

Copyright is owned by the Author of the thesis. Permission is given for a copy to be downloaded by an individual for the purpose of research and private study only. The thesis may not be reproduced elsewhere without the permission of the Author.

Optimization of the rearing environment for
the black soldier fly, *Hermetia illucens* L.
(Diptera: Stratiomyidae), a promising agent
for organic waste bioconversion



A thesis presented in partial fulfilment of the requirements for the degree of

Doctor of Philosophy

in

Zoology

at Massey University, Manawatū,

New Zealand

Zhongyi Liu

2022

Acknowledgements

I would like to express my appreciation to my supervisors Dr Maria Minor, Dr Adriana Najjar-Rodriguez, and Prof Patrick Morel for their support and encouragement throughout my doctoral journey. I remember every time you helped me step out of a depressing situation and guided me back on to the right track. I have learned to be patient, self-motivated, courageous, and most importantly, humble. Your professional advice has helped me pave the way for becoming a responsible and dedicated researcher.

I would like to thank the founders of Prescient Nutrition, Bruce Miller and Dennis Hucker, and my friends and colleagues Dr Kambiz Esfandi and Mari Nakano for their assistance in my research. Bruce and Dennis, thank you for kindly and generously providing insects for my experiments. Talking with you, I learned that rearing black soldier fly at large scales is challenging, which in turn has motivated me to devote efforts to research and development. Kambiz, we had become friends even before I came to New Zealand. Thank you for always being there when I needed you and showing me how excellent a young researcher could be. Mari, thank you for helping me with my experiments, sharing your knowledge, insights, and ideas, and just listening.

My gratitude goes to Tracy Harris, Shaun Nielsen, Paul Barrett, Steven Burgess, and Martin Hunt, who provided technical support for my research, and taught me techniques that eased experimental handlings. They also encouraged me to think about multiple technical routes that could be applied to test the same hypothesis; this mentality will benefit my future career. Many thanks to Neil Birrell and Dr Jessica Devitt, who helped me improve several sections of my thesis.

My sincere appreciation to Kai Qi, Dr Boyang Jia, and Jian Wang for their recognition of my achievements and potential. Your generous offers and encouragement have helped

Acknowledgements

me build my confidence, find my career path, and strengthen my dedication to research and development in insect farming and organic waste management.

Finally, I would like to express my appreciation to my dear friends and my family. Lulu, Hossein, Kris, Dennis, Christin, Song, Tina, and Jacky, thank you for lighting up my life and becoming my family in New Zealand. Mum and dad, thank you for supporting me during my PhD. Your son will soon be able to look after you. Fang, thank you for loving, accepting, and marrying me, and giving birth to our lovely son. Eason, my beautiful boy, thank you for coming into my life and loving me. You taught me how to be a patient and responsible father, how to deliver my knowledge in simple languages, and how to always be curious about the world and be keen to look for answers.

This research was partially funded by the Bioresource Processing Alliance in New Zealand.

Abstract

Bioconversion is an environmentally, socially and economically sound measure for organic waste management. The black soldier fly (BSF), *Hermetia illucens* L. (Diptera Stratiomyidae), is one of the promising species for organic waste bioconversion. Larvae of BSF (BSFL) can feed on multiple types of organic waste, and the harvested larvae could be processed into valuable products such as animal feed, chitin, and organic fertilizer. To maximize the efficiency of a bioconversion system using BSFL, it is necessary to optimize the rearing environment of the insects, which requires knowledge of the impact of environmental factors on BSF at different life stages. This thesis reports my investigations on (1) the effect of diet on selected BSF larval (BSFL) life history and physiological traits and bioconversion efficiency, (2) the effect of substrate type and moisture content on BSF pupation, and (3) the effect of artificial light type, light regime, and adult density on BSF reproduction.

First of all, I tested three types of organic waste (brewer's waste, pig manure, and semidigested grass) against a standard diet (broll; a mixture of wheat bran and wheat flour). Among the organic wastes tested, brewer's waste led to the fastest larval growth and highest larval weight gain. However, protein conversion efficiency of BSFL fed on brewer's waste was lower than the larvae fed on the standard diet, which may be due to the high protein content in brewer's waste. A meta-analysis revealed that dietary lignocellulosic content has an adverse impact on BSFL weight gain.

Second, I investigated the effects of two pupation substrates (vermiculite and wood chips) and nine moisture levels on BSF pupation rate and depth. Using moist pupation substrate could reduce prepupal water loss, improve prepupal survival rate, and move forward the onset of pupation. BSF prepupae were found to stay at shallower depth levels when

moisture content exceeded 20% and 70% for vermiculite and wood chips, respectively, which may be due to reduced oxygen availability.

Third, I assessed four types of artificial light for their suitability of supporting mating of BSF from two different colonies. The artificial light that matches the spectral sensitivity of photoreceptors of BSF adults led to the highest mating success. Interestingly, artificial light resembling summer sunlight failed to support BSF mating, which may be due to its flickering. Colony effect was also significant on BSF mating success.

Moreover, I found an interaction between the light regime and adult density on mean individual female reproductive output and adult survival rate. To optimize the reproductive output of a BSF colony, 8 h of photoperiod would be sufficient for a low adult density (e.g., lower than $800 \text{ individuals}\cdot\text{m}^{-3}$), whereas longer photoperiods may be needed for higher adult densities.

Finally, I present the implications that can be drawn from my findings, and my recommendations for future research and the relevant industry.

Table of Contents

Acknowledgements	i
Abstract	iii
Table of Contents	v
List of Tables.....	ix
List of Figures	xi
List of Supplementary Tables	xv
List of Supplementary Figures	xvii
List of Abbreviations.....	xviii
Chapter 1 General introduction.....	1
1.1 Organic waste bioconversion by insects	2
1.2 Study species – the black soldier fly	6
1.2.1 Geographic distribution and general biology	6
1.2.2 Uses of the black soldier fly in organic waste bioconversion.....	10
1.3 Thesis aims and layout	15
1.4 References	17
Chapter 2 Bioconversion of three organic wastes by black soldier fly (Diptera: Stratiomyidae) larvae	35
Abstract	36
2.1 Introduction	37
2.2 Materials and methods.....	39
2.2.1 Black soldier fly colony	39
2.2.2 Organic wastes used for the larval diet experiment	40
2.2.3 Experimental design.....	41
2.2.4 Chemical analyses.....	42
2.2.5 Calculations and statistics	43

Table of contents

2.2.6 Impact of lignin on larval growth: a meta-analysis..... 45

2.3 Results 46

2.3.1 Chemical composition of the selected organic wastes..... 46

2.3.2 Larval development and survival 47

2.3.3 BSFL chemical composition..... 52

2.3.4 Bioconversion and Waste Reduction 55

2.4 Discussion 57

2.5 Acknowledgments 62

2.6 References 62

2.7 Appendix 67

Chapter 3 Substrate and moisture content effects on pupation of the black soldier fly
..... 73

Abstract 74

3.1 Introduction 75

3.2 Materials and methods..... 76

3.2.1 Pupation substrate 76

3.2.2 Source of insects 78

3.2.3 Experimental design..... 79

3.2.4 Statistical analysis 82

3.3 Results 84

3.3.1 Prepupal mortality rate..... 84

3.3.2 Pupation rate 85

3.3.3 Fresh and dry weight loss..... 87

3.3.4 Pupation depth..... 88

3.4 Discussion 89

3.5 Acknowledgments 94

3.6	References	94
3.7	Appendix	101
Chapter 4	Mating success of the black soldier fly, <i>Hermetia illucens</i> (Diptera: Stratiomyidae), under four artificial light sources	107
	Abstract	108
4.1	Introduction	109
4.2	Materials and methods.....	111
4.2.1	Source of insects	111
4.2.2	Artificial light sources.....	112
4.2.3	Experimental design.....	112
4.2.4	Calculations and statistics	116
4.3	Results	117
4.3.1	Selected optical properties of the tested artificial light sources.....	117
4.3.2	Effects of artificial light on BSF mating.....	120
4.4	Discussion	123
4.5	Acknowledgments	129
4.6	References	129
4.7	Appendix	133
Chapter 5	Reproduction of the black soldier fly (Diptera: Stratiomyidae) under different adult densities and light regimes	137
	Abstract	138
5.1	Introduction	139
5.2	Materials and methods.....	141
5.2.1	Experimental design.....	141
5.2.2	Calculations and statistics	143
5.3	Results	146
5.3.1	Temporal traits in BSF oviposition.....	146

Table of contents

5.3.2	Reproductive output.....	147
5.3.3	Mating success	152
5.3.4	Egg fertility, hatching rate, and insect survival rate	154
5.4	Discussion	156
5.5	Acknowledgments	162
5.6	References	162
5.7	Appendix	168
Chapter 6	General discussion	171
6.1	Introduction	172
6.2	Optimization of BSFL diet.....	173
6.2.1	Chemical composition.....	174
6.2.2	Lignocellulose in BSFL diet	176
6.2.3	Dietary moisture content	178
6.3	Optimization of BSF pupation substrate	180
6.4	Optimization of visual environment for BSF mating.....	182
6.5	Evaluating the effects of environmental factors on the entire BSF life history	185
6.6	Maintaining a genetically healthy BSF colony	186
6.7	Summary of recommendations.....	187
6.7.1	Recommendations for practical applications	187
6.7.2	Recommendations for future research	189
6.8	Conclusion.....	190
6.9	References	190
Appendices	197

List of Tables

Table 1.1 Insect species that could be used as bioconversion agents for various types of organic waste.....	5
Table 1.2 Maximum suggested inclusion ratio (%) of full fat or partially defatted black soldier fly (<i>Hermetia illucens</i>) larval meal (BSFLM) in feeds for different domestic animal species.	13
Table 2.1 Chemical composition of a standard diet (broll), brewer’s waste, pig manure, and semidigested grass used in this study as feeding substrates for black soldier fly (<i>Hermetia illucens</i> L.) larvae.	48
Table 2.2 Survival, developmental time, larval weight gain, average weight gaining rate, and prepupal weight for black soldier fly (<i>Hermetia illucens</i> L.) larvae fed a standard diet (broll), brewer’s waste, pig manure, and semidigested grass.....	49
Table 2.3 Summary of the general linear model conducted as part of the meta-analysis carried out in this study to test the impact of lignin on black soldier fly (<i>Hermetia illucens</i> L.) larval growth rate.	50
Table 2.4 Meta-analysis testing the impact of diet lignin content and experimental setup on black soldier fly (<i>Hermetia illucens</i> L.) larval growth rate.....	51
Table 2.5 Chemical composition of black soldier fly (<i>Hermetia illucens</i> L.) larvae at the start of the experiment (baseline larvae) and of resulting prepupae after feeding the larvae on a standard diet (broll), brewer’s waste, pig manure, or semidigested grass....	54
Table 2.6 Waste reduction and bioconversion by black soldier fly (<i>Hermetia illucens</i> L.) larvae when fed a standard diet (broll), brewer’s waste or pig manure.	56
Table 3.1 Selected physical characteristics of the pupation substrates tested in the present study. Vermiculite was soaked with water and then oven-dried, while wood chips were oven-dried without soaking. Both substrates were allowed to naturally restore moisture content at $20 \pm 1^\circ\text{C}$ and $50 \pm 5\%$ relative humidity for 2 d.	78
Table 3.2 Different moisture levels and the corresponding amount of water added to each type of the pupation substrate tested in the present study.....	80

Table 3.3 Type I Wald χ^2 tests for the effects of substrate type, moisture content, and their interaction on prepupal mortality rate of the black soldier fly *Hermetia illucens*. 85

Table 3.4 Type I Wald χ^2 tests for the effects of substrate type, moisture content, and their interaction on pupation rate of black soldier fly *Hermetia illucens* prepupae. 86

Table 3.5 Effect of wood chips substrate moisture content on black soldier fly *Hermetia illucens* fresh and dry weight loss after pupation. 87

Table 4.1 The absolute irradiance ($\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) across different wavebands (from 300 to 885 nm) of four artificial light sources: a 500-W halogen lamp (HALO), a combination of a 50-W white LED lamp and a 50-W compact fluorescent black light (LEDUV), a 400-W metal halide lamp (MH), a 20-W LED lamp designed for black soldier fly, *Hermetia illucens*, breeding (BSFLED). Sunlight is included as a reference. The proportional irradiance (%) of each light is shown in parentheses. 118

Table 4.2 Accumulated deviance analyses conducted based on generalized linear models to test the effects of insect colony, artificial light source, and time on the number of egg clutches, the percentage of fertile egg clutches, and the percentage of inseminated females. 121

Table 5.1 The 3×3 -factorial block design used to test the effects of adult density and light regime on the selected life history traits of black soldier fly (*Hermetia illucens* L.) adults. 144

Table 5.2 Selected life history traits of black soldier fly (*Hermetia illucens* L.) adults analyzed with general linear models to test the effects of adult density and light regime. 145

Table 5.3 Reproductive output of all the female black soldier flies (*Hermetia illucens* L.) from an experimental cage in response to different adult densities. 151

List of Figures

- Figure 1.1** Greenhouse gasses and leachate generated from landfill of organic waste. GWP₁₀₀ (100-year global warming potential) describes the heat that is absorbed by the greenhouse gases over 100 years, in comparison with the heat that would be absorbed by the same weight of CO₂ over the same period. Image by Z. Liu, 2022; CC BY-NC. .2
- Figure 1.2** Morphology of the black soldier fly *Hermetia illucens*: (A) an egg clutch, (B) eggs (arrow indicates an ocellus), (C) a 4-day-old larva, (D) a prepupa, (E) a pupa, (F) a male (arrow indicates the genitalia), (G) a female (arrow indicates the genitalia). Images by Z. Liu, 2016; CC BY-NC. 8
- Figure 1.3** Some of the businesses established to convert local organic wastes into black soldier fly (*Hermetia illucens*) larval biomass. Numbers next to the business logos indicate the year when the businesses were founded. Image by Z. Liu, 2022; CC BY.. 12
- Figure 1.4** An illustration of the research conducted in this thesis to optimize the rearing environment for the black soldier fly (BSF; *Hermetia illucens*) at different life stages. Image by Z. Liu, 2022; CC BY. 15
- Figure 2.1** Scatter plot of the actual black soldier fly (BSF) (*Hermetia illucens* L.) larval growth rates observed in this study (our data) and those by Rehman *et al.* (2017a, b) versus the predictions based on our meta-analysis of the impact of lignin content on BSF larval growth. 51
- Figure 2.2** Growth rates of black soldier fly (BSF) (*Hermetia illucens* L.) larvae as related to the lignin content in the diets used in this study (our data) and those used by Rehman *et al.* (2017a, b). Fitted lines are those estimated by linear regression. 52
- Figure 3.1** Mortality rate of black soldier fly *Hermetia illucens* prepupae with and without pupation substrates and at different substrate moisture content. Moisture content is calculated as (weight loss during drying/oven-dry weight) × 100%. Error bars indicate standard errors of means (n = 6). The means and standard errors were back transformed from the log scale outputs. Non-significant differences between no substrate and substrate treatments are indicated as ‘n.s.’. Solid and dashed lines are the binomial logit regression curves for vermiculite and wood chips, respectively — vermiculite: $\text{Logit}(y) = -3.0563 - 0.0140x$; wood chips: $\text{Logit}(y) = -2.5994 - 0.0140x$ 84

Figure 3.2 Pupation rate of black soldier fly *Hermetia illucens* prepupae with and without pupation substrates and at different substrate moisture content. Moisture content is calculated as (weight loss during drying/oven-dry weight) × 100%. Error bars indicate standard errors of means (n = 6). The means and standard errors were back transformed from the log scale outputs. Non-significant differences between substrate and no substrate treatments are indicated as ‘n.s.’. Solid and dashed lines are the binomial logit regression curves for vermiculite and wood chips, respectively — vermiculite: $\text{Logit}(y) = 1.5427 + 0.0028x$; wood chips: $\text{Logit}(y) = 1.3342 + 0.0028x$ 86

Figure 3.3 Fresh and dry weight loss of black soldier fly *Hermetia illucens* after pupation in no substrate and in wood chips at different moisture content. Moisture content is calculated as (weight loss during drying/oven-dry weight) × 100%. Error bars indicate standard errors of means (n = 6). The value next to a fresh weight data point shows the difference between the mean of a wood chips treatment and the no substrate treatment; an asterisk indicates significant difference (two-sided Dunnett’s test; $P < 0.05$). 88

Figure 3.4 Proportion of black soldier fly *Hermetia illucens* pupae presented deeper than 2 cm in different pupation substrates and at different moisture levels. Moisture content is calculated as (weight loss during drying/oven-dry weight) × 100%. Error bars indicate standard errors of means (n = 6). Solid and dashed lines are nonlinear regression curves for vermiculite and wood chips, respectively. 89

Figure 4.1 (A) Reproductive organs of a black soldier fly, *Hermetia illucens*, female — arrows indicate spermathecae. (B) A spermatheca of a virgin female. A spermatheca of a newly inseminated female is shown in (C), which is ruptured in (D) — arrow indicates sperm. 115

Figure 4.2 The absolute spectral irradiance of four artificial light sources tested for their effects on black soldier fly, *Hermetia illucens*, mating success. (A) a 500-W halogen lamp (HALO), (B) a combination of a 50-W white LED lamp and a 50-W compact fluorescent black light (LEDUV), (C) a 400-W metal halide lamp (MH), (D) a 20-W LED lamp designed for black soldier fly, *H. illucens*, breeding (BSFLED). The measurement for the artificial light sources was conducted at the center of the cage containing *H. illucens* adult flies. The grey area indicates wavelengths (> 650 nm) that

are invisible to the *H. illucens* adult flies (Oonincx *et al.*, 2016). The red dotted curves represent sunlight radiation. 119

Figure 4.3 Number of black soldier fly, *Hermetia illucens*, egg clutches collected, percentage of fertile egg clutches, and percentage of inseminated females from the BROL colony (A–C) and the PRST colony (D–F) under different artificial lights: a 500-W halogen lamp (HALO), a combination of a 50-W white LED lamp and a 50-W compact fluorescent black light (LEDUV), a 400-W metal halide lamp (MH), and a 20-W LED lamp designed for *H. illucens* breeding (BSFLED). Data points represent means, error bars denote the standard error of the means. The number of eggs did not significantly differ across light treatments (A and D). Pairwise comparisons were not conducted for the BROL colony regarding the percentage of fertile egg clutches (B) or inseminated females (C). Different letters above the error bars in E and F indicate significant differences across light treatments ($P < 0.05$). 122

Figure 4.4 Scatter plots of (A) the number of inseminated black soldier fly, *Hermetia illucens* females against the number of fertile egg clutches, and (B) the percentage of inseminated *H. illucens* females against the percentage of fertile egg clutches. Data from both colonies were used. 123

Figure 5.1 Daily oviposition pattern of black soldier fly (*Hermetia illucens* L.) housed in $30 \times 30 \times 30$ cm mesh cages and under different combinations of adult densities and light regimes. Grey and white parts of the bars represent the number of fertile and infertile eggs, respectively. The squares on solid lines indicate the cumulative number of fertile eggs, and the triangles on dash lines indicate the cumulative number of all eggs. 370, 926, and 1,852 pairs·m⁻³ indicate 10, 25, and 50 pairs of flies per $30 \times 30 \times 30$ cm cage, respectively. 148

Figure 5.2 The timing of the first oviposition peak of female black soldierfly (*Hermetia illucens* L.) in response to different combinations of adult densities and light regimes. The tested densities were 370, 926, and 1,852 pairs·m⁻³, indicating 10, 25, and 50 pairs of flies per $30 \times 30 \times 30$ cm cage, respectively. The values shown are means \pm SE. 149

Figure 5.3 Black soldier fly (*Hermetia illucens* L.) female reproductive output from an experimental cage used in response to different combinations of adult densities and light regimes. (A) Total number of eggs, (B) total number of fertile eggs, and (C) total number

of neonates. The tested densities were 370, 926, and 1,852 pairs·m⁻³, indicating 10, 25, and 50 pairs of flies per 30 × 30 × 30 cm cage, respectively. The values shown are means ± SE..... 150

Figure 5.4 Black soldier fly (*Hermetia illucens* L.) mean individual female reproductive output in response to different combinations of adult densities and light regimes. (A) Average number of eggs laid per female, (B) Average number of fertile eggs laid per inseminated female. The tested densities were 370, 926, and 1,852 pairs·m⁻³, indicating 10, 25, and 50 pairs of flies per 30 × 30 × 30 cm cage, respectively. The values shown are means ± SE..... 153

Figure 5.5 Black soldier fly (*Hermetia illucens* L.) 10-d survival rate (in percentage) for adult (A) males and (B) females in response to different combinations of adult densities and light regimes. The tested densities were 370, 926, and 1,852 pairs·m⁻³, indicating 10, 25, and 50 pairs of flies per 30 × 30 × 30 cm cage, respectively. The values shown are means ± SE..... 155

List of Supplementary Tables

Table S 2.1 Summary of the general linear model conducted as part of the meta-analysis carried out in this study to test the impact of neutral detergent fiber (NDF) on black soldier fly (<i>Hermetia illucens</i> L.) larval growth rate. Data from this study and Rehman <i>et al.</i> (2017a, b) was used to build the general linear model, with experiment being a fixed factor and NDF content as a covariate.	67
Table S 2.2 Meta-analysis testing the impact of diet neutral detergent fiber (NDF) content and experimental setup on black soldier fly (<i>Hermetia illucens</i> L.) larval growth rate. Data from this study and Rehman <i>et al.</i> (2017a, b) was used to build the general linear model, with experimental setup as a fixed factor and NDF content as a covariate.	67
Table S 2.3 Summary of the general linear model conducted as part of the meta-analysis carried out in this study to test the impact of acid detergent fiber (ADF) on black soldier fly (<i>Hermetia illucens</i> L.) larval growth rate. Data from this study and Rehman <i>et al.</i> (2017a, b) was used to build the general linear model, with experiment being a fixed factor and ADF content as a covariate.	68
Table S 2.4 Meta-analysis testing the impact of diet acid detergent fiber (ADF) content and experimental setup on black soldier fly (<i>Hermetia illucens</i> L.) larval growth rate. Data from this study and Rehman <i>et al.</i> (2017a, b) was used to build the general linear model, with experimental setup as a fixed factor and ADF content as a covariate.	68
Table S 2.5 Summary of the general linear model conducted as part of the meta-analysis carried out in this study to test the impact of hemicellulose (calculated as neutral detergent fiber - acid detergent fiber) on black soldier fly (<i>Hermetia illucens</i> L.) larval growth rate. Data from this study and Rehman <i>et al.</i> (2017a, b) was used to build the general linear model, with experiment being a fixed factor and hemicellulose content as a covariate.	69
Table S 2.6 Meta-analysis testing the impact of diet hemicellulose (calculated as neutral detergent fiber - acid detergent fiber) content and experimental setup on black soldier fly (<i>Hermetia illucens</i> L.) larval growth rate. Data from this study and Rehman <i>et al.</i> (2017a, b) was used to build the general linear model, with experimental setup as a fixed factor and hemicellulose content as a covariate.	69

Table S 2.7 Summary of the general linear model conducted as part of the meta-analysis carried out in this study to test the impact of cellulose (calculated as acid detergent fiber - lignin) on black soldier fly (*Hermetia illucens* L.) larval growth rate. Data from this study and Rehman *et al.* (2017a, b) was used to build the general linear model, with experiment being a fixed factor and cellulose content as a covariate..... 70

Table S 2.8 Meta-analysis testing the impact of diet cellulose (calculated as acid detergent fiber - lignin) content and experimental setup on black soldier fly (*Hermetia illucens* L.) larval growth rate. Data from this study and Rehman *et al.* (2017a, b) was used to build the general linear model, with experimental setup as a fixed factor and cellulose content as a covariate..... 70

Table S 3.1 Percentage of black soldier fly *Hermetia illucens* pupae presented at different depth ranges in vermiculite at different moisture content. Standard errors are shown in parentheses. 102

Table S 3.2 Percentage of black soldier fly *Hermetia illucens* pupae presented at different depth ranges in wood chips at different moisture content. Standard errors are shown in parentheses. 103

Table S 3.3 Nonlinear models fitted to describe the effect of moisture content on the proportion of black soldier fly *Hermetia illucens* pupated deeper than 2 cm in different substrates..... 104

Table S 5.1 Coefficient table of the general linear model conducted to analyze factors that could affect the temperature in the experiment environment in the present study. 168

Table S 5.2 Coefficient table of the general linear model conducted to analyze factors that could affect the relative humidity in the experiment environment in the present study. 168

Table S 5.3 Selected black soldier fly (BSF; *Hermetia illucens* L.) life history traits in response to different light regimes used in the present experiment. All BSF adults were housed in 30 × 30 × 30 cm mesh cages. A 20-watt LED BSF breeding lamp was set 1 cm above each cage to induce BSF mating..... 169

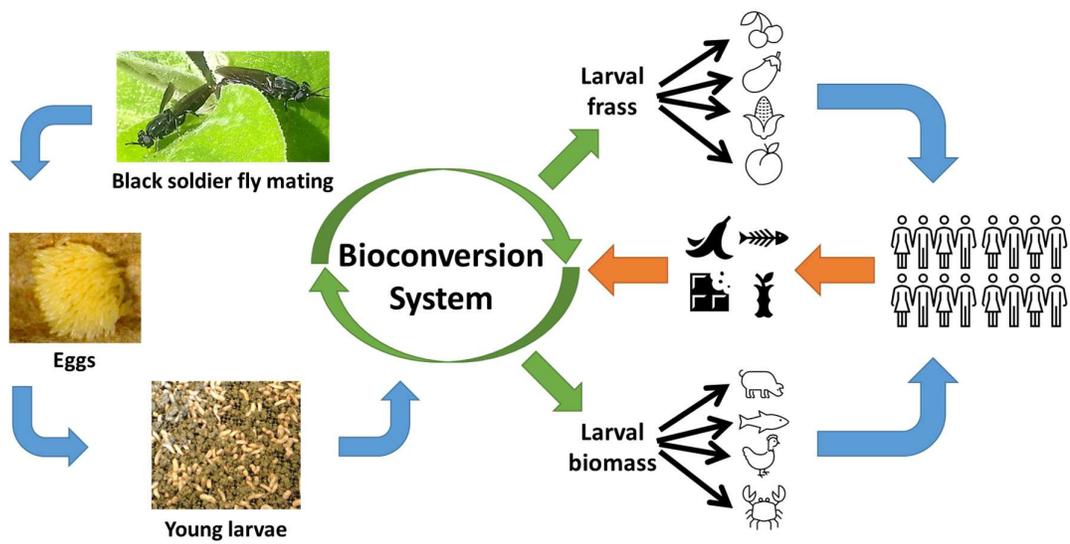
List of Supplementary Figures

Figure S 3.1 Differences in vermiculite particle shapes before (A) and after (B) a soaking-and-drying cycle.	101
Figure S 3.2 Samples of black soldier fly <i>Hermetia illucens</i> pupae collected from substrate and no substrate treatments. Moisture levels 2, 3, 4, 5, 6, 7, 8, and 9 were for 18.8%, 37.5%, 56.3%, 75.0%, 93.8%, 112.5%, 131.3%, and 150%, respectively; moisture level 1 was 4.3% and 5.9% for vermiculite and wood chips, respectively. Moisture content is calculated as (weight loss during drying/oven-dry weight) × 100%.	101
Figure S 4.1 Cage setup used in this study to test the effect of different lighting systems on the mating success of black soldier fly, <i>Hermetia illucens</i> , adults.	133

List of Abbreviations

Abbreviation	Meaning
ADF	acid detergent fiber
ANOVA	analysis of variance
AOAC	Association of Official Analytical Chemists
BROL	a black soldier fly laboratory colony, of which larvae were fed with broil
BSF	black soldier fly
BSFL	black soldier fly larvae
BSFLM	black soldier fly larval meal
BSFLED	a light-emitting diode lamp specially designed based on the visual spectral sensitivity of black soldier fly adults
CORS	conversion of organic refuse by saprophages
DC	direct current
GLM	general linear model
GLiM	generalized linear model
GWP ₁₀₀	100-year global warming potential
HALO	a 500-W halogen lamp
IL	Illinois
LED	light-emitting diode
LEDUV	a combination of a 50-W white light-emitting diode lamp and a 50-W compact fluorescent black light
LSD	least significant difference
MA	Massachusetts
MH	a 400-W metal halide lamp
MI	Michigan
NDF	neutral detergent fiber
NFC	nonfiber carbohydrates
PRST	a black soldier fly commercial colony kept by Prescient Nutrition
R.H.	relative humidity
U.S.	the United States of America
UV	ultraviolet
UVA	ultraviolet type A

Chapter 1 General introduction



A bioconversion system that helps form a circular economy (image by Z. Liu, 2022; CC BY-NC)

1.1 Organic waste bioconversion by insects

Organic waste is generated from household activities, agriculture and forestry, animal husbandry and fishery, and post-harvest food processing (National Research Council (U.S.), 1983; Polprasert & Koottatep, 2017). Globally, about 880 million tonnes of food and garden waste are generated per year, and this rate is expected to increase to about 1.5 billion tonnes per year by 2050 (Kaza *et al.*, 2018).

In many parts of the world, landfill still acts as the main route for organic waste disposal, which leads to problems such as greenhouse gas emission and leachate generation, though the latter could be collected to avoid soil and groundwater pollution (Christensen *et al.*, 2010; Figure 1.1). A comprehensive life-cycle assessment revealed that landfilling a tonne of organic waste could result in emitting greenhouse gases equivalent to nearly 400 kg of carbon dioxide (Nordahl *et al.*, 2020). Worse still, people in low-income countries often dump or burn organic waste in open areas, resulting in disease transmission and severe environmental pollution (Kaza *et al.*, 2018; Polprasert & Koottatep, 2017).

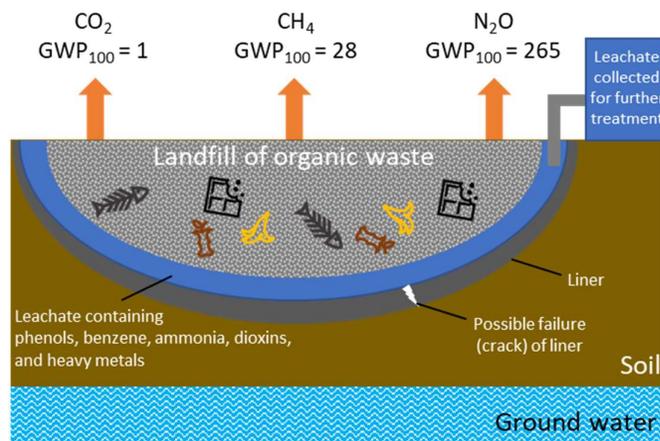


Figure 1.1 Greenhouse gasses and leachate generated from landfill of organic waste. GWP₁₀₀ (100-year global warming potential) describes the heat that is absorbed by the greenhouse gases over 100 years, in comparison with the heat that would be absorbed by the same weight of CO₂ over the same period. Image by Z. Liu, 2022; CC BY-NC.

Proper organic waste management approaches, such as pyrolysis, incineration, and bioconversion, not only reduce the quantity of organic waste, but also valorize the waste by recovering energy and/or nutrients from underutilized organic matter (Polprasert & Koottatep, 2017). Pyrolysis and incineration facilities require considerable upfront and running costs, therefore these methods may not be economically viable in all places. In contrast, bioconversion systems could be established and operated in simple ways and thus would be more feasible for low- and middle-income countries or regions. For instance, subject to local legislation, defatted bran and some broken rice could be consumed by pigs, chickens, and ducks, and the manure of these animals could be fed to fish ponds where fish is produced and the effluent is used to grow plants (Polprasert & Koottatep, 2017). The process forms a simple and circular bioconversion system that can be easily established and operated in a low- and middle-income rural area.

In addition to their vast adoptability, bioconversion systems could return wasted nutrients (e.g., proteins, lipids, and polysaccharides) to the food supply chain in the form of food or feed (Polprasert & Koottatep, 2017; Surendra *et al.*, 2020). Globally, food waste from post-harvest to consumption accounts for about 30% of the amount produced (Food and Agriculture Organization of the United Nations, 2019; United Nations Environment Programme, 2021). The wasted food represents an unnecessary loss of resources (i.e., land, water, energy, fertilizer, etc.) put into and an excessive amount of greenhouse gases emitted from the food supply chain (Shafiee-Jood and Cai, 2016). Thus, recovery of nutrients through bioconversion could improve the overall efficiency of the food supply chain and remove the unnecessary burden on the environment.

One of the bioconversion systems is the CORS (conversion of organic refuse by saprophages) system, where saprophagous invertebrates (especially insects) and their

symbiotic microorganisms turn organic waste into invertebrate biomass and fertile residue (Diener & Zurbrügg, 2008). The invertebrate biomass produced from the CORS system could then be further processed into food or feed ingredients (Parolini *et al.*, 2020; Sogari *et al.*, 2019; Tang *et al.*, 2019), chitin- or protein-based bioplastics (Nuvoli *et al.*, 2021; Pasquier *et al.*, 2021; Sanandiya *et al.*, 2020), or fat-based products (Franco *et al.*, 2022; Xiong *et al.*, 2020).

Although many invertebrate species may be potential candidates for organic waste bioconversion, research and application have been mainly focusing on insects (Table 1.1), among which the most extensively studied species are the mealworm *Tenebrio molitor* (L.) (Coleoptera: Tenebrionidae), housefly *Musca domestica* (L.) (Diptera: Muscidae), and the black soldier fly *Hermetia illucens* (L.) (Diptera: Stratiomyidae). This thesis is focused on optimizing the rearing environment for the black soldier fly at different life stages.

Table 1.1 Insect species that could be used as bioconversion agents for various types of organic waste.

Order	Species	Waste type
Blattodea	<i>Periplaneta americana</i> (L.)	food waste ¹
Coleoptera	<i>Attagenus unicolor</i> (Brahm)	feathers ^{2,3}
	<i>Tenebrio molitor</i> (L.)	spent mushroom substrate ⁴ , vegetable pomace ^{5,6} , polylactic acid ⁷ , poultry litter ⁸
	<i>Protaetia brevitarsis</i> (Lewis)	crop straw and sawdust ⁹ , spent mushroom substrate ¹⁰
Diptera	<i>Chrysomya chloropyga</i> (Wiedemann)	abattoir waste ¹¹
	<i>Hermetia illucens</i> (L.)	animal manure ¹² , restaurant waste ¹³ , fish offal ¹⁴ , brewery by-products ¹⁵ , vegetable and fruit waste ¹⁵ , abattoir waste ¹⁶
	<i>Musca domestica</i> (L.)	animal manure ¹⁷ , food waste ¹⁸
Isoptera	<i>Coptotermes formosanus</i> (Shiraki)	paper waste ¹⁹
	<i>Reticulitermes speratus</i> (Kolbe)	paper waste ¹⁹
Orthoptera	<i>Acheta domesticus</i> (L.)	food waste ²⁰ , tofu house by-product ²¹

¹⁻²⁰ References:

¹ (Addy *et al.*, 2021), ² (Keefe & Lei, 2021), ³ (Keefe & Lei, 2022), ⁴ (Song & Kim, 2014), ⁵ (Ruschioni *et al.*, 2020), ⁶ (Rovai *et al.*, 2021), ⁷ (Peng *et al.*, 2021), ⁸ (Silva *et al.*, 2021), ⁹ (Li *et al.*, 2019), ¹⁰ (Wei *et al.*, 2020), ¹¹ (Parry *et al.*, 2020), ¹² (Oonincx *et al.*, 2015), ¹³ (Zheng *et al.*, 2012), ¹⁴ (St-Hilaire *et al.*, 2007), ¹⁵ (Meneguz *et al.*, 2018), ¹⁶ (Lalander *et al.*, 2019), ¹⁷ (Wang *et al.*, 2013), ¹⁸ (Niu *et al.*, 2017), ¹⁹ (Lenz *et al.*, 2011), ²⁰ (Lundy & Parrella, 2015), ²¹ (Quek *et al.*, 2020).

1.2 Study species – the black soldier fly

1.2.1 Geographic distribution and general biology

The black soldier fly (BSF) *H. illucens* (Diptera: Stratiomyidae) is believed to originate from Latin America (Guilliet *et al.*, 2021). Although the species may have been introduced to Europe about five centuries ago (Benelli *et al.*, 2014), it was not recorded on the European continent until 1926 (Lindner, 1919). Further range expansion of BSF was mainly due to human transportation since the 1940s, especially during World War II (Barbier, 1952; Leclercq, 1997). Today, BSF is a cosmopolitan species distributed across a vast latitudinal range (between Canada and New Zealand; Maquart *et al.*, 2020).

BSF has four life stages (Figure 1.2): egg, larva, pupa, and adult. Eggs are laid in clutches (Figure 1.2A). The eggs are elongate-oval, transparent to ivory, with a smooth surface. Fertile eggs develop ocelli (also called “eye spots” in some articles) that are visible through the chorion (Figure 1.2B) and can thus be easily distinguished from infertile eggs. Larvae pass through six instars (May, 1961). The newly hatched larvae are milky white in color, about 1.8 mm in length and 0.4 mm in width. Each larva has two black ocelli, eleven obvious segments, and bristles on each segment. The larval cuticle keeps the original color (Figure 1.2C) from the first to the fourth instar, starts to tan in the fifth instar, and is highly darkened and hardened in the sixth instar (May, 1961). A sixth instar larva (Figure 1.2D) may be up to 27 mm in length and 6 mm in width, and is also called a “prepupa”, as it stops feeding at this stage (May, 1961). BSF pupae are coarctate pupae. The color of the pupae is similar to that of the prepupae, and the puparium is rigid. The last two segments of the pupae bend ventrally (Figure 1.2E), which can be used to differentiate pupae and prepupae. Except for the difference in pupal size between females and males (female pupae are normally bigger than male pupae), there is no obvious

morphological feature that can be used to sex pupae (May, 1961). Adults (Figures 1.2F and G) are bright black in color, 13 to 20 mm in body length, and resemble wasps in appearance. There are some variable yellowish spots near the compound eyes and two flattened tubercles on the broad vertex. Each adult has two translucent cuticle patches on the first abdominal segment, which are normally called “windows” (Barry, 2004). Each leg has a conspicuous creamy white coloration from tibia to tarsus. Females are on average bigger than males (Gobbi *et al.*, 2013; Tomberlin *et al.*, 2002). The two sexes can be easily identified by the morphology of the genitalia.

In its natural environments, BSF can colonize decaying organic materials such as animal manure (Booth & Sheppard, 1984; Tomberlin & Sheppard, 2001), animal (including human) carrion (Barros *et al.*, 2019; Kotzé & Tomberlin, 2020; Martínez-Sánchez *et al.*, 2011), and decaying vegetation (Kotzé & Tomberlin, 2020). Female BSF tend to deposit eggs into dry crevices above potential larval feeding substrate that is nutritious and moist (Booth & Sheppard, 1984), where hatchlings would easily fall onto the substrate and start feeding. When reaching the prepupal stage, the insects may migrate from diet to seek suitable pupation sites (Craig Sheppard *et al.*, 1994), or bury themselves into spent diet to pupate (Barros *et al.*, 2019). In pupation substrates, BSF pupae are oriented vertically with heads facing upwards (May, 1961). To allow adult emergence, an opening on the puparium is created by an adult pushing off the head capsule and the thoracic segment, and creating a mid-dorsal crack in the second and third segments (May, 1961).



Figure 1.2 Morphology of the black soldier fly *Hermetia illucens*: (A) an egg clutch, (B) eggs (arrow indicates an ocellus), (C) a 4-day-old larva, (D) a prepupa, (E) a pupa, (F) a male (arrow indicates the genitalia), (G) a female (arrow indicates the genitalia). Images by Z. Liu, 2016; CC BY-NC.

Male BSF could be sexually mature and ready to mate as early as less than 24h post emergence (Malawey *et al.*, 2021), while the age of sexual maturity for female BSF could be less than 48h post emergence (Tomberlin & Sheppard, 2002). Feeding is not necessary for BSF adults to achieve reproduction (Sheppard *et al.*, 2002), but increases adult longevity and fecundity (Bertinetti *et al.*, 2019). BSF tend to mate earlier in the day (e.g., before 15:00) and under bright light (e.g., sunlight exceeding $200 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) (Tomberlin & Sheppard, 2002; Zhang *et al.*, 2010). Oonincx *et al.* (2016) showed that light spectral composition also influences BSF reproduction, and that the ultraviolet-B band seems to be a key component that improves BSF reproductive success. To initiate mating, a BSF male grasps a female in flight and attempts to copulate with her; if the male succeeds, the two insects would land *in copula*, otherwise they land with the male persisting in his copulation attempts. While *in copula*, the male and female may face opposite directions or form a male-mounting position (Julita *et al.*, 2020). About two days post mating, inseminated BSF females start to lay eggs (Tomberlin & Sheppard, 2002).

BSF life history traits are influenced by various environmental factors, such as temperature (Chia *et al.*, 2018; Tomberlin *et al.*, 2009), humidity (Holmes *et al.*, 2012), and larval diet (Gobbi *et al.*, 2013; Tomberlin *et al.*, 2002). When reared at 27°C, 60% relative humidity with Gainesville diet (wheat bran: alfalfa meal: corn meal = 5: 3: 2; 70% moisture), it takes about 24 d for BSF to develop from hatchlings to prepupae, and about 18 d for pupal development (Tomberlin *et al.*, 2009). In the same environment, adult longevity is about 14 days for females and 17 days for males (Tomberlin *et al.*, 2009).

1.2.2 Uses of the black soldier fly in organic waste bioconversion

BSF larvae (hereafter BSFL) are able to voraciously feed on many types of organic waste (Table 1.1). Generally, BSFL prefer wastes that are moderately moist, rich in organic matter, and with low lignocellulose contents, such as abattoir waste, animal manure, fruit and vegetable waste, food waste, or their mixtures (Barragán-Fonseca *et al.*, 2018; Dzepe *et al.*, 2020; Lalander *et al.*, 2019). Depending on waste type, a 10-d old BSFL could grow 200–300 mg of body weight in 15 d, converting 10–15% of waste dry matter into its body biomass (Lalander *et al.*, 2019). After BSFL consumption, the residue of organic waste contains un-ingested organic matter and larval excreta, and is normally called “frass” (e.g., Beesigamukama *et al.*, 2021; Tanga *et al.*, 2022). Studies have shown that BSFL frass could be used as a fertilizer or soil amendment for cultivation of various plant species, such as basil (*Ocimum basilicum* L. [Lamiales: Lamiaceae]), French bean (*Phaseolus vulgaris* L. [Fabales: Fabaceae]), kale (*Brassica oleracea* L. var. *acephala* [Brassicales: Brassicaceae]), komatsuna (*B. rapa* L. var. *perviridis* [Brassicales: Brassicaceae]), lettuce (*Lactuca sativa* L. [Asterales: Asteraceae]), maize (*Zea mays* L. [Poales: Poaceae]), pak choi (*B. rapa* L. subsp. *chinensis*), parsley (*Petroselinum crispum* (Mill.) Fuss [Apiales: Apiaceae]), ryegrass (*Lolium multiflorum* Lam. [Poales: Poaceae]), Swiss chard (*Beta vulgaris* L. [Caryophyllales: Amaranthaceae]), and tomato (*Solanum lycopersicum* L. [Solanales: Solanaceae]) (Anyega *et al.*, 2021; Beesigamukama *et al.*, 2021; Borkent & Hodge, 2021; Chirere *et al.*, 2021; Kawasaki *et al.*, 2020; Klammsteiner *et al.*, 2020; Menino *et al.*, 2021; Setti *et al.*, 2019; Tan *et al.*, 2021).

BSFL biomass harvested from bioconversion systems contains 37–63% of crude protein, 7–39% of crude fat, 1–9% of crude fiber, and 9–28% of minerals on a dry matter basis (Barragan-Fonseca *et al.*, 2017; Józefiak *et al.*, 2016). A large body of research has been

conducted to evaluate the potential of using BSFL meal as a protein source in animal feeds (Table 1.2). In most studies reviewed here, BSFL meal could only replace a proportion of conventional protein sources, such as fish meal or soybean meal. The factors limiting the use of BSF meal are its high crude fat content and high proportion of saturated fatty acids, and thus further processing (e.g., defatting) of BSFL meal is preferable (Barragan-Fonseca *et al.*, 2017; Zarantoniello *et al.*, 2020; Zhou *et al.*, 2018). Fortunately, BSFL fatty acid profile is changeable through dietary modification (Barroso *et al.*, 2017; Erbland *et al.*, 2020). Therefore, BSFL meal with more desirable fatty acid profiles could be derived from larvae fed with diets enriched with wastes or supplements rich in unsaturated fatty acids (Agbohessou *et al.*, 2021; Bruni *et al.*, 2020). In addition, providing live BSFL to farmed animals is suggested where applicable, as live larvae not only provide nutrients, but also serve as environmental enrichment for animals to improve their welfare (Ipema *et al.*, 2020, 2021). Nevertheless, a factor that currently limits the use of BSFL biomass in animal feed is its high production costs (Ffoulkes *et al.*, 2021). Therefore, there is an urgent need to reduce operational costs for producing BSFL biomass through optimizing BSF rearing environments.

BSFL-derived nutrients could also be used to support growth of animals that are mass produced not for human consumption. For example, in sterile insect technique programs, *Aedes aegypti* Meigen (Diptera: Culicidae) and *A. albopictus* Skuse (Diptera: Culicidae) larval diets could comprise 50% of BSFL meal (Mamai *et al.*, 2019). Dindo *et al.* (2016) showed that adding BSFL hemolymph in artificial diet improved development of *Exorista larvarum* L. (Diptera: Tachinidae), a larval parasitoid of Lepidoptera. Moreover, wounded BSFL may be live hosts to produce entomopathogenic nematodes (Tourtois *et al.*, 2017).

Subject to local legislations, BSFL fed with abattoir waste, animal manure, or restaurant waste may not be allowed as a feed ingredient for farmed animals (Bosch *et al.*, 2019). In such circumstances, BSFL may be used to feed animals not purposed for food (e.g., pets or the animals mentioned above) or to be processed into other products. The protein fraction of BSFL biomass could be processed into a bioplastic that may be used to produce biodegradable pots, mulching films, or packaging (Nuvoli *et al.*, 2021; Setti *et al.*, 2020); whereas the lipid fraction could be used to produce bio-fuel or detergents (Franco *et al.*, 2022; Kamarulzaman & Abdullah, 2020). The remaining fraction of BSFL, as well as some by-products from BSF farming such as puparia and dead adults, would contain mostly chitin, which could be extracted and used in agricultural, healthcare, or waste management sectors (Pasquier *et al.*, 2021).

Globally, there is an increasing number of businesses being established to manage locally generated organic waste using bioconversion systems employing BSFL (Figure 1.3).

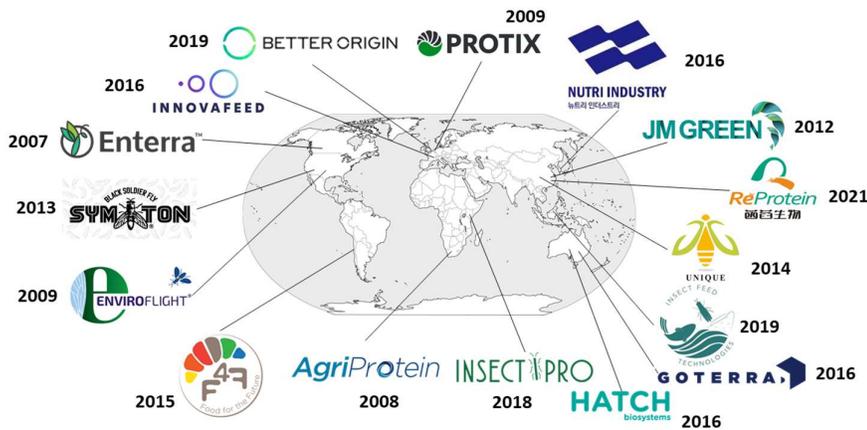


Figure 1.3 Some of the businesses established to convert local organic wastes into black soldier fly (*Hermetia illucens*) larval biomass. Numbers next to the business logos indicate the year when the businesses were founded. Image by Z. Liu, 2022; CC BY.

Table 1.2 Maximum suggested inclusion ratio (%) of full fat or partially defatted black soldier fly (*Hermetia illucens*) larval meal (BSFLM) in feeds for different domestic animal species.

Class	Species	Type	Full-fat BSFLM	Partially defatted BSFLM
Mammalia	Pig (<i>Sus domesticus</i> Erxleben)	Weaner	2–18.5 ¹⁻⁴	5.4–10 ^{4,5}
		Growing	30.5 ⁶	
		Finishing	8–14 ^{7,8}	
Aves	Dog (<i>Canis familiaris</i> L.)	Adult beagle		20 ⁹
	Chicken (<i>Gallus domesticus</i> L.)	Broiler	0.2–20 ¹⁰⁻¹²	7.5–15 ¹³⁻¹⁵
		Layer	3–10 ^{16,17}	4–17 ¹⁸⁻²⁰
	Quail (<i>Coturnix coturnix</i> L.)	Broiler		5.4–15 ^{21,22}
		Layer	13.2 ²³	15 ²⁴
Reptilia	Chinese soft-shell turtle (<i>Pelodiscus sinensis</i> Wiegmann)	Juvenile		4.2 ²⁵
		Adult		8.5 ²⁶
Actinopterygii	Atlantic salmon (<i>Salmo salar</i> L.)	Pre-smolt	16.1 ²⁷	
		Post-smolt	14.8 ²⁸	
	Rainbow trout (<i>Oncorhynchus mykiss</i> Walbaum)	Juvenile	8–10.5 ^{29,30}	6.2–40 ³¹⁻³³
	European sea bass (<i>Dicentrarchus labrax</i> L.)	Juvenile	14.8 ³⁴	19.5 ³⁵

(continued on next page)

Table 1.2 (continued) Maximum suggested inclusion ratio (%) of full fat or partially defatted black soldier fly (*Hermetia illucens*) larval meal (BSFLM) in feeds for different domestic animal species.

Class	Species	Type	Full-fat BSFLM	Partially defatted BSFLM
Actinopterygii	Nile tilapia (<i>Oreochromis niloticus</i> L.)	Juvenile	8–33.6 ^{36–38}	18.5 ³⁹
	Siberian sturgeon (<i>Acipenser baerii</i> Brandt)	Juvenile	15–30 ^{40, 41}	18.5 ⁴²
	Jian carp (<i>Cyprinus carpio</i> var. <i>jian</i>)	Juvenile		7.9 ⁴³
	Minor carp (<i>Cyprinus carpio</i> var. <i>specularis</i>)	Juvenile	13.1 ⁴⁴	
	African catfish (<i>Clarias gariepinus</i> Burchell)	Juvenile	7.5 ⁴⁵	17.2 ⁴⁶
Malacostraca	Pacific white shrimp (<i>Litopenaeus vannamei</i> Boone)	Post larvae	9.5–29 ^{47, 48}	10.5 ⁴⁹

^{1–48} References:

¹ (Chia *et al.*, 2019), ² (Yu *et al.*, 2020), ³ (Tang *et al.*, 2022), ⁴ (Spranghers *et al.*, 2018), ⁵ (Biasato *et al.*, 2020a), ⁶ (Kar *et al.*, 2021), ⁷ (Chia *et al.*, 2021), ⁸ (Yu *et al.*, 2019), ⁹ (Freel *et al.*, 2021), ¹⁰ (Murawska *et al.*, 2021), ¹¹ (Józefiak *et al.*, 2018), ¹² (Onsongo *et al.*, 2018), ¹³ (Dabbou *et al.*, 2018), ¹⁴ (Kim *et al.*), ¹⁵ (Biasato *et al.*, 2020b), ¹⁶ (Liu *et al.*, 2021), ¹⁷ (Bejaei & Cheng, 2020), ¹⁸ (Park *et al.*, 2021), ¹⁹ (Moniello *et al.*, 2019), ²⁰ (Secci *et al.*, 2018), ²¹ (Mbhele *et al.*, 2019), ²² (Cullere *et al.*, 2018), ²³ (Harlystiarini *et al.*, 2020), ²⁴ (Dalle Zotte *et al.*, 2019), ²⁵ (Shang *et al.*, 2022), ²⁶ (Li *et al.*, 2021), ²⁷ (Weththasinghe *et al.*, 2021), ²⁸ (Bruni *et al.*, 2020), ²⁹ (Hossain *et al.*, 2021), ³⁰ (Cardinaletti *et al.*, 2019), ³¹ (Renna *et al.*, 2017), ³² (Caimi *et al.*, 2021), ³³ (Bordignon *et al.*, 2022), ³⁴ (Abdel-Latif *et al.*, 2021), ³⁵ (Magalhães *et al.*, 2017), ³⁶ (Wachira *et al.*, 2021), ³⁷ (Devic *et al.*, 2018), ³⁸ (Agbohessou *et al.*, 2021), ³⁹ (Dietz & Liebert, 2018), ⁴⁰ (Rawski *et al.*, 2021), ⁴¹ (Rawski *et al.*, 2020), ⁴² (Caimi *et al.*, 2020), ⁴³ (Li *et al.*, 2017), ⁴⁴ (Xu *et al.*, 2020), ⁴⁵ (Adeoye *et al.*, 2020), ⁴⁶ (Fawole *et al.*, 2020), ⁴⁷ (Chen *et al.*, 2021), ⁴⁸ (Cummins *et al.*, 2017), ⁴⁹ (Richardson *et al.*, 2021)

1.3 Thesis aims and layout

The present work aims to optimize the BSF rearing environment through studying larval nutritional requirements and factors influencing pupation and reproduction (Figure 1.4). Chapters 2, 3, 4, and 5 are presented as manuscripts, which are published in peer-reviewed international journals. I have tried to minimize repetition between chapters, although some repetition was still necessary to form stand-alone works. The published or submitted chapters were reformatted in style to achieve cohesion of the thesis.

