The effect of sustainably-sourced waste food diets on yellow mealworm beetle (*Tenebrio molitor*) larvae

Project Sponsorship:
Langara College Research and Scholarly Activity Fund (RSAF),
Colleges and Institutes Canada (CICan), Langara College Biology and Chemistry Departments

Project Supervisor:
Mário Moniz de Sá, PhD, Instructor, Langara College

Figure 1: Scholarship Café announcement for this project and three of four life stages of the yellow mealworm beetle (*Tenebrio molitor*) as photographed in the lab, Langara College, Vancouver, BC. Top: larvae, bottom right: pupae, bottom left: beetle

Prepared by:

Roshan Noronha, A.S and Maggie G. Stewart, A.S. and Dipl T.

27 March 2018

Abstract

The agricultural industry contributes significantly to deforestation, pollution, climate change, and other deleterious global phenomena. As the world population grows, the demand for protein-rich foods, and thus the effects of their production, will dramatically increase. Insect species use less space and water, cost less to feed, and produce fewer emissions than traditional livestock (e.g. beef cattle). This project assessed the feasibility of feeding *T. molitor* three diets (i.e. diet 1 - oatmeal, diet 2 - waste food, and diet 3 - animal protein and brewer's spent grain). Number of alive and dead individuals of each life stage were recorded. Diet 3 had the lowest mortality and highest pupation rates but had low recruitment numbers. Low mortality amongst all three diets suggests that all diets provided *T. molitor* with sufficient nutrients for survival. This supports *T. molitor*'s ability to survive on a multitude of diets and that they can be grown on sustainably-sourced waste food diets.

Table of Contents

List of Figures

List of Tables

Acknowledgements

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.

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, Instructor, Chemistry, 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.

Additional appreciation is awarded to our volunteers and student staff: We would like to recognize our Student Lab Supervisors, Prerna Bedi and Jocelyn O'Brien, for their commitment to the project, their dedication in coordinating, and participating in, data collection, and for providing other perspectives when we needed them most. 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.

Langara.
THE COLLEGE OF HIGHER LEARNING.

careerlauncher
CLEANTECH INTERNSHIPS

FACULTY
BREWING
CO.

Introduction

Our need for sustainably-produced food

The agricultural industry contributes significantly to deforestation, pollution, climate change, and other deleterious global phenomena. 75% of deforestation results from cultivation of crops and the agricultural process (Colombo et al. 2017). Maintenance of these crops leads to land and water degradation (e.g. through the use of pesticides, fertilizers, and animal waste; Colombo et al. 2017). The agricultural industry is responsible for approximately 33% of methane emissions, 50% of carbon dioxide emissions and 60% of nitrous oxide emissions (Colombo et al. 2017). As the world population grows from 7.3 billion to 9.8 billion by the year 2050 (United Nations 2017), the demand for protein-rich foods, and thus the effects of their production, will dramatically increase (Oonincx 2015).

Waste to worms: Using insects to solve these problems

Insect species (e.g. yellow mealworm beetle [*Tenebrio molitor],* hereafter referred to as *T. molitor*) use less space and water, cost less to feed, and produce fewer emissions than traditional livestock (e.g. beef cattle; Figure 2). Soybeans, a crop commonly used as cattle feed, produces about 70 g/m^2 of protein (Kaldy 1972), compared to 250 g/m^2 for *T. molitor* (Halloran et al. 2013). 1 kg of beef requires 22,000 L of water (Pimentel et al. 2004), whereas *T. molitor* do not directly require a water supply. 3 kg of carrot can provide enough water for 1 kg of *T. molitor* larvae (Oonincx et al. 2015).

Cattle ranching has faced extensive criticism for emissions that affect climate change (EPA 2017). *T. molitor* larvae produce only a small amount of carbon dioxide (i.e. 2.2 g of CO_2 per kg of body mass; Oonincx et al. 2010), while cattle produce 34.6 kg of CO_2 per kg of meat (Foster et al. 2006).

T. molitor can be fed a variety of different waste products from low nutrient waste food (Ramos-Elorduy et al. 2002; Oonincx et al. 2015) to manure (Halloran et al. 2013). With nearly half of all Canadian food waste originating in consumer homes (Metro Vancouver 2017), *T. molitor* could transform this waste into protein and effectively close the loop between food production and waste.

