Effect of Food Availability on the Rate of *Zophobas morio* (Coleoptera: Tenebrionidae) (F.) Pupation

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Abstract: *Zophobas morio* (Coleoptera: Tenebrionidae) (F.) larvae are insects that can be found at most pet stores. The larvae are common sources of food for insectivorous pets, but there is a new interest in the potential for these as sources food for humans. The aim of this study was to determine whether the absence of food would affect the time it takes for the larvae to pupate. Larvae were divided into two groups: one with food and one without food. The days it took the larvae to reach the pupal stage were monitored and noted. By the end of the thirty days that were dedicated to this study, only six of the fourteen larvae had pupated. Larvae that either did not pupate or died during the length of the experiment were recorded with a value of zero. Raw data indicated that the group without food pupated nearly twice as fast as the group that did have food. However, the results of a one-tail independent t-test showed that there was no statistically significant difference between the two groups. The t stat value for this test, 0.46, was not greater than the t critical one-tail value, 1.81, indicating that the larvae did not pupate faster in either group and the null hypothesis could not be rejected. The results of this test could have been a due to the small sample size in this experiment as four larvae from one group and two larvae from another group are not a large enough sample to gather accurate data.

Keywords: *Zophobas morio*, pupation, starvation, Tenebrionidae

Zophobas morio (Coleoptera: Tenebrionidae) (F.), more affectionately known as morio worms or superworms, are a species of readily available darkling beetle larvae that can be found at most pet stores. These insects are unique in that they can be easily reared, are a staple in the diet for insectivorous pets, are integrated in feed for poultry and fish, and are even consumed by people around the world (Finke 2002, Soares Araújo et al. 2019). Furthermore, these larvae serve as model organisms in experiments that range from extracting antimicrobial peptides to analyzing anticancer peptides extracted from the larvae (Yusof et al. 2017, Mohtar 2014).

Concerns regarding food security in the future have continued to rise over the years. Projections from as recently as 2019 predict that the consumption of animal protein will continue to grow as the years go on, increasing by as much as 22% by 2030 and 25% by 2050. However, there simply are not enough resources to support this growth and current food industry and agricultural practices are not sustainable enough to support this growth either (Soares Araujo 2019). This is more concerning when considering that malnutrition and lack of food security is a problem in many nations across the world, showing that production needs are barely being met as it is (Gahukar 2011). There are also environmental consequences of increasing meat production, such as increased gas production and the increased amount of water needed to sustain these animals (Soares Araujo 2019). Furthermore, there are many other issues affecting food security, including things such as pests, disease, and soil fertility that affect crops (Gahukar 2011).

With issues such as these, people have begun to turn to alternative and more sustainable food sources, such as insects. Insects do have some notable advantages when considering their sustainability including that they: reproduce quickly, have high growth and feed conversion ratios, and cause very little environmental impact (Saores Araujo 2019). As of 2011, insects were consumed by people in Asia, Africa, Mexico, and South America (Gahukar 2011). *Z. morio* is one of the two most common insects reared for animal feed in Brazil, but a study was conducted to analyze the nutritional composition of them along with the Jamaican field cricket, *Gryllus assimilis* (Orthoptera: Gryllidae) (Fabricius). The researchers found that *Z. morio* were actually better supplemented in areas that included mineral content, fatty acid concentrations, and protein content when compared to other sources of oil and animal food sources. For example, *Z. morio* contained about twice as much as chicken, rump beef, loin pork, and hake fillet in this study (Soares Araujo 2019).

The aim of this study is to determine whether the presence of food for *Z. morio* larvae will affect the rates at which they pupate. In order to create colonies quickly and minimize the costs for rearing, it is important to know how to get adult *Z. morio* the fastest. The added stress of starvation in the larval stage will most likely cause the larvae to pupate faster than if they were offered food.