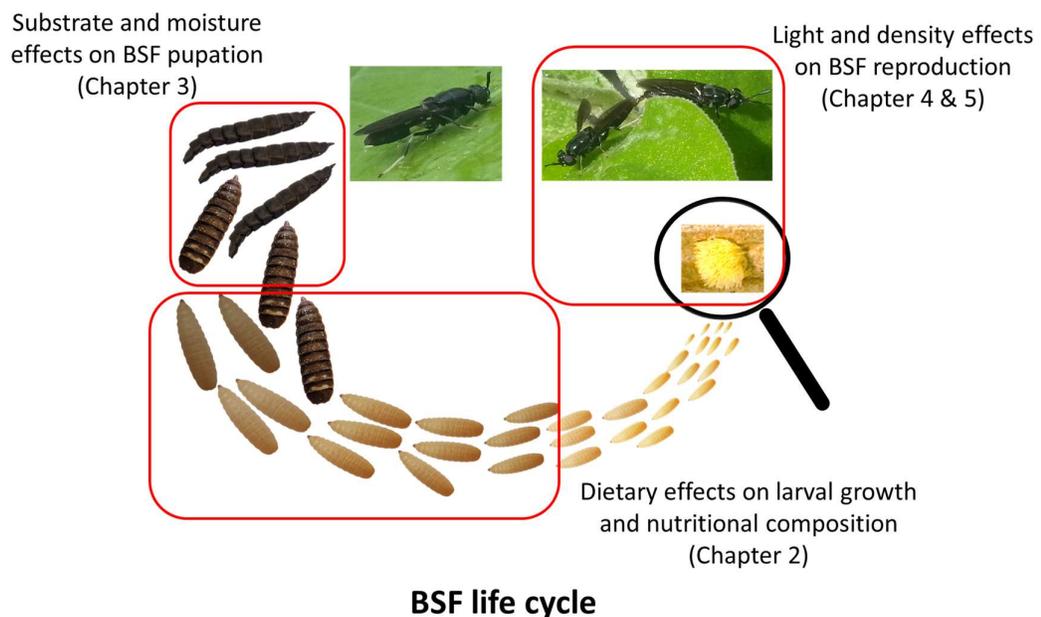


Figure 1.4 An illustration of the research conducted in this thesis to optimize the rearing environment for the black soldier fly (BSF; *Hermetia illucens*) at different life stages. Image by Z. Liu, 2022; CC BY.

In Chapter 2, I tested BSFL with four types of substrates and examined larval life history traits, nutritional composition at the harvest stage (i.e., prepupae), diet consumption rates, and nutrient conversion ratios. This experiment was a part of a commercial project, and the substrates were selected by the clients. In addition, a meta-analysis was performed to understand the impact of dietary lignocellulosic content on BSFL development. This work contributes to the knowledge on why and how waste nutrient composition would influence the efficiency of a bioconversion system employing BSFL, and helps to select or combine suitable waste types to be consumed by BSFL.

The objective of Chapter 3 was to optimize the substrate environment for BSF pupation. I determined prepupal mortality, pupation rate, and pupation depth in response to two pupation substrates (vermiculite and wood chips) and at nine moisture content levels. While providing pupation substrate may fulfil thigmotactic requirement by BSF prepupae, adjusting substrate moisture level would balance the availability of both water and oxygen for the insects.

Successful operation of a bioconversion system employing BSFL requires year-round and consistent supply of neonatal larvae. Because BSF mating is affected by temperature and light, the most reliable measure to achieve this goal would be breeding BSF in temperature controlled indoor spaces using artificial lighting. The objective of Chapter 4 was to test four artificial light sources for their effects on supporting BSF mating success. Irradiance across different wavebands was measured for each light source. BSF mating success was indicated by the presence of sperm in BSF female spermathecae. Moreover, two BSF colonies were compared under the same types of lights, highlighting importance of maintaining a healthy BSF stock colony for improving neonatal supply.

In Chapter 5, I studied possible effects of increasing adult density and reducing photoperiod on BSF reproductive output, with the aim of optimizing BSF neonatal production within a limited indoor space and reducing costs of artificial lighting. Combinations of three different adult densities and three light regimes were tested. While the total reproductive output directs the selection of adult density and light regime, mean individual reproductive output and adult mortality revealed the effects of adult density and light regime on BSF adult fitness.

In Chapter 6, I addressed the major findings of my research and implications for future research to optimize BSFL diet, BSF pupation substrate, visual environment for BSF mating. Also discussed are the potential use of life tables and the importance of maintaining a genetically healthy BSF colony for organic waste bioconversion. Finally, I presented a summary of practical recommendations based on my study, which could be directly applied to BSF farming.

1.4 References

- Abdel-Latif, H. M. R., Abdel-Tawwab, M., Khalil, R. H., Metwally, A. A., Shakweer, M. S., Ghetas, H. A., & Khallaf, M. A. (2021). Black soldier fly (*Hermetia illucens*) larvae meal in diets of European seabass: Effects on antioxidative capacity, non-specific immunity, transcriptomic responses, and resistance to the challenge with *Vibrio alginolyticus*. *Fish & Shellfish Immunology*, *111*, 111–118. <https://doi.org/10.1016/j.fsi.2021.01.013>
- Addy, M., Huo, S., Liu, J., Li, K., Cheng, P., Schiappacasse, C., Chen, D., Cheng, Y., Liu, Y., Ma, Y., Wang, L., Wang, Y., Cobb, K., Chen, P., & Ruan, R. (2021). Bioconversion technologies: Insect and worm farming. In J. Wong, G. Kaur, M. Taherzadeh, A. Pandey, & K. Lasaridi (Eds.), *Current developments in biotechnology and bioengineering* (pp. 235–256). Elsevier. <https://doi.org/10.1016/B978-0-12-819148-4.00008-7>

- Adeoye, A. A., Akegbejo-Samsons, Y., Fawole, F. J., & Davies, S. J. (2020). Preliminary assessment of black soldier fly (*Hermetia illucens*) larval meal in the diet of African catfish (*Clarias gariepinus*): Impact on growth, body index, and hematological parameters. *Journal of the World Aquaculture Society*, 51(4), 1024–1033. <https://doi.org/10.1111/jwas.12691>
- Agbohessou, P. S., Mandiki, S. N. M., Gougbedji, A., Megido, R. C., Hossain, M. S., De Jaeger, P., Larondelle, Y., Francis, F., Lalèyè, P. A., & Kestemont, P. (2021). Total replacement of fish meal by enriched-fatty acid *Hermetia illucens* meal did not substantially affect growth parameters or innate immune status and improved whole body biochemical quality of Nile tilapia juveniles. *Aquaculture Nutrition*, 27(3), 880–896. <https://doi.org/10.1111/anu.13232>
- Anyega, A. O., Korir, N. K., Beesigamukama, D., Changeh, G. J., Nkoba, K., Subramanian, S., van Loon, J. J. A., Dicke, M., & Tanga, C. M. (2021). Black soldier fly-composted organic fertilizer enhances growth, yield, and nutrient quality of three key vegetable crops in Sub-Saharan Africa. *Frontiers in Plant Science*, 12. <https://doi.org/10.3389/fpls.2021.680312>
- Barbier, J. (1952). Introduction en France d'un Diptère Stratiomyide américain. *Bulletin de la Société entomologique de France*, 57(7), 108. https://www.persee.fr/doc/bsef_0037-928x_1952_num_57_7_18587
- Barragan-Fonseca, K. B., Dicke, M., & Loon, J. J. A. v. (2017). Nutritional value of the black soldier fly (*Hermetia illucens* L.) and its suitability as animal feed – a review. *Journal of Insects as Food and Feed*, 3(2), 105–120. <https://doi.org/10.3920/jiff2016.0055>
- Barragán-Fonseca, K. B., Dicke, M., & van Loon, J. J. A. (2018). Influence of larval density and dietary nutrient concentration on performance, body protein, and fat contents of black soldier fly larvae (*Hermetia illucens*). *Entomologia Experimentalis et Applicata*, 166(9), 761–770. <https://doi.org/10.1111/eea.12716>
- Barros, L. M., Ferreira-Kepler, R. L., Martins, R. T., & Gutjahr, A. L. N. (2019). Bionomy of *Hermetia illucens* (Diptera: Stratiomyidae) on decomposing swine carcass in an urban area of Central Amazon. *Journal of Medical Entomology*, 56(3), 681–689. <https://doi.org/10.1093/jme/tjz005>
- Barroso, F. G., Sánchez-Muros, M. J., Segura, M., Morote, E., Torres, A., Ramos, R., & Guil, J. L. (2017). Insects as food: Enrichment of larvae of *Hermetia illucens* with omega 3 fatty acids by means of dietary modifications. *Journal of Food Composition and Analysis*, 62, 8–13. <https://doi.org/10.1016/j.jfca.2017.04.008>
- Barry, T. (2004). *Evaluation of the economic, social, and biological feasibility of bioconverting food wastes with the black soldier fly (Hermetia illucens)* [Doctoral thesis, University of North Texas]. UNT Digital Library. <https://digital.library.unt.edu/ark:/67531/metadc4639/>
- Beesigamukama, D., Mochoge, B., Korir, N., Ghemoh, C. J., Subramanian, S., & Tanga, C. M. (2021). In situ nitrogen mineralization and nutrient release by soil amended with black soldier fly frass fertilizer. *Scientific Reports*, 11(1), 14799. <https://doi.org/10.1038/s41598-021-94269-3>

- Bejaei, M., & Cheng, K. M. (2020). The effect of including full-fat dried black soldier fly larvae in laying hen diet on egg quality and sensory characteristics. *Journal of Insects as Food and Feed*, 6(3), 305–314. <https://doi.org/10.3920/jiff2019.0045>
- Benelli, G., Canale, A., Raspi, A., & Fornaciari, G. (2014). The death scenario of an Italian Renaissance princess can shed light on a zoological dilemma: Did the black soldier fly reach Europe with Columbus? *Journal of Archaeological Science*, 49, 203–205. <https://doi.org/10.1016/j.jas.2014.05.015>
- Bertinetti, C., Samayoa, A. C., & Hwang, S. (2019). Effects of feeding adults of *Hermetia illucens* (Diptera: Stratiomyidae) on longevity, oviposition, and egg hatchability: Insights into optimizing egg production. *Journal of Insect Science*, 19(1). <https://doi.org/10.1093/jisesa/iez001>
- Biasato, I., Ferrocino, I., Colombino, E., Gai, F., Schiavone, A., Cocolin, L., Vincenti, V., Capucchio, M. T., & Gasco, L. (2020a). Effects of dietary *Hermetia illucens* meal inclusion on cecal microbiota and small intestinal mucin dynamics and infiltration with immune cells of weaned piglets. *Journal of Animal Science and Biotechnology*, 11(1), 64. <https://doi.org/10.1186/s40104-020-00466-x>
- Biasato, I., Ferrocino, I., Dabbou, S., Evangelista, R., Gai, F., Gasco, L., Cocolin, L., Capucchio, M. T., & Schiavone, A. (2020b). Black soldier fly and gut health in broiler chickens: Insights into the relationship between cecal microbiota and intestinal mucin composition. *Journal of Animal Science and Biotechnology*, 11(1), 11. <https://doi.org/10.1186/s40104-019-0413-y>
- Booth, D. C., & Sheppard, C. (1984). Oviposition of the black soldier fly, *Hermetia illucens* (Diptera: Stratiomyidae): Eggs, masses, timing, and site characteristics. *Environmental Entomology*, 13(2), 421–423. <https://doi.org/10.1093/ee/13.2.421>
- Bordignon, F., Gasco, L., Birolo, M., Trocino, A., Caimi, C., Ballarin, C., Bortoletti, M., Nicoletto, C., Maucieri, C., & Xiccato, G. (2022). Performance and fillet traits of rainbow trout (*Oncorhynchus mykiss*) fed different levels of *Hermetia illucens* meal in a low-tech aquaponic system. *Aquaculture*, 546, 737279. <https://doi.org/10.1016/j.aquaculture.2021.737279>
- Borkent, S., & Hodge, S. (2021). Glasshouse evaluation of the black soldier fly waste product HexaFrass™ as an organic fertilizer. *Insects*, 12(11), 977. <https://doi.org/10.3390/insects12110977>
- Bosch, G., van Zanten, H. H. E., Zamprogna, A., Veenenbos, M., Meijer, N. P., van der Fels-Klerx, H. J., & van Loon, J. J. A. (2019). Conversion of organic resources by black soldier fly larvae: Legislation, efficiency and environmental impact. *Journal of Cleaner Production*, 222, 355–363. <https://doi.org/10.1016/j.jclepro.2019.02.270>
- Bruni, L., Belghit, I., Lock, E. J., Secci, G., Taiti, C., & Parisi, G. (2020). Total replacement of dietary fish meal with black soldier fly (*Hermetia illucens*) larvae does not impair physical, chemical or volatile composition of farmed Atlantic salmon (*Salmo salar* L.). *Journal of the Science of Food and Agriculture*, 100(3), 1038–1047. <https://doi.org/10.1002/jsfa.10108>
- Caimi, C., Biasato, I., Chemello, G., Oddon, S. B., Lussiana, C., Malfatto, V. M., Capucchio, M. T., Colombino, E., Schiavone, A., Gai, F., Trocino, A.,

- Brugiapaglia, A., Renna, M., & Gasco, L. (2021). Dietary inclusion of a partially defatted black soldier fly (*Hermetia illucens*) larva meal in low fishmeal-based diets for rainbow trout (*Oncorhynchus mykiss*). *Journal of Animal Science and Biotechnology*, *12*(1), 50. <https://doi.org/10.1186/s40104-021-00575-1>
- Caimi, C., Renna, M., Lussiana, C., Bonaldo, A., Gariglio, M., Meneguz, M., Dabbou, S., Schiavone, A., Gai, F., Elia, A. C., Prearo, M., & Gasco, L. (2020). First insights on black soldier fly (*Hermetia illucens* L.) larvae meal dietary administration in Siberian sturgeon (*Acipenser baerii* Brandt) juveniles. *Aquaculture*, *515*, 734539. <https://doi.org/10.1016/j.aquaculture.2019.734539>
- Cardinaletti, G., Randazzo, B., Messina, M., Zarantoniello, M., Giorgini, E., Zimbelli, A., Bruni, L., Parisi, G., Olivotto, I., & Tulli, F. (2019). Effects of graded dietary inclusion level of full-fat *Hermetia illucens* prepupae meal in practical diets for rainbow trout (*Oncorhynchus mykiss*). *Animals*, *9*(5), 251. <https://doi.org/10.3390/ani9050251>
- Chen, Y., Chi, S., Zhang, S., Dong, X., Yang, Q., Liu, H., Tan, B., & Xie, S. (2021). Effect of black soldier fly (*Hermetia illucens*) larvae meal on lipid and glucose metabolism of Pacific white shrimp *Litopenaeus vannamei*. *British Journal of Nutrition*, 1–15. <https://doi.org/10.1017/S0007114521004670>
- Chia, S. Y., Tanga, C. M., Khamis, F. M., Mohamed, S. A., Salifu, D., Sevgan, S., Fiaboe, K. K. M., Niassy, S., van Loon, J. J. A., Dicke, M., & Ekesi, S. (2018). Threshold temperatures and thermal requirements of black soldier fly *Hermetia illucens*: Implications for mass production. *PLOS ONE*, *13*(11), e0206097. <https://doi.org/10.1371/journal.pone.0206097>
- Chia, S. Y., Tanga, C. M., Osuga, I. M., Alaru, A. O., Mwangi, D. M., Githinji, M., Dubois, T., Ekesi, S., Loon, J. J. A. v., & Dicke, M. (2021). Black soldier fly larval meal in feed enhances growth performance, carcass yield and meat quality of finishing pigs. *Journal of Insects as Food and Feed*, *7*(4), 433–447. <https://doi.org/10.3920/jiff2020.0072>
- Chia, S. Y., Tanga, C. M., Osuga, I. M., Alaru, A. O., Mwangi, D. M., Githinji, M., Subramanian, S., Fiaboe, K. K. M., Ekesi, S., van Loon, J. J. A., & Dicke, M. (2019). Effect of dietary replacement of fishmeal by insect meal on growth performance, blood profiles and economics of growing pigs in Kenya. *Animals*, *9*(10), 705. <https://doi.org/10.3390/ani9100705>
- Chirere, T. E. S., Khalil, S., & Lalander, C. (2021). Fertiliser effect on Swiss chard of black soldier fly larvae-frass compost made from food waste and faeces. *Journal of Insects as Food and Feed*, *7*(4), 457–469. <https://doi.org/10.3920/jiff2020.0120>
- Christensen, T. H., Scharff, H., & Hjelmar, O. (2010). Landfilling: Concepts and challenges. In T. H. Christensen (Ed.), *Solid waste technology & management* (pp. 683–694). Blackwell Publishing Ltd. <https://doi.org/10.1002/9780470666883.ch44>
- Craig Sheppard, D., Larry Newton, G., Thompson, S. A., & Savage, S. (1994). A value added manure management system using the black soldier fly. *Bioresource Technology*, *50*(3), 275–279. [https://doi.org/10.1016/0960-8524\(94\)90102-3](https://doi.org/10.1016/0960-8524(94)90102-3)

- Cullere, M., Tasoniero, G., Giaccone, V., Acuti, G., Marangon, A., & Dalle Zotte, A. (2018). Black soldier fly as dietary protein source for broiler quails: Meat proximate composition, fatty acid and amino acid profile, oxidative status and sensory traits. *Animal*, *12*(3), 640–647. <https://doi.org/10.1017/S1751731117001860>
- Cummins, V. C., Rawles, S. D., Thompson, K. R., Velasquez, A., Kobayashi, Y., Hager, J., & Webster, C. D. (2017). Evaluation of black soldier fly (*Hermetia illucens*) larvae meal as partial or total replacement of marine fish meal in practical diets for Pacific white shrimp (*Litopenaeus vannamei*). *Aquaculture*, *473*, 337–344. <https://doi.org/10.1016/j.aquaculture.2017.02.022>
- Dabbou, S., Gai, F., Biasato, I., Capucchio, M. T., Biasibetti, E., Dezzutto, D., Meneguz, M., Plachà, I., Gasco, L., & Schiavone, A. (2018). Black soldier fly defatted meal as a dietary protein source for broiler chickens: Effects on growth performance, blood traits, gut morphology and histological features. *Journal of Animal Science and Biotechnology*, *9*(1), 49. <https://doi.org/10.1186/s40104-018-0266-9>
- Dalle Zotte, A., Singh, Y., Michiels, J., & Cullere, M. (2019). Black soldier fly (*Hermetia Illucens*) as dietary source for laying quails: Live performance, and egg physico-chemical quality, sensory profile and storage stability. *Animals*, *9*(3), 115. <https://doi.org/10.3390/ani9030115>
- Devic, E., Leschen, W., Murray, F., & Little, D. C. (2018). Growth performance, feed utilization and body composition of advanced nursing Nile tilapia (*Oreochromis niloticus*) fed diets containing black soldier fly (*Hermetia illucens*) larvae meal. *Aquaculture Nutrition*, *24*(1), 416–423. <https://doi.org/10.1111/anu.12573>
- Diener, S., & Zurbrügg, C. (2008). Conversion of organic refuse by saprophages (CORS). *Sandec News*, *9*, 10–11. https://www.dora.lib4ri.ch/eawag/islandora/object/eawag%3A9522/datastream/PDF/Diener-2008-Conversion_of_organic_refuse_by-%28published_version%29.pdf
- Dietz, C., & Liebert, F. (2018). Does graded substitution of soy protein concentrate by an insect meal respond on growth and N-utilization in Nile tilapia (*Oreochromis niloticus*)? *Aquaculture Reports*, *12*, 43–48. <https://doi.org/10.1016/j.aqrep.2018.09.001>
- Dindo, M. L., Vandicke, J., Marchetti, E., Spranghers, T., Bonte, J., & De Clercq, P. (2016). Supplementation of an artificial medium for the parasitoid *Exorista larvarum* (Diptera: Tachnidae) with hemolymph of *Hermetia illucens* (Diptera: Stratiomyidae) or *Antheraea pernyi* (Lepidoptera: Saturniidae). *Journal of Economic Entomology*, *109*(2), 602–606. <https://doi.org/10.1093/jee/tow024>
- Dzepe, D., Nana, P., Fotso, A., Tchuinkam, T., & Djouaka, R. (2020). Influence of larval density, substrate moisture content and feedstock ratio on life history traits of black soldier fly larvae. *Journal of Insects as Food and Feed*, *6*(2), 133–140. <https://doi.org/10.3920/jiff2019.0034>
- Erbland, P., Alyokhin, A., Perkins, L. B., & Peterson, M. (2020). Dose-dependent retention of omega-3 fatty acids by black soldier fly larvae (Diptera: Stratiomyidae). *Journal of Economic Entomology*, *113*(3), 1221–1226. <https://doi.org/10.1093/jee/toaa045>

- Fawole, F. J., Adeoye, A. A., Tihamiyu, L. O., Ajala, K. I., Obadara, S. O., & Ganiyu, I. O. (2020). Substituting fishmeal with *Hermetia illucens* in the diets of African catfish (*Clarias gariepinus*): Effects on growth, nutrient utilization, haemato-physiological response, and oxidative stress biomarker. *Aquaculture*, *518*, 734849. <https://doi.org/10.1016/j.aquaculture.2019.734849>
- Ffoulkes, C., Illman, H., O'Connor, R., Lemon, F., Behrendt, K., Wynn, S., Wright, P., Godber, O., Ramsden, M., Adams, J., Metcalfe, P., Walker, L., Gittins, J., Wickland, K., Nanua, S. and Sharples, B. (2021). *Development of a roadmap to scale up insect protein production in the U.K. for use in animal feed*. WWF-U.K. https://www.wwf.org.uk/sites/default/files/2021-06/the_future_of_feed_technical_report.pdf
- Food and Agriculture Organization of the United Nations. (2019). *The State of Food and Agriculture 2019. Moving forward on food loss and waste reduction*. The United Nations.
- Franco, A., Salvia, R., Scieuzo, C., Schmitt, E., Russo, A., & Falabella, P. (2022). Lipids from insects in cosmetics and for personal care products. *Insects*, *13*(1), 41. <https://doi.org/10.3390/insects13010041>
- Freel, T. A., McComb, A., & Koutsos, E. A. (2021). Digestibility and safety of dry black soldier fly larvae meal and black soldier fly larvae oil in dogs. *Journal of Animal Science*, *99*(3). <https://doi.org/10.1093/jas/skab047>
- Gobbi, P., Martínez-Sánchez, A., & Rojo, S. (2013). The effects of larval diet on adult life-history traits of the black soldier fly, *Hermetia illucens* (Diptera: Stratiomyidae). *EJE*, *110*(3), 461–468. <https://doi.org/10.14411/eje.2013.061>
- Guilliet, J., Baudouin, G., Pollet, N., & Filée, J. (2021). The natural history of the black soldier fly, *Hermetia illucens*: Insights from complete mitochondrial genome sequences. *bioRxiv*, 2021.2010.2021.465311. <https://doi.org/10.1101/2021.10.21.465311>
- Harlystiarini, H., Mutia, R., Wibawan, I. W. T., & Astuti, D. A. (2020). Immune responses and egg productions of quails fed rations supplemented with larvae meal of black soldier fly (*Hermetia illucens*). *Tropical Animal Science Journal*, *43*(1), 43–49. <https://doi.org/10.5398/tasj.2020.43.1.43>
- Holmes, L. A., Vanlaerhoven, S. L., & Tomberlin, J. K. (2012). Relative humidity effects on the life history of *Hermetia illucens* (Diptera: Stratiomyidae). *Environmental Entomology*, *41*(4), 971–978. <https://doi.org/10.1603/en12054>
- Hossain, M. S., Fawole, F. J., Labh, S. N., Small, B. C., Overturf, K., & Kumar, V. (2021). Insect meal inclusion as a novel feed ingredient in soy-based diets improves performance of rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*, *544*, 737096. <https://doi.org/10.1016/j.aquaculture.2021.737096>
- Ipema, A. F., Bokkers, E. A. M., Gerrits, W. J. J., Kemp, B., & Bolhuis, J. E. (2020). Long-term access to live black soldier fly larvae (*Hermetia illucens*) stimulates activity and reduces fearfulness of broilers, without affecting health. *Scientific Reports*, *10*(1), 17428. <https://doi.org/10.1038/s41598-020-74514-x>
- Ipema, A. F., Bokkers, E. A. M., Gerrits, W. J. J., Kemp, B., & Bolhuis, J. E. (2021). Providing live black soldier fly larvae (*Hermetia illucens*) improves welfare while

- maintaining performance of piglets post-weaning. *Scientific Reports*, 11(1), 7371. <https://doi.org/10.1038/s41598-021-86765-3>
- Józefiak, A., Kierończyk, B., Rawski, M., Mazurkiewicz, J., Benzertiha, A., Gobbi, P., Nogales-Merida, S., Świątkiewicz, S., & Józefiak, D. (2018). Full-fat insect meals as feed additive – the effect on broiler chicken growth performance and gastrointestinal tract microbiota. *Journal of Animal and Feed Sciences*, 27(2), 131–139. <https://doi.org/10.22358/jafs/91967/2018>
- Józefiak, D., Józefiak, A., Kierończyk, B., Rawski, M., Świątkiewicz, S., Długosz, J., & Engberg, R. M. (2016). Insects – A natural nutrient source for poultry – A Review. *Annals of Animal Science*, 16(2), 297–313. <https://doi.org/10.1515/aoas-2016-0010>
- Julita, U., Fitri, L., Putra, R., & Permana, A. (2020). Mating success and reproductive behavior of black soldier fly *Hermetia illucens* L. (Diptera, Stratiomyidae) in tropics. *Journal of Entomology*, 17(3), 117–127. <https://doi.org/10.3923/je.2020.117.127>
- Kamarulzaman, M. K., & Abdullah, A. (2020). Multi-objective optimization of diesel engine performances and exhaust emissions characteristics of *Hermetia illucens* larvae oil-diesel fuel blends using response surface methodology. *Energy Sources, Part A: Recovery, Utilization, and Environmental Effects*, (Online). <https://doi.org/10.1080/15567036.2020.1849450>
- Kar, S. K., Schokker, D., Harms, A. C., Kruijt, L., Smits, M. A., & Jansman, A. J. M. (2021). Local intestinal microbiota response and systemic effects of feeding black soldier fly larvae to replace soybean meal in growing pigs. *Scientific Reports*, 11(1), 15088. <https://doi.org/10.1038/s41598-021-94604-8>
- Kawasaki, K., Kawasaki, T., Hirayasu, H., Matsumoto, Y., & Fujitani, Y. (2020). Evaluation of fertilizer value of residues obtained after processing household organic waste with black soldier fly larvae (*Hermetia illucens*). *Sustainability*, 12(12), 4920. <https://doi.org/10.3390/su12124920>
- Kaza, S., Yao, L. C., Bhada-Tata, P., & Van Woerden, F. (2018). *What a waste 2.0 : A global snapshot of solid waste management to 2050*. World Bank. <https://openknowledge.worldbank.org/handle/10986/30317>
- Keefe, M., & Lei, X. G. (2021). Unveiling the keratinolytic transcriptome of the black carpet beetle (*Attagenus unicolor*) for sustainable poultry feather recycling. *Applied Microbiology and Biotechnology*, 105(13), 5577–5587. <https://doi.org/10.1007/s00253-021-11427-3>
- Keefe, M., & Lei, X. G. (2022). Growth and survivability of the black carpet beetle fed a whole feather diet. *Journal of Applied Entomology*, 146(1–2), 158–167. <https://doi.org/10.1111/jen.12943>
- Kim, B., Kim, H. R., Baek, Y. C., Ryu, C. H., Ji, S. Y., Jeong, J. Y., Kim, M., Jung, H., & Park, S. H. (2021). Evaluation of microwave-dried black soldier fly (*Hermetia illucens*) larvae meal as a dietary protein source in broiler chicken diets. *Journal of Insects as Food and Feed*, in press. <https://doi.org/10.3920/jiff2021.0113>
- Klammsteiner, T., Turan, V., Fernández-Delgado Juárez, M., Oberegger, S., & Insam, H. (2020). Suitability of black soldier fly frass as soil amendment and implication for

- organic waste hygienization. *Agronomy*, 10(10), 1578. <https://doi.org/10.3390/agronomy10101578>
- Kotzé, Z., & Tomberlin, J. K. (2020). Influence of substrate age and interspecific colonization on oviposition behavior of a generalist feeder, black soldier fly (Diptera: Stratiomyidae), on carrion. *Journal of Medical Entomology*, 57(4), 987–993. <https://doi.org/10.1093/jme/tjaa021>
- Lalander, C., Diener, S., Zurbrügg, C., & Vinnerås, B. (2019). Effects of feedstock on larval development and process efficiency in waste treatment with black soldier fly (*Hermetia illucens*). *Journal of Cleaner Production*, 208, 211–219. <https://doi.org/10.1016/j.jclepro.2018.10.017>
- Leclercq, M. (1997). A propos de *Hermetia illucens* (Linnaeus, 1758) (“soldier fly”) (Diptera: Stratiomyidae: Hermetiinae). *Bulletin et Annales de la Societe Royale Belge d'Entomologie*, 133(2), 275–282. <https://agris.fao.org/agris-search/search.do?recordID=BE1998001184>
- Lenz, M., Lee, C. Y., Lacey, M. J., Yoshimura, T., & Tsunoda, K. (2011). The potential and limits of termites (Isoptera) as decomposers of waste paper products. *Journal of Economic Entomology*, 104(1), 232–242. <https://doi.org/10.1603/ec10155>
- Li, M., Li, M., Wang, G., Liu, C., Shang, R., Chen, Y., & Li, L. (2021). Defatted black soldier fly (*Hermetia illucens*) larvae meal can partially replace fish meal in diets for adult Chinese soft-shelled turtles. *Aquaculture*, 541, 736758. <https://doi.org/10.1016/j.aquaculture.2021.736758>
- Li, S., Ji, H., Zhang, B., Zhou, J., & Yu, H. (2017). Defatted black soldier fly (*Hermetia illucens*) larvae meal in diets for juvenile Jian carp (*Cyprinus carpio* var. Jian): Growth performance, antioxidant enzyme activities, digestive enzyme activities, intestine and hepatopancreas histological structure. *Aquaculture*, 477, 62–70. <https://doi.org/10.1016/j.aquaculture.2017.04.015>
- Li, Y., Fu, T., Geng, L., Shi, Y., Chu, H., Liu, F., Liu, C., Song, F., Zhang, J., & Shu, C. (2019). *Protaetia brevitarsis* larvae can efficiently convert herbaceous and ligneous plant residues to humic acids. *Waste Management*, 83, 79–82. <https://doi.org/10.1016/j.wasman.2018.11.010>
- Lindner, P. (1919). Zur fettgewinnung aus kleintieren. *Zeitschrift für Technische Biologie*, 7, 213–220. <https://archive.org/details/zeitschriftftec8leip>
- Liu, X., Liu, X., Yao, Y., Qu, X., Chen, J., Xie, K., Wang, X., Qi, Y., Xiao, B., & He, C. (2021). Effects of different levels of *Hermetia illucens* larvae meal on performance, egg quality, yolk fatty acid composition and oxidative status of laying hens. *Italian Journal of Animal Science*, 20(1), 256–266. <https://doi.org/10.1080/1828051X.2021.1878946>
- Lundy, M. E., & Parrella, M. P. (2015). Crickets are not a free lunch: Protein capture from scalable organic side-streams via high-density populations of *Acheta domesticus*. *PLOS ONE*, 10(4), e0118785. <https://doi.org/10.1371/journal.pone.0118785>
- Magalhães, R., Sánchez-López, A., Leal, R. S., Martínez-Llorens, S., Oliva-Teles, A., & Peres, H. (2017). Black soldier fly (*Hermetia illucens*) pre-pupae meal as a fish

- meal replacement in diets for European seabass (*Dicentrarchus labrax*). *Aquaculture*, 476, 79–85. <https://doi.org/10.1016/j.aquaculture.2017.04.021>
- Malawey, A. S., Zhang, H., McGuane, A. S., Walsh, E. M., Rusch, T. W., Hjelman, C. E., Delclos, P. J., Rangel, J., Zheng, L., Cai, M., Yu, Z., Tarone, A. M., Zhang, J., & Tomberlin, J. K. (2021). Interaction of age and temperature on heat shock protein expression, sperm count, and sperm viability of the adult black soldier fly (Diptera: Stratiomyidae). *Journal of Insects as Food and Feed*, 7(1), 21–33. <https://doi.org/10.3920/jiff2020.0017>
- Mamai, W., Bimbilé Somda, N. S., Maiga, H., Konczal, A., Wallner, T., Bakhoun, M. T., Yamada, H., & Bouyer, J. (2019). Black soldier fly (*Hermetia illucens*) larvae powder as a larval diet ingredient for mass-rearing *Aedes* mosquitoes. *Parasite*, 26, 57. <https://doi.org/10.1051/parasite/2019059>
- Maquart, P. O., Richard, D., & Willems, J. (2020). First record of the black soldier fly, *Hermetia illucens*, in the western regions of France (Vendée, Loire-Atlantique, Ille-et-Vilaine) with notes on its worldwide repartition (Diptera, Stratiomyidae). *Bulletin de la Société entomologique de France*, 125(1), 13–18. https://doi.org/10.32475/bsef_2104
- Martínez-Sánchez, A., Magaña, C., Saloña, M., & Rojo, S. (2011). First record of *Hermetia illucens* (Diptera: Stratiomyidae) on human corpses in Iberian Peninsula. *Forensic Science International*, 206(1), e76–e78. <https://doi.org/10.1016/j.forciint.2010.10.021>
- May, B. M. (1961). The occurrence in New Zealand and the life-history of the soldier fly *Hermetia illucens* (L.) (Diptera: Stratiomyidae). *New Zealand Journal of Science*, 4, 55–65.
- Mbhele, F. G. T., Mnisi, C. M., & Mlambo, V. (2019). A nutritional evaluation of insect meal as a sustainable protein source for jumbo quails: Physiological and meat quality responses. *Sustainability*, 11(23), 6592. <https://doi.org/10.3390/su11236592>
- Meneguz, M., Schiavone, A., Gai, F., Dama, A., Lussiana, C., Renna, M., & Gasco, L. (2018). Effect of rearing substrate on growth performance, waste reduction efficiency and chemical composition of black soldier fly (*Hermetia illucens*) larvae. *Journal of the Science of Food and Agriculture*, 98(15), 5776–5784. <https://doi.org/10.1002/jsfa.9127>
- Menino, R., Felizes, F., Castelo-Branco, M. A., Fareleira, P., Moreira, O., Nunes, R., & Murta, D. (2021). Agricultural value of black soldier fly larvae frass as organic fertilizer on ryegrass. *Heliyon*, 7(1), e05855. <https://doi.org/10.1016/j.heliyon.2020.e05855>
- Moniello, G., Ariano, A., Panettieri, V., Tulli, F., Olivotto, I., Messina, M., Randazzo, B., Severino, L., Piccolo, G., Musco, N., Addeo, N. F., Hassoun, G., & Bovera, F. (2019). Intestinal morphometry, enzymatic and microbial activity in laying hens fed different levels of a *Hermetia illucens* larvae meal and toxic elements content of the insect meal and diets. *Animals*, 9(3), 86. <https://doi.org/10.3390/ani9030086>

- Murawska, D., Daszkiewicz, T., Sobotka, W., Gesek, M., Witkowska, D., Matusevičius, P., & Bakula, T. (2021). Partial and total replacement of soybean meal with full-fat black soldier fly (*Hermetia illucens* L.) larvae meal in broiler chicken diets: Impact on growth performance, carcass quality and meat quality. *Animals*, *11*(9), 2715. <https://doi.org/10.3390/ani11092715>
- National Research Council (U.S.). (1983). *Underutilized resources as animal feedstuffs*. The National Academies Press. <https://doi.org/10.17226/41>
- Niu, Y., Zheng, D., Yao, B., Cai, Z., Zhao, Z., Wu, S., Cong, P., & Yang, D. (2017). A novel bioconversion for value-added products from food waste using *Musca domestica*. *Waste Management*, *61*, 455–460. <https://doi.org/10.1016/j.wasman.2016.10.054>
- Nordahl, S. L., Devkota, J. P., Amirebrahimi, J., Smith, S. J., Breunig, H. M., Preble, C. V., Satchwell, A. J., Jin, L., Brown, N. J., Kirchstetter, T. W., & Scown, C. D. (2020). Life-cycle greenhouse gas emissions and human health trade-offs of organic waste management strategies. *Environmental Science & Technology*, *54*(15), 9200–9209. <https://doi.org/10.1021/acs.est.0c00364>
- Nuvoli, D., Montevecchi, G., Lovato, F., Masino, F., Van Der Borgh, M., Messori, M., & Antonelli, A. (2021). Protein films from black soldier fly (*Hermetia illucens*, Diptera: Stratiomyidae) prepupae: Effect of protein solubility and mild crosslinking. *Journal of the Science of Food and Agriculture*, *101*(11), 4506–4513. <https://doi.org/10.1002/jsfa.11091>
- Onsongo, V. O., Osuga, I. M., Gachuri, C. K., Wachira, A. M., Miano, D. M., Tanga, C. M., Ekesi, S., Nakimbugwe, D., & Fiaboe, K. K. M. (2018). Insects for income generation through animal feed: Effect of dietary replacement of soybean and fish meal with black soldier fly meal on broiler growth and economic performance. *Journal of Economic Entomology*, *111*(4), 1966–1973. <https://doi.org/10.1093/jee/toy118>
- Oonincx, D. G. A. B., van Huis, A., & van Loon, J. J. A. (2015). Nutrient utilisation by black soldier flies fed with chicken, pig, or cow manure. *Journal of Insects as Food and Feed*, *1*(2), 131–139. <https://doi.org/10.3920/jiff2014.0023>
- Oonincx, D. G. A. B., Volk, N., Diehl, J. J. E., van Loon, J. J. A., & Belušič, G. (2016). Photoreceptor spectral sensitivity of the compound eyes of black soldier fly (*Hermetia illucens*) informing the design of LED-based illumination to enhance indoor reproduction. *Journal of Insect Physiology*, *95*, 133–139. <https://doi.org/10.1016/j.jinsphys.2016.10.006>
- Park, S. H., Kim, H. R., Baek, Y. C., Ryu, C. H., Ji, S. Y., Jeong, J. Y., Kim, M., Jung, H., & Kim, B. (2021). Effects of dietary inclusion level of microwave-dried and press-defatted black soldier fly (*Hermetia illucens*) larvae meal on productive performance, cecal volatile fatty acid profile, and egg quality in laying hens. *Animals*, *11*(6), 1486. <https://doi.org/10.3390/ani11061486>
- Parolini, M., Ganzaroli, A., & Bacenetti, J. (2020). Earthworm as an alternative protein source in poultry and fish farming: Current applications and future perspectives. *Science of The Total Environment*, *734*, 139460. <https://doi.org/10.1016/j.scitotenv.2020.139460>

- Parry, N. J., Pieterse, E., & Weldon, C. W. (2020). Stocking rate and organic waste type affect development of three *Chrysomya* species and *Lucilia sericata* (Diptera: Calliphoridae): Implications for bioconversion. *Journal of Applied Entomology*, *144*(1–2), 94–108. <https://doi.org/10.1111/jen.12712>
- Pasquier, E., Beaumont, M., Mattos, B. D., Otoni, C. G., Winter, A., Rosenau, T., Belgacem, M. N., Rojas, O. J., & Bras, J. (2021). Upcycling byproducts from insect (fly larvae and mealworm) farming into chitin nanofibers and films. *ACS Sustainable Chemistry & Engineering*, *9*(40), 13618–13629. <https://doi.org/10.1021/acssuschemeng.1c05035>
- Peng, B., Chen, Z., Chen, J., Zhou, X., Wu, W., & Zhang, Y. (2021). Biodegradation of polylactic acid by yellow mealworms (larvae of *Tenebrio molitor*) via resource recovery: A sustainable approach for waste management. *Journal of Hazardous Materials*, *416*, 125803. <https://doi.org/10.1016/j.jhazmat.2021.125803>
- Polprasert, C., & Koottatep, T. (2017). *Organic waste recycling: Technology, management and sustainability* (4th ed.). IWA Publishing. <https://doi.org/10.2166/9781780408217>
- Quek, X. T., Liang, L., Tham, H. H., Yeo, H., Tan, M. K., & Tan, H. T. W. (2020). Are the growth and survival of *Acheta domesticus* comparable when reared on okara, waste vegetables and premium animal feed? *Journal of Insects as Food and Feed*, *6*(2), 161–168. <https://doi.org/10.3920/jiff2019.0039>
- Rawski, M., Mazurkiewicz, J., Kierończyk, B., & Józefiak, D. (2020). Black soldier fly full-fat larvae meal as an alternative to fish meal and fish oil in Siberian sturgeon nutrition: The effects on physical properties of the feed, animal growth performance, and feed acceptance and utilization. *Animals*, *10*(11), 2119. <https://doi.org/10.3390/ani10112119>
- Rawski, M., Mazurkiewicz, J., Kierończyk, B., & Józefiak, D. (2021). Black soldier fly full-fat larvae meal is more profitable than fish meal and fish oil in Siberian sturgeon farming: The effects on aquaculture sustainability, economy and fish GIT development. *Animals*, *11*(3), 604. <https://doi.org/10.3390/ani11030604>
- Renna, M., Schiavone, A., Gai, F., Dabbou, S., Lussiana, C., Malfatto, V., Prearo, M., Capucchio, M. T., Biasato, I., Biasibetti, E., De Marco, M., Brugiapaglia, A., Zoccarato, I., & Gasco, L. (2017). Evaluation of the suitability of a partially defatted black soldier fly (*Hermetia illucens* L.) larvae meal as ingredient for rainbow trout (*Oncorhynchus mykiss* Walbaum) diets. *Journal of Animal Science and Biotechnology*, *8*(1), 57. <https://doi.org/10.1186/s40104-017-0191-3>
- Richardson, A., Dantas-Lima, J., Lefranc, M., & Walraven, M. (2021). Effect of a black soldier fly ingredient on the growth performance and disease resistance of juvenile pacific white shrimp (*Litopenaeus vannamei*). *Animals*, *11*(5), 1450. <https://doi.org/10.3390/ani11051450>
- Rovai, D., Ortgies, M., Amin, S., Kuwahara, S., Schwartz, G., Lesniasuskas, R., Garza, J., & Lammert, A. (2021). Utilization of carrot pomace to grow mealworm larvae (*Tenebrio molitor*). *Sustainability*, *13*(16), Article 9341. <https://doi.org/10.3390/su13169341>

- Ruschioni, S., Loreto, N., Foligni, R., Mannozi, C., Raffaelli, N., Zamporlini, F., Pasquini, M., Roncolini, A., Cardinali, F., Osimani, A., Aquilanti, L., Isidoro, N., Riolo, P., & Mozzon, M. (2020). Addition of olive pomace to feeding substrate affects growth performance and nutritional value of mealworm (*Tenebrio Molitor* L.) larvae. *Foods*, *9*(3), Article 317. <https://doi.org/10.3390/foods9030317>
- Sanandiyana, N. D., Ottenheim, C., Phua, J. W., Caligiani, A., Dritsas, S., & Fernandez, J. G. (2020). Circular manufacturing of chitinous bio-composites via bioconversion of urban refuse. *Scientific Reports*, *10*(1), 4632. <https://doi.org/10.1038/s41598-020-61664-1>
- Secci, G., Bovera, F., Nizza, S., Baronti, N., Gasco, L., Conte, G., Serra, A., Bonelli, A., & Parisi, G. (2018). Quality of eggs from Lohmann Brown Classic laying hens fed black soldier fly meal as substitute for soya bean. *Animal*, *12*(10), 2191–2197. <https://doi.org/10.1017/S1751731117003603>
- Setti, L., Francia, E., Pulvirenti, A., De Leo, R., Martinelli, S., Maistrello, L., Macavei, L. I., Montorsi, M., Barbi, S., & Ronga, D. (2020). Bioplastic film from black soldier fly prepupae proteins used as mulch: Preliminary results. *Agronomy*, *10*(7), 933. <https://doi.org/10.3390/agronomy10070933>
- Setti, L., Francia, E., Pulvirenti, A., Gigliano, S., Zaccardelli, M., Pane, C., Caradonia, F., Bortolini, S., Maistrello, L., & Ronga, D. (2019). Use of black soldier fly (*Hermetia illucens* (L.), Diptera: Stratiomyidae) larvae processing residue in peat-based growing media. *Waste Management*, *95*, 278–288. <https://doi.org/10.1016/j.wasman.2019.06.017>
- Shafiee-Jood, M. & Cai, X. (2016). Reducing food loss and waste to enhance food security and environmental sustainability. *Environmental Science & Technology*, *50*(16), 8432–8443. <https://doi.org/10.1021/acs.est.6b01993>
- Shang, R., Man, L., Wang, G., Li, M., Liu, C., & Li, L. (2022). Influences of partial substitution of fish meal with defatted black soldier fly (*Hermetia illucens*) larvae meal in diets on growth performance, biochemical parameters, and body composition of juvenile Chinese soft-shelled turtles (*Pelodiscus sinensis*). *Aquaculture Nutrition*, *2022*, 4278137. <https://doi.org/10.1155/2022/4278137>
- Sheppard, D. C., Tomberlin, J. K., Joyce, J. A., Kiser, B. C., & Sumner, S. M. (2002). Rearing methods for the black soldier fly (Diptera: Stratiomyidae). *Journal of Medical Entomology*, *39*(4), 695–698. <https://doi.org/10.1603/0022-2585-39.4.695>
- Silva, L. B., de Souza, R. G., da Silva, S. R., Feitosa, A. d. C., Lopes, E. C., Lima, S. B. P., Dourado, L. R. B., & Pavan, B. E. (2021). Development of *Tenebrio molitor* (Coleoptera: Tenebrionidae) on poultry litter-based diets: Effect on chemical composition of larvae. *Journal of Insect Science (Online)*, *21*(1). <https://doi.org/10.1093/jisesa/ieaa145>
- Sogari, G., Amato, M., Biasato, I., Chiesa, S., & Gasco, L. (2019). The potential role of insects as feed: A multi-perspective review. *Animals*, *9*(4), 119. <https://doi.org/10.3390/ani9040119>

- Song, S., & Kim, N. (2014). Recycling agricultural wastes as feed for mealworm (*Tenebrio molitor*). *Korean Journal of Applied Entomology*, 53(4), 367–373. <https://doi.org/10.5656/KSAE.2014.10.0.043>
- Spranghers, T., Michiels, J., Vrancx, J., Owyn, A., Eeckhout, M., De Clercq, P., & De Smet, S. (2018). Gut antimicrobial effects and nutritional value of black soldier fly (*Hermetia illucens* L.) prepupae for weaned piglets. *Animal Feed Science and Technology*, 235, 33–42. <https://doi.org/10.1016/j.anifeedsci.2017.08.012>
- St-Hilaire, S., Cranfill, K., McGuire, M. A., Mosley, E. E., Tomberlin, J. K., Newton, L., Sealey, W., Sheppard, C., & Irving, S. (2007). Fish offal recycling by the black soldier fly produces a foodstuff high in omega-3 fatty acids. *Journal of the World Aquaculture Society*, 38(2), 309–313. <https://doi.org/10.1111/j.1749-7345.2007.00101.x>
- Surendra, K. C., Tomberlin J. K., van Huis, A., Cammack, J. A., Heckmann, L.-H. L., & Khanal, S. K. (2020). Rethinking organic wastes bioconversion: Evaluating the potential of the black soldier fly (*Hermetia illucens* (L.)) (Diptera: Stratiomyidae) (BSF). *Waste Management*, 117, 58–80. <https://doi.org/10.1016/j.wasman.2020.07.050>
- Tan, J. K. N., Lee, J. T. E., Chiam, Z., Song, S., Arora, S., Tong, Y. W., & Tan, H. T. W. (2021). Applications of food waste-derived black soldier fly larval frass as incorporated compost, side-dress fertilizer and frass-tea drench for soilless cultivation of leafy vegetables in biochar-based growing media. *Waste Management*, 130, 155–166. <https://doi.org/10.1016/j.wasman.2021.05.025>
- Tang, C., Yang, D., Liao, H., Sun, H., Liu, C., Wei, L., & Li, F. (2019). Edible insects as a food source: A review. *Food Production, Processing and Nutrition*, 1(1), 8. <https://doi.org/10.1186/s43014-019-0008-1>
- Tang, Q., Xu, E., Wang, Z., Xiao, M., Cao, S., Hu, S., Wu, Q., Xiong, Y., Jiang, Z., Wang, F., Yang, G., Wang, L., & Yi, H. (2022). Dietary *Hermetia illucens* larvae meal improves growth performance and intestinal barrier function of weaned pigs under the environment of enterotoxigenic *Escherichia coli* K88. *Frontiers in Nutrition*, 8. <https://doi.org/10.3389/fnut.2021.812011>
- Tanga, C. M., Beesigamukama, D., Kassie, M., Egonyu, P. J., Ghemoh, C. J., Nkoba, K., Subramanian, S., Anyega, A. O., & Ekesi, S. (2022). Performance of black soldier fly frass fertiliser on maize (*Zea mays* L.) growth, yield, nutritional quality, and economic returns. *Journal of Insects as Food and Feed*, 8(2), 185–196. <https://doi.org/10.3920/jiff2021.0012>
- Tomberlin, J. K., Adler, P. H., & Myers, H. M. (2009). Development of the black soldier fly (Diptera: Stratiomyidae) in relation to temperature. *Environmental Entomology*, 38(3), 930–934. <https://doi.org/10.1603/022.038.0347>
- Tomberlin, J. K., & Sheppard, D. C. (2001). Lekking behavior of the black soldier fly (Diptera: Stratiomyidae). *The Florida Entomologist*, 84(4), 729–730. <https://doi.org/10.2307/3496413>
- Tomberlin, J. K., & Sheppard, D. C. (2002). Factors influencing mating and oviposition of black soldier flies (Diptera: Stratiomyidae) in a colony. *Journal of*

- Entomological Science*, 37(4), 345–352. <https://doi.org/10.18474/0749-8004-37.4.345>
- Tomberlin, J. K., Sheppard, D. C., & Joyce, J. A. (2002). Selected life-history traits of black soldier flies (Diptera: Stratiomyidae) reared on three artificial diets. *Annals of the Entomological Society of America*, 95(3), 379–386. [https://doi.org/10.1603/0013-8746\(2002\)095\[0379:Slhtob\]2.0.Co;2](https://doi.org/10.1603/0013-8746(2002)095[0379:Slhtob]2.0.Co;2)
- Tourtois, J., Ali, J. G., & Grieshop, M. J. (2017). Susceptibility of wounded and intact black soldier fly *Hermetia illucens* (L.) (Diptera: Stratiomyidae) to entomopathogenic nematodes. *Journal of Invertebrate Pathology*, 150, 121–129. <https://doi.org/10.1016/j.jip.2017.10.002>
- United Nations Environment Programme. (2021). *Food Waste Index Report 2021*. The United Nations.
- Wachira, M. N., Osuga, I. M., Munguti, J. M., Ambula, M. K., Subramanian, S., & Tanga, C. M. (2021). Efficiency and improved profitability of insect-based aquafeeds for farming Nile tilapia fish (*Oreochromis niloticus* L.). *Animals*, 11(9), 2599. <https://doi.org/10.3390/ani11092599>
- Wang, H., Zhang, Z. J., Czapar, G. F., Winkler, M. K. H., & Zheng, J. G. (2013). A full-scale house fly (Diptera: Muscidae) larvae bioconversion system for value-added swine manure reduction. *Waste Management & Research*, 31(2), 223–231. <https://doi.org/10.1177/0734242x12469431>
- Wei, P., Li, Y., Lai, D., Geng, L., Liu, C., Zhang, J., Shu, C., & Liu, R. (2020). *Protaetia brevitarsis* larvae can feed on and convert spent mushroom substrate from *Auricularia auricula* and *Lentinula edodes* cultivation. *Waste Management*, 114, 234–239. <https://doi.org/10.1016/j.wasman.2020.07.009>
- Weththasinghe, P., Hansen, J. Ø., Nøkland, D., Lagos, L., Rawski, M., & Øverland, M. (2021). Full-fat black soldier fly larvae (*Hermetia illucens*) meal and paste in extruded diets for Atlantic salmon (*Salmo salar*): Effect on physical pellet quality, nutrient digestibility, nutrient utilization and growth performances. *Aquaculture*, 530, 735785. <https://doi.org/10.1016/j.aquaculture.2020.735785>
- Xiong, J., Mao, J., Wang, T., Feng, W., Wang, W., Yang, C., Miao, X., & Wang, C. (2020). Refining and sulfurization of oil from black soldier fly and its application as biodegradable lubricant additive. *Journal of the American Oil Chemists' Society*, 97(11), 1243–1251. <https://doi.org/10.1002/aocs.12403>
- Xu, X., Ji, H., Yu, H., & Zhou, J. (2020). Influence of dietary black soldier fly (*Hermetia illucens* Linnaeus) pulp on growth performance, antioxidant capacity and intestinal health of juvenile mirror carp (*Cyprinus carpio* var. *specularis*). *Aquaculture Nutrition*, 26(2), 432–443. <https://doi.org/10.1111/anu.13005>
- Yu, M., Li, Z., Chen, W., Rong, T., Wang, G., Li, J., & Ma, X. (2019). Use of *Hermetia illucens* larvae as a dietary protein source: Effects on growth performance, carcass traits, and meat quality in finishing pigs. *Meat Science*, 158, 107837. <https://doi.org/10.1016/j.meatsci.2019.05.008>
- Yu, M., Li, Z., Chen, W., Rong, T., Wang, G., Wang, F., & Ma, X. (2020). Evaluation of full-fat *Hermetia illucens* larvae meal as a fishmeal replacement for weanling piglets: Effects on the growth performance, apparent nutrient digestibility, blood

parameters and gut morphology. *Animal Feed Science and Technology*, 264, 114431. <https://doi.org/10.1016/j.anifeedsci.2020.114431>

- Zarantoniello, M., Zimbelli, A., Randazzo, B., Compagni, M. D., Truzzi, C., Antonucci, M., Riolo, P., Loreto, N., Osimani, A., Milanović, V., Giorgini, E., Cardinaletti, G., Tulli, F., Cipriani, R., Gioacchini, G., & Olivotto, I. (2020). Black soldier fly (*Hermetia illucens*) reared on roasted coffee by-product and *Schizochytrium sp.* as a sustainable terrestrial ingredient for aquafeeds production. *Aquaculture*, 518, 734659. <https://doi.org/10.1016/j.aquaculture.2019.734659>
- Zhang, J., Huang, L., He, J., Tomberlin, J. K., Li, J., Lei, C., Sun, M., Liu, Z., & Yu, Z. (2010). An artificial light source influences mating and oviposition of black soldier flies, *Hermetia illucens*. *Journal of Insect Science*, 10(1), 202. <https://doi.org/10.1673/031.010.20201>
- Zheng, L., Hou, Y., Li, W., Yang, S., Li, Q., & Yu, Z. (2012). Biodiesel production from rice straw and restaurant waste employing black soldier fly assisted by microbes. *Energy*, 47(1), 225–229. <https://doi.org/10.1016/j.energy.2012.09.006>
- Zhou, J. S., Liu, S. S., Ji, H., & Yu, H. B. (2018). Effect of replacing dietary fish meal with black soldier fly larvae meal on growth and fatty acid composition of Jian carp (*Cyprinus carpio* var. Jian). *Aquaculture Nutrition*, 24(1), 424–433. <https://doi.org/10.1111/anu.12574>



GRADUATE
RESEARCH
SCHOOL

STATEMENT OF CONTRIBUTION DOCTORATE WITH PUBLICATIONS/MANUSCRIPTS

We, the candidate and the candidate's Primary Supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the candidate's contribution as indicated below in the *Statement of Originality*.

