

Population Assay of *Tenebrio molitor* (Coleoptera: Tenebrionidae) (Linnaeus) Growth and Development Analysis

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Edited by Erika Davila

Abstract: *Tenebrio molitor* (Coleoptera: Tenebrionidae) is a common grain pest distributed throughout the U.S. It is also a common source of food for many animals and humans alike. Due to its usefulness in bio regenerative life support system studies, the goal of this experiment is explore *Tenebrio molitor* changes during development. This experiment will also explore simple and easy dietary options for rearing *Tenebrio molitor*. Measurements of the front of head capsule to the end of abdomen, as well as body weight, were taken from larvae, pupae, and adult stages. It appears that variation in development of *T. molitor* occurs from the larval to pupal stage where most of the fatalities occurred. It was also determined that there is no correlation between length and weight of the larvae. Data for adult *Tenebrio molitor* show that length and weight are inversely related to overall density. A higher weight in *T. molitor* adults would yield better results for rearing as a food source.

Keywords: Population assay, Growth, Diet, Development, *Tenebrio molitor*, Nutrition

Tenebrio molitor (Coleoptera: Tenebrionidae), also known as mealworms, are an abundant stored-grain pest in the United States (Schroeckenstein et al. 1990). Their abundance also makes them one of the most common foods for birds, reptiles, and even humans (Davis 1978). Insects are commonly eaten in poor countries, where there is a shortage of nutritious food. Mealworms provide high protein values, and amino acids which make them a good alternative food source (Ewa Siemianowska 2013). In addition, mealworms have a relatively high growth rate and require only light labor and materials for rearing, which

lowers working time and costs. *Tenebrio molitor* are omnivorous, in the larval and adult form, which means they can get their nutrients from plant material as well as animal products (Ramos-Elorduy et al., 2002). Most importantly, this insect can be fed and survive off of decaying food sources. *Tenebrio molitor* are becoming useful in the study of bio regenerative life support systems (BLSS) for future long-term and far-distance manned space missions. This species of insect will likely serve as a source of food for astronauts (Li et al. 2016). The overall goal of this study was to explore *Tenebrio molitor* changes during development to contribute to

the understanding of variation within life cycles and diet. This information will be valuable to BLSS research by showing if *T. molitor* can be sustained by only feeding on oats, apples and potatoes. Specific objectives were to use statistical analysis with measurements and weight of *T. molitor* to explore similarities and differences within each life cycle stage.

Materials and Methods

This study was conducted over a 12 week time period. One hundred mealworms (Droll Yankees MW Mealworm, Plainfield, CT) were ordered for observation; however, only 75 mealworms were used for this study. A habitat consisting of eleven mealworms was then set up using plastic containers with dimensions $17.5 \times 9.3 \times 6.3$ cm (Sterilite, Townsend, MA). For this experiment, oats (Quaker Oats, Leicester, Great Britain), potatoes (Idaho Potato Commission, Eagle, ID) and apples (Granny Smith, USA) were used as sources of food for *Tenebrio molitor* development. There were approximately four cups of oats placed in the bottom of each container along with half a potato and apple sliced into fourths. The apple and potato were placed on separate sides of the containers to observe what source would be eaten most often. Larvae were allowed to feed *ad libitum* and diet was replenished once a week or as needed. As larvae began to mature into pupal, and adult forms they were placed in separate designated containers (Sterilite Townsend, MA). Each container was given the same proportions of food. During food renewal, *Tenebrio* populations were assayed at weekly intervals and the length, width and weight of the larva, pupa and adult populations were documented. The pupal stage of *T. molitor* does not eat so although the diet observations were not relevant, data on growth and behavior was

still observed. When not being observed, the container was covered with a punctured lid and placed in a low-lit area.

For each stage of *Tenebrio molitor*, data on length, weight and written observations were recorded. Length was logged using a standard 12 inch ruler (Westcott Wood Ruler, Acme United). Larvae were measured one at a time from mandible to spine. This length was collected in centimeters and rounded to the nearest millimeter. In this experiment, coffee filters were used to mimic weigh boats. A coffee filter (Bunn Home Brewer, Springfield, Illinois) was placed on the gram scale, zeroed out, then larvae were weighed individually. The weight was calibrated using an electronic gram scale to the nearest hundredth gram (American Weigh Scale, Norcross, GA). After all measurements were complete, the larvae were placed in a container that would solely contain a stage of *T. molitor* per stage. These processes were repeated for all larvae, pupae, and adults within the initial colony.

Written observations consisted of documenting fatalities, growth development, and food preferences. The standard deviation (SD), average, and range of each stage was calculated.

$$\text{Percent error of mean} = \frac{|\text{calculated mean} - \text{true or target value}|}{\text{true or target value}} \times 100$$

Results

Variation among length and mass comparisons in the larval and pupal stages showed the most precision of measurements and lowest standard deviation. Diet affected mealworm development and larval development time. Larvae and adult *T. molitor* who fed on a diet containing potatoes and apples showed increases of 0.13g and 0.05g times their initial weight during the 12-weeks, respectively (Figure 1). Larvae weights ranged from 0.13 g – 0.21 g. The standard deviation for weight was $p=0.022$ g which was not a significant value ($p>0.05$) (Figure 1). At the conclusion of this experiment, pupae showed a 63% population loss (Figure 2). For this matter, direct comparison of pupal to adult values for developmental times are difficult because of mortality as pupae. Consequently, mean values can appear higher for pupae (Figure 2) than for adults (Figure 3). *Tenebrio* larvae are able to utilize the small amounts of water held in dry feeds, apples and potatoes. However, the productivity and water-deprived mealworms is lower than those who received proper nutrients (Murray 1968) (Urs, K. C. D. 1973).