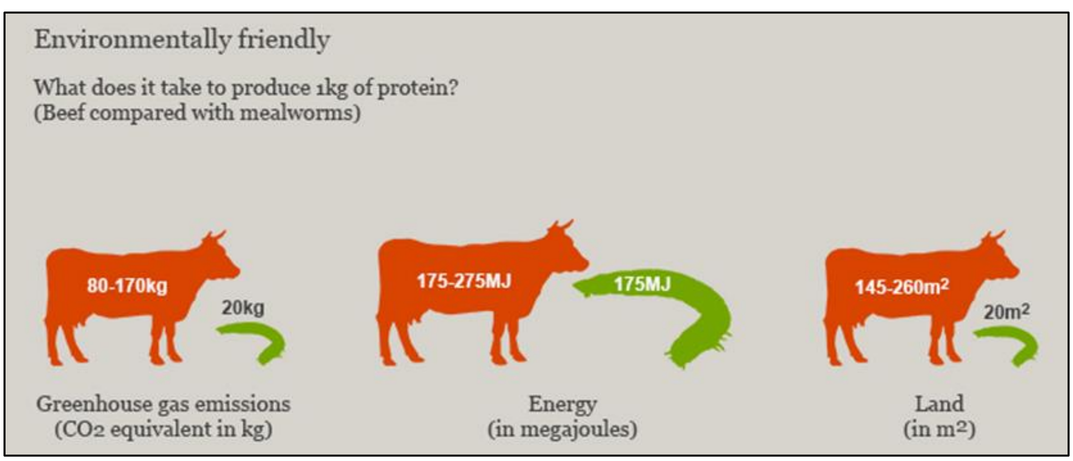

Figure 2: Comparison of beef cattle and yellow mealworm beetle (*Tenebrio molitor*) greenhouse gas emission, energy, and land requirements to produce 1 kg protein (UN 2014).

Objectives

The objectives of this research project were the following:

1. To assess the feasibility of growing *T. molitor* on three diets:
    a. Control diet: oatmeal (as fed when cultured for pet feed) and carrot
    b. Waste Food diet: kitchen scraps or other food waste and carrot
    c. Animal Protein & BSG diet: waste chicken, pork and/or beef, brewer's spent grain (BSG), and carrot
2. To evaluate if these 3 diets have an effect on mortality rate, and/or life stage transition (i.e. pupation) rates.
3. To provide innovative and high-quality experiential learning opportunities for Langara Science and Technology students.

As previously demonstrated by Oonincx et al. (2015), an increase in available protein and starch should increase the nutritive quality of the diet. As such, we predicted that the animal protein and BSG diet would perform the best (i.e. lower mortality and higher pupation rates). The waste food diet had a more varied composition, so we were unsure as to how it would perform relative to the other two diets. However, as *T. molitor* are known to consume a variety of food types, we expected all three diets to sustain populations of mealworms.

Methods

The research lab, provided by the Langara College Biology Department, is located in room A 330 at Langara College (49^{th} Avenue Campus, Vancouver, BC). The lab maintained an average temperature of 22 degrees Celsius over the study period.

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

Each life stage was raised in separate Sterlite containers. Initial density was 500 larvae per bin, with additions to each bin from reproduction or when requited (i.e. to bolster low population numbers). If density exceeded 500 individuals, a new bin was created. *T. molitor* were grown for an entire generation on the control diet (i.e. oatmeal) to allow for acclimatization and to perfect research methods. All *T. molitor* bins were stored in cabinets under each bench in the lab and removed only for data collection or maintenance procedures.

To create consistent food pellet size and characteristics, waste food, meat and BSG were dehydrated, ground into powder, bound with potato starch, baked into sheets, and then broken into approximately the same pellet size. Oatmeal did not require dehydration but was otherwise, processed similarly. Ingredients for each diet were sourced locally, with the support of community partners (Table 1). Carrot slices (30 g per container) were provided to *T. molitor* as a water source.

Table 1: Food pellet ingredient sources for each diet type fed to yellow mealworm beetle (*Tenebrio molitor*) from 23 October 2017 to 16 March 2018 at Langara College, Vancouver, BC.

