SFU SURJ | SIMON FRASER UNIVERSITY SCIENCE UNDERGRADUATE RESEARCH JOURNAL
Vol. 4 (2018-19)

The Effect of Sustainably-Sourced Waste Food Diets on Yellow Mealworm Larvae (*Tenebrio molitor*)

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Abstract

As the world's population continues to increase, the demand for protein-rich foods will increase commensurately as well. Currently, a large amount of deforestation, pollution, climate change, and other deleterious global phenomena occurs to meet current demand. Deforestation in particular is most concerning as large amounts of land is used to produce food for livestock instead of people. With respect to the latter, current food production systems result in a large amount of food that goes to waste. As such, using insects to address these issues is a promising avenue of research. Insect species use less space and water to grow, have significantly reduced feeding costs, and produce fewer emissions than traditional livestock (e.g. beef cattle). In addition to these benefits, a variety of insects such as flies, have the potential to consume items like waste food without any detriment to their health or growth. This project assessed the feasibility of feeding Tenebrio molitor (*T. molitor*) larvae three diets - oatmeal, waste food, and animal protein/brewer's spent grain. Population numbers, mortality rate and pupation rate at each stage of the *T. molitor* lifecycle were recorded for each diet. Low mortality among other observed metrics suggest that *T. molitor* can grow on a waste food diet.

Keywords — Entomophagy, Waste-Food, Sustainability, Yellow Mealworm

1. Introduction

With the world's population projected to reach nine billion by 2020 [1], there is an ever-increasing demand for land and for protein-rich foods. Currently, global issues such as deforestation, pollution and climate change are due in part, to this demand. Maintenance of agricultural crops leads to land and water degradation via the use of pesticides, fertilizers, and animal waste [2].

Globally, the agricultural industry is responsible for approximately 50% of methane emissions and 60% of nitrous oxide emissions [3]. With respect to a rising population, the increased demand for protein-rich foods will dramatically increase these negative effects [4, 5].

A large amount of land will be required to feed these livestock as well and indeed, according to Cassidy et al [6], "in developing countries with high rates of increasing animal product demands, a greater proportion of cereals are being directed to animals."

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This land will be required in the years to come to sustain an increased population either with food or housing.

Insect species such as yellow mealworm beetle larvae (*Tenebrio molitor*), hereafter referred to as *T. molitor*, are a realistic solution to these problems. Compared to other livestock species, raising insects as a source of food features significant benefits.

Firstly, compared to cattle ranching, insects produce fewer emissions during their growth. On average, cattle produce 67.8 kg of CO_2 per kg [7] in contrast to *T. molitor* which produce 2.2 g of CO_2 per kg [8]. Secondly, insects require significantly less water. For example, 22,000 L of water is required to produce 1 kg of beef whereas insects like *T. molitor* do not directly require a water supply [9]. Lastly, insect protein can be grown in a small area [10] and with further research could be used to potentially supplement the diet of livestock [11, 12] as well as humans [13]. This nutritional density means that the land previously used to grow feed crops for livestock could be repurposed for housing, solar panels or even be reforested.

The ability of many insects to consume a variety of products from low nutrient waste food [8, 14, 15] to manure is also important. Globally, waste food contributes 3.3 Gtonnes of CO_2 into the environment every year [16]. This waste food could instead be used to feed different insect species which in turn could be used as a high-quality protein source. This would effectively close the loop between food production and food waste.

With respect to the objectives of this research project, we chose *T. molitor* larvae as our model organism. For research purposes, they were they easy to procure and required very little maintenance.

The objectives of this research project were as follows. First, we wanted to see if *T. molitor* could successfully grow on a waste food diet. As such, we used three different diets during this experiment - oatmeal, waste food and animal protein/ brewer's spent grain.

The oatmeal diet is what most home and commercial growers feed to mealworms and it served as our control diet. We hypothesized that the animal protein/brewer's spent grain diet would be higher in protein and starch and therefore be more nutrient dense than the other diets.

