Styrofoam Pacmans: The Degradation of Polystyrene (Styrofoam) By Tenebrio Beetles and Their Larvae

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Abstract

To reduce styrofoam pollution, Tenebrio beetles and their larvae, mealworms, are very effective. They can digest and biodegrade polystyrene plastic, or styrofoam, into released carbon dioxide, feces, and biomass. This project aims to investigate the best temperature and food condition for these mealworms to degrade the styrofoam. Also, another goal is to image the gut bacteria to see how Tenebrio beetles can digest it, noting the differences in imaging between experimental groups. To obtain this information, three aquariums were set up at each of these temperatures: 15°C, 20°C, and 30°C. One aquarium at each temperature had a 5 kg bag of bran, another had 5 g of bran and 40 g of styrofoam, and a third had just 40 g of styrofoam. After periodically taking the mass of the styrofoam, the worms eating just styrofoam, compared to bran and styrofoam, consumed the most mass. Worms stored at 20°C were most effective at breaking down styrofoam as this is the ideal temperature for mealworms. Mealworms at a high temperature (30°C) broke down more styrofoam than those at the lower temperature (15°C). The lengths of each mealworm were also measured and averaged to identify any differences. Styrofoam seemed to negatively affect growth as the mealworms only eating styrofoam were much shorter than those having bran available to consume. Comparing this data to the imaging data using confocal microscopy, there were tighter actin coils in the gut of styrofoam fed mealworms, which might have a correlation with the shorter length of the mealworms.

Introduction:

Background Research:

Styrofoam and Its Effect on the Environment

Plastic pollution is a global issue that has arisen within the last century, that is devastating Earth and its environment. The United States produces about 33 million tons of plastic every year, with less than 10% being recycled (Imam, 2016). Plastic is so common, especially styrofoam, one of the most environmentally harmful variants, is found in many everyday products. Styrofoam is a trademarked, common name for polystyrene plastic (Britannica, 2018). Its chemical formula is $(C_8H_8)_n$. Polystyrene is a hydrocarbon, making it insoluble in water. As polystyrene has a high boiling point (430°C) and melting point (240°C), it has strong intramolecular bonds. Polystyrene is very slow to degrade, therefore any littering would keep it in the environment much longer. It is also a carcinogen, which can be fatal (CEHN, 2019). Even though styrofoam is fatal, its slow degradation process drastically reduce available measures to reduce its pollution.

Mealworms and Tenebrio Beetles

Tenebrio beetles (Tenebrio molitor) and their larvae, have an innate capacity to eat plastic and styrofoam, reducing the impact of styrofoam pollution that humans contribute to (Imam, 2016). They have microorganisms in their gut that enables them to digest polystyrene, a common component of plastic, and change it to carbon dioxide, biomass and biodegradable wastes. The approximate breakdown of each product is "48 percent of the carbon from the polystyrene into carbon dioxide gas and about 49 percent of the carbon into feces" (Yang et al, 2015). The remainder of the carbon yields biomass. In this experiment, the Tenebrio beetles were stored at 20°C because the ideal temperature for mealworms is 23°C. In previous experiments, 100 mealworms were able to digest 34-39 mg of styrofoam daily (Jordan, 2015). Since the styrofoam can be broken down effectively into more environmentally friendly products, these Tenebrio beetles are a functional small-scale solution for styrofoam pollution. No evidence available shows any harm to the beetles after styrofoam consumption.

Rationale/Application:

Due to an increased rate of styrofoam use and its long degradation time, Styrofoam pollution has become an issue that needs to be addressed in an innovative, effective and sustainable way. One organism, the Tenebrio beetle and its larvae, known as a mealworm, can digest and convert styrofoam into carbon dioxide, feces, and biomass (Yang, 2015). This is not a full-scale solution to the worldwide problem, just an inexpensive and effective way for many people and organizations to get involved in curbing plastic waste. Investigating the optimal temperature and food supply for the Tenebrio beetles and mealworms can best identify how to store them when ingesting styrofoam.