Name of candidate:	Zhongyi Liu
Name/title of Primary Supervisor:	Dr Maria Minor
In which chapter is the manuscript /published work: Chapter 2	
<p>Please select one of the following three options:</p> <p><input checked="" type="radio"/> The manuscript/published work is published or in press</p> <ul style="list-style-type: none"> • Please provide the full reference of the Research Output: Liu, Zhongyi, Maria Minor, Patrick C. H. Morel, and Adriana J. Najar-Rodriguez. 2018. Bioconversion of three organic wastes by black soldier fly (Diptera: Stratiomyidae) larvae. <i>Environmental Entomology</i>, 47(6): 1609–1617. <p><input type="radio"/> The manuscript is currently under review for publication – please indicate:</p> <ul style="list-style-type: none"> • The name of the journal: • The percentage of the manuscript/published work that was contributed by the candidate: 85.00 • Describe the contribution that the candidate has made to the manuscript/published work: Zhongyi Liu performed the majority of the work for the article that forms Chapter 2 of this thesis, including experimental design, data collection, data analysis, and manuscript writing, with assistance from the co-authors of the article. <p><input type="radio"/> It is intended that the manuscript will be published, but it has not yet been submitted to a journal</p>	
Candidate's Signature:	Zhongyi Liu <small>Digitally signed by Zhongyi Liu Date: 2021.10.12 21:05:19 +13'00'</small>
Date:	12-Oct-2021
Primary Supervisor's Signature:	Maria Minor <small>Digitally signed by Maria Minor Date: 2021.10.13 17:44:42 +13'00'</small>
Date:	13-Oct-2021

This form should appear at the end of each thesis chapter/section/appendix submitted as a manuscript/publication or collected as an appendix at the end of the thesis.

Chapter 2 Bioconversion of three organic wastes by black soldier fly (Diptera: Stratiomyidae) larvae



Five-day-old larvae of the black soldier fly (*Hermetia illucens*) (image by Z. Liu, 2016; CC BY-NC)

This chapter has been published as:

Liu, Zhongyi, Maria Minor, Patrick C. H. Morel, and Adriana J. Najar-Rodriguez. 2018. Bioconversion of three organic wastes by black soldier fly (Diptera: Stratiomyidae) larvae. *Environmental Entomology*, 47(6): 1609–1617.

Reproduced here with some minor modifications in style and formatting.

The original publication is available at: <https://doi.org/10.1093/ee/nvy141>

Copyright agreement/license can be found in the Appendices section of this thesis.

Abstract

This study aimed to determine the suitability of several organic waste substrates to be processed by the larvae of the black soldier fly, *Hermetia illucens* (L.) (Diptera: Stratiomyidae) (BSFL) in a value-added bioconversion system. Three types of organic waste (brewer's waste, solid phase of pig manure, and semidigested grass) were tested and compared with a standard larval diet, broil (wheat middling). Larval survival and growth, chemical composition of the resulting prepupae, conversion ratios of nutrients and waste dry matter, and waste reduction rate were measured. Larval survival was high in all tested substrates. Compared with the larvae fed pig manure or semidigested grass, those fed standard diet or brewer's waste showed shorter development time, higher weight gain, and higher prepupal crude protein and crude fat content. BSFL also reduce more dry matter in the standard diet or in brewer's waste than in the other two substrates. On the other hand, larvae fed semidigested grass took 70 d to complete development and suffered fat loss. Thus, we suggest that brewer's waste is the most suitable substrate among the selected wastes for being processed by BSFL, whereas semidigested grass is an unsuitable substrate. We found that lignin had a significantly negative effect on larval growth, and emphasize the importance of applying lignin-digesting microorganisms to lignin-rich substrates being converted by BSFL. Moreover, a protein:fat:digestible carbohydrate ratio of 2:1:2 was hypothesized to benefit larval development.

Keywords: black soldier fly, larval development, organic waste bioconversion, nutritional composition, lignin

2.1 Introduction

Organic waste is generated worldwide at a rapid rate. Annual organic waste generation was about 600 million tons within the first decades of this century, and is projected to be approximately one billion tons by 2025 (Hoorweg & Bhada-Tata, 2012). The current prevailing approach for organic waste disposal is landfill, which can potentially contribute to global warming and soil and water pollution, thus becoming a growing concern for society (Christensen *et al.*, 2010; Hoorweg & Bhada-Tata, 2012).

A promising alternative solution for organic waste management is the use of insects to convert organic waste into insect biomass, which can then be used as an animal feed ingredient (El Boushy, 1991; Elissen *et al.*, 2006; Makkar *et al.*, 2014; Ramos-Elorduy *et al.*, 2002). Black soldier fly (BSF) *Hermetia illucens* (L.) (Diptera: Stratiomyidae) larvae have been suggested as effective organisms for converting many types of organic waste, such as food waste, waste plant tissues, animal offal, and animal manure, into insect biomass (Kalová & Borkovcová, 2013; Myers *et al.*, 2008; Nguyen *et al.*, 2015; St-Hilaire *et al.*, 2007a). The resulting larval biomass has been proven to be a useful feed ingredient for farmed animals such as pigs, chickens, and fish (Hale, 1973; Lock *et al.*, 2016; Newton *et al.*, 1977; St-Hilaire *et al.*, 2007b).

In this study, the performance of BSF larvae (BSFL) in converting locally-generated organic waste into insect biomass was assessed in a bench-top laboratory experiment, where larval feeding rate (i.e., the amount of diet offered to an individual larva at a certain frequency) was fixed, and all the prepupae generated by the experiment were harvested. Three types of organic waste, i.e., brewer's waste, semidigested grass, and the solid phase of pig manure, were selected based on their current management in relevant businesses in New Zealand, and the associated economic and environmental costs. Brewer's waste

is the spent barley from beer production. In New Zealand, some quantity of it is collected by pig farmers, while the rest, especially that generated by urban craft brewers, is dumped to landfill. Semidigested grass, which is the paunch waste from deer slaughtering, is all dumped to landfill. Organic waste was reported to account for the largest proportion of landfill in New Zealand (Ministry for the Environment, 2009), with the single landfill (Victoria Flats landfill) in the South Island reporting to have received 152 tons of organic waste per week (Waste Not Consulting, 2016). Thus, landfilling of brewer's waste and semidigested grass does not only increase financial costs in the relevant industries, but also increases the burden of organic waste management in New Zealand. The solid phase of pig manure (hereafter, pig manure) is often used as an organic fertilizer after being composted (Hjorth *et al.*, 2010). However, during conventional composting, carbon and nitrogen in pig manure are converted into carbon dioxide, methane, nitrous oxide, and nitrate, leading to greenhouse gas emission and a waste of nutrients (Sommer, 2001; Tiquia *et al.*, 2002).

The selected organic wastes were tested in comparison with broll — a mixture of wheat bran and wheat flour. Broll is normally used for chicken feed and has been used as a standard diet to rear BSFL in our laboratory for several generations. Larval development, prepupal chemical composition, and bioconversion efficiency of BSF were recorded for each type of diet.

BSFL can barely degrade lignin (Zheng *et al.*, 2012), which makes it difficult for them to break down and utilize lignin-protected hemicellulose and cellulose for their growth (Ohkuma, 2003). Therefore, we also conducted a meta-analysis combining the data obtained in the current study with data from published sources, in order to investigate the effect of lignin content on BSF larval growth.

2.2 Materials and methods

2.2.1 Black soldier fly colony

The insects used in this experiment were derived from a colony maintained in a glasshouse at the Plant Growth Unit, Massey University, Palmerston North, New Zealand. The colony was initiated from larvae collected from New Zealand wild populations at Thames, Coromandel Peninsula (37°03'S, 175°52'E), Pukenui, Northland (34°82'S, 173°15'E), and Palmerston North, Manawatu (40°36'S, 175°64'E). The colony had been maintained using food scraps in a BioPod Plus (Prota Culture Ltd.) for 1 yr, and then it was transferred to broll, referred to as the standard diet hereafter, with a similar method as described by Sheppard *et al.* (2002). At the time the experiments described here were conducted, the colony had been maintained on broll for 2 mo. Briefly, while the colony was maintained using BioPod Plus, female flies laid eggs in the pod, where food scraps were fed to the larvae *ad libitum*. The prepupae were collected daily and then transferred into a tray containing vermiculite to facilitate pupation and adult emergence. By contrast, when the colony was maintained on broll, females laid into flutes of corrugated cardboard, where they were attracted to lay eggs by fermented larval-consumed diet (Nakamura *et al.*, 2016). The eggs were removed from the flutes and taken to an insect rearing room (24.5 ± 1.5°C, 40 ± 10% relative humidity [R.H.], 12L:12D) for hatching. Ten egg clutches were placed on 300 g of standard diet in a 500-ml glass jar (ca. 8.5 cm in diameter, 8 cm in depth) sealed with a layer of paper towel and a metal lid frame to initiate a larval 'batch'. The standard diet was prepared by adding 200 ml tap water to 100 g of the diet. Egg clutches were placed on the moist diet during their hatching period, so the humidity of the microenvironment inside the jar was kept high to allow egg hatching (Holmes *et al.*, 2012). It took 2–3 d for egg hatching, and all the fertile clutches

hatched. After 7 d, the larval batch was fed 1,500 g of standard diet for the first 2 wk, and after that 750 g of standard diet weekly until more than 70% of the larvae had reached the prepupal stage. The larvae and prepupae were then left in the consumed diet for 7 d, after which they were taken to the glasshouse to complete metamorphosis.

2.2.2 Organic wastes used for the larval diet experiment

Broll was supplied by the Animal Science Department, Massey University, New Zealand. Brewer's waste was provided by Garage Project, Wellington, New Zealand. Pig manure, i.e., solid phase that had been isolated by a pig-effluent solid-liquid separation system, was collected from Ratanui Development Co. Ltd., Feilding, New Zealand. Semidigested grass was acquired from Ovation New Zealand Ltd., Feilding, New Zealand. Each type of diet was homogenized, randomly divided into 50-g packs, and kept frozen at -20°C for further use. One pack per diet was sent for chemical analyses.

Prior to feeding the different diets to the BSFL, a pack of each diet was removed from the freezer and maintained at room temperature for 12 h to thaw. BSFL diets with a moisture content between 70 and 90% have been shown to benefit larval development (He, 2010). Therefore, the moisture content of the standard diet and pig manure was adjusted to 75 and 79%, respectively. The brewer's waste and semidigested grass diets were not adjusted, as their original moisture content was 78 and 88%, respectively.

2.2.3 Experimental design

More than 10 egg clutches were obtained from the glasshouse within a 24-h period, and transferred to the insect rearing room, as mentioned above. All the egg clutches were placed in a 500-ml glass jar (ca. 8.5 cm in diameter, 8 cm in depth) sealed with a layer of paper towel. The glass jar was placed into a plastic storage box (25 × 25 × 25 cm) with two vented walls. Because high humidity levels are preferable for BSF egg hatching (Holmes *et al.*, 2012), a layer of wet cotton wool was placed at the bottom of the box to increase the humidity within the storage box to $92 \pm 3\%$.

The eggs were observed daily. On the day of hatching, 25 groups of 50 neonatal larvae were each transferred onto 15 g of the standard diet in separate 30-ml plastic jars (2.5 cm in diameter, 7 cm in depth). The jars were then each sealed with a layer of paper towel to prevent larval escape. These 25 jars are hereafter referred to as ‘nursery’ jars. The larvae were then reared in these jars for 7 d.

Thirty 7-d-old larvae were randomly selected from a nursery jar, weighed as a group, and placed into a clean 70-ml plastic jar (4.5 cm in diameter, 5.5 cm in depth) containing either brewer’s waste, semidigested grass, pig manure, or the standard diet. These larvae, which were used to initialize the experiment, are referred to as ‘initial larvae’. Each jar was then sealed with a layer of paper towel. The same method was repeated five times to have five replicates per diet (i.e., in total four diets with five jars per diet). The larvae were supplied with fresh diet at a rate of $200 \text{ mg} \cdot \text{larva}^{-1} \text{ day}^{-1}$. The larvae from the remaining five nursery jars were pooled together and 150 larvae were randomly selected as ‘baseline larvae’. The baseline larvae were weighed as a group, stored at -20°C , and then further analyzed to represent the chemical composition of the initial larvae.

Larval development in all replicate jars was observed every 5 d. During each observation, prepupae were collected while the remaining larvae were provided with fresh diet for the next 5 d, which resembled prepupal self-harvesting (Sheppard *et al.*, 1994). Prepupae do not have gut content because 1) they empty their gut during the last molt, and 2) they do not feed due to their reduced and immobile mouthparts (May, 1961). The prepupae and the consumed diet were stored at -20°C for further analyses. The amount of diet supplied for the next 5 d was adjusted according to the feed rate mentioned above, the number of remaining larvae, and the number of days until the next feeding. Therefore, the total diet supplied per replicate declined with time, which reduced the overall water retention capacity of the diet. Thus, to avoid dehydration of the diet, smaller containers with narrower openings were used to accommodate the remaining larvae as their numbers declined, i.e., 70-ml plastic jars for 15–30 larvae, 30-ml plastic jars for 6–14 larvae, and 6-ml plastic vials (1 cm in diameter, 7.5 cm in depth) for individual larva when less than six larvae were left in a replicate. After the last surviving larvae per diet had turned into prepupae, they were pooled together with those that had emerged earlier and were then sent for chemical analyses.

2.2.4 Chemical analyses

The chemical analyses of all the diets, baseline larvae, and prepupae collected per diet were conducted by the Nutrition Laboratory, Massey University, Palmerston North, New Zealand.

Sample dry weight was determined after drying the samples at 105°C using a Contherm Thermotec 2000 oven (Contherm Scientific Ltd., Lower Hutt, New Zealand), according

to the method 930.15 of the Association of Official Analytical Chemist (Association of Official Analytical Chemists (AOAC), 2005). Ash (inorganic matter) was determined by complete combustion at 550°C in an Elecfurn furnace (Electric Furnace Ltd., Auckland, New Zealand) (AOAC, 2005; method 942.05). Total nitrogen was determined by combustion using a Leco CNS 200 Analyzer (Leco Corporation, St. Joseph, MI, U.S.) (AOAC, 2005; Dumas Method, method 968.06). Fat content was determined by ether extraction using a Tecator Soxtec System HT 1043 Extraction Unit (Foss Analytical, Slangerupgade 69, 3400 Hillerød, Denmark) (AOAC, 2005; method 991.36). Larval diets were analyzed for neutral detergent fiber (NDF), acid detergent fiber (ADF), and lignin content using the Fibretec 2010 Auto Fiber Analysis System (Foss Analytical, Slangerupgade 69, 3400 Hillerød, Denmark) (AOAC, 2005; method 2002.04). The gross energy in all diets was determined using a Leco AC-350 Calorimeter (Leco Corporation).

2.2.5 Calculations and statistics

The nonfiber carbohydrates (NFC) content of all diets was calculated by mass balance. Crude protein was calculated from the total nitrogen in each diet using the generic nitrogen-to-protein conversion factor of 6.25 (Moore *et al.*, 2010).

For each diet, initial larval dry weight was calculated as initial larval fresh weight multiplied by the average dry matter content of the baseline larvae (23.67%). Prepupal dry weight was calculated by dividing the total dry weight of prepupae by the number of prepupae. Larval weight gain was determined using prepupal dry weight minus initial larval dry weight. Average weight gaining rate was calculated as larval weight gain divided by developmental time.

The mineral, crude protein, and crude fat content in the initial larvae were calculated using the nutritional profile of the baseline larvae. The content of these nutrients in the resulting prepupae per diet was measured as aforementioned. The weight of mineral and crude protein in the initial larvae and prepupae was calculated using their fresh weights and nutritional profiles. The conversion ratios of mineral and crude protein in each diet were calculated as the difference in weights between the prepupae in each diet and the initial larvae, divided by the total input of these two components (mineral and crude protein), respectively.

The ratios of waste dry matter that had been reduced, converted into insect biomass, or metabolized were calculated for each diet using equation (1), (2), or (3), respectively, whereby W is the total dry weight of the organic waste material supplied during the experiment, R is the total dry weight of the residue after the experiment and G is the difference between prepupal and initial larval dry weight. The initial larval dry weight was estimated using the fresh weight of the initial larvae and the dry matter content of the baseline larvae.

$$\text{Ratio of reduced waste dry matter} = \frac{W - R}{W} \times 100\% \quad (1)$$

$$\text{Ratio of converted waste dry matter} = \frac{G}{W} \times 100\% \quad (2)$$

$$\text{Ratio of metabolized waste dry matter} = \frac{R - G}{W} \times 100\% \quad (3)$$

Waste conversion efficiency measures the percentage of diet dry matter loss that was converted to insect biomass. This parameter was calculated for each diet using equation (4). Following Diener *et al.* (2009), waste reduction rate was calculated using equation

(5), whereby T is the average developmental time.

$$\text{Waste conversion efficiency} = \frac{G}{W - R} \times 100\% \quad (4)$$

$$\text{Waste reduction rate} = \frac{W - R}{T} \times 100\% \quad (5)$$

Average prepupal dry weight, conversion ratios of mineral, crude protein, and ratio of converted waste dry matter were analyzed using one-way analyses of variances (ANOVAs), followed by Tukey's honestly significant differences post-hoc tests if the overall *F*-test was significant. Survival, developmental time, weight gaining rate, and waste conversion efficiency were analyzed using Welch ANOVAs followed by Games–Howell tests, because the assumption of homogeneity of variance for standard ANOVA was violated for these parameters. Outliers were found in waste reduction rate and ratios of reduced and metabolized waste dry matter. Therefore, the Kruskal–Wallis *H* tests were used for these parameters, followed by Mann–Whitney *U* tests with Bonferroni corrections for multiple comparisons. General linear models (using normal distribution) were used in the meta-analysis. Calculations and statistical analyses were performed using SPSS Statistics v. 23 (SPSS Inc., Chicago, IL), with the significance level for all tests set at $\alpha = 0.05$.

2.2.6 Impact of lignin on larval growth: a meta-analysis

To analyze the impact of lignin on larval growth, the data from the current study and from two published studies by Rehman *et al.* (2017a, b) were used. Due to the different definitions of final larval dry weight used in the three studies, and the lack of initial larval

dry weight in Rehman *et al.* (2017a, b), larval growth rate was used in the meta-analysis; this was calculated by dividing final larval dry weight by developmental time. Considering possible effects from not only lignin content, but also from the experimental setup (e.g., black soldier fly strain, temperature, larval density, etc.) *per se*, and the interaction between the experimental setup and lignin content, we combined the observations of the three experiments and fitted the data to a general linear model, with experiment as a fixed factor and lignin content as a covariate. A scatter plot between actual and predicted larval growth rate was drawn to illustrate the fit of the model with the data.

The same meta-analysis was also conducted for NDF ($R^2 = 81.00\%$; Table S2.1), ADF ($R^2 = 94.22\%$; Table S2.3), hemicellulose (calculated as $\text{NDF} - \text{ADF}$; $R^2 = 35.11\%$; Table S2.5), and cellulose (calculated as $\text{ADF} - \text{lignin}$; $R^2 = 90.72\%$; Table S2.7). Hemicellulose did not have a significant effect on larval growth (Tables S2.5 and S2.6), while all the other types of fiber showed significant negative effects on larval growth (Tables S2.2, S2.4, and S2.8). Moreover, among all the types of fiber addressed by our meta-analyses, lignin ($R^2 = 95.40\%$; Table 2.3) had the strongest impact on larval growth (Table 2.4, Tables S2.2, S2.4, and S2.8).

2.3 Results

2.3.1 Chemical composition of the selected organic wastes

The organic wastes used in this study varied in their chemical composition and were shown to have a considerable amount of nutrients that could be potentially converted into insect biomass (Table 2.1). For instance, the highest ash content was found in pig manure.

Both brewer's waste and pig manure were rich in protein, whereas brewer's waste had the highest fat content among the tested diets (Table 2.1). Fiber composition varied across diets. Approximately 60 to 75% of the dry matter in the selected organic wastes was comprised of plant fiber (Table 2.1). The NFC content of the diets was not determined by our chemical analyses, but instead was calculated by mass balance. The standard diet had the highest NFC content, followed by brewer's waste and semidigested grass. Pig manure contained no NFC (Table 2.1).

2.3.2 Larval development and survival

Larval survival was not significantly different across diets ($F_{3, 8.5} = 3.61$; $P = 0.062$) (Table 2.2). About 98% of the larvae fed the standard diet, brewer's waste, and pig manure survived during the experiment, while a lower percentage of larvae survived on semidigested grass. Developmental time was, by contrast, significantly affected by the diet ($F_{3, 7.45} = 61.97$; $P < 0.001$) (Table 2.2). Larvae fed brewer's waste took significantly less time to develop into prepupae than those fed the standard diet and pig manure ($F_{2, 12} = 21.73$; $P < 0.001$), with no significant differences found between the latter two diets. The longest larval developmental time (70 d) was recorded in semidigested grass. Average weight gaining rate of larvae significantly differed across the different diets ($F_{3, 7.27} = 1120.83$; $P < 0.001$), with the highest weight gaining rate observed in the standard diet, followed by brewer's waste, pig manure, and semidigested grass (Table 2.2). The same pattern was observed for prepupal weight ($F_{3, 16} = 419.58$; $P = 0.001$) (Table 2.2). The diets in which larvae had a significantly higher weight gaining rate also resulted in significantly higher prepupal weight (Table 2.2). Given the excessively long developmental time of larvae fed semidigested grass and their little weight gain, this diet was considered as unsuitable to be converted by BSFL, and thus was excluded from further analyses regarding waste reduction and bioconversion.

Chapter 2

Table 2.1 Chemical composition of a standard diet (broll), brewer's waste, pig manure, and semidigested grass used in this study as feeding substrates for black soldier fly (*Hermetia illucens* L.) larvae.

Chemical composition	Standard diet	Brewer's waste	Pig manure	Semidigested grass
Moisture content (%)	75	78	79	88
Ash (g/100 g)	4.6	3.7	13.7	7.5
Crude protein (g/100 g)	15.6	22.6	26.6	15.0
Crude fat (g/100 g)	2.9	5.8	1.2	1.4
Neutral detergent fiber (g/100 g)	39.7	59.0	61.2	75.1
Acid detergent fiber (g/100 g)	12.8	20.3	33.3	44.7
Lignin (g/100 g)	3.6	4.4	11.4	17.7
NFC (g/100 g)	37.2	8.9	0	1
Gross energy (kj/100 g)	1887.0	2084.0	1837.3	1932.1

The values are presented on a dry matter basis except for moisture content.

Table 2.2 Survival, developmental time, larval weight gain, average weight gaining rate, and prepupal weight for black soldier fly (*Hermetia illucens* L.) larvae fed a standard diet (broll), brewer's waste, pig manure, and semidigested grass.

Parameters	Standard diet	Brewer's waste	Pig manure	Semidigested grass
Survival (%)	98.00 ± 0.82	98.00 ± 1.33	98.67 ± 0.82	88.67 ± 2.71
Developmental time (days)	17.32 ± 0.24 ^b	14.97 ± 0.10 ^a	17.30 ± 0.43 ^b	70.00 ± 5.09 ^c
Larval weight gain (mg)	48.48 ± 1.62 ^d	34.54 ± 0.73 ^c	13.98 ± 1.19 ^b	2.63 ± 0.79 ^a
Average weight gaining rate (mg·day ⁻¹)	2.80 ± 0.06 ^d	2.31 ± 0.04 ^c	0.80 ± 0.07 ^b	0.04 ± 0.01 ^a
Prepupal dry weight (mg)	58.70 ± 1.47 ^d	45.48 ± 0.67 ^c	24.16 ± 0.90 ^b	12.98 ± 0.80 ^a

Different letters (^{a,b,c,d}) within a row indicate significant differences across diets ($P < 0.05$). Larval weight gain, average weight gaining rate and prepupal weight are presented on a dry matter basis.

The values are means ± SE, $n = 5$.

In the meta-analysis of the impact of diet lignin content, experimental setup, and their interaction on larval growth, it was found that all three factors had significant effects on larval growth rate, and the effect of experimental setup was greater than that of the other two sources of variation (Tables 2.3 and 2.4). The general linear model appeared to be robust (Table 2.3, Figure 2.1). Lignin content of the diet was negatively correlated with larval growth rate (Table 2.4, Figure 2.2).

Table 2.3 Summary of the general linear model conducted as part of the meta-analysis carried out in this study to test the impact of lignin on black soldier fly (*Hermetia illucens* L.) larval growth rate.

Source	df	Sequential SS	<i>F</i>	<i>P</i>
Lignin content	1	4.56	94.11	<0.001
Experimental setup	2	3.02	31.11	<0.001
Lignin content × experimental setup	2	2.47	25.48	<0.001
Error	10	0.48		
Total	15	10.54		

Data from this study and Rehman *et al.* (2017a, b) was used to build the general linear model, with experiment being a fixed factor and lignin content as a covariate.

$R^2 = 95.40\%$, adjusted $R^2 = 93.10\%$, predicted $R^2 = 83.78\%$.

Table 2.4 Meta-analysis testing the impact of diet lignin content and experimental setup on black soldier fly (*Hermetia illucens* L.) larval growth rate.

Term	Coefficient	SE	<i>T</i>	<i>P</i>
Intercept	2.53	0.19	13.27	<0.001
Lignin content	-0.12	0.02	-6.75	<0.001
Experimental setup				
This study	1.55	0.23	6.89	<0.001
Rehman <i>et al.</i> 2017a	-0.39	0.35	-1.13	0.28
Lignin content × experimental setup				
This study	-0.10	0.02	-4.88	0.001
Rehman <i>et al.</i> 2017a	0.01	0.03	0.16	0.88

Data from this study and Rehman *et al.* (2017a, b) was used to build the general linear model, with experimental setup as a fixed factor and lignin content as a covariate.

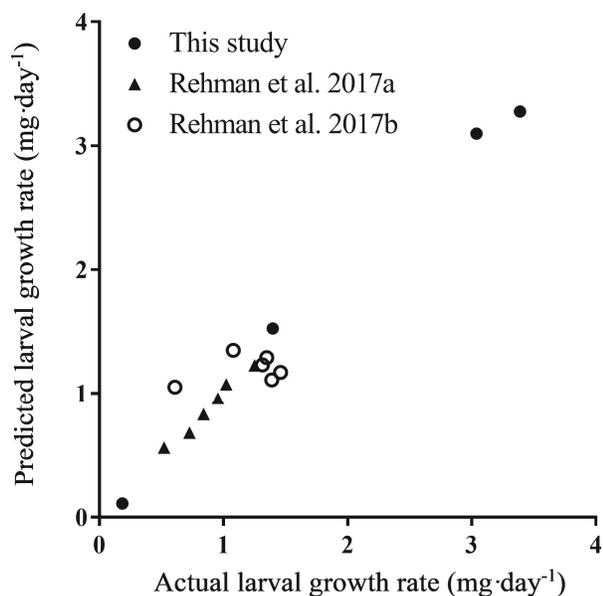


Figure 2.1 Scatter plot of the actual black soldier fly (BSF) (*Hermetia illucens* L.) larval growth rates observed in this study (our data) and those by Rehman *et al.* (2017a, b) versus the predictions based on our meta-analysis of the impact of lignin content on BSF larval growth.

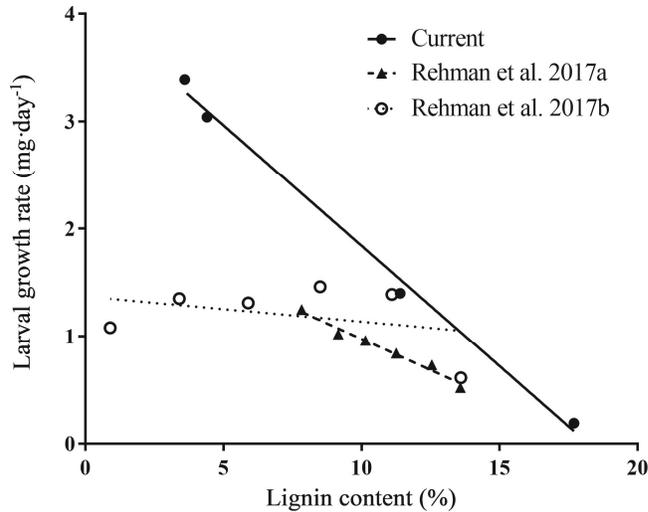


Figure 2.2 Growth rates of black soldier fly (BSF) (*Hermetia illucens* L.) larvae as related to the lignin content in the diets used in this study (our data) and those used by Rehman *et al.* (2017a, b). Fitted lines are those estimated by linear regression.

2.3.3 BSFL chemical composition

The different types of diet fed to the BSFL resulted in significant differences in the chemical composition of the resulting prepupae. There were no significant differences in prepupal crude protein content either between the standard diet and brewer's waste, or between pig manure and semidigested grass, but the prepupae from the former two diets had significantly higher crude protein content than those from the latter two diets ($F_{3, 16} = 42.83$; $P < 0.001$) (Table 2.5). All diets were significantly different from each other for mineral (i.e., ash) ($F_{3, 7.89} = 1705.28$; $P < 0.001$) and crude fat ($F_{3, 16} = 1430.25$; $P < 0.001$) content (Table 2.5). The highest mineral content was found in the prepupae fed semidigested grass, followed by pig manure, brewer's waste, and the standard diet. The mineral content of the prepupae fed pig manure and semidigested grass was three to seven times higher than that of the prepupae fed the standard diet or brewer's waste. The crude

fat content showed an opposite pattern to the mineral content, with the highest crude fat content found in the prepupae fed the standard diet and the lowest in the prepupae fed semidigested grass.

Changes in BSFL chemical composition were observed during the experiment. The crude protein content of BSFL biomass decreased by 21–28% during larval development, while the crude fat content increased by 41–193%, except when larvae were fed semidigested grass. BSFL mineral content decreased when larvae were fed the standard diet or brewer's waste, but increased by more than 260% when fed pig manure or semidigested grass (Table 2.5).

Table 2.5 Chemical composition of black soldier fly (*Hermetia illucens* L.) larvae at the start of the experiment (baseline larvae) and of resulting prepupae after feeding the larvae on a standard diet (broll), brewer's waste, pig manure, or semidigested grass.

Chemical composition	Baseline larvae	Prepupae			
		Standard diet	Brewer's waste	Pig manure	Semidigested grass
Ash content (g/100 g)	6.52	3.97 ± 0.23 ^a	5.72 ± 0.10 ^b	23.95 ± 0.24 ^c	29.56 ± 0.72 ^d
Crude protein content (g/100 g)	64.05	50.55 ± 0.25 ^b	49.89 ± 0.21 ^b	45.94 ± 0.48 ^a	46.39 ± 0.42 ^a
Crude fat content (g/100 g)	12.42	36.38 ± 0.43 ^d	33.71 ± 0.31 ^c	17.53 ± 0.40 ^b	5.51 ± 0.37 ^a

Different letters (^{a,b,c,d}) within a row indicate significant differences across diets ($P < 0.05$). The values are means ± SE presented on a dry matter basis, $n = 5$.

2.3.4 Bioconversion and Waste Reduction

BSFL performed significantly differently in terms of reducing and recovering dry matter when fed different diets (Table 2.6). BSFL recovered a significantly higher proportion ($F_{2,12} = 234.67$; $P < 0.001$) and metabolized a significantly higher proportion ($H_2 = 11.58$; $P = 0.003$) of dry matter when fed the standard diet or brewer's waste, resulting in significantly higher dry matter reduction ratios ($H_2 = 11.58$; $P = 0.003$) compared to when fed pig manure, with no significant differences between the former two diets. BSFL reduced dry matter from the standard diet and brewer's waste at similar rates, but reduced dry matter significantly more slowly when fed pig manure ($H_2 = 11.58$; $P = 0.008$). However, there were no significant differences in waste conversion efficiency among the three diets ($F_{2,12} = 4.41$; $P = 0.063$). During the experiment, and for all diets (except semidigested grass), 13–40% of waste dry matter was reduced, but only up to about 17% of the reduced dry matter was converted into insect biomass.

BSFL converted a significantly higher proportion of minerals from brewer's waste than from the standard diet or pig manure ($F_{2,12} = 409.13$; $P < 0.001$) (Table 2.6). Mineral conversion ratio did not differ significantly between the standard diet and pig manure. All diets differed significantly from each other for crude protein conversion ratio ($F_{2,12} = 35.60$; $P < 0.001$), with the highest crude protein conversion ratio in larvae fed the standard diet, followed by brewer's waste and pig manure (Table 2.6).

Table 2.6 Waste reduction and bioconversion by black soldier fly (*Hermetia illucens* L.) larvae when fed a standard diet (broll), brewer's waste or pig manure.

Parameters	Standard diet	Brewer's waste	Pig manure
Total dry matter per replicate			
Waste fed to larvae (g)	26.12 ± 0.36 ^c	19.66 ± 0.16 ^a	21.97 ± 0.56 ^b
Residue (g)	17.28 ± 0.22 ^b	12.05 ± 0.25 ^a	18.91 ± 0.37 ^c
Proportion of waste dry matter			
Reduced (%)	33.76 ± 1.51 ^b	38.69 ± 1.24 ^b	13.81 ± 1.56 ^a
Converted (%)	5.43 ± 0.12 ^b	5.13 ± 0.12 ^b	1.86 ± 0.15 ^a
Metabolized (%)	28.34 ± 1.50 ^b	33.56 ± 1.29 ^b	11.95 ± 1.66 ^a
Waste			
Conversion efficiency (%)	16.19 ± 0.69	13.33 ± 0.65	14.65 ± 2.73
Reduction rate (mg/day)	509.41 ± 23.58 ^b	508.01 ± 14.87 ^b	175.32 ± 19.85 ^a
Conversion ratios			
Mineral (%)	4.02 ± 0.38 ^a	7.60 ± 0.27 ^b	5.02 ± 0.26 ^a
Crude protein (%)	16.59 ± 0.48 ^c	10.30 ± 0.25 ^b	2.26 ± 0.30 ^a

Different letters (^{a,b,c}) within a row indicate significant differences across diets ($P < 0.05$). The values are means ± SE on a dry matter basis, $n = 5$.

2.4 Discussion

Three selected organic wastes (brewer's waste, pig manure, and semidigested grass) were assessed in this study, in comparison with the standard diet (broll), for their suitability to be converted into BSFL biomass. During our experiment, more than 88% of the BSFL survived on the selected diets. BSFL gained significantly more weight when fed the standard diet or brewer's waste, compared to when fed pig manure or semidigested grass, resulting in significantly higher prepupal weights. Comparable crude protein and fat content was measured in the prepupae fed the standard diet and brewer's waste, which was significantly higher than that in the prepupae fed pig manure and semidigested grass. Moreover, BSFL were able to reduce significantly more dry matter from the standard diet and brewer's waste than from the other two diets. Hence, among the selected organic wastes, brewer's waste is the most suitable to be processed by the BSFL.

BSFL fed semidigested grass took 70 d to develop into prepupal stage and gained negligible weight (0.04 mg/day). Moreover, it was noted that these larvae survived on their stored fat, as their fat content at the prepupal stage was lower than that of the baseline larvae (Table 2.5) (Tschirner & Simon, 2015). Therefore, semidigested grass is considered unsuitable for BSF-bioconversion. The poor larval growth on semidigested grass could be due to its high lignin content (17.7%), which is barely degradable by the BSF-bioconversion system (Zheng *et al.*, 2012). Indeed, the meta-analysis of the impact of lignin on larval growth showed that larval growth rate, in terms of dry weight accumulation, is generally negatively correlated with lignin content in larval diet (Table 2.4, Figure 2.2). Moreover, among the types of fiber addressed by our meta-analyses (ADF, NDF, hemicellulose, cellulose, lignin) all except hemicellulose had negative effects on larval growth, but lignin ($R^2 = 95.40\%$; Table 2.3) had the strongest impact.

The underlying mechanism is that, in nature, lignin is the least biodegradable plant fiber, and it protects hemicellulose and cellulose from being degraded (Brune, 2014; Ohkuma, 2003; Ribeiro *et al.*, 2016; Vanholme *et al.*, 2010). Thus, the degradation of lignin in a BSF-bioconversion system limited the usage of plant fiber as a carbohydrate source for the larvae.

A possible solution to this issue is co-conversion by microorganisms and BSFL. In Zheng *et al.* (2012), without the aid of microorganisms BSFL only degraded 0.8% of lignin in a mixture of rice straw and restaurant waste, whereas the addition of a commercial microorganism product (Rid-X) boosted the lignin degradation rate by 10 times. Microorganisms can also be obtained from natural resources, such as dairy manure (Ozbayram *et al.*, 2018). Indeed, the lignin degradation rates by BSFL in substrates including dairy manure ranged from 25 to 66% (Li *et al.* 2011, Rehman *et al.* 2017a, b). The degradation of lignin exposes hemicellulose and cellulose, which can be further broken down (Pérez *et al.*, 2002) and then utilized by BSFL. Some white-rot fungi (e.g., *Phanerochaete chrysosporium* (Burds.) (Polyporales: Phanerochaetaceae), *Pleurotus ostreatus* (Jacq.) (Agaricales: Pleurotaceae), and *Ceriporiopsis subvermispora* ((Pilát Gilb. & Ryvardeen) (Polyporales: Meruliaceae) have been shown to selectively degrade lignin (Cullen, 1997; Vicuña, 2000), and thus may be useful for pretreatment of lignin-rich substrates before BSF-bioconversion.

The differences in BSFL life history traits across the diets tested here might also result from the differences in the composition of the digestible macronutrients in each diet. Oonincx *et al.* (2015) reported that a protein to fat ratio of 2.3:1 led to the fastest BSFL development in their experiment. Cammack and Tomberlin (2017) tested BSFL on diets with different protein (a mixture of casein, peptone, and egg albumen) to digestible

carbohydrate (a mixture of sucrose and white dextrin) ratios. They suggested that a protein to digestible carbohydrate ratio of 1:1 could significantly benefit BSFL development. Based on these two studies, we surmised that the balance of protein:fat:digestible carbohydrate ratio of BSFL diet is roughly 2:1:2. As discussed above, without effective removal of lignin, BSFL does not seem to be able to use plant fiber as a major source of carbohydrates for development. Thus, the prevailing digestible carbohydrate present in our larval diets was most likely NFC. The protein:fat:NFC ratios for the standard diet, brewer's waste, pig manure, and semidigested grass were approximately 5:1:13, 4:1:1.5, 22:1:0, and 10:1:0.7, respectively. Given that the most balanced digestible macronutrient ratio was found in brewer's waste, it is not surprising that the fastest BSFL development was recorded in this diet.

In this study, the chemical composition of BSF prepupae was found to be affected by the larval diet. The protein content of the resulting prepupae ranged from 45.9 to 50.6%, which is consistent with previous studies (reviewed by Makkar *et al.*, 2014). Interestingly, the protein content of the prepupae was not directly related to that of the larval diet. For example, the protein content of the standard diet was only 70% of that of brewer's waste, but the prepupae fed those two diets had similar protein content. A possible explanation is that compared with the larvae fed brewer's waste, those fed the standard diet had increased protein assimilation efficiency to maintain protein homeostasis (Simpson & Simpson, 1990). This is supported by the evidence that BSFL showed similar waste dry matter conversion ratios when fed the standard diet and on brewer's waste, but converted a significantly higher proportion of protein into larval biomass when fed the standard diet than when fed brewer's waste (Table 2.6). Such phenomenon is referred to as a compensatory response to nutritional deficiency and/or imbalance, which is mainly found in phytophagous insect species (Simpson & Simpson, 1990). For example, fifth instar

Locusta migratoria (L.) (Orthoptera: Acrididae) tended to assimilate more nitrogen and oxidize excess carbohydrate when fed a diet with high protein:digestible carbohydrate ratio (Zanotto *et al.*, 1993). In our experiment, conversion ratios of protein were higher than those of dry matter for all diets (except semidigested grass, as its dry matter conversion ratio was not determined), suggesting that dietary protein is well assimilated by BSFL, which is consistent with Oonincx *et al.* (2015).

The mineral content in BSFL is generally higher than in other insects investigated for their potential as food and/or feed ingredients, such as housefly larvae, mealworm larvae, grasshoppers, crickets, and silkworm (Makkar *et al.*, 2014). In this study, the mineral content of the prepupae ranged from 3.97 to 29.56% (Table 2.5). The prepupae reared on pig manure and semidigested grass had notably high mineral content, which might be a result of the high mineral content in these substrates (Liland *et al.*, 2017). Another possibility is that the BSFL reared on pig manure and semidigested grass had ingested extra diet for energy homeostasis as a compensation due to the paucity of digestible carbohydrate in these diets, which might have eventually led to an excessive mineral intake.

The efficiencies of waste reduction and bioconversion are of primary concern for the development of a BSF-bioconversion system. In the present study, up to 38.7% of the dry matter was reduced during larval feeding, which is relatively lower than reported values found in the literature. For example, Diener *et al.* (2009) reported a maximum dry matter reduction ratio of 44.1%, while Myers *et al.* (2008) found that BSFL could reduce more than 50% of waste dry matter. We found that less than 16.2% of the reduced dry matter had been converted into insect biomass, while more than 24.4% was reported in Diener *et al.* (2009). A possible explanation for the lower efficiencies of waste reduction and

bioconversion recorded in our study is that the feeding rate used for each diet was not optimal. During our study, an oversupply of the standard diet or brewer's waste was always observed, indicated by the presence of high amounts of unconsumed substrate, whereas the larvae fed pig manure and semidigested grass probably suffered food deficiency judging by the low larval weight gain when fed these diets. Therefore, further studies should address the optimal feeding rate for each of these diets, as per Diener *et al.* (2009) and Myers *et al.* (2008). Also, the ambient relative humidity in our study ($40 \pm 10\%$ R.H.) was lower than that used by Myers *et al.* (2008) (60% R.H.) and Diener *et al.* (2009) (67% R.H.), which might have led to lower waste reduction than in previous studies. The ambient temperature in our study was lower than those in Myers *et al.* (2008) and Diener *et al.* (2009), which could have had detrimental effects on larval growth (Harnden & Tomberlin, 2016). Higher temperatures have been found to increase feeding rates and metabolic rate of the larvae (Bjørge *et al.*, 2018; Lactin & Johnson, 1995), which would result in higher waste reduction.

Conclusively, the results of this study suggest that BSFL could convert both brewer's waste and pig manure into insect biomass, with brewer's waste being a more suitable diet, while semidigested grass being considered an unsuitable diet. Larval development, prepupal chemical composition, and conversion of protein and mineral are shown to depend on the chemical composition of the diets. Based on our meta-analysis, lignin has a significantly negative effect on larval weight gain. Thus, we suggest using lignin-digesting microorganisms to aid BSF-bioconversion systems in dealing with lignin-rich substrates. We also hypothesize that a protein:fat:digestible carbohydrate ratio of 2:1:2 could benefit larval development. To achieve higher conversion efficiency and waste reduction rates by BSFL, further investigations of the effect of optimal feeding rates, mixed wastes, and temperature and humidity during larval feeding would be warranted.

2.5 Acknowledgments

Z.L. was partially funded by the Bioresource Processing Alliance in New Zealand. We would like to thank Prescient Nutrition Ltd. for assistance with establishing the black soldier fly colony and the two anonymous reviewers for their helpful suggestions.

2.6 References

- Association of Official Analytical Chemists (AOAC). (2005). *Official methods of analysis of AOAC international* (18th ed.). AOAC International.
- Bjørge, J. D., Overgaard, J., Malte, H., Gianotten, N., & Heckmann, L. H. (2018). Role of temperature on growth and metabolic rate in the tenebrionid beetles *Alphitobius diaperinus* and *Tenebrio molitor*. *Journal of Insect Physiology*, *107*, 89–96. <https://doi.org/10.1016/j.jinsphys.2018.02.010>
- Brune, A. (2014). Symbiotic digestion of lignocellulose in termite guts. *Nature Reviews Microbiology*, *12*(3), 168–180. <https://doi.org/10.1038/nrmicro3182>
- Cammack, J. A., & Tomberlin, J. K. (2017). The impact of diet protein and carbohydrate on select life-history traits of the black soldier fly *Hermetia illucens* (L.) (Diptera: Stratiomyidae). *Insects*, *8*(2), 56. <https://doi.org/10.3390/insects8020056>
- Christensen, T. H., Scharff, H., & Hjelmar, O. (2010). Landfilling: Concepts and challenges. In T. H. Christensen (Ed.), *Solid waste technology & management* (pp. 683–694). Blackwell Publishing Ltd. <https://doi.org/10.1002/9780470666883.ch44>
- Cullen, D. (1997). Recent advances on the molecular genetics of ligninolytic fungi. *Journal of Biotechnology*, *53*(2), 273–289. [https://doi.org/10.1016/S01681656\(97\)01684-2](https://doi.org/10.1016/S01681656(97)01684-2)
- Diener, S., Zurbrugg, C., & Tockner, K. (2009). Conversion of organic material by black soldier fly larvae: Establishing optimal feeding rates. *Waste Management & Research*, *27*(6), 603–610. <https://doi.org/10.1177/0734242x09103838>
- El Boushy, A. R. (1991). House-fly pupae as poultry manure converters for animal feed: A review. *Bioresource Technology*, *38*(1), 45–49. [https://doi.org/10.1016/0960-8524\(91\)90220-E](https://doi.org/10.1016/0960-8524(91)90220-E)
- Elissen, H. J. H., Hendrickx, T. L. G., Temmink, H., & Buisman, C. J. N. (2006). A new reactor concept for sludge reduction using aquatic worms. *Water Research*, *40*(20), 3713–3718. <https://doi.org/10.1016/j.watres.2006.08.029>
- Hale, O. M. (1973). Dried *Hermetia illucens* larvae (Diptera: Stratiomyidae) as a feed additive for poultry. *Journal of the Georgia Entomological Society*, *8*, 16–20.

- Harnden, L. M., & Tomberlin, J. K. (2016). Effects of temperature and diet on black soldier fly, *Hermetia illucens* (L.) (Diptera: Stratiomyidae), development. *Forensic Science International*, 266, 109–116. <https://doi.org/10.1016/j.forsciint.2016.05.007>
- He, G. (2010). *Research on the artificial diet water content optimization and enzyme-secreting Bacillus subtilis of black soldier fly (Hermetia illucens)* [Master's thesis, Sun Yat-sen University]. CNKI. <http://cdmd.cnki.com.cn/article/cdmd-10558-2010219038.htm>
- Hjorth, M., Christensen, K. V., Christensen, M. L., & Sommer, S. G. (2010). Solid-liquid separation of animal slurry in theory and practice. A review. *Agronomy for Sustainable Development*, 30(1), 153–180. <https://doi.org/10.1051/agro/2009010>
- Holmes, L. A., Vanlaerhoven, S. L., & Tomberlin, J. K. (2012). Relative humidity effects on the life history of *Hermetia illucens* (Diptera: Stratiomyidae). *Environmental Entomology*, 41(4), 971–978. <https://doi.org/10.1603/en12054>
- Hoornweg, D., & Bhada-Tata, P. (2012). *What a waste: A global review of solid waste management. Urban development series; knowledge papers no. 15*. World Bank.
- Kalová, M., & Borkovcová, M. (2013). Voracious larvae *Hermetia illucens* and treatment of selected types of biodegradable waste. *Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis*, 61(1), 77–83. <https://doi.org/10.11118/actaun201361010077>
- Lactin, D. J., & Johnson, D. L. (1995). Temperature-dependent feeding rates of *Melanoplus sanguinipes* nymphs (Orthoptera: Acrididae) laboratory trials. *Environmental Entomology*, 24(5), 1291–1296. <https://doi.org/10.1093/ee/24.5.1291>
- Li, Q., Zheng, L., Qiu, N., Cai, H., Tomberlin, J. K., & Yu, Z. (2011). Bioconversion of dairy manure by black soldier fly (Diptera: Stratiomyidae) for biodiesel and sugar production. *Waste Management*, 31(6), 1316–1320. <https://doi.org/10.1016/j.wasman.2011.01.005>
- Liland, N. S., Biancarosa, I., Araujo, P., Biemans, D., Bruckner, C. G., Waagbø, R., Torstensen, B. E., & Lock, E. J. (2017). Modulation of nutrient composition of black soldier fly (*Hermetia illucens*) larvae by feeding seaweed-enriched media. *PLOS ONE*, 12(8), e0183188. <https://doi.org/10.1371/journal.pone.0183188>
- Lock, E. R., Arsiwalla, T., & Waagbø, R. (2016). Insect larvae meal as an alternative source of nutrients in the diet of Atlantic salmon (*Salmo salar*) postsmolt. *Aquaculture Nutrition*, 22(6), 1202–1213. <https://doi.org/10.1111/anu.12343>
- Makkar, H. P. S., Tran, G., Heuzé, V., & Ankers, P. (2014). State-of-the-art on use of insects as animal feed. *Animal Feed Science and Technology*, 197, 1–33. <https://doi.org/10.1016/j.anifeedsci.2014.07.008>
- May, B. M. (1961). The occurrence in New Zealand and the life-history of the soldier fly *Hermetia illucens* (L.) (Diptera: Stratiomyidae). *New Zealand Journal of Science*, 4, 55–65.
- Ministry for the Environment. (2009). *Environmental report card: Solid waste composition*. New Zealand Ministry for the Environment.