Figure 1. *Tenebrio* larvae changes over time in average population and growth rate. Data is the variance \pm SD of 11 individual larvae.

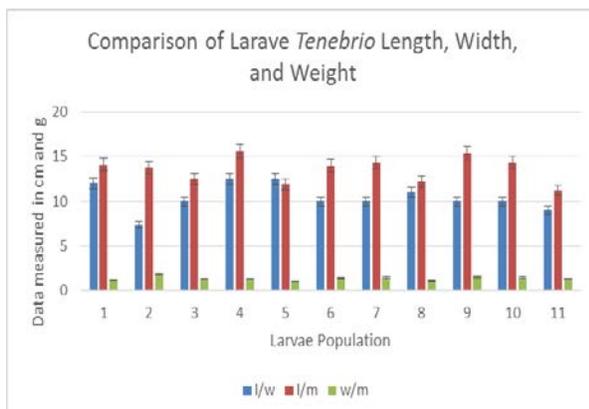


Figure 2. *T. molitor* from larval to pupal stage experienced seven fatalities. Data is the variance \pm SD of 11 individual pupae.

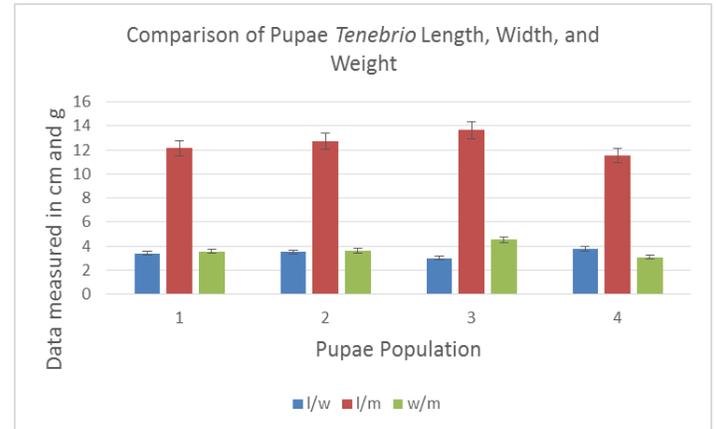
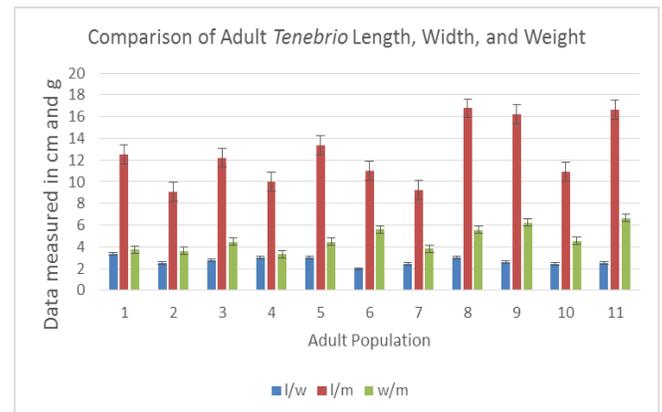


Figure 3. Adult *Tenebrio* showed the most significant differences between length and weight of individuals. Data is the variance \pm SD of 11 individual adults.



Composition of Nutrients Potato vs Apple vs Oats

Nutrient	Potato	Apple	Oats
Water	79.25 g	85.56 g	6.55 g
Protein	2.05 g	0.26 g	17.30 g
Lipid	0.09 g	0.17 g	7.03 g
Sugars	0.82 g	10.39 g	1.45 g

Table 1. *All dietary components obtained from ICN Nutritional Biochemicals Corporation, Cleveland, Ohio, except as otherwise noted.

Discussion

This study demonstrated that population quantity and *T. molitor* characteristics varied across metamorphosis. Larvae weights and appeared to show very little distribution between growths however, when comparing it to the SD for length $p=0.230$ cm (2.300 mm) it can be determined that there is not a direct correlation between the length and weight of the larvae. The increase in weight was partially attributed to an increase in stored lipids, there being little difference in total body water (URS and HOPKINS, 1973). Similar studies have been completed (Dunford et al. 2005.) to find that the larval form can grow up to 3.5 cm (35 mm) long. The *Tenebrio* in this experiment grew from larval to adult form in as little as 84 days. Out of the initial 11 larvae observed, only four made it to the pupation stage. Because pupae

lie dormant during this stage, they lack many of the nutrients found during other life stages including proteins, lipids, sugars and hydration. The larval and adult stages showed the most activity. Completion of the larval stage took approximately 6 weeks. Larvae burrowed below the surface of the oats and underwent a series of 8-12 molts each before transitioning into the final adult stage (Baek et al. 2015). After several weeks, they emerged to the surface and wriggled out of their exoskeletons. Pupae showed no indication of food or shelter preferences. These preferences were based on observations of the amount of food consumed. The uniformity of colony size for larvae and adults may be attributed to the amino acids other nutrients found in their diets. Pupae closely resembled the beetle in that it had defined body segmentation (head, thorax, and abdomen). The development from pupae to adult took approximately 6 days to complete and showed the least variation among length, weight and mass than any other life stage. Data for adult *Tenebrio molitor* show that length and weight are inversely related to overall density. A small length and high weight will result in a relatively high density while large length and small weight produce lower results. A higher weight in *T. molitor* adults would yield better results for rearing as a food source. In summary, this study presents a basis for interpreting *Tenebrio* variations in growth in relation to nutritional values.

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