations and throughout the world. Recalcitrant plastic materials made of polyethylene (PE) or polystyrene (PS) are ubiquitous, with breakdown times for many plastics estimated at 400 years (Parker 2019). PE contains any combination of long and/or branched chains of ethylene, while PS (Styrofoam) contains any combination of styrene in long or branched chains. PS is among the most common and least sustainable plastics (Brandon et al. 2020), and it is bulky and difficult to transport. Because many recycling centers do not accept PS, it is often landfilled where it persists as a recalcitrant waste product (Brandon et al. 2020).

Recycling plastics is an energy intensive process, but there is a unique and largely unexplored niche for entomoremediation and insect-driven plastivory. PS and some PE materials are amenable to degradation by either darkling ground beetles (Coleoptera: Tenebrionidae: *Tenebrio molitor* and *Zophobas morio*), in both the larval (mealworms) or adult stage, or waxworm moth larvae (Lepidoptera: Pyralidae: *Achroia grisella* and *Galleria mellonella*) (Yang et al. 2015b; Yang et al. 2014; Urbanek et al. 2020; Yang et al. 2018; Yang et al. 2020; Yang et al. 2015a; Brandon et al. 2018; Kundungal et al. 2019). Mandibular activity of the insects chewing on plastics serves to increase the surface area of the polymers that can be attacked by insect digestive enzymes and by gut bacteria that can degrade the polymers into CO_2 and low molecular weight compounds (Brandon et al. 2018; Yang et al. 2015a).

Although mealworms have been shown to degrade various styrene polymers, there appears to be more limitations on the types of PE materials they can degrade (as reviewed by Pham et al. 2023). In a short experiment using commercial PE material (plastic grocery store bag coupons), the PE was largely ignored by the mealworms even when introduced as the sole food source, while commercial PS in the form of shipping foam was eagerly consumed (C. M. Jung, unpublished data). In another experiment, simple monocompound PE and PS were introduced to mealworms separately, resulting in high-level feeding and degradation of both plastics (Brandon et al. 2018). However, when introduced in combination, the mealworms showed a preference for PE. Shifts in the gut microbiome community structure when insects were moved from one food source to the next were observed, but in general, the major bacterial phyla were unchanged. Minor microbial phyla in the gut were more variable between PE and PS diets and it appeared that numerous bacteria worked in concert to degrade the plastics in a nonspecific fashion (Brandon et al. 2018).

Interestingly, the native gut microbiome of the *Tenebrio* species from 22 geographic locations was found to have an intrinsic ability to degrade PS (Yang et al. 2018). Popular science outlets through internet searches indicate current work is focused on finding ways to increase feeding rates of different larvae in larger scale to take advantage of their unique degradative ability, but finding trusted literature has proven challenging. Work is also being conducted with isolated cultures of gut bacteria, but utilization of these bacteria has confounding limitations for practical waste-reduction purposes. For instance, plastics would need to be separated by types and class so that similar chemistries would be acted upon by each specific bacterial degrader or syntrophic bacterial consortium. Furthermore, plastic-degrading cultures would need to be maintained in liquid reactors and would require significant oversight and monitoring. In contrast, utilizing a holistic insect system as an autonomous, self-regulating minireactor would be more robust. Intact insect gut microbiomes may support any number of bacteria that could feasibly degrade numerous types of plastic polymers. Darkling beetles/mealworms are further well-suited for use in plastics-degrading systems because they continue to eat throughout their larval and adult stages, are prolific breeders, are flightless and docile, have minimal water and food requirements, are extremely low maintenance, and are environmentally benign. In contrast, the waxworm is not a reasonable model because it has more strict nutrient requirements, only feeds in larval stage, and emerges as an adult moth that is a honeybee pest.

In the case of entomoremediation, the Environmental Lab's (EL) Environmental Microbiology Team has had some success transplanting soil bacteria into the larvae of a green June beetle for the degradation of phenanthrene and the explosive RDX (Jung, Carr, Fleischman, and Roesch 2021; Jung, Carr, Lindsay, et al. 2021). Transmitting microbial consortia, and thus PE degrading functionality, from the waxworm gut to the gut of the more manageable darkling beetle/mealworm could result in a plastic super-degrader that survives as both a juvenile and adult in a compost ecosystem, with minimal escape concerns, population reproduction for many generations (i.e., self-perpetuation), and minimal oversight. It is envisioned that these super-beetles could then serve as keystone, foundational elements in a dynamic plastics-composting ecology that can exist at a small, contained scale (e.g., installation composting bins). This novel objective, of super-enriching darkling beetle gut communities, is certainly innovative and within the scope of this research team. Furthermore, the analytical capabilities proposed herein would lend insight to the nature of the frass produced as end products of plastic degradation by super-beetles. Even if the mealworm treatment is only the initial treatment step, the resultant degradation products could be more amenable to additional downstream steps. The frass itself, however, may be safe for common garden compost or broken down into feedstock for plastics or other carbonaceous material; either outcome would be a potential value-added benefit of this work.