Materials and Methods

Fourteen large *Z. morio* (Timberline Fisheries Corporation, Marion, IL) were placed individually in one-ounce sized cups (Dart Container Corporation, Mason, MI). Seven of the *Z. morio* were designated as the control and, as per Carolina Biological Supply Company (2010), were given dried oats and baby carrots. The other seven worms were placed in cups that had no oats or carrots or any other materials. The dried, Old Fashioned oats (HEB, San Antonio, TX) were to serve primarily as a food source, but also as substrate and the carrots (Bolthouse Farms, Inc., Bakersfield, CA) were to give the worms a source of hydration. The carrot slices were removed every evening and replaced with a fresh slice. To avoid possible variation in treatments, the cups in which the worms were offered no oats or carrots were opened and closed so that they would experience the same disturbance that the other group did. The oats were replaced once a week on average to combat the growth of mold. The cups that contained the larvae were separated into their respective groups and placed in a cardboard egg carton to prevent them from falling over. Larvae were reared in a closet in which the door remained partially open at all times so as to ensure 12:12 L:D ratio of indirect sunlight via a window in the room. Temperatures of the general vicinity and of the cups themselves were taken twice daily, once in the morning and once at night, using an infrared digital thermometer (PetSmart, Phoenix, AZ). Temperatures were maintained at 25°C (± 2° C) using a small ceramic heater (Ningbo Singfun Electric Appliance Co., LTD., Cixi, Zhejiang) placed a meter away from the larvae. As carrots were being replaced and temperatures were being recorded, any pupations or adult emergences were noted.

Results

Table 1 shows the raw data for the amount of time it took the larvae from each group to pupate.

Table 1. The larvae from each group, those that received food and those who did not, were assigned a number. The days it took that larva to pupate, if pupation occurred, were recorded.

Days for Larvae to pupate		
Assigned Number to *Z. morio*	No Food Group	Food Group
1.00	11.00	27.00
2.00	15.00	30.00
3.00	15.00	0.00
4.00	17.00	0.00
5.00	0.00	0.00
6.00	0.00	0.00
7.00	0.00	0.00

Zeros were assigned to larvae that either did not pupate or died before they were able to pupate. A one-tail independent t-test was performed to determine if these results were statistically significant using the results of a descriptive statistics test seen in table 2.

Table 2. A descriptive statistics test was done in order to run a one-tail independent t-test.

No Food Group		Food Group	
Mean	7.83	Mean	5.00
Standard Error	3.52	Standard Error	5.00
Median	7.50	Median	0.00
Mode	0.00	Mode	0.00
Standard Deviation	8.61	Standard Deviation	12.25
Sample Variance	74.17	Sample Variance	150.00
Kurtosis	-3.24	Kurtosis	6.00
Skewness	0.03	Skewness	2.45
Range	17.00	Range	30.00
Minimum	0.00	Minimum	0.00
Maximum	17.00	Maximum	30.00
Sum	47.00	Sum	30.00
Count	6.00	Count	6.00

Table 3 shows the results of the t-test. For there to be a significant difference between the two groups, the *t* Stat value would have needed to have been greater than 1.81. The days it took the group without food (M = 7.83, SD = 8.61, n = 6) was not significantly different than the days it took the group with food (M = 5, SD = 12.25, n = 6).

Table 3. The results of the t-test show that there was no significant difference between the two groups as the t Stat value, 0.46, was not greater than the t Critical one-tail, 1.81.

t-Test: Two-Sample Assuming Equal Variances	No Food Group	Food Group
Mean	7.83	5.00
Variance	74.17	150.00
Observations	6.00	6.00
Pooled Variance	112.08	
Hypothesized Mean Difference	0.00	
df	10.00	
t Stat	0.46	
P(T<=t) one-tail	0.33	
t Critical one-tail	1.81	
P(T<=t) two-tail	0.65	
t Critical two-tail	2.23	

Discussion

For pupations to take place in the *Z. morio* larvae used in this experiment, it was necessary to first isolate the larvae. Several studies have been conducted on the effects of larval crowding on *Tribolium freemani* (Coleoptera: Tenebrionidae) (Hinton) have revealed that juvenile hormone may be the primary factor that inhibits the pupation of *T. freeman* in crowded settings. The exact reason that this occurs is not known in this species, but it is assumed that the amount of juvenile hormone secreted by the larvae may halt development and extend the period of time required for the larvae to pupate (Kotaki et al. 1993, Nakakita 1982). The direct effect this had was that the *T. freeman* larvae continued to feed and molts in crowded settings. This revealed a difference in physiological responses to crowding as *Trogoderma granarium* (Coleoptera: Dermestidae) (Everts) continued to gain weight when experiencing crowded environments (Nakakita 1982). This is important to note because, while there may not be data showing this is the same case for *Z. morio*, it can be assumed that this species experiences similar effects of crowding. Other studies have shown similar effects of crowding in other tenebrionids, *Zophobas rugipes* (Coleoptera: Tenebrionidae) (Kirsch) and *Tenebrio molitor* (Coleoptera: Tenebrionidae) (Linnaeus), so *Z. morio* may experience these effects as well (Nakakita 1982). This is why larvae had to be separated into individual cups in order for pupation to be observed.

