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The Effects of Post-Consumer Waste Polystyrene on the Rate of Mealworm Consumption

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Summary

Polymer debris is estimated to pollute approximately 40% of the planet's oceans, posing a great threat to marine ecosystems globally. A study released by Stanford scientists Yang *et al.* in 2015 found that mealworms consume and biodegrade expanded polystyrene (EPS), or Styrofoam, a polymer that is responsible for a large percentage of ocean pollution. However, this study only tested the mealworm consumption rates for virgin white EPS. If mealworms are to be used by consumers or the recycling industry to biodegrade EPS pollution, post-consumer use and colored EPS must be tested. This study investigates these new variables by testing different types of post-consumer EPS (highly pigmented, white, high-density, and low-density) to determine if mealworm consumption is affected. The measured results of 3.79 mg EPS consumed per mealworm life cycle for high-density EPS confirm *Yang et al.*'s measurements of 3.60 mg EPS per mealworm life cycle for the consumption rate of white, high-density EPS. Furthermore, the results indicate that whether the EPS was virgin or post-consumer did not affect the consumption rates. It was found that low-density EPS is consumed at a 56% higher rate than high-density EPS. Consumption rates for non-waxy and white EPS were slightly higher than those of waxy and colored EPS respectively but were within one standard deviation of difference. From this data we can calculate that it would take 4,720 mealworms to consume 17 grams of EPS, equivalent to a single serving clamshell container, consumed over the course of the mealworms' 30-day lifespan.

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Introduction

Expanded polystyrene (EPS) is a polystyrene foam found commonly in cups, egg cartons, take-out containers, soup bowls, packing peanuts, and meat trays (1). It is made from petroleum which is refined into polystyrene beads. A blowing agent, commonly pentane, is then passed through these beads, and they are heated by steam and molded into their commercial form (2). Due to its light polymer chain and brittle nature, it is difficult to recycle without crumbling and clogging the machinery. Additionally, it is typically contaminated by food products or may contain extra additives or fillers (3). Thus, EPS is commonly not recycled and, along with other plastics, makes up 30% of landfill waste annually. Due to its light weight, it is easily blown from landfills into waterways, and it is the top source of contamination for America's rivers and bays (4). EPS also photodegrades quickly, breaking down into smaller particles that can be ingested by animals, causing death due to starvation and poisoning from the harmful additives that are released when it begins to degrade (4).

Until recently, EPS was not classified as biodegradable, meaning that it could not be broken down biologically due to its long polymer chains (1). However, a 2015 study found that mealworms (*Tenebrio molitor*), the larvae of darkling beetles, could digest EPS in small quantities (5). When tested, the fecal matter of the mealworms was found free of all chemicals originally used to create the EPS. No changes were found in the life cycle of the mealworms fed with EPS, showing that they can survive sustainably on an EPS diet (5). However, this study was only conducted on unused or "virgin" white EPS, and it did not include highly pigmented or post-consumer use materials.

Other studies done by Yoshida *et al.* (6) and Bombelli *et al.* (7) discovered that bacteria and wax worms may also be able to consume plastic. The bacteria *Idonella sakaiensis*, discovered in landfills by Yoshida *et al.*, consumes polyethylene terephthalate (PET) by cutting the polymer chains into smaller, easily consumed sizes before breaking them down (6). Despite being a major breakthrough in biodegrading plastics, the current form of this bacteria consumes PET at a rate that is too slow to be efficient (6). Following Yang *et al.* (5), Bombelli *et al.* (7) investigated wax worms' consumption of polyethylene (PE) and found that its consumption rate, unlike that of the bacteria, was extremely efficient, devouring 92 milligrams of plastic every 12 hours per 100 wax worms (7). However, a follow-up study done by Weber *et al.* (8) was unable to reproduce the degradation of the PE into ethylene glycol with a homogenized mass of wax worms, suggesting that the PE merely sat in the gut of the wax worms instead of fully biodegrading.

The research presented here intends to corroborate and expand the results discovered in the study by Yang *et al.* (5) by testing post-consumer, virgin white, and colored polystyrene to determine if the mealworm consumption will remain at the same rate. It was hypothesized that the consumption rate would not change if mealworms were fed white and colored post-consumer waste EPS as compared to white "virgin" unused EPS as the chemical structure of the EPS did not vary with use or coloration (though there were dyes added to colored EPS). This hypothesis was found to be correct, as there were no distinguishable differences in consumption rate between colored and white EPS and virgin and post-consumer waste EPS. Whereas experimental data corroborated the consumption rate found in Yang *et al.* for virgin white EPS, a noteworthy observation was that the density of the EPS affected mealworm consumption rates.