The primary objective of this reported work was to determine the most beneficial modes of rearing the mealworms and raising them as both adults and larvae, the basic rates of PS degradation, the rate of LDPE and HDPE degradation, if at all, and the typical survival rate throughout a feeding experiment.

MATERIALS AND METHODS: Bran feed/bedding and five mealworm containers (of approximately 100 worms each) were ordered from Carolina Biological Supply Company (Burlington, North Carolina) (Figure 1). Mealworms were split (n = 100 [approximately]) into separate large containers with 1 in.[*] layer of bran that would be used as either a control group (no plastics) or experimental groups that would receive plastics. PS foam blocks (Uline Catalog No. s-23554, Pleasant Prairie, Wisconsin) were cut (5 × 5 × 2 cm) using a hot foam knife (Figure 2), marked, and weighed. A single PS block was placed into each experimental group container.

Figure 1. (*A*) Mealworm containers as ordered from Carolina Biological Supply, and (*B*) individual mealworms with metric ruler (in millimeters) for reference.

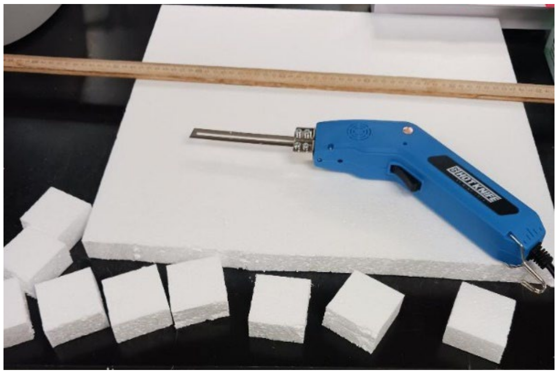

Figure 2. Hot foam knife used to cut blocks of polystyrene foam from sheets.

After 4 days of exposure to the experimental group, the PS block was well received by the mealworm population. Pupae and any emergent adults were sorted from both the experimental and control groups and added to a new, large experimental or control container, respectively. The adults from the experimental container were given a fresh PS block, which they immediately began to eat, climb on, and hide under (Figure 3).

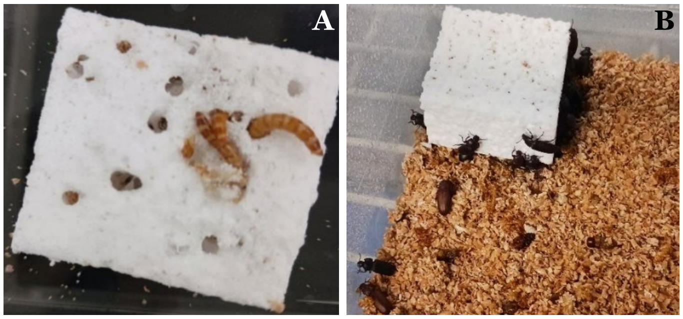

Figure 3. (*A*) Mealworms (larvae) and (*B*) adults actively eating and moving about on PS foam.

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[*] For a full list of the spelled-out forms of the units of measure used in this document and their conversions, please refer to *US Government Publishing Office Style Manual*, 31st ed. (Washington, DC: US Government Publishing Office, 2016), 248–52 and 345–47, https://www.govinfo.gov/content/pkg/GPO-STYLEMANUAL-2016/pdf/GPO-STYLEMANUAL-2016.pdf.

An LDPE experimental group was also established using a PS-fed group that now contained approximately 500 mealworms that were mostly the result of emergent adults and thus a first generation of PS-fed, lab-raised mealworms. LDPE foam sheets (Uline Catalog No. s-19363) were cut into 5 × 5 cm squares, marked, and weighed (Figure 4); and as a quick check, LDPE Square #1 was added to the mealworm colony. After 15 days of eating, LDPE Square #1 was retrieved, cleaned of debris, and weighed. Only 55% of the original material (0.126 g) remained by weight (0.0692 g) (Figure 5).

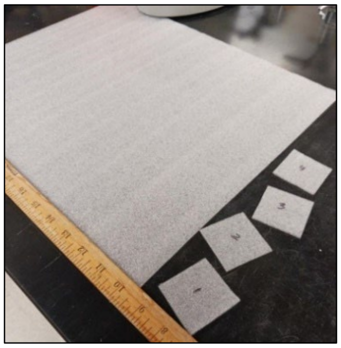

Figure 4. LDPE foam sheets cut into squares for feeding.

