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EFFECTS OF POLYSTYRENE FOAM ON THE GROWTH AND DEVELOPMENT OF DARKLING BEETLES (*TENEBRIO MOLITOR*)

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Acknowledgements

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ABSTRACT

The growing field of entomoremediation explores the use of insects and other invertebrates as biological agents for the natural removal of hazardous substances, and recent research has investigated the potential for using mealworms (*Tenebrio molitor*) to digest and decompose polystyrene. Two experiments conducted over two years tested the tolerance of mealworm populations to the inclusion of polystyrene in their diet, and the ability of these diets to support multi-generational populations. The first experiment compared mealworms raised on a diet of 100% polystyrene foam with mealworms raised on a control diet of wheat bran and potatoes. The second experiment involved four treatments that mixed polystyrene foam with wheat bran and potatoes, each incorporating 0% polystyrene, 50% polystyrene, 75% polystyrene, or 90% polystyrene. Once beetles began to emerge, data were collected on the number of beetles emerging over time, as well as the mass and length of these beetles. No beetles emerged from the replicates with 100% polystyrene, suggesting that this diet was unable to support a sustainable population. Beetles in the control treatment had significantly smaller masses than the 50% and 75% polystyrene treatments, and also had significantly shorter lengths than beetles from the 90% polystyrene treatments. Regression analysis found a significant correlation between length and mass in all treatments except the 75% polystyrene treatment. These results suggest that some diets with polystyrene may be able to support populations of mealworms, and may result in larger, more successful beetles.

Introduction

Human activity places great pressure on the biodiversity and productivity of natural systems. According to Primack (2014), the loss of biodiversity of an ecosystem is afflicted by five major pressures; habitat loss, overconsumption by humans, overpopulation of humans, pollution, and introduction of invasive species. Pollution is one of the most quantifiable pressures, in that the effects of exposure to a foreign contaminate can be measured by the response from the organisms in an ecosystem. Developing methods of waste disposal or mitigation is important as a means of reducing human impact on the environment.

One such approach that has been adopted for pollution management is bioremediation, which involves removing hazardous materials from a habitat using biological agents. One well-known application of this process is the use of microorganisms to break down crude oil in soil or oceans. In many cases microorganisms successfully removed hazardous hydrocarbons such as benzene and xylene from the environment. The alternatives to this are physical or chemical processes, which tend to be more expensive and physically demanding (Maaroof & Dursun, 2018).

Microbes are often used in bioremediation, but many studies have investigated the potential for multicellular organisms to dispose of environmental hazards. For example, Yousefi-Garakouei et al. (2019) investigated the ability of a population of polychaetes (*Nereis diversicolor*) to consume the solid organic wastes from rainbow trout (*Oncorhynchus mykiss*) hatcheries. Higher densities of polychaetes were able to more effectively remove waste materials such as nitrite, ammonia, and phosphate, mitigating the hatchery’s waste production. Boughattas et al. (2019) investigated the use of earthworms (*Eisenia andrei*) for the bioremediation of heavy metals in soil, allowing them to accumulate in worms instead of remaining in the soil. Indeed, the opportunities for discovering new species to use for bioremediation purposes seems limitless, especially as new techniques are developed.

Entomoremediation is bioremediation that applies insects and other invertebrates for the breakdown of previously inert materials, or the removal of hazardous substances from bio-waste. This field has great potential in removing hazardous materials that would otherwise remain as waste for years. In Bulak et al. (2018), black soldier fly larvae (*Hermetia illucens*) were used to remove hazardous Cd and Zn ions from agricultural waste. Forty-nine percent of their polluted substrate was consumed after 36 days, and the hazardous ions had bioaccumulated in the tissues of pupae and adults. The fecal matter left by the larvae was significantly lower in pollutants but was nitrogen-rich and could be reused in agriculture. This represents a post-harvest management strategy that is more effective than the previous strategy of composting. Bombelli et al. (2017) investigated the degradation of polyethylene using wax worm (*Galleria mellonella*) larvae. Wax worms ingested plastic shopping bags and chemically decomposed polyethylene. A wax worm paste (homogenate) was smeared onto bags, which resulted in a decomposition rate of 13% after 14 hours – this suggests that breakdown is a chemical process that takes place in the guts of larvae, instead of through mechanical chewing (Bombelli et al., 2017). These two strategies are promising, and emphasize the need to discover methods for decomposing as many different materials as possible.

In this study, I examined the bioremediation of polystyrene, which is a widely manufactured, environmentally hazardous, and non-biodegradable material. Polystyrene is a polymer consisting of a chain of styrene molecules ($C_6H_5CH=CH_2$) bound together with a methyl group at the end (Yoon et al., 1975). This plastic compound has seen widespread commercial use, from cups to packaging material to yogurt containers. Its versatility and popularity are owed to the three major forms it is processed into: its hard plastic form, its extruded foam form, and its thermally expanded form that results in a soft, workable material known as Styrofoam. Although it has been useful for humans, it is also a common waste material that can have serious negative implications for the environment. In 2015, the Environmental Protection Agency reported that plastics and similar materials constituted about 13.1% of all waste discarded into USA landfills for that year. This equates to about 34.5 million tons of waste, which is an increase of 4.9% since 1990. No data are present on what percent of this waste is attributed to polystyrene, although it was noted in this report that polystyrene was observed frequently among the waste (EPA, 2017). This figure is also likely not reflective of the actual plastic waste generated, as it does not account for plastics dumped outside of landfills.

Even when polystyrene or other plastics are buried in landfills, decomposition will not occur for years. Several decades of being buried will result in little decomposition, if any (Otake et al., 1995). Often plastics and similar waste are discarded in the ocean, where light and heat exposure causes them to break apart into smaller and smaller pieces, becoming tiny fragments of microplastics. These microplastics have since become the main source of persistent organic pollutants in the ocean (Andrady, 2011). The scope and severity of their presence in the ocean is not fully understood, but they have become increasingly more concentrated in marine systems, especially in the Pacific Ocean (Kwon et al., 2017). Eriksen et al. (2014) estimated a total of about 5.25 trillion pieces of plastics distributed throughout all oceans – these pieces range from microplastics to large (>4.75mm) particles.