Second, to evaluate if these three diets influence mortality rate, and/or life stage transition (i.e. pupation) rates. Lastly, to explore novel possibilities of applying the potential of insects in our communities. For example, previous studies have shown that mealworms are capable of safely breaking down Styrofoam [17, 18] (Fig. 1). Additionally, the shells of the mealworm beetle, which contain chitin, can be used to create a biodegradable plastic substitute [19] (Fig. 2) among other novel products [20]. For the purposes of our research we wanted to explore ways in which the community or local companies could contribute and make a difference.

2. Materials and Methods

All *T. molitor* were obtained from Wild Birds Unlimited in Surrey, B.C., Canada. Their supplier, Little Fish Company, is also located in Surrey, B.C., Canada and typically supplies *T. molitor* to be fed to pet fish or reptiles.

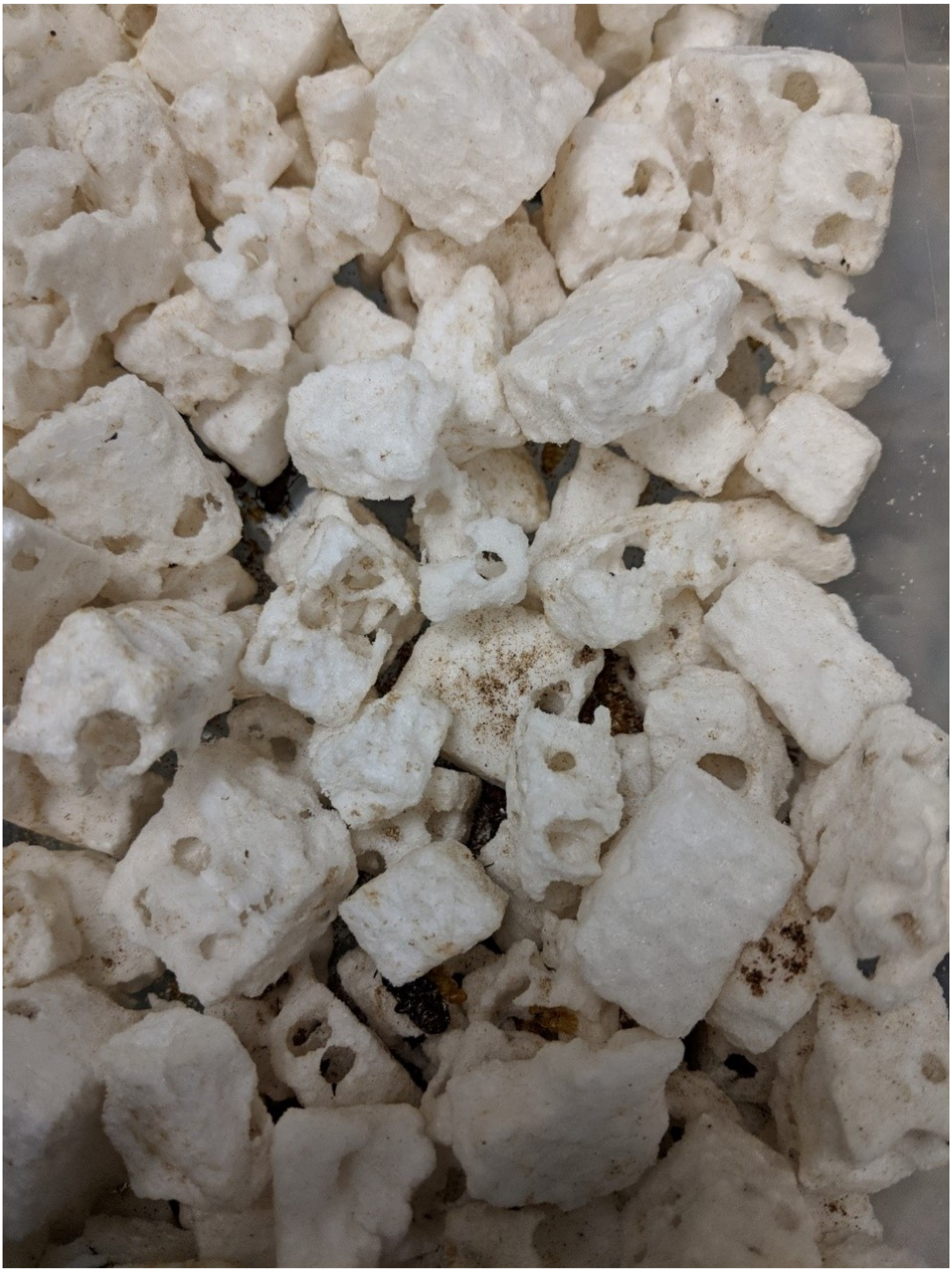

Figure 1: *Styrofoam that has been consumed by T. molitor. This photo was taken in our lab.*

Despite looking at a number of research papers, there were no established methods to raise *T. molitor* larvae for research purposes. As such, the methods outlined below were developed by our team.