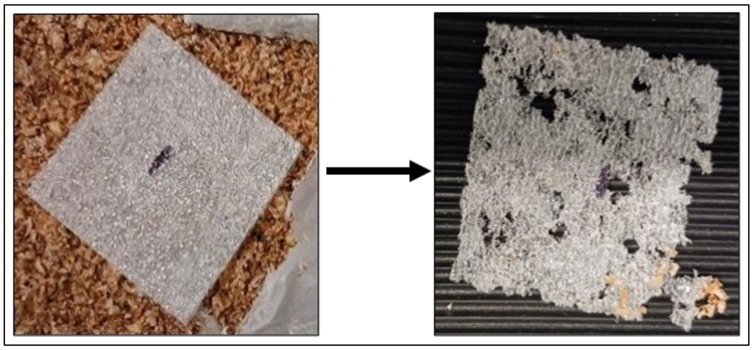

Figure 5. Evidence of LDPE feeding activity within 15 days by approximately 500 mealworms.

Range-Finding Feeding Study: A range-finding feeding study was prepared in deli cups (*n* = 20; in triplicate) (Berry Global, Evansville, Indiana; polypropylene #5) with 150 g Ottawa sand (approximately 5/8 in. [1.65 cm]) (Figure 6). Those that received bran as a food source were allotted 50 mL bran that was placed on top of the sand. The preweighed (2.5 × 2.5 cm) PS, PE, or PS and PE were added to the appropriate treatment containers and three squirts (approximately 5 mL) of water from a household spray bottle were added to each twice weekly. The setup was as follows with 20 larvae in each condition, in triplicate: Control (no food), Control + Bran, PS, PS + Bran, PE, PE + Bran, PS + PE, and PS + PE + Bran.

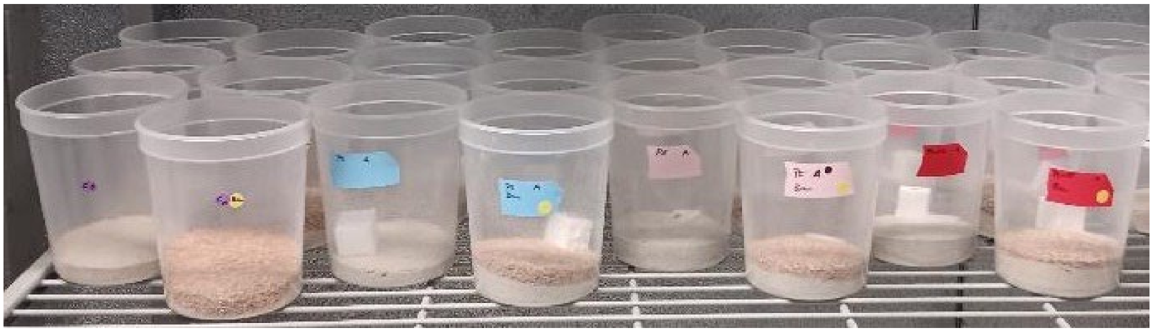

Figure 6. Feeding setup in deli cups.

Final Feeding Experiment: A final feeding experiment was prepared in deli cups containing 50 mL fine pine shavings (Queen Horse Bedding, Ulmer, South Carolina [approximately 1.5 cm]) instead of sand and larger numbers of mealworms (*n* = 50; in triplicate) to account for any attrition. Those that received bran as a food source were allotted 50 mL bran that was placed on top of the shavings. The preweighed (2.5 × 2.5 cm) PS, PE, or PS and PE were added to the appropriate treatment containers and three squirts of water were added to each twice weekly. Measurements and replacement of the plastics were done ad hoc, and the mealworms were counted and weighed at 20-day intervals.

HDPE and Mixed Plastics Degradation: As a point of interest, HDPE in the form of an orange biohazard bag was cut into 5 × 7.6 cm pieces and added to the large brood chambers containing greater than 1,000 beetles at various stages of development.

RESULTS AND DISCUSSION

Range-Finding Feeding Study: The initial range-finding feeding study was broken down after 51 days. Mass attrition was observed in every condition without bran. This was likely due to the abrasiveness and desiccating nature of the sand rather than starvation, as a similar test with only a fine layer of sand had been performed previously with much less attrition (data not shown). Mealworms typically burrow, and this sand was not conducive to that activity. However, data were still obtained and, in each case, those with bran had close to 100% survival ($n = 20$) while those without had no more than 10% survival (Figure 7). Again, this likely had more to do with the inability to properly burrow than the lack of bran as a food source. These data do show that within 51 days, the eating of different plastics was not detrimental to mealworm survival or growth (as determined by weight). The larval body weight from each treatment without bran was significantly less ($p < 0.02$) than its counterpart with bran, but all the plastics-only treatments or plastics with bran treatments when compared to other data without or with bran, respectively, were similar ($p > 0.02$).