- Moore, J. C., DeVries, J. W., Lipp, M., Griffiths, J. C., & Abernethy, D. R. (2010). Total protein methods and their potential utility to reduce the risk of food protein adulteration. *Comprehensive Reviews in Food Science and Food Safety*, 9(4), 330–357. <https://doi.org/10.1111/j.1541-4337.2010.00114.x>
- Myers, H. M., Tomberlin, J. K., Lambert, B. D., & Kattes, D. (2008). Development of black soldier fly (Diptera: Stratiomyidae) larvae fed dairy manure. *Environmental Entomology*, 37(1), 11–15. <https://doi.org/10.1093/ee/37.1.11>
- Nakamura, S., Ichiki, R. T., Shimoda, M., & Morioka, S. (2016). Small-scale rearing of the black soldier fly, *Hermetia illucens* (Diptera: Stratiomyidae), in the laboratory: Low-cost and year-round rearing. *Applied Entomology and Zoology*, 51(1), 161–166. <https://doi.org/10.1007/s13355-015-0376-1>
- Newton, G. L., Booram, C. V., Barker, R. W., & Hale, O. M. (1977). Dried *Hermetia Illucens* larvae meal as a supplement for swine. *Journal of Animal Science*, 44(3), 395–400. <https://doi.org/10.2527/jas1977.443395x>
- Nguyen, T. T. X., Tomberlin, J. K., & Vanlaerhoven, S. (2015). Ability of black soldier fly (Diptera: Stratiomyidae) larvae to recycle food waste. *Environmental Entomology*, 44(2), 406–410. <https://doi.org/10.1093/ee/nvv002>
- Ohkuma, M. (2003). Termite symbiotic systems: Efficient bio-recycling of lignocellulose. *Applied Microbiology and Biotechnology*, 61(1), 1–9. <https://doi.org/10.1007/s00253-002-1189-z>
- Oonincx, D. G. A. B., van Broekhoven, S., van Huis, A., & van Loon, J. J. A. (2015). Feed conversion, survival and development, and composition of four insect species on diets composed of food by-products. *PLOS ONE*, 10(12), e0144601. <https://doi.org/10.1371/journal.pone.0144601>
- Ozbayram, E. G., Ince, O., Ince, B., Harms, H., & Kleinsteuber, S. (2018). Comparison of rumen and manure microbiomes and implications for the inoculation of anaerobic digesters. *Microorganisms*, 6(1), 15. <https://doi.org/10.3390/microorganisms6010015>
- Pérez, J., Muñoz-Dorado, J., de la Rubia, T., & Martínez, J. (2002). Biodegradation and biological treatments of cellulose, hemicellulose and lignin: An overview. *International Microbiology*, 5(2), 53–63. <https://doi.org/10.1007/s10123-002-0062-3>
- Ramos-Elorduy, J., González, E. A., Hernández, A. R., & Pino, J. M. (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. <https://doi.org/10.1603/0022-0493-95.1.214>
- Rehman, K. u., Cai, M., Xiao, X., Zheng, L., Wang, H., Soomro, A. A., Zhou, Y., Li, W., Yu, Z., & Zhang, J. (2017a). Cellulose decomposition and larval biomass production from the co-digestion of dairy manure and chicken manure by mini-livestock (*Hermetia illucens* L.). *Journal of Environmental Management*, 196, 458–465. <https://doi.org/10.1016/j.jenvman.2017.03.047>
- Rehman, K. u., Rehman, A., Cai, M., Zheng, L., Xiao, X., Somroo, A. A., Wang, H., Li, W., Yu, Z., & Zhang, J. (2017b). Conversion of mixtures of dairy manure and soybean curd residue by black soldier fly larvae (*Hermetia illucens* L.). *Journal*

- of Cleaner Production*, 154, 366–373. <https://doi.org/10.1016/j.jclepro.2017.04.019>
- Ribeiro, G. O., Gruninger, R. J., Badhan, A., & McAllister, T. A. (2016). Mining the rumen for fibrolytic feed enzymes. *Animal Frontiers*, 6(2), 20–26. <https://doi.org/10.2527/af.2016-0019>
- Sheppard, D. C., Newton, G. L., Thompson, S. A., & Savage, S. (1994). A value added manure management system using the black soldier fly. *Bioresource Technology*, 50(3), 275–279. [https://doi.org/10.1016/0960-8524\(94\)90102-3](https://doi.org/10.1016/0960-8524(94)90102-3)
- Sheppard, D. C., Tomberlin, J. K., Joyce, J. A., Kiser, B. C., & Sumner, S. M. (2002). Rearing methods for the black soldier fly (Diptera: Stratiomyidae). *Journal of Medical Entomology*, 39(4), 695–698. <https://doi.org/10.1603/0022-2585-39.4.695>
- Simpson, S., & Simpson, C. (1990). The mechanisms of nutritional compensation by phytophagous insects. In E. A. Bernays (Ed.), *Insect-plant interactions* (pp. 111–160). CRC Press.
- Sommer, S. G. (2001). Effect of composting on nutrient loss and nitrogen availability of cattle deep litter. *European Journal of Agronomy*, 14(2), 123–133. [https://doi.org/10.1016/S1161-0301\(00\)00087-3](https://doi.org/10.1016/S1161-0301(00)00087-3)
- St-Hilaire, S., Cranfill, K., McGuire, M. A., Mosley, E. E., Tomberlin, J. K., Newton, L., Sealey, W., Sheppard, C., & Irving, S. (2007a). Fish offal recycling by the black soldier fly produces a foodstuff high in omega-3 fatty acids. *Journal of the World Aquaculture Society*, 38(2), 309–313. <https://doi.org/10.1111/j.1749-7345.2007.00101.x>
- St-Hilaire, S., Sheppard, C., Tomberlin, J. K., Irving, S., Newton, L., McGuire, M. A., Mosley, E. E., Hardy, R. W., & Sealey, W. (2007b). Fly prepupae as a feedstuff for rainbow trout, *Oncorhynchus mykiss*. *Journal of the World Aquaculture Society*, 38(1), 59–67. <https://doi.org/10.1111/j.1749-7345.2006.00073.x>
- Tiquia, S. M., Richard, T. L., & Honeyman, M. S. (2002). Carbon, nutrient, and mass loss during composting. *Nutrient Cycling in Agroecosystems*, 62(1), 15–24. <https://doi.org/10.1023/A:1015137922816>
- Tschirner, M., & Simon, A. (2015). Influence of different growing substrates and processing on the nutrient composition of black soldier fly larvae destined for animal feed. *Journal of Insects as Food and Feed*, 1(4), 249–259. <https://doi.org/10.3920/jiff2014.0008>
- Vanholme, R., Van Acker, R., & Boerjan, W. (2010). Potential of *Arabidopsis* systems biology to advance the biofuel field. *Trends in Biotechnology*, 28(11), 543–547. <https://doi.org/10.1016/j.tibtech.2010.07.008>
- Vicuña, R. (2000). Ligninolysis. A very peculiar microbial process. *Molecular Biotechnology*, 14(2), 173–176. <https://doi.org/10.1385/MB:14:2:173>
- Waste Not Consulting. (2016). *Analysis of the composition of solid waste in Queenston Lakes District*. Waste Not Consulting.
- Zanotto, F. P., Simpson, S. J., & Raubenheimer, D. (1993). The regulation of growth by locusts through post-ingestive compensation for variation in the levels of dietary

protein and carbohydrate. *Physiological Entomology*, 18(4), 425–434. <https://doi.org/10.1111/j.1365-3032.1993.tb00617.x>

Zheng, L., Hou, Y., Li, W., Yang, S., Li, Q., & Yu, Z. (2012). Biodiesel production from rice straw and restaurant waste employing black soldier fly assisted by microbes. *Energy*, 47(1), 225–229. <https://doi.org/10.1016/j.energy.2012.09.006>

2.7 Appendix

Table S 2.1 Summary of the general linear model conducted as part of the meta-analysis carried out in this study to test the impact of neutral detergent fiber (NDF) on black soldier fly (*Hermetia illucens* L.) larval growth rate. Data from this study and Rehman *et al.* (2017a, b) was used to build the general linear model, with experiment being a fixed factor and NDF content as a covariate.

Source	df	Sequential SS	<i>F</i>	<i>P</i>
NDF content	1	1.61	8.02	0.018
Experimental setup	2	4.19	10.45	0.004
NDF content × experimental setup	2	2.74	6.84	0.013
Error	10	2.00		
Total	15	10.54		

$R^2 = 81.00\%$, adjusted $R^2 = 71.49\%$, predicted $R^2 = 25.56\%$.

Table S 2.2 Meta-analysis testing the impact of diet neutral detergent fiber (NDF) content and experimental setup on black soldier fly (*Hermetia illucens* L.) larval growth rate. Data from this study and Rehman *et al.* (2017a, b) was used to build the general linear model, with experimental setup as a fixed factor and NDF content as a covariate.

Term	Coefficient	SE	<i>T</i>	<i>P</i>
Intercept	3.80	0.72	5.27	< 0.001
NDF content	-0.04	0.01	-3.32	0.008
Experimental setup				
This study	3.50	0.95	3.70	0.004
Rehman <i>et al.</i> 2017a	-0.93	1.17	-0.79	0.45
NDF content × experimental setup				
This study	-0.05	0.02	-2.89	0.02
Rehman <i>et al.</i> 2017a	0.005	0.02	0.25	0.81

Table S 2.3 Summary of the general linear model conducted as part of the meta-analysis carried out in this study to test the impact of acid detergent fiber (ADF) on black soldier fly (*Hermetia illucens* L.) larval growth rate. Data from this study and Rehman *et al.* (2017a, b) was used to build the general linear model, with experiment being a fixed factor and ADF content as a covariate.

Source	df	Sequential SS	<i>F</i>	<i>P</i>
ADF content	1	4.12	67.65	< 0.001
Experimental setup	2	2.16	17.74	0.001
ADF content × experimental setup	2	3.65	29.92	< 0.001
Error	10	0.61		
Total	15	10.54		

$R^2 = 94.22\%$, adjusted $R^2 = 91.33\%$, predicted $R^2 = 84.86\%$.

Table S 2.4 Meta-analysis testing the impact of diet acid detergent fiber (ADF) content and experimental setup on black soldier fly (*Hermetia illucens* L.) larval growth rate. Data from this study and Rehman *et al.* (2017a, b) was used to build the general linear model, with experimental setup as a fixed factor and ADF content as a covariate.

Term	Coefficient	SE	<i>T</i>	<i>P</i>
Intercept	2.66	0.22	11.79	< 0.001
ADF content	-0.04	0.01	-7.12	< 0.001
Experimental setup				
This study	2.25	0.29	7.84	< 0.001
Rehman <i>et al.</i> 2017a	-0.80	0.34	-2.31	0.04
ADF content × experimental setup				
This study	-0.06	0.01	-7.03	< 0.001
Rehman <i>et al.</i> 2017a	0.02	0.01	1.61	0.14

Table S 2.5 Summary of the general linear model conducted as part of the meta-analysis carried out in this study to test the impact of hemicellulose (calculated as neutral detergent fiber - acid detergent fiber) on black soldier fly (*Hermetia illucens* L.) larval growth rate. Data from this study and Rehman *et al.* (2017a, b) was used to build the general linear model, with experiment being a fixed factor and hemicellulose content as a covariate.

Source	df	Sequential SS	<i>F</i>	<i>P</i>
Hemicellulose content	1	2.80	4.09	0.07
Experimental setup	2	0.78	0.57	0.58
Hemicellulose content × experimental setup	2	0.12	0.09	0.91
Error	10	6.84		
Total	15	10.54		

$R^2 = 35.11\%$, adjusted $R^2 = 2.67\%$, predicted $R^2 < 0.01\%$.

Table S 2.6 Meta-analysis testing the impact of diet hemicellulose (calculated as neutral detergent fiber - acid detergent fiber) content and experimental setup on black soldier fly (*Hermetia illucens* L.) larval growth rate. Data from this study and Rehman *et al.* (2017a, b) was used to build the general linear model, with experimental setup as a fixed factor and hemicellulose content as a covariate.

Term	Coefficient	SE	<i>T</i>	<i>P</i>
Intercept	0.78	4.63	0.17	0.87
Hemicellulose content	0.01	0.28	0.04	0.97
Experimental setup				
This study	-0.67	4.91	-0.14	0.89
Rehman <i>et al.</i> 2017a	-2.35	5.11	-0.46	0.66
Hemicellulose content × experimental setup				
This study	0.05	0.28	0.18	0.86
Rehman <i>et al.</i> 2017a	0.12	0.30	0.40	0.70

Table S 2.7 Summary of the general linear model conducted as part of the meta-analysis carried out in this study to test the impact of cellulose (calculated as acid detergent fiber - lignin) on black soldier fly (*Hermetia illucens* L.) larval growth rate. Data from this study and Rehman *et al.* (2017a, b) was used to build the general linear model, with experiment being a fixed factor and cellulose content as a covariate.

Source	df	Sequential SS	<i>F</i>	<i>P</i>
Cellulose content	1	1.89	19.32	0.001
Experimental setup	2	2.67	13.48	0.001
Cellulose content × experimental setup	2	5.03	25.71	< 0.001
Error	10	0.98		
Total	15	10.54		

$R^2 = 90.72\%$, adjusted $R^2 = 86.07\%$, predicted $R^2 < 0.01\%$.

Table S 2.8 Meta-analysis testing the impact of diet cellulose (calculated as acid detergent fiber - lignin) content and experimental setup on black soldier fly (*Hermetia illucens* L.) larval growth rate. Data from this study and Rehman *et al.* (2017a, b) was used to build the general linear model, with experimental setup as a fixed factor and cellulose content as a covariate.

Term	Coefficient	SE	<i>T</i>	<i>P</i>
Intercept	2.77	0.28	9.97	< 0.001
Cellulose content	-0.07	0.01	-6.44	< 0.001
Experimental setup				
This study	2.70	0.39	7.00	< 0.001
Rehman <i>et al.</i> 2017a	-0.99	0.41	-2.42	0.04
Cellulose content × experimental setup				
This study	-0.11	0.02	-6.37	< 0.001
Rehman <i>et al.</i> 2017a	0.04	0.02	2.07	0.07



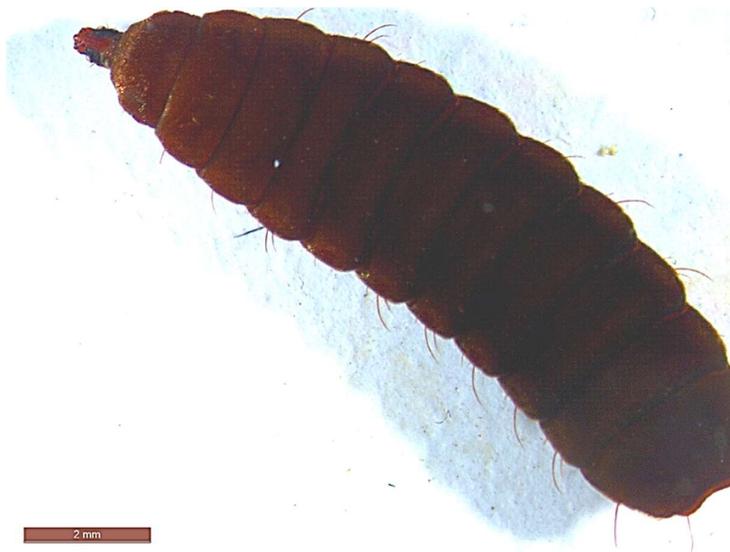
STATEMENT OF CONTRIBUTION DOCTORATE WITH PUBLICATIONS/MANUSCRIPTS

We, the candidate and the candidate's Primary Supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the candidate's contribution as indicated below in the *Statement of Originality*.

Name of candidate:	Zhongyi Liu
Name/title of Primary Supervisor:	Dr Maria Minor
In which chapter is the manuscript /published work: Chapter 3	
Please select one of the following three options:	
<input checked="" type="radio"/> The manuscript/published work is published or in press <ul style="list-style-type: none"> • Please provide the full reference of the Research Output: Liu, Zhongyi, Patrick C. H. Morel, and Maria A. Minor. 2022. Substrate and moisture content effects on pupation of the black soldier fly (Diptera: Stratiomyidae). <i>Journal of Insects as Food and Feed</i>, https://doi.org/10.3920/JIFF2022.0057 	
<input type="radio"/> The manuscript is currently under review for publication – please indicate: <ul style="list-style-type: none"> • The name of the journal: • The percentage of the manuscript/published work that was contributed by the candidate: 85.00 • Describe the contribution that the candidate has made to the manuscript/published work: Zhongyi Liu performed the majority of the work for the article that forms Chapter 3 of this thesis, including experimental design, data collection, data analysis, and manuscript writing, with assistance from the co-authors of the article. 	
<input type="radio"/> It is intended that the manuscript will be published, but it has not yet been submitted to a journal	
Candidate's Signature:	Zhongyi Liu <small>Digitally signed by Zhongyi Liu Date: 2022.12.08 11:21:51 +13'00'</small>
Date:	08-Dec-2022
Primary Supervisor's Signature:	Maria Minor <small>Digitally signed by Maria Minor Date: 2022.12.12 13:21:46 +13'00'</small>
Date:	12-Dec-2022

This form should appear at the end of each thesis chapter/section/appendix submitted as a manuscript/ publication or collected as an appendix at the end of the thesis.

Chapter 3 Substrate and moisture content effects on pupation of the black soldier fly (Diptera: Stratiomyidae)



A black soldier fly (*Hermetia illucens*) prepupa (image by Z. Liu, 2016; CC BY-NC)

This chapter has been published as:

Liu, Zhongyi, Patrick C. H. Morel, and Maria A. Minor. 2022. Substrate and moisture content effects on pupation of the black soldier fly (Diptera: Stratiomyidae). *Journal of Insects as Food and Feed*, <https://doi.org/10.3920/JIFF2022.0057>

Presented here as the original author-created version, i.e., prior to peer review.

The original publication is available at: <https://doi.org/10.3920/JIFF2022.0057>

Copyright agreement/license can be found in the Appendices section of this thesis.

Abstract

Pupation is a critical biological process in the life cycle of the black soldier fly (BSF) *Hermetia illucens* (L.) (Diptera: Stratiomyidae), a beneficial insect species of great environmental and economic potential. In this study, we tested the effects of two pupation substrates (vermiculite and wood chips) and nine moisture content levels on BSF pupation rate and depth. Substrate type and moisture content both affected BSF pupation rate and depth. In all substrate treatments almost all the pupae were found in the top 4 cm depth. At very low moisture content, neither of the pupation substrates tested reduced BSF prepupal mortality, but the presence of vermiculite advanced BSF pupation. Within the range used in this study, increasing moisture content not only reduced BSF prepupal mortality but also brought forward the onset of pupation. We hypothesize that the difference between substrates in their effects on BSF pupation rate lies on the differences in their density and texture, and that moisture content affects BSF pupation rate through changing substrate penetrability and water availability. The tendency of BSF to pupate deeper depended on both substrate type and moisture content, and the effect of moisture content on the pupation depth followed a parabolic trend — BSF tended to pupate shallower when substrate was either too dry or too wet. Our results provide clues for selecting suitable substrate type, moisture content, and substrate depth to facilitate pupation of BSF.

Keywords: *Hermetia illucens*, vermiculite, wood chips, water, metamorphosis

3.1 Introduction

The black soldier fly (BSF) *Hermetia illucens* (L.) (Diptera: Stratiomyidae) is of great environmental and economic potential in dealing with a broad range of organic wastes and by-products (e.g., Bohm *et al.*, 2022; Cai *et al.*, 2019; Cammack *et al.*, 2021; Gao *et al.*, 2019; Mahmood *et al.*, 2021), developing sustainable feed products for different animals (e.g., Dörper *et al.*, 2021; Hu *et al.*, 2020; Kar *et al.*, 2021; Richardson *et al.*, 2021), and producing organic fertilizer (Beesigamukama *et al.*, 2020; Beesigamukama *et al.*, 2021; Cai *et al.*, 2019). Driven by increasing demand for BSF-relevant services (e.g., Cammack *et al.*, 2021; Li *et al.*, 2019), interest in BSF-relevant products (e.g., Antarest *et al.*, 2020; Ffoulkes *et al.*, 2021), and government support (Meticulous Research, 2021), an increasing number of BSF farms have been set up throughout the world, creating a need to optimize the mass-rearing environment for all BSF life stages.

Pupation is a critical transitioning process in BSF life cycle, which links the larval feeding stage to the adult reproductive stage. In nature, BSF prepupae bury themselves into a pupation substrate (e.g., soil and spent larval diet) prior to pupation (Barros *et al.*, 2019; Sheppard *et al.*, 1994). Laboratory experiments have demonstrated that suitable substrates (e.g., wood shavings or potting soil) can shorten prepupal and pupal stages of BSF and increase its pupation rate and adult emergence success (Dzepe *et al.*, 2020; Holmes *et al.*, 2013), suggesting a potential application of optimizing pupation substrates in BSF mass-rearing.

Moisture content is an important physical property to be considered in the use of pupation substrates for insects. Several studies have shown that moist pupation substrates generally perform better than their dry counterparts, but, on the other hand, extremely wet substrates (e.g., 90% moisture content) may hamper insect pupation (Chen & Shelton, 2014;

Hulthen & Clarke, 2006; Kökdener & Şahin Yurtgan, 2022; Rickelmann & Bach, 1991; Wang *et al.*, 2017). Pupation substrate moisture may also affect insect pupation depth (Dimou *et al.*, 2003; Jackson *et al.*, 1998; Wang *et al.*, 2017), which further determines the amount of pupation substrate needed for a given area in an insect mass-rearing facility. Moreover, the effects of substrate moisture on insect pupation may depend on substrate type (Dimou *et al.*, 2003; Hulthen & Clarke, 2006; Kökdener & Şahin Yurtgan, 2022; Wang *et al.*, 2017). Holmes *et al.* (2013) indicated that BSF pupation may be affected by substrate moisture and its possible interaction with substrate type, but, to the best of our knowledge, relevant research has not been conducted.

The present study aimed to investigate the effects of substrate type and moisture content on BSF pupation rate and depth, and to discuss the underlying mechanisms. We tested two substrates: (1) vermiculite, which is widely used as a pupation substrate for mass rearing of several brachycerans, including BSF (e.g., Bernard *et al.*, 2020; Generalovic *et al.*, 2021; Incho, 1954; Liu *et al.*, 2018; Reynolds *et al.*, 2010; Vargas *et al.*, 1987), and (2) wood chips, which are similar to a substrate suggested for BSF pupation in a previous study (Holmes *et al.*, 2013).

3.2 Materials and methods

3.2.1 Pupation substrate

Vermiculite (Egmont Vermiculite Seed Cover, Egmont Commercial Ltd., Christchurch, New Zealand) and wood chips (manuka wood; Big Smoke, Riverlea Group Ltd., Hamilton, New Zealand) were purchased from local stores.

In a preliminary trial, particles of vermiculite were broken and flattened after a soaking-and-drying cycle (Figure S3.1) and the new particle shape did not change in subsequent soaking-and-drying cycles. Such changes in vermiculite particle shapes result in an increase in its density from 0.12 to 0.43 g/cm³. Although obvious shape changes do not happen to wood chips after soaking-and-drying cycles, some volatile compounds in wood chips may evaporate at a certain temperature when moisture content is determined using an oven-drying method (Forest Products Laboratory, 2021), causing an overestimation of moisture content. Therefore, as a pre-treatment of the pupation substrates used in the present experiment, soaked vermiculite and newly bought wood chips were dried in an oven (Contherm Thermotec 2000; Contherm Scientific Ltd., Lower Hutt, New Zealand) at 105°C until constant weight (Forest Products Laboratory, 2021; O'Kelly, 2005; Pedišius *et al.*, 2021). After the pre-treatment, the substrates were allowed to naturally restore moisture content in a lab environment (20 ± 1 °C, 50 ± 5 % relative humidity [R.H.]) for 2 d. Then, both substrates were homogenized by shaking in respective 20-L buckets for 2 min and stored in the buckets sealed with airtight lids. Some physical characteristics of the two pupation substrates after moisture restoration are shown in Table 3.1.

Table 3.1 Selected physical characteristics of the pupation substrates tested in the present study. Vermiculite was soaked with water and then oven-dried, while wood chips were oven-dried without soaking. Both substrates were allowed to naturally restore moisture content at $20 \pm 1^\circ\text{C}$ and $50 \pm 5\%$ relative humidity for 2 d.

Physical characteristics	Vermiculite	Wood chips
Density (g/cm^3)	0.43	0.31
Moisture content (%) ^a	4.34	5.94
Particle size > 2 mm	0.5	13.6
distribution 1–2 mm	37.4	53.3
(gravimetric; %) 0.5–1 mm	52.4	32.0
< 0.5 mm	9.7	1.1

^a Moisture content is calculated as (weight loss during drying/oven-dry weight) \times 100% (Cammack *et al.*, 2010).

3.2.2 Source of insects

BSF pupae were obtained from a mass-reared lab colony, which had been maintained in captivity for about 2 yr prior to the present experiment. Adult cages (BugDorm, 4M4545, MegaView Science Co. Ltd., Taiwan) were kept indoors ($28 \pm 1^\circ\text{C}$, $50 \pm 5\%$ R.H.) and illuminated using artificial light sources (BSF-4C-200-3030, HK SPR AgTech Trading Ltd., Hong Kong, China) with a 8 : 16 (L : D) h light regime (Liu *et al.*, 2020). Eggs were collected daily from adult cages and incubated at $28 \pm 0.5^\circ\text{C}$ and $80 \pm 2\%$ R.H. for 2 d (Holmes *et al.*, 2012). To start a batch of larvae, approximately 125 mg of fertile egg clutches, recognized by the presence of eye spots, were transferred to a larval rearing container and were provided with 2.1 kg of broil diet mix (broil : water = 1 : 2, gravimetric ratio) (Liu *et al.*, 2018). Larvae were reared at $28 \pm 1^\circ\text{C}$, $70 \pm 5\%$ R.H.; the high relative humidity level reduced water loss from newly added diet. Seven days later, each batch of

larvae was fed 600 g of broil diet three times a week until about 50% individuals had grown into prepupae. In this way unnecessary overfeeding was avoided (Barragán-Fonseca *et al.*, 2018; Diener *et al.*, 2015). The container was then left in the rearing environment to allow growth of the remaining larvae. When about 80% of the individuals in a batch had grown into prepupae, all the prepupae were collected, washed with cold running tap water, and then dried with paper towels. Each batch of prepupae was allocated to one replicate of the experiment described below.

3.2.3 Experimental design

Pupation substrates and insects were placed in paper cups (top diameter 9 cm, bottom diameter 6 cm, height 10.8 cm; BC-12-ART 390ml, BioPak Pty. Ltd., Bondi Junction, Australia). Each cup was filled with a substrate (vermiculite or wood chips) to a depth of 8 cm; this depth has been shown to be sufficient to study pupation depth of brachyceran species in different substrates and at different moisture levels, such as *Anastrepha suspensa* Loew (Diptera: Tephritidae), *Bactrocera spp.* (Diptera: Tephritidae), *Ceratitis capitata* Wiedemann (Diptera: Tephritidae), *Lucilia sericata* Meigen (Diptera: Calliphoridae), and *Drosophila suzukii* Matsunura (Diptera: Drosophilidae) (Ballman *et al.*, 2017; Cammack *et al.*, 2010; Hennessey, 1994; Hou *et al.*, 2006; Jackson *et al.*, 1998). The substrate in cups was not compacted, but the bottom of each cup was gently tapped during filling to minimize pore space. The weight of vermiculite and wood chips per cup was standardized to be 131 g and 95 g, respectively. Water was added at the bottom of the cups using plastic straws connected to syringes to achieve nine moisture levels (Table 3.2). The highest moisture content was set at 150% because vermiculite used here was almost saturated at this level. The lower moisture levels were selected by equally dividing

the range between 0% and 150% into eight intervals. Because vermiculite shrinks while wood chips expand after absorbing water, the actual depth of substrate in a cup varied depending on both substrate type and the amount of water added, and was in a range of 8 ± 1 cm. To evaluate the improvement in BSF pupation rate by using a pupation substrate and to understand the underlying mechanism of the improvement, one cup without any substrate or water was added as a ‘no substrate’ treatment. Therefore, a total of eighteen treatments with substrates and a treatment without substrate were set up for the experiment. The experiment was replicated six times.

Table 3.2 Different moisture levels and the corresponding amount of water added to each type of the pupation substrate tested in the present study.

Moisture level	Moisture content (%) ^a	Water added (ml)	
		Vermiculite	Wood chips
1	Dry ^b	0	0
2	18.8	18.1	11.5
3	37.5	41.7	28.3
4	56.3	65.2	45.2
5	75.0	88.8	62.0
6	93.8	112.3	78.8
7	112.5	135.9	95.6
8	131.3	159.4	112.4
9	150.0	183.0	129.3
Substrate placed in a cup (g)		131.0	95.0

^a Moisture content is calculated as (weight loss during drying/oven-dry weight) \times 100% (Cammack *et al.*, 2010).

^b Substrate oven-dried then allowed to rehydrate for 2 d in a lab environment at $50 \pm 5\%$ R.H.; this moisture content was 4.34% for vermiculite and 5.94% for wood chips.

Fifty prepupae were weighed in groups, and in total nineteen groups of prepupae were prepared for the nineteen treatments. The prepupae were then transferred to the cups. A perforated lid was used to cover each cup to prevent larvae from escaping and to allow air exchange. The cups were kept at $28 \pm 1^\circ\text{C}$ and $50 \pm 5\%$ R.H. in complete darkness. All the treatments were terminated after 10 d, which maximized pupation rate in a suitable environment, while minimizing the number of adults emerged (Holmes *et al.*, 2013). An additional group of fifty prepupae (referred to as ‘representative prepupae’) was sampled from the same batch, weighed, and stored in a sample bag at -20°C for future reference.

Upon termination of the experiment, a thin bamboo skewer was inserted into each cup to help measure the actual content depth, and to divide the content into four different depth ranges (0–2 cm, 2–4 cm, 4–6 cm, and > 6 cm). The substrate containing insects within each of the four depth ranges was quickly lifted and transferred to separate sample bags. Then, the number of prepupae and pupae in each sample bag were counted. This technique allowed collecting the prepupae or pupae presenting at different depth ranges, while avoiding vertical migration of live prepupae. Although the duration of the experiment was set to minimize the chance of adult emergence, there were still up to three adult flies emerged from a treatment. Both pupae and adults were considered as pupated individuals for the calculation of pupation rate. The numbers of dead prepupae were also counted.

Because vermiculite adhered to the cuticle of pupae and could not be completely removed even by rinsing with water (Figure S3.2), BSF fresh and dry weight changes after pupation could not be accurately determined for vermiculite treatments, and thus weights were only investigated for the wood chips and no substrate treatments. Pupae collected from each cup were washed with running tap water, dried with paper towels, and then

weighed as a group. The treatment pupae and the representative prepupae were dried at 105°C until constant weight. The dry matter content of the representative prepupae was calculated as $(\text{dry weight}/\text{fresh weight}) \times 100\%$, and was used to represent that of the prepupae used in the experiment. Dry weight of the prepupae used in the experiment was calculated as $\text{prepupal fresh weight} \times \text{dry matter content}$. BSF fresh weight loss after pupation was calculated as the difference between the average fresh weight of prepupae before the experiment and that of pupae after the experiment. BSF dry weight loss was calculated in the same way.

3.2.4 Statistical analysis

Statistical analyses were conducted using SPSS Statistics v. 23 (SPSS Inc., Chicago, IL, U.S.) and OriginPro 2016 (OriginLab Corp., Northampton, MA, U.S.), with the significance level for all tests set at $\alpha = 0.05$.

Prepupal mortality rates across all treatments were analyzed with a generalized linear model (GLiM) with a binomial distribution (logit link) using the GENLIN procedure (event/trial mode). If treatment effect was significant, a Fisher's least significant difference (LSD) test was performed, where only the differences between no substrate and substrate treatments were of interest. Another generalized linear model with a binomial distribution (logit link) was conducted to analyze the effects of substrate type, moisture content, and their possible interaction on prepupal mortality rate. The same methods were used to analyze pupation rate of survived prepupae (referred to as 'pupation rate' hereafter).

Because mortality and pupation rates may change in parabolic fashion in response to increasing moisture content (Chen & Shelton, 2014; Hulthen & Clarke, 2006; Kökdener & Şahin Yurtgan, 2022; Rickelmann & Bach, 1991; Wang *et al.*, 2017), a quadratic term of moisture content was initially included in relevant models to address possible curvature (Osborne, 2015). Where the inclusion of a quadratic term did not significantly improve the model based on a likelihood ratio test, it was removed to avoid overfitting (Lewis *et al.*, 2011). Non-significant interaction terms were removed to obtain the final models (Engqvist, 2005), where predicted values of mortality and pupation rates were obtained to plot regression curves.

Differences in BSF fresh and dry weight loss between the no substrate treatment and each wood chips treatment were analyzed using a one-way analysis of variance (ANOVA) followed by two-sided Dunnett's tests. Linear regressions were conducted to analyze the effect of substrate moisture content on BSF fresh and dry weight loss.

The tendency of BSF to pupate deeper in substrate (at depths over 2 cm) in response to moisture level was investigated for each substrate. Preliminary data analyses revealed that this tendency followed substrate-specific parabolic patterns, therefore, nonlinear models based on quadratic functions, reciprocal density-yield functions, and probability density functions were fitted to the data for each substrate. The models were conducted using OriginPro 2016. Final models were selected based on fit.

3.3 Results

3.3.1 Prepupal mortality rate

Prepupal mortality rate in no substrate treatment did not differ significantly from those in substrate treatments when moisture content was low (Figure 3.1). Prepupal mortality rate in vermiculite was lower than that in wood chips, and decreased as moisture content declined; the interaction between substrate type and moisture content was not significant (Figure 3.1, Table 3.3).

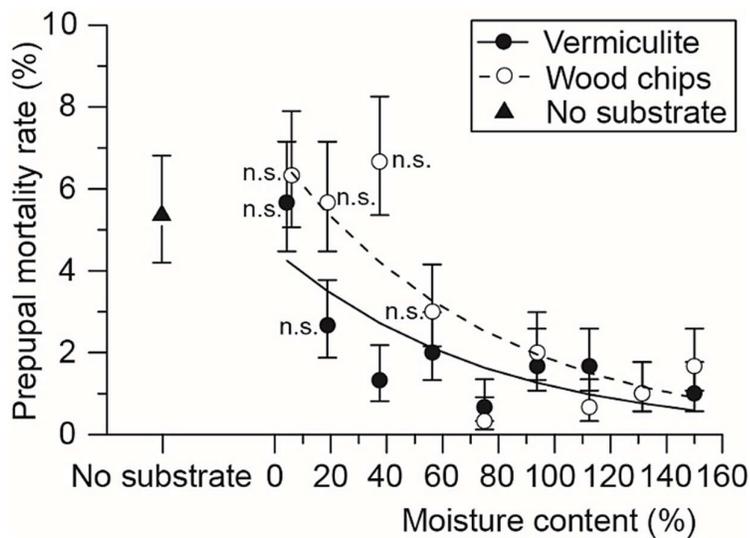


Figure 3.1 Mortality rate of black soldier fly *Hermetia illucens* prepupae with and without pupation substrates and at different substrate moisture content. Moisture content is calculated as (weight loss during drying/oven-dry weight) \times 100%. Error bars indicate standard errors of means (n = 6). The means and standard errors were back transformed from the log scale outputs. Non-significant differences between no substrate and substrate treatments are indicated as 'n.s.'. Solid and dashed lines are the binomial logit regression curves for vermiculite and wood chips, respectively — vermiculite: $\text{Logit}(y) = -3.0563 - 0.0140x$; wood chips: $\text{Logit}(y) = -2.5994 - 0.0140x$.

Table 3.3 Type I Wald χ^2 tests for the effects of substrate type, moisture content, and their interaction on prepupal mortality rate of the black soldier fly *Hermetia illucens*.

Source	Wald χ^2	df	<i>P</i>
Intercept	1604.1	1	< 0.001
Substrate type	8.0	1	= 0.005
Moisture content	42.3	1	< 0.001
Substrate type \times moisture content	1.0	1	= 0.3

3.3.2 Pupation rate

BSF pupation rate was affected by the presence of pupation substrate, substrate type and moisture content, but not by the interaction between substrate type and moisture content (Figure 3.2, Table 3.4). Compared to the no substrate treatment, vermiculite improved pupation rate of survived prepupae, but wood chips did not achieve the same at low moisture content ($\leq 18.8\%$). Moisture content had a significant positive effect on BSF pupation rate. At the same moisture content, BSF pupation rate in vermiculite was higher than that in wood chips.

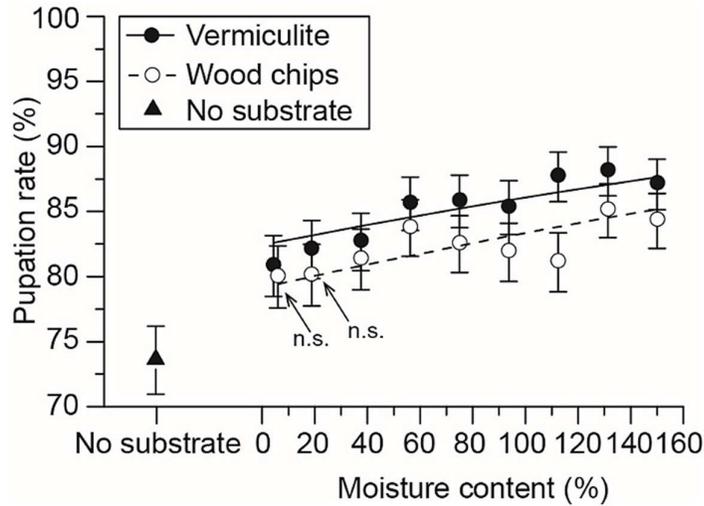


Figure 3.2 Pupation rate of black soldier fly *Hermetia illucens* prepupae with and without pupation substrates and at different substrate moisture content. Moisture content is calculated as (weight loss during drying/oven-dry weight) \times 100%. Error bars indicate standard errors of means ($n = 6$). The means and standard errors were back transformed from the log scale outputs. Non-significant differences between substrate and no substrate treatments are indicated as ‘n.s.’. Solid and dashed lines are the binomial logit regression curves for vermiculite and wood chips, respectively — vermiculite: $\text{Logit}(y) = 1.5427 + 0.0028x$; wood chips: $\text{Logit}(y) = 1.3342 + 0.0028x$.

Table 3.4 Type I Wald χ^2 tests for the effects of substrate type, moisture content, and their interaction on pupation rate of black soldier fly *Hermetia illucens* prepupae.

Source	Wald χ^2	df	P
Intercept	1901.2	1	< 0.001
Substrate type	7.1	1	= 0.008
Moisture content	12.3	1	< 0.001
Substrate type \times moisture content	1.5	1	= 0.23

3.3.3 Fresh and dry weight loss

Pupation caused a reduction of both BSF fresh and dry weight (Figure 3.3). No significant difference was found for dry weight loss across treatments (ANOVA; $F_{9, 50} = 0.95$; $P = 0.49$). In comparison with the insects that pupated without substrate, those in wood chips with a moisture content between 5.9 and 37.5% lost more fresh weight, whereas at higher moisture content they lost less fresh weight. BSF fresh weight loss decreased with increasing moisture content, while dry weight loss was not affected by moisture content (Table 3.5).

Table 3.5 Effect of wood chips substrate moisture content on black soldier fly *Hermetia illucens* fresh and dry weight loss after pupation.

Response	Term	Coefficient	Standard error	<i>t</i>	<i>P</i>
Fresh weight loss	Intercept	31.8	1.0	30.3	< 0.001
	Moisture content	-2.5	0.2	-13.6	< 0.001
Dry weight loss	Intercept	4.5	0.4	12.0	< 0.001
	Moisture content	0.03	0.07	0.5	0.61

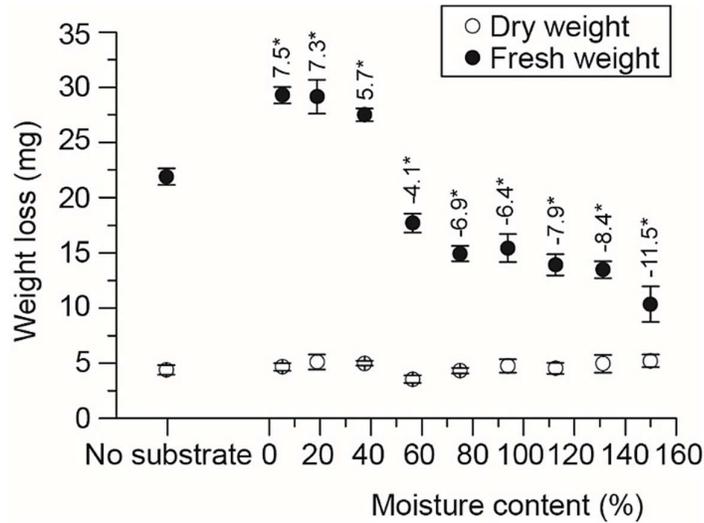


Figure 3.3 Fresh and dry weight loss of black soldier fly *Hermetia illucens* after pupation in no substrate and in wood chips at different moisture content. Moisture content is calculated as (weight loss during drying/oven-dry weight) \times 100%. Error bars indicate standard errors of means ($n = 6$). The value next to a fresh weight data point shows the difference between the mean of a wood chips treatment and the no substrate treatment; an asterisk indicates significant difference (two-sided Dunnett's test; $P < 0.05$).

3.3.4 Pupation depth

More than 85% of pupae were found in the top 2 cm of substrate for both pupation substrates tested (Tables S3.1 and S3.2). The proportion of BSF pupated deeper than 2 cm depended on both substrate type and moisture content (Figure 3.4). Among the nonlinear model functions tested, the lognormal distribution density function fitted vermiculite data the best, while the Holliday model (Holliday, 1960) was the best for wood chips (Table S3.3). The proportion of BSF that pupated deeper than 2 cm first increased then declined with increasing moisture content within the range used here; the predicted maximum was at about 20 and 70% moisture content for vermiculite and wood chips, respectively (Figure 3.4).

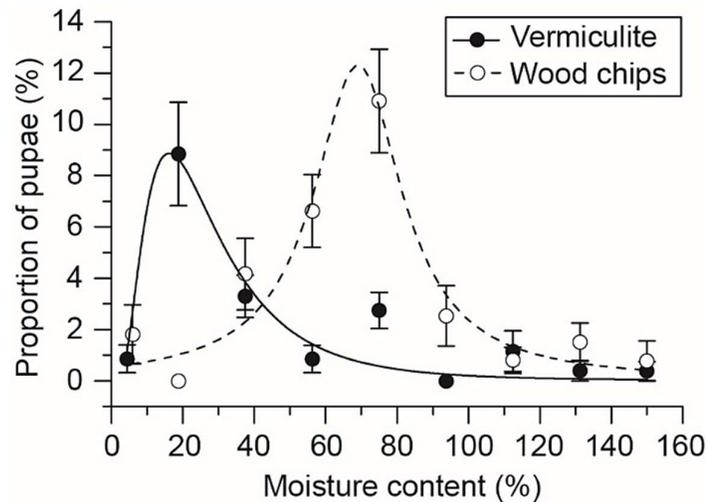


Figure 3.4 Proportion of black soldier fly *Hermetia illucens* pupae presented deeper than 2 cm in different pupation substrates and at different moisture levels. Moisture content is calculated as (weight loss during drying/oven-dry weight) \times 100%. Error bars indicate standard errors of means (n = 6). Solid and dashed lines are nonlinear regression curves for vermiculite and wood chips, respectively.

3.4 Discussion

It is a common practice to use pupation substrates for mass rearing of insects that are of economic and/or environmental importance, such as those used in the sterile insect technique (Pascacio-Villafán *et al.*, 2021; Steinitz *et al.*, 2016), in silk production (pupation substrate is called mountage in this case; Sangappa *et al.*, 2010), in waste management (Holmes *et al.*, 2013; Parry *et al.*, 2017), and as biocontrol agents (Halbritter & Wheeler, 2019; Muratori *et al.*, 2009). Indeed, using a suitable substrate material and optimizing physiochemical parameters therein (i.e., moisture content, compaction, and possibly temperature) could shorten metamorphosis duration, reduce mortality, improve adult fitness, and reduce production costs (Aceituno-Medina *et al.*, 2017; Dzepe *et al.*, 2020; Holmes *et al.*, 2013; Parry *et al.*, 2017; Weston & Desurmont, 2008). Here, we

show that vermiculite or wood chips with adjusted moisture content could potentially be used as a pupation substrate for BSF mass rearing. Currently, some BSF farmers do not use any pupation substrate (unpublished data). In an industrial scenario, the added costs of using a pupation substrate need to be balanced with the improvement in BSF pupation rate (and perhaps emergence rate as well). Now that we have the comparison between substrate and no substrate treatments, readers could use our results in their business models.

Dry vermiculite performed better than no substrate in terms of supporting pupation of BSF prepupae, but the same was not true for wood chips. Because pupation rates presented here are that of the survived prepupae, the results suggested that vermiculite, but not wood chips, brought forward the onset of BSF pupation. The underlying mechanisms may be that vermiculite reduced surrounding disturbance and provided thigmotactic stimuli to the insects. The wood chip substrate, due to its more rigid texture and lower density than vermiculite, may not have reduced disturbance or provided thigmotactic stimuli as much as vermiculite.

The effects of surrounding disturbance on insect pupation have been reported for different insects. For instance, pupation of *Zophobas rugipes* (Kirsch) (Coleoptera: Tenebrionidae) and *Tenebrio molitor* (L.) (Coleoptera: Tenebrionidae) was delayed by direct contact with moving conspecifics or with moving metal chains, and *Z. rugipes* pupation was also retarded by the vibration of Petri dishes (Tschinkel & Willson, 1971). Kotaki & Fujii (1995) suggested that both mechanical and chemical stimuli from conspecifics were involved in delaying pupation of *Tribolium freemani* (Hinton) (Coleoptera: Tenebrionidae). To remove most of surrounding disturbance, Finlayson (1967) and Žďárek & Denlinger (1991) suspended larvae of tsetse flies *Glossina morsitans morsitans*

(West) (Diptera: Glossinidae) in the mid-air and found that pupation in such circumstance was the quickest among all treatments in their studies. In BSF, isolated prepupae took on average 9.5 d to pupate at 25°C (70% R.H., 14L:10D) (Holmes *et al.*, 2012), whereas prepupae accommodated together at 28°C took on average 10.3 d to pupate under similar conditions (Holmes *et al.*, 2013). Since the higher temperature in the latter study is expected not to prolong but rather to shorten the BSF prepupal stage (Chia *et al.*, 2018), these results indicate the effect of conspecific disturbance on delaying BSF pupation.

Positive thigmotaxis, which refers to the tendency to remain close or move towards thigmotactic stimuli (i.e., physical pressure), is a conservative risk-avoiding strategy among animals (Hill & Lodge, 1994; Klosinski *et al.*, 2022; Mashoodh *et al.*, 2009; Starry *et al.*, 1998). Last instar insect larvae and mobile pupae tend to choose crevices, corners, or penetrable substrates as suitable pupation sites to reduce the risks from predators or parasitoids (Hodgson *et al.*, 1998; Lindstedt *et al.*, 2019; Okuyama, 2019). Žďárek & Denlinger (1991) found that providing thigmotactic stimuli to *G. morsitans* larvae by wrapping them with Parafilm or wedging them into pipette tips accelerated their pupation. To our best knowledge, there is no evidence thus far showing that thigmotactic stimuli benefit BSF pupation, but the aggregation behavior of BSF prepupae may suggest positive thigmotaxis (Holmes *et al.*, 2013).

Increasing moisture content improved BSF pupation rate through advancing the onset of pupation and reducing prepupal mortality. Our results agree with those in a previous study, where desiccation delayed BSF pupation and increased prepupal mortality (Holmes *et al.*, 2012). As moisture content increases, pupation substrates may become more penetrable (Cairns *et al.*, 2011; Vaz *et al.*, 2011), and thus may lead to earlier BSF pupation. This assumption is supported by our results regarding pupation depth when moisture content

ranged from dry to a certain level (e.g., 20% and 70% for vermiculite and wood chips, respectively). Moreover, our results regarding fresh weight changes after pupation indicate that BSF pupated in a substrate with higher moisture content are likely to retain more water. Because water serves as both a medium and a participant of biological processes (Frenkel-Pinter *et al.*, 2021), BSF prepupae in higher moisture content treatments may have pupated more quickly and were less prone to death caused by physiological malfunction. Desiccated prepupae were found in low moisture content treatments, but we could not determine whether desiccation was the direct cause of their death or happened after death.

Notably, when the moisture content of wood chips was lower than 56.3%, BSF water loss in wood chip treatments was larger than that without any substrate. Most of the water added to wood chips stayed at the bottom of the cups. Thus, we speculate that, when little or no water was added, the wood chips in the top 2 cm, where most pupae presented, were so dry that they absorbed water from the air in the pore space and headspace, creating a drying microenvironment.

Moisture content of a pupation substrate affects the availability of not only water but also oxygen. Pupation substrates at extremely high moisture content would have pore space taken by water, creating hypoxic environments for insects (Kökdener & Şahin Yurtgan, 2022; Shi *et al.*, 2021). In the present study, adverse effects of high moisture content on BSF pupation rate or mortality rate were not observed. However, as moisture content went from a certain level (e.g., 20% and 70% for vermiculite and wood chips, respectively) to higher levels, more BSF tended to pupate shallower, which may indicate avoiding oxygen deficiency.

Pupation substrates that are too shallow (e.g., less than 1 cm) may not be enough to provide thigmotactic stimuli or to retain water during BSF pupation, whereas those deeper than necessary would lead to a waste of material. In the present study, almost all the pupae were found at the top 4 cm of both pupation substrates (Tables S3.1 and S3.2), suggesting that a 4-cm of substrate would be sufficient for BSF pupation.

Pupation rate is but one factor to consider when choosing a suitable pupation substrate for BSF farming, with other factors including but not limiting to accessibility, reusability, and price. Both substrates used here are easily accessible from local stores, and can be reused after being separated from BSF pupae through sieving. In New Zealand, the price of vermiculite and wood chips used here are currently 9 and 7.5 dollars per kilogram, respectively. The price of wood chips could be lower if it is derived from other types of wood or wood by-products. Moreover, vermiculite that stuck on BSF puparia may lead to extra cleaning steps before the puparia are used for chitin extraction (Hahn *et al.*, 2022). Therefore, wood chips or other lignocellulosic materials may be better options than vermiculite in industrial scenarios. We encourage further exploration for alternative materials with low cost, soft texture, and high density to facilitate BSF pupation.

As one of the first attempts to optimize BSF pupation environment, the present study provides clues for selecting suitable substrates and moisture content for BSF pupation. We hypothesize that pupation substrates assist BSF pupation by reducing surrounding disturbance and providing thigmotactic stimuli, and thus softer and denser substrates may be more favorable. Moreover, wetting pupation substrate is necessary to reduce prepupal mortality and to help BSF pupate early. Considering the trade-off between water and oxygen levels in the substrate, extremely wet conditions should be avoided.

3.5 Acknowledgments

The authors would like to thank Duncan Hedderley for his suggestions on statistics. We also want to acknowledge Dr Kambiz Esfandi for providing equipment and insect rearing room for the experiment.