Diet Number	Diet Composition	Source
1 - Control	Oatmeal	• Purchased from assorted grocery outlets
2 - Waste Food	Assorted kitchen scraps	• Chartwells (Langara College; donation) • Research team members personal homes (donation)
3 - Animal Protein & Brewer's Spent Grain	Expired meat (within a week) Brewer's spent grain	• Grocery store donations • Faculty Brewing (donation)

Data was collected every Monday, Wednesday, and Friday from 23 October 2017 to 16 March 2018, with minimal exceptions due to holiday closures or scheduling conflicts. Feed pellets (500 g) and carrot slices (30 g) were topped up during each data collection session. Six categories of metrics were collected for each bin and for each life stage to assess the research objectives (Table 2).

Table 2: Metrics collected for each life stage and diet type fed to yellow mealworm beetle (*Tenebrio molitor*) from 23 October 2017 to 16 March 2018 at Langara College, Vancouver, BC.

Metric	Rationale
Number of alive individuals	Track population numbers
Number of dead individuals	Assess mortality rate
Weight of individuals *alive larvae only	Assess average gain
Initial weight of feed (g) & amount of remaining feed (g)	Assess feed consumption rate
Initial weight of carrot (g) and amount of remaining carrot (g)	Track any differences
Whether or not mold was present *complete re-set if present	Assess correlations with feed type or mortality

Results

All three diets sustained populations over the course of the study (Figure 3). Diet 3 (i.e. animal protein and brewer's spent grain) had the lowest population numbers for a good portion of the study period with diet 1 (i.e. oatmeal) and diet 2 (i.e. waste food) at higher population numbers throughout the study.

Mortality never exceeded 15% of the total population (Figure 4). All three diets experienced significant mortality over the winter break (i.e. from 29 November 2017 to 3 January 2018).

The control diet (i.e. diet 1 - oatmeal) experienced the highest overall mortality rate (14.1%) on 15 January 2018, and had the highest average mortality rate (3.0 % ± SD 2.9). Diet two and three had lower average mortality rates (2.2% ± SD 2.5 and 2.2% ± SD 2.0, respectively).

Transition from larvae to pupae (i.e. pupation) rate was significantly higher for diet 3 (i.e. animal protein and brewer's spent grain) than the other two diets. Diet 3 demonstrated the three highest pupation rates (27.7% on 3 January 2018, 21.2% on 8 January 2018, and 21.0% on 15 January 2018; Figure 5).

Over the study period, new larvae hatched from eggs produced by *T. molitor* beetles. Hatching numbers were low for diet 3, resulting in a lower overall population number. In total, a larger amount of new individuals were added to diet 1 (i.e. control - oatmeal; 2292 larvae) than the waste food (1545 larvae) or animal protein and brewer's spent grain (1295 larvae) diets (Figure 6).

Discussion

Low mortality amongst all three diets suggests that all diets provided *T. molitor* with sufficient nutrients for survival. This supports *T. molitor*'s ability to survive on a multitude of diets and that they can be grown on sustainably-sourced waste food diets.

There was a period of high mortality for all three diets over winter break (i.e. from 29 November 2017 to 3 January 2018) when access to the lab was limited due to holiday closures.

Diet 3 had the lowest mortality and highest pupation rates but had low recruitment numbers. We recommend further study into beetle fecundity, hatching rates, and offspring survival as it may explain why this may have happened. In the event of this study's replication, we recommend maintaining a closed population within each bin (i.e. introducing the hatchings and new worms into separate bins to eliminate any effects this may have had).

It is possible that a combination of diets or a sequential feeding of the diets may perform better than the diets on their own. We recommend researching each component of the diets, in addition to studying when each diet may perform best (e.g. at each life stage).

Diet preparation made it difficult to assess feed consumption. We found that the pellets absorbed a significant amount of water weight and thus, gained weight between data collection intervals. Additionally, scales that are accurate to 0.01 g are essential as each worm weighs only 0.09 g (± SD 0.01) on average. Future studies on feed consumption and conversion would provide further insight into the performance of each diet.