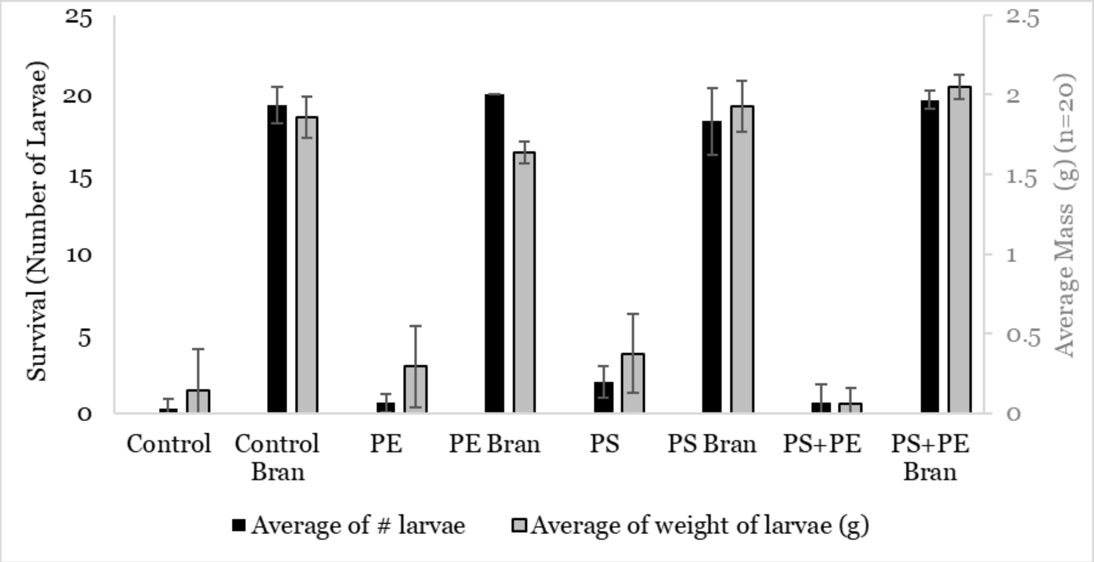

Figure 7. Number and normalized weight ($n = 20$) of larvae after 51 days of growth.

The degradation of plastics was compared against one another and with and without the addition of bran as a food source (Figures 8 and 9). The raw weight measurements indicate there are significant ($p < 0.01$) differences between the plastics degradation when comparing bran and plastics-only fed groups except in the PS group and this was due to high variability in the samples. There were no differences between the same plastic (either PS or PE) degraded singularly or in concert with the other plastic for either the bran or plastics-only fed treatments.

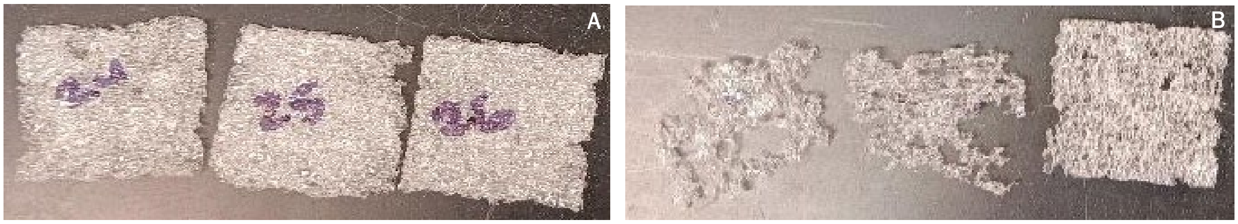

Figure 8. LDPE after 51 days of larval feeding as (*A*) a sole food source, or (*B*) with bran as an alternative food source.