3.6 References

- Aceituno-Medina, M., Rivera-Ciprian, J. P. & Hernández, E. (2017). Influence of pupation substrate on mass production and fitness of adult *Anastrepha obliqua* Macquart (Diptera: Tephritidae) for sterile insect technique application. *Journal of Economic Entomology*, 110(6), 2397–2405. <https://doi.org/10.1093/jee/tox279>
- Antarest, E., Egger, J., Virard, G., Dortmans, B. & Zurbrügg, C. (2020). *Market assessment for BSF products*. Eawag. https://www.eawag.ch/fileadmin/Domain1/Abteilungen/sandec/schwerpunkte/swm/Practical_knowhow_on_BSF/market_assessment_report_bsf.pdf
- Ballman, E. S., Collins, J. A. & Drummond, F. A. (2017). Pupation behavior and predation on *Drosophila suzukii* (Diptera: Drosophilidae) pupae in maine wild blueberry fields. *Journal of Economic Entomology*, 110(6), 2308–2317. <https://doi.org/10.1093/jee/tox233>
- Barragán-Fonseca, K. B., Dicke, M. & van Loon, J. J. A. (2018). Influence of larval density and dietary nutrient concentration on performance, body protein, and fat contents of black soldier fly larvae (*Hermetia illucens*). *Entomologia Experimentalis et Applicata*, 166(9), 761–770. <https://doi.org/10.1111/eea.12716>
- Barros, L. M., Ferreira-Keppeler, R. L., Martins, R. T. & Gutjahr, A. L. N. (2019). Bionomy of *Hermetia illucens* (Diptera: Stratiomyidae) on decomposing swine carcass in an urban area of central amazon. *Journal of Medical Entomology*, 56(3), 681–689. <https://doi.org/10.1093/jme/tjz005>
- Beesigamukama, D., Mochoge, B., Korir, N. K., Fiaboe, K. K. M., Nakimbugwe, D., Khamis, F. M., Subramanian, S., Dubois, T., Musyoka, M. W., Ekesi, S., Kelemu, S. & Tanga, C. M. (2020). Exploring black soldier fly frass as novel fertilizer for improved growth, yield, and nitrogen use efficiency of maize under field conditions. *Frontiers in Plant Science*, 11, 574592. <https://doi.org/10.3389/fpls.2020.574592>
- Beesigamukama, D., Mochoge, B., Korir, N. K., Fiaboe, K. K. M., Nakimbugwe, D., Khamis, F. M., Subramanian, S., Wangu, M. M., Dubois, T., Ekesi, S. & Tanga, C. M. (2021). Low-cost technology for recycling agro-industrial waste into nutrient-rich organic fertilizer using black soldier fly. *Waste Management*, 119, 183–194. <https://doi.org/10.1016/j.wasman.2020.09.043>

- Bernard, E., Villazana, J., Alyokhin, A. & Rose, J. (2020). Colonisation of finfish substrate inhabited by black soldier fly larvae by blow flies, bacteria, and fungi. *Journal of Insects as Food and Feed*, 6(3), 291–304. <https://doi.org/10.3920/jiff2019.0044>
- Bohm, K., Hatley, G. A., Robinson, B. H. & Gutiérrez-Ginés, M. J. (2022). Black soldier fly-based bioconversion of biosolids creates high-value products with low heavy metal concentrations. *Resources, Conservation and Recycling*, 180, 106149. <https://doi.org/10.1016/j.resconrec.2021.106149>
- Cai, M., Zhang, K., Zhong, W., Liu, N., Wu, X., Li, W., Zheng, L., Yu, Z. & Zhang, J. (2019). Bioconversion-composting of golden needle mushroom (*Flammulina velutipes*) root waste by black soldier fly (*Hermetia illucens*, Diptera: Stratiomyidae) larvae, to obtain added-value biomass and fertilizer. *Waste and Biomass Valorization*, 10(2), 265–273. <https://doi.org/10.1007/s12649-017-0063-2>
- Cairns, J. E., Impa, S. M., O'Toole, J. C., Jagadish, S. V. K. & Price, A. H. (2011). Influence of the soil physical environment on rice (*Oryza sativa* L.) response to drought stress and its implications for drought research. *Field Crops Research*, 121(3), 303–310. <https://doi.org/10.1016/j.fcr.2011.01.012>
- Cammack, J. A., Adler, P. H., Tomberlin, J. K., Arai, Y. & Bridges Jr, W. C. (2010). Influence of parasitism and soil compaction on pupation of the green bottle fly, *Lucilia sericata*. *Entomologia Experimentalis et Applicata*, 136(2), 134–141. <https://doi.org/10.1111/j.1570-7458.2010.01019.x>
- Cammack, J. A., Miranda, C. D., Jordan, H. R. & Tomberlin, J. K. (2021). Upcycling of manure with insects: Current and future prospects. *Journal of Insects as Food and Feed*, 7(5), 605–619. <https://doi.org/10.3920/jiff2020.0093>
- Chen, M. & Shelton, A. M. (2014). Impact of soil type, moisture, and depth on swede midge (Diptera: Cecidomyiidae) pupation and emergence. *Environmental Entomology*, 36(6), 1349–1355. <https://doi.org/10.1093/ee/36.6.1349>
- Chia, S. Y., Tanga, C. M., Khamis, F. M., Mohamed, S. A., Salifu, D., Sevgan, S., Fiaboe, K. K. M., Niassy, S., van Loon, J. J. A., Dicke, M. & Ekesi, S. (2018). Threshold temperatures and thermal requirements of black soldier fly *Hermetia illucens*: Implications for mass production. *PLOS ONE*, 13(11), e0206097. <https://doi.org/10.1371/journal.pone.0206097>
- Diener, S., Zurbrügg, C. & Tockner, K. (2015). Bioaccumulation of heavy metals in the black soldier fly, *Hermetia illucens* and effects on its life cycle. *Journal of Insects as Food and Feed*, 1(4), 261–270. <https://doi.org/10.3920/jiff2015.0030>
- Dimou, I., Koutsikopoulos, C., Economopoulos, A. P. & Lykakis, J. (2003). Depth of pupation of the wild olive fruit fly, *Bactrocera (Dacus) oleae* (Gmel.) (Dipt., Tephritidae), as affected by soil abiotic factors. *Journal of Applied Entomology*, 127(1), 12–17. <https://doi.org/10.1046/j.1439-0418.2003.00686.x>
- Dörper, A., Veldkamp, T. & Dicke, M. (2021). Use of black soldier fly and house fly in feed to promote sustainable poultry production. *Journal of Insects as Food and Feed*, 7(5), 761–780. <https://doi.org/10.3920/jiff2020.0064>

- Dzepe, D., Nana, P., Kuietche, H. M., Kuate, A. F., Tchuinkam, T. & Djouaka, R. (2020). Role of pupation substrate on post-feeding development of black soldier fly larvae, *Hermetia illucens* (Diptera: Stratiomyidae). *Journal of Entomology and Zoology Studies*, 8(2), 760–764. <https://www.entomoljournal.com/archives/2020/vol8issue2/PartN/8-1-393-704.pdf>
- Engqvist, L. (2005). The mistreatment of covariate interaction terms in linear model analyses of behavioural and evolutionary ecology studies. *Animal Behaviour*, 70(4), 967–971. <https://doi.org/10.1016/j.anbehav.2005.01.016>
- Ffoulkes, C., Illman, H., O'Connor, R., Lemon, F., Behrendt, K., Wynn, S., Wright, P., Godber, O., Ramsden, M., Adams, J., Metcalfe, P., Walker, L., Gittins, J., Wickland, K., Nanua, S. & Sharples, B. (2021). *Development of a roadmap to scale up insect protein production in the U.K. for use in animal feed*. WWF-U.K. https://www.wwf.org.uk/sites/default/files/2021-06/the_future_of_feed_technical_report.pdf
- Finlayson, L. H. (1967). Behaviour and regulation of puparium formation in the larva of the tsetse fly *Glossina morsitans orientalis* Vanderplank in relation to humidity, light and mechanical stimuli. *Bulletin of Entomological Research*, 57(2), 301–313. <https://doi.org/10.1017/s000748530005001x>
- Forest Products Laboratory (2021). *Wood handbook—wood as an engineering material*. (General technical report FPL-GTR-282.) U.S. Department of Agriculture. https://www.fpl.fs.fed.us/documnts/fplgtr/fplgtr282/fpl_gtr282.pdf
- Frenkel-Pinter, M., Rajaei, V., Glass, J. B., Hud, N. V. & Williams, L. D. (2021). Water and life: The medium is the message. *Journal of Molecular Evolution*, 89(1), 2–11. <https://doi.org/10.1007/s00239-020-09978-6>
- Gao, Z., Wang, W., Lu, X., Zhu, F., Liu, W., Wang, X. & Lei, C. (2019). Bioconversion performance and life table of black soldier fly (*Hermetia illucens*) on fermented maize straw. *Journal of Cleaner Production*, 230, 974–980. <https://doi.org/10.1016/j.jclepro.2019.05.074>
- Generalovic, T. N., McCarthy, S. A., Warren, I. A., Wood, J. M. D., Torrance, J., Sims, Y., Quail, M., Howe, K., Pipan, M., Durbin, R. & Jiggins, C. D. (2021). A high-quality, chromosome-level genome assembly of the black soldier fly (*Hermetia illucens* L.). *G3 Genes|Genomes|Genetics*, 11(5), jkab085. <https://doi.org/10.1093/g3journal/jkab085>
- Hahn, T., Tafi, E., von Seggern, N., Falabella, P., Salvia, R., Thomä, J., Febel, E., Fijalkowska, M., Schmitt, E., Stegbauer, L. & Zibek, S. (2022). Purification of chitin from pupal exuviae of the black soldier fly. *Waste and Biomass Valorization*, 13(4), 1993–2008. <https://doi.org/10.1007/s12649-021-01645-1>
- Halbritter, D. A. & Wheeler, G. S. (2019). Organic mulch can increase the survival of a weed biological control agent during laboratory mass rearing. *Biocontrol Science and Technology*, 29(9), 852–859. <https://doi.org/10.1080/09583157.2019.1608510>
- Hennessey, M. K. (1994). Depth of pupation of caribbean fruit fly (Diptera: Tephritidae) in soils in the laboratory. *Environmental Entomology*, 23(5), 1119–1123. <https://doi.org/10.1093/ee/23.5.1119>

- Hill, A. M. & Lodge, D. M. (1994). Diel changes in resource demand: competition and predation in species replacement among crayfishes. *Ecology*, 75(7), 2118–2126. <https://doi.org/10.2307/1941615>
- Hodgson, P. J., Sivinski, J., Quintero, G., & Aluja, M. (1998). Depth of pupation and survival of fruit fly (*Anastrepha spp.*: Tephritidae) pupae in a range of agricultural habitats. *Environmental Entomology*, 27(6), 1310–1314. <https://doi.org/10.1093/ee/27.6.1310>
- Holliday, R. (1960). Plant population and crop yield: Part I. *Field Crop Abstracts*, 13(3), 159–167. <https://doi.org/10.1079/cabireviews/20063048313>
- Holmes, L. A., Vanlaerhoven, S. L. & Tomberlin, J. K. (2012). Relative humidity effects on the life history of *Hermetia illucens* (Diptera: Stratiomyidae). *Environmental Entomology*, 41(4), 971–978. <https://doi.org/10.1603/en12054>
- Holmes, L. A., Vanlaerhoven, S. L. & Tomberlin, J. K. (2013). Substrate effects on pupation and adult emergence of *Hermetia illucens* (Diptera: Stratiomyidae). *Environmental Entomology*, 42(2), 370–374. <https://doi.org/10.1603/en12255>
- Hou, B., Xie, Q. & Zhang, R. (2006). Depth of pupation and survival of the Oriental fruit fly, *Bactrocera dorsalis* (Diptera: Tephritidae) pupae at selected soil moistures. *Applied Entomology and Zoology*, 41(3), 515–520. <https://doi.org/10.1303/aez.2006.515>
- Hu, Y., Huang, Y., Tang, T., Zhong, L., Chu, W., Dai, Z., Chen, K. & Hu, Y. (2020). Effect of partial black soldier fly (*Hermetia illucens* L.) larvae meal replacement of fish meal in practical diets on the growth, digestive enzyme and related gene expression for rice field eel (*Monopterus albus*). *Aquaculture Reports*, 17, 100345. <https://doi.org/10.1016/j.aqrep.2020.100345>
- Hulthen, A. D. & Clarke, A. R. (2006). The influence of soil type and moisture on pupal survival of *Bactrocera tryoni* (Froggatt) (Diptera: Tephritidae). *Australian Journal of Entomology*, 45(1), 16–19. <https://doi.org/10.1111/j.1440-6055.2006.00518.x>
- Incho, H. H. (1954). A rapid method for obtaining clean house fly pupae. *Journal of Economic Entomology*, 47(5), 938–939. <https://doi.org/10.1093/jee/47.5.938>
- Jackson, C. G., Long, J. P. & Klungness, L. M. (1998). Depth of pupation in four species of fruit flies (Diptera: Tephritidae) in sand with and without moisture. *Journal of Economic Entomology*, 91(1), 138–142. <https://doi.org/10.1093/jee/91.1.138>
- Kar, S. K., Schokker, D., Harms, A. C., Kruijt, L., Smits, M. A. & Jansman, A. J. M. (2021). Local intestinal microbiota response and systemic effects of feeding black soldier fly larvae to replace soybean meal in growing pigs. *Scientific Reports*, 11(1), 15088. <https://doi.org/10.1038/s41598-021-94604-8>
- Klosinski, P., Kobak, J., Augustyniak, M., Pawlak, R., Jermacz, L., Poznanska-Kakareko, M. & Kakareko, T. (2022). Behavioural responses to con- and heterospecific alarm cues by an alien and a coexisting native fish. *Hydrobiologia*, 849(4), 985–1000. <https://doi.org/10.1007/s10750-021-04761-0>

- Kökdener, M. & Şahin Yurtgan, M. (2022). The effect of soil type and moisture level on the development of *Lucilia sericata* (Diptera: Calliphoridae). *Journal of Medical Entomology*, 59(2), 508–513. <https://doi.org/10.1093/jme/tjab229>
- Kotaki, T. & Fujii, H. (1995). Crowding inhibits pupation in *Tribolium freemani*: Contact chemical and mechanical stimuli are involved. *Entomologia Experimentalis et Applicata*, 74(2), 145–149. <https://doi.org/10.1111/j.1570-7458.1995.tb01886.x>
- Lewis, F., Butler, A. & Gilbert, L. (2011). A unified approach to model selection using the likelihood ratio test. *Methods in Ecology and Evolution*, 2(2), 155–162. <https://doi.org/10.1111/j.2041-210X.2010.00063.x>
- Li, Y., Jin, Y., Borrion, A. & Li, H. (2019). Current status of food waste generation and management in China. *Bioresource Technology*, 273, 654–665. <https://doi.org/10.1016/j.biortech.2018.10.083>
- Lindstedt, C., Murphy, L. & Mappes, J. (2019). Antipredator strategies of pupae: How to avoid predation in an immobile life stage? *Philosophical Transactions of the Royal Society B: Biological Sciences*, 374(1783), 20190069. <https://doi.org/10.1098/rstb.2019.0069>
- Liu, Z., Minor, M., Morel, P. C. H. & Najar-Rodriguez, A. J. (2018). Bioconversion of three organic wastes by black soldier fly (Diptera: Stratiomyidae) larvae. *Environmental Entomology*, 47(6), 1609–1617. <https://doi.org/10.1093/ee/nvy141>
- Liu, Z., Najar-Rodriguez, A. J., Minor, M. A., Hedderley, D. I. & Morel, P. C. H. (2020). Mating success of the black soldier fly, *Hermetia illucens* (Diptera: Stratiomyidae), under four artificial light sources. *Journal of Photochemistry and Photobiology B: Biology*, 205, 111815. <https://doi.org/10.1016/j.jphotobiol.2020.111815>
- Mahmood, S., Zurbrügg, C., Tabinda, A. B., Ali, A. & Ashraf, A. (2021). Sustainable waste management at household level with black soldier fly larvae (*Hermetia illucens*). *Sustainability*, 13(17), 9722. <https://doi.org/10.3390/su13179722>
- Mashoodh, R., Sinal, C. J. & Perrot-Sinal, T. S. (2009). Predation threat exerts specific effects on rat maternal behaviour and anxiety-related behaviour of male and female offspring. *Physiology & Behavior*, 96(4–5), 693–702. <https://doi.org/10.1016/j.physbeh.2009.01.001>
- Meticulous Research (2021). *Black soldier fly market by product type (protein meal, biofertilizer, whole dried larvae, larvae oil, and other BSF products), application (animal feed, agriculture, pet food, and others), and geography- global forecast to 2030*. Meticulous Market Research Pvt. Ltd. <https://www.marketresearch.com/Meticulous-Research-v4061/Black-Soldier-Fly-Product-Type-12979571/>
- Muratori, F. B., Gagne, R. J. & Messing, R. H. (2009). Ecological traits of a new aphid parasitoid, *Endaphis fugitiva* (Diptera: Cecidomyiidae), and its potential for biological control of the banana aphid, *Pentalonia nigronervosa* (Hemiptera: Aphididae). *Biological Control*, 50(2), 185–193. <https://doi.org/10.1016/j.biocontrol.2009.04.007>
- O'Kelly, B. C. (2005). Oven-drying characteristics of soils of different origins. *Drying Technology*, 23(5), 1141–1149. <https://doi.org/10.1081/drt-200059149>

- Okuyama, T. (2019). Density-dependent distribution of parasitism risk among underground hosts. *Bulletin of Entomological Research*, 109(4), 528–533. <https://doi.org/10.1017/s0007485318000871>
- Osborne, J. W. (2015). *Best practices in logistic regression*, SAGE Publications, Ltd. <https://doi.org/10.4135/9781483399041>
- Parry, N. J., Pieterse, E. & Weldon, C. W. (2017). Longevity, fertility and fecundity of adult blow flies (Diptera: Calliphoridae) held at varying densities: Implications for use in bioconversion of waste. *Journal of Economic Entomology*, 110(6), 2388–2396. <https://doi.org/10.1093/jee/tox251>
- Pascacio-Villafán, C., Quintero-Fong, L., Guillén, L., Rivera-Ciprian, J. P., Aguilar, R. & Aluja, M. (2021). Pupation substrate type and volume affect pupation, quality parameters and production costs of a reproductive colony of *Ceratitis capitata* (Diptera: Tephritidae) VIENNA 8 genetic sexing strain. *Insects*, 12(4), 337. <https://doi.org/10.3390/insects12040337>
- Pedišius, N., Praspaliauskas, M., Pedišius, J. & Dzenajavičienė, E. F. (2021). Analysis of wood chip characteristics for energy production in Lithuania. *Energies*, 14(13), 3931. <https://doi.org/10.3390/en14133931>
- Reynolds, O. L., Dominiak, B. C. & Orchard, B. A. (2010). Pupal release of the Queensland fruit fly, *Bactrocera tryoni* (Froggatt) (Diptera: Tephritidae), in the sterile insect technique: Seasonal variation in eclosion and flight. *Australian Journal of Entomology*, 49(2), 150–159. <https://doi.org/10.1111/j.1440-6055.2009.00740.x>
- Richardson, A., Dantas-Lima, J., Lefranc, M. & Walraven, M. (2021). Effect of a black soldier fly ingredient on the growth performance and disease resistance of juvenile pacific white shrimp (*Litopenaeus vannamei*). *Animals*, 11(5), 1450. <https://doi.org/10.3390/ani11051450>
- Rickelmann, K. M. & Bach, C. E. (1991). Effects of soil moisture on the pupation behavior of *Altica subplicata* (Coleoptera: Chrysomelidae). *The Great Lakes Entomologist*, 24(4), 231–237. <https://scholar.valpo.edu/tgle/vol24/iss4/4>
- Sangappa, S., Raj, B., Srinivasa G. S. & Dandin, S. B. (2010). Impact of different types of mountages on raw silk production and quality. *The Journal of Silk Science and Technology of Japan*, 18, 15–19. <https://doi.org/10.11417/silk.18.15>
- Sheppard, C. D., Newton, L. G., Thompson, S. A. & Savage, S. (1994). A value added manure management system using the black soldier fly. *Bioresource Technology*, 50(3), 275–279. [https://doi.org/10.1016/0960-8524\(94\)90102-3](https://doi.org/10.1016/0960-8524(94)90102-3)
- Shi, Y., Li, L., Shahid, S., Smagghe, G. & Liu, T. (2021). Effect of soil moisture on pupation behavior and inhabitation of *Spodoptera frugiperda* (Lepidoptera: Noctuidae). *Applied Entomology and Zoology*, 56(1), 69–74. <https://doi.org/10.1007/s13355-020-00709-3>
- Starry, O., Wanzenböck, J. & Danielopol, D. L. (1998). Tendency of the amphipod *Gammarus roeseli* Gervais to colonize coarse sediment habitats under fish predation pressure. *International Review of Hydrobiology*, 83(5–6), 371–380. <https://doi.org/10.1002/iroh.19980830505>

- Steinitz, H., Sadeh, A., Tremmel, M. & Harari, A. R. (2016). Methods to separate *Lobesia botrana* (Lepidoptera: Tortricidae) males from females for the implementation of sterile insect-inherited sterility technique control tactics. *Florida Entomologist*, 99(sp1), 192–199. <https://doi.org/10.1653/024.099.sp123>
- Tschinkel, W. R. & Willson, C. D. (1971). Inhibition of pupation due to crowding in some tenebrionid beetles. *Journal of Experimental Zoology*, 176(2), 137–145. <https://doi.org/10.1002/jez.1401760203>
- Vargas, R. I., Chang, H. B., Komura, M. & Kawamoto, D. (1987). Mortality, stadia duration, and weight loss in three species of mass-reared fruit fly pupae (Diptera: Tephritidae) held with and without vermiculite at selected relative humidities. *Journal of Economic Entomology*, 80(4), 972–974. <https://doi.org/10.1093/jee/80.4.972>
- Vaz, C. M. P., Manieri, J. M., de Maria, I. C. & Tuller, M. (2011). Modeling and correction of soil penetration resistance for varying soil water content. *Geoderma*, 166(1), 92–101. <https://doi.org/10.1016/j.geoderma.2011.07.016>
- Wang, H., Ma, T., Xiao, Q., Cao, P., Chen, X., Wen, Y., Xiong, H., Qin, W., Liang, S., Jian, S., Li, Y., Sun, Z., Wen, X. & Wang, C. (2017). Pupation behaviors and emergence successes of *Ectropis grisescens* (Lepidoptera: Geometridae) in response to different substrate types and moisture contents. *Environmental Entomology*, 46(6), 1365–1373. <https://doi.org/10.1093/ee/nvx168>
- Weston, P. A. & Desurmont, G. A. (2008). Pupation by viburnum leaf beetle (Coleoptera: Chrysomelidae): Behavioral description and impact of environmental variables and entomopathogenic nematodes. *Environmental Entomology*, 37(4), 845–849. <https://doi.org/10.1093/ee/37.4.845>
- Žďárek, J. & Denlinger, D. L. (1991). Wandering behaviour and pupariation in tsetse larvae. *Physiological Entomology*, 16(4), 523–529. <https://doi.org/10.1111/j.1365-3032.1991.tb00591.x>

3.7 Appendix

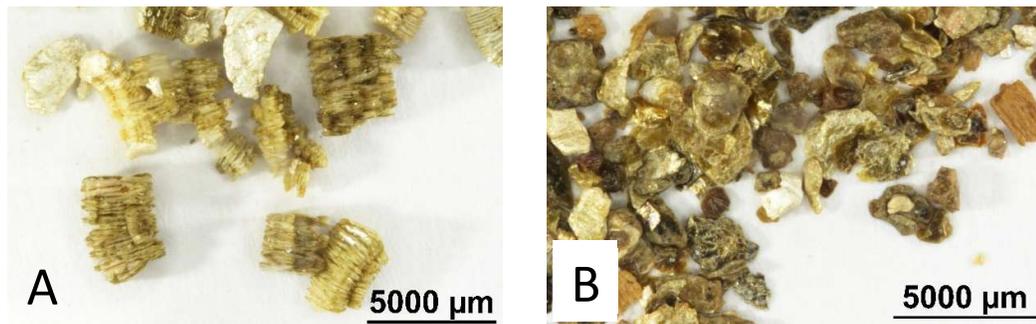


Figure S 3.1 Differences in vermiculite particle shapes before (A) and after (B) a soaking-and-drying cycle.

Substrate	Moisture level									No substrate
	1	2	3	4	5	6	7	8	9	
Vermiculite										
Wood chips										

Figure S 3.2 Samples of black soldier fly *Hermetia illucens* pupae collected from substrate and no substrate treatments. Moisture levels 2, 3, 4, 5, 6, 7, 8, and 9 were for 18.8%, 37.5%, 56.3%, 75.0%, 93.8%, 112.5%, 131.3%, and 150%, respectively; moisture level 1 was 4.3% and 5.9% for vermiculite and wood chips, respectively. Moisture content is calculated as (weight loss during drying/oven-dry weight) \times 100%.

Chapter 3

Table S 3.1 Percentage of black soldier fly *Hermetia illucens* pupae presented at different depth ranges in vermiculite at different moisture content. Standard errors are shown in parentheses.

Depth range (cm)	Moisture content (%)								
	4.3	18.8	37.5	56.3	75	93.8	112.5	131.3	150
0–2	99.14 (0.54)	91.15 (0.02)	96.71 (0.01)	99.15 (0.01)	97.25 (0.01)	100 (0)	98.84 (0.01)	99.60 (0.004)	99.63 (0.004)
2–4	0.86 (0.54)	5.01 (0.02)	3.29 (0.01)	0.85 (0.01)	1.95 (0.01)	0 (0)	0.78 (0.01)	0.40 (0.004)	0.37 (0.004)
4–6	0 (0)	2.89 (0.01)	0 (0)	0 (0)	0.40 (0.004)	0 (0)	0.39 (0.004)	0 (0)	0 (0)
> 6	0 (0)	0.95 (0.01)	0 (0)	0 (0)	0.40 (0.004)	0 (0)	0 (0)	0 (0)	0 (0)

Moisture content is calculated as (weight loss during drying/oven-dry weight) × 100%.

Table S 3.2 Percentage of black soldier fly *Hermetia illucens* pupae presented at different depth ranges in wood chips at different moisture content. Standard errors are shown in parentheses.

Depth range (cm)	Moisture content (%)								
	5.9	18.8	37.5	56.3	75	93.8	112.5	131.3	150
0–2	98.19 (0.01)	100 (0)	95.84 (0.01)	93.38 (0.01)	89.08 (0.02)	97.47 (0.01)	99.20 (0.01)	98.49 (0.01)	99.22 (0.01)
2–4	1.81 (0.01)	0 (0)	4.16 (0.01)	6.62 (0.01)	10.92 (0.02)	2.53 (0.01)	0.80 (0.01)	1.51 (0.01)	0.78 (0.01)
4–6	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
> 6	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

Moisture content is calculated as (weight loss during drying/oven-dry weight) × 100%.

Table S 3.3 Nonlinear models fitted to describe the effect of moisture content on the proportion of black soldier fly *Hermetia illucens* pupated deeper than 2 cm in different substrates.

Substrate	Nonlinear function *	Model parameters	Parameter estimate \pm SE	Adjusted R^2
Vermiculite	$y = \frac{A}{\sqrt{2\pi wx}} e^{-\frac{(\ln \frac{x}{xc})^2}{2w^2}}$	A	287.97 ± 37.96	0.556
		xc	24.66 ± 2.85	
		w	0.65 ± 0.07	
Wood chips	$y = (a + bx + cx^2)^{-1}$	a	1.78 ± 0.51	0.553
		b	-0.049 ± 0.016	
		c	0.00036 ± 0.00012	

* In the functions, y stands for the proportion of black soldier fly pupated deeper than 2 cm, and x stands for moisture content.



GRADUATE
RESEARCH
SCHOOL

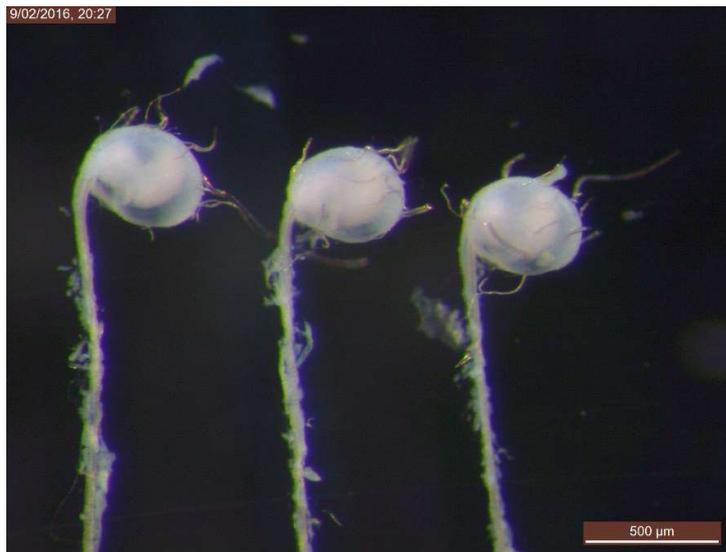
STATEMENT OF CONTRIBUTION DOCTORATE WITH PUBLICATIONS/MANUSCRIPTS

We, the candidate and the candidate's Primary Supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the candidate's contribution as indicated below in the *Statement of Originality*.

Name of candidate:	Zhongyi Liu
Name/title of Primary Supervisor:	Dr Maria Minor
In which chapter is the manuscript /published work:	Chapter 4
Please select one of the following three options:	
<input checked="" type="radio"/> The manuscript/published work is published or in press <ul style="list-style-type: none"> • Please provide the full reference of the Research Output: Liu, Zhongyi, Adriana J. Najar-Rodriguez, Maria A. Minor, Duncan I. Hedderley, and Patrick C. H. Morel. 2020. Mating success of the black soldier fly, <i>Hermetia illucens</i> (Diptera: Stratiomyidae), under four artificial light sources. <i>Journal of Photochemistry & Photobiology, B: Biology</i>, 205: 111815. 	
<input type="radio"/> The manuscript is currently under review for publication – please indicate: <ul style="list-style-type: none"> • The name of the journal: • The percentage of the manuscript/published work that was contributed by the candidate: 85.00 • Describe the contribution that the candidate has made to the manuscript/published work: Zhongyi Liu performed the majority of the work for the article that forms Chapter 4 of this thesis. This including experimental design, data collection, data analysis, and manuscript writing, with assistance from the co-authors of the article. 	
<input type="radio"/> It is intended that the manuscript will be published, but it has not yet been submitted to a journal	
Candidate's Signature:	Zhongyi Liu <small>Digitally signed by Zhongyi Liu Date: 2022.06.08 22:25:42 +1200</small>
Date:	08-Jun-2022
Primary Supervisor's Signature:	Maria Minor <small>Digitally signed by Maria Minor Date: 2022.06.08 15:12:38 +1200</small>
Date:	9-Jun-2022

This form should appear at the end of each thesis chapter/section/appendix submitted as a manuscript/ publication or collected as an appendix at the end of the thesis.

Chapter 4 Mating success of the black soldier fly, *Hermetia illucens* (Diptera: Stratiomyidae), under four artificial light sources



Spermathecae of a newly inseminated black soldier fly (*Hermetia illucens*) female (image by Z. Liu, 2016; CC BY-NC)

This chapter has been published as:

Liu, Zhongyi, Adriana J. Najar-Rodriguez, Maria A. Minor, Duncan I. Hedderley, and Patrick C. H. Morel. 2020. Mating success of the black soldier fly, *Hermetia illucens* (Diptera: Stratiomyidae), under four artificial light sources. *Journal of Photochemistry & Photobiology, B: Biology*, 205: 111815.

Reproduced here with some minor modifications in style and formatting.

The original publication is available at: <https://doi.org/10.1016/j.jphotobiol.2020.111815>

Copyright agreement/license can be found in the Appendices section of this thesis.

Abstract

Larvae of the black soldier fly (BSF) *Hermetia illucens* (L.) (Diptera: Stratiomyidae) are promising organisms to be used for organic waste bioconversion. Breeding BSF indoors has been suggested as a cost-effective approach for countries with long winters or low sunlight levels through the year. As the BSF mating is visually mediated, artificial illumination conditions are critical to a successful indoor breeding system. In this study, we tested four different types of artificial light sources for their effects on BSF mating success. They included: (1) a halogen lamp; (2) a combination of a white light-emitting diode (LED) lamp and a fluorescent ultraviolet lamp; (3) a metal halide lamp, and (4) a specially designed light-emitting diode (BSFLED) lamp, whose design was based on the specific BSF adult visual spectral sensitivity. We determined the spectra of four artificial light sources, compared their spectral composition in relation to the BSF-visible spectrum, and compared their effects on the mating success of two different BSF colonies. BSFLED was the most energy efficient light source in spectral composition and led to the highest mating success in terms of the percentage of inseminated females and fertile clutches. Thus, BSFLED is the most suitable light source tested in our experiment for breeding BSF indoors. The colony effect and possible light flickering effect on BSF mating success were also detected. The implications of these findings are discussed.

Keywords: light spectrum, indoor breeding, mating success, female reproductive organs, colony effect, organic waste bioconversion

4.1 Introduction

The black soldier fly (BSF) *Hermetia illucens* (L.) (Diptera: Stratiomyidae) has been identified as a promising species in organic waste management. BSF larvae can convert a wide range of organic wastes into larval biomass, which can be further used as an animal feed ingredient (Bosch *et al.*, 2014; Makkar *et al.*, 2014; Meneguz *et al.*, 2018; Nguyen *et al.*, 2015; Oonincx *et al.*, 2015; St-Hilaire *et al.*, 2007). After being processed by BSF larvae, the organic waste residues can be used as organic fertilizers (Cai *et al.*, 2019; Choi *et al.*, 2009; Setti *et al.*, 2019). Moreover, it is also feasible to use the lipids extracted from BSF larvae to produce biodiesel, thereby adding more value to the BSF-bioconversion system (Li *et al.*, 2011).

Year-round production of fertile BSF eggs is a prerequisite for using this species as a bioconversion agent (Čičková *et al.*, 2015; Pastor *et al.*, 2015). Therefore, achieving a consistent and high level BSF mating success is of primary concern. Previous studies suggested that BSF mating is visually mediated (Nakamura *et al.*, 2016; Oonincx *et al.*, 2016; Sheppard *et al.*, 2002; Tingle *et al.*, 1975; Tomberlin & Sheppard, 2002; Zhang *et al.*, 2010). Like other animals, BSF visual system has evolved to optimally function under natural illumination (Kelber & Osorio, 2010; Nakamura *et al.*, 2016; Osorio & Vorobyev, 2008; Zhang *et al.*, 2010). Therefore, BSF adults are predominantly kept in glasshouses with sunlight, which is for many countries, available to breed this species year round (Sheppard *et al.*, 2002).

BSF mating decreases when sunlight is limited due to poor weather conditions (Tingle *et al.*, 1975) or in temperate regions due to long winters or low sunlight levels throughout the year, which leads to inconsistent production of fertile eggs. In addition to light, BSF has also optimal temperature requirements (Chia *et al.*, 2018a), which in many instances

are provided through heating. The high costs of insulation and heating make it financially unsustainable to breed BSF in a glasshouse year round, in some countries (Sheppard *et al.*, 2002). An alternative solution is to develop a well-insulated indoor BSF breeding system, where temperature is effectively controlled and an artificial light source is provided to support the consistent production of fertile eggs (Sheppard *et al.*, 2002).

Several artificial light sources have been reported to support BSF mating indoors with some degree of success, including quartz-iodine (i.e., halogen) lamps (Heussler *et al.*, 2018; Zhang *et al.*, 2010), fluorescent lamps (Heussler *et al.*, 2018; Oonincx *et al.*, 2016), light-emitting diode (LED) lamps (Heussler *et al.*, 2018), a combination of an LED lamp and two fluorescent lamps (Nakamura *et al.*, 2016), and an innovative LED lighting system based on the BSF adults' visual spectrum (Oonincx *et al.*, 2016). These artificial light sources have been compared to determine their effects on BSF oviposition, adult longevity, and the resulting number of young larvae (Heussler *et al.*, 2018; Oonincx *et al.*, 2016). However, the influence of artificial light sources on BSF mating success *per se* is unclear.

In this study, we compared the effects of four different artificial light sources on BSF mating success using two different BSF colonies. We also measured the spectra of the tested four artificial light sources, calculated their spectral composition in relation to the BSF-visible spectrum, and compared their energy efficiency in terms of their output spectra.

4.2 Materials and methods

4.2.1 Source of insects

Two BSF colonies were used in this experiment. One colony, referred to as “BROL” hereafter, was established from two thousand prepupae obtained from Birdwatch Rotorua Ltd. (Rotorua), New Zealand in 2017. The larvae of this colony were reared in our laboratory on broll — a by-product of wheat flour production (Liu *et al.*, 2018). Due to our limited rearing capacity, two thousand BROL prepupae were assigned for breeding at each generation. The other colony, referred to as “PRST” hereafter, originated from more than ten thousand larvae collected from Thames, Pukenui, and Palmerston North, New Zealand. The larvae of the PRST colony were fed with food waste collected from restaurants located in Palmerston North, New Zealand. This colony was kept by Prescient Nutrition Ltd. (New Zealand) at a commercial scale, where hundreds of thousands of prepupae were used for breeding at each generation. The adult flies of both colonies were kept in their respective cages (each cage 180 × 90 × 90 cm; L × W × H) in the same glasshouse, and sunlight was the only light source for maintaining both colonies. Prior to this experiment, the BROL and PRST colonies had been in captivity for two and about 14 generations, respectively. To obtain adult flies for each replicate in the present experiment, two hundred pupae were collected from our BROL colony, and two hundred pupae were obtained from the PRST colony. All pupae were kept at 27°C and 80% R.H. (relative humidity), and adults that emerged within a 24-h window with fully expanded wings were collected.

4.2.2 Artificial light sources

Four artificial light sources were included here based on the following criteria:

1. A 500-W halogen lamp (HL110, Arlec Australia Pty. Ltd., Australia) further referred to as “HALO”. Halogen radiation has been reported to successfully facilitate BSF mating (Zhang *et al.*, 2010).
2. A combination of a 50-W white LED lamp (WLED100, Arlec Australia Pty. Ltd., Australia) and a 50-W compact fluorescent black light (LT50G, Mayo Hardware Pty. Ltd., Australia) further referred to as “LEDUV”. This light source is similar to that tested by Nakamura *et al.* (2016), and follows earlier suggestions that ultraviolet (UV) radiation is crucial for BSF mating (Oonincx *et al.*, 2016).
3. A 400-W metal halide lamp (MF400DL, Eye Lighting International of North America Inc., U.S.) referred to as “MH”. This light was included in our study because its spectral pattern resembles close to that of sunlight.
4. A 20-W LED lamp (BSF-4C-20-3900A, JM Green Co. Ltd., China) further referred to as “BSFLED”. This light was included because it was designed to match the visual spectral sensitivity of BSF adults (Oonincx *et al.*, 2016).

4.2.3 Experimental design

The experiment was originally subject to a randomized block design (blocking by time) to test the HALO, LEDUV, and MH lights with the BROL colony only. However, no fertile egg clutches were obtained from any of these lights after two replicates. After that,

a trial with adults from the PRST colony under the BSFLED light revealed successful reproduction. Therefore, a new randomized block design with (a) three replicates for the PRST colony and all four types of lights, (b) three replicates for the BROL colony and the BSFLED light, and (c) one more replicate for BROL colony and the HALO, LEDUV and MH lights was set up. Due to spatial and facility constraints, only one replicate per treatment could be run at a time. The entire experiment was carried out from September 2017 to November 2018.

For each artificial light and colony, a $30 \times 30 \times 30$ cm BugDorm cage (DP-1000, MegaView Science Co. Ltd., Taiwan) was used to accommodate 20 BSF adults with a 1 : 1 sex ratio (Ooninx *et al.*, 2016). To provide water for the flies, a piece of paper towel was folded into a fan to wick water up through a hole on the lid of a 250-mL plastic container (Cuisine Queen FS3320, Seymour Distributing Co. Ltd., New Zealand). Two sugar cubes were placed on the cage floor (Nakamura *et al.*, 2016). Another 250-mL plastic container was used to hold fermented larvae-consumed broil to attract oviposition (Nakamura *et al.*, 2016), and covered with a mesh lid to allow volatile emission. Three pieces of 3×6 cm corrugated cardboard strips (flute length: 3 cm; flute opening: ca. 2×3 mm) were glued together and placed on the mesh lid to provide oviposition sites. The cage setup is illustrated in Figure S4.1.

The artificial light source to be tested each time was set up above the cage. The illumination regime was set as 12 L : 12 D (Ooninx *et al.*, 2016). During the scotophase, when the light was off, the temperature in the experimental room was maintained at $27 \pm 0.5^\circ\text{C}$ by using an air conditioner (CS-E7PKR, Panasonic Corp., Japan). During the photophase, the infrared radiation from the HALO and MH lights brought the temperature in the experimental room to $29 \pm 1^\circ\text{C}$. Thus, to assure the same temperature regime for

all the light treatments, supplementary heating was provided in the LEDUV and BSFLED light treatments during the photophase. Because higher light intensity (i.e., irradiance) could benefit BSF mating (Oonincx *et al.*, 2016; Tingle *et al.*, 1975; Tomberlin & Sheppard, 2002), the distance from the bottom of each type of light to the top of the cage was set as small as possible, as long as the temperature could be controlled within the aforementioned range during the photophase. The irradiance (300–885 nm) of each artificial light source at the center of the cage was measured with a spectrometer (USB4000, Ocean Optics, Inc., U.S.), with the sensor facing upwards.

Sunlight irradiance was also measured on five sunny southern hemisphere summer afternoons, during January 8th–31st, 2018 at the center of a glasshouse (40°22'41.3"S, 175°36'48.13"E), where the two BSF adult colonies were kept. The sunlight spectrum was averaged over the five measurements. Because BSF mating prefers sunny days and higher light intensities (Oonincx *et al.*, 2016; Tingle *et al.*, 1975), our measured sunlight represents the most possibly suitable natural illumination for BSF mating. Relative humidity in the experimental room was kept at $60 \pm 10\%$.

Each artificial light and colony was replicated three times. For each replicate, the BSF adults were kept in the cage with a particular type of light for 10 d. This timing was selected because (1) light type does not affect BSF fecundity or oviposition temporal pattern (Nakamura *et al.*, 2016; Oonincx *et al.*, 2016), and (2) in preliminary trials, females from both colonies laid more than 80% eggs within the first 10 d.

During the course of each replicate, egg clutches were collected and counted, and dead adults were removed from the cage and stored at -20°C every day. It was difficult to determine the true size of a clutch, because (1) it depends on female fecundity and the

size of the crevice, where the eggs are laid, (2) females may lay their eggs into a single egg clutch, and (3) a female may deposit her eggs into multiple clutches at different locations (Heussler *et al.*, 2018; Tomberlin *et al.*, 2002). Therefore, an egg clutch was arbitrarily defined as a group of more than 200 eggs that were laid together. The collected egg clutches were incubated at 27°C and 80% R.H. for 5 d to determine their fertility (Holmes *et al.*, 2012). Fertile eggs showed eye spots during the incubation period. The percentage of fertile clutches was used to estimate mating success (Nakamura *et al.*, 2016). In a preliminary experiment, females that had laid fertile eggs were found to also carry sperm in their spermathecae after death, while sperm was not found in females that laid infertile eggs (Figure 4.1). Therefore, the presence of sperm in BSF female spermathecae was used as a sign of successful mating. All the females were dissected after each replicate, and the percentage of inseminated females was also used as an indicator of mating success.

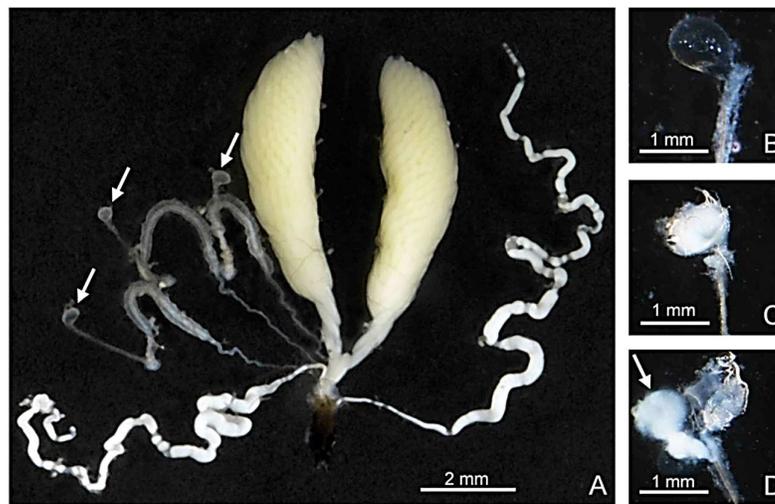


Figure 4.1 (A) Reproductive organs of a black soldier fly, *Hermetia illucens*, female — arrows indicate spermathecae. (B) A spermatheca of a virgin female. A spermatheca of a newly inseminated female is shown in (C), which is ruptured in (D) — arrow indicates sperm.

4.2.4 Calculations and statistics

The absolute spectral irradiance were integrated into 300–400 nm, 400–500 nm, 500–650 nm, and 650–885 nm using OriginPro 2016 (OriginLab Corp., U.S.) (Ooninx *et al.*, 2016). For each light source, the percentage of irradiance within each selected waveband out of the total measured irradiance was calculated.

Generalized linear models (GLiMs) with a Poisson distribution (logarithm link) for count data, or a binomial distribution (logit link) for percentage data were used to determine the effects of colony and artificial light source on the number of egg clutches, the percentage of fertile egg clutches, and the percentage of inseminated females. The experiment was run over a considerable time. Thus, to account for a possible drift over time, the time when each replicate was started (time lapse) was fitted in all models. Accumulated deviance analysis was conducted based on the GLiMs. Pairwise likelihood ratio tests with Bonferroni corrections were used for multiple comparisons. Pearson correlations were used to analyze the correlation between the number of inseminated females and the number of fertile egg clutches per light type and colony, and the correlation between the percentage of inseminated females and the percentage of fertile egg clutches per light type and colony.

All statistical analyses were performed using Genstat 18th Edition (VSN International Ltd., U.K.), with the significance level for all tests being set at $\alpha = 0.05$.

4.3 Results

4.3.1 Selected optical properties of the tested artificial light sources

The light intensity and spectral pattern of the artificial light sources used in this study were found to be highly specific and distinct (Table 4.1, Figure 4.2). The highest BSF-visible irradiance (i.e. light intensity) was observed under the BSFLED light, followed by the MH light (Table 4.1). The BSF-visible irradiance under the HALO and the LEDUV lights was only 6% and 19%, respectively, of that under the BSFLED light (Table 4.1). The highest UV (300–400 nm) irradiance was observed under the MH light, while the highest irradiance at blue (400–500 nm) and long-wavelength (500–650 nm) wavebands were both observed under the BSFLED light (Table 4.1). Except for the HALO light, all the artificial light sources provided higher UV irradiance than sunlight (Table 4.1 and Figure 4.2A–D). Regarding the non-UV BSF-visible irradiance (i.e., 400–650 nm), the MH light was found to be similar to sunlight, while the BSFLED light was found to be much stronger than sunlight (Table 4.1 and Figure 4.2C, D).

Along the selected waveband (300–885 nm), the LEDUV and BSFLED lights had 89.6 and 95.5% of their radiation output within the BSF-visible spectrum, respectively; whereas only 22% of the HALO light radiation was within the BSF-visible spectrum (Table 4.1). Across the tested artificial light sources, the BSF-visible spectral composition of the MH light had the most similar pattern to that of sunlight (Table 4.1).

Table 4.1 The absolute irradiance ($\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) across different wavebands (from 300 to 885 nm) of four artificial light sources: a 500-W halogen lamp (HALO), a combination of a 50-W white LED lamp and a 50-W compact fluorescent black light (LEDUV), a 400-W metal halide lamp (MH), a 20-W LED lamp designed for black soldier fly, *Hermetia illucens*, breeding (BSFLED). Sunlight is included as a reference. The proportional irradiance (%) of each light is shown in parentheses.

Waveband	Visibility to <i>H. illucens</i> adults ^a	HALO	LEDUV	MH	BSFLED	Sunlight
300–400 nm	Visible	0.9 (0.3)	26.8 (13.1)	42.5 (5.9)	28.2 (2.9)	20.2 (1.9)
400–500 nm	Visible	7.8 (3.0)	45.2 (22.1)	186.8 (25.8)	457.4 (46.3)	188.6 (18.1)
500–650 nm	Visible	49.6 (18.7)	111.6 (54.5)	301.8 (41.7)	459.2 (46.4)	336.9 (32.4)
650–885 nm	Invisible	206.2 (78.0)	21.2 (10.4)	193.0 (26.7)	44.1 (4.5)	493.6 (47.5)

^aThe wavebands visible and invisible to *H. illucens* adults are indicated according to Ooninx *et al.* (2016).

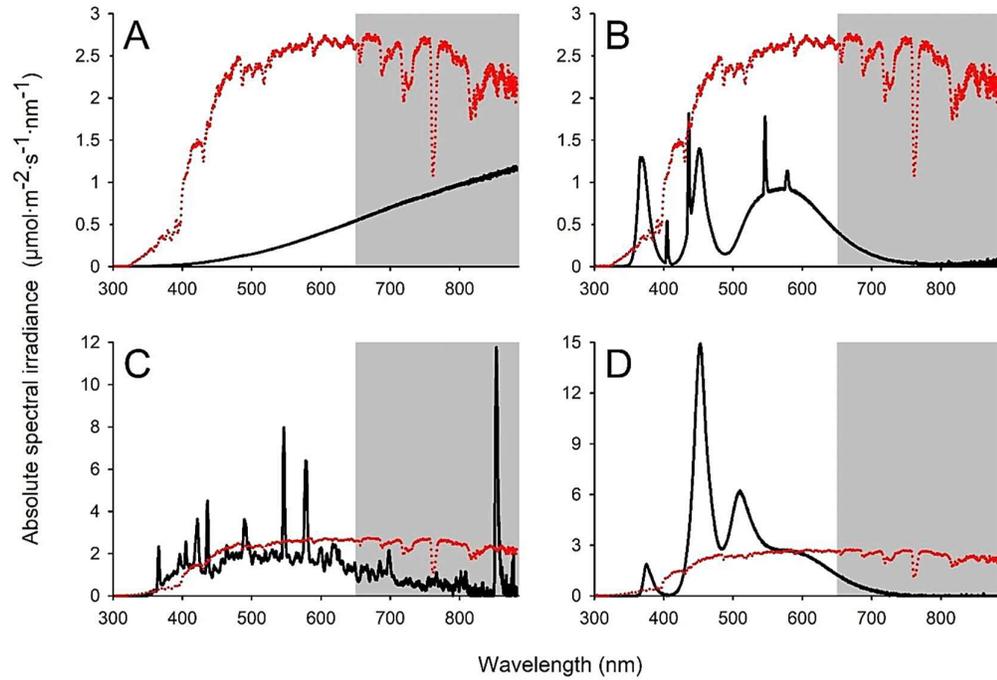


Figure 4.2 The absolute spectral irradiance of four artificial light sources tested for their effects on black soldier fly, *Hermetia illucens*, mating success. (A) a 500-W halogen lamp (HALO), (B) a combination of a 50-W white LED lamp and a 50-W compact fluorescent black light (LEDUV), (C) a 400-W metal halide lamp (MH), (D) a 20-W LED lamp designed for black soldier fly, *H. illucens*, breeding (BSFLED). The measurement for the artificial light sources was conducted at the center of the cage containing *H. illucens* adult flies. The grey area indicates wavelengths (> 650 nm) that are invisible to the *H. illucens* adult flies (Oonincx *et al.*, 2016). The red dotted curves represent sunlight radiation.

4.3.2 Effects of artificial light on BSF mating

Both insect colony and artificial light source had significant effects on the percentage of fertile egg clutches and inseminated females, but these two factors did not affect the number of egg clutches (Table 4.2, Figure 4.3). Time (i.e. number of days after the first day of the experiment) did not affect the number of egg clutches or the percentage of inseminated females; however, the percentage of fertile egg clutches changed significantly with time (Table 4.2).

For the BROL colony, fertile egg clutches and inseminated females were only observed when BSF adults were exposed to the BSFLED light (Figure 4.3B, C). Therefore, pairwise comparisons were not conducted for this colony.

For the PRST colony, adults exposed to the BSFLED light had the highest mating success ($90.0 \pm 5.8\%$) and the highest production of fertile egg clutches ($91.4 \pm 5.3\%$), followed by the HALO and the LEDUV lights (Figure 4.3E, F). No fertile egg clutches or inseminated females were collected when adults were exposed to the MH light (Figure 4.3E, F).

There were significant differences between the two colonies in terms of mating success. Under the BSFLED light, successful reproduction was observed in all replicates when the PRST colony was used, and on average more than eight females mated; when the BROL colony was used, mating success was only observed in one replicate, where only four inseminated females were found. Moreover, mating success was observed under the HALO and LEDUV lights with the PRST colony but not with the BROL colony (Figure 4.3B, C, E, F).

Table 4.2 Accumulated deviance analyses conducted based on generalized linear models to test the effects of insect colony, artificial light source, and time on the number of egg clutches, the percentage of fertile egg clutches, and the percentage of inseminated females.

Parameter	Factor	df	Deviance	Deviance ratio	<i>P</i>
Number of egg clutches	Colony	1	1.56	1.56	0.21
	Artificial light source	3	2.92	0.97	0.40
	Colony × Artificial light source	3	4.82	1.61	0.19
	Time lapse	1	0.05	0.05	0.82
	Residual	15	3.19		
	Total	23	12.55		
Percentage of fertile egg clutches	Colony	1	38.48	38.48	< 0.001
	Artificial light source	3	108.54	36.18	< 0.001
	Colony × Artificial light source	3	0.11	0.04	0.99
	Time lapse	1	6.43	6.43	0.01
	Residual	15	8.73		
	Total	23	162.28		
Percentage of inseminated females	Colony	1	36.12	36.12	< 0.001
	Artificial light source	3	90.37	30.12	< 0.001
	Colony × Artificial light source	3	0.41	0.14	0.94
	Time lapse	1	1.60	1.60	0.21
	Residual	15	15.81		
	Total	23	144.31		

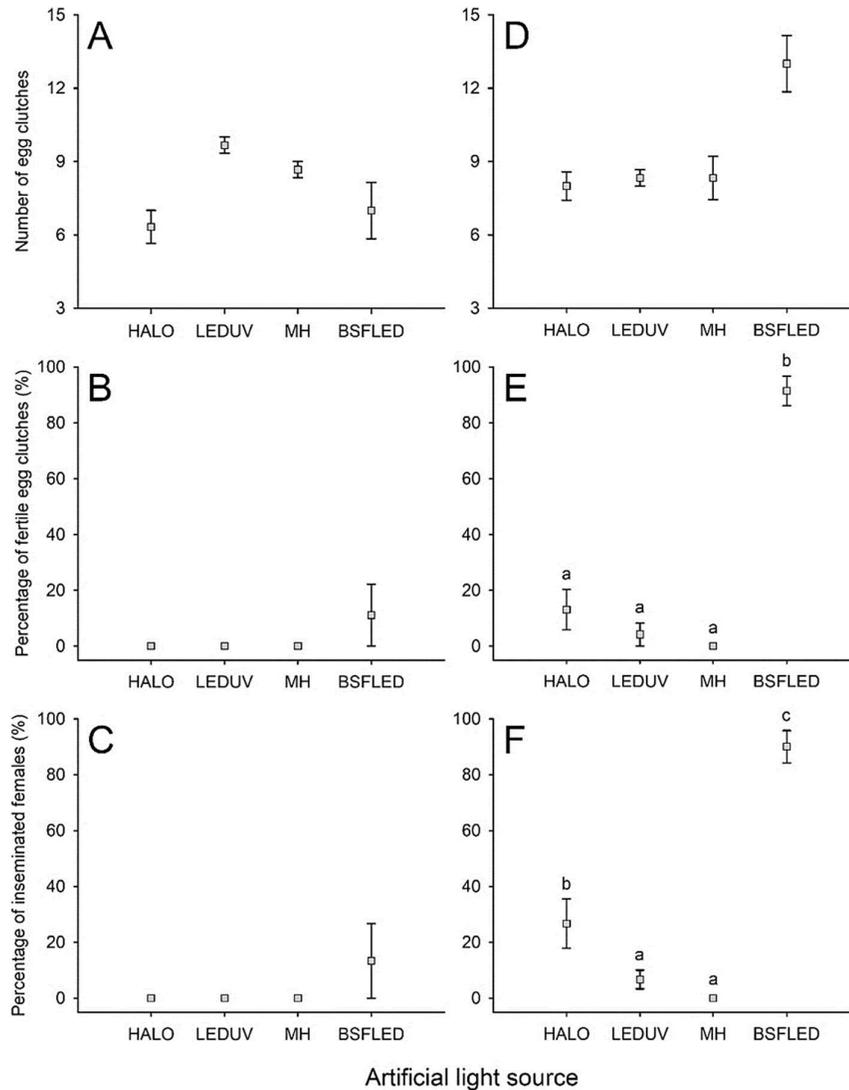


Figure 4.3 Number of black soldier fly, *Hermetia illucens*, egg clutches collected, percentage of fertile egg clutches, and percentage of inseminated females from the BROL colony (A–C) and the PRST colony (D–F) under different artificial lights: a 500-W halogen lamp (HALO), a combination of a 50-W white LED lamp and a 50-W compact fluorescent black light (LEDUV), a 400-W metal halide lamp (MH), and a 20-W LED lamp designed for *H. illucens* breeding (BSFLED). Data points represent means, error bars denote the standard error of the means. The number of eggs did not significantly differ across light treatments (A and D). Pairwise comparisons were not conducted for the BROL colony regarding the percentage of fertile egg clutches (B) or inseminated females (C). Different letters above the error bars in E and F indicate significant differences across light treatments ($P < 0.05$).