Burying polystyrene or dumping it in the ocean have proved ineffective in the long run, as neither results in polystyrene decomposition. In fact, the generation of microplastics in oceans has widespread negative impacts for the ecology of these systems. Exposure to microplastics, especially through ingestion, has negative impacts on numerous marine species: brine shrimp (*Artemia parthenogenetica*) experienced intestinal damage after ingestion, and chronic exposure altered behavior and negatively impacted growth and fecundity of fish and other large marine animals (Wang et al., 2019, Galloway et al., 2017). Polystyrene (Styrofoam) has the ability to bind to dioxin-like chemicals while in the ocean, which may contribute to a toxic marine environment and another source of persistent organic pollutants in the oceans (Chen et al., 2019).

The hazards presented by the high volume of plastics in the ocean and in landfills highlights the need for their control and mitigation. Although it would be extremely difficult to remove all the plastics from the ocean, preventative steps can be taken to mitigate plastics from being dumped into oceans and landfills in the first place. Instead of being dumped, materials such as plastics could be broken down via biological agents and converted into biodegradable or recyclable byproducts. For polystyrene, this process could take place using mealworms (*Tenebrio molitor*).

Mealworms are a valuable species for processing agricultural or industrial waste, and have great potential for entomoremediation. These larvae have ravenous appetites and are useful in recycling plant matter into fecal matter that can be used as fertilizer (Ramos-Elorduy et al., 2002). Abeolkheir et al. (2019) investigated their ability to decompose the vulcanized rubber used in tires. After an exposure time of three weeks, all mealworms were dead, and surface-level chemical changes were observed on the rubber after three weeks, suggesting that mealworms can decompose rubber to some extent. Although this substance was hazardous for mealworm health, this study as well as Ramos-Elorduy et al. (2002) highlight the versatility of using mealworms for decomposing waste materials.

Insect larvae have potential to decompose numerous inert materials, and polystyrene is no exception. Yang et al. (2015a) studied the use of mealworms in this process by placing mealworms on a large ($1m^2$) block of polystyrene. After an exposure period of 30 days mealworms had consumed polystyrene, and they concluded that larvae were able to digest and survive off of polystyrene. Polystyrene samples were marked with ^{13}C, and these carbon atoms were found in the lipid profiles of larvae. The conclusion was that polystyrene was being metabolized with some carbon being incorporated into mealworm biomass, and the rest being converted into carbon dioxide and carbon-based residuals (the residuals composing their fecal matter were not identified). Mealworms exposed to antibiotics were no longer able to digest polystyrene, which provided evidence that styrene metabolism is possible using a symbiotic gut bacteria (Yang et al., 2015b). This presents an exciting method for sustainably decomposing polystyrene into a fully biodegradable form, using a process that takes months rather than years. However, further research must be done on mealworms, to determine their ability to decompose and survive on a polystyrene diet.

The results of both of these studies were promising, but present a few gaps that provoke further investigation. Neither of these studies exposed mealworms to polystyrene for longer than one month – this brings into question if a population could survive in an environment consuming polystyrene, and therefore if using mealworms in biodegradation is efficient and viable. Additionally, there was no investigation into the health effects on these larvae; the potential damage that could have been caused to their digestive tracts and the effects on their growth, body condition, or fecundity in later stages of their life were not evaluated. Rather, researchers simply observed to see if the mealworms were still alive after exposure to the treatments, and if metabolism had occurred. Determining if mealworms can survive in a habitat where polystyrene is a major food source could prove invaluable, as it would determine if this diet can support an entire population. Long-term exposure would likely be a much more efficient means of degrading polystyrene, as this could result in a higher rate of consumption and thus a larger volume of polystyrene being decomposed.

The goal of this study was to rear populations of mealworms on diets containing polystyrene to analyze effects on the growth and development of mealworms into their adult life stage: darkling beetles. To do this, two experiments were conducted that compared mealworms reared on a traditional diet with mealworms reared on diets incorporating polystyrene. Diet is an important factor in adult morphology, which in turn may significantly affect survivorship and fecundity (Hanks et al., 2005, Moczek, 1998). Morales-Ramos et al. (2012) found that as average weight of a group of darkling beetles decreases, so does the number of offspring they produce. This relationship was not linear, and they could not conclude what the trend between weight and fecundity was. Although the relationship is not fully understood, the morphology of beetles is influenced by their diet as larvae, and affects their reproductive success.

The first experiment compared the growth and emergence rate of mealworms reared on a control diet, with mealworms reared on a diet of pure polystyrene. The prediction was that a diet of only polystyrene would result in slower growth times and fewer mealworms reaching adulthood, as it is less nutritious than a traditional diet. The second experiment included four treatments that mixed polystyrene at different ratios with wheat bran and potatoes. I predicted that more polystyrene in a mealworm’s diet would result in slower emergence rates, and smaller adults.

Methods

Treatment design and environment

To evaluate the effect of ingesting polystyrene on the growth and development of *Tenebrio molitor* larvae, larvae were reared in treatments with different percentages of polystyrene in their substrate. The substrates in each treatment contained different ratios of polystyrene, so that more or less polystyrene would be incorporated into larvae's diets. Polystyrene foam was used, as this form was easier for mealworms ingest than solid or extruded polystyrene; it was acquired from leftover shipping materials at local retailers in Westerville, Ohio.