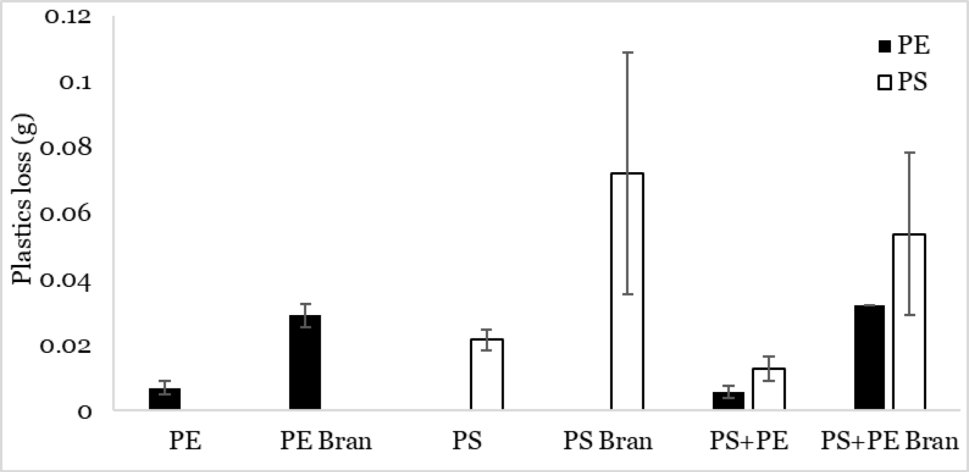

Figure 9. Average plastic mass loss over 51 days.

Due to the massive attrition (Figure 7), and therefore differences in numbers of mealworms and total body weight, it was important to look at rates of degradation as normalized by grams of mealworm body weight per day (Figure 10). This analysis shows a very different picture of similar degradation ($p > 0.1$) between paired bran and plastics-only treatments, except for PS degradation when it was the only food source available. This datum reveals that within 51 days, before their death, the mealworms in the PS only treatment ate more PS than any other treatment.

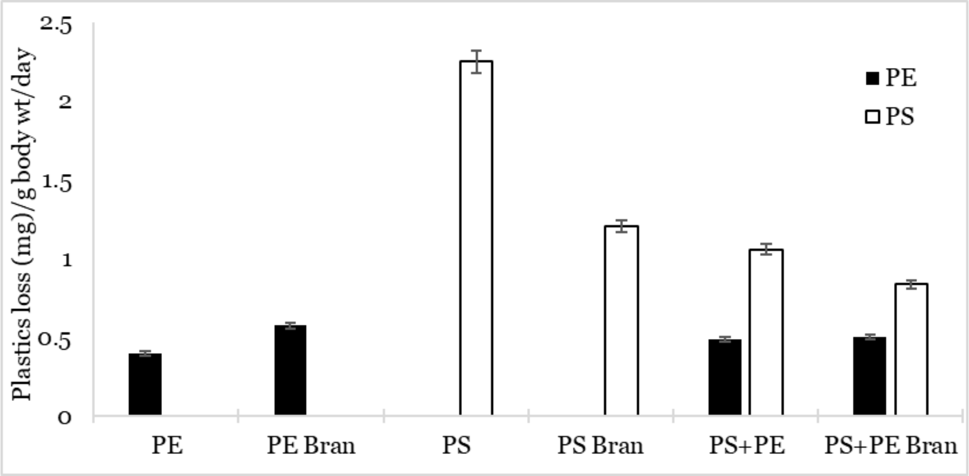

Figure 10. Plastics degradation per gram mealworm body weight per day.

Final Feeding Experiment: Mealworms were counted and weighed on days 20, 40, and 59 of the final feeding experiment. Survivors included larvae, pupae, and adults (Figure 11). By day 40, all the nonbran-fed conditions (Figure 11*A*; dashed lines) were showing attrition around 50% and around 90% by 59 days, which was a significant decrease in survival compared to all the bran-fed treatments and control ($p < 0.05$) (Table 1). There was no difference between the control that was completely starved and the other plastics-fed only conditions. This demonstrated that when fed plastics with bran, the plastics had no negative impact on survival compared to bran alone. This should not be too surprising since these plastics are carbon polymers, often without any other nutrients. Interestingly, the larvae began to pupate and even emerge as adults almost exclusively in all the conditions containing bran while no pupation occurred when fed on plastics alone. This lends further support of the lack of toxicity of plastics to insect development but highlights the lack of nutrients available with a diet containing carbon polymers alone.