Figure 2: *A chitosan based plastic substitute made from T. molitor beetle shells. On Top: The finished biodegradable plastic substitute. On Bottom, from left to right. a) Breaking down T. molitor beetle shells by grinding. b) Removal of organic matter. c) Deprotonation of T. molitor beetle shells. d) Evaporating excess liquid to form chitosan sheets.*

The research lab in which the *T. molitor* were grown in maintained an average temperature of 22 degrees Celsius during the study period. Each life stage was raised in food safe Sterilite containers. Initial density consisted of 500 *T. molitor* per container and the first generation was raised on the control diet to allow for acclimatization and to improve the research methodology.

Post generation one, 3000 *T. molitor* were randomly assigned into three different diet groups. Each group consisted of two containers each containing 500 *T. molitor* and were manually checked to ensure that density did not exceed this number. In cases where this did occur, the excess was added to a new container.

As *T. molitor* continued to develop past the larvae stage, they were separated into their respective life stages - pupae and then beetles. The beetle eggs were collected and the container monitored until new *T. molitor* larvae were seen. In general, the stages of the *T. molitor* lifecycle occur at set times.

All life stages were raised in a dark environment and containers were removed only for data collection or maintenance procedures. The removal times did not exceed more than one hour.

To create consistent food pellets for each diet the raw components of each diet were processed separately albeit in the same fashion (Tab. 1). Oatmeal did not require dehydration but was otherwise processed in the same fashion.

Table 1: *Diet processing procedure.*

Dehydration	Each diet component was dehydrated to prevent formation of mold and pathogens.
Pulverize	Dehydrated diet components were ground to a fine powder. This ensured that each pellet was consistent.
Binding Agent	Potato starch was added to bind components together. It was chosen as it had little to no nutrition.
Baked	The components were mixed together with water and baked at a low temperature until hard.

We were able to collaborate with several supermarkets and a local brewery for waste food and spent grain respectively. To mimic real world conditions, where waste food composition would be continually changing, a new batch of waste food was obtained biweekly. As the waste food provided varied in composition between batches, we recorded the composition of each batch as well. In the event that a *T. molitor* group being fed on waste food was affected, we could see which specific batch was used.

Ingredients for each diet were sourced locally, with the support of community partners (Tab. 2). Carrot slices (30 g per container) were included in each container and served as a water source.

Table 2: *Diet composition.*

Diet Type	Diet Composition	Source
Control	Oatmeal	Supermarkets
Waste Food	Waste Food	College cafeteria, personal homes, supermarkets
Animal Protein and BSG	Animal Protein and Brewer's Spent Grain	Supermarkets, Faculty Brewing: Local brewery located in Vancouver, British Columbia

Containers were checked at the end of each data collection session. Old feed was removed and replaced with 500 g of fresh feed along with 30 g of fresh carrot slices.

Due to logistical issues we were only able to start data collection at the end of generation two - October 23, 2017. In this period, the population of *T. molitor* for each diet was around 200 larvae and new hatchings were being introduced.

Data was collected every Monday, Wednesday, and Friday for six months (Tab. 3), with minimal exceptions due to holiday closures or scheduling conflicts.

Table 3: *Recorded metrics.*

Metric	Rationale
Number of alive/dead *T. molitor* larvae	Track population numbers/mortality
Number of new *T. molitor* larvae added	Population growth
Number of alive/dead *T. molitor* pupae	Track pupation rate

3. Results

From October 23, 2017 to November 20th, 2017 the increase in population size was due to *T. molitor* larvae being added (Fig. 3). Compared to the oatmeal diet, the *T. molitor* on the waste food and animal protein/brewer's spent grain diets were able to introduce more new larvae into the population (Fig. 6).