The mealworms were housed in plastic bins with dimensions of 35 cm x 58 cm x 30 cm. Both experiments used eight replicates per treatment, with a total of 16 replicates in the first experiment, and 32 replicates in the second. This number of replicates was enough for statistical analysis, but was also limited by the space available and the time required to collect data from each replicate. The total volume of each replicate was 5 L, with a substrate depth of about 5cm. Three hundred mealworms were introduced into each replicate - this number was sufficient to produce robust results but was not enough to oversaturate the substrates and significantly reduce survival rates (Weaver & McFarlane, 1990). Mealworms were ordered online from rainbowmealworms.net, and the smallest available size was chosen so that the mealworms would be exposed to each treatment in their larval stage for as long as possible. The mealworms that arrived were estimated to be < 1cm long on average.

Wheat bran was incorporated into the diets of each treatment alongside polystyrene. This plant-based source of nutrients is commonly used for commercial rearing of mealworms, and was also relatively inexpensive to some alternatives (Aguilar-Miranda et al., 2001, Urs &

Hopkins, 1973). This or other cereal brans are often used in lab conditions to rear groups of mealworms, but fruits and vegetables are often supplemented to serve as a source of moisture and prevent desiccation; a lack of moisture results in slow growth rates, and low activity of adults (Urs & Hopkins, 1973). As a result, each treatment was supplied with a constant amount of potatoes, which were cut into sizes approximately 64 cubic centimeters in volume. Each replicate was given 4-5 of these pieces, making the total mass of potatoes given to be about 100 grams per replicate. Potatoes were replaced once a week, due to their eventual drying out or decomposition.

Replicates were placed randomly on several shelves in a single room, which was usually kept dark unless data were being collected (Figure 1). Insects such as these are usually more active in dark conditions, and so the lights were never left on for an extended period of time. Temperature is important for insect growth, with the optimal range of 22-38°C (Gordon, 1999); this room was kept at a constant 23°C. Atmospheric humidity was less important as the food provided was able to provide a majority of an individual's moisture (Gordon, 1999). Pielou and Gunn (1940) found that darkling beetles tend to aggregate in drier atmospheric conditions, implying that these conditions may be more favorable. Once introduced to their replicates, each population of mealworms was left to consume the substrate, mature, and metamorphose.

Experiment 1

This experiment compared mealworms reared on a control diet to mealworms reared on a treatment diet of pure polystyrene. Polystyrene foam was broken in pieces approximately 64cm^3 in size, and no other substance as a food source. No potatoes were provided, and instead moisture was added every two days via a misting spray; all moisture was free-standing or atmospheric, and offered no other nutritional impact. The control diet was a more traditional diet, with a substrate of wheat bran and potatoes.

Once every week, each treatment was sampled to observe the number of adults present in each replicate. This was performed once every week to display their growth over time. Data collection was halted on each replicate after about 25% of all larvae showed metamorphosis, as this many adults was determined to be able to replace the current population in the next generation. After reaching this point, final measurements were sampled from that replicate, and all remaining mealworms were euthanized and dried down.

Experiment 2

Four treatments were created that implemented polystyrene into the substrates in different ratios. Styrofoam blocks were shredded using a potato peeler, which allowed for easy measurement and integration with the rest of the substrate. The first treatment acted as a control diet consisting of 5 L of wheat bran (0% polystyrene), the second treatment consisted of 2.5 L of wheat bran with 2.5 L of polystyrene foam (50% polystyrene), treatment 3 consisted of 1.25 L of wheat bran with 3.75 L of polystyrene foam (75% polystyrene), and treatment 4 consisted of 0.5 L of wheat bran and 4.5 L of polystyrene foam (90% polystyrene).

Once beetles started to emerge, each replicate was scanned by hand every three days to count the number of new beetles. These beetles were then marked with a color combination of nail polish that translated to a specific day, preventing recounting of beetles. This allowed observation of how many beetles emerged from each replicate over time, and calculate the rate at which adults emerged for each treatment (Figure 2).

For six collection times over the course of two weeks, the individuals that were counted were also photographed and weighed, so that their lengths and masses could be determined.

Features such as these are easily comparable between treatments, as there is no sexual dimorphism among darkling beetles, meaning any significant differences are the result of genotype or environment (Rantala et al., 2003). The total beetle mass for each sample was recorded, and was divided by the number of beetles present to find the average mass on that day. Lengths were measured using ImageJ software, which allowed for more accurate measurements than those done by hand – taking measuring with calipers would be less accurate due to their small size and constant movements. These individual lengths could then be used to calculate the average length of the beetles in a replicate on a specific day, and plotted with the average mass for the same replicate. This comparison to the lengths determined if there is a correlation between the length and mass of beetles within treatments. The pictures taken were also used to observe the number of beetles being damaged as adults, either from cannibalism or experiencing defects from metamorphosis (Figure 3).

Analysis between treatments for the average mass and length of beetles was conducted using a univariate general linear model in SPSS. Regression analysis was also performed between the lengths and mass of each replicate to determine a correlation between the average length and average mass of beetles in a replicate. A univariate ANCOVA test was used to determine if there is a significant difference between the regression lines for each of these treatments. A univariate general linear model was also used to compare the frequency of damaged beetles between treatments. Tank effects were also analyzed, so that heteroscedasticity between replicates in each treatment could be ruled out.

Figure 1. Setup of bins in laboratory space. Replicates for each treatment were stored in plastic bins, each with the same volume of 60900 cm^3. Each replicate was placed randomly on the shelves.

Figure 2. Darkling beetles (*Tenebrio molitor*) marked with nail polish. Emergent beetles were marked with a specific color combination of nail polish that designated them to a specific day. Nail polish is a safe, durable substance that was used to prevent beetles from being recounted.

Figure 3. Damaged darkling beetle (*Tenebrio molitor*). One of the primary causes of damage done to some beetles was suspected to be cannibalism from larvae, as some beetle's bodies appeared to have been partially eaten.

Results

Experiment 1

The mealworms exposed to a substrate of only polystyrene showed a very slow growth rate over time, with no adults emerging after 11 weeks. In contrast, the control replicates showed faster growth and maturation, with an average of 31.79% of individuals reaching their pupal stage 4 weeks into the experiment (Figure 4). The mealworms in the polystyrene diet had a higher rate of mortality, and their exposure to this diet was halted after 11 weeks, when the sharp decrease in their population size was observed. As a result, there was no treatment data on adult beetles to compare to the control.