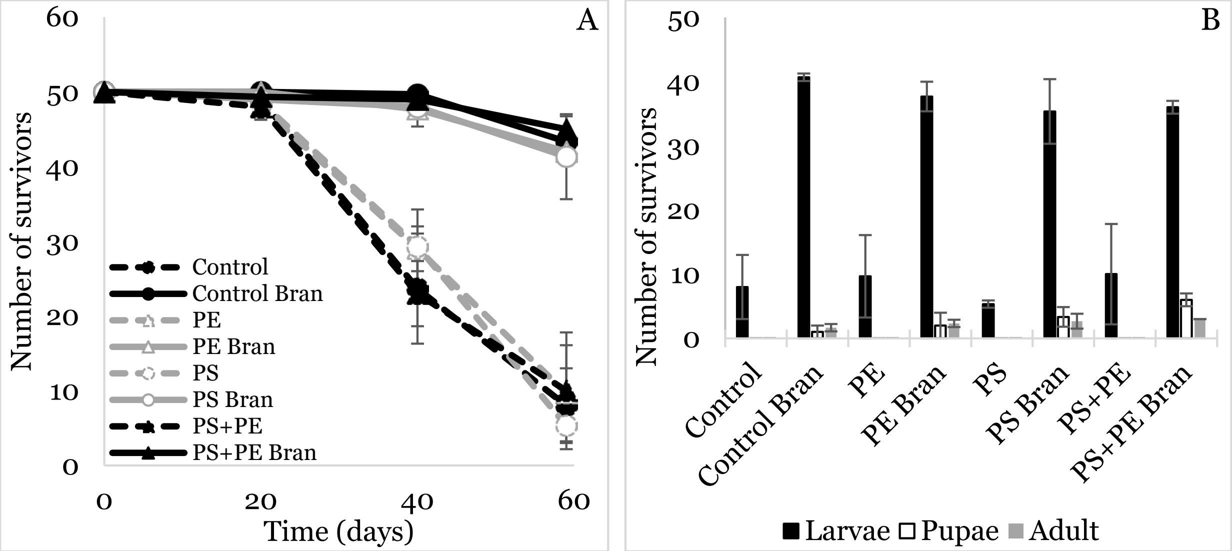

Figure 11. (*A*) Number of survivors during a 59-day feeding experiment, and (*B*) breakdown of different developmental stages of the survivors that all began as early instar larvae.

Table 1. Summary of t-test results of pairwise comparisons for Figure 11*A*. Significant data points at $p < 0.05$ are in bold.

	Survivors			
t-test ($p < 0.05$)	**Control Bran**	**PS Bran**	**PE Bran**	**PS+PE Bran**
Control	**0.00800**	**0.02798**	**0.00882**	**0.00315**
PS	**0.00000**	**0.00788**	**0.00008**	**0.00113**
PE	**0.00995**	**0.01442**	**0.01015**	**0.01725**
PS+PE	**0.01904**	0.05612	**0.01928**	**0.01386**

The body weight per larvae was markedly different ($p < 0.05$) between those fed with bran and those not (Figure 12 and Table 2). There was an approximately 50% decrease in body weight by day 59 in the plastics-only fed treatments compared to their plastics and bran-fed counterpart treatment, further indicating that plastics alone may not support complete development of the mealworms in the long term. Absolute plastics consumed (Figure 13*A*) were significantly higher for both plastics when bran was present ($p < 0.01$), and the low-density PE used in this feeding trial was preferred over PS (Table 3). As shown with the previous data sets, it is important to look at both the total plastics degraded over the experimental period as well as plastics degradation normalized by mealworm body weight.

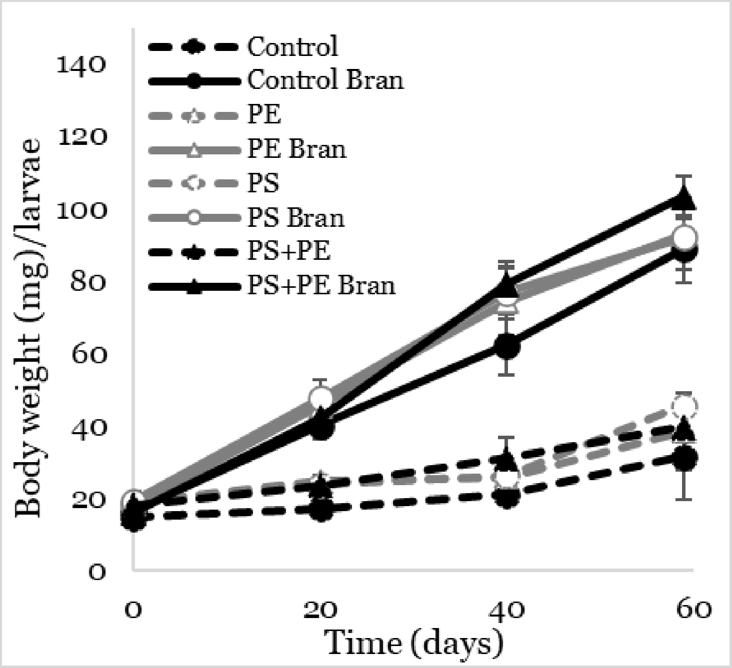

Figure 12. Body weight per larvae during the 59-day feeding trial.

Table 2. Summary of t-test results of pairwise comparisons for Figure 12. Significant data points at $p < 0.05$ are in bold.