Figure 3: *Impact of diet on T. molitor larvae population from October 23, 2017 to March 16, 2018.*

From November 20th, 2017 to January 1st, 2018, population size started to decrease through a combination of *T. molitor* death and pupation (Fig. 4 and Fig. 5). The *T. molitor* on the oatmeal diet displayed a sharp drop in population and there was a corresponding spike in both mortality and pupation rate. After November 20th, the population of this group increased due to new larvae hatching. In contrast, the animal protein/brewer's spent grain group showed a more consistent population decrease with mortality and pupation increasing towards the end of this period. Interestingly, the waste food population was able to maintain the larval stage more consistently compared to the other two diets. While there was a small decrease in this group's population due to *T. molitor* death, the pupation rate of the waste food group decreased as well.

From January 1st, 2018 to January 31st, 2018 all three populations displayed a sharp drop in population. Both pupation rates and mortality rate increased concurrently during this period as well. Around January 31st, 2018, the population sizes of all three diets was as the lowest. The population of the waste food and oatmeal diets were similar and were slightly higher in density compared to the size seen start of the generation. In contrast, the animal protein/brewer's spent grain diet had the lowest

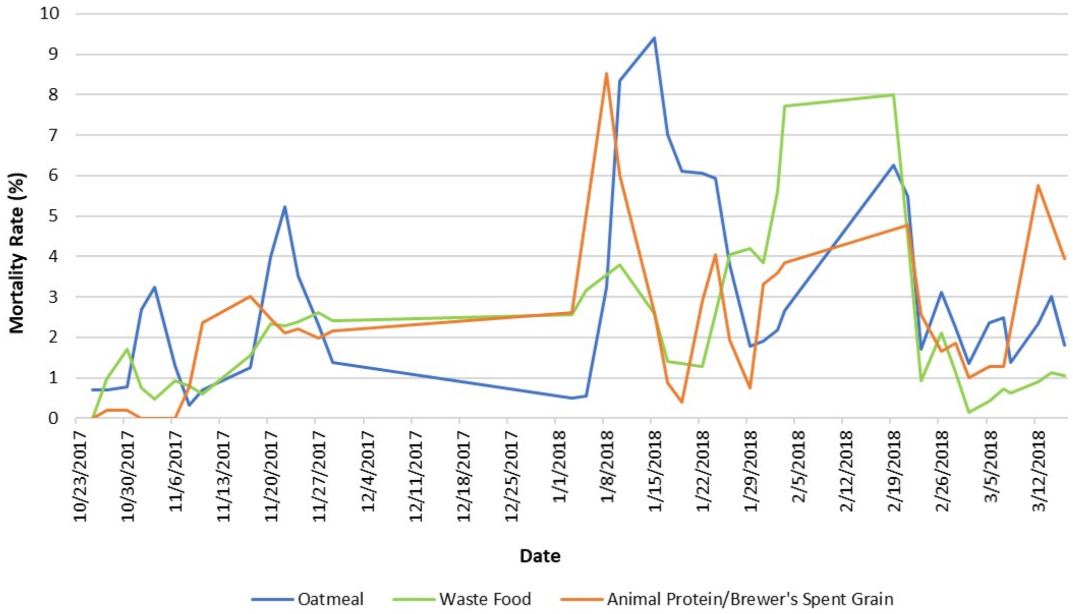

Figure 4: *Impact of diet on T. molitor larvae mortality rate from October 23, 2017 to March 16, 2018.*

population size.

From January 31st, 2018 to February 10th, 2018 the next generation of *T. molitor* larvae emerged and were added. Unlike the previous generation, the waste food diet was the only diet to have the largest population size.