An independent samples t-test was conducted with a 95% confidence interval to determine if the percent survivorship between these two samples was significantly different. The 0% polystyrene treatment had a significantly higher percent survivorship (94.9 ± 2.18) than the 100% polystyrene treatment (10.9 ± 1.78) ($t_{14} = -58.48$, $p < 0.0001$) (Figure 5).

Cannibalism by larvae was a likely the reason for the low survivorship in the 100% polystyrene treatment, as few corpses were observed in any of the 100% polystyrene replicates. It is entirely possible that some mealworms were able to reach their pupal stage, but became easy prey for mealworms due to their limited mobility. Pupae are able to rotate their bodies in response to disturbance as an anti-predator behavior, and Ichikawa & Kurauchi (2009) found that this behavior can prevent larvae from cannibalizing them. However, a slow rate of pupation and many larvae present increases the chance for larvae to cannibalize pupae, decreasing survivorship. The 0% polystyrene diet had beetles emerge after about 4 weeks, and therefore suggests that the 100% polystyrene treatment inhibits larval growth. These results provides ample reason to provide more biologically appropriate food sources alongside polystyrene, and create a more complete and balanced diet.

Experiment 2: Rate of emergence

After about 12 weeks – the average number of emerged beetles were 106.1 in the 0% polystyrene treatment, 136.5 in the 50% polystyrene treatment, 135.3 in the 75% polystyrene treatment, and 130.9 in the 90% polystyrene treatment. Analysis using a general linear model found that the total number of beetles emerging changed over time ($F_{9, 252} = 645.3$, $p < 0.001$), but there was no significant difference between any of the treatments ($F_{3, 28} = 1.797$, $p = 0.171$). (Figure 6).

Although mealworms do not appear to be able to survive long-term on a 100% polystyrene diet, the presence of polystyrene at lower percentages did not significantly impact the rate at which larvae pupated into adulthood. The similarity in these trends suggest that for each treatment, it will take roughly 12 weeks for 1/3 of the larvae present in these conditions to reach adulthood, given the volume of substrate and density of mealworms. The total number of beetles per treatment increasing over time is salient because it implies that mealworms were able to consistently grow over time in these conditions, and each treatment was able to produce a significant population of beetles.

Had data been collected on beetle emergence until no new beetles were be observed, the predicted shape of the graph would resemble a logistic function – the rate would likely slow as the smallest mealworms mature, resulting in a shallower slope that eventually reaches a plateau. This shape assumes that the sizes and relative age of the mealworms in a sample are normally distributed, but being able to map out this curve could help create a function that describes the time required for a certain percentage of a population of mealworms to reach adulthood.

Experiment 2: Mass and length of beetles

For beetle mass, there was no significant tank effect ($F_{28,\,160}$ = 1.036, p = 0.425). There was a significant difference between treatments ($F_{3,\,28}$ = 5.694, p = 0.004). A Tukey HSD post hoc test found that the average mass in the 0% polystyrene treatment was significantly smaller than the 50% polystyrene treatment (p = 0.001), and the 75% polystyrene treatment (p = 0.025). All other treatments were not significantly different (Figure 7).

No significant tank effect was found for the length of beetles ($F_{28,\,160}$ = 1.348, p = 0.131), but there was a significant treatment effect ($F_{3,28}$ = 3.059, p = 0.044). A Tukey HSD test found the average length in the 0% polystyrene treatment to be significantly smaller than the 90% polystyrene treatment (p = 0.006), but no significant difference between any other treatments (Figure 8).

It was surprising to see that beetles in the control treatments had smaller average masses, at least compared to the treatments with 50% and 75% polystyrene. This evidence suggest that beetles raised in an environment with polystyrene in these concentrations can lead to beetles with greater mass overall, which could result in differences in survivorship and fecundity. It is unclear why these beetles have a significantly higher mass; polystyrene could act as a supply of macromolecules to mealworms, with the 50% and 75% polystyrene diets being more nutritious than the traditional diet presented in the control. Since the lengths of beetles between these three treatments were not significantly different, beetles in the 50% and 75% polystyrene treatments are significantly denser. More dense beetles could have consequences for their health, and greater body mass may be linked to being able to produce more offspring and greater survivability.

Beetles in the 90% polystyrene treatment were significantly less dense than the beetles in the 0% polystyrene treatment, since they were significantly longer but had no difference in mass. This suggests that inclusion of polystyrene in a mealworm larvae's diet can increase their mass as adults, but too much polystyrene and not enough wheat bran can result in a loss of this effect and a potentially detrimental loss of density. This outlines that wheat bran is essential to the diets of mealworms in this study, especially since the 100% polystyrene treatment in experiment 1 resulted in slower growth and lower survivorship. An optimal range of polystyrene composition is likely between 50-75%, as this allows beetles to consume polystyrene while still being provided a healthy amount of wheat bran. Exceeding this range may produce less dense, potentially less healthy beetles. Regardless, the 90% polystyrene diet was still able to produce the same amount of adults as the other treatments, and further studies would need to be performed to determine if this treatment results in a lower survivorship, or other negative effects over time.

A lack of a significant tank effect is useful in verifying the results that were found from each treatment. No significant effect helps rule out the presence of heteroscedasticity among replicates, and allows the effects of the treatments to be considered more closely linked to the differences observed in beetle morphology.

There was a significant correlation between length and mass of all beetles in this study, ($R^2 = 0.145$, $F_{1, 182} = 30.92$, $p < 0.0001$) (Figure 9). This R^2 value suggests that 14.5% of a beetle's mass was directly influenced by its length. For the individual treatments, there was a significant correlation for the 0% polystyrene treatment, 50% polystyrene treatment, and 90% polystyrene treatment, but no significant correlation for the 75% polystyrene treatment (Table 1).

Table 1. Regression analysis between average length and mass of beetles results for each treatment (Experiment 2).

