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The Influence of Visible Light on the Consumption Rate of Expanded Polystyrene by *Zophobas morio* Larvae

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Abstract

The process of disposing of expanded polystyrene (EPS) is by burning it in municipal incinerators. This process gives off EPS microplastics, which can find their way into water, food, blood, and major organ systems. *Zophobas morio* larvae are capable of consuming and breaking down EPS within their digestive tracts by minimizing the spread of microplastics. Studies of the consumption of EPS by *Z. morio* larvae are typically conducted under white or no visible light treatments. This study tested whether the color of visible light influenced the consumption rate of EPS by *Z. morio* larvae. If *Z. morio* larvae consume EPS under visible light, then visible light will influence the amount of EPS consumed. If results suggest that the consumption rate is influenced by visible light colors, then *Z. morio* larvae could be a solution for recycling EPS. This study's procedure placed *Z. morio* larvae into 25 jars under one of six visible light treatments of red, yellow, green, blue, white, and no visible light. Each jar contained a pre-weighed block of EPS and six *Z. morio* larvae. After two weeks, the *Z. morio* larvae were removed from the jars, and the difference between each pre-weighed EPS block and the weight of the same partially consumed block was recorded in three trials. The data indicates that green and blue visible light treatments resulted in the greatest amount of EPS consumed by *Z. morio* larvae while the red and yellow had the least amount of EPS consumed by the *Z. morio* larvae. In conclusion, results indicate that green and blue visible light, compared to the no visible light treatment, could be used to influence the *Z. morio* larvae to consume more EPS. Green and blue visible light and *Z. morio* larvae could make the recycling process of EPS more environmentally friendly when used in households or by local environmental organizations.

Keywords

Zophobas morio, *Z. morio*, Superworm, Larvae, Visible Light, Expanded Polystyrene, EPS, Styrofoam, Consumption Rate, Pollution, Recycling, Nanoplastics, Microplastics

1. Introduction

The purpose of this study was to determine if visible light had an influence on the consumption rate of expanded polystyrene by *Zophobas morio* larvae, also known as superworms. In recent years, microplastic and nanoplastic particles from expanded polystyrene (EPS) have contaminated the environment, clothing, and food [1]-[3]. Recycling EPS is very difficult, as microplastic or nanoplastic particles of EPS often escape into the environment [2]. Because EPS does not bio-degrade, EPS is recycled in incinerators at high temperatures [1] [3] [4]. The superworm, *Zophobas morio* is capable of digesting high amounts of polystyrene [5]. This current study discusses the consumption of EPS by *Zophobas mori*o (*Z. morio*) larvae under various visible light treatments, which could pose a solution to better the process of recycling EPS, and reduce the amount of microplastic and nanoplastic particles in the environment.

Everyday items, such as food containers, and drinking bottles contain particles of EPS [1] [2]. Fabrics can also contain these particles [2] [3]. Direct contact with these particles can pose certain problems. Kik et al. (2020) explain this process by stating, "Plastic NPs (nanoparticles) may enter living organisms with air, food, and water, and also through the skin. They can accumulate in various organs" ([1]: p. 4). The polystyrene particles are in your bloodstream, and can sometimes travel through the bloodstream into the intestines [1] [2]. Polystyrene particles and many other nanoplastics give off toxins once mixed with biomolecules and other chemicals. This can lead to allergic reactions and inflammation [1].

Polystyrene can also be toxic and damaging to the environment. Landfills are the primary sites for recycling EPS. Recycling EPS in landfills, however, can create large amounts of pollution. In a study by Afrin *et al.* (2020), soil from a landfill in Bangladesh was extracted, tested, and showed heavy amounts of microplastics, which suggests that landfills are a source of microplastic pollution [4]. However, landfills are not the only places that are contaminated by EPS. The ocean is another example of a source of microplastic pollution [1]-[3]. According to Kik *et al.* (2020), "Plastic is responsible for 70% of sea and ocean pollution. Every year, 8 million tons of plastic are released into the sea" ([1]: p. 3). The microplastics in the ocean are harmful to aquatic life and to the people who consume the aquatic life in their diets [1]-[3].