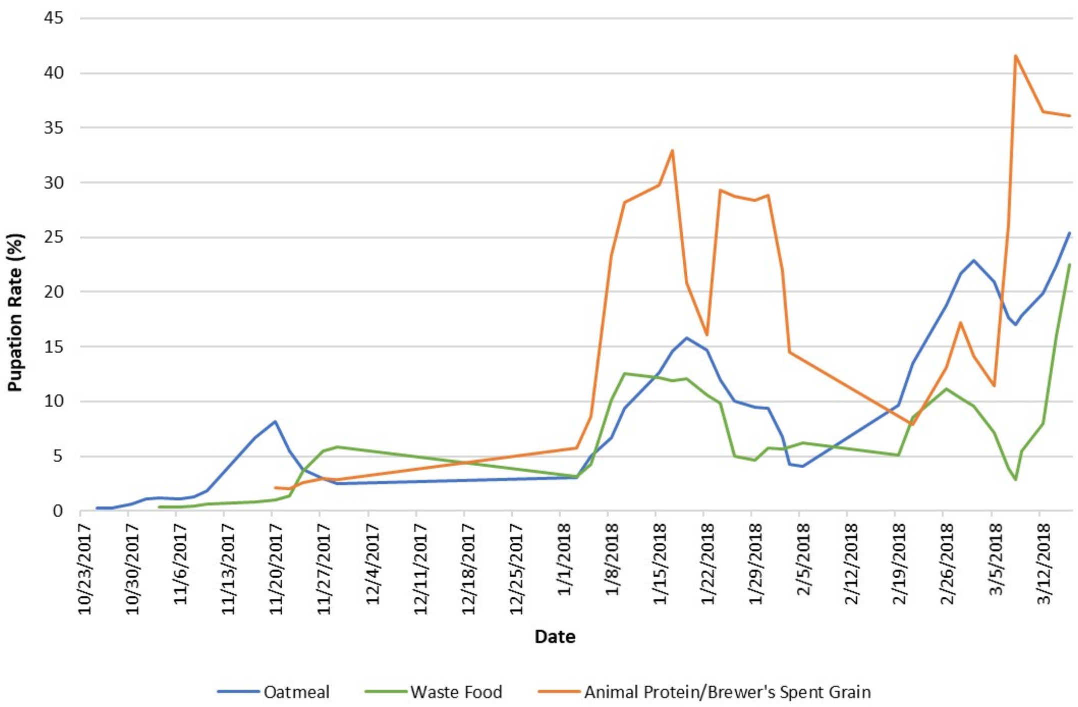

Figure 5: *Impact of diet on T. molitor larvae pupation rate from October 23, 2017 to March 16, 2018.*

The oatmeal diet produced roughly the same number of new larvae as the previous generation while the animal protein/brewer's spent grain diet was the lowest. This was interesting as the animal protein/brewer's spent grain diet had the highest pupation rate compared to the other two diets.

From February 10th, 2018 to February 20th, 2018 - the conclusion of the experiment, all *T. molitor* populations decreased in size. During this period the animal protein/brewer's spent grain diet again had the highest pupation rate however the mortality rate for all three diets was much more consistent.

Although the experiment concluded on February 20th, 2018 data collection continued until March 16th, 2018. Due to construction noise and reduced maintenance during this time period, the results are likely not accurate.

Overall, mortality never exceeded 10% of the total population. The oatmeal diet experienced the highest overall mortality rate (14.1%) on January 15, 2018 and had the highest average mortality rate (3.0% $\pm$ 2.9%). Diets 2 and 3 had lower average mortality rates (2.2% $\pm$ 2.5% and 2.2% $\pm$ 2.0%).

With respect to the pupation rate, the animal protein and brewer's spent grain diet was much higher than the other two diets. This diet displayed the three highest pupation rates (27.7% on January 3, 2018, 21.2% on January 8, 2018 and 21.0% on January 15, 2018).

Over the study period, new larvae hatched from eggs produced by *T. molitor* beetles. Compared to the other two diets hatching numbers were low for the animal protein/brewer's spent grain diet. (Fig. 6).