Treatment	R^2	*F*	*p*	*y = mx +b*
0% Polystyrene	0.132	6.663	0.013	y = 0.0053x + 0.0271
50% Polystyrene	0.257	15.257	< 0.001	y = 0.0078x - 0.0014
75% Polystyrene	0.083	3.968	0.053	y = 0.0054x + 0.0285
90% Polystyrene	0.211	11.767	0.001	y = 0.0078x - 0.0049

The univariate ANCOVA test revealed no significant tank effect ($F_{28, 151} = 0.823$, $p = 0.721$), but a significant difference in the mean mass between treatments ($F_{3, 30} = 5.869$, $p = 0.003$). The pairwise comparisons post hoc test that was run found that the intercept for the 0% polystyrene was significantly smaller than the 50% polystyrene treatment ($p = 0.006$). The intercept for the 90% polystyrene treatment was also significantly lower than the 50% polystyrene treatment ($p < 0.0005$) (Figure 10).

The 50% polystyrene treatment having a significantly higher trend line than the 0% and 90% polystyrene treatments suggests that this treatment's trend line has a significantly higher intercept, and mealworms raised on a 50% polystyrene diet are more likely to have a higher mass relative to length than either the 0% or 90% polystyrene treatments. This provides further evidence that for any given length, beetles in the 50% polystyrene treatment are more likely to have a greater mass than beetles in the 0% polystyrene and 90% polystyrene diets. A lack of significant differences between the 75% polystyrene treatment and any other treatment could be due to the insignificant correlation between length and mass in this treatment. This correlation was very close to being significant, however, and future replications of this study could show a trend that is significant.

Experiment 2: Damaged beetles

A univariate general linear model found that there was no significant tank effect for the percentage of damaged beetles ($F_{28, 152} = 1.320$, $p = 0.147$). This same analysis found that there was a significant effect of treatment ($F_{3, 28} = 11.608$, $p < 0.0001$).The 0% polystyrene treatment was significantly different from the 50% polystyrene ($p < 0.0001$), 75% polystyrene ($p < 0.0001$), and 90% polystyrene ($p < 0.0001$) treatments, but the 50% polystyrene, 75% polystyrene, 90% polystyrene are not significantly different from each other (Figure 11).

Control beetles were more likely to experience damage than beetles in the polystyrene treatments. The majority of damages observed were the results of partial cannibalism from larvae on adults or from complications that arose during metamorphosis; beetles were observed with bite marks or with incomplete development of their abdomen. These lead to injuries or defects that may make it more difficult for a beetle to survive or reproduce. A higher percentage of damage among control beetles is interesting because this was the only treatment with no polystyrene included in the substrate, which suggests that this higher rate is because of a lack of polystyrene foam. However, this conclusion cannot be made from the given information alone, as there may be other factors present that influence the rate of damage. Polystyrene makes for a loose substrate that could make escape from predatory larvae more difficult. This could result in a higher catch rate, and fewer damaged beetles being observed as they would be fully consumed by larvae.

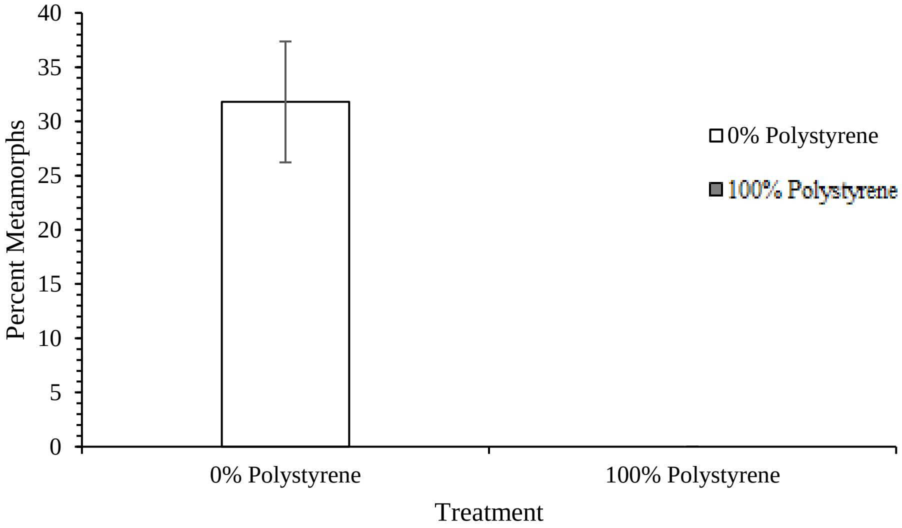

Figure 4. Percent of population metamorphosed into pupae or beetles after 4 weeks. No larvae from the 100% polystyrene treatment were able to metamorphose after 4 weeks, and by then the 0% polystyrene treatment had surpassed the 25% metamorphosis threshold. To reach this threshold, at least 75 of the 300 total larvae in a treatment had to metamorphose into pupae or beetles. Error bars signify standard deviation between replicates.

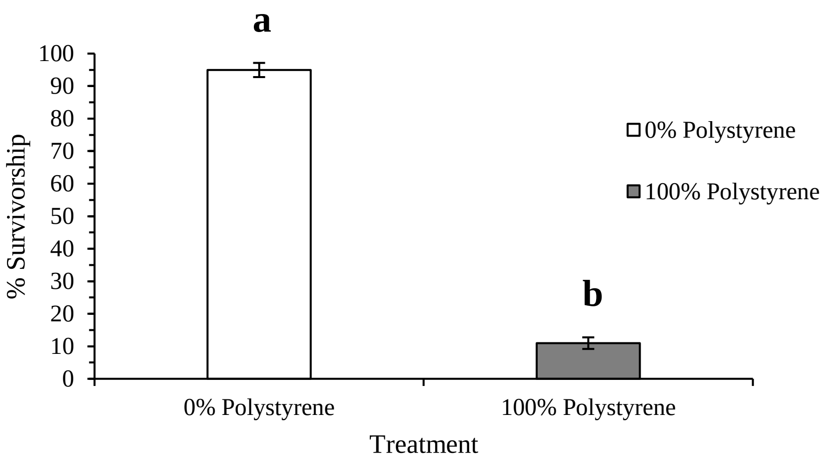

Figure 5. Percent survivorship of all individuals between treatments. Sixteen populations containing 300 mealworms each were exposed to a substrate of 0% polystyrene or 100% polystyrene for a period of 11 weeks. The survivorship in the 0% polystyrene treatment was significantly higher than the survivorship in the 100% polystyrene treatment. Error bars represent a 95% confidence interval.