Results

The initial experimental setup consisted of mealworms fed with bran (control), virgin white EPS, post-consumer use EPS, and colored EPS. These four experiments, along with those conducted in the second experimental setup, are summarized by mealworm food source in **Table 1** and will henceforth be referred to by their corresponding food source number.

1	Control (bran)
2	Unused White, Low Density EPS (cup)
3	Used White, Waxy, High Density EPS (clamshell)
4	Used Green High Density EPS (meat tray)
5	Used Yellow High Density EPS (meat tray)
6	Used Black High Density EPS (meat tray)
7	Used White Low Density EPS (cup)
8	Used White Waxy Low Density EPS (packing peanuts)

Table 1: Mealworm Food Source

Over the span of 30 days, the food source consumption rate of the mealworms was measured in each experiment. The EPS and mealworm feces were weighed every other day to determine the amount of EPS consumed, and dead mealworms were removed from each experimental setup. The amount of EPS consumed was then totaled and divided by the number of living mealworms to provide the number of milligrams per mealworm life cycle (**Table 2**).

Next, the data was grouped into different categories to explore the effects of pigmentation, use, density, and coating on the mealworm consumption rate. Additionally, all of the EPS was averaged in order to compare it to the control. The data was then plotted with one standard deviation above and below the mean in order to quantify the variation associated with the measurement set.

Overall, there was a similar average consumption rate for all the post-consumer use EPS (food sources 3-8) of 4.60 mg/mealworm life cycle (standard deviation (SD) = 1.76) as compared to the average consumption rate of 5.20 mg/mealworm life cycle (SD = 0.50) for the virgin EPS (food source 2) (**Figure 1**). Furthermore, mealworms consumed pigmented EPS (food sources 4-6) at a similar average rate of 4.01 mg/mealworm life cycle (SD = 1.19) compared to the average rate of the white EPS (food sources 2, 3, 7 and 8) of 5.09 mg/mealworm life cycle (SD = 1.69) (**Figure 2**).

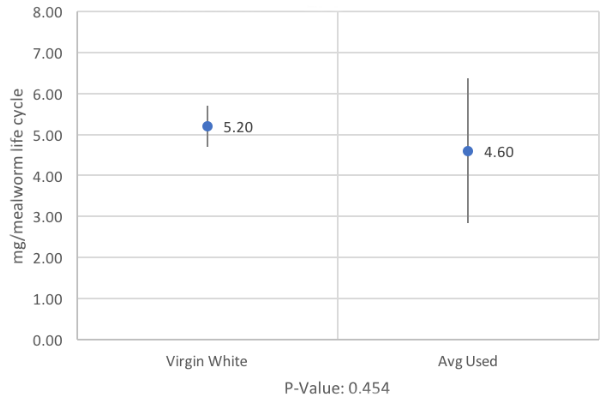

Figure 1: Consumption of Virgin White vs. Post-consumer (USED) EPS. The consumption rates and standard deviations of the virgin white EPS (food source 2) and the post-consumer use EPS (food sources 3-8).

When analyzed by color, the black EPS had a slightly higher consumption rate than both the average of the white EPS and the other two colors, and green EPS had the lowest average of all the colors (**Figure 3**). However, as each of the different colors was only tested once, more experiments are needed to validate this trend. Additionally, all of these results are comparable within one standard deviation of the mean, thereby illustrating that they do not have a large impact on the mealworms' consumption rate. In order to establish this, the *t*-test was performed and groups with a *p*-value greater than or equal to 0.05 were considered to have an insignificant impact on the consumption rate. Therefore, the color, use and coating were found to have a negligible impact on the consumption rate as they had *p*-values of 0.458, 0.454, and 0.321 respectively.