Methods

Experimental Procedures:

1. 9 10-gallon glass aquariums were set up with 1 thermometer in each.
2. 3 aquariums were left at 15°C, 3 aquariums set to 20°C using 1 heating pad, and 3 aquariums set to 30°C using 2 heating pads.
3. For each temperature, bran was placed in 1 aquarium, bran and 40 g of styrofoam in another, and just 40 g of styrofoam in the last.
4. 500 mealworms were placed in each aquarium.
5. 100 mL of water was sprayed onto any aquarium containing

bran to ensure the mealworms came up to the surface to be observed.
6. Periodically, each aquarium was carefully observed.
7. Every few weeks, all the styrofoam was taken out of the aquariums, weighed, and the decrease in mass was calculated.
8. Before returning the styrofoam to the aquarium, another 100 mL of water was sprayed in the aquariums containing bran.
9. At the end of the experiment, 10 mealworms were taken from each aquarium, placed in the freezer for 10 minutes, and the length was measured.
10. 3 samples of mealworms from each aquarium, 3 beetles from the 30°C aquariums (since these were the only aquariums that contained beetles), and 3 pupas from the 30°C aquariums (since these were the only aquariums that contained pupas), were taken for cryosectioning and imaging.

Cryosectioning Procedures:

1. Each sample was placed in a test tube containing 2% paraformaldehyde and left for 24 hours.
2. The paraformaldehyde was removed from each test tube and the samples were washed with PBS.
3. Using a dissecting microscope, each sample was dissected to obtain the body.
4. Tissue-tek was placed in cryomolds to cover the bottom.
5. The bodies of the sample were then carefully positioned in the cryomolds containing tissue-tek.
6. Using tweezers, the cryomolds were placed in -80°C ethanol, in order for the tissue-tek to freeze over.
7. The plastic molds were removed, and placed in the cryostat and sectioned.
8. All sections with tissue were placed and labeled on slides.
9. Of the 70 slides, 28 were chosen using light microscopes to be imaged

Imaging Procedures:

Image data was collected using a Leica TCS SP5 LSCM [NIH SIG award 1 S10 RR027154-01A1] housed in the Regenerative Medicine Imaging Facility at Arizona State University.

1. Each slide was washed three times in antibody wash.
2. 500 µl of the primary antibody was placed using a micropipette on each slide covering all the tissues and left for 24 hours.
3. After 24 hours, the primary antibody was removed and the slides were washed 3 more times with antibody wash.
4. 500 µl of the secondary antibody was placed on each slide covering all the tissues and left for 24 hours.
5. After 24 hours, the secondary antibody was removed and the slides were washed 3 times with PBS.
6. 300 µl of DAPI was placed on each slide covering all the tissues and left for 15 minutes.
7. After 15 minutes, the DAPI was removed and the slides were washed in PBS.
8. The cover slides were placed on each slide using vector shield.
9. Each slide was then imaged using a confocal microscope with fluorescent light.

Results

See next page.

Confocal imaging of the gut: All data was collected using a Confocal microscope

Imaging stains: Red stains are actin, from a phalloidin stain. Blue stains are DNA, from a nuclear stain called DAPI. Green stains are tubulin. All were performed 20X + 3 times zoom.

Discussion of Results:

Data Table and Graph 1

- Styrofoam consumption based on temperature: 20°C mealworms ate the most styrofoam, followed by mealworms at 30°C, and finally mealworms in 15°C ate the least percent of styrofoam.
- Styrofoam consumption based on food supply: Mealworms eating styrofoam ate the most styrofoam. Adding bran to the aquarium with styrofoam decreases the consumption of styrofoam.
- Overall, mealworms in 20°C with only styrofoam, consumed the most styrofoam out of all treatments. These mealworms ate 21.20 g of styrofoam, around 53% of the 40 grams given at set-up. At 20°C with bran and styrofoam, the mealworms ate 9.6 g, 24% of the styrofoam.
- Following this, mealworms at 30°C consumed 11.7 g, 29.25% of the total. With bran and styrofoam, they ate 8.3 g, 20.75% of the total mass of styrofoam.
- And finally, mealworms at 15°C ate 11.4 g, 28.5% of the total styrofoam. In bran and styrofoam, mealworms ate 8.2 g, 20.5% of the total.
- Trends for Styrofoam Consumption: All mealworm treatments only eating styrofoam consumed more styrofoam than the treatments with both bran and styrofoam. For optimal styrofoam consumption, only giving styrofoam is the most effective set-up.
- Mealworm Length (Data Table 2 and Graph 2): The length of mealworms was different based on presence of styrofoam. These were measured after 1 month in the specific treatment and food supply. Typically, when styrofoam was in the treatment, then the mealworms were shorter.
- Mealworm Length by Food: The average length of each treatment was measured using 5 mealworms from each aquarium. The average lengths for all mealworms in styrofoam was 1.72 cm. For mealworms in bran and styrofoam, they averaged 2.35 cm long. Mealworms in bran averaged 2.55 cm long.
- Mealworm Length by Temperature: Temperature seemed to have no effect on the length of the mealworms. The averages were very precise.