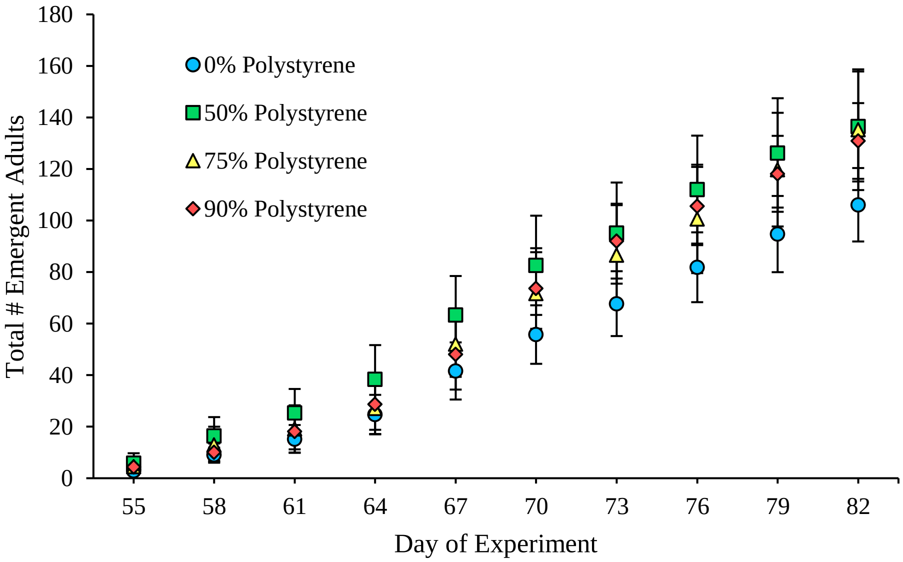

Figure 6. Total number of emergent adults over time. Four treatments incorporating difference percentages of polystyrene were provided as the food source for 32 populations of mealworm larvae. Beetles started to emerge from each treatment after about 8 weeks, and the total number of beetles from each replicate was recorded. Although there was a significant difference in the number of beetles over time, there was no significant difference between the emergence rates of treatments. Error bars represent 95% confidence intervals.

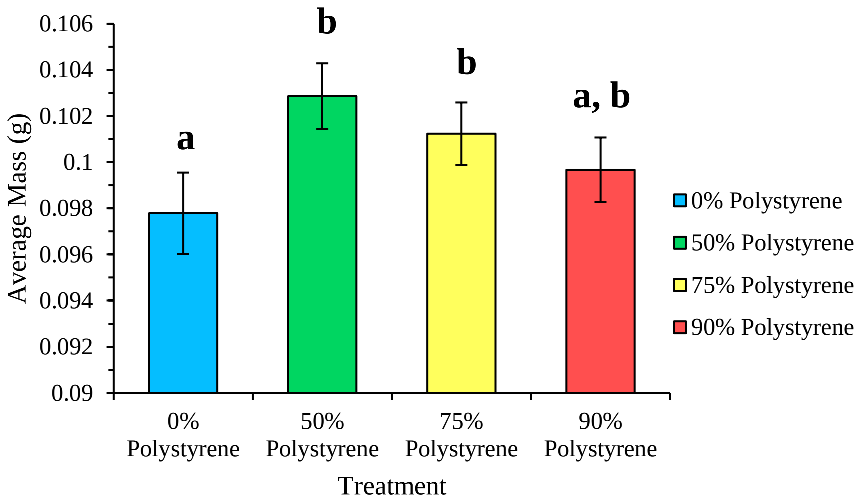

Figure 7. Average mass of *Tenebrio molitor* beetles. The amount of polystyrene present in a mealworm larvae's diet affected its mass at later life stages. Letters above bars represent statistically significant differences. Error bars represent 95% confidence intervals.

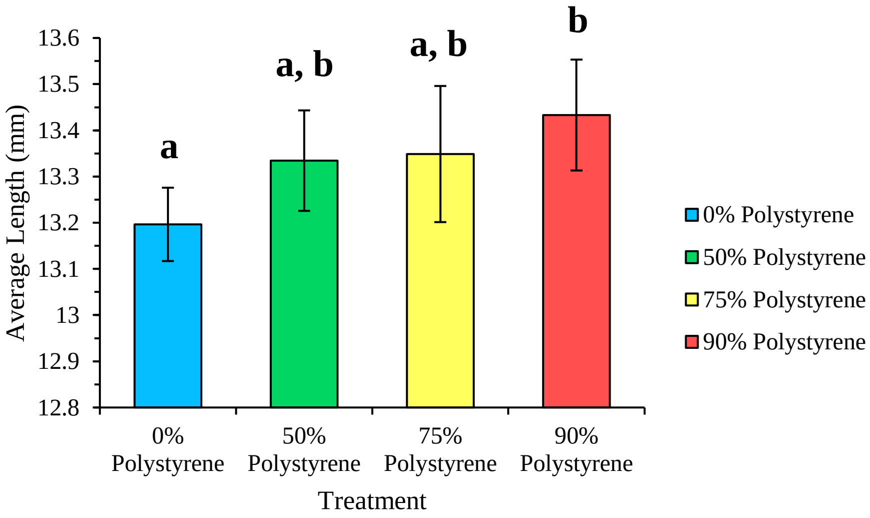

Figure 8. Average length of *Tenebrio molitor* beetles. The amount of polystyrene present in a mealworm larvae's diet was determined to have some effect on its length at later life stages. Letters above bars represent statistically significant differences. Error bars represent 95% confidence intervals.