The number of inseminated females had a significant positive correlation with the number of fertile clutches ($R = 0.95$; $P < 0.001$; Figure 4.4A). Similarly, the percentage of inseminated females was positively correlated to the percentage of fertile clutches ($R = 0.98$; $P < 0.001$; Figure 4.4B).

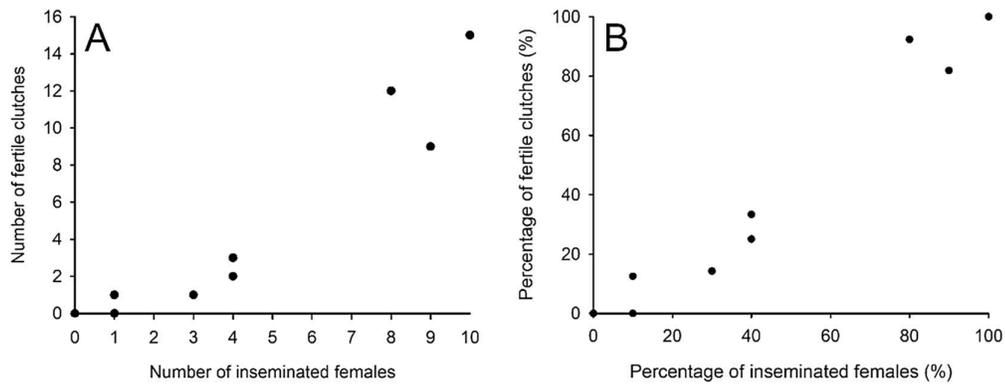


Figure 4.4 Scatter plots of (A) the number of inseminated black soldier fly, *Hermetia illucens* females against the number of fertile egg clutches, and (B) the percentage of inseminated *H. illucens* females against the percentage of fertile egg clutches. Data from both colonies were used.

4.4 Discussion

Breeding BSF indoors has been suggested as an energy-efficient approach to consistently obtain fertile eggs of this species year round (Sheppard *et al.*, 2002). This would be particularly beneficial in regions with low sunlight levels or prolonged winters. However, a key challenge is to optimize the artificial illumination conditions. In this experiment, we determined the spectra and calculated the spectral energy allocations of four artificial light sources, and then tested their effects on BSF mating success.

Early studies using a halogen lamp or a combination of LED and fluorescent lamps revealed some success at supporting BSF mating (Nakamura *et al.*, 2016; Zhang *et al.*, 2010). However, until the spectral sensitivity of the photoreceptors of the compound eyes of the adult BSF was reported, it was unclear why different light spectra led to different mating success (Oonincx *et al.*, 2016). In our study, the BSFLED light, which was designed according to the spectral sensitivity of the BSF adult photoreceptors, yielded the highest number of inseminated females and fertile egg clutches. For the PRST colony, adults exposed to this type of light had the highest mating success ($90.0 \pm 5.8\%$) and the highest production of fertile egg clutches ($91.4 \pm 5.3\%$). Such a high level of mating success has never been reported before and clearly suggested that BSFLED is a very suitable light for successful BSF indoor breeding.

From an industrial perspective, energy efficiency is also a critical parameter when selecting an artificial light source for BSF breeding. By investigating the spectra of the tested lights, we found that the BSFLED light emitted more than 95% of its radiation within the BSF-visible waveband (300–650 nm), whereas the HALO and MH lights emitted, respectively, 78.0% and 26.7% of their radiation in the BSF-invisible range (> 650 nm). Thus, our results suggest that an artificial light spectrum that matches the BSF visible sensitivity does not only increase BSF mating success, but also improves energy use efficiency. However, we also found that the BSF-visible light intensity of the BSFLED light extends beyond that of bright sunlight. Such high light intensity may not be necessary for an optimized BSF mating system, and thus may represent a waste of energy. Future studies are needed to optimize the light intensity emitted by the BSFLED light or light sources with similar spectra, thereby minimizing power consumption while still maximizing BSF mating success.

Higher light intensity (i.e., higher irradiance) has been reported to be more optimal for BSF reproduction (Oonincx *et al.*, 2016; Tingle *et al.*, 1975; Tomberlin & Sheppard, 2002). In our experiment, the higher BSF-visible irradiance was measured under the MH light than under the LEDUV or the HALO lights (Table 4.1). However, while the PRST colony successfully reproduced under the HALO and the LEDUV lights, no fertile egg clutches or inseminated females were collected under the MH light. Moreover, significantly more inseminated PRST females were found under the HALO light than under the LEDUV light, in spite of a lower BSF-visible irradiance measured under the former light. A possible reason is that apart from light intensity, light flickering also affects BSF mating (Oonincx *et al.*, 2016). Eichorn *et al.* (2017) found that casting a flickering light on the wings of female *Lucilia sericata* (Meigen) could attract mate-seeking males, and that male response was significantly different with different flickering frequencies, as wing beat frequency represents both sex and age in this species. BSF males also use female wing beats as a visual cue to recognize potential mates (Giunti *et al.*, 2018). Flickering light casting on female wings may interfere with the males' ability to recognize the females' wing beat frequency, and thus could lead to reducing mate recognition. In our experiments, this variable differed across the tested artificial light sources. Flickering could be observed with the naked eyes in the MH (personal observation), while the same was not true for the other light sources. However, we were unable to quantify the exact frequency of flickering for each tested artificial light source, and thus cannot conclude if flickering effects do affect BSF mating success. Oonincx *et al.* (2016) suggested that light flickering could be avoided by using direct current (DC) power supply or high-frequency ballasts. This aspect warrants further investigation.

Oonincx *et al.* (2016) emphasized the importance of light spectral composition in supporting BSF mating. These authors suggested that UV radiation (< 400 nm) is crucial

for BSF mating success. However, while BSF successfully mated under a white LED lamp (400–800 nm) without any UV radiation (Heussler *et al.*, 2018), mating was not observed under a rear-earth lamp with a spectrum between 350 and 450 nm (Zhang *et al.*, 2010). Therefore, non-UV radiation may also play a key role in BSF mating. In our experiment, different UV and non-UV irradiance were measured under different artificial light sources (Table 4.1). However, due to the possible flickering effect, it was difficult to interpret the influence of radiation at different wavebands on BSF mating success.

Previous studies used the number of mating pairs (e.g., Zhang *et al.*, 2010), the number and percentage of fertile egg clutches (e.g., Nakamura *et al.*, 2016), or the number of resulting young larvae (e.g., Oonincx *et al.*, 2016), to estimate the effect of an artificial light source on BSF mating success. However, acquiring the number of mating pairs requires a regular and precise counting, which could be difficult at high adult densities. Because a fertile female may deposit her eggs at various locations, and a fertile clutch may be attributed to multiple females, the number and percentage of fertile clutches may not be a precise indicator of mating success (Heussler *et al.*, 2018; Tomberlin *et al.*, 2002). Similarly, the number of young larvae might be influenced by egg hatching rate and larval mortality, and thus is not fully indicative of mating success. Inspecting spermathecae allowed us to determine the exact number of females that had mated successfully when exposed to each particular light type. We were able to dissect a female and inspect spermathecae within as little as 30 s. We found a significantly positive correlation between the percentage of inseminated females and the percentage of fertile egg clutches, which indicated that the latter is a reliable parameter to estimate BSF mating success in our experiment. However, we suggest the percentage of fertile egg clutches to be used with low adult densities (e.g., 10 pairs), because different egg clutches may cluster together under the oviposition attractant container when higher adult densities are used

(Heussler *et al.*, 2018).

Interestingly, we found differences in reproductive performance and response to the artificial lights tested according to the BSF colony used. The number and percentage of inseminated females and fertile egg clutches were significantly higher for the PRST colony compared to the BROL colony, regardless of the type of light used. Because all BSF adults were only fed with water and sugar in our experiment, the nutritional resource allocated to reproduction at the adult stage should have originated mainly (if not all) from resources accumulated during the larval stage. Quantity and quality of larval diet have been shown to affect reproductive fitness of adult insects, including female fecundity (Telang *et al.*, 2006), male mating success and spermatophore size (Delisle & Hardy, 1997). Female fecundity of BSF can also be affected by larval diet (Chia *et al.*, 2018b), especially by its nutrient concentration and protein content (Barragán-Fonseca, 2018). Compared with the diet fed to the BROL colony, i.e. broil only, which is a by-product of wheat flour production, the diet fed to the PRST colony included a broader variety of nutritional sources including meat, grain, vegetables, and fruits, and was probably a more balanced BSF larval diet. Nevertheless, Barragán-Fonseca (2018) reported that BSF larval diet did not affect the percentage of fertile egg clutches. Given that the definition of an egg clutch is still ambiguous, we suggest using the percentage of inseminated females to further investigate the effects of larval diet on BSF mating success.

Genetic effects may have also affected mating success in the colonies used in our experiment. The BROL colony originated from only one geographic population (i.e., Rotorua), while the PRST colony originated from three geographic populations in New Zealand (i.e., Thames, Pukenui, and Palmerston North). Moreover, the adult population size of the BROL colony was much smaller ($< 1/100$) than the PRST colony. Thus, even

though the BROL colony have been kept in captivity for fewer generations than the PRST colony, the BROL colony is still more prone to loss of genetic diversity and possible inbreeding depression than the PRST colony (Falconer & Mackay, 1996; Frankham *et al.*, 2010). Studies have revealed that inbreeding could affect insect mating success through deteriorating fitness (Rantala *et al.*, 2011), impaired mating behaviors (Miller *et al.*, 1993), and changing sexually selected traits (Drayton *et al.*, 2007). Since an increasing number of BSF colonies are being kept in captivity for commercial feed production and waste management, inbreeding is likely to happen in poorly managed colonies, and its potential consequences and ways of avoidance should be addressed by future studies.

In summary, among the four types of artificial light sources tested here, the BSFLED light was the most efficient in terms of energy allocation to the BSF-visible waveband, and it led to the highest mating success. We recommend the use of this light type or similar ones for BSF-indoor breeding. We also identified a colony effect on BSF mating success, which warrants future studies and consideration. To the best of our knowledge, this is the first study that shows the morphology of BSF female reproductive organs with some detail. We recommend using the presence of sperm in the spermathecae as a reliable indicator of mating success. Now that we have identified a very effective artificial light source for BSF breeding, in-detail reproductive studies of this species could be conducted under easily controllable indoor environments.

4.5 Acknowledgments

This research was partially funded by the Bioresource Processing Alliance in New Zealand. The authors would like to thank Neil Birrell for his help with an earlier version of this manuscript. We want to acknowledge Dr. Simon Deroles for his assistance with using a spectrometer and Fang Zeng for her assistance with experiments. We would like to thank the three anonymous reviewers for their helpful suggestions.

4.6 References

- Barragán-Fonseca, K. B. (2018). *Flies are what they eat: Tailoring nutrition of black soldier fly (Hermetia illucens L.) for larval biomass production and fitness* [Doctoral thesis, Wageningen University]. Wageningen University & Research. <https://doi.org/10.18174/449739>
- Bosch, G., Zhang, S., Oonincx, D. G. A. B., & Hendriks, W. H. (2014). Protein quality of insects as potential ingredients for dog and cat foods. *Journal of Nutritional Science*, 3, e29, Article e29. <https://doi.org/10.1017/jns.2014.23>
- Cai, M., Zhang, K., Zhong, W., Liu, N., Wu, X., Li, W., Zheng, L., Yu, Z., & Zhang, J. (2019). Bioconversion-composting of golden needle mushroom (*Flammulina velutipes*) root waste by black soldier fly (*Hermetia illucens*, Diptera: Stratiomyidae) larvae, to obtain added-value biomass and fertilizer. *Waste and Biomass Valorization*, 10(2), 265–273. <https://doi.org/10.1007/s12649-017-0063-2>
- Chia, S. Y., Tanga, C. M., Khamis, F. M., Mohamed, S. A., Salifu, D., Sevgan, S., Fiaboe, K. K. M., Niassy, S., van Loon, J. J. A., Dicke, M., & Ekesi, S. (2018a). Threshold temperatures and thermal requirements of black soldier fly *Hermetia illucens*: Implications for mass production. *PLOS ONE*, 13(11), e0206097. <https://doi.org/10.1371/journal.pone.0206097>
- Chia, S. Y., Tanga, C. M., Osuga, I. M., Mohamed, S. A., Khamis, F. M., Salifu, D., Sevgan, S., Fiaboe, K. K., Niassy, S., & van Loon, J. J. (2018b). Effects of waste stream combinations from brewing industry on performance of black soldier fly, *Hermetia illucens* (Diptera: Stratiomyidae). *PeerJ*, 6, e5885. <https://doi.org/10.7717/peerj.5885>
- Choi, Y. C., Choi, J. Y., Kim, J. G., Kim, M. S., Kim, W. T., Park, K. H., Bae, S. W., & Jeong, G. S. (2009). Potential usage of food waste as a natural fertilizer after digestion by *Hermetia illucens* (Diptera: Stratiomyidae). *International journal of industrial entomology*, 19(1), 171–174.

- Čičková, H., Newton, G. L., Lacy, R. C., & Kozánek, M. (2015). The use of fly larvae for organic waste treatment. *Waste Management*, *35*, 68–80. <https://doi.org/10.1016/j.wasman.2014.09.026>
- Delisle, J., & Hardy, M. (1997). Male larval nutrition influences the reproductive success of both sexes of the Spruce Budworm, *Choristoneura fumiferana* (Lepidoptera: Tortricidae). *Functional Ecology*, *11*(4), 451–463. <https://doi.org/10.1046/j.1365-2435.1997.00114.x>
- Drayton, J. M., Hunt, J., Brooks, R., & Jennions, M. D. (2007). Sounds different: Inbreeding depression in sexually selected traits in the cricket *Teleogryllus commodus*. *Journal of Evolutionary Biology*, *20*(3), 1138–1147. <https://doi.org/10.1111/j.1420-9101.2006.01286.x>
- Eichorn, C., Hrabar, M., Van Ryn, E. C., Brodie, B. S., Blake, A. J., & Gries, G. (2017). How flies are flirting on the fly. *BMC Biology*, *15*(1), 2. <https://doi.org/10.1186/s12915-016-0342-6>
- Falconer, D. S., & Mackay, T. F. C. (1996). *Introduction to quantitative genetics* (4th ed.). Pearson.
- Frankham, R., Ballou, J. D., & Briscoe, D. A. (2010). *Introduction to Conservation Genetics* (2nd ed.). Cambridge University Press.
- Giunti, G., Campolo, O., Laudani, F., & Palmeri, V. (2018). Male courtship behaviour and potential for female mate choice in the black soldier fly *Hermetia illucens* L. (Diptera: Stratiomyidae). *Entomologia Generalis*, *38*(1), 29–46. <https://doi.org/10.1127/entomologia/2018/0657>
- Heussler, C. D., Walter, A., Oberkofler, H., Insam, H., Arthofer, W., Schlick-Steiner, B. C., & Steiner, F. M. (2018). Influence of three artificial light sources on oviposition and half-life of the black soldier fly, *Hermetia illucens* (Diptera: Stratiomyidae): Improving small-scale indoor rearing. *PLOS ONE*, *13*(5), e0197896. <https://doi.org/10.1371/journal.pone.0197896>
- Holmes, L. A., Vanlaerhoven, S. L., & Tomberlin, J. K. (2012). Relative humidity effects on the life history of *Hermetia illucens* (Diptera: Stratiomyidae). *Environmental Entomology*, *41*(4), 971–978. <https://doi.org/10.1603/en12054>
- Kelber, A., & Osorio, D. (2010). From spectral information to animal colour vision: experiments and concepts. *Proceedings of the Royal Society B: Biological Sciences*, *277*(1688), 1617–1625. <https://doi.org/10.1098/rspb.2009.2118>
- Li, Q., Zheng, L., Cai, H., Garza, E., Yu, Z., & Zhou, S. (2011). From organic waste to biodiesel: Black soldier fly, *Hermetia illucens*, makes it feasible. *Fuel*, *90*(4), 1545–1548. <https://doi.org/10.1016/j.fuel.2010.11.016>
- Liu, Z., Minor, M., Morel, P. C. H., & Najar-Rodriguez, A. J. (2018). Bioconversion of three organic wastes by black soldier fly (Diptera: Stratiomyidae) larvae. *Environmental Entomology*, *47*(6), 1609–1617. <https://doi.org/10.1093/ee/nvy141>
- Makkar, H. P. S., Tran, G., Heuzé, V., & Ankers, P. (2014). State-of-the-art on use of insects as animal feed. *Animal Feed Science and Technology*, *197*, 1–33. <https://doi.org/10.1016/j.anifeedsci.2014.07.008>

- Meneguz, M., Schiavone, A., Gai, F., Dama, A., Lussiana, C., Renna, M., & Gasco, L. (2018). Effect of rearing substrate on growth performance, waste reduction efficiency and chemical composition of black soldier fly (*Hermetia illucens*) larvae. *Journal of the Science of Food and Agriculture*, *98*(15), 5776–5784. <https://doi.org/10.1002/jsfa.9127>
- Miller, P. S., Glasner, J., & Hedrick, P. W. (1993). Inbreeding depression and male-mating behavior in *Drosophila melanogaster*. *Genetica*, *88*(1), 29. <https://doi.org/10.1007/BF02424449>
- Nakamura, S., Ichiki, R. T., Shimoda, M., & Morioka, S. (2016). Small-scale rearing of the black soldier fly, *Hermetia illucens* (Diptera: Stratiomyidae), in the laboratory: Low-cost and year-round rearing. *Applied Entomology and Zoology*, *51*(1), 161–166. <https://doi.org/10.1007/s13355-015-0376-1>
- Nguyen, T. T. X., Tomberlin, J. K., & Vanlaerhoven, S. (2015). Ability of black soldier fly (Diptera: Stratiomyidae) larvae to recycle food waste. *Environmental Entomology*, *44*(2), 406–410. <https://doi.org/10.1093/ee/nvv002>
- Oonincx, D. G. A. B., van Huis, A., & van Loon, J. J. A. (2015). Nutrient utilisation by black soldier flies fed with chicken, pig, or cow manure. *Journal of Insects as Food and Feed*, *1*(2), 131–139. <https://doi.org/10.3920/jiff2014.0023>
- Oonincx, D. G. A. B., Volk, N., Diehl, J. J. E., van Loon, J. J. A., & Belušič, G. (2016). Photoreceptor spectral sensitivity of the compound eyes of black soldier fly (*Hermetia illucens*) informing the design of LED-based illumination to enhance indoor reproduction. *Journal of Insect Physiology*, *95*, 133–139. <https://doi.org/10.1016/j.jinsphys.2016.10.006>
- Osorio, D., & Vorobyev, M. (2008). A review of the evolution of animal colour vision and visual communication signals. *Vision Research*, *48*(20), 2042–2051. <https://doi.org/10.1016/j.visres.2008.06.018>
- Pastor, B., Velasquez, Y., Gobbi, P., & Rojo, S. (2015). Conversion of organic wastes into fly larval biomass: Bottlenecks and challenges. *Journal of Insects as Food and Feed*, *1*(3), 179–193. <https://doi.org/10.3920/jiff2014.0024>
- Rantala, M. J., Viitaniemi, H., & Roff, D. A. (2011). Effects of inbreeding on potential and realized immune responses in *Tenebrio molitor*. *Parasitology*, *138*(7), 906–912. <https://doi.org/10.1017/S0031182011000473>
- Setti, L., Francia, E., Pulvirenti, A., Gigliano, S., Zaccardelli, M., Pane, C., Caradonia, F., Bortolini, S., Maistrello, L., & Ronga, D. (2019). Use of black soldier fly (*Hermetia illucens* (L.), Diptera: Stratiomyidae) larvae processing residue in peat-based growing media. *Waste Management*, *95*, 278–288. <https://doi.org/10.1016/j.wasman.2019.06.017>
- Sheppard, D. C., Tomberlin, J. K., Joyce, J. A., Kiser, B. C., & Sumner, S. M. (2002). Rearing methods for the black soldier fly (Diptera: Stratiomyidae). *Journal of Medical Entomology*, *39*(4), 695–698. <https://doi.org/10.1603/0022-2585-39.4.695>
- St-Hilaire, S., Cranfill, K., McGuire, M. A., Mosley, E. E., Tomberlin, J. K., Newton, L., Sealey, W., Sheppard, C., & Irving, S. (2007). Fish offal recycling by the black soldier fly produces a foodstuff high in omega-3 fatty acids. *Journal of the World*

- Aquaculture Society*, 38(2), 309–313. <https://doi.org/10.1111/j.1749-7345.2007.00101.x>
- Telang, A., Li, Y., Noriega, F. G., & Brown, M. R. (2006). Effects of larval nutrition on the endocrinology of mosquito egg development. *Journal of Experimental Biology*, 209(4), 645–655. <https://doi.org/10.1242/jeb.02026>
- Tingle, F. C., Mitchell, E. R., & Copeland, W. W. (1975). The soldier fly, *Hermetia illucens* in poultry houses in north central Florida. *The Journal of the Georgia Entomological Society*, 10(2), 179–183.
- Tomberlin, J. K., & Sheppard, D. C. (2002). Factors influencing mating and oviposition of black soldier flies (Diptera: Stratiomyidae) in a colony. *Journal of Entomological Science*, 37(4), 345–352. <https://doi.org/10.18474/0749-8004-37.4.345>
- Tomberlin, J. K., Sheppard, D. C., & Joyce, J. A. (2002). Selected life-history traits of black soldier flies (Diptera: Stratiomyidae) reared on three artificial diets. *Annals of the Entomological Society of America*, 95(3), 379–386. [https://doi.org/10.1603/0013-8746\(2002\)095\[0379:Slhtob\]2.0.Co;2](https://doi.org/10.1603/0013-8746(2002)095[0379:Slhtob]2.0.Co;2)
- Zhang, J., Huang, L., He, J., Tomberlin, J. K., Li, J., Lei, C., Sun, M., Liu, Z., & Yu, Z. (2010). An artificial light source influences mating and oviposition of black soldier flies, *Hermetia illucens*. *Journal of Insect Science*, 10(1), 202. <https://doi.org/10.1673/031.010.20201>

4.7 Appendix

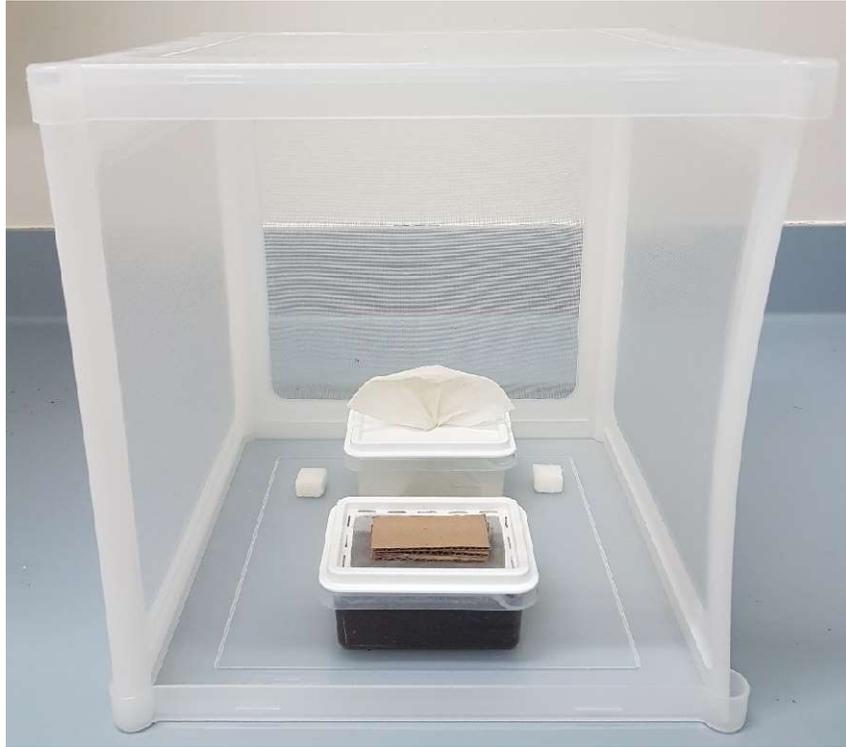


Figure S 4.1 Cage setup used in this study to test the effect of different lighting systems on the mating success of black soldier fly, *Hermetia illucens*, adults.



GRADUATE
RESEARCH
SCHOOL

STATEMENT OF CONTRIBUTION DOCTORATE WITH PUBLICATIONS/MANUSCRIPTS

We, the candidate and the candidate's Primary Supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the candidate's contribution as indicated below in the *Statement of Originality*.

Name of candidate:	Zhongyi Liu
Name/title of Primary Supervisor:	Dr Maria Minor
In which chapter is the manuscript /published work:	Chapter 5
<p>Please select one of the following three options:</p> <p><input checked="" type="radio"/> The manuscript/published work is published or in press</p> <ul style="list-style-type: none"> Please provide the full reference of the Research Output: Liu, Zhongyi, Adriana J. Najar-Rodriguez, Patrick C. H. Morel, and Maria A. Minor. 2022. Reproduction of black soldier fly (Diptera: Stratiomyidae) under different adult densities and light regimes. <i>Journal of Economic Entomology</i>, 115(1): 37–45. <p><input type="radio"/> The manuscript is currently under review for publication – please indicate:</p> <ul style="list-style-type: none"> The name of the journal: The percentage of the manuscript/published work that was contributed by the candidate: 85.00 Describe the contribution that the candidate has made to the manuscript/published work: Zhongyi Liu performed the majority of the work for the article that forms Chapter 5 of this thesis, including experimental design, data collection, data analysis, and manuscript writing, with assistance from the co-authors of the article. <p><input type="radio"/> It is intended that the manuscript will be published, but it has not yet been submitted to a journal</p>	
Candidate's Signature:	Zhongyi Liu <small>Digitally signed by Zhongyi Liu Date: 2022.06.08 22:27:45 +1200</small>
Date:	08-Jun-2022
Primary Supervisor's Signature:	Maria Minor <small>Digitally signed by Maria Minor Date: 2022.06.08 15:13:32 +1200</small>
Date:	9-Jun-2022

This form should appear at the end of each thesis chapter/section/appendix submitted as a manuscript/publication or collected as an appendix at the end of the thesis.

Chapter 5 Reproduction of the black soldier fly (Diptera: Stratiomyidae) under different adult densities and light regimes



Black soldier fly (*Hermetia illucens*) mating (images by Z. Liu, 2016; CC BY-NC)

This chapter has been published as:

Liu, Zhongyi, Adriana J. Najar-Rodriguez, Patrick C. H. Morel, and Maria A. Minor. 2022. Reproduction of black soldier fly (Diptera: Stratiomyidae) under different adult densities and light regimes. *Journal of Economic Entomology*, 115(1): 37–45.

Reproduced here with some minor modifications in style and formatting.

The original publication is available at: <https://doi.org/10.1093/jee/toab225>

Copyright agreement/license can be found in the Appendices section of this thesis.

Abstract

The black soldier fly (BSF) *Hermetia illucens* (L.) (Diptera: Stratiomyidae) has been recognized as a promising insect species for sustainable management of organic waste and by-products. Indoor breeding of BSF with artificial lighting has been proved successful, but efforts are still needed to optimize BSF reproductive output. Increasing adult density seems an option to exploit space, whereas decreasing artificial lighting duration may reduce unnecessary power consumption. This study aimed at investigating the effects of adult density (10, 25, and 50 pairs per $30 \times 30 \times 30$ cm cage; i.e., 370, 926, and $1,852 \text{ pairs}\cdot\text{m}^{-3}$), light regime (8 : 16, 12 : 12, and 16 : 8 [L:D] h), and their possible interactions, on some BSF life history traits relevant to reproduction. The results show that the overall BSF reproductive output increased with increasing adult density but was not affected by light regimes *per se*. With the highest BSF adult density tested, an average of more than 20,000 neonate larvae were produced from a cage within 10 d. At this density, increasing photoperiod increased neonate production, but also decreased the number of neonates per watt used for artificial illumination. The temporal oviposition patterns, mean individual female reproductive output, mating success, egg hatching rate, and insect survival rate were not affected by adult density or light regime as simple effects. However, the interaction between adult density and light regime was significant for the first oviposition peak, mean individual female reproductive output, and insect survival rate. The possible mechanisms behind our results are discussed.

Keywords: egg hatching rate, indoor breeding, mating success, oviposition rate, photoperiod

5.1 Introduction

The black soldier fly (BSF) *Hermetia illucens* (L.) (Diptera: Stratiomyidae) possesses great potential to upcycle organic waste and by-products in circular bioeconomy. The BSF larvae can consume animal manure (e.g., Oonincx *et al.*, 2015), agricultural and food-industrial by-products (e.g., Lim *et al.*, 2019; Palma *et al.*, 2018), municipal food waste (e.g., Diener *et al.*, 2011; Nguyen *et al.*, 2015), and their combinations (e.g., Barragán-Fonseca *et al.*, 2018; Rehman *et al.*, 2017; Zheng *et al.*, 2012), thereby reducing the amount of organic substrates in landfills. The harvested BSF larval biomass can be used to feed different animals (Barragán-Fonseca *et al.*, 2017) or be processed into industrial products such as biodiesel and chitin, which are potential substitutions for petroleum and plastic, respectively (Li *et al.*, 2011; Rehman *et al.*, 2018; Sanandiya *et al.*, 2020). The compost derived from the larval feeding activities can also be used to substitute for or supplement artificial fertilizers (Chirere *et al.*, 2021; Sarpong *et al.*, 2019; Setti *et al.*, 2019).

Worldwide, the amount of organic waste is growing rapidly due to increasing human population (Berendes *et al.*, 2018; Hoornweg & Bhada-Tata, 2012), thus a year-round demand for young BSF larvae (i.e., neonate larvae) for sustainable organic waste management would be expected. To ensure the supply of BSF neonates, the BSF breeding system must be efficient in producing viable eggs year-round (Pastor *et al.*, 2015). Because BSF reproduction requires both special illumination (Klüber *et al.*, 2020; Liu *et al.*, 2020; Macavei *et al.*, 2020; Oonincx *et al.*, 2016) and temperature ranges not reached in every part of the world (Chia *et al.*, 2018), it could be cost-efficient to use artificial-light-assisted and temperature-controlled indoor breeding systems for year-around BSF neonate production (Sheppard *et al.*, 2002). Increasing adult population density could

potentially increase the production of BSF neonates within an indoor breeding space (Pastor *et al.*, 2011), while shortening photophase (i.e., hours of artificial illumination) could potentially reduce running costs.

Successful BSF reproduction has been observed at adult densities ranging from 350 to 2550 pairs·m⁻³ (Heussler *et al.*, 2018; Nakamura *et al.*, 2016; Ooninx *et al.*, 2016), but, to the best of our knowledge, the effect of adult density *per se* on BSF reproduction has been investigated by only two studies thus far (Hoc *et al.*, 2019; Park *et al.*, 2016). However, both Park *et al.* (2016) and Hoc *et al.* (2019) used only the number of prepupae or pupae to represent adult densities, without considering adult emergence rates (i.e., losses during pupation or eclosion). Therefore, the actual adult densities used in the Park *et al.* (2016) and Hoc *et al.* (2019) experiments are unclear.

BSF can successfully reproduce under a wide range of artificial light regimes, such as 2:22, 6:18, 9:15, 12:12, 16:8, and 18:6 h L:D (Heussler *et al.*, 2018; Hoc *et al.*, 2019; Klüber *et al.*, 2020; Liu *et al.*, 2020; Nakamura *et al.*, 2016; Ooninx *et al.*, 2016; Zhang *et al.*, 2010). However, one of the few (if not the only) studies available thus far exploring the effects of light regime on BSF reproduction was conducted with female dominant populations (Hoc *et al.*, 2019). Thus, the effects of light regime on BSF reproduction and neonate production remain largely unexplored.

In the present study, we investigated the effects of adult density, light regime, and their possible interactions on selected BSF life history traits, including temporal oviposition patterns, female reproductive output from a breeding cage, mean individual female reproductive output, mating success, egg hatching rate, and insect survival rate. We compared the relative importance of manipulating adult density and photoperiod in

maximizing BSF neonate production, and discussed the possible underlying mechanisms and the implications of our results for optimized BSF indoor breeding.

5.2 Materials and methods

5.2.1 Experimental design

The insects used in this experiment were provided by Prescient Nutrition Ltd., New Zealand in 2019. The larvae were fed with food waste collected from local restaurants in Palmerston North, New Zealand. The adults were kept in breeding cages (each cage 180 × 90 × 90 cm; L × W × H) in a glasshouse. For each experimental run, 1 kg of BSF pupae were collected and kept at 27°C and 80% R.H. (relative humidity) for adult emergence. Adults that emerged within a 24-h window with fully expanded wings were used for the experiment described here.

The newly emerged flies were separated by sex, and the same number of males and females were assigned to one of the treatments (Table 5.1). Regardless of the light regime or adult density tested, all adults were housed in 30 × 30 × 30 cm mesh cages (BugDorm, DP-1000, MegaView Science Co. Ltd., Taiwan). A 20-watt LED BSF breeding lamp (BSF-4C-20-3900A, JM Green Co. Ltd., China) was set 1 cm above each cage to induce BSF mating. The choice of experimental cage size and BSF breeding lamps were based on our previous success at breeding BSF indoors (Liu *et al.*, 2020).

Three different light regimes (8 : 16, 12 : 12, and 16 : 8 h L:D) and three different adult densities (10, 25, and 50 pairs per cage; i.e., 370, 926, and 1,852 pairs·m⁻³, respectively) were tested.

Due to equipment availability, three different incubators (Incubator A: Contherm Series Five, Contherm Scientific Ltd., New Zealand; Incubator B: Qualtex Thermostat, Watson Victor Ltd., Australia; Incubator C: Sanyo MOV-212F, SANYO Electric Biomedical Ltd., Japan) were covered with black cloth and used to accommodate different light regimes within an experimental run. A 3×3 -factorial resolvable block design was generated by CycDesign 5.1 (VSN International Ltd. Hertfordshire, U.K.) to resolve all the nine combinations of the two factors to be tested. Three replicates were conducted during the experiment. The experimental design is shown in Table 5.1.

For each adult density and light regime combination tested, eggs and dead adults were collected daily, if present. The sex and number of dead adults were determined, and all dead females were stored at -20°C for later dissections. Any eggs collected from the cages were transferred to a Petri dish and kept at 27°C and 80% R.H. for 36 h to allow any fertile eggs to develop eye spots. Eggs were then separated into fertile or infertile groups under a stereomicroscope (Leica M80, Leica Microsystems GmbH, Germany) based on the presence of eye spots, and were weighed as respective groups (i.e., fertile vs. infertile). Finally, one hundred fertile or infertile eggs were counted from each group and weighed. This weight was then used to estimate the respective number of fertile or infertile eggs collected daily from each experimental cage. The number of fertile or infertile eggs that were fewer than one hundred was directly obtained by counting. All fertile eggs were returned to the hatching environment for another 3 d, and then the number of unhatched eggs was recorded. Each experimental run lasted 10 d, which was consistent with our previous experiments testing the best type of artificial light to be used in a BSF indoor breeding system (Liu *et al.*, 2020).

On the last day of each experimental run, all remaining live females were transferred to

0°C while the dead females already collected from the same run were thawed at the same temperature. After the live females had reached cold-induced dormancy for at least 5 minutes, all the females from the same experimental run were dissected to determine their insemination status as per Liu *et al.* (2020).

5.2.2 Calculations and statistics

All statistical analyses were performed using Minitab 18 (Minitab, LLC), with $\alpha = 0.05$ for all tests. All graphical work was conducted with OriginPro 2016 (OriginLab Corp.).

The number of eggs collected daily from a cage during each 10-d experimental run was plotted against time to identify the preoviposition period and the oviposition peaks.

General linear models (GLMs; GLM procedure) were fitted to the data to analyze their effects on different insect life history traits (Table 5.2). Elapsed time (days between the start date of the first experimental run and the start date of each following experimental run) was treated as a covariate, while incubator, adult density, light regime, and the interaction between adult density and light regime were treated as fixed effects. If adult density or light regime effect was significant, Tukey's honestly significant difference test was performed for multiple comparisons.

Table 5.1 The 3×3 -factorial block design used to test the effects of adult density and light regime on the selected life history traits of black soldier fly (*Hermetia illucens* L.) adults.

Replicate	Experimental run	Incubator A	Incubator B	Incubator C
1	1	370 pairs·m ⁻³ , 8L : 16D	926 pairs·m ⁻³ , 16L : 8D	1852 pairs·m ⁻³ , 12L : 12D
	2	370 pairs·m ⁻³ , 16L : 8D	1852 pairs·m ⁻³ , 8L : 16D	926 pairs·m ⁻³ , 12L : 12D
	3	1852 pairs·m ⁻³ , 16L : 8D	926 pairs·m ⁻³ , 8L : 16D	370 pairs·m ⁻³ , 12L : 12D
2	4	926 pairs·m ⁻³ , 12L : 12D	370 pairs·m ⁻³ , 16L : 8D	1852 pairs·m ⁻³ , 8L : 16D
	5	370 pairs·m ⁻³ , 12L : 12D	1852 pairs·m ⁻³ , 16L : 8D	926 pairs·m ⁻³ , 8L : 16D
	6	1852 pairs·m ⁻³ , 12L : 12D	370 pairs·m ⁻³ , 8L : 16D	926 pairs·m ⁻³ , 16L : 8D
3	7	926 pairs·m ⁻³ , 16L : 8D	370 pairs·m ⁻³ , 12L : 12D	1852 pairs·m ⁻³ , 16L : 8D
	8	926 pairs·m ⁻³ , 8L : 16D	1852 pairs·m ⁻³ , 12L : 12D	370 pairs·m ⁻³ , 16L : 8D
	9	1852 pairs·m ⁻³ , 8L : 16D	926 pairs·m ⁻³ , 12L : 12D	370 pairs·m ⁻³ , 8L : 16D

Three different adult densities, 370, 926, and 1852 pairs·m⁻³ were achieved by housing 10, 25, and 50 pairs of adults in a 30 × 30 × 30 cm mesh cage, respectively. A 20-watt lamp was set 1 cm above each cage to induce black soldier fly mating.

Table 5.2 Selected life history traits of black soldier fly (*Hermetia illucens* L.) adults analyzed with general linear models to test the effects of adult density and light regime.

Category	Life history trait measured
Temporal oviposition pattern	Length of pre-oviposition period Day of oviposition peak
Female reproductive output from an experimental cage used	Total number of eggs Total number of fertile eggs Total number of neonates
Mean individual female reproductive output	Average number of eggs laid per female ^a Average number of fertile eggs laid per inseminated female ^b
Mating success	Percentage of inseminated females
Egg fertility and hatching rate	Percentage of fertile eggs Hatching rate of fertile eggs
Insect survival rate	Adult male Adult female

a. Calculated as the total number of eggs divided by the total number of females placed in an experimental cage.

b. Calculated as the total number of fertile eggs divided by the total number of inseminated females obtained in a cage.

In preliminary data analyses, temperature and R.H. were found to be significantly affected by incubator, elapsed time, and photoperiod (Tables S5.1 and S5.2). Therefore, temperature and R.H. were not fitted in the GLMs to avoid multicollinearity. However, possible temperature and R.H. effects are discussed later.

Paired t-tests were performed to analyze (1) the differences between the average number of eggs laid per female and the average number of fertile eggs laid per inseminated female and (2) the differences between male and female survival rate.

5.3 Results

5.3.1 Temporal traits in BSF oviposition

Overall, BSF females started to lay eggs after a preoviposition period of 2.7 ± 0.1 (mean \pm SE) d. The first and the second oviposition peaks appeared 4.5 ± 0.1 and 7.3 ± 0.2 d after the placement of adult flies in the experimental cages, respectively. The daily oviposition patterns of BSF under different adult densities and light regimes are shown in Figure 5.1. Preoviposition and the timing of the second oviposition peak were not significantly affected by any factor fitted in the model. The timing of the first oviposition peak was not significantly affected by adult density ($F_{2,15} = 1.21$, $P = 0.33$) or light regime ($F_{2,15} = 0.42$, $P = 0.66$), but was significantly affected by their interaction ($F_{4,15} = 3.71$, $P = 0.027$) (Figure 5.2). Under 8- and 12-h photoperiods, BSF reached the first oviposition peak earlier at $926 \text{ pairs} \cdot \text{m}^{-3}$ than at the other adult densities tested. The opposite trend was found under 16-h photoperiod. Moreover, at lower adult densities the timing of the first oviposition period varied more widely in response to light regime. Incubator effect

was significant for the timing of the first oviposition peak ($F_{2,15} = 5.34$, $P = 0.018$), but not for other temporal oviposition traits.

5.3.2 Reproductive output

Adult density had significant positive effects on the total number of eggs, fertile eggs, and neonates (Figures 5.1 and 5.3; Table 5.3). On the contrary, the light regimes used did not affect the total numbers of eggs ($F_{2,15} = 2.52$, $P = 0.11$), fertile eggs ($F_{2,15} = 0.14$, $P = 0.87$), or neonates ($F_{2,15} = 0.12$, $P = 0.89$). There was a significant interaction between adult density and light regime in the total number of eggs ($F_{4,15} = 5.43$, $P = 0.007$) (Figure 5.3A), with longer photoperiods resulting in higher total egg production when adult density was high, but not when adult density was low. There was no significant interaction between adult density and light regime for the total numbers of fertile eggs laid ($F_{4,15} = 1.3$, $P = 0.32$) or neonates produced ($F_{4,15} = 1.36$, $P = 0.29$), though the trends (Figure 5.3B and C) were similar to that for the total number of eggs.

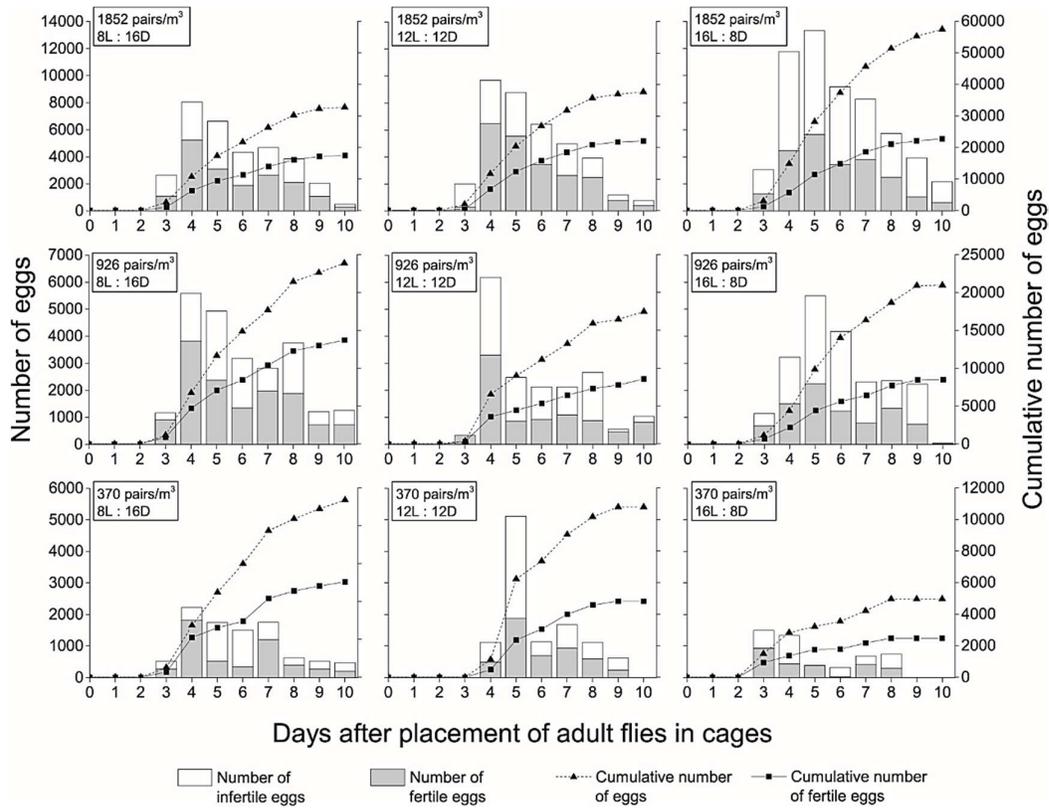


Figure 5.1 Daily oviposition pattern of black soldier fly (*Hermetia illucens* L.) housed in $30 \times 30 \times 30$ cm mesh cages and under different combinations of adult densities and light regimes. Grey and white parts of the bars represent the number of fertile and infertile eggs, respectively. The squares on solid lines indicate the cumulative number of fertile eggs, and the triangles on dash lines indicate the cumulative number of all eggs. 370, 926, and 1,852 $\text{pairs} \cdot \text{m}^{-3}$ indicate 10, 25, and 50 pairs of flies per $30 \times 30 \times 30$ cm cage, respectively.

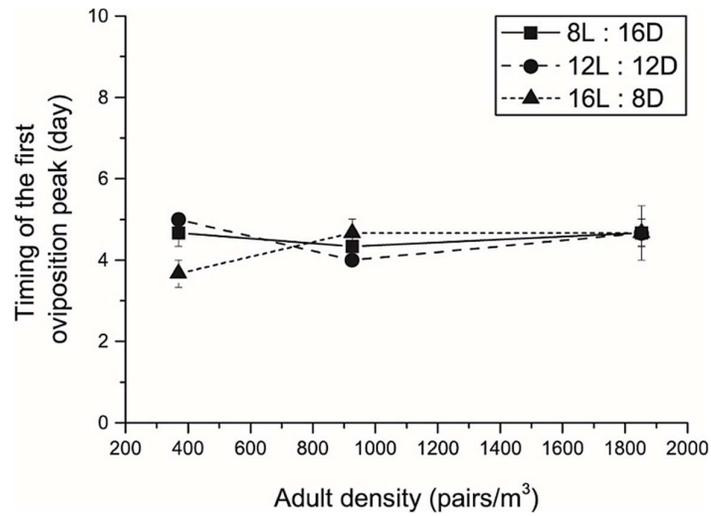


Figure 5.2 The timing of the first oviposition peak of female black soldier fly (*Hermetia illucens* L.) in response to different combinations of adult densities and light regimes. The tested densities were 370, 926, and 1,852 pairs·m⁻³, indicating 10, 25, and 50 pairs of flies per 30 × 30 × 30 cm cage, respectively. The values shown are means ± SE.

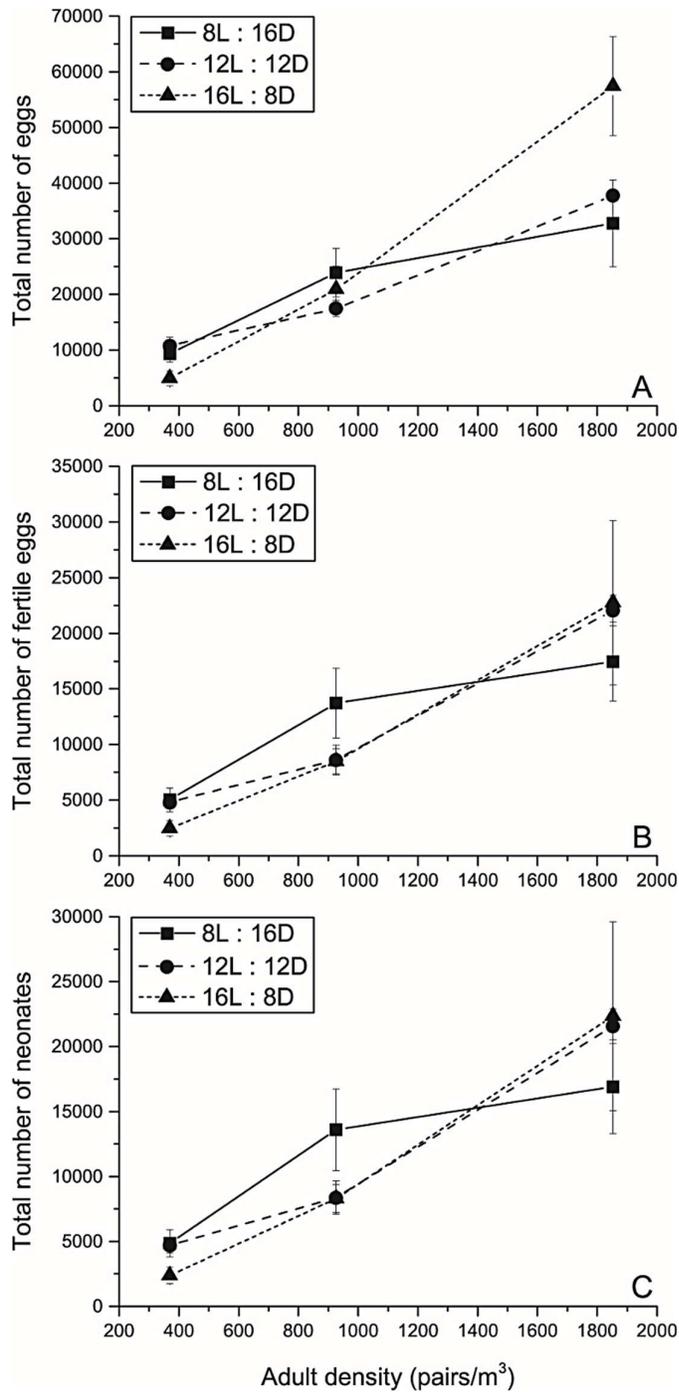


Figure 5.3 Black soldier fly (*Hermetia illucens* L.) female reproductive output from an experimental cage used in response to different combinations of adult densities and light regimes. (A) Total number of eggs, (B) total number of fertile eggs, and (C) total number of neonates. The tested densities were 370, 926, and 1,852 pairs·m⁻³, indicating 10, 25, and 50 pairs of flies per 30 × 30 × 30 cm cage, respectively. The values shown are means ± SE.

Table 5.3 Reproductive output of all the female black soldier flies (*Hermetia illucens* L.) from an experimental cage in response to different adult densities.

Adult density (pairs·m ⁻³)	Total number of eggs (mean ± SE)	Total number of fertile eggs (mean ± SE)	Total number of neonates (mean ± SE)
370	8,366 ± 1,136a	4,108 ± 575a	3,974 ± 558a
926	20,772 ± 2,008b	10,280 ± 1,346b	10,085 ± 1,353b
1852	42,633 ± 5,089c	20,743 ± 2,537c	20,271 ± 2,523c
<i>F</i> _{2,15}	63.68	29.34	28.52
<i>P</i>	< 0.001	< 0.001	< 0.001

The data from all the experimental runs were used to calculate the means and standard errors presented (i.e., $n = 9$). The *F* and *P* values are extracted from the general linear models described in the Calculations and Statistics section. Different letters within a column indicate significant differences in Tukey's honestly significant difference tests ($\alpha = 0.05$). Three different BSF adult densities, 370, 926, and 1852 pairs·m⁻³ were achieved by housing 10, 25, and 50 pairs of adults in a 30 × 30 × 30 cm mesh cage, respectively. A 20-watt lamp was set 1 cm above each cage to induce black soldier fly mating.

For mean individual reproductive output, adult density did not affect the average number of eggs laid per female ($F_{2,15} = 0.03$, $P = 0.97$) or the average number of fertile eggs laid per inseminated female ($F_{2,15} = 0.62$, $P = 0.55$). Light regime did not affect the average number of eggs laid per female ($F_{2,15} = 0.01$, $P = 0.99$) or the average number of fertile eggs laid per inseminated female ($F_{2,15} = 2.29$, $P = 0.14$). The interaction between adult density and light regime was significant for both the average number of eggs laid per female ($F_{4,15} = 7.08$, $P = 0.002$; Figure 5.4A) and the average number of fertile eggs laid per inseminated female ($F_{4,15} = 3.64$, $P = 0.029$; Figure 5.4B). Under the 8-h photoperiod, individual BSF females tended to have higher reproductive output when adult density was low. Under the 16-h photoperiod, higher individual reproductive output was found with

higher adult densities. Overall, the average number of fertile eggs per inseminated female (607 ± 35) was significantly lower than the average number of eggs laid per female (840 ± 55) ($t = 5.45, P < 0.001$).