Bacterial Imaging

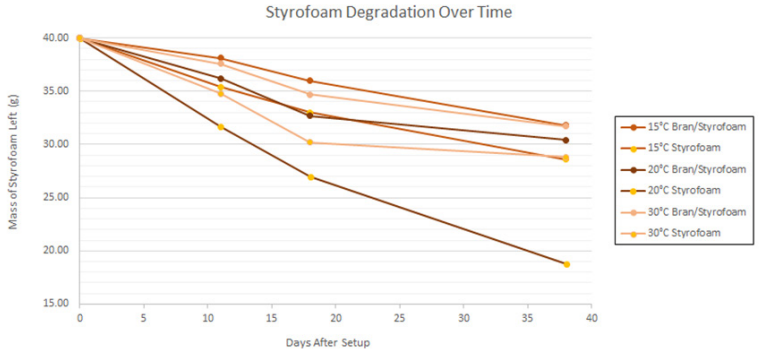
- The mealworms were imaged and stained using DAPI, a nuclear DNA stain; phalloidin, actin stain; and a primary tubulin antibody. Main trend: mealworms in only styrofoam

Styrofoam Degredation Over Time						
Temperature + Food Source	Styrofoam (g)					
	12/20/2019	12/31/2019	1/7/2020	1/27/2020	Mass Change	Percent Change (%)
15°C Bran	0.00	0.00	0.00	0.00	0.00	0.00
15°C Bran/Styrofoam	40.00	38.10	36.00	31.80	8.20	20.50
15°C Styrofoam	40.00	35.40	33.00	28.60	11.40	28.50
20°C Bran	0.00	0.00	0.00	0.00	0.00	0.00
20°C Bran/Styrofoam	40.00	36.20	32.70	30.40	9.60	24.00
20°C Styrofoam	40.00	31.70	27.00	18.80	21.20	53.00
30°C Bran	0.00	0.00	0.00	0.00	0.00	0.00
30°C Bran/Styrofoam	40.00	37.60	34.70	31.70	8.30	20.75
30°C Styrofoam	40.00	34.80	30.20	28.30	11.70	29.25

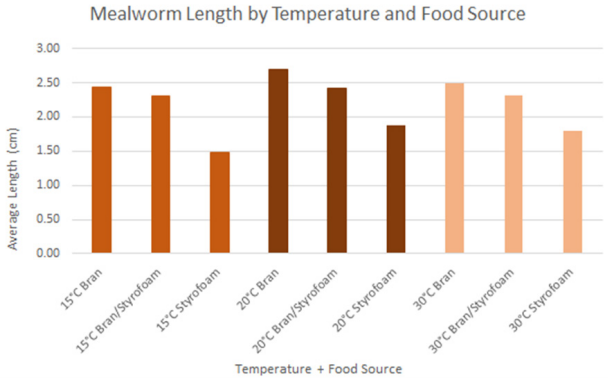
Data Table 1: % consumption of Styrofoam

Mealworm Length by Temperature and Food Source						
Temperature + Food Source	Length (cm)					
	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Average
15°C Bran	2.20	2.50	2.40	2.50	2.60	2.44
15°C Bran/Styrofoam	2.30	1.90	2.40	2.50	2.40	2.30
15°C Styrofoam	1.10	1.60	1.40	1.60	1.70	1.48
20°C Bran	2.60	2.70	2.50	2.90	2.80	2.70
20°C Bran/Styrofoam	2.40	2.50	2.20	2.40	2.60	2.42
20°C Styrofoam	2.00	1.60	2.10	1.80	1.90	1.88
30°C Bran	2.40	2.50	2.60	2.40	2.60	2.50
30°C Bran/Styrofoam	2.30	2.20	2.50	2.20	2.40	2.32
30°C Styrofoam	1.90	1.70	2.00	1.50	1.90	1.80