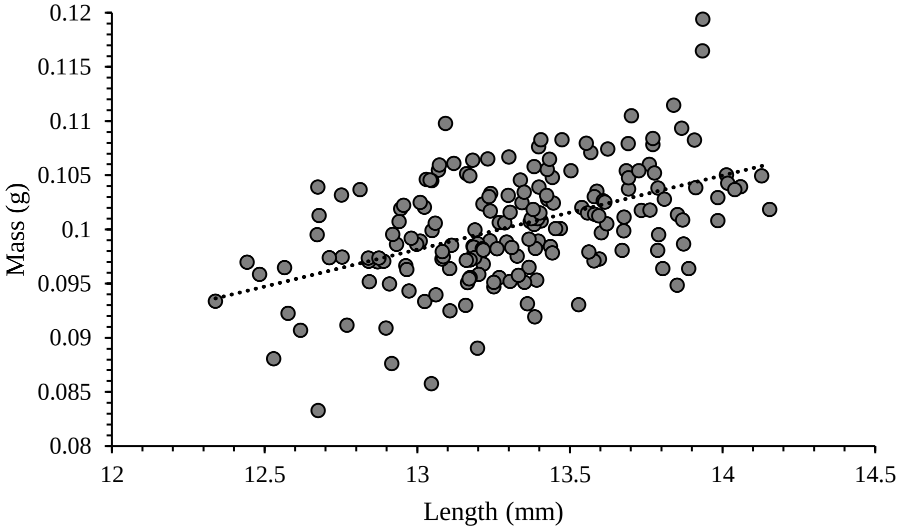

Figure 9. Relationship between length and mass of *Tenebrio molitor* adults. Analysis was conducted on the lengths and masses of beetles in experiment 2, in order to confirm a correlation between the size of a beetle and its mass. The results of regression analysis are significant ($R^2 = 0.145$, $F_{1, 182} = 30.92$, $p < 0.0001$). There was a weak positive linear correlation but shows a trend that about 14.5% of a beetle's mass was influenced by its length, and a trend formula of $y = 0.12 + 0.007x$.

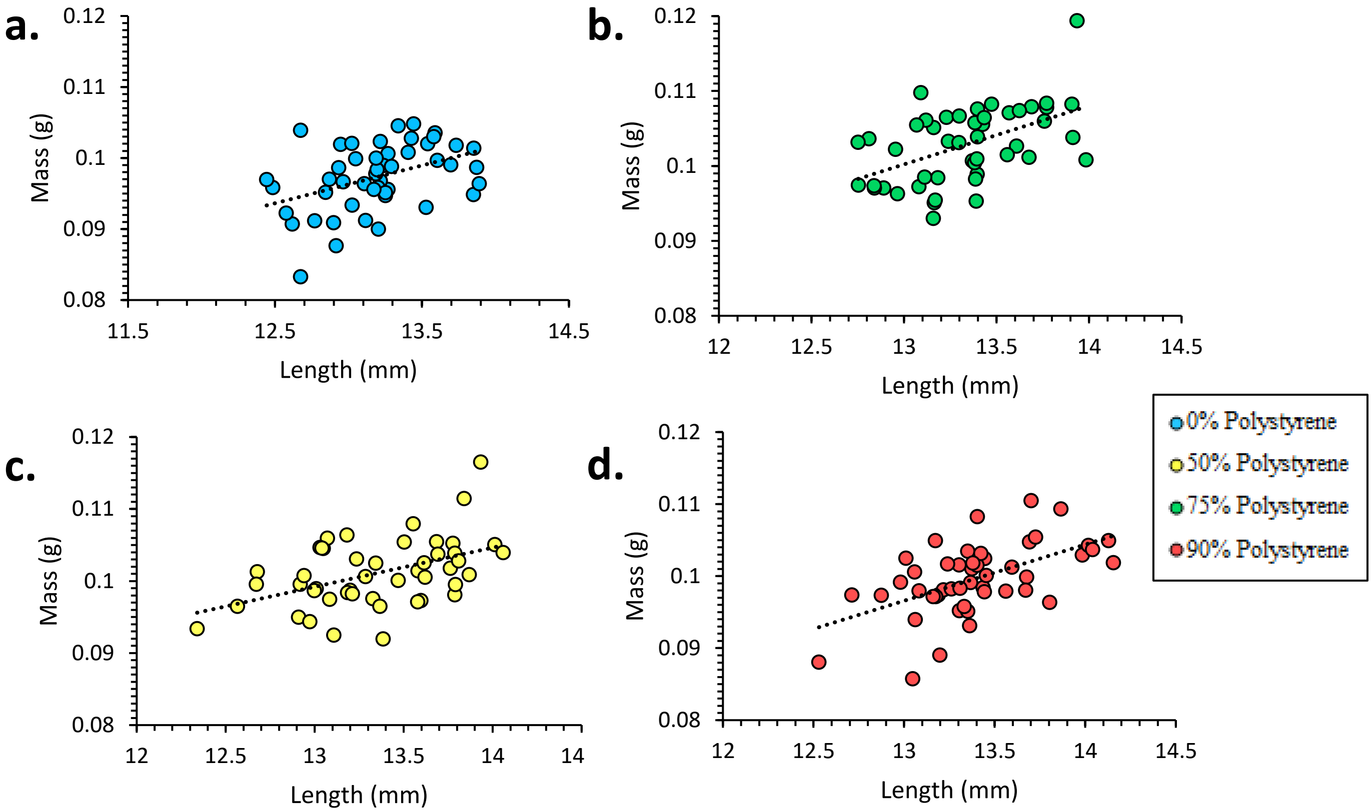

Figure 10. Relationship between length and mass of *Tenebrio molitor* adults between treatments. The size of a darkling beetle in most treatments has a significant positive correlation that beetle’s mass. Each treatment varied in its composition, in which each treatment contained (a) 0% polystyrene, (b) 50% polystyrene, (c) 75% polystyrene, or (d) 90% polystyrene. The ANCOVA test run between each relationship found that the 0% polystyrene and 90% polystyrene treatments have significantly lower intercepts than the 50% polystyrene treatment.