Effect of incubator was significant for the total number of eggs ($F_{2,15} = 5.4, P = 0.017$) and the average number of eggs laid per female ($F_{2,15} = 6.92, P = 0.007$), but not for the total number of fertile eggs ($F_{2,15} = 3.47, P = 0.06$), the total number of neonates ($F_{2,15} = 3.48, P = 0.06$), or the average number of fertile eggs laid per inseminated female ($F_{2,15} = 0.63, P = 0.55$).

5.3.3 Mating success

The percentage of inseminated females ($68.15 \pm 3.04\%$) was not affected by adult density ($F_{2,15} = 2.29, P = 0.14$), light regime ($F_{2,15} = 0.89, P = 0.43$), or their interaction ($F_{4,15} = 0.22, P = 0.92$). However, the percentage of inseminated females decreased with time (coefficient = $-0.11, F_{1,15} = 16.03, P = 0.001$). Incubator had significant effect on the percentage of inseminated females ($F_{2,15} = 13.46, P < 0.001$).

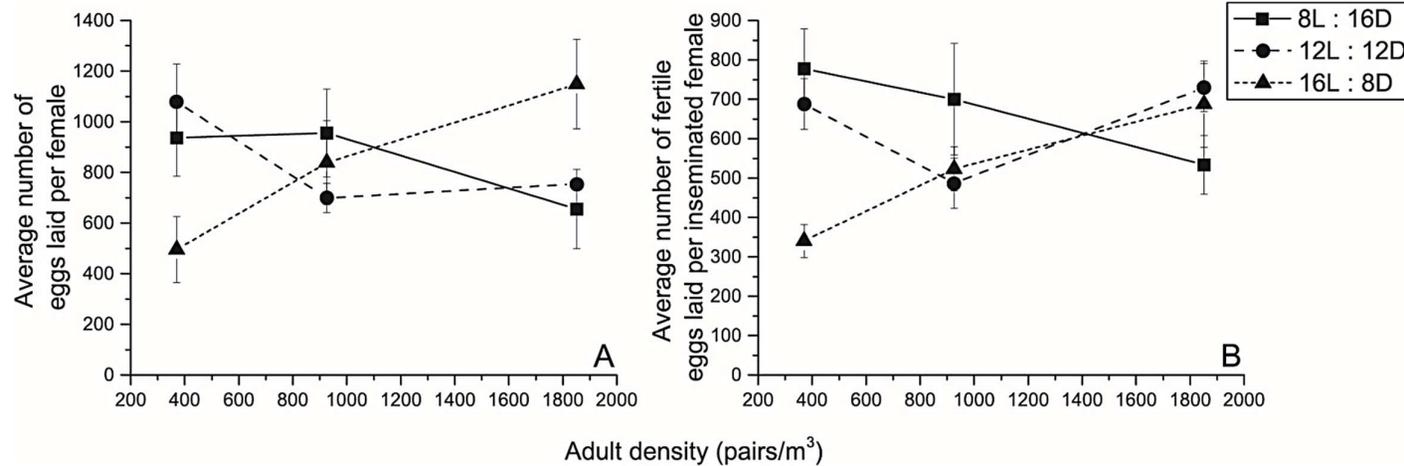


Figure 5.4 Black soldier fly (*Hermetia illucens* L.) mean individual female reproductive output in response to different combinations of adult densities and light regimes. (A) Average number of eggs laid per female, (B) Average number of fertile eggs laid per inseminated female. The tested densities were 370, 926, and 1,852 pairs·m⁻³, indicating 10, 25, and 50 pairs of flies per 30 × 30 × 30 cm cage, respectively. The values shown are means ± SE.

5.3.4 Egg fertility, hatching rate, and insect survival rate

The hatching rate of fertile eggs ($97.4 \pm 0.4\%$) was not affected by any factor fitted in the model. The percentage of fertile eggs decreased with longer photoperiod ($F_{2,15} = 4.29$, $P = 0.034$), but was not affected by adult density ($F_{2,15} = 0.003$, $P = 0.997$) or by the interaction between adult density and light regime ($F_{4,15} = 0.96$, $P = 0.46$). The percentage of fertile eggs ($50.19 \pm 2.58\%$) declined with time (coefficient = -0.11 , $F_{1,15} = 13.53$, $P = 0.002$). Incubator did not have significant effect on the percentage of fertile eggs ($F_{2,15} = 0.83$, $P < 0.46$).

The 10-d survival rates of BSF males ($75.85 \pm 2.89\%$) and females ($73.63 \pm 3.23\%$) did not differ significantly ($t = 0.86$; $P = 0.397$). BSF male and female survival rates were not affected by adult density or light regime as simple effects. The interaction between adult density and light regime on the survival rates of BSF was significant for males ($F_{4,15} = 7.29$, $P = 0.002$) but marginally nonsignificant for females ($F_{4,15} = 2.91$, $P = 0.058$) (Figure 5.5). For both sexes, survival rates tended to decrease with higher adult densities under 8- and 12-h photoperiods, but the trend was the opposite under a 16-h photoperiod. BSF survival rate did not vary significantly with time or across incubators.

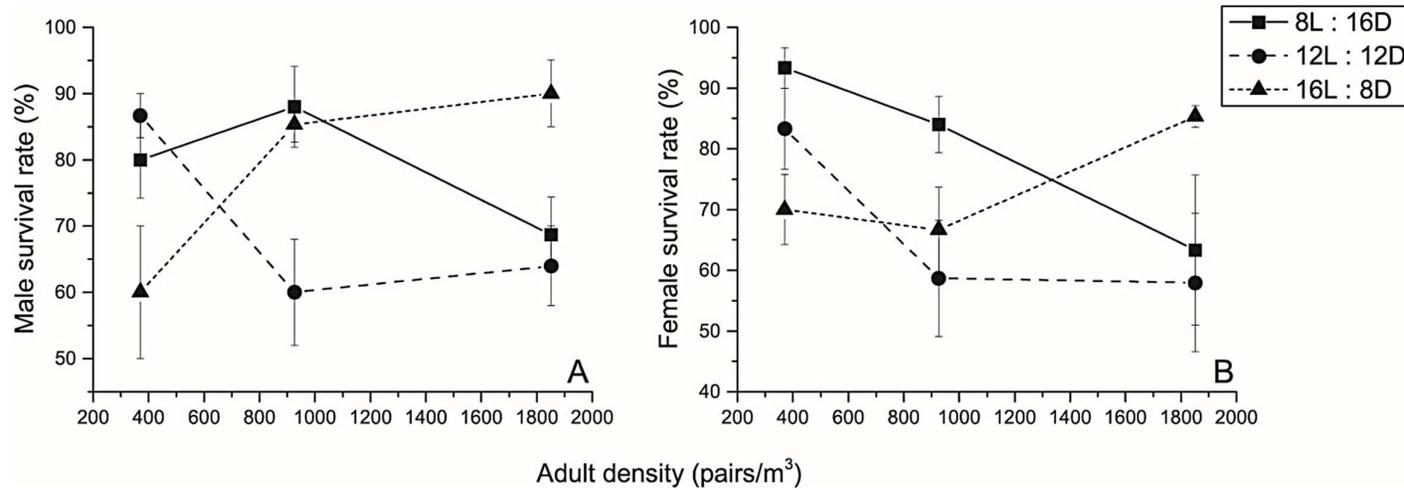


Figure 5.5 Black soldier fly (*Hermetia illucens* L.) 10-d survival rate (in percentage) for adult (A) males and (B) females in response to different combinations of adult densities and light regimes. The tested densities were 370, 926, and 1,852 pairs·m⁻³, indicating 10, 25, and 50 pairs of flies per 30 × 30 × 30 cm cage, respectively. The values shown are means ± SE.

5.4 Discussion

Understanding the effects of different environmental variables on BSF reproduction underlies the efficient production of BSF neonate larvae, which is a prerequisite for the use of this species in organic waste management. In the present study, we tested the effects of adult density, light regime, and their possible interactions, on selected life history traits that are relevant to BSF reproduction.

Within the density range used in the present study, increasing adult density significantly increased the overall BSF reproductive output (Table 5.3). The highest BSF adult density (i.e., $1,852 \text{ pairs}\cdot\text{m}^{-3}$) resulted in a neonate production of $20,271 \pm 2,523$ from a $30 \times 30 \times 30$ cm cage. Similar results were also reported by Park *et al.* (2016) and Hoc *et al.* (2019) — higher number of BSF pupae loaded in a breeding cage resulted in higher egg production. Adult density *per se* did not significantly affect individual reproductive output of BSF females. Similarly, in a previous study, increasing the number of BSF pupae loaded in a breeding cage did not significantly affect the weight of eggs laid per female on average (Hoc *et al.*, 2019). Therefore, higher reproductive output at higher adult density in our experiment seemed to be the result of the increase in adult numbers. Our results suggest that adult density could be increased further (i.e., above $1,852 \text{ pairs}\cdot\text{m}^{-3}$) to exploit the limited breeding space without compromising individual reproductive output. However, a threshold of adult density for BSF reproductive output is likely to exist, beyond which individual reproductive output may be impaired due to competition for food (Mamai *et al.*, 2017) or oviposition sites (Delves & Browne, 1989). Because BSF copulation occurs in flight (Tomberlin & Sheppard, 2001), crowding would reduce flight space, and thus affect copulation success. Moreover, when a large number of BSF are in flight within a limited space under artificial lighting, shading would possibly

occur, reducing the overall light intensity and likely affecting visually mediated cues needed for successful BSF mating (Oonincx *et al.*, 2016; Tomberlin & Sheppard, 2002). Although the increased number of adults beyond the density threshold may compensate for the loss of individual reproductivity, the efficiency of utilizing each pair loaded in a breeding cage is expected to decline with increasing density; and as the individual reproductivity continues to decline, increasing adult density could eventually result in a drop of overall reproductive output from a breeding cage. For instance, female fecundity of *Tenebrio molitor* (L.) (Coleoptera: Tenebrionidae) started to decrease from 1.4 adults·dm⁻², whereas the total egg yield increased until 14 adults·dm⁻² and then decrease with higher adult densities tested (Morales-Ramos *et al.*, 2012).

Light regime (i.e., hours of light per day or photoperiod) represents a daily and seasonal environmental variation known to have a significant effect on insect reproduction (Bradshaw & Holzapfel, 2007; de Wilde, 1962). For instance, a short photoperiod (e.g., 8 to 13 h), which represents winter conditions, inhibited ovarian development of *Eysarcoris ventralis* (Westwood) (Hemiptera: Pentatomidae) (Noda & Ishii, 1981), and suppressed oviposition of *Pissodes strobi* (Peck) (Coleoptera: Curculionidae) (Trudel *et al.*, 2002). Long photoperiods (e.g., 16 h or longer), on the other hand, induced reproductive diapause of *Oedipoda miniata* (Pallas) (Orthoptera: Acrididae), because it represents the dry summer conditions in the natural habitat of this species (Orshan & Pener, 1979). The effect of light regime on BSF reproduction has been scarcely reported in literature. One of the few studies (if not the only) published on this topic thus far showed that photoperiods shorter than 6 h·d⁻¹ could drastically hamper both individual BSF female fecundity and egg production from breeding cages (Hoc *et al.*, 2019). In our experiment, light regime did not affect BSF reproductive output as a simple effect, probably because all photoperiods tested were longer than 6 h·d⁻¹; and reproductive

diapause seemed to have not taken place under any light regime tested here. Longer photoperiods resulted in lower percentage of fertile eggs, which may be due to lower percentage of inseminated females under longer photoperiods and reduction (though not significant in our experiment) of the average number of fertile eggs laid by inseminated females (Table S5.3 and Equation S5.1). Future studies on the effects of light regime on BSF physiological (e.g., hormone and pheromone production, development of reproductive systems, sperm viability) and behavioral traits (e.g., flight activity, female calling, and male responses) are required to unveil the underlying mechanisms (Gao *et al.*, 2022; Gemeno & Haynes, 2001; Nakamura *et al.*, 2016; Orshan & Pener, 1979).

Light regime has been observed to affect the temporal oviposition patterns in different insects (e.g., Jacobson, 1960; Kawano & Ando, 1997; Reznik & Vaghina, 2007). In a female dominated BSF population, the oviposition peak appeared 5.7 d after the first oviposition when exposed to a short photoperiod (6:18 h L:D); whereas longer photoperiods (12 : 12 and 18 : 6 h L:D) brought the oviposition peak forward by about 2 d (Hoc *et al.*, 2019). In the present study, the temporal BSF oviposition patterns remained stable under different light regimes, most likely because the photoperiods used were all longer than 6 h·d⁻¹.

The interaction between adult density and light regime significantly affected BSF reproductive output and survival (Figures 5.3–5.5). Interestingly, the interaction between adult density and light regime on BSF female fecundity and adult survival rate showed similar trends. At the lowest adult density tested, female fecundity and survival rate of both sexes were higher under shorter photoperiods (i.e., 8 and 12 h), whereas at the highest adult density tested, the same responses were only higher under a longer 16-h photoperiod. These results indicate that the interaction between adult density and light

regime possibly reflects their roles in overall BSF fitness. Energy balance may be a contributing factor in this interaction. Holmes *et al.* (2017) reported that BSF adult longevity decreased with longer exposure to light, which was likely due to more adult activity under light than in darkness. This may partly explain the higher BSF adult survival found with shorter photoperiods at a density of 370 pairs·m⁻³ in the present study. Moreover, energy deprivation in the adult diet seems to adversely affect both fecundity and adult life span in BSF (Macavei *et al.*, 2020). BSF adults have energy-consuming and space-requiring reproductive behaviors such as courtship flights (Tomberlin & Sheppard, 2001). Because the cage size and the amount of adult diet (sugar cubes) did not change throughout our experiment, high adult density could have potentially increased the competition for flight space and diet availability. As BSF seems to be a diurnal species (Tomberlin & Sheppard, 2002), longer photoperiods could increase the total time window available for both reproduction and feeding behaviors to take place, therefore reducing the intensity of competition at high adult densities. Since we did not directly measure the flying or feeding behaviors of the flies, we cannot attest to the actual behaviors displayed, and therefore suggest further experimentation to unveil the mechanisms behind our results.

Increasing adult density appears to be more effective than changing light regime for maximizing neonate production. Doubling adult density from 926 to 1,852 pairs·m⁻³ (i.e., from 25 to 50 pairs per cage) doubled the neonate production from 10,085 ± 1,353 to 20,271 ± 2,523. Longer photoperiods could benefit neonate production under the highest adult density (Figure 5.3C). However, while prolonging photoperiod from 8 to 16 h·d⁻¹ increased neonate production by 32%, it would also reduce power efficiency of artificial illumination (i.e., number of neonates produced per watt used for artificial illumination) by 34%. Similarly, Hoc *et al.* (2019) found that increasing BSF prepupae loaded in a

breeding cage from 4,500 to 8,500 per m³ almost doubled overall reproductive output (from 4.2 to 7.8 g per cage). However, according to Hoc *et al.* (2019), increasing photoperiod from 6 to 18 h·d⁻¹ did not improve BSF reproductive output of female-dominant populations, resulting in a waste of energy. Therefore, to develop a cost-effective BSF neonate production system, more importance should be given to increasing adult density, as to maximize neonate production, while the selection of a suitable light regime should depend on the balance between neonate production and power efficiency.

The percentage of inseminated females and the percentage of fertile eggs both decreased by 28% during the present study (244 d). Moreover, the hatching rate of eggs was about 50% on average in the present study, which is lower than that reported in previous studies (Bertinetti *et al.*, 2019; Hoc *et al.*, 2019; Permana *et al.*, 2020). Since the BSF colony used here had been kept in captivity for almost 3 yr without genetic refreshment with wild individuals (Z. Liu personal communication with Prescient Nutrition Ltd.), low egg viability and decline in the percentage of inseminated females with time may be attributed to inbreeding (Hoffmann *et al.*, 2021; Rhode *et al.*, 2020). Inbreeding could reduce mating propensity (Meffert & Bryant, 1991), production of sex pheromone (van Bergen *et al.*, 2013), impair mating behaviors (Miller *et al.*, 1993) and affect sexually-selected traits (Drayton *et al.*, 2007).

The incubator effect had significant effects on the timing of the first oviposition peak, the total number of eggs produced, the average number of eggs laid per female, and the percentage of inseminated females. We hypothesize that this effect may be partly attributed to humidity, because relative humidity could vary up to 30% across incubators (Table S5.2). Tomberlin and Sheppard (2002) reported that BSF oviposition positively correlated with relative humidity. Temperature also has been reported to affect BSF

preoviposition period, female fecundity, and adult longevity (Chia *et al.*, 2018; Shumo *et al.*, 2019). In our experiment, temperature varied only about 1–2°C subject to each influencing factor (Table S5.1). Nevertheless, because of the dependency of temperature and relative humidity on other variables, they were not statistically analyzed in our models.

The present study is only one of the first attempts towards optimizing BSF indoor breeding environments, and our results may not be directly applied to different breeding scales (i.e., different sizes of cages used for BSF breeding). For instance, if the volume of the cage is increased by 1000 times while fixing the density of BSF adult flies, the landing surface available for each insect will be reduced by 90% due to the reduction of surface-to-volume ratio, which may result in crowding and competition for landing surface. Moreover, light distribution in the cage and water and nutrient (e.g., sugar cubes) availability may also differ with scales. Therefore, studies investigating BSF adult performance in response to different breeding cage sizes will be needed.

In conclusion, our study is among the first to investigate BSF indoor reproductive performance under different environmental conditions, aiming at efficiently maximizing BSF neonate production for sustainable management of organic wastes and by-products. We demonstrated that, within the range used in our experiment, adult density was the most important variable for total BSF reproductive output. A BSF adult density of 1,852 pairs·m⁻³ (i.e., 50 pairs per 30 × 30 × 30 cm cage) could be used without compromising individual female fecundity, and a neonate production of 7.5 × 10⁵·m⁻³ (i.e., about 20,000 neonates per 30 × 30 × 30 cm cage) on average could be achieved within 10 d under the presented experimental conditions. Due to possible adverse effect of increasing case size while keeping adult density, replication of the present study or any previous similar ones

is recommended prior to implementation at larger breeding scales. Future studies are warranted to determine the maximum adult density suitable for neonate production. Increasing photoperiod could benefit neonate production when adult density is high, however, this would come at a cost for power efficiency in terms of artificial illumination. Therefore, a trade-off between neonate production and power consumption would need to be considered when choosing a suitable light regime for BSF indoor breeding.

5.5 Acknowledgments

We would like to thank Duncan Hedderley for his suggestions on statistics. We also want to acknowledge Paul Barrett for his technical support and Mari Nakano for her assistance with the experiments.

5.6 References

- Barragán-Fonseca, K., Pineda-Mejia, J., Dicke, M., & van Loon, J. J. A. (2018). Performance of the black soldier fly (Diptera: Stratiomyidae) on vegetable residue-based diets formulated based on protein and carbohydrate contents. *Journal of Economic Entomology*, *111*(6), 2676–2683. <https://doi.org/10.1093/jee/toy270>
- Barragán-Fonseca, K. B., Dicke, M., & van Loon, J. J. A. (2017). Nutritional value of the black soldier fly (*Hermetia illucens* L.) and its suitability as animal feed – a review. *Journal of Insects as Food and Feed*, *3*(2), 105–120. <https://doi.org/10.3920/jiff2016.0055>
- Berendes, D. M., Yang, P. J., Lai, A., Hu, D., & Brown, J. (2018). Estimation of global recoverable human and animal faecal biomass. *Nature Sustainability*, *1*(11), 679–685. <https://doi.org/10.1038/s41893-018-0167-0>
- Bertinetti, C., Samayoa, A. C., & Hwang, S. (2019). Effects of feeding adults of *Hermetia illucens* (Diptera: Stratiomyidae) on longevity, oviposition, and egg hatchability: Insights into optimizing egg production. *Journal of insect science (Online)*, *19*(1), 19. <https://doi.org/10.1093/jisesa/iez001>

- Bradshaw, W. E., & Holzapfel, C. M. (2007). Evolution of animal photoperiodism. *Annual Review of Ecology, Evolution, and Systematics*, 38(1), 1–25. <https://doi.org/10.1146/annurev.ecolsys.37.091305.110115>
- Chia, S. Y., Tanga, C. M., Khamis, F. M., Mohamed, S. A., Salifu, D., Sevgan, S., Fiaboe, K. K. M., Niassy, S., van Loon, J. J. A., Dicke, M., & Ekesi, S. (2018). Threshold temperatures and thermal requirements of black soldier fly *Hermetia illucens*: Implications for mass production. *PLOS ONE*, 13(11), e0206097. <https://doi.org/10.1371/journal.pone.0206097>
- Chirere, T. E. S., Khalil, S., & Lalander, C. (2021). Fertiliser effect on Swiss chard of black soldier fly larvae-frass compost made from food waste and faeces. *Journal of Insects as Food and Feed*, 7(4), 457–469. <https://doi.org/10.3920/jiff2020.0120>
- de Wilde, J. (1962). Photoperiodism in insects and mites. *Annual Review of Entomology*, 7(1), 1–26. <https://doi.org/10.1146/annurev.en.07.010162.000245>
- Delves, R. I., & Browne, L. B. (1989). Changes in reproductive performance of the Australian sheep blowfly, *Lucilia cuprina* (Wied.) (Dipt., Calliphoridae) following laboratory colonization. *Journal of Applied Entomology*, 107(1-5), 228–237. <https://doi.org/10.1111/j.1439-0418.1989.tb00253.x>
- Diener, S., Studt Solano, N. M., Roa Gutiérrez, F., Zurbrügg, C., & Tockner, K. (2011). Biological treatment of municipal organic waste using black soldier fly larvae. *Waste and Biomass Valorization*, 2(4), 357–363. <https://doi.org/10.1007/s12649-011-9079-1>
- Drayton, J. M., Hunt, J., Brooks, R., & Jennions, M. D. (2007). Sounds different: Inbreeding depression in sexually selected traits in the cricket *Teleogryllus commodus*. *Journal of Evolutionary Biology*, 20(3), 1138–1147. <https://doi.org/10.1111/j.1420-9101.2006.01286.x>
- Gao, Q., Li, B., Wei, B., Liu, W., Wang, P., Wang, J., Zhou, X., & Wang, X. (2022). Juvenile hormone regulates photoperiod-mediated male reproductive diapause via the methoprene-tolerant gene in the ladybeetle *Harmonia axyridis*. *Insect Science*, 29(1), 139–150. <https://doi.org/10.1111/1744-7917.12918>
- Gemeno, C., & Haynes, K. F. (2001). Impact of photoperiod on the sexual behavior of the black cutworm moth (Lepidoptera: Noctuidae). *Environmental Entomology*, 30(2), 189–195. <https://doi.org/10.1603/0046-225x-30.2.189>
- Heussler, C. D., Walter, A., Oberkofler, H., Insam, H., Arthofer, W., Schlick-Steiner, B. C., & Steiner, F. M. (2018). Influence of three artificial light sources on oviposition and half-life of the black soldier fly, *Hermetia illucens* (Diptera: Stratiomyidae): Improving small-scale indoor rearing. *PLOS ONE*, 13(5), e0197896. <https://doi.org/10.1371/journal.pone.0197896>
- Hoc, B., Noël, G., Carpentier, J., Francis, F., & Caparros Megido, R. (2019). Optimization of black soldier fly (*Hermetia illucens*) artificial reproduction. *PLOS ONE*, 14(4), e0216160. <https://doi.org/10.1371/journal.pone.0216160>
- Hoffmann, L., Hull, K. L., Bierman, A., Badenhorst, R., Bester-van der Merwe, A. E., & Rhode, C. (2021). Patterns of genetic diversity and mating systems in a mass-reared black soldier fly colony. *Insects*, 12(6), 480. <https://doi.org/10.3390/insects12060480>

- Holmes, L. A., VanLaerhoven, S. L., & Tomberlin, J. K. (2017). Photophase duration affects immature black soldier fly (Diptera: Stratiomyidae) development. *Environmental Entomology*, *46*(6), 1439–1447. <https://doi.org/10.1093/ee/nvx165>
- Hoornweg, D., & Bhada-Tata, P. (2012). *What a waste: A global review of solid waste management. Urban development series; knowledge papers no. 15*. World Bank.
- Jacobson, L. A. (1960). Influence of photoperiod on oviposition by the army cutworm, *Chorizagrotis auxiliaris* (Lepidoptera: Noctuidae), in an insectary. *Annals of the Entomological Society of America*, *53*(4), 474–475. <https://doi.org/10.1093/aesa/53.4.474>
- Kawano, S., & Ando, Y. (1997). Effects of photoperiod on nymphal development, pre-oviposition period and egg diapause in the subtropical rice grasshopper, *Oxya chinensis formosana* Shiraki (Orthoptera: Catantopidae). *Applied Entomology and Zoology*, *32*(3), 465–470. <https://doi.org/10.1303/aez.32.465>
- Klüber, P., Bakonyi, D., Zorn, H., & Rühl, M. (2020). Does light color temperature influence aspects of oviposition by the black soldier fly (Diptera: Stratiomyidae)? *Journal of Economic Entomology*, *113*(5), 2549–2552. <https://doi.org/10.1093/jee/toaa182>
- Li, Q., Zheng, L., Cai, H., Garza, E., Yu, Z., & Zhou, S. (2011). From organic waste to biodiesel: Black soldier fly, *Hermetia illucens*, makes it feasible. *Fuel*, *90*(4), 1545–1548. <https://doi.org/10.1016/j.fuel.2010.11.016>
- Lim, J. W., Mohd-Noor, S. N., Wong, C. Y., Lam, M. K., Goh, P. S., Beniers, J. J. A., Oh, W. D., Jumbri, K., & Ghani, N. A. (2019). Palatability of black soldier fly larvae in valorizing mixed waste coconut endosperm and soybean curd residue into larval lipid and protein sources. *Journal of Environmental Management*, *231*, 129–136. <https://doi.org/10.1016/j.jenvman.2018.10.022>
- Liu, Z., Najar-Rodriguez, A. J., Minor, M. A., Hedderley, D. I., & Morel, P. C. H. (2020). Mating success of the black soldier fly, *Hermetia illucens* (Diptera: Stratiomyidae), under four artificial light sources. *Journal of Photochemistry and Photobiology B: Biology*, *205*, 111815. <https://doi.org/10.1016/j.jphotobiol.2020.111815>
- Macavei, L. I., Benassi, G., Stoian, V., & Maistrello, L. (2020). Optimization of *Hermetia illucens* (L.) egg laying under different nutrition and light conditions. *PLOS ONE*, *15*(4), e0232144. <https://doi.org/10.1371/journal.pone.0232144>
- Mamai, W., Bimbile-Somda, N. S., Maiga, H., Juarez, J. G., Muosa, Z. A. I., Ali, A. B., Lees, R. S., & Gilles, J. R. L. (2017). Optimization of mosquito egg production under mass rearing setting: Effects of cage volume, blood meal source and adult population density for the malaria vector, *Anopheles arabiensis*. *Malaria Journal*, *16*(1), 41. <https://doi.org/10.1186/s12936-017-1685-3>
- Meffert, L. M., & Bryant, E. H. (1991). Mating propensity and courtship behavior in serially bottlenecked lines of the housefly. *Evolution*, *45*(2), 293–306. <https://doi.org/10.1111/j.1558-5646.1991.tb04404.x>

- Miller, P. S., Glasner, J., & Hedrick, P. W. (1993). Inbreeding depression and male-mating behavior in *Drosophila melanogaster*. *Genetica*, 88(1), 29. <https://doi.org/10.1007/BF02424449>
- Morales-Ramos, J. A., Rojas, M. G., Kay, S., Shapiro-Ilan, D. I., & Tedders, W. L. (2012). Impact of adult weight, density, and age on reproduction of *Tenebrio molitor* (Coleoptera: Tenebrionidae). *Journal of Entomological Science*, 47(3), 208–220, 213. <https://doi.org/10.18474/0749-8004-47.3.208>
- Nakamura, S., Ichiki, R. T., Shimoda, M., & Morioka, S. (2016). Small-scale rearing of the black soldier fly, *Hermetia illucens* (Diptera: Stratiomyidae), in the laboratory: Low-cost and year-round rearing. *Applied Entomology and Zoology*, 51(1), 161–166. <https://doi.org/10.1007/s13355-015-0376-1>
- Nguyen, T. T. X., Tomberlin, J. K., & Vanlaerhoven, S. (2015). Ability of black soldier fly (Diptera: Stratiomyidae) larvae to recycle food waste. *Environmental Entomology*, 44(2), 406–410. <https://doi.org/10.1093/ee/nvv002>
- Noda, H., & Ishii, T. (1981). Effect of photoperiod and temperature on the ovarian development of the white-spotted stink bug, *Eysarcoris ventralis* (Heteroptera: Pentatomidae). *Japanese Journal of Applied Entomology and Zoology*, 25(1), 33–38.
- Ooninx, D. G. A. B., van Huis, A., & van Loon, J. J. A. (2015). Nutrient utilisation by black soldier flies fed with chicken, pig, or cow manure. *Journal of Insects as Food and Feed*, 1(2), 131–139. <https://doi.org/10.3920/jiff2014.0023>
- Ooninx, D. G. A. B., Volk, N., Diehl, J. J. E., van Loon, J. J. A., & Belušič, G. (2016). Photoreceptor spectral sensitivity of the compound eyes of black soldier fly (*Hermetia illucens*) informing the design of LED-based illumination to enhance indoor reproduction. *Journal of Insect Physiology*, 95, 133–139. <https://doi.org/10.1016/j.jinsphys.2016.10.006>
- Orshan, L., & Pener, M. P. (1979). Repeated reversal of the reproductive diapause by photoperiod and temperature in males of the grasshopper, *Oedipoda miniata*. *Entomologia Experimentalis et Applicata*, 25(3), 219–226. <https://doi.org/10.1111/j.1570-7458.1979.tb02874.x>
- Palma, L., Ceballos, S. J., Johnson, P. C., Niemeier, D., Pitesky, M., & VanderGheynst, J. S. (2018). Cultivation of black soldier fly larvae on almond byproducts: Impacts of aeration and moisture on larvae growth and composition. *Journal of the Science of Food and Agriculture*, 98(15), 5893–5900. <https://doi.org/10.1002/jsfa.9252>
- Park, K., Kim, W., Kim, E., Choi, J. Y., & Kim, S. H. (2016). Effect of adult population density on egg production in the black soldier fly, *Hermetia illucens* (Diptera: Stratiomyidae). *International journal of industrial entomology*, 33(2), 92–95. <https://doi.org/10.7852/ijie.2016.33.2.92>
- Pastor, B., Čičková, H., Kozánek, M., Martínez-Sánchez, A., Takáč, P., & Rojo, S. (2011). Effect of the size of the pupae, adult diet, oviposition substrate and adult population density on egg production in *Musca domestica* (Diptera: Muscidae). *EJE*, 108(4), 587–596. <https://doi.org/10.14411/eje.2011.076>

- Pastor, B., Velasquez, Y., Gobbi, P., & Rojo, S. (2015). Conversion of organic wastes into fly larval biomass: Bottlenecks and challenges. *Journal of Insects as Food and Feed*, 1(3), 179–193. <https://doi.org/10.3920/jiff2014.0024>
- Permana, A. D., Fitri, L. L., & Julita, U. (2020). Influence of mates virginity on black soldier fly, *Hermetia illucens* L. (Diptera: stratiomyidae) mating performance. 2020, 5(2), 8. <https://doi.org/10.15575/biodjati.v5i2.9049>
- Rehman, K. u., Liu, X., Wang, H., Zheng, L., Rehman, R. u., Cheng, X., Li, Q., Li, W., Cai, M., Zhang, J., & Yu, Z. (2018). Effects of black soldier fly biodiesel blended with diesel fuel on combustion, performance and emission characteristics of diesel engine. *Energy Conversion and Management*, 173, 489–498. <https://doi.org/10.1016/j.enconman.2018.07.102>
- Rehman, K. u., Rehman, A., Cai, M., Zheng, L., Xiao, X., Somroo, A. A., Wang, H., Li, W., Yu, Z., & Zhang, J. (2017). Conversion of mixtures of dairy manure and soybean curd residue by black soldier fly larvae (*Hermetia illucens* L.). *Journal of Cleaner Production*, 154, 366–373. <https://doi.org/10.1016/j.jclepro.2017.04.019>
- Reznik, Y. S., & Vaghina, P. N. (2007). Effect of photoperiod on parasitization by *Trichogramma principium* (Hymenoptera: Trichogrammatidae). *EJE*, 104(4), 705–713. <https://doi.org/10.14411/eje.2007.089>
- Rhode, C., Badenhorst, R., Hull, K. L., Greenwood, M. P., Bester-van der Merwe, A. E., Andere, A. A., Picard, C. J., & Richards, C. (2020). Genetic and phenotypic consequences of early domestication in black soldier flies (*Hermetia illucens*). *Animal Genetics*, 51(5), 752–762. <https://doi.org/10.1111/age.12961>
- Sanandiya, N. D., Ottenheim, C., Phua, J. W., Caligiani, A., Dritsas, S., & Fernandez, J. G. (2020). Circular manufacturing of chitinous bio-composites via bioconversion of urban refuse. *Scientific Reports*, 10(1), 4632. <https://doi.org/10.1038/s41598-020-61664-1>
- Sarpong, D., Oduro-Kwarteng, S., Gyasi, S. F., Buamah, R., Donkor, E., Awuah, E., & Baah, M. K. (2019). Biodegradation by composting of municipal organic solid waste into organic fertilizer using the black soldier fly (*Hermetia illucens*) (Diptera: Stratiomyidae) larvae. *International Journal of Recycling of Organic Waste in Agriculture*. <https://doi.org/10.1007/s40093-019-0268-4>
- Setti, L., Francia, E., Pulvirenti, A., Gigliano, S., Zaccardelli, M., Pane, C., Caradonia, F., Bortolini, S., Maistrello, L., & Ronga, D. (2019). Use of black soldier fly (*Hermetia illucens* (L.), Diptera: Stratiomyidae) larvae processing residue in peat-based growing media. *Waste Management*, 95, 278–288. <https://doi.org/10.1016/j.wasman.2019.06.017>
- Sheppard, D. C., Tomberlin, J. K., Joyce, J. A., Kiser, B. C., & Sumner, S. M. (2002). Rearing methods for the black soldier fly (Diptera: Stratiomyidae). *Journal of Medical Entomology*, 39(4), 695–698. <https://doi.org/10.1603/0022-2585-39.4.695>
- Shumo, M., Khamis, F. M., Tanga, C. M., Fiaboe, K. K. M., Subramanian, S., Ekesi, S., van Huis, A., & Borgemeister, C. (2019). Influence of temperature on selected life-history traits of black soldier fly (*Hermetia illucens*) reared on two common

urban organic waste streams in Kenya. *Animals*, 9(3), 79. <https://doi.org/10.3390/ani9030079>

- Tomberlin, J. K., & Sheppard, D. C. (2001). Lekking behavior of the black soldier fly (Diptera: Stratiomyidae). *The Florida Entomologist*, 84(4), 729–730. <https://doi.org/10.2307/3496413>
- Tomberlin, J. K., & Sheppard, D. C. (2002). Factors influencing mating and oviposition of black soldier flies (Diptera: Stratiomyidae) in a colony. *Journal of Entomological Science*, 37(4), 345–352. <https://doi.org/10.18474/0749-8004-37.4.345>
- Trudel, R., Lavallée, R., Bauce, É. r., & Guertin, C. (2002). The effect of cold temperature exposure and long-day photoperiod on the termination of the reproductive diapause of newly emerged female *Pissodes strobi* (Coleoptera: Curculionidae). *Agricultural and Forest Entomology*, 4(4), 301–308. <https://doi.org/10.1046/j.1461-9563.2002.00155.x>
- van Bergen, E., Brakefield, P. M., Heuskin, S., Zwaan, B. J., & Nieberding, C. M. (2013). The scent of inbreeding: A male sex pheromone betrays inbred males. *Proceedings of the Royal Society B: Biological Sciences*, 280(1758), 20130102. <https://doi.org/10.1098/rspb.2013.0102>
- Zhang, J., Huang, L., He, J., Tomberlin, J. K., Li, J., Lei, C., Sun, M., Liu, Z., & Yu, Z. (2010). An artificial light source influences mating and oviposition of black soldier flies, *Hermetia illucens*. *Journal of Insect Science*, 10(1), 202. <https://doi.org/10.1673/031.010.20201>
- Zheng, L., Hou, Y., Li, W., Yang, S., Li, Q., & Yu, Z. (2012). Biodiesel production from rice straw and restaurant waste employing black soldier fly assisted by microbes. *Energy*, 47(1), 225–229. <https://doi.org/10.1016/j.energy.2012.09.006>

5.7 Appendix

Table S 5.1 Coefficient table of the general linear model conducted to analyze factors that could affect the temperature in the experiment environment in the present study.

Term	Coefficient	SE	<i>t</i>	<i>P</i>
Incubator				
B ^a	-1.28	0.38	-3.34	0.003
C ^a	-0.76	0.38	-1.98	0.06
A ^b	1.28	0.38	3.34	0.003
C ^b	0.52	0.38	1.36	0.19
Elapsed time	-0.01	0.002	-4.96	< 0.001
Photoperiod	0.16	0.05	3.30	0.003

a. Incubator A was set as a reference for comparisons between incubators.

b. Incubator B was set as a reference for comparisons between incubators.

Table S 5.2 Coefficient table of the general linear model conducted to analyze factors that could affect the relative humidity in the experiment environment in the present study.

Term	Coefficient	SE	<i>t</i>	<i>P</i>
Incubator				
B ^a	-9.13	1.58	-5.79	< 0.001
C ^a	-29.45	1.58	-18.69	< 0.001
A ^b	9.13	1.58	5.79	< 0.001
C ^b	-20.32	1.58	-12.9	< 0.001
Elapsed time	-0.04	0.009	-4.04	0.001
Photoperiod	-0.58	0.2	-2.9	0.008

a. Incubator A was set as a reference for comparisons between incubators.

b. Incubator B was set as a reference for comparisons between incubators.

Table S 5.3 Selected black soldier fly (BSF; *Hermetia illucens* L.) life history traits in response to different light regimes used in the present experiment. All BSF adults were housed in 30 × 30 × 30 cm mesh cages. A 20-watt LED BSF breeding lamp was set 1 cm above each cage to induce BSF mating.

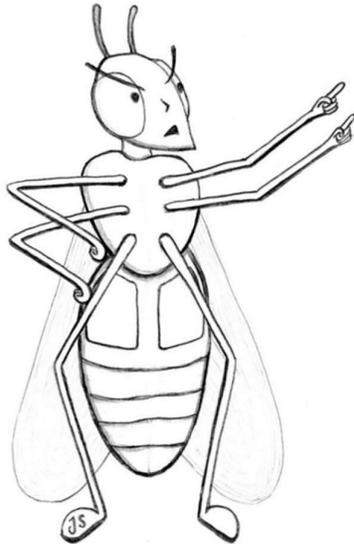
Response	8L : 16D	12L : 12D	16L : 8D
Average number of fertile eggs laid per inseminated female	670 ± 65	635 ± 49	517 ± 63
Percentage of inseminated females (%)	70.4 ± 6.6	67.1 ± 2.7	66.9 ± 6.2

Data from all the observations were used (i.e., n = 9). The values are means ± SE.

Equation S 5.1

$$\begin{aligned}
 \text{Percentage of fertile eggs} &= \frac{\text{Number of fertile eggs}}{\text{Number of all eggs}} \\
 &= \frac{\text{Average number of fertile eggs laid per inseminated female} \times \text{Number of inseminated females}}{\text{Average number of eggs laid per female} \times \text{Number of females}} \\
 &= \frac{\text{Average number of fertile eggs laid per inseminated female}}{\text{Average number of eggs laid per female}} \times \text{Percentage of inseminated females}
 \end{aligned}$$

Chapter 6 General discussion



- My babies like soft food with balanced nutrients, and they need to be hugged before sleeping
- My partner and I like bright sunlight, but artificial light is also fine if you make its spectrum and intensity right
- We like to live in a large community with hundreds of thousands of neighbors

“My family needs a good work environment.” (picture of a black soldier fly by J. Shailer, 2016; reproduced with permission)

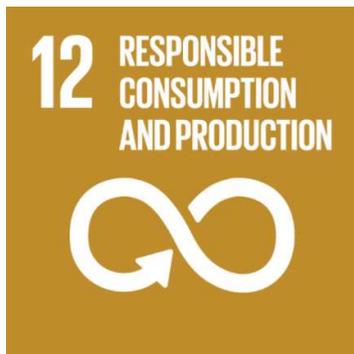
6.1 Introduction

Ending hunger and achieving environmentally sound waste management are two targets for sustainable development (Figure 6.1; United Nations, 2015). Compared to landfill and composting, bioconversion of organic waste upcycles underutilized nutrients back to the human food chain and reduces leachate and greenhouse gas production, thereby assisting the achievement of the two sustainable development objectives (Christensen *et al.*, 2010; Pang *et al.*, 2020).



Goal 2. End hunger, achieve food security and improved nutrition and promote sustainable agriculture

Target 2.1. By 2030, end hunger and ensure access by all people, in particular the poor and people in vulnerable situations, including infants, to safe, nutritious and sufficient food all year round



Goal 12. Ensure sustainable consumption and production patterns

Target 12.4. By 2020, achieve the environmentally sound management of chemicals and all wastes throughout their life cycle, in accordance with agreed international frameworks, and significantly reduce their release to air, water and soil in order to minimize their adverse impacts on human health and the environment

Figure 6.1 Goals and targets announced by the United Nations showing the urgent need of ending hunger and achieving environmentally sound waste management for global sustainable development (United Nations, 2015).

The black soldier fly (BSF), *Hermetia illucens* L. (Diptera: Stratiomyidae), is one of the promising candidates for organic waste bioconversion. BSF larvae (referred to as "BSFL" hereafter) could be used to convert multiple types of organic waste into organic fertilizer, animal feed, precursors of bioplastics, biofuel, and detergents (Chapter 1). To effect bioconversion of organic waste employing BSFL at large scales, efforts are needed to understand environmental factors that could influence the fitness of this species at different life stages, based on which optimization of its rearing environment would be possible. My research project was designed to fill knowledge gaps regarding the effect of (1) diet on selected BSFL life history and physiological traits, (2) substrate type and moisture level on BSF pupation, and (3) illumination and adult density on selected life history traits of BSF adults. Based on my results, I discuss aspects that need to be considered when optimizing BSF rearing environments, and present my recommendations for future research and practical applications.

6.2 Optimization of BSFL diet

In bioconversion systems employing BSFL, the quality of the organic waste fed to the larvae affects the performance of the bioconversion system. In Chapter 2, more suitable diets were found to be generally associated with higher rates of larval weight gain (i.e., daily dry weight increase per larva), shorter time needed for the larvae to reach the harvestable stage, and higher proportions of waste dry matter converted to larval biomass. Such trends were also found in other studies (e.g., Adebayo *et al.*, 2021; Lalander *et al.*, 2019; Scala *et al.*, 2020). Although not observed in my study, suitable diets may also increase larval survival rate (Lalander *et al.*, 2019), and thus would increase larval biomass production. Moreover, BSF that feed on suitable diets during the larval stage

have higher fecundity (Barragan-Fonseca *et al.*, 2021). Because some of the larvae harvested from the bioconversion system may be used to breed neonatal larvae of the next generation, increasing adult fecundity would reduce the number of larvae allocated to producing the next generation neonates, thus increasing system biomass output. Overall, optimizing BSFL diet is essential to improve the efficiency of the bioconversion system.

6.2.1 Chemical composition

A large body of research, including the work presented in Chapter 2, was conducted to select locally generated organic waste types that are suitable for BSFL rearing. However, the fact that BSFL can grow well on a particular kind of waste does not necessarily mean that the chemical composition of that waste is optimal (Barragan-Fonseca *et al.*, 2021; Lalander *et al.*, 2019). Feeding on an imbalanced diet, BSFL would need to compromise between insufficient intake of nutrients that are in deficit and excessive intake of nutrients that are abundant; the former case leads to sub-optimal fitness such as low larval weight and adult fecundity, whereas in the latter case, excessive nutrients could be respired (e.g., carbohydrates) through catabolism or excreted (e.g., proteins) (Barragan-Fonseca *et al.*, 2021; Chapman *et al.*, 2013). For instance, brewer's waste was the most suitable diet for BSFL rearing among all the waste types tested in Chapter 2. However, because protein : digestible carbohydrate ratio in brewer's waste is about six times higher than that in broll, the larvae fed on brewer's waste had a shorter development time and lower lipid store in their body at pupation than those fed on broll (a mixture of wheat bran and wheat flour). Thus, the larvae fed on brewer's waste would have lower fecundity than the broll-fed ones (Barragan-Fonseca *et al.*, 2021; Jucker *et al.*, 2020). Moreover, larvae fed on brewer's waste converted a lower proportion of crude protein into larval biomass than

those fed on broil, indicating a possible underutilization of crude protein in brewer's waste. Therefore, to improve the performance of the bioconversion system, it is necessary to understand the effects of dietary chemical composition on BSF fitness at different life stages (e.g., Barragan-Fonseca *et al.*, 2021) and nutrient metabolism; based on these findings, diets with balanced chemical composition can be formulated.

Thus far, several semi- and fully-synthetic diets have been used to investigate BSFL nutritional requirements (e.g., Barragán-Fonseca, 2018; Cammack & Tomberlin, 2017; Chia *et al.*, 2018b; Ooninx *et al.*, 2015). Similar methods could be used to test the hypothesis raised in Chapter 2 — that a protein : fat : digestible carbohydrate ratio of 2 : 1 : 2 benefits BSFL development. When designing synthetic diets for BSFL, it should be noted that dietary ingredients with similar nutritional functions may differ in their physical characteristics owing to the differences in the degree of polymerization or pre-treatment, and that synthetic diets with similar chemical compositions may have different textures (Woods *et al.*, 2019). For instance, both sucrose and starch can serve as a sole source of digestible carbohydrate, but their differences in water solubility would lead to different dietary textures; while albumin is water soluble in its natural form, heating could change its molecular structure and reduce its solubility. Therefore, future studies should consider both chemical and physical characteristics of dietary ingredients when formulating artificial diets for BSFL and interpreting dietary effects on BSFL fitness traits (e.g., Woods *et al.*, 2019).

6.2.2 Lignocellulose in BSFL diet

Lignocellulose is the major component of the plant cell wall, which includes hemicellulose, cellulose, and lignin. In Chapter 2, dietary lignocellulosic content was found to have an adverse effect on BSFL weight gain. Evidence has shown that BSFL cannot consume most of the lignocellulose component in their diet (Wang *et al.*, 2017; Zheng *et al.*, 2012), but the underlying mechanism has not been addressed yet.

The function of BSFL mouthparts is to scrape small particles off the diet surface before ingestion (Bruno *et al.*, 2020) or to collect nutrients suspended or dissolved in liquid (Popa & Green, 2012). Therefore, suitable BSFL diets or diet ingredients could be either solids that are tender enough for BSFL to mechanically break down (e.g., animal manure and fish offal), or liquid (e.g., leachate and expired milk), or their mixture (e.g., abattoir waste) (Lalander *et al.*, 2019; Popa & Green, 2012; St-Hilaire *et al.*, 2007). My research used broil (a mixture of wheat bran and wheat flour) as a standard BSFL diet for colony maintenance. BSFL were able to consume wheat flour but left most of the lignocellulosic component in wheat bran unconsumed (Figure 6.2), indicating that the lignocellulose component is hard for BSFL to break down with their mouthparts.

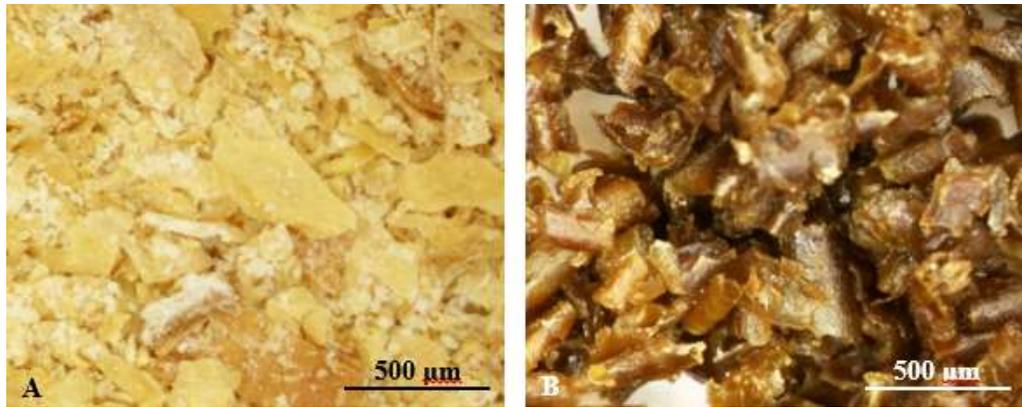


Figure 6.2 Broll (a mixture of wheat bran and wheat flour), before (A) and after (B) consumption by larvae of the black soldier fly, *Hermetia illucens*. The white particles in (A) are wheat flour, and the yellow flakes are wheat bran. The substrate in (B) mainly consists of larval excreta and the uningested lignocellulosic component of wheat bran. Images by Z. Liu, 2019; CC BY-NC.

Although most of the lignocellulose component is retained in spent BSFL diets, some hemicellulose and cellulose can still be degraded during BSFL consumption (Wang *et al.*, 2017; Zheng *et al.*, 2012). Understanding whether or to what extent hemicellulose and cellulose may be used by BSFL as nutrient sources would help to better define what constitutes digestible carbohydrate for the larvae, which could improve the accuracy of diet formulation. Exploration of the ingestibility and digestibility of hemicellulose and cellulose could be achieved by analyzing their quantities in original, ingested, semidigested, and fully digested diets, in comparison with that of a marker (e.g., titanium dioxide). Ingested and semidigested diets could be obtained from the foregut and midgut of BSFL, respectively, whereas fully digested diets could be collected from larval hindgut or as excreta according to a novel method developed by Gold *et al.* (2020). Because the quantity of the samples collected from larval guts would be very small, the analytical techniques described in Chapter 2 are not feasible. It may be better to use instrumental

analytical methods such as near-infrared and Fourier-transform infrared spectroscopy (Abidi *et al.*, 2014; Ai *et al.*, 2022), which do not require large quantities of samples.

Although it is difficult for BSFL to consume lignocellulose, dietary inclusion of lignocellulose is not always detrimental to BSFL growth. For example, lignocellulose in BSFL diets can bind to lipids, reducing diet stickiness and allowing larval movements (Woods *et al.*, 2019). Lignocellulose also “connects” dietary ingredients that otherwise are not inter-soluble, such as water- and lipid-soluble vitamins, thereby improving the consistency of the diet (Cohen, 2015).

6.2.3 Dietary moisture content

Moisture content is a critical factor in BSFL diet and should be neither too low nor too high. Diets with unsuitably low moisture content may form crust or clumps at the surface (Bekker *et al.*, 2021), which are hard for BSFL to break down with their mouthparts, reducing nutrient accessibility for the larvae. Cammack and Tomberlin (2017) reported that when dietary moisture content is reduced from 70% to 50%, the amount of diet required for BSFL development could double due to poor ingestion rates. In addition, diet serves as a substrate which BSFL inhabit. Thus, diets that are too dry may become dehydrating environments to the larvae, which impedes their growth and survival. Studies on different diet types have revealed that dietary moisture content below 60% is unfavorable for BSFL (Bekker *et al.*, 2021; Cammack & Tomberlin, 2017; Chen *et al.*, 2019; Dzepe *et al.*, 2020a; Palma *et al.*, 2018).

On the other hand, if dietary moisture content is too high, a liquid phase may form above

the solids; if the liquid phase is too thick, BSFL will float on the surface with no access to the solids – the primary nutrient source (Bekker *et al.*, 2021; Chen *et al.*, 2019). Because water holding capacity is different among substances, the optimum dietary moisture content is not universal and should be determined separately for each type of diet. For instance, when fed to BSFL, the optimum moisture content for almond by-products is 66%, whereas that for pig manure is 75% (Chen *et al.*, 2019; Palma *et al.*, 2018). According to Bekker *et al.* (2021), who used chicken feed as BSFL diet, the optimum moisture content seems achieved when the diet almost reached its water holding capacity (i.e., cannot absorb more water). Although not mentioned before, the diets tested in Chapter 2 were almost reaching their water holding capacities, and they were at different moisture levels due to their varying water holding capacities.

Dietary moisture content is affected by the ambient relative humidity if water is not replenished. In Chapter 2, the ambient relative humidity was $40 \pm 10\%$. In this dry environment, diets dried faster than would be expected in more humid environments, leading to lower waste reduction than in previous studies (e.g., Diener *et al.*, 2009; Myers *et al.*, 2008). In future studies and in practice, if BSFL are reared in dry environments, it is suggested that water should be replenished daily based on the water loss rate (e.g., Bekker *et al.*, 2021). Since diet dehydration normally starts from its surface (e.g., Bekker *et al.*, 2021), misting water on diet surface may be a better way of water replenishment than pouring liquid water into the diet. However, keeping dietary moisture content high would make it difficult to separate larvae from the spent diet by sieving (Cheng *et al.*, 2017). Therefore, if sieving is used to separate larvae from spent diet, water replenishment should be gradually reduced and eventually ceased towards the time of harvest; if relative humidity is high, increasing aeration of the larval rearing space would be necessary to carry moisture away (Cheng *et al.*, 2017).