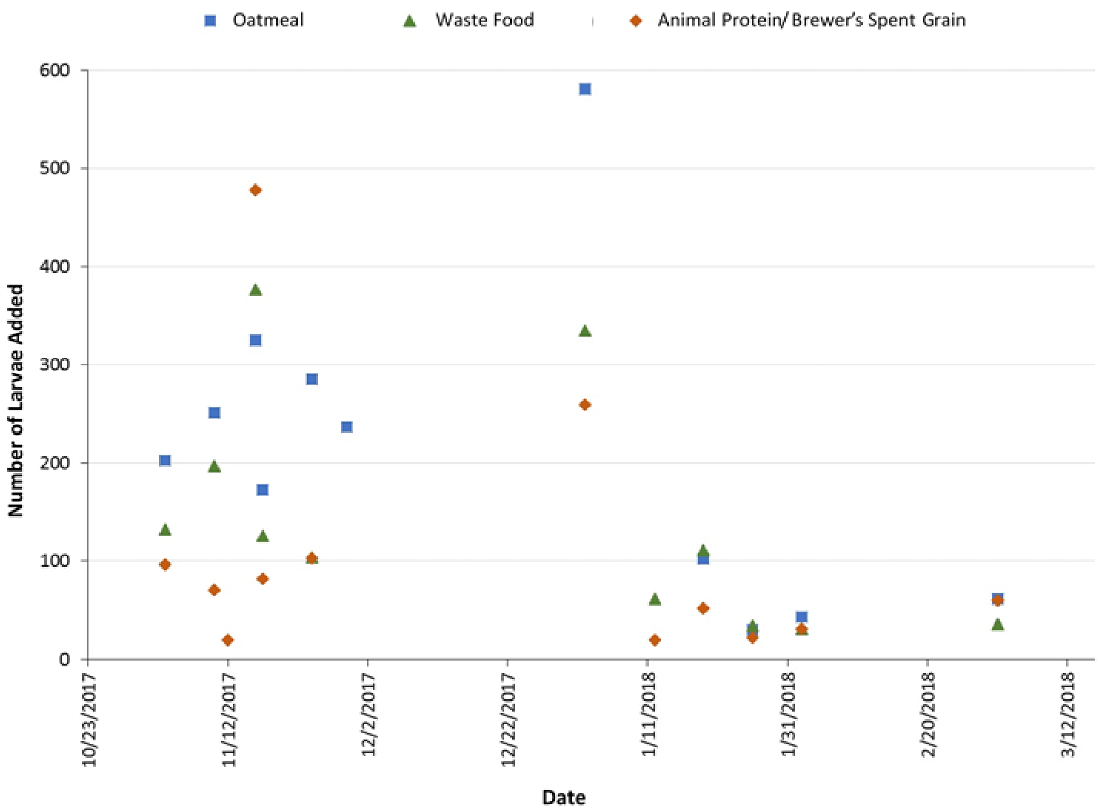

Figure 6: *Impact of diet on T. molitor larvae pupation rate from October 23, 2017 to March 16, 2018.*

4. Discussion

The goal of this experiment was to see if *T. molitor* could successfully grow on a waste food diet. In addition to serving as an environmentally friendly source of protein, this would allow for large amounts of agricultural land to be repurposed.

Throughout the course of this experiment *T. molitor* were able to sustain populations. However, there were differences for each group that provided insight into the effectiveness of each diet.

With respect to the other two diets, the animal protein/brewer's spent grain displayed the largest differences. During the first generation, this diet was able to introduce a similar number of new larvae as the waste food diet. However, unlike the waste food diet, the population of this group decreased over time. In contrast, the waste food diet, had a more consistent population with a drop only seen during pupation.

During the transition to the next generation, the animal protein/brewer's spent grain diet displayed much higher pupation rates than the other diets. This increased rate did not translate into a larger population size as this diet group also had an increase in larval mortality. This could suggest that the animal protein/brewer's spent grain diet lacks key nutritive components that are needed as *T. molitor* develops from new to adult larvae. The brewer's spent grain could be suspect as many of the complex carbohydrates would have likely been extracted during the brewing process.

To see if the possible lack of carbohydrates was affecting these metrics we looked at the oatmeal diet. While the mortality rate for this diet was similar to the animal protein/brewer's spent grain diet, the oatmeal diet had a pupation rate comparable to the waste food diet.

The high mortality and low pupation rate seen in the oatmeal diet was interesting as this group was able to produce a higher population at the start of the second generation. We have two theories why this may be the case. The first is that diet affects the ability of the *T. molitor* pupae to transition into the final life stage of beetles. Despite a high pupation rate, if a fewer number of beetles are present then this would impact the number of offspring produced. The second is that diet affects the ability of *T. molitor* beetles to produce eggs or for the eggs to hatch into new larvae. It could also be that results we saw are due to a combination of both theories.