The *Z. morio* larvae can help reduce the amount of EPS in the environment. *Z. morio* larvae are capable of surviving purely on a diet of EPS [5]. The *Z. morio* larvae can digest polystyrene, without damaging their organs and can live to

pupation. The *Z. morio* larvae can degrade EPS and place it back into the environment in the form of white pellets. A study by Sun *et al.* (2022) shows that "faeces changed colour from light brown to white pellets in the first 24 - 48 hrs… suggesting that the worms had started to consume and egest PS" ([5]: p. 5). In that study, *Z. morio* larvae were observed for their consumption rate of EPS. The authors of that journal article created a study feeding EPS to a group of *Z. morio* larvae. After three weeks of EPS consumption, electron microscopy indicated that the white particles found compacted in the guts of the *Z. morio* larvae were partially degraded showing that *Z. morio* larvae can ingest EPS [5].

Z. morio larvae are capable of consuming polystyrene due to the bacteria that lie within the *Z. morio* larvae's guts [5] [6]. Once the EPS enters the digestive tracts of the *Z. morio* larvae, the EPS begins to degrade. Lee *et al.* (2020) discuss the efficiency of EPS degradation in the guts of *Z. morio* larvae in their research report [6]. The authors of that article created an experiment to show how the *Z. morio* larvae can break down plastic waste by depolymerizing the polystyrene. The authors provided polystyrene as a main source of food to fill the guts of the *Z. morio* larvae. They dissected the *Z. morio* larvae to isolate the digestive tract from the rest of the internal organs of the larvae. Two types of bacteria were found after the guts had been incubated. The most common bacteria found in the *Z. morio* larvae system is known as *P. aeruginosa* [6]. This bacteria degrades the polystyrene in the guts of the *Z. morio* larvae [6]. Lee *et al.* (2020) report that "*P. aeruginosa* can survive in various places, including soil, wood, water, dumpsites, the deep sea, and the guts of larvae of superworms, suggesting that it is highly adaptive. It not only degrades plastic in the guts of larvae but also in our in vitro systems with plastic added LCDBM media" ([6]: p. 8). The bacteria in the guts of the *Z. morio* larvae can help reduce the amount of polystyrene in the environment [6]. Lee *et al.* (2020) also note that "Recently, several plastic-ingesting worms capable of removing plastic wastes have been reported, including waxworms (*Galleria mellonella*), mealworms (*Tenebrio molitor*), and superworms (*Zophobas atratus*), which can depolymerize polyethylene (PE) and polystyrene (PS) upon ingesting" ([2]: p. 6).

Visible light is the segment of the electromagnetic spectrum to which the human eye can view [7]. The main colors of the visible light spectrum are red, yellow, green, blue, and white. White light holds every color of the visible light spectrum. Visible light can change moods or even affect behaviors. This is due to the circadian rhythms. A change in light will cause a phase shift in the circadian rhythm [7]. The circadian rhythm is the matching of an organism's bodily functions with the patterns of the light-dark cycles during 24 hours [7]. In this study, the author states that "Although circadian clocks are self-sustained, a change in the timing of the light exposure will result in a phase shift of circadian rhythms" ([7]: p. 3). This is what causes the change in moods and behaviors.

In the studies mentioned above, the authors tested the consumption rates of polystyrene under certain conditions that did not involve visible light other than white visible light. There tends to be a gap in research, including the studies

mentioned in this report, which omits the factor of visible light. Due to this gap in research, it was easy to develop the following research question: To what extent does visible light affect the consumption rate of EPS by the *Z. morio* larvae*?* By asking this question, the following hypothesis for this current research experiment was posed: If *Z. morio* larvae consume EPS under visible light, then visible light will influence the amount of EPS consumed.

2. Materials and Methods

2.1. Introduction

The purpose of this study is to determine if visible light influences the consumption rate of polystyrene by *Z. morio* larvae. The pending research question is, "To what extent does visible light affect the consumption rate of EPS by the *Z. morio* larvae*?"* Therefore, the hypothesis for the research is, If *Z. morio* larvae consume EPS under visible light, then visible light will influence the amount of EPS consumed. The primary data needed to rule out the null hypothesis would be the difference in weight measured in grams, from the initial block of EPS to the partially consumed block of EPS.