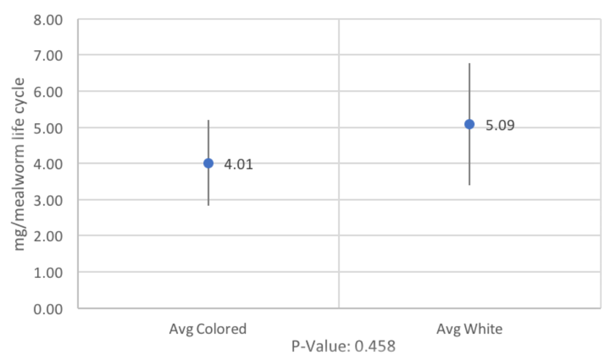

Figure 2: Consumption Rates of White vs Pigmented EPS. The consumption rate and standard deviation of colored EPS (food sources 4-6) and the rate of consumption and standard deviation of white EPS (food sources 2 ,3, 7, and 8)

Though the data was found to support the original hypothesis in the first experimental setup, this experiment still showed a large variation in consumption rate between the different types of EPS. In a more detailed analysis, differences in the coating and density of the EPS were observed, leading to a second experimental setup to test these constraints (**Table 2**).

Figure 3: Consumption Rate by Color. The difference in the consumption rates of the average white EPS (food sources 2, 3, 7, and 8) and the green, yellow, black EPS (food sources 4, 5, and 6 respectively).

Food Source ID	**1**	**2**	**3**	**4**
Waxy	Control	no	yes	no
Density	(Bran)	low	high	high
Use		no	yes	yes
Color		white	white	green
Initial Weight (g)	2.80	3.35	3.50	2.75
Final Weight (g)	1.95	2.73	3.10	2.35
Grams Eaten	0.85	0.62	0.40	0.40
% Consumed	30.4	18.5	11.4	14.5
# of Mealworms	110	128	125	129
% Mealworms Dead	7.3	2.3	9.6	2.3
mg/mealworm life cycle	7.73	4.84	3.20	3.10

Table 2A: Raw Data from Experimental Setup 1.

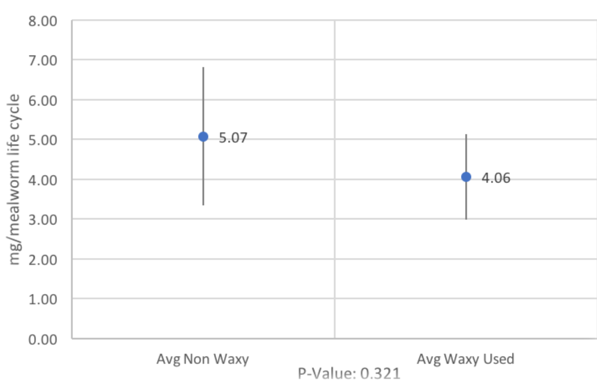

Figure 4: Consumption Rates of Waxy and Non-Waxy EPS. The consumption rates and standard deviations of the waxy EPS (food sources 3 and 8) and the non-waxy EPS (food sources 2, 4, 5, 6, and 7).

However, similar to the variables of use and color, coating was also found to have a negligible effect on the consumption rate of mealworms. The 5.07 mg/mealworm life cycle consumption rate for the non-waxy EPS (food sources 2, 4, 5, 6, and 7) was slightly higher than the 4.06 mg/mealworm consumption rate of the waxy EPS (sources 3 and 8) (**Figure 4**). Yet, as this result was within both the 1.74 mg/mealworm life cycle standard deviation for non-waxy EPS and the 1.08 standard deviation for the waxy EPS, its effect on mealworm consumption rate was considered negligible. Conversely, the final factor evaluated, density, was found to have a profound impact on the mealworm consumption rate. The less dense types of EPS such as a cup and packing peanuts (food sources 2, 7, and 8) had a higher consumption rate of 5.92 mg EPS/mealworm life cycle versus the consumption rate of 3.79 mg EPS/mealworm life cycle of the denser EPS found in the various meat trays and clamshell takeout containers (food sources 3-6) (**Figure 5**). The consumption rate of the low-density EPS was found to be 56 % higher than the value of the high-density EPS, showing the significant impact that density has on the consumption rate.

1	**2**	**3**	**7**	**5**	**6**	**8**
Control	no	yes	no	no	no	yes
(Bran)	low	high	low	high	high	low
	no	yes	yes	yes	yes	yes
	white	white	white	yellow	black	white
1.50	1.50	1.50	1.50	1.50	1.50	1.50
1.00	1.15	1.30	1.10	1.30	1.20	1.20
0.50	0.35	0.20	0.40	0.20	0.30	0.30
33.3	23.3	13.3	26.7	13.3	20.0	20.0
64	63	54	50	56	56	57
4.7	3.2	3.7	8.0	16.1	8.9	21.1
7.81	5.56	3.70	8.00	3.57	5.36	5.26

Table 2B: Raw Data from Experimental Setup 2.