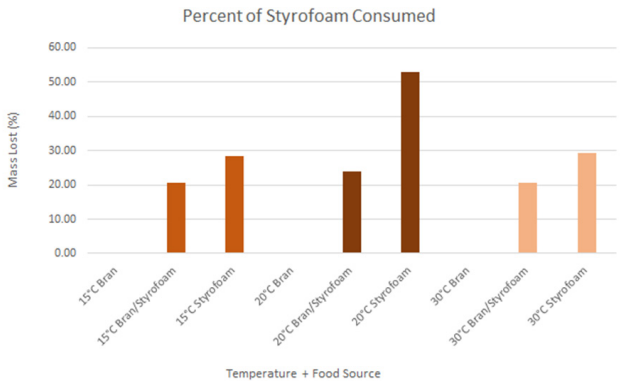
Data Table 2: Body length of the mealworms



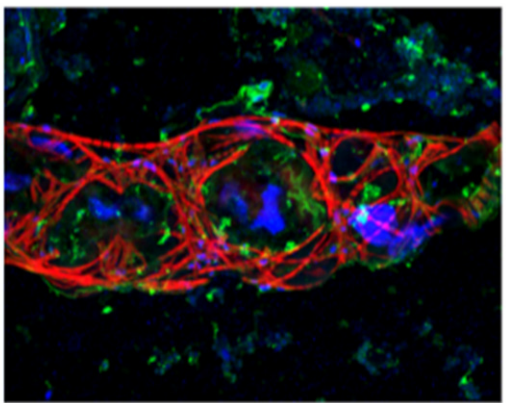
Graph 1: Shows the degradation of styrofoam: Over time, the styrofoam degraded by the mealworms steadily increased, with mealworms given only styrofoam to eat consuming styrofoam quicker than mealworms with both bran and styrofoam.



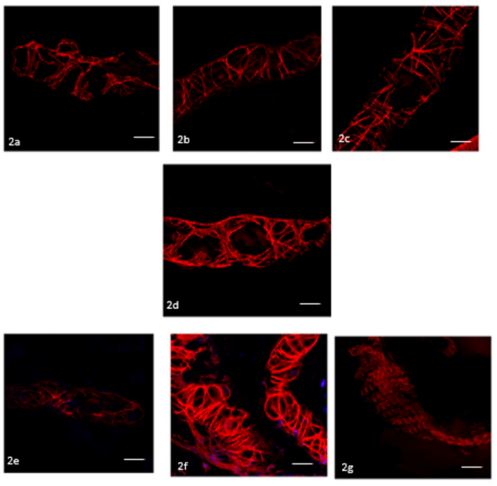
Graph 2: Shows the percent styrofoam consumed by the beetles. Beetles only eating styrofoam correlates to a significantly higher percent of styrofoam that they consumed; the bran seems to deter the beetles from eating styrofoam.



Graph 3: Shows the average length of the mealworms by food source and temperature. Mealworms eating styrofoam were shorter than those with bran and styrofoam, and mealworms with just bran to eat were longer for all temperatures, suggesting that styrofoam consumption correlates with a slower growth rate.



Picture 1: Shows a mealworm at 20 Degrees temperature with both Bran and Styrofoam diet.



Picture 2: a-g shows actin rings in the gut of the mealworm: 2a shows is at 15°C and on Bran diet; 2b is at 15°C and on Styrofoam+bran diet; 2c is at 15°C and just on Styrofoam; 2d is at 20°C on styrofoam and bran diet; 2e is at 30°C with just bran diet; 2f is at 30°C with bran and styrofoam diet, and 2g is at 30°C with just styrofoam diet. Scale Bar = 10 um

had tightly coiled actin. The actin, stained red, was less coiled in mealworms with styrofoam and bran, and just bran.

- Picture 1 shows the larger actin fragments that are loosely coiled. This was taken from the gut of a mealworm eating bran and styrofoam. Green phalloidin and blue DAPI stains are present in the image.
- Pictures 2a-2g only show the actin in the mealworm guts. In Pictures 2a (15°C, bran) and 2b (15°C, bran/styrofoam), there are gaps in the actin, and less coils. Picture 2c, of a mealworm in 15°C and styrofoam, has more condensed actin filaments and less space in between. Pictures 2d (20°C, bran/styrofoam) and 2e (30°C, bran), show similar findings to those in 2a and 2b. The actin is not as condensed as those in just styrofoam. Picture 2f (30°C, bran/styrofoam) shows the same actin structure as other guts of mealworms living in bran or bran and styrofoam. Picture 2g (30°C, styrofoam) definitively shows the condensed actin from mealworms in styrofoam. There are few gaps in between actin filaments compared to the others.