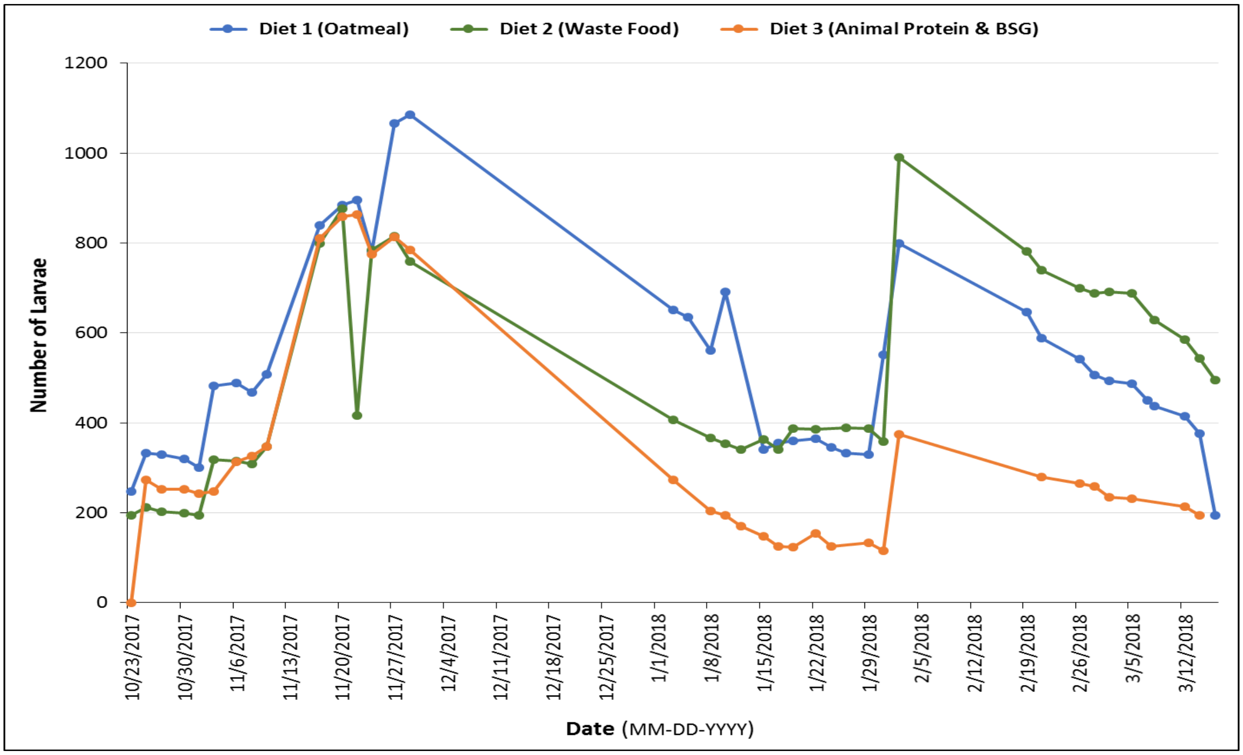

Figure 3: Number of larvae (yellow mealworm beetle; *Tenebrio molitor*) fed 3 diets (i.e. diet 1 - oatmeal, diet 2 - waste food/kitchen scraps, and diet 3 - animal protein and brewer's spent grain) from 23 October 2017 to 16 March 2018 at Langara College, Vancouver, BC.