Based on the population increase seen in generation one, none of the diets appeared to affect the new mealworms added. However, the start of generation two saw marked differences with respect to population size. Both the oatmeal and the animal protein/brewer's spent grain diets were able to add new larvae to the population. However, the oatmeal diet population increase occurred over a longer period of time - approximately two weeks. In contrast, the increase seen in animal protein/brewer's spent grain diet occurred in less than a week. As such, it seems that diet plays a role in the long-term development of *T. molitor*.

With respect to long term growth, it would appear that the waste food diet minimizes the risks seen with the other two diets. Although mortality and pupation rates were similar to the oatmeal diet, the overall population of the waste food group was higher. As such, even though the waste food diet changed biweekly, there is evidence to suggest that *T. molitor* would do well on it.

While these results are promising they are not definitive and further research is required. In retrospect, we realize that gathering data on *T. molitor* beetles and eggs would have been valuable. As such a task would have increased the timeline of our experiment, we were unable to do so. We would also recommend that future studies be longer in length as we were only able to collect just over one generation's worth of *T. molitor* data.

Additionally, future studies that cover feed consumption and feed conversion ratios would also be insightful. We did measure the amount of feed consumed as well each weight of each *T. molitor* colony, however the scales we used were not precise enough. For future studies, we recommend using scales that are accurate to 0.01 g to measure both. We also found that the food pellets absorbed a significant amount of water weight between data collection intervals. Storing the pellets with a desiccant would help to alleviate this issue.

Lastly, experiments that also incorporate research into the nutritional aspects of each diet type would help to shed further light. It is possible that a combination of diets or a sequential feeding of the diets may perform better than the diets on their own. Researching each component of the diets, in addition to studying when each diet may perform best (e.g. at each life stage) merits further study.

The results of our research strongly suggest that *T. molitor* can be raised on a variety of different diets. However, diets that are varied in composition will likely result in more robust *T. molitor* populations.

5. Conclusion

There is evidence that raising *T. molitor* on a waste food diet is feasible, however further research is needed. As such, this research is a promising first step in using insects to address global issues like deforestation, waste food and climate change.

6. Acknowledgments

We would like to recognize Dr. Mário Moniz de Sá for supervising this project, providing unwavering support throughout the research design and coordination process and for his contagious enthusiasm for student development and innovation. We would also like to extend gratitude to Kelly Sveinson for supporting us with equipment acquisition and project guidance, and for his passion for driving student research at Langara College. We give special thanks to Faculty Brewing Co. for their donation of brewer's spent grain and for their advancement of local science initiatives.

We would like to recognize our lab supervisors, Prerna Bedi and Jocelyn O'Brien, for their commitment to the project and for providing their perspectives. Finally, we would like to thank our research assistants - Brendon Henry, Nhat Nguyen and HanBin Sung - for their attention to detail, and assistance with the collection of data.

7. Grant Information

We are grateful to the Langara College Research and Scholarly Activity Fund (RSAF) awarded to Dr. Mário Moniz de Sá and to the Career Launcher Clean Tech Internship Program through Colleges and Institutes Canada (CICan) for generous financial contributions to this project in the form of an apprenticeship awarded to Roshan Noronha.

Additionally, we extend our appreciation to the Langara College Biology and Chemistry departments for funding provided to hire students involved in this project, through the SWAP and WOC programs.

References

[1] World population prospects - population division. URL https://population.un.org/wpp/Publications/Files/WPP2019_Highlights.pdf.

[2] Adrian Muller, Christian Schader, Nadia El-Hage Scialabba, Judith Brüggemann, Anne Isensee, Karl-Heinz Erb, Pete Smith, Peter Klocke, Florian Leiber, Matthias Stolze, et al. Strategies for feeding the world more sustainably with organic agriculture. *Nature communications*, 8(1):1290, 2017.

[3] B Colombo, P West, Pete Smith, FN Tubiello, J Gerber, P Engstrom, A Urevig, and Eva K Wollenberg. How does agriculture change our climate? 2016.

[4] Maeve Henchion, Maria Hayes, Anne Mullen, Mark Fenelon, and Brijesh Tiwari. Future protein supply and demand: strategies and factors influencing a sustainable equilibrium. *Foods*, 6(7):53, 2017.