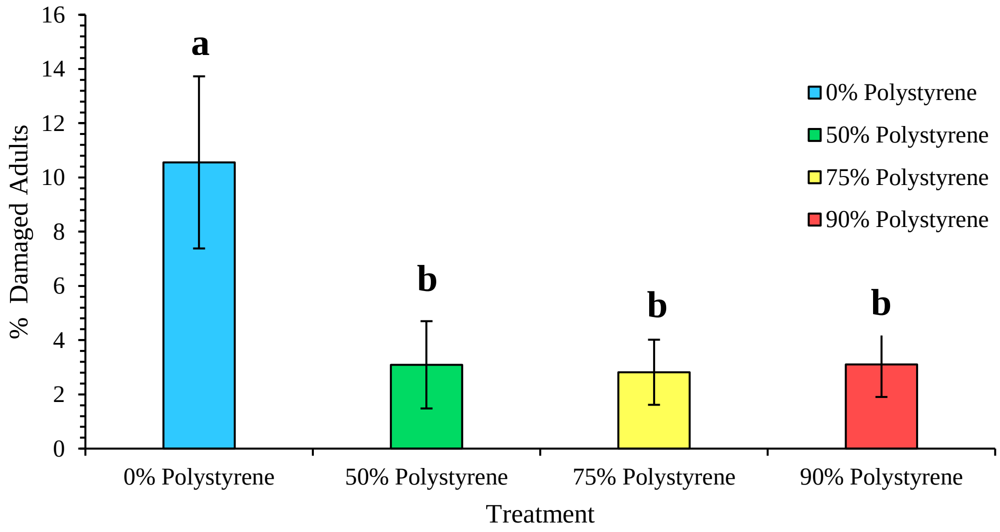

Figure 11. Percent of damaged adults per treatment. Mealworm larvae were exposed to four treatments with different percentages of polystyrene in the substrate, and were able to grow and develop into beetles. Some of those beetles that emerged observed were damaged either through metamorphosis, cannibalism, or other means. Letters above bars represent statistically significant differences. Error bars represent 95% confidence intervals.

Discussion

This study presents compelling evidence for mealworms to be able to survive long-term with polystyrene included in their diet. Contrary to Yang et al. (2015a), polystyrene foam was not suitable as the only food source for a population of mealworms. Mealworms may be able to survive on a diet of 100% polystyrene for a month, but are unable to complete a full life cycle in these conditions. Even the inclusion of a small percentage of a grain-based food source showed a significant increase in the success of mealworm larvae and their ability to reach adulthood. Other food sources besides bran, such as the agricultural waste used in Ramos-Elorduy et al. (2002) could be used instead as a supplementary food source. This would allow more waste products to be processed, and a more effective use of mealworms. Although this provides strong evidence that polystyrene is not fully adequate as a food source, this study describes a series of diets that are able to support a population of mealworms.

The increase in beetle mass with the 50% and 75% polystyrene treatments may be a result of the consumption of polystyrene. Beetles in these treatments may have higher lipid contents than beetles in the other treatments, as Yang et al. (2015a) found that carbons from digested polystyrene were incorporated into the lipids of larvae. Polystyrene consumption may increase fatty acid synthesis in mealworms, and produce heavier beetles. This may result in more offspring being produced, and a more successful mealworm population overall (Morales-Ramos et al., 2012). More offspring being produced may result in more polystyrene being consumed, and a greater quantity being decomposed. This may also result in more intraspecific competition among mealworms in future generations, as the larval density in the substrate may reach a point that negatively affects growth and development rate (Weaver & MacFarlane, 1990).

Research can also be done to examine the internal physiology of these mealworms and beetles, in order to observe both short-term and long-term effects of exposure to polystyrene. Much like in Wang et al. (2019), the digestive tract of mealworms can be observed to determine the effects and implications of exposing a mealworm's gut to polystyrene. Features such as microvilli density and gut length can be compared, as gut plasticity may help describe how polystyrene affects mealworm physiology, as well as how polystyrene is digested. The biochemical pathway for the decomposition of polystyrene is not yet fully understood (Yang et al., 2015b). The composition of the protein content, lipid content, and chemical energy in mealworms and beetles can also be compared, to provide more information on how polystyrene affects body condition. The byproducts of polystyrene metabolism must also be identified, so that fecal analysis can measure how much of each polystyrene is being consumed over time.

Information such as this would help determine how much polystyrene is being consumed, and determine the magnitude at which polystyrene impacts a mealworm's diet. If the amount of polystyrene ingested is lower than expected, further studies could investigate other techniques that may incorporate the polystyrene into the substrate more effectively, and maximize the ingestion and processing of polystyrene. Mealworms consumed polystyrene, but other methods can develop techniques that more closely and uniformly combine wheat bran and polystyrene. This could create an environment where mealworms are not eating bran or polystyrene, but are forced to consume both at the same time.

The study of bioremediation is an area of study that is full of opportunity, and demands constant exploration of new techniques that can uncover new means of handling materials that were previously resigned to take decades or centuries to remove. Abilities latent in different species to decompose materials are being discovered at an astounding rate, which speaks to the importance of exploring and refining new solutions in innovative ways. The growing threat of pollution calls for more action not just for the natural world, but also for humanity as a whole. Improving the natural world and finding solutions for such inescapable problems is an endeavor that will prevent further harm to be done to the systems that countless species are dependent on, and are integral in providing invaluable goods and services to all of humanity. Further development of these pathways could potentially even help reverse the damage done, and remediate the consequences brought on by years of neglect and indifference.

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