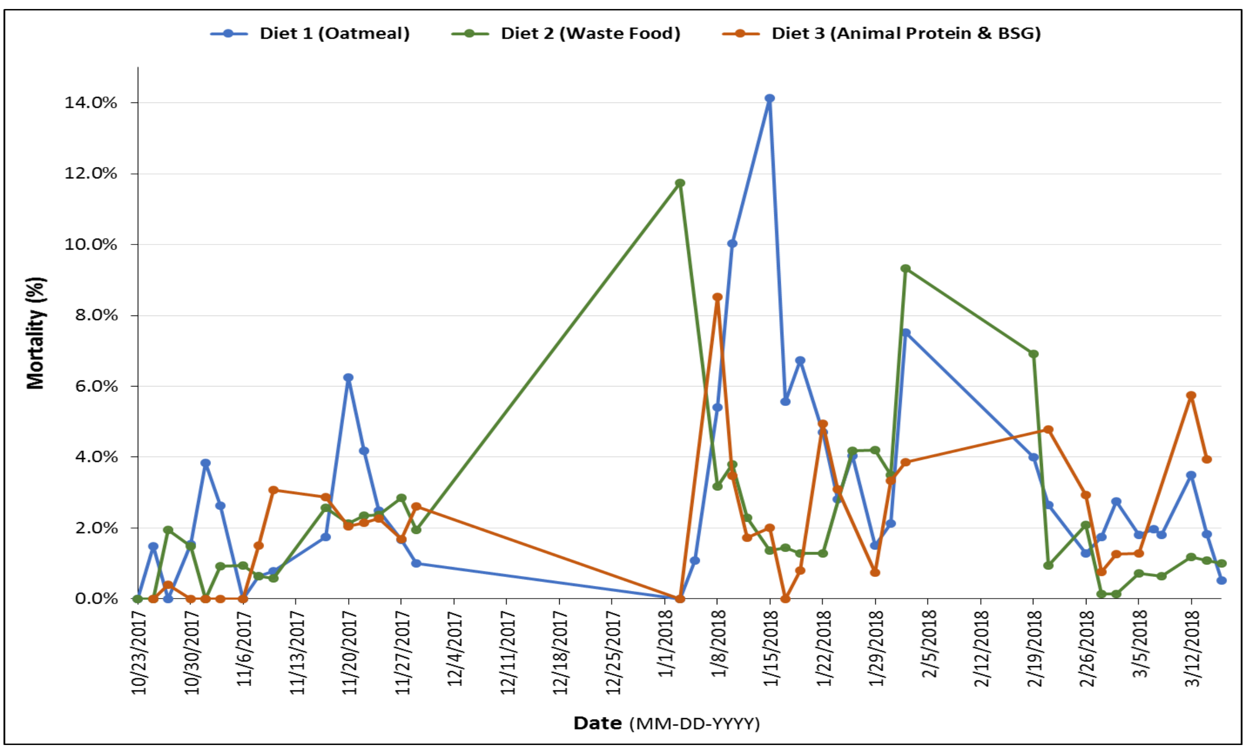

Figure 4: Larval mortality (%) for yellow mealworm beetle (*Tenebrio molitor*) fed 3 diets (i.e. diet 1 - oatmeal, diet 2 - waste food/kitchen scraps, and diet 3 - animal protein and brewer's spent grain) from 23 October 2017 to 16 March 2018 at Langara College, Vancouver, BC.