2.2. Methodology and Process

This current research study is a quantitative study with an experimental control group and pretest-post-test design. The study was conducted in three trials, each lasting two weeks, where the *Z. morio* larvae were under one of six visible light treatments (red, yellow, green, blue, white, and no visible light). The research study evaluated numerical data to establish whether or not visible light influenced the consumption rate of EPS by *Z. morio* larvae. This current research study was experimental since it attempted to establish a relationship between two variables. The two variables were the independent variable of the visible light treatments and the dependent variable of the amount of EPS consumed by the *Z. morio* larvae. This study also established an experimental group and a control group. The experimental groups were the samples of *Z. morio* larvae and EPS blocks placed under the various visible light treatments of red, yellow, green, blue, and white. The control group placed *Z. morio* larvae and the EPS blocks under no visible light treatments. The pretest aspect of this study was the weight of the EPS blocks before the beginning of each trial. The posttest aspect of this study was the weight of the partially consumed EPS block at the conclusion of each trial.

2.3. Environmental Setup

The materials that were used to create the experimental environment for the *Z. morio* larvae and EPS blocks are seen in **Figure 1**. The housing storage containers for this study measured 32.3"L × 20.4"W × 16.7"H in dimension. The hygrometers and probes were placed into the same locations of each container to keep each environment consistent. A rectangular section was cut from the lids of the containers, to allow for the access and viewing of the jars. Sixty-watt, light-emitting

diode (LED) strips (17.5 feet of 90 diodes) were placed inside the containers around the perimeter of the rectangular window of the lids. The LED strips emitted one of each visible color light treatment directly into each housing container's 25 jars. The jars as seen in Figure 2 were 16 oz/473 ml in volume. The bases of the jars measured 2.5 inches in diameter whereas the openings of the jars measured 2.76 inches in diameter. Each jar was 5.25 inches in height. The metal lids of the jars were removed and replaced with an aluminum screening. The screening was held into place by the metal rings of the jar lids to allow visible light to enter each jar and to keep the larvae from escaping. Twenty-five, 2"L × 2"W × 2"H blocks of EPS for each visible light treatment and for the control group were weighed by a high-precision milligram scale. After being weighed, one EPS block was placed inside each jar. Each jar was randomly assigned six *Z. morio* larvae that were picked from a total of 900 larvae. Picking randomly from a large population allows for a fair representation of the physical characteristics of that population. A spray bottle was used to hydrate the Z. morio larvae. Each jar received one spray in the evening delivering 2 ml to the *Z morio* larvae.

Figure 1. Exterior and interior views of the testing environment. Note. The six containers were used to each house 25 jars. Each jar contained one 2"L × 2"W × 2"H block of EPS and six *Z. morio* larvae. The container with the no visible light treatment is located to the right of the container with the red visible light treatment. Picture taken by Tyler Ferenz.

Figure 2. One of the 25 jars housed in each container of each visible light treatment. Note: Each jar contained one 2"L × 2"W × 2"H block of EPS and six *Z. morio* larvae. Picture taken by Tyler Ferenz.

2.4. Data Source and Sampling

This study used purposive sampling since the population type was selected specifically for this study. Before starting the experiment, over 900 *Z. morio* larvae were placed in one large holding container. Six *Z. morio larvae* were randomly selected from this container, and placed randomly into one jar housing one already-weighed block of EPS. The jars were then placed into one of the six visible light treatment containers until 25 jars were placed under each visible light treatment.

The number of *Z. morio* larvae placed in the jar was determined carefully. The Z. *morio* larvae are territorial insects. Cannibalism occurs in overcrowding environments. Placing an excessive amount of Z. *morio* larvae into the close quarters of any small container would encourage the larvae to consume each other. Cannibalism would keep the larvae from consuming the EPS, hence confounding the study. When *Z. morio* larvae are separated from other larvae, pupation occurs. Having a small number of larvae in the jars could potentially encourage pupation. During the stage of pupation, the *Z. morio* larvae are in a dormant state and do not consume food. As a result, EPS would not be consumed placing a confounding variable into the study.