[5] David Pimentel, Bonnie Berger, David Filiberto, Michelle Newton, Benjamin Wolfe, Elizabeth Karabinakis, Steven Clark, Elaine Poon, Elizabeth Abbett, and Sudha Nandagopal. Water resources: agricultural and environmental issues. *BioScience*, 54(10):909–918, 2004.

[6] Emily S Cassidy, Paul C West, James S Gerber, and Jonathan A Foley. Redefining agricultural yields: from tonnes to people nourished per hectare. *Environmental Research Letters*, 8(3):034015, 2013.

[7] P Gerber, C Opio, T Vellinga, A Falcucci, G Tempio, G Gianni, B Henderson, M MacLeod, H Makkar, A Mottet, et al. Greenhouse gas emissions from ruminant supply chains–a global life cycle assessment. *Food and Agriculture Organization, Rome, Italy*, 2013.

[8] Dennis GAB Oonincx, Joost Van Itterbeeck, Marcel JW Heetkamp, Henry Van Den Brand, Joop JA Van Loon, and Arnold Van Huis. An exploration on greenhouse gas and ammonia production by insect species suitable for animal or human consumption. *PloS one*, 5(12):e14445, 2010.

[9] Arnold Van Huis, Joost Van Itterbeeck, Harmke Klunder, Esther Mertens, Afton Halloran, Giulia Muir, and Paul Vantomme. *Edible insects: future prospects for food and feed security*. Number 171. Food and Agriculture Organization of the United Nations, 2013.

[10] Dennis GAB Oonincx and Imke JM De Boer. Environmental impact of the production of mealworms as a protein source for humans–a life cycle assessment. *PloS one*, 7(12):e51145, 2012.

[11] Alexandre Thévenot, Julio L Rivera, Aurélie Wilfart, Frédéric Maillard, Melynda Hassouna, Tristan Senga-Kiesse, Samuel Le Féon, and Joël Aubin. Mealworm meal for animal feed: Environmental assessment and sensitivity analysis to guide future prospects. *Journal of Cleaner Production*, 170:1260–1267, 2018.

[12] Julieta Ramos-Elorduy, Ernesto Avila González, Alma Rocha Hernández, and José Manuel Pino. Use of tenebrio molitor (coleoptera: Tenebrionidae) to recycle organic wastes and as feed for broiler chickens. *Journal of economic entomology*, 95 (1):214–220, 2002.

[13] Ariana Vieira Alves, Eliana Janet Sanjinez-Argandoña, Adelita Maria Linzmeier, Claudia Andrea Lima Cardoso, and Maria Lígia Rodrigues Macedo. Food value of mealworm grown on acrocomia aculeata pulp flour. *PLoS One*, 11(3):e0151275, 2016.

[14] Dennis GAB Oonincx, Sarah Van Broekhoven, Arnold Van Huis, and Joop JA van Loon. Feed conversion, survival and development, and composition of four insect species on diets composed of food by-products. *PLoS One*, 10(12):e0144601, 2015.

[15] Afton Halloran, Nanna Roos, Jørgen Eilenberg, Alessandro Cerutti, and Sander Bruun. Life cycle assessment of edible insects for food protein: a review. *Agronomy for Sustainable Development*, 36(4):57, 2016.

[16] N Scialabba, J Olivier, C Tostivint, A Turbé, C O'Connor, P Lavelle, and et al. Food wastage footprint: Impacts on natural resources. *Summary Report*, page 63, 2013.

[17] John A Glaser. Biological degradation of polymers in the environment. In *Plastics in the Environment*. IntechOpen, 2019.

[18] Yu Yang, Jun Yang, Wei-Min Wu, Jiao Zhao, Yiling Song, Longcheng Gao, Ruifu Yang, and Lei Jiang. Biodegradation and mineralization of polystyrene by plastic-eating mealworms: Part 1. chemical and physical characterization and isotopic tests. *Environmental science & technology*, 49(20):12080–12086, 2015.

[19] Javier G Fernandez and Donald E Ingber. Manufacturing of large-scale functional objects using biodegradable chitosan bioplastic. *Macromolecular Materials and Engineering*, 299(8):932–938, 2014.

[20] Florence Croisier and Christine Jérôme. Chitosan-based biomaterials for tissue engineering. *European polymer journal*, 49(4):780–792, 2013.