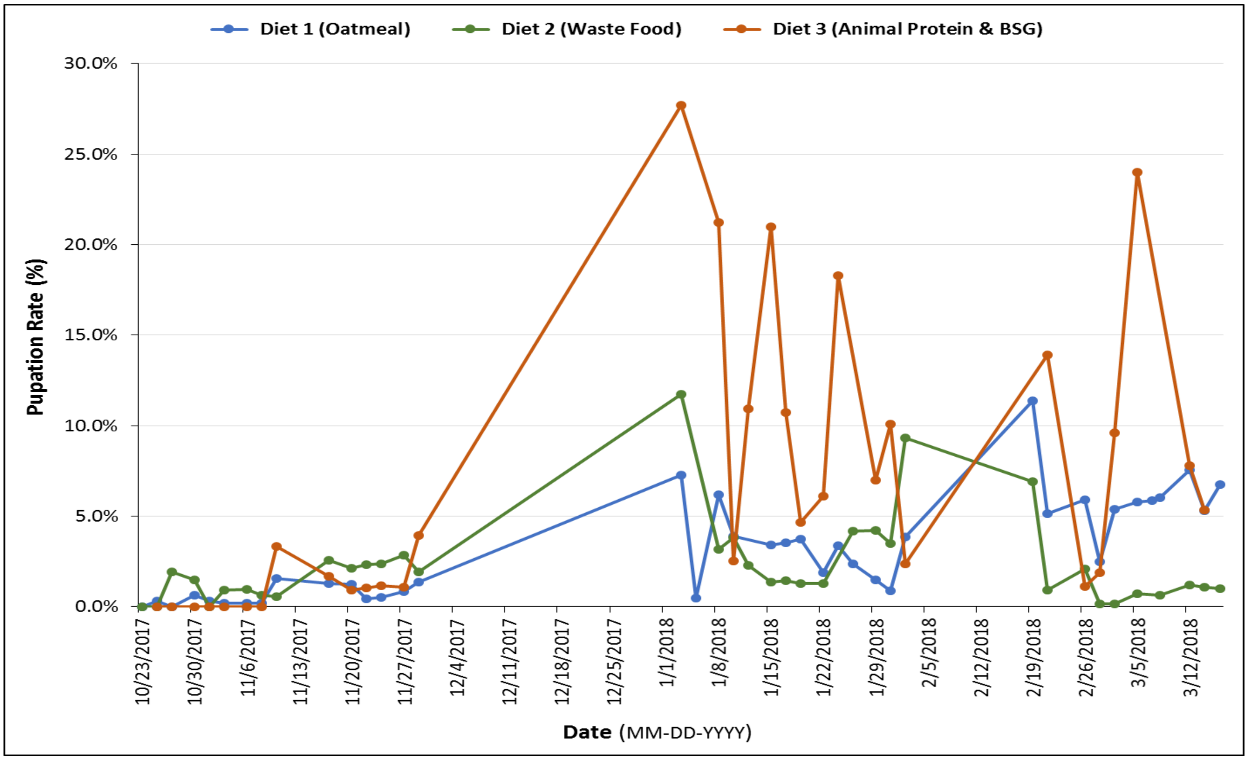

Figure 5: Pupation rate (%) for yellow mealworm beetle (*Tenebrio molitor*) fed 3 diets (i.e. diet 1 - oatmeal, diet 2 - waste food/kitchen scraps, and diet 3 - animal protein and brewer's spent grain) from 23 October 2017 to 16 March 2018 at Langara College, Vancouver, BC.