To keep the conditions constant between all visible light treatments, temperature and humidity were recorded. Temperature and humidity were recorded in the morning and the evening. Each jar received the same amount of water as well to control the variables for an equal testing environment between all treatment and control groups.

2.5. Instruments Used for Data Collection

A high-precision milligram weighing scale was the primary instrument used in this research study. This type of scale weighs items in grams up to the thousandth of a decimal. The blocks of EPS were individually measured using this weighing scale before each trial and after each trial. The difference between the initial and end weights was calculated. EPS is a very light material and requires weight measurements to the thousandth of a decimal point to show a difference in weight. The initial weight of the EPS blocks, the partially consumed weight of the EPS blocks, and the difference between the two weights were recorded on spreadsheets using the Google Sheets platform. Temperature and humidity taken twice daily for each visible light treatment in all trials were also recorded on spreadsheets using Google Sheets.

2.6. Data Analysis

To obtain the average amount of EPS consumed by one larva, all jars with at least one dead larva were eliminated from the calculations. The study was not able to detect the timing of the larvae's deaths. Therefore, the duration of EPS consumed by the dead larvae would not be equal to the duration of EPS consumed by the living larvae. Only jars with six living larvae were considered for data analysis. To determine the average amount of EPS consumed by one larva in each of the jars,

the calculated difference of the consumed weight from the initial weight of each EPS block was divided by the number of living larvae for each of the jars. The calculations to obtain the base unit of the average amount of EPS consumed by one larva were conducted for each block separately. The sum of the average amount of EPS consumed per larva for each EPS block was then calculated and divided by the amount of blocks of all eligible jars for all three trials under each visible light condition to obtain the mean of the mean amount of EPS consumed by one larva for each visible light condition. From these calculations, the standard error of measure (SEM) was calculated using a Google Sheets calculator. The SEM compared the results of each visible light treatment to the projected population mean of that same visible color light treatment. A one-way ANOVA was then conducted through the Google Sheet calculator, to determine which visible light color treatment had the most profound influence on the amount of EPS consumed by *Z. morio* larvae.

To find the average temperature and humidity, temperature and humidity were collected twice a day. At the end of all trials, the sum of all temperatures and the sum of all humidity measurements was calculated, and divided by the number of times the temperatures and humidity measurements were recorded to find the average temperature and humidity measurements for each visible light treatment. The SEM was calculated to determine the level of confidence that the same temperatures and humidity readings would occur if the test was repeated in the same way.

3. Results

The primary focus of the study was to analyze the amount of EPS consumed by the *Z. morio* larvae for each visible light treatment so that it can be compared to the findings of the control group of no visible light treatment. To determine the average amount of EPS consumed by a single larva, the jars containing at least one dead larva were excluded from the calculations. Since the study couldn't determine when the larvae died, the duration of EPS consumption between dead and living larvae was not equal. For the data analysis, only the jars with six living larvae were considered. Table 1 shows the count of jars without any dead larvae for each visible light treatment throughout the three trials.

Table 1. The number of eligible jars and larvae for each visible light treatment. Note: Jars were considered eligible for data analysis only if the jars contained no dead larvae from the six randomly assigned living larvae delivered to each jar.

Color of Light Treatment	Number of Jars	Number of Living Larvae
Red Visible Light Treatment	57	342
Yellow Visible Light Treatment	51	306
Green Visible Light Treatment	51	306
Blue Visible Light Treatment	55	330
White Visible Light Treatment	54	324
No Visible Light Treatment	52	312

Figure 3 illustrates the average amount of EPS consumed per *Z. morio* larva for each visible light treatment. The green visible light treatment yielded the highest average amount of EPS consumed per *Z. morio* larva (0.023 g) second to the blue visible light treatment (0.022 g). The red visible light, white light, and no visible light visible light treatments yielded a lower average amount of EPS consumed per *Z. morio* larva of 0.017 g each. The yellow visible light treatment yielded the lowest average amount of EPS consumed per *Z. morio* larva at 0.015 g.