The main factor being tested, the effect of starvation on the rates of pupation in *Z. morio*, did not yield the expected results. While the raw data in table 1 shows that the group that received no food and was starved pupated in nearly half the time as those that did receive food, the t-test indicated that there was no significant difference. This could be due to the small sample size, but the results of the statistical analysis correspond to a similar study done on *Attagenus megatoma* (Coleoptera: Dermestidae) (Latreille) in which it was found that starved groups of larvae pupated at the same time as fed groups of larvae (Baker 1977). This is a stark comparison to *Dacne picta* (Coleoptera: Erotylidae) (Crotch). *D. picta* is a pest species that targets shiitake mushrooms and it was found that the pupation of the larvae was most likely triggered by starvation, but the possibility that a deterrent stimulus from the mushrooms cannot be removed (Sato and Suzuki 2001). Furthermore, a study conducted on *Psacothe hilaris* (Coleoptera: Carambycidae) (Fairmaire) showed that the larvae of this species will actually shorten their larval life stage and pupate at a smaller size when food is scarce or no existent (Munyiri et al. 2003). This indicates that larvae will pupate faster than they normally would when given an abundance of resources.

Investigation what can make *Z. morio* larvae pupate at faster rates is of interest to those that use these species as a sustainable food source for both humans and animals. In cultures where alternative sources for animal protein are being investigated and explored, insects stand out as being ideal when looking at the space needed to rear them. Furthermore, while *Z. morio* larvae cannot be considered as the ideal insect in a diet as they have a high saturated fatty acid content, the larvae were found to have protein content similar to that of a roast pork (Adamkova et al. 2016). For pets, it was found that *Z. morio* can make good additions to the ingredients in commercial dog and cat foods as their nitrogen content is highly digestible and had the second highest crude protein content when compared to several other species of insects. However, care does need to take place when supplementing insects in general to cat and dog foods as there is variability in the amount of the amino acids methionine and cysteine, which are the first limiting amino acids in other ingredients to diets (Bosch et al. 2014). Furthermore, a study looking at the effects of including *Z. morio* meal in the diets of Nile tilapia found that a 25% and 50% inclusion of the meal in the diet lead to improved growth performance. However, it is important to note that this study found that 25% is the optimal inclusion percentage as weight gain was reduced when the percentage of meal in the diet was over 50% (Jabir et al. 2012). All of these studies together show that *Z. morio* has the potential to become part of the diets of humans and other animals.

Future work needs to be done on the rates of pupation when looking at starvation versus fed groups. Firstly, this study needs to be re-conducted with a much larger sample size in order to obtain statistically significant results. A larger sample size will also decrease the effects that deaths of specimens will have on the data. One factor that needs to be investigated is the effect of the quality of the food the *Z. morio* larvae are offered and how this affects the rates at which they pupate. In a study looking at the effect of nutritional quality in *Anthenus verbasci* (Coleoptera: Tenebrionidae) (Linnaeus), it was determined that larval development rate of this species was dependent on the nutritional quality of the food the larvae consumed (Miyazaki et al. 2009). Another factor that needs to be investigated is whether or not the quantity of food given to the larvae has an effect on the amount of time spent in the pupal stage. In *Culex nigripalpus* (Diptera: Culicidae) (Theobald), it was found that the quantity of food offered to the larvae at a constant 27°C did not affect when the larvae pupated, but significantly affected how long it took for the insects to emerge from their pupae. Larvae offered twice as much food as the control group emerged from their pupae nearly twice as fast as the group that received a normal amount of food in the study (Nayar 1968). Finally, this experiment needs to be repeated but using *Z. morio* larvae that are different larval instars. One study looking at the effects of *T. molitor* found that feeding is crucial for growth between larval instars but becomes facultative in the last larval instar. It was reported that the last larval instar is not the stage where the larvae feed the most in *T. molitor* (Connat et al. 1991). This would be important to consider when re-conducting this experiment as the effects of starvation in the last larval instar would have little to no effect on *Z. morio* if they are not obligately feeding in this instar.

In summary, while there was an apparent difference between the times that the groups with and without food pupated, there was no significant difference between the two groups. This may be due to the low sample size that was a result of deaths and larvae that failed to pupate. This experiment should be redone in order to develop a greater understanding of the factors that affect the rate at which *Z. morio* pupate. It is important to know how to develop colonies in a timely manner for human, pet, and livestock consumption.

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