Conclusion

- This project investigates how temperature and food source affects the styrofoam degradation by Tenebrio beetles and their mealworm larvae. It was hypothesized that mealworms in 20°C with only styrofoam to eat will degrade the most styrofoam in grams because if these mealworms only have one food source, they will eat the styrofoam. Also, digestive enzymes work best at 20°C, causing quicker degradation. Following this, mealworms in the 30°C temperature environment will degrade the styrofoam less rapidly, but still faster than mealworms in 15°C. Digestive enzymes in mealworms work best near 25°C, but they can metabolize in warmer temperatures at a higher rate than in colder temperatures. Based on the data collected, the initial hypothesis was supported.
- This is because mealworms kept at 20°C, only having access to styrofoam, digested the most styrofoam. Out of the 40 grams provided for the mealworms, those at 20°C with styrofoam consumed 21.2 g, 52.6% of the styrofoam. Following the hypothesis, the beetles at 30°C digested 11.7 g, then those at 15°C digested 11.4 g.
- Mealworms eating bran and styrofoam still degraded the styrofoam, but not as rapidly as those with only styrofoam. The same trend existed where 20°C stored mealworms ate the most, followed by 30°C, then 15°C. 20°C mealworms consumed 24.00% of the styrofoam, a total of 9.6 grams. Then, mealworms at 30°C consumed 20.75%, and finally, mealworms at 15°C ate 20.5% of the styrofoam.
- When mealworms' sole food source is styrofoam, the most styrofoam was consumed. However, these exact mealworms only eating styrofoam are shorter than the others. Out of the 15 mealworms measured from the worms only eating styrofoam, 5 from each aquarium, the average length is 1.72 cm. Compared to the 2.55 cm average length for mealworms in bran and 2.35 cm average for mealworms in bran and styrofoam, this is markedly distinct. In this experiment, access to styrofoam correlates with a shorter length of mealworms.
- Imaging the mealworms' gut was done to pinpoint differences in mealworms when they have access to different foods. Although there was minimal distinguishable difference when analyzing the DAPI or nuclear stain, the actin stain was more conclusive. The actin stain, phalloidin, stains the actin a dark red color, making them easily visible under a microscope. In all pictures taken, there was actin visible in the tissue. But, the main difference was observed when viewing the coil tightness of the actin protein. Tissue taken from Tenebrio beetles that only ate styrofoam had noticeably tighter coiled actin. As stated earlier, these same mealworms had a shorter length than others.
- Thus, Tenebrio beetles are an effective way as a small-scale solution at degrading styrofoam. Storing them at 20°C yields the most degradation, and no other food source besides styrofoam needs to be provided.
- Comparison to Other Studies: In a study investigating styrofoam degradation on mealworms, researchers say that "mealworms fed ~10% w/w PS and ~90% bran, an agricultural byproduct, rates of PS degradation at 25 "C nearly doubled compared to mealworms fed PS alone" (Yang et al. 2015). However, in the data collected in this experiment, styrofoam degradation was much more rapid without bran in the treatment. The bran to styrofoam ratio conducted in Yang's experiment was 9:1, but in this experiment, it was 125:1. Providing too much bran can detract from styrofoam degradation, which was likely evident in this experiment.
- Sources of Error: Repeatedly, the beetles urinated on the styrofoam, which can increase the mass of the styrofoam measured. That error source underrepresents the amount of styrofoam eaten.
- The mealworms tend to burrow into the styrofoam and consume the styrofoam from the inside, and they would also pupate. If these mealworms were not seen when styrofoam was measured, then the styrofoam mass collected would have been higher. This also underrepresents styrofoam consumption.
- The temperatures were kept constant using heating pads. Although the setting of the pads was kept constant and the temperature was consistently checked for precision, there could have been possible temperature variations.
- Future Research Studies: These can include having a smaller mealworm colony and examining how the different food sources and temperature affect mortality. This can show how populations are dying with the temperature difference, a chi-squared analysis can show whether the number of deaths is due to random chance or that other environmental factors are killing them.

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Methods and Materials: Image data was collected using a Leica SP5 confocal microscope system, housed in the Regenerative Medicine and Bioimaging Facility at Arizona State University, and was acquired by the NIH SIG award 1 S10 RR027154-01A1.

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