6.3 Optimization of BSF pupation substrate

It is a common behavior among terrestrial brachycerans to stay in pupation substrates during metamorphosis, as has been observed in *Chrysomya megacephala* (Fabricius) (Diptera: Calliphoridae), *Cochliomyia macellaria* (Fabricius) (Diptera: Calliphoridae) (Godoy *et al.*, 1995), *Protopiophila litigate* (Bonduriansky) (Diptera: Piophilidae) (Bonduriansky, 1995), and *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae) (Ballman *et al.*, 2017), and BSF is no exception (Barros *et al.*, 2019; Sheppard *et al.*, 1994). Insects benefit from this behavior by reducing risks of predation, parasitism, and dehydration (Hodgson *et al.*, 1998; Okuyama, 2019; Wen *et al.*, 2017). In a well-managed BSF rearing facility, although predation and parasitism on BSF pupae are not likely to happen, providing suitable pupation substrates to BSF prepupae can improve pupation rate and reduce prepupal duration (Dzepe *et al.*, 2020b; Holmes *et al.*, 2013). As pupation rate increases, fewer resources are needed to produce prepupae for colony maintenance, and more can be used for larval biomass production. Shorter prepupal periods could lead to less stored energy wasted during the wandering stage and more allocated to adult fitness (e.g., longevity and reproduction) (Holmes *et al.*, 2013), and thus may further reduce the number of prepupae required for colony maintenance. Therefore, using suitable pupation substrates can improve the performance of the bioconversion system.

So far, several types of substrates have been tested for their suitability to support BSF pupation, including sand, topsoil, potting soil, vermiculite, wood shavings, wood chips, and wheat bran (Dzepe *et al.*, 2020b; Holmes *et al.*, 2013). According to Dzepe *et al.* (2020b), Holmes *et al.* (2013) and my results (Chapter 3), potting soil, vermiculite, wood shavings, and wood chips can be all considered as potentially suitable substrates for BSF pupation. While topsoil may become a suitable BSF pupation substrate after adjusting its

moisture content, sand and wheat bran are not likely to improve BSF pupation based on my hypothesis – that pupation substrates assist BSF pupation by reducing surrounding disturbance (e.g., vibration) and providing thigmotactic stimuli (i.e., physical pressure). Wet wheat bran is soft and can absorb vibration, but it probably will not offer sufficient thigmotactic stimuli due to its low density. Sand, on the other hand, is dense enough to give sufficient pressure to BSF prepupae, but its texture is too rigid to absorb vibration. Nevertheless, this hypothesis still needs verification.

Probably because of its origin and evolutionary history in humid Latin America (Guilliet *et al.*, 2021), BSF is susceptible to dry environments (Holmes *et al.*, 2012). In Chapter 3, BSF pupae provided with pupation substrates at higher moisture levels had lower mortality rates and higher pupation rates, which indicates the importance of regulating moisture in BSF pupation substrate. On the other hand, very high moisture content affects oxygen availability in pupation substrates, and adding too much water to pupation substrates would create an oxygen-depleting environment for insects. In Chapter 3, although BSF prepupae started to avoid burrowing deeper when moisture content exceeded 20% and 70% for vermiculite and wood chips, respectively, higher moisture content in these substrates did not show detrimental effects on prepupal survival or pupation rate. This may be because of the low pupation density (fifty prepupae per 390-ml cup) used in the experiment, where most (if not all) prepupae could inhabit depths with sufficient oxygen. At higher pupation densities, more individuals would be forced to move to deeper levels, in which case increased mortality or delayed pupation may be caused by excessive moisture content. In this regard, it is necessary to evaluate the effects of substrate moisture content on BSF pupation at different pupation densities.

6.4 Optimization of visual environment for BSF mating

Studies have shown that BSF mating requires bright sunlight or artificial light with a special spectrum (Ooninx *et al.*, 2016; Tingle *et al.*, 1975; Tomberlin & Sheppard, 2002). Since strong sunlight is not always available, developing artificial lights suitable for BSF mating is necessary to ensure a stable year-round supply of neonatal larvae for organic waste management. Among all the artificial lights that have been tested (e.g., halogen, fluorescent, light-emitting diode), combining different light-emitting diode (LED) chips seems the easiest way to achieve the optimal spectral recipe (Heussler *et al.*, 2018; Ooninx *et al.*, 2016). Moreover, since LED lights can be powered by direct current (DC), possible detrimental effects of light flickering on BSF mating can be avoided (Chapter 4).

Even though the BSFLED light the best result in supporting BSF mating among the light sources tested in Chapter 4 had, there are still several aspects where the design and the use of the light can be improved.

First of all, although the spectrum of BSFLED peaked at wavelengths to which BSF photoreceptors are most sensitive (Ooninx *et al.*, 2016), the irradiance ratio between the three key wavebands (ultraviolet/UV: 300–400, blue: 400–500, and long-wavelength: 500–650 nm) may not be optimal. The irradiance ratio UV : blue : long-wavelength for sunlight is 1 : 9.3 : 16.7, whereas that for BSFLED it is 1 : 16.2 : 16.3. Since BSF has evolved to adapt to sunlight, the blue irradiance emitted from BSFLED may be excessive, and thus may represent a waste of energy in a BSF production system. Klüber *et al.* (2020) found that artificial lights with warmer color temperatures are more favorable for BSF mating, with 3000K (also known as warm white) being the best among all the color temperatures tested. Therefore, having a high proportion of blue radiation from artificial

light for BSF mating may be unnecessary; efforts are still needed to explore energy-saving and effective artificial light spectrum for BSF mating.

Secondly, Heussler *et al.* (2018) reported that BSF can reproduce under an LED light with a spectrum ranging from 400–750 nm, which indicates that UV may not be necessary for the optimal BSF mating environment. The UV radiation emitted from BSFLED ranged between 350–400 nm and peaked at 357 nm, and is therefore within the range of UVA (315–400 nm) (Sinha & Häder, 2002). UVA can induce the generation of reactive oxygen species in cells, which can cause oxidative stress to insects, or cause malfunction of antioxidant enzymes; these effects can lead to reduced adult longevity and fecundity, and reduced fitness of the F₁ generation (Ali *et al.*, 2016, 2017; Cui *et al.*, 2021; Khan *et al.*, 2021; Meng *et al.*, 2009; Sinha & Häder, 2002; Tariq *et al.*, 2015; Zhang *et al.*, 2011). Therefore, further research is needed to verify the role that UVA radiation plays in BSF mating and its possible harmful effects to the BSF adults; if UVA radiation is unnecessary, it should be avoided.

Third, light intensity decreases in proportion to the square of the distance from the light source (Brownson, 2014). Since BSF mating requires bright light, the radiation of artificial light should increase as BSF breeding enclosure (e.g., cage or room) gets larger to ensure sufficient irradiance received by the adults at the farthest corner (Schneider, 2020). On the other hand, Schneider (2020) reported that the effect of light intensity on BSF mating follows a logarithm function, and thus it is unnecessary to have too intense radiation. According to Schneider (2020), when using a white LED light to support BSF mating, the irradiance received by the adults should be at least $0.92 \text{ W}\cdot\text{m}^2$, but it does not need to exceed $157 \text{ W}\cdot\text{m}^2$. Nevertheless, this range may not suit other light sources due to spectral differences. Hence, replication of the work by Schneider (2020) is necessary

after the optimal light spectrum for BSF mating is confirmed.

Another factor that needs to be considered is the background reflection of the breeding enclosure. For instance, increasing the reflectance of the walls of BSF breeding enclosure would reduce the radiation needed from artificial light (Schneider, 2020), thereby reducing energy consumed by the facility. In addition, the contrast between background and insects also affects their mating. In *Lispe cana* Walker (Diptera: Muscidae), males prefer to display themselves against backgrounds that contrast highly with their own colors, which increases female attention and leads to higher mating success (White *et al.*, 2020). In *Asterocampa leilia* Edwards (Lepidoptera: Nymphalidae), males tend to perch where they are more likely to detect their potential mates, and they are more likely to take off from their perching sites when potential mates in flight have higher contrast against the background (Bergman *et al.*, 2015). Since BSF also use visual cues to detect potential mates (Giunti *et al.*, 2018), increasing the contrast between BSF adults and their backgrounds may improve mating success; this is something that could be investigated.

Finally, if artificial light is the sole light source to support BSF mating, photoperiod would determine both power consumption used for lighting and the time window during which BSF mating could happen. A shorter artificial photoperiod means less power consumption, but it also means less chance for BSF adults to mate. When BSF adult density is low (e.g., less than 800 individuals per cubic meter), a photoperiod of 8 h is enough for BSF adults to mate (Julita *et al.*, 2020; Zhang *et al.*, 2010; Chapter 5). However, when BSF adult density is high (e.g., more than 2000 individuals per cubic meter), prolonging photoperiod could increase BSF adult longevity and female fecundity (Hoc *et al.*, 2019; Chapter 5). Since high adult density benefits the production of neonatal larvae within a given breeding space, photoperiods of longer than 8 h would be needed in

practice, and the trade-off between neonate production and power consumption would need to be considered.

6.5 Evaluating the effects of environmental factors on the entire BSF life history

So far, optimization of BSF rearing environment has been discussed for each life stage separately. However, an environmental factor that has been optimized for one life stage may not be optimal for some other life stages of the same insect. For instance, the ratio between digestible carbohydrate and protein in BSF larval diet optimal for larval survival is not the ratio optimal for adult longevity or female fecundity (Barragan-Fonseca *et al.*, 2021). In another example, 35°C is the optimal temperature for BSF larval growth when feeding on brewer's waste, but the same temperature is too hot for pupal development (Chia *et al.*, 2018a). Therefore, the effects of environmental factors on BSF fitness should not only be examined for a specific life stage, but also over the entire life history of the insect.

Life tables, which record important life history parameters such as age, survival rate, and the number of progeny (Portilla *et al.*, 2014), are a valuable tool for an overall evaluation of the impact of an environmental factor on the entire BSF life history. With life tables constructed, the intrinsic rate of increase can be calculated, which simultaneously evaluates multiple life history parameters across different life stages, including development period, immature survivorship, adult longevity, and fecundity (Portilla *et al.*, 2014). With this tool, the optimal content of digestible carbohydrate and protein in BSF larval diet are estimated to be 60% and 15%, respectively (Barragan-Fonseca *et al.*,

2021). Similarly, if only one temperature could be selected over an entire bioconversion system, the BSF population would need to be maintained at 30°C to achieve the highest intrinsic rate of increase (Chia *et al.*, 2018a).

6.6 Maintaining a genetically healthy BSF colony

The phenotypic performance of a BSF colony depends on both the environment and its genetic quality (e.g., genotype and genetic diversity) (Rhode *et al.*, 2020; Zhou *et al.*, 2013; Chapter 4). In an artificially managed BSF colony, mating among close relatives is inevitable, which could result in obvious inbreeding depression when a population is founded by a small number of individuals or has been kept for a long time without outcrossing (Eriksson & Picard, 2021). In my research, there was evidence that inbreeding depression may be acting to reduce mating success in a small BSF colony, where two thousand prepupae were used to breed neonatal larvae at each generation (Chapter 4), and in a bigger colony, where hundreds of thousands of prepupae were used for breeding each generation yet with no outcrossing for 3 yr (Chapter 5). In a recent study by Rhode *et al.* (2020), where the census population size (i.e., number of adults used for breeding) of a BSF colony was below 4000, the colony collapsed in a short time due to the failure of adult eclosion at the end of the fourth generation. Based on the results of their genetic analysis, Rhode *et al.* (2020) suggested that the colony collapse was most likely due to inbreeding depression.

Generally, to avoid inbreeding, a captive BSF colony should be started with a large number of founders, preferably from multiple genetically distant groups, and the colony should be maintained with a large effective population size at each generation with regular

outcrossing (Eriksson & Picard, 2021). Nevertheless, outcrossing a captive BSF colony with random wildtypes may result in outbreeding depression through introduction of deleterious alleles or genotypes which are less compatible with the rearing environment and/or less productive. Therefore, it may be less risky and more desirable to separate a stabilized stock colony into several genetic lines and cross them regularly (Eriksson & Picard, 2021; Peng *et al.*, 2015). Detailed methods of establishing and managing genetically healthy BSF colonies warrant future studies.

6.7 Summary of recommendations

6.7.1 Recommendations for practical applications

Brewer's waste is a suitable diet for BSFL and can be mixed with wastes rich in carbohydrates (such as expired bread or cookies) to improve the chemical composition of BSFL diet. Lignocellulolytic bacteria or enzymes could be added to BSFL diet to increase the digestibility of the lignocellulosic component.

BSFL diet should not be too dry or too wet. Dietary moisture content (calculated as water / diet wet weight) should not be below 60%, and the optimal moisture level seems to be when the diet reaches its water holding capacity. Misting water onto the diet surface could help to maintain dietary moisture content during larval growth. If larvae are separated from the spent diet by sieving, it would be necessary to reduce dietary moisture content towards larval harvest.

Potting soil, vermiculite, wood shavings, or wood chips can be used as a pupation substrate for BSF to improve prepupal survival and reduce prepupal duration. With the

substrate depth and pupation density used in Chapter 3, the recommended moisture content (calculated as water / substrate dry weight) is 150% for vermiculite or wood chips. For higher pupation densities, it may be necessary to reduce moisture content.

To ensure a stable year-round supply of neonatal BSFL, artificial lights could be used to facilitate BSF mating indoors or to supplement sunlight in glasshouses. DC-powered LED lamps with spectra similar to BSFLED (see Chapter 4) are recommended, although efforts are still needed to optimize artificial light spectrum for BSF mating. Increasing background reflection of BSF breeding enclosure may enhance light intensity, which is beneficial for BSF mating. Replication of the work by Schneider (2020) is necessary to determine the maximum size of BSF breeding space illuminated by the artificial light. A photoperiod of 8 h is sufficient when BSF adult density is low (e.g., less than 800 individuals per cubic meter), but longer photoperiods may be needed for higher adult densities.

Life tables are a valuable tool to record important life history parameters of BSF, based on which the intrinsic rate of increase can be calculated to monitor colony fitness or examine the effects of optimization measures (e.g., changing diet composition or changing artificial light spectrum) on the entire BSF life history.

To avoid inbreeding depression in a captive BSF colony, a large number of founders originating from genetically distinct groups should be used to initiate the colony, and the effective population size of the colony should be kept as high as possible. Measures to maintain genetic diversity (e.g., outcrossing) should be performed regularly.

6.7.2 Recommendations for future research

Semi- and fully-synthetic diets are recommended for investigating BSFL nutritional requirements and the effects of the chemical composition of larval diet on BSFL fitness at different life stages. In order to improve the accuracy of diet formulation, it is crucial to understand the ingestibility and digestibility of hemicellulose and cellulose by BSFL. Moreover, the physical characteristics of dietary ingredients should also be considered when formulating BSFL larval diets or interpreting dietary effects on BSFL fitness traits.

In Chapter 3, we tried to analyze the underlying mechanisms of improving BSFL pupation with suitable substrates from three aspects: (1) avoiding desiccation, (2) reducing surrounding disturbance, and (3) providing thigmotactic stimuli. The first mechanism has been revealed by our study, whereas the other two warrant further investigation. A better understanding of these (possible) mechanisms will help develop novel methods to assist BSFL pupation, where pupation substrates might not be necessary.

The visual environment for BSFL mating should be further optimized by understanding the roles that different wavebands play in BSFL mate recognition, especially for the UV (300–400 nm) and blue (400–500 nm) wavebands. In addition, it would be interesting to investigate the effects of background reflection and color on BSFL mating.

Establishing and maintaining genetically healthy BSFL colonies is vital to the consistent and effective production of neonates that are used for organic waste bioconversion. Efforts are needed to develop detailed protocols to avoid both inbreeding and outbreeding depression and assist the management of BSFL brood stocks with genetic tools (e.g., DNA markers).

6.8 Conclusion

Bioconversion of organic waste fulfils sustainable development targets of enhancing food security and reducing environmental impacts. The improvement of bioconversion systems employing BSFL relies on optimizing the rearing environment for this species. This thesis provides knowledge and insights that can be used to optimize larval diet, pupation substrate and its moisture content, and adult mating environment for BSF. Studying the effects of an environmental factor on a single life stage of BSF has helped to improve our understanding of the underlying mechanisms, whereas in order to improve the fitness of a mass-reared population, it is necessary to view environmental impacts across the entire life history of BSF.

6.9 References

- Abidi, N., Cabrales, L., & Haigler, C. H. (2014). Changes in the cell wall and cellulose content of developing cotton fibers investigated by FTIR spectroscopy. *Carbohydrate Polymers*, *100*, 9–16. <https://doi.org/10.1016/j.carbpol.2013.01.074>
- Adebayo, H. A., Kemabonta, K. A., Ogbogu, S. S., Elechi, M. C., & Obe, M. T. (2021). Comparative assessment of developmental parameters, proximate analysis and mineral compositions of black soldier fly (*Hermetia illucens*) prepupae reared on organic waste substrates. *International Journal of Tropical Insect Science*, *41*(3), 1953–1959. <https://doi.org/10.1007/s42690-020-00404-4>
- Ai, N., Jiang, Y., Omar, S., Wang, J., Xia, L., & Ren, J. (2022). Rapid measurement of cellulose, hemicellulose, and lignin content in *Sargassum horneri* by near-infrared spectroscopy and characteristic variables selection methods. *Molecules*, *27*(2), 335. <https://doi.org/10.3390/molecules27020335>
- Ali, A., Rashid, M. A., Huang, Q. Y., & Lei, C. (2016). Effect of UV-A radiation as an environmental stress on the development, longevity, and reproduction of the oriental armyworm, *Mythimna separata* (Lepidoptera: Noctuidae). *Environmental Science and Pollution Research*, *23*(17), 17002–17007. <https://doi.org/10.1007/s11356-016-6865-0>
- Ali, A., Rashid, M. A., Huang, Q. Y., & Lei, C. (2017). Influence of UV-A radiation on oxidative stress and antioxidant enzymes in *Mythimna separata* (Lepidoptera:

- Noctuidae). *Environmental Science and Pollution Research*, 24(9), 8392–8398. <https://doi.org/10.1007/s11356-017-8514-7>
- Ballman, E. S., Collins, J. A., & Drummond, F. A. (2017). Pupation behavior and predation on *Drosophila suzukii* (Diptera: Drosophilidae) pupae in maine wild blueberry fields. *Journal of Economic Entomology*, 110(6), 2308–2317. <https://doi.org/10.1093/jee/tox233>
- Barragán-Fonseca, K. B. (2018). *Flies are what they eat: Tailoring nutrition of black soldier fly (Hermetia illucens L.) for larval biomass production and fitness* [Doctoral thesis, Wageningen University]. Wageningen University & Research. <https://doi.org/10.18174/449739>
- Barragan-Fonseca, K. B., Gort, G., Dicke, M., & Loon, J. J. A. v. (2021). Nutritional plasticity of the black soldier fly (*Hermetia illucens*) in response to artificial diets varying in protein and carbohydrate concentrations. *Journal of Insects as Food and Feed*, 7(1), 51–61. <https://doi.org/10.3920/jiff2020.0034>
- Barros, L. M., Ferreira-Keppler, R. L., Martins, R. T., & Gutjahr, A. L. N. (2019). Bionomy of *Hermetia illucens* (Diptera: Stratiomyidae) on decomposing swine carcass in an urban area of Central Amazon. *Journal of Medical Entomology*, 56(3), 681–689. <https://doi.org/10.1093/jme/tjz005>
- Bekker, N. S., Heidebach, S., Vestergaard, S. Z., Nielsen, M. E., Riisgaard-Jensen, M., Zeuner, E. J., Bahrndorff, S., & Eriksen, N. T. (2021). Impact of substrate moisture content on growth and metabolic performance of black soldier fly larvae. *Waste Management*, 127, 73–79. <https://doi.org/10.1016/j.wasman.2021.04.028>
- Bergman, M., Lessios, N., Seymoure, B. M., & Rutowski, R. L. (2015). Mate detection in a territorial butterfly—the effect of background and luminance contrast. *Behavioral Ecology*, 26(3), 851–860. <https://doi.org/10.1093/beheco/arv020>
- Bonduriansky, R. (1995). A new Nearctic species of *Protopiophila* Duda (Diptera: Piophilidae), with notes on its behaviour and comparison with *P. latipes* (Meigen). *The Canadian Entomologist*, 127(6), 859–863. <https://doi.org/10.4039/Ent127859-6>
- Brownson, J. R. S. (2014). Laws of light. In J. R. S. Brownson (Ed.), *Solar Energy Conversion Systems* (pp. 41–66). Academic Press.
- Bruno, D., Bonacci, T., Reguzzoni, M., Casartelli, M., Grimaldi, A., Tettamanti, G., & Brandmayr, P. (2020). An in-depth description of head morphology and mouthparts in larvae of the black soldier fly *Hermetia illucens*. *Arthropod Structure & Development*, 58, 100969. <https://doi.org/10.1016/j.asd.2020.100969>
- Cammack, J. A., & Tomberlin, J. K. (2017). The impact of diet protein and carbohydrate on select life-history traits of the black soldier fly *Hermetia illucens* (L.) (Diptera: Stratiomyidae). *Insects*, 8(2), 56. <https://doi.org/10.3390/insects8020056>
- Chapman, R. F., Simpson, S. J., & Douglas A. E. (Eds.). (2013). *The insects: Structure and function*. (5th ed.). Cambridge University Press.
- Chen, J., Hou, D., Pang, W., Nowar, E. E., Tomberlin, J. K., Hu, R., Chen, H., Xie, J., Zhang, J., Yu, Z., & Li, Q. (2019). Effect of moisture content on greenhouse gas and NH₃ emissions from pig manure converted by black soldier fly. *Science of*

- The Total Environment*, 697, 133840. <https://doi.org/10.1016/j.scitotenv.2019.133840>
- Cheng, J. Y. K., Chiu, S. L. H., & Lo, I. M. C. (2017). Effects of moisture content of food waste on residue separation, larval growth and larval survival in black soldier fly bioconversion. *Waste Management*, 67, 315–323. <https://doi.org/10.1016/j.wasman.2017.05.046>
- Chia, S. Y., Tanga, C. M., Khamis, F. M., Mohamed, S. A., Salifu, D., Sevgan, S., Fiaboe, K. K. M., Niassy, S., van Loon, J. J. A., Dicke, M., & Ekesi, S. (2018a). Threshold temperatures and thermal requirements of black soldier fly *Hermetia illucens*: Implications for mass production. *PLOS ONE*, 13(11), e0206097. <https://doi.org/10.1371/journal.pone.0206097>
- Chia, S. Y., Tanga, C. M., Osuga, I. M., Mohamed, S. A., Khamis, F. M., Salifu, D., Sevgan, S., Fiaboe, K. K. M., Niassy, S., van Loon, J. J. A., Dicke, M., & Ekesi, S. (2018b). Effects of waste stream combinations from brewing industry on performance of black soldier fly, *Hermetia illucens* (Diptera: Stratiomyidae). *PeerJ*, 6, e5885. <https://doi.org/10.7717/peerj.5885>
- Christensen, T. H., Scharff, H., & Hjelmar, O. (2010). Landfilling: Concepts and challenges. In T. H. Christensen (Ed.), *Solid waste technology & management* (pp. 683–694). Blackwell Publishing Ltd. <https://doi.org/10.1002/9780470666883.ch44>
- Cohen, A. C. (2015). *Insect diets: Science and technology* (2nd ed.). CRC press.
- Cui, H., Zeng, Y., Reddy, G. V. P., Gao, F., Li, Z., & Zhao, Z. (2021). UV radiation increases mortality and decreases the antioxidant activity in a tephritid fly. *Food and Energy Security*, 10(3), e297. <https://doi.org/10.1002/fes3.297>
- Diener, S., Zurbrugg, C., & Tockner, K. (2009). Conversion of organic material by black soldier fly larvae: Establishing optimal feeding rates. *Waste Management & Research*, 27(6), 603–610. <https://doi.org/10.1177/0734242x09103838>
- Dzepe, D., Nana, P., Fotso, A., Tchuinkam, T., & Djouaka, R. (2020a). Influence of larval density, substrate moisture content and feedstock ratio on life history traits of black soldier fly larvae. *Journal of Insects as Food and Feed*, 6(2), 133–140. <https://doi.org/10.3920/jiff2019.0034>
- Dzepe, D., Nana, P., Kuintche, H. M., Kuate, A. F., Tchuinkam, T., & Djouaka, R. (2020b). Role of pupation substrate on post-feeding development of black soldier fly larvae, *Hermetia illucens* (Diptera: Stratiomyidae). *Journal of Entomology and Zoology Studies*, 8(2), 760–764. <https://www.entomoljournal.com/archives/?year=2020&vol=8&issue=2&ArticleId=6520>
- Eriksson, T., & Picard, C. J. (2021). Genetic and genomic selection in insects as food and feed. *Journal of Insects as Food and Feed*, 7(5), 661–682. <https://doi.org/10.3920/jiff2020.0097>
- Giunti, G., Campolo, O., Laudani, F., & Palmeri, V. (2018). Male courtship behaviour and potential for female mate choice in the black soldier fly *Hermetia illucens* L. (Diptera: Stratiomyidae). *Entomologia Generalis*, 38(1), 29–46. <https://doi.org/10.1127/entomologia/2018/0657>

- Godoy, W. A. C., Fowler, H. G., von Zuben, C. J., Ziti, L., & Ribeiro, O. B. (1995). Larval dispersion in *Chrysomya megacephala*, *Chrysomya putoria* and *Cochliomyia macellaria* (Dipt., Calliphoridae). *Journal of Applied Entomology*, *119*(1–5), 263–266. <https://doi.org/10.1111/j.1439-0418.1995.tb01283.x>
- Gold, M., Binggeli, M., Kurt, F., de Wouters, T., Reichlin, M., Zurbrügg, C., Mathys, A., & Kreuzer, M. (2020). Novel experimental methods for the investigation of *Hermetia illucens* (Diptera: Stratiomyidae) larvae. *Journal of Insect Science*, *20*(3). <https://doi.org/10.1093/jisesa/ieaa057>
- Guilliet, J., Baudouin, G., Pollet, N., & Filée, J. (2021). The natural history of the black soldier fly, *Hermetia illucens*: Insights from complete mitochondrial genome sequences. *bioRxiv*, 2021.2010.2021.465311. <https://doi.org/10.1101/2021.10.21.465311>
- Heussler, C. D., Walter, A., Oberkofler, H., Insam, H., Arthofer, W., Schlick-Steiner, B. C., & Steiner, F. M. (2018). Influence of three artificial light sources on oviposition and half-life of the black soldier fly, *Hermetia illucens* (Diptera: Stratiomyidae): Improving small-scale indoor rearing. *PLOS ONE*, *13*(5), e0197896. <https://doi.org/10.1371/journal.pone.0197896>
- Hoc, B., Noël, G., Carpentier, J., Francis, F., & Caparros Megido, R. (2019). Optimization of black soldier fly (*Hermetia illucens*) artificial reproduction. *PLOS ONE*, *14*(4), e0216160. <https://doi.org/10.1371/journal.pone.0216160>
- Hodgson, P. J., Sivinski, J., Quintero, G., & Aluja, M. (1998). Depth of pupation and survival of fruit fly (*Anastrepha spp.*: Tephritidae) pupae in a range of agricultural habitats. *Environmental Entomology*, *27*(6), 1310–1314. <https://doi.org/10.1093/ee/27.6.1310>
- Holmes, L. A., Vanlaerhoven, S. L., & Tomberlin, J. K. (2012). Relative humidity effects on the life history of *Hermetia illucens* (Diptera: Stratiomyidae). *Environmental Entomology*, *41*(4), 971–978. <https://doi.org/10.1603/en12054>
- Holmes, L. A., Vanlaerhoven, S. L., & Tomberlin, J. K. (2013). Substrate effects on pupation and adult emergence of *Hermetia illucens* (Diptera: Stratiomyidae). *Environmental Entomology*, *42*(2), 370–374. <https://doi.org/10.1603/en12255>
- Jucker, C., Leonardi, M. G., Rigamonti, I., Lupi, D., & Savoldelli, S. (2020). Brewery's waste streams as a valuable substrate for black soldier fly *Hermetia illucens* (Diptera: Stratiomyidae). *Journal of Entomological and Acarological Research*, *51*(3). <https://doi.org/10.4081/jear.2019.8876>
- Julita, U., Fitri, L., Putra, R., & Permana, A. (2020). Mating success and reproductive behavior of black soldier fly *Hermetia illucens* L. (Diptera, Stratiomyidae) in tropics. *Journal of Entomology*, *17*(3), 117–127. <https://doi.org/10.3923/je.2020.117.127>
- Khan, M. M., Fan, Z., O'Neill Rothenberg, D., Peng, J., Hafeez, M., Chen, X., Pan, H., Wu, J., & Qiu, B. (2021). Phototoxicity of ultraviolet-A against the whitefly *Bemisia tabaci* and its compatibility with an entomopathogenic fungus and whitefly parasitoid. *Oxidative Medicine and Cellular Longevity*, *2021*, 2060288. <https://doi.org/10.1155/2021/2060288>

- Klüber, P., Bakonyi, D., Zorn, H., & Rühl, M. (2020). Does light color temperature influence aspects of oviposition by the black soldier fly (Diptera: Stratiomyidae)? *Journal of Economic Entomology*, *113*(5), 2549–2552. <https://doi.org/10.1093/jee/toaa182>
- Lalander, C., Diener, S., Zurbrügg, C., & Vinnerås, B. (2019). Effects of feedstock on larval development and process efficiency in waste treatment with black soldier fly (*Hermetia illucens*). *Journal of Cleaner Production*, *208*, 211–219. <https://doi.org/10.1016/j.jclepro.2018.10.017>
- Meng, J., Zhang, C., Zhu, F., Wang, X., & Lei, C. (2009). Ultraviolet light-induced oxidative stress: Effects on antioxidant response of *Helicoverpa armigera* adults. *Journal of Insect Physiology*, *55*(6), 588–592. <https://doi.org/10.1016/j.jinsphys.2009.03.003>
- Myers, H. M., Tomberlin, J. K., Lambert, B. D., & Kattes, D. (2008). Development of black soldier fly (Diptera: Stratiomyidae) larvae fed dairy manure. *Environmental Entomology*, *37*(1), 11–15. <https://doi.org/10.1093/ee/37.1.11>
- Okuyama, T. (2019). Density-dependent distribution of parasitism risk among underground hosts. *Bulletin of Entomological Research*, *109*(4), 528–533. <https://doi.org/10.1017/S0007485318000871>
- Oonincx, D. G. A. B., van Broekhoven, S., van Huis, A., & van Loon, J. J. A. (2015). Feed conversion, survival and development, and composition of four insect species on diets composed of food by-products. *PLOS ONE*, *10*(12), e0144601. <https://doi.org/10.1371/journal.pone.0144601>
- Oonincx, D. G. A. B., Volk, N., Diehl, J. J. E., van Loon, J. J. A., & Belušič, G. (2016). Photoreceptor spectral sensitivity of the compound eyes of black soldier fly (*Hermetia illucens*) informing the design of LED-based illumination to enhance indoor reproduction. *Journal of Insect Physiology*, *95*, 133–139. <https://doi.org/10.1016/j.jinsphys.2016.10.006>
- Palma, L., Ceballos, S. J., Johnson, P. C., Niemeier, D., Pitesky, M., & VanderGheynst, J. S. (2018). Cultivation of black soldier fly larvae on almond byproducts: Impacts of aeration and moisture on larvae growth and composition. *Journal of the Science of Food and Agriculture*, *98*(15), 5893–5900. <https://doi.org/10.1002/jsfa.9252>
- Pang, W., Hou, D., Chen, J., Nowar, E. E., Li, Z., Hu, R., Tomberlin, J. K., Yu, Z., Li, Q., & Wang, S. (2020). Reducing greenhouse gas emissions and enhancing carbon and nitrogen conversion in food wastes by the black soldier fly. *Journal of Environmental Management*, *260*, 110066. <https://doi.org/10.1016/j.jenvman.2020.110066>
- Peng, L., Zou, M., Ren, N., Xie, M., Vasseur, L., Yang, Y., He, W., Yang, G., Gurr, G. M., Hou, Y., You, S., & You, M. (2015). Generation-based life table analysis reveals manifold effects of inbreeding on the population fitness in *Plutella xylostella*. *Scientific Reports*, *5*(1), 12749. <https://doi.org/10.1038/srep12749>
- Popa, R., & Green, T. R. (2012). Using black soldier fly larvae for processing organic leachates. *Journal of Economic Entomology*, *105*(2), 374–378. <https://doi.org/10.1603/ec11192>

- Portilla, M., Morales-Ramos, J. A., Rojas, M. G., & Blanco, C. A. (2014). Life tables as tools of evaluation and quality control for arthropod mass production. In J. A. Morales-Ramos, M. G. Rojas, & D. I. Shapiro-Ilan (Eds.), *Mass Production of Beneficial Organisms* (pp. 241–275). Academic Press.
- Rhode, C., Badenhorst, R., Hull, K. L., Greenwood, M. P., Bester-van der Merwe, A. E., Andere, A. A., Picard, C. J., & Richards, C. (2020). Genetic and phenotypic consequences of early domestication in black soldier flies (*Hermetia illucens*). *Animal Genetics*, *51*(5), 752–762. <https://doi.org/10.1111/age.12961>
- Scala, A., Cammack, J. A., Salvia, R., Scieuzo, C., Franco, A., Bufo, S. A., Tomberlin, J. K., & Falabella, P. (2020). Rearing substrate impacts growth and macronutrient composition of *Hermetia illucens* (L.) (Diptera: Stratiomyidae) larvae produced at an industrial scale. *Scientific Reports*, *10*(1), 19448. <https://doi.org/10.1038/s41598-020-76571-8>
- Schneider, J. C. (2020). Effects of light intensity on mating of the black soldier fly (*Hermetia illucens*, Diptera: Stratiomyidae). *Journal of Insects as Food and Feed*, *6*(2), 111–119. <https://doi.org/10.3920/jiff2019.0003>
- Sheppard, C. D., Newton, L. G., Thompson, S. A., & Savage, S. (1994). A value added manure management system using the black soldier fly. *Bioresource Technology*, *50*(3), 275–279. [https://doi.org/10.1016/0960-8524\(94\)90102-3](https://doi.org/10.1016/0960-8524(94)90102-3)
- Sinha, R. P., & Häder, D. P. (2002). UV-induced DNA damage and repair: A review. *Photochemical & Photobiological Sciences*, *1*(4), 225–236. <https://doi.org/10.1039/B201230H>
- St-Hilaire, S., Cranfill, K., McGuire, M. A., Mosley, E. E., Tomberlin, J. K., Newton, L., Sealey, W., Sheppard, C., & Irving, S. (2007). Fish offal recycling by the black soldier fly produces a foodstuff high in omega-3 fatty acids. *Journal of the World Aquaculture Society*, *38*(2), 309–313. <https://doi.org/10.1111/j.1749-7345.2007.00101.x>
- Tariq, K., Noor, M., Saeed, S., & Zhang, H. (2015). The effect of ultraviolet-A radiation exposure on the reproductive ability, longevity, and development of the *Dialeurodes citri* (Homoptera: Aleyrodidae) F1 generation. *Environmental Entomology*, *44*(6), 1614–1618. <https://doi.org/10.1093/ee/nvv133>
- Tingle, F. C., Mitchell, E. R., & Copeland, W. W. (1975). The soldier fly, *Hermetia illucens* in poultry houses in north central Florida. *Journal of the Georgia Entomological Society*, *10*(2), 179–183.
- Tomberlin, J. K., & Sheppard, D. C. (2002). Factors influencing mating and oviposition of black soldier flies (Diptera: Stratiomyidae) in a colony. *Journal of Entomological Science*, *37*(4), 345–352. <https://doi.org/10.18474/0749-8004-37.4.345>
- United Nations. (2015). *Transforming our world: The 2030 agenda for sustainable development (A/Res/70/1) - Resolution adopted by the General Assembly on 25 September 2015*. United Nations. https://www.un.org/en/development/desa/population/migration/generalassembly/docs/globalcompact/A_RES_70_1_E.pdf
- Wang, H., Rehman, K. u., Liu, X., Yang, Q., Zheng, L., Li, W., Cai, M., Li, Q., Zhang, J., & Yu, Z. (2017). Insect biorefinery: A green approach for conversion of crop

- residues into biodiesel and protein. *Biotechnology for Biofuels*, 10(1), 304. <https://doi.org/10.1186/s13068-017-0986-7>
- Wen, Y., Qin, W., Chen, X., Wen, X., Ma, T., Dong, X., Lin, S., Sun, Z., Zeng, S., & Wang, C. (2017). Soil moisture effects on pupation behavior, physiology, and morphology of *Heortia vitessoides* (Lepidoptera: Crambidae). *Journal of Entomological Science*, 52(3), 229–238, 210. <https://doi.org/10.18474/JES16-12PT.1>
- White, T. E., Vogel-Ghibely, N., & Butterworth, N. J. (2020). Flies exploit predictable perspectives and backgrounds to enhance iridescent signal salience and mating success. *The American Naturalist*, 195(4), 733–742. <https://doi.org/10.1086/707584>
- Woods, M. J., Hoffman, L. C., & Pieterse, E. (2019). Artificial diets for neonatal black soldier fly (*Hermetia illucens*) larvae. *Journal of Insects as Food and Feed*, 5(2), 99–105. <https://doi.org/10.3920/jiff2018.0028>
- Zhang, C., Meng, J., Wang, X., Zhu, F., & Lei, C. (2011). Effects of UV-A exposures on longevity and reproduction in *Helicoverpa armigera*, and on the development of its F1 generation. *Insect Science*, 18(6), 697–702. <https://doi.org/10.1111/j.1744-7917.2010.01393.x>
- Zhang, J., Huang, L., He, J., Tomberlin, J. K., Li, J., Lei, C., Sun, M., Liu, Z., & Yu, Z. (2010). An artificial light source influences mating and oviposition of black soldier flies, *Hermetia illucens*. *Journal of Insect Science*, 10(1), 202. <https://doi.org/10.1673/031.010.20201>
- Zheng, L., Hou, Y., Li, W., Yang, S., Li, Q., & Yu, Z. (2012). Biodiesel production from rice straw and restaurant waste employing black soldier fly assisted by microbes. *Energy*, 47(1), 225–229. <https://doi.org/10.1016/j.energy.2012.09.006>
- Zhou, F., Tomberlin, J. K., Zheng, L., Yu, Z., & Zhang, J. (2013). Developmental and waste reduction plasticity of three black soldier fly strains (Diptera: Stratiomyidae) raised on different livestock manures. *Journal of Medical Entomology*, 50(6), 1224–1230. <https://doi.org/10.1603/me13021>

Appendices

Appendix 1: List of conference talks given during this study

- Liu, Z., Minor, M., Morel, P. C. H., Najar-Rodriguez, A. J. (2017). Using black soldier fly *Hermetia illucens* (Diptera: Stratiomyidae) for organic waste conversion. *The 66th Entomological Society of New Zealand Conference*, Wellington, New Zealand.
- Liu, Z., Minor, M., Morel, P. C. H., Najar-Rodriguez, A. J. (2018). Bio-conversion of three organic wastes by black soldier fly (*Hermetia illucens* L.) larvae in New Zealand. *The 2nd International Conference Insects to Feed the World*. Wuhan, China.

Appendix 2: Copyright agreements and licenses of the published chapters

Chapter 2

Journal: Environmental Entomology

DOI: 10.1093/ee/nvy141

Title: Bioconversion of Three Organic Wastes by Black Soldier Fly (Diptera: Stratiomyidae) Larvae

Standard Licence

You hereby grant to Oxford University Press an exclusive licence for the full period of copyright throughout the world:

- to publish the final version of the Article in the above Journal, and to distribute it and/or to communicate it to the public, either within the Journal, on its own, or with other related material throughout the world, in printed, electronic or any other format or medium whether now known or hereafter devised;
- to make translations and abstracts of the Article and to distribute them to the public;
- to authorize or grant licences to third parties to do any of the above;
- to deposit copies of the Article in online archives maintained by OUP or by third parties authorized by OUP.

You authorize us to act on your behalf to defend the copyright in the Article if anyone should infringe it and to register the copyright of the Article in the US and other countries, if necessary.

In the case of a multi authored article, you confirm that you are authorized by your co-authors to enter the licence on their behalf.

You confirm to OUP that the Article

- is your original work;
- has not previously been published (in print or electronic format), is not currently under consideration by another journal, or if it has already been submitted to other journal, it will be immediately withdrawn;
- will not be submitted for publication to any other journal following acceptance in the above Journal; and
- OUP will be the first publisher of the Article.

You warrant to OUP that

- no part of the Article is copied from any other work,
- you have obtained ALL the permissions required (for print and electronic use) for any material you have used from other copyrighted publications in the Article; and
- you have exercised reasonable care to ensure that the Article is accurate and does not contain anything which is libellous, or obscene, or infringes on anyone's copyright, right of privacy, or other rights.

Further Information

(Full details of OUP's publication rights policies, including author rights can be found at https://academic.oup.com/journals/pages/access_purchase/rights_and_permissions/publication_rights)

Author Self-Archiving Policy

On publication of your Article in the Journal you are not required to remove any previously posted ORIGINAL VERSIONS from your own personal website or that of your employer or free public servers of articles in your subject area, provided (1) you include a link (url) to the VERSION OF RECORD on the Journal's website; AND (2) the Journal is attributed as the original place of publication with the correct citation details given.

You may post the ACCEPTED MANUSCRIPT of the Article (but not the published version itself) onto your own website, your institution's website and in institutional or subject-based repositories, PROVIDED THAT it is not made publicly available until 12 MONTHS after the online date of publication, and that: (1) you include a link (url) to the VERSION OF RECORD on the Journal's website; (2) the Journal is attributed as the original place of publication with the correct citation details given.

Notes

Author's Original Version (AOV): an unrefereed manuscript version of the article, as submitted for review by a journal

Accepted Manuscript (AM): the final draft author manuscript, as accepted for publication by a journal, including modifications based on referees' suggestions but before it has undergone copyediting and proof correction

Version of Record (VOR): the final published version of the article.

Free Link to Published Article

On publication, authors will be sent an online offprint link allowing access to their article on the OUP website without subscription. For authors of non- Open Access articles, this link may be shared directly with interested colleagues, but is not intended for mass distribution on websites, repositories, or through social media. If you wish to share links or draw attention to your article we would ask that you instead distribute a link to the abstract of the article. Authors of Open Access articles are free to post and distribute their links anywhere immediately upon publication.

Educational Use

You may use the Article within your employer's institution or company for educational or research purposes only, including use in course-packs, as long as: (1) you do not use it for commercial purposes or re-distribution outside of the institution/company; (2) you acknowledge the Journal as the original place of publication with the correct citation details given.

zhy.liu@hotmail.com signed this licence on 2018-09-02 06:11:43 GMT.



Copyright Transfer Agreement – Journal of Insects as Food and Feed

The copyright to:

Manuscript title: substrate and moisture content effects on pupation of the black soldier
Manuscript ID: JIFF2022.0057
Authors: Zhongyi Liu, Patrick C.H. Morel, and Maria A. Minor

is hereby transferred to Wageningen Academic Publishers, publisher of *Journal of Insects as Food and Feed*, (respective to owner if other than Wageningen Academic Publishers and for U.S. government employees: to the extent transferable) effective if and when the article is accepted for publication. If the submitted manuscript is not accepted by *Journal of Insects as Food and Feed*, all rights will be returned to the author(s) of the work.

The author warrants that his/her contribution to *Journal of Insects as Food and Feed* is original and has not been published before (except in the form of an abstract or as part of a published lecture, review or thesis); that it is not under consideration for publication elsewhere; and that its publication has been approved by all co-authors, if any, as well as tacitly or explicitly by the responsible authorities at the institution where the work was carried out. The author affirms that the article contains no unfounded or unlawful statements and does not violate the rights of others. If copyrighted works are included, the author bears responsibility to obtain written permission from the copyright owners and to appropriately credit the sources.

The copyright to this article is transferred to Wageningen Academic Publishers. The author warrants that he/she has full power to make this grant. The author(s) retain all proprietary rights, other than copyright, such as patent and trademark rights. The author signs for and accepts responsibility for releasing this material on behalf of any and all co-authors. The copyright transfer covers the exclusive and unlimited rights to reproduce and distribute the article in any form of reproduction (reprints, photographic reproductions, printing, electronic media (offline, online) or any other form), as well as the translation rights for all languages and countries, for the full term (including extensions and renewals). No material published in *Journal of Insects as Food and Feed* may be reproduced without first obtaining written permission from the publisher.

The author may send or transmit individual copies of the final published version of their manuscript in any format, including the publisher's PDF version, to colleagues upon their specific request provided no fee is charged, and further-provided that there is no systematic distribution of the manuscript, e.g. posting on a listserve, website or automated delivery. However posting the article on a secure network, not accessible to the public, is permitted. For other purposes, e.g. publication on his/her own website, the author must use an author-created version of his/her article, i.e. the original submitted file prior to review. He/she may not use the publisher's PDF version, with the exception if their manuscript is published under 'Your Choice for Open Access' ([Creative Commons Attribution CC BY license](#)). Furthermore, the author may only post his/her version provided acknowledgement is given to the original source of publication and a link (DOI) is inserted to the published article on the *Journal of Insects as Food and Feed* website. The link must be accompanied by the following text: 'The original publication is available at <insert website link here>'.

I choose to publish my article under 'Your Choice for Open Access' (Creative Commons Attribution CC BY Licence) and pay the one-off fee of 1400 Euro (ex. VAT) to the publisher.¹

Name Zhongyi Liu

Date August 27th, 2022

(Corresponding) author's signature:

¹ Authors from 'low income' and 'lower middle income' countries can apply for a reduced fee (www.wageningenacademic.com/jiff).

Chapter 4

ELSEVIER

Publishing Agreement

Elsevier B.V.

Mating success of the black soldier fly, *Hermetia illucens* (Diptera: Stratiomyidae), under four artificial light sources

Corresponding author	Mr. Zhongyi Liu
E-mail address	z.liu1@massey.ac.nz
Journal	Journal of Photochemistry & Photobiology, B: Biology
Article number	111815
Our reference	JPB_111815
P II	S1011-1344(19)30780-8
DOI	10.1016/j.jphotobiol.2020.111815

Your Status

- I am one author signing on behalf of all co-authors of the manuscript
- I am signing on behalf of the corresponding author.
 - **Name/Job title/Company:** Zhongyi Liu
 - **E-mail address:** z.liu1@massey.ac.nz

Assignment of Copyright

I hereby assign to Elsevier B.V. the copyright in the manuscript identified above (where Crown Copyright is asserted, authors agree to grant an exclusive publishing and distribution license) and any tables, illustrations or other material submitted for publication as part of the manuscript (the "Article"). This assignment of rights means that I have granted to Elsevier B.V., the exclusive right to publish and reproduce the Article, or any part of the Article, in print, electronic and all other media (whether now known or later developed), in any form, in all languages, throughout the world, for the full term of copyright, and the right to license others to do the same, effective when the Article is accepted for publication. This includes the right to enforce the rights granted hereunder against third parties.

Supplemental Materials

"Supplemental Materials" shall mean materials published as a supplemental part of the Article, including but not limited to graphical, illustrative, video and audio material.

With respect to any Supplemental Materials that I submit, Elsevier B.V. shall have a perpetual worldwide, non-exclusive right and license to publish, extract, reformat, adapt, build upon, index, redistribute, link to and otherwise use all or any part of the Supplemental Materials in all forms and media (whether now known or later developed), and to permit others to do so.

Research Data

"Research Data" shall mean the result of observations or experimentation that validate research findings and that are published separate to the Article, which can include but are not limited to raw data, processed data, software, algorithms, protocols, and methods.

With respect to any Research Data that I wish to make accessible on a site or through a service of Elsevier B.V., Elsevier B.V. shall have a perpetual worldwide, non-exclusive right and license to publish, extract, reformat, adapt, build upon, index, redistribute, link to and otherwise use all or any part of the Research Data in all forms and media (whether now known or later developed) and to permit others to do so. Where I have selected a specific end user license under which the Research Data is to

be made available on a site or through a service, the publisher shall apply that end user license to the Research Data on that site or service.

Reversion of rights

Articles may sometimes be accepted for publication but later rejected in the publication process, even in some cases after public posting in "Articles in Press" form, in which case all rights will revert to the author (see <https://www.elsevier.com/about/our-business/policies/article-withdrawal>).

Revisions and Addenda

I understand that no revisions, additional terms or addenda to this Journal Publishing Agreement can be accepted without Elsevier B.V.'s express written consent. I understand that this Journal Publishing Agreement supersedes any previous agreements I have entered into with Elsevier B.V. in relation to the Article from the date hereof.

Author Rights for Scholarly Purposes

I understand that I retain or am hereby granted (without the need to obtain further permission) the Author Rights (see description below), and that no rights in patents, trademarks or other intellectual property rights are transferred to Elsevier B.V.

The Author Rights include the right to use the **Preprint**, **Accepted Manuscript** and the **Published Journal Article for Personal Use and Internal Institutional Use**. They also include the right to use these different versions of the Article for **Scholarly Sharing** purposes, which include sharing:

- the Preprint on any website or repository at any time;
- the Accepted Manuscript on certain websites and usually after an embargo period;
- the Published Journal Article only privately on certain websites, unless otherwise agreed by Elsevier B.V.

In the case of the Accepted Manuscript and the Published Journal Article the Author Rights exclude Commercial Use (unless expressly agreed in writing by Elsevier B.V.), other than use by the author in a subsequent compilation of the author's works or to extend the Article to book length form or re-use by the author of portions or excerpts in other works (with full acknowledgment of the original publication of the Article).

Author Representations / Ethics and Disclosure / Sanctions

I affirm the Author Representations noted below, and confirm that I have reviewed and complied with the relevant Instructions to Authors, Ethics in Publishing policy, Declarations of Interest disclosure and information for authors from countries affected by sanctions (Iran, Cuba, Sudan, Burma, Syria, or Crimea). Please note that some journals may require that all co-authors sign and submit Declarations of Interest disclosure forms. I am also aware of the publisher's policies with respect to retractions and withdrawal (<https://www.elsevier.com/about/our-business/policies/article-withdrawal>).

For further information see the publishing ethics page at <https://www.elsevier.com/about/our-business/policies/publishing-ethics> and the journal home page. For further information on sanctions, see <https://www.elsevier.com/about/our-business/policies/trade-sanctions>

Author representations

- The Article I have submitted to the journal for review is original, has been written by the stated authors and has not been previously published.
- The Article was not submitted for review to another journal while under review by this journal and will not be submitted to any other journal.
- The Article and the Supplemental Materials do not infringe any copyright, violate any other intellectual property, privacy or other rights of any person or entity, or contain any libellous or other unlawful matter.
- I have obtained written permission from copyright owners for any excerpts from copyrighted works that are included and have credited the sources in the Article or the Supplemental Materials.
- Except as expressly set out in this Journal Publishing Agreement, the Article is not subject to any prior rights or licenses and, if my or any of my co-authors' institution has a policy that might restrict my ability to grant the rights required by this Journal Publishing Agreement (taking into account the Author Rights permitted hereunder, including Internal Institutional Use), a written waiver of that policy has been obtained.

- If I and/or any of my co-authors reside in Iran, Cuba, Sudan, Burma, Syria, or Crimea, the Article has been prepared in a personal, academic or research capacity and not as an official representative or otherwise on behalf of the relevant government or institution.
- If I am using any personal details or images of patients, research subjects or other individuals, I have obtained all consents required by applicable law and complied with the publisher's policies relating to the use of such images or personal information. See <https://www.elsevier.com/about/our-business/policies/patient-consent> for further information.
- Any software contained in the Supplemental Materials is free from viruses, contaminants or worms.
- If the Article or any of the Supplemental Materials were prepared jointly with other authors, I have informed the co-author(s) of the terms of this Journal Publishing Agreement and that I am signing on their behalf as their agent, and I am authorized to do so.

Governing Law and Jurisdiction

This Agreement will be governed by and construed in accordance with the laws of the country or state of Elsevier B.V. ("the Governing State"), without regard to conflict of law principles, and the parties irrevocably consent to the exclusive jurisdiction of the courts of the Governing State.

For information on the publisher's copyright and access policies, please see <http://www.elsevier.com/copyright>.
For more information about the definitions relating to this agreement click [here](#).

I have read and agree to the terms of the Journal Publishing Agreement.

10 February 2020

T-copyright-v22/2017



Journal: Journal of Economic Entomology

DOI: 10.1093/jee/toab225

Title: Reproduction of Black Soldier Fly (Diptera: Stratiomyidae) under Different Adult Densities and Light Regimes

Standard Licence

You hereby grant to Oxford University Press an exclusive licence for the full period of copyright throughout the world:

- to publish the final version of the Article in the above Journal, and to distribute it and/or to communicate it to the public, either