Figure 3 also shows similar variances from the mean of the project population throughout the averages of all trials of the same visible light treatment: Red visible light (SEM= +/− 0.0016 g), yellow visible light (SEM= +/− 0.0015 g), white visible light (SEM= +/− 0.0014 g), and no visible light (SEM= +/− 0.0014 g). The green (SEM= +/− 0.0018 g) and blue (SEM= +/− 0.0020 g) visible light treatments yielded the highest average of EPS consumed by one *Z. morio* larva in all of the trials. The green visible light treatment had a lower variance from the mean of the projected green visible light population than the blue visible light condition from the mean of the blue visible light projected population for the average amount of EPS consumed by one *Z. morio* larva.

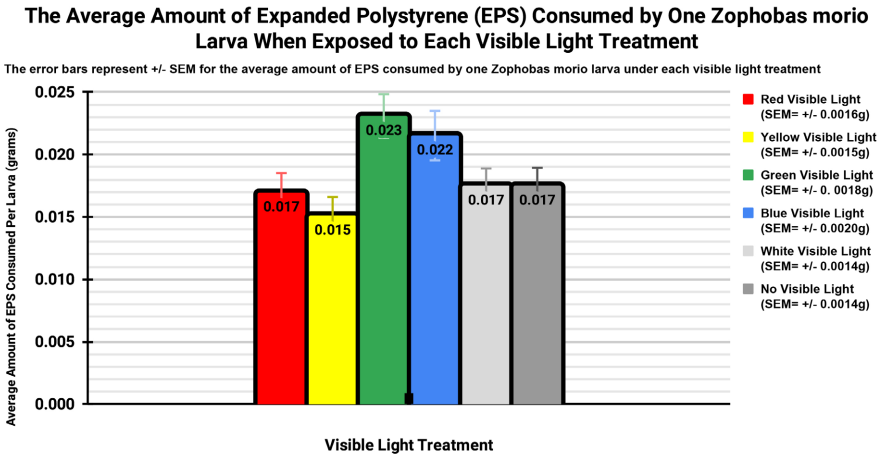

Figure 3. The average amount of expanded polystyrene consumed by one *Zophobas morio* larva when exposed to each visible light treatment. Graph created by Tyler Ferenz.

A one-way ANOVA was conducted at a .05 alpha level to determine whether visible light influenced the EPS consumption rate by *Z. morio* larvae. The one-way ANOVA showed an influence of visible light on the amount of EPS consumed by the *Z. morio* larvae. ($F(5, 314) = [4.06]$, $p = 0.001$). The red, yellow, and white visible light treatments showed a lesser difference from the no visible light control group compared to the green and blue visible light treatments. The green and blue visible light suggest a significant difference in the average amount of EPS consumed by one *Z. morio* larva.

3.1. Controlled Variable of Temperature

Temperature was recorded twice daily for each trial of each light treatment to

ensure the accuracy of the data through controlled conditions of regulated room temperatures. Figure 4 illustrates the inverse proportion of the average temperatures recorded for each visible light to the average amount of EPS consumed by one *Z. morio* larva for each visible light treatment as seen in Figure 3. The highest average temperatures for all trials of each visible light treatment were noted in the red visible light (64.3˚F), yellow visible light (64.3˚F), white visible light (64.1˚F), and no visible light (64.0˚F) treatments. The average amount of EPS consumed by one *Z. morio* larva of red, yellow, white, and no visible light treatments, was lower than the green and blue visible light treatments as indicated in Figure 3. The inverse proportion was also noted for the average temperatures recorded for the green and blue visible light treatments in Figure 4 compared to the average amount of EPS consumed by one *Z. morio* larva of the red, yellow, white, and no visible light treatment groups illustrated in Figure 3. As the green visible light (63.6˚F) and blue visible light (63.5˚F) treatments average temperatures were lower than the other visible light treatments. The average amount of EPS consumed by one *Z. morio* larva in the green and blue visible light conditions was higher than the other visible light treatments seen in Figure 3. The average temperature measurements for each visible light group varied very little from the mean of the projected population of the same visible light group (Red SEM = +/− 0.17˚F; yellow SEM = +/− 0.17˚F; green SEM = +/− 0.13˚F; blue SEM = +/− 0.13˚F; white SEM = +/− 0.15˚F; and no visible light SEM = +/− 0.16˚F).