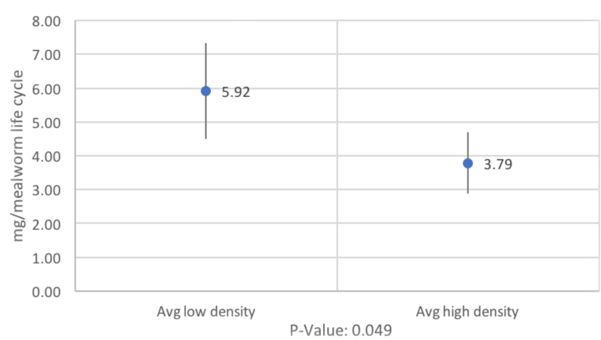

Figure 5: Consumption Rates of Low and High Density EPS. The consumption rates and standard deviations of the low-density EPS (food sources 2, 7, and 8) and the high-density EPS (food sources 3, 4, 5, and 6).

Additionally, the difference between densities had a *p*-value of 0.049, meaning the difference in consumption rates was statistically significant.

The average consumption rate of all polystyrene, 4.73 mg EPS/mealworm life cycle (food sources 2-8), was lower than the average consumption rate of the control (food source 1) which was 7.77 mg bran/

mealworm life cycle (**Figure 6**). The death rate and total weight of mealworms appeared to have little or no correlation with the type or amount of EPS consumed, as they fluctuated throughout the experiments (Table 2). The consumption rate of the control (food source 1) was similar for both experimental setups, 7.81 mg bran/mealworm life cycle and 7.73 mg bran/mealworm life cycle, showing reproducibility in the test results. Overall, the consumption rate of virgin EPS was similar to or higher than most types of post-consumer EPS, with the exception of the low density EPS tested (**Figure 7**).

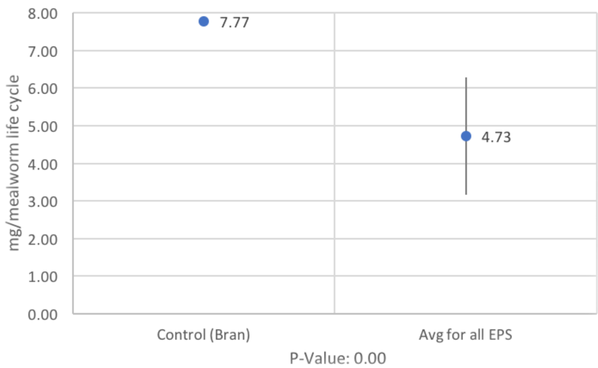

Figure 6: Consumption Rate of Control vs. Average EPS. The standard deviation and consumption rate of the control (food source 1) and the average of all EPS experiments (food sources 2-8), regardless of density, coating, use or color.

Finally, the consumption rate of 3.79 mg/mealworm life cycle taken for the high density EPS (sources 3-6) is comparable to the rate stated in Yang *et al.* (2015) of 3.60 mg/mealworm life cycle (2). As the use, coating, and coloration of the EPS was found to be negligible, they were combined in this measurement to provide the most accurate average of the mealworm consumption rate. Therefore, the accuracy and reproducibility of the data taken in the study published by Yang *et al.* (2015) is supported, and the data taken and conclusions made in this research can be viewed as accurate relative to the other study.

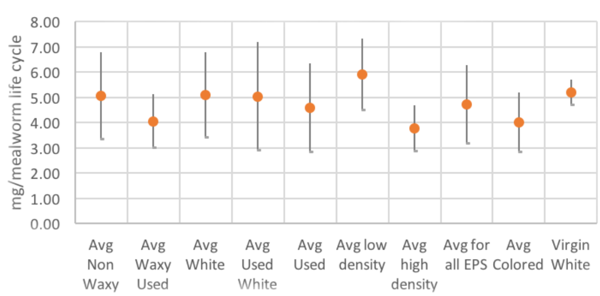

Figure 7: Groupings of combined results. The average results for the different groupings and the virgin white EPS (food source 2).