	Body Weight per Larvae			
t-test ($p < 0.05$)	**Control Bran**	**PS Bran**	**PE Bran**	**PS+PE Bran**
Control	**0.04231**	**0.01706**	**0.03522**	**0.00337**
PS	**0.01470**	**0.00057**	**0.01347**	**0.00351**
PE	**0.01544**	**0.01193**	**0.03193**	**0.01874**
PS+PE	**0.00862**	**0.00129**	**0.00433**	**0.00495**

Because the mealworms were larger and thus consumed more plastics when bran was available as a food source, it makes sense that they could eat more plastics with bran. Therefore, the trends for both plastics were reversed when the rate of plastic consumption per day was normalized to larval body weight (Figure 13*B*). Interestingly, when both plastics were offered in the presence of bran, PE was preferred over PS as both total consumption ($p = 0.0079$) (Table 3) and rate of consumption per body weight per day ($p = 0.0015$) (Table 4). Without bran, consumption of the two plastics when offered together were similar.

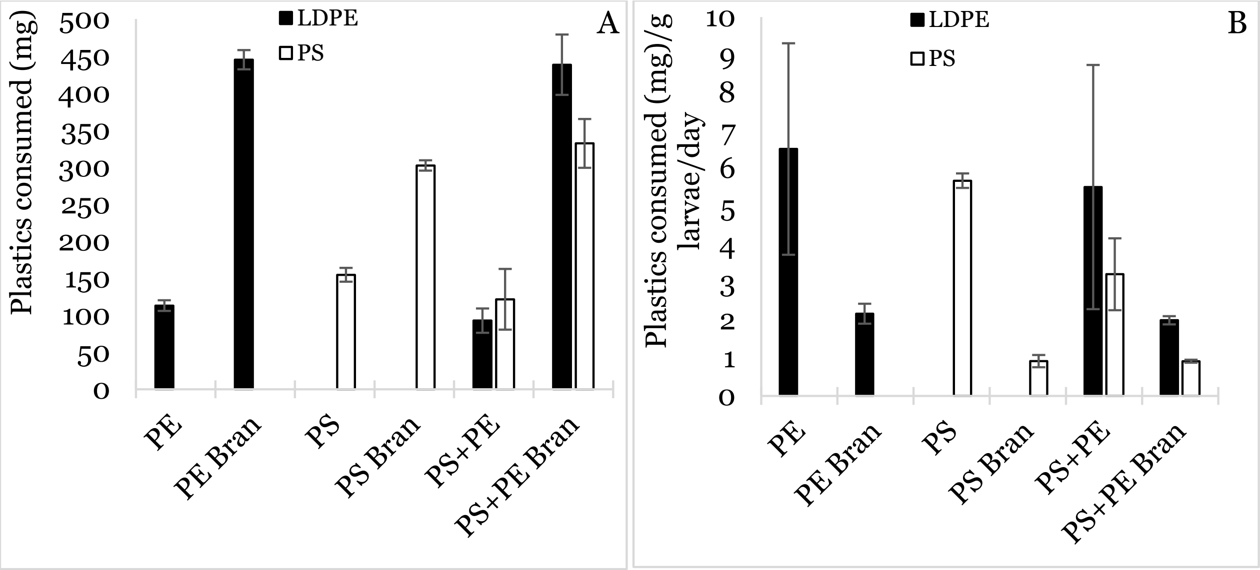

Figure 13. (*A*) Average plastics mass loss over 59 days, and (*B*) total plastics consumed as normalized to mealworm body weight per day.

Table 3. Summary of t-test results of pairwise comparisons for Figure 13*A*. Significant data points at $p < 0.01$ are in bold. *PS- is the PS portion of the mixed PS+PE, and #PE- is the PE portion of the mixed PS+PE plastics conditions.

t-tests ($p < 0.01$)	**Total Plastics Consumed over 59 Days**			
		PS Bran	**PS- PS+PE**	**PS- PS+PE Bran**
PS		**0.00142**	0.34546	**0.00790**
PS Bran		—	0.02250	0.19307
*PS- PS+PE		—	—	0.03847
		PE Bran	**PE- PS+PE**	**PE- PS+PE Bran**
PE		**0.00017**	0.07984	**0.00630**
PE Bran		—	**0.00003**	0.81156
#PE- PS+PE		—	—	**0.00569**
	PE	**PE Bran**	**PE- PS+PE**	**PE- PS+PE Bran**
PS	0.04479	**0.00144**	0.04161	**0.00433**
PS Bran	**0.00069**	**0.00149**	**0.00126**	0.02295
*PS- PS+PE	0.75689	**0.00747**	0.42943	0.01979
*PS- PS+PE Bran	**0.00833**	0.02435	**0.00597**	**0.00791**