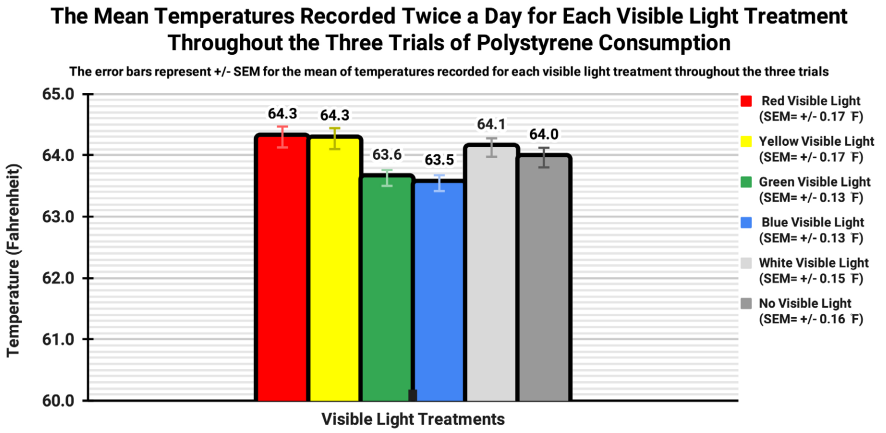

Figure 4. The mean temperatures were recorded twice a day for each visible light treatment throughout the three trials of polystyrene consumption. Graph created by Tyler Ferenz

3.2. Controlled Variable of Humidity

Similar to temperature, humidity measurements were also recorded twice daily for each trial of each light treatment to ensure the accuracy of the data through controlled conditions. Figure 5 illustrates the direct proportion of the average humidity recorded for each visible light condition throughout all trials to the average amount of EPS consumed by *one Z. morio* larva for each visible light treatment as indicated in Figure 3. The average humidity measurements for all visible light

treatments were higher for the green (55.76%) and blue (55.71%) visible light treatments than the other visible light treatments. Likewise, the average amount of EPS consumed by one *Z. morio* larva in the green and blue visible light treatments was higher than the average amount of EPS consumed by one *Z. morio* larva in all of the remaining visible light treatments as indicated in Figure 3. The average humidity measurements for each visible light group varied very little from the mean of the projected population of the same visible light group (Red SEM= +/− 0.72%; yellow SEM= +/− 0.70%; green SEM= +/− 0.71%; blue SEM= +/− 0.67%; white SEM= +/− 0.72%; and no visible light SEM= +/− 0.70%).

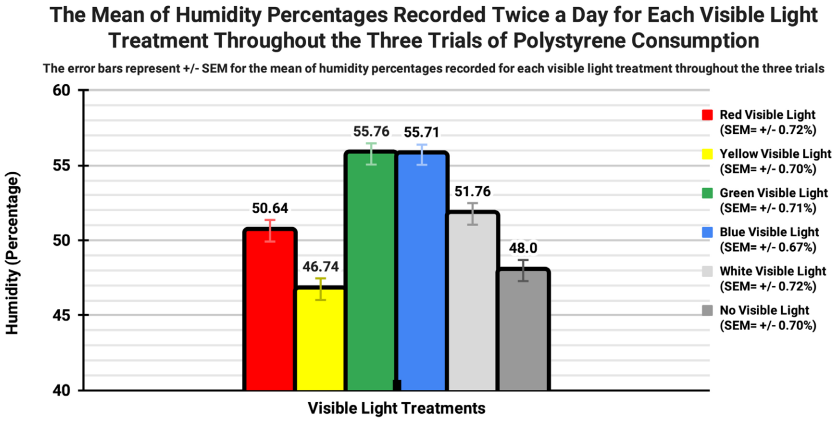

Figure 5. The mean humidity was recorded twice a day for each visible light treatment throughout the three trials of polystyrene consumption. Graph created by Tyler Ferenz.