Discussion

This research reaffirms the conclusions found by Yang *et al.* (2015) that mealworms consume virgin white EPS (5). These results support the hypothesis that mealworms consume a similar amount of virgin, post-consumer use white, and post-consumer use colored EPS. Additionally, this study found that the pigmentation, coating, and usage of the EPS did not have a significant impact on the mealworm consumption rate. The density of the EPS, which had a p-value of 0.049, did reach statistical significance. The consumption rate for the high-density EPS was found to be lower than that of the low-density EPS.

The molecular weight was unknown in all samples because they were taken from commonly found commercial use items such as a coffee cup, packing peanuts, takeout clamshell containers, and various colored meat trays. Likewise, the precise range of the EPS foam density is unknown. Future work should therefore be conducted regarding the sensitivity of the mealworms' consumption rate to varying densities and molecular weight. Furthermore, as it was only tested once, the rate for non-waxy white EPS (food source 7) may have been skewed due to more active mealworms, as it is much larger than the average of the non-used white EPS of the same material. This result should be tested again in future work for reproducibility. Other experimental challenges were noted in the weighing of the EPS, as mealworms would burrow inside the EPS, making it difficult to extract them and gain an accurate measurement of the EPS alone. Moreover, the dead mealworms were excluded from calculations of average consumption, which may have led to consumption rates being slightly lower than represented in this paper.

Through this experiment, it was found that at the current EPS consumption rate, it would take approximately 375 mealworms to consume one EPS cup, 4,720 mealworms per clamshell takeout container, and 1,500 mealworms per meat tray. Thus, commercial applications must address the large mass fraction of mealworms required to biodegrade EPS. If mealworm composting bins were to be implemented, methods of handling the adult darkling beetle would also have to be established. Ways of increasing mealworm consumption, such as increasing the temperature of the mealworm container, prolonging the larvae cycle of the mealworms, or boosting their consumption rate through genetic modification could be explored in future work. Additionally, Yang *et al.* extracted the gut bacteria responsible for the degradation of EPS, *Exiguobacterium* strain YT2, but found it to be less effective when applied directly to the EPS (5). Therefore, more research should be conducted on an efficient way of chemically manufacturing this bacterium and increasing its consumption rate when extracted so that EPS can be degraded as part of a bacterial recycling process.

Methods

During both experimental setups, 50 to 129 giant mealworms were placed in separate containers, each containing 1.50 to 3.35 grams of different types of EPS. All of the mealworms were obtained from PetCo from the manufacturer Timberline Live Pet Foods. The EPS was collected from various sources: the waxy white polystyrene was taken from a clamshell container; the white EPS (non-waxy) and virgin white EPS were taken from EPS cups; used low-density white EPS came from packing peanuts; and colored EPS in the colors black, yellow, and green were taken from meat trays. To qualify as a "used" item, the polystyrene had to be used in its commercial application (e.g., packing peanuts had to have been put in a box and shipped, EPS meat trays had to have held packaged meat purchased from a grocery store). Two experiments were conducted, each over the span of one month (30 days), a standard mealworm life cycle. In the first experimental setup (food sources 1-4, **Table 2**), there was one control set where mealworms were fed bran, one where mealworms were fed virgin white polystyrene as in Yang *et al.*, one where mealworms were fed green used high-density EPS, and one where they were fed used white waxy high-density EPS. The second experimental setup contained seven different tests (**Table 2**): control (bran), virgin white polystyrene, used white waxy high-density EPS, used yellow high-density EPS, used black high-density EPS, used white non-waxy low-density EPS, and used white low-density waxy EPS (packing peanuts). Next, the containers were stored at room temperature (around 23°C) to simulate the environment of a mealworm composting bin as it would be used commercially. Every other day, the mealworms were removed and weighed, along with their feces, and mass of EPS or bran, using a standard Ohaus triple beam balance scale, which measured to three significant figures. The dead mealworms were also removed and counted, and any mealworms that had pupated were noted. After the thirtieth day had passed, the EPS was removed from the container and weighed one last time, ensuring that all of the mealworms were removed from the EPS before it was weighed. After the experiment, the values were computed by subtracting the initial amount of EPS from the final amount, and dividing it by the number of living mealworms (dead mealworms were excluded from data calculations to accurately represent consumption rates) in the container to get the amount consumed per mealworm life cycle. Mealworm feces did not factor into this calculation, but were weighed to ensure that the recorded amount of EPS decreased consistently with the amount the mealworms consumed (measured by the weight of the mealworm feces).

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