Table 4. Summary of t-test results of pairwise comparisons for Figure 13*B*. Significant data points at $p < 0.01$ are in bold. *PS- is the PS portion of the mixed PS+PE, and #PE- is the PE portion of the mixed PS+PE plastics conditions.

t-tests ($p < 0.01$)	**Plastic Consumed per Gram Body Weight per Day**			
		PS Bran	**PS- PS+PE**	**PS- PS+PE Bran**
PS		**0.00103**	0.05446	**0.00060**
PS Bran		—	0.06756	0.99405
*PS- PS+PE		—	—	0.04842
		PE Bran	**PE- PS+PE**	**PE- PS+PE Bran**
PE		0.11835	0.71360	0.11189
PE Bran		—	0.23844	0.46776
#PE- PS+PE		—	—	0.46776
	PE	**PE Bran**	**PE- PS+PE**	**PE- PS+PE Bran**
PS	0.63333	**0.00361**	0.93568	**0.00177**
PS Bran	0.07410	**0.00238**	0.14291	0.01747
*PS- PS+PE	0.23175	0.26041	0.25169	0.13012
Bran	0.07429	0.01848	0.12952	**0.00151**

However, after 69 days, some noticeable eating had begun (Figure 14). As with the LDPE, given time the mealworms appear to have the ability to degrade HDPE. Other researchers have recently begun to document similar findings (Pham et al. 2023). Lessons learned from these feeding experiments will greatly enhance future feeding and degradation studies. Also, important to note is that some plastics may require an acclimation period, which by itself is a possible area for study; this possible acclimation period is important for proper timing when looking at degradation during the mealworm larval stage of this beetle.

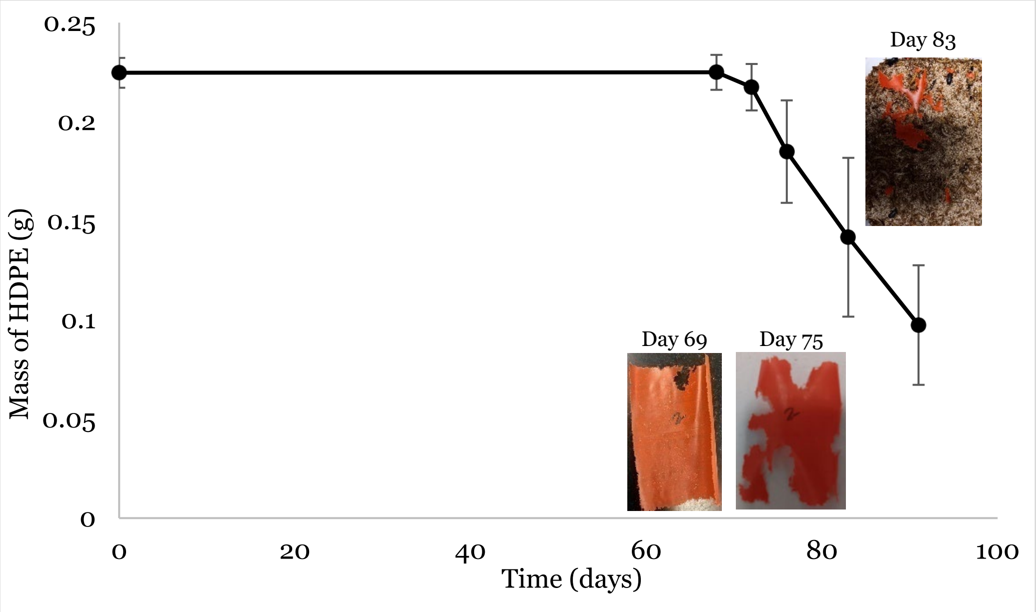

Figure 14. Degradation of HDPE by greater than 1,000 larvae over 91 days.

CONCLUSIONS: Although the primary objective of this research was to determine if plastics-degrading gut bacterial communities from a nonoptimal insect host can be successfully transplanted into the gut of the optimal mealworm host for large scale composting, numerous fundamental questions needed to be addressed first. Proper housing and feeding regimes, expected plastics degradation rates, and survivability on plastics as a food source were unknown for our research team. It was determined that plastics alone did not support long-term survival of the mealworm larvae (typically beyond 60 days), as massive attrition was observed in plastics-only fed groups compared to groups receiving bran as an alternate food source. Of greatest interest was the observation that given enough time (greater than 60 days in the reported observation), the mealworm larvae and adults will adapt to feeding on a previously undesirable plastic substrate. Bacterial gut microbiome communities are likely driving this adaptation and perhaps leading to behavioral changes in feeding response of the host animal. Numerous questions have arisen from this phenomenon of beetles eating plastics and this document serves as a starting point for future research efforts.

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