4. Discussion

4.1. Consumption Rate under Visible Light Condition

At the end of the study, the green and blue visible light treatments had the greatest influence on the consumption rate of EPS. Data analysis shows that the green visible light treatment had a 26% chance and the blue visible light treatment had a 23% likelihood of increasing EPS consumption compared to the control group. Consumption of EPS under green and blue visible light can be explained by the insects' attraction toward the green and blue visible light wavelengths. According to Wakefield *et al.* (2016), "Different wavelengths of light vary in their attractiveness to an insect order and families of moths, with shorter wavelengths including ultraviolet being more attractive for macro-moth species than longer wavelengths" ([8]: p. 1). Green visible light wavelengths are between 520 - 565 nanometers and blue visible light wavelengths are between 445 - 520 nanometers [9]. Because the green and blue visible lights have short wavelengths, the number of waves that pass through a point in space is high. This places the green and blue visible lights on the high or upper end of the visible light spectrum due to this high frequency. The wavelength frequencies of green and blue visible lights are greater than the other colors visible light colors of the spectrum [9]. Wakefield *et al.* (2016) note that, "Insect vision is either di or trichromatic, with peak sensitivities

shifted toward the UV end of the EM spectrum (<380 nm)" ([8]: p. 6). Insects are trichromatic, which is a sensitivity to color [8]. Trichromatic UV-blue-green vision is regularly observed with insects, suggesting that the *Z. morio* larvae could be attracted towards the upper end of the visible light spectrum, where the green and blue visible lights are found [8]. The evidence from these sources supports that green and blue visible light can affect the amount of EPS consumed by the *Z. morio* larvae. One can conclude that there is a trending correlation between the green and blue visible light and the amount of EPS consumed by the *Z. morio* larvae.

4.2. Limitations

In this study, there was a slight variance in the average temperatures and average humidity between the six visible light groups. The lower temperatures and the higher humidity levels could have influenced the amount of EPS consumed by the *Z. morio* larvae. The higher average temperatures yielded lower average amounts of EPS consumed per *Z. morio* larva. Overall, the blue visible light was found to have the lowest average temperatures, and the green visible light was noted to have the second lowest average temperatures. Despite having the lowest average temperatures compared to the other visible light treatments, the *Z. morio* larvae under green and blue visible light treatments consumed the largest amounts of EPS.

Humidity was also recorded for each visible light treatment. There was a relationship of direct proportion between the average amount of EPS consumed per *Z. morio* larva and the average humidity recorded for each visible light treatment. Green visible light was noted to have the highest average and the blue visible light was noted to have the second highest average humidity and average amount of EPS consumed by a *Z. morio* respectively

4.3. Conclusions

Because there was a gap in research to determine if visible light influenced EPS consumption by *Z. morio* larvae, the following research question was created: To what extent does visible light affect the consumption rate of EPS of the *Z. Morio* larvae? The following hypothesis was then subsequently created: If *Z. morio* larvae consume EPS under visible light, then visible light will influence the amount of EPS consumed. The goal of this research project was to determine if the colors of visible light on the visible light spectrum would affect the amount of EPS consumed by the superworm, *Zophobas morio*. The results of this research would also address whether or not *Z. morio* larvae could be used as a tool to help make the recycling of EPS more sustainable for the environment. The results of this research study lean toward what was predicted in the hypothesis. Through extensive data analysis, the result of this research showed that visible light may influence the amount of EPS consumed. Green and blue visible light treatments had the greatest influence on the amount of EPS consumed, while yellow visible light treatment had the lowest influence on the amount of EPS consumed by the *Z. morio* larvae.

With these results in mind, it is safe to conclude that the *Z. morio* larvae under green and blue visible lights could be used as a tool to help make the recycling process of EPS healthier for the environment. However, for the time that it takes for the *Z. morio* larvae to consume EPS, a large number of *Z. morio* larvae would be required to consume the large amounts of EPS found in landfills. This would require manpower for the upkeep of the larvae, which would make the process of using *Z. morio* costly. The ideal place for recycling EPS would be in home environments, small offices, or with environmental groups that would recycle EPS on a small scale within small communities.

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Conflicts of Interest

The author declares no conflicts of interest regarding the publication of this paper.

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