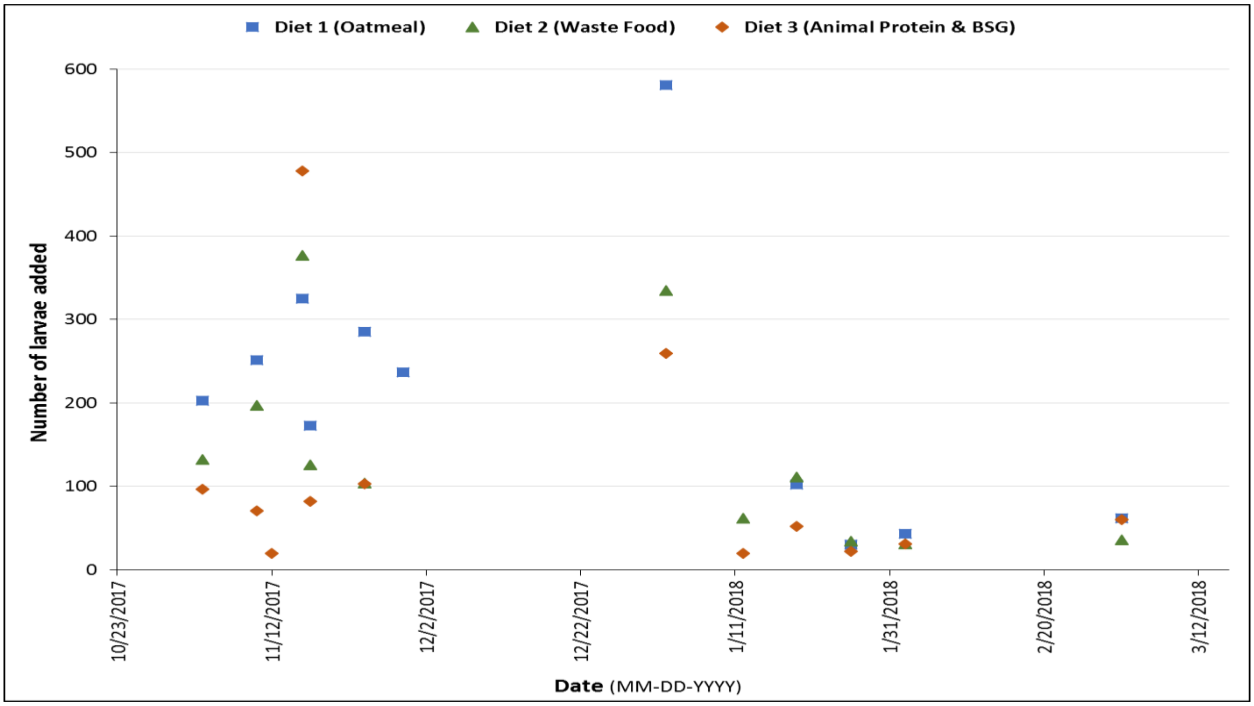

Figure 6: New larvae added from reproduction (i.e. hatchings) or other events from 23 October 2017 to 16 March 2018 for yellow mealworm beetle (*Tenebrio molitor*) larvae fed 3 diets (i.e. diet 1 - oatmeal, diet 2 - waste food/kitchen scraps, and diet 3 - animal protein and brewer's spent grain) at Langara College, Vancouver, BC.

References Cited

Colombo, B., P. West, P. Smith, F.N. Tubiello, J. Gerber, P. Engstrom, A. Urevig and E. Wollenberg. 2017. How does agriculture change our climate? Environment Reports: Food Matters. Retrieved 18 September 2017 from http://www.environmentreports.com/how-does-agriculture-change/#section2

[EPA] United States Environmental Protection Agency. 2017. Overview of greenhouse gases. Retrieved 21 March 2018. https://www.epa.gov/ghgemissions/overview-greenhouse-gases

Foster, C., K. Green, M. Bleda, P. Dewick, B. Evans, A. Flynn, and J. Mylan. 2006. Environmental impacts of food production and consumption: A report to the Department for Environment, Food and Rural Affairs. Manchester Business School, London pp. 199.

Halloran, A., H. Klunder, E. Mertens, G. Muir, A. Van Huis, J. Van Itterbeeck, and P. Vantomme. 2013. Edible insects: Future prospects for food and feed security. Retrieved 19 September 2017 from http://www.fao.org/docrep/018/i3253e/i3253e06.pdf

Kaldy, M.S. 1972. Protein yield of various crops as related to protein value. *Economic Botany* 26(2): 142-144. DOI: 10.1007/bf02860775

Metro Vancouver. 2017. Why food waste matters. Retrieved 19 September 2017 from http://www.lovefoodhatewaste.ca/why-it-matters/Pages/default.aspx

Oonincx, D.G., J. Van Itterbeeck, M.J. Heetkamp, H.Van Brand, J.J. Loon, and A.Van Huis. 2010. An exploration on greenhouse gas and ammonia production by insect species suitable for animal or human consumption. *PLoS ONE* 5(12). DOI: 10.1371/journal.pone.0014445

Oonincx, D.G. and I.J.M. de Boer. 2012. Environmental impact of the production of mealworms as a protein source for humans: A life cycle assessment. *PLoS One* 7(12): e51145. Retrieved 19 September 2017 from http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0051145

Oonincx, D. G., S. Van Broekhoven, A. Van Huis, and J.J. Loon. 2015. Feed conversion, survival and development, and composition of four insect species on diets composed of food by-products. *PloS One* 10(12). DOI: 10.1371/journal.pone.0144601

Pimentel, D., B. Berger, D. Filiberto, M. Newton, B. Wolfe, E. Karabinakis, S. Clark, E. Poon, E. Abbett, and S. Nandagopal. 2004. Water resources: Agricultural and environmental issues. *BioScience* 54(10): 909-918.

Ramos-Elorduy, J., E.A. González, A.R. Hernández, and J.M. Pino. 2002. Use of *Tenebrio molitor* (Coleoptera: Tenebrionidae) to recycle organic wastes and as feed for broiler chickens. *Journal of Economic Entomology* 95(1): 214-220.

[UN DESA] United Nations Department of Economic and Social Affairs. World population projected to reach 9.8 billion in 2050, and 11.2 billion in 2100. 2017, June 21. https://www.un.org/development/desa/en/news/population/world-population-prospects-2017.html

[UN] United Nations Food and Agriculture Organization. 2014. Environmentally friendly: What does it take to produce 1kg of protein? Retrieved 20 March 2018 from http://www.dw.com/en/locusts-are-the-tastiest-insects/a-17641920.

The World Bank Group. 2014. Agricultural land (% of land area). Retrieved 18 September 2017 from https://data.worldbank.org/indicator/AG.LND.AGRI.ZS?end=2014&start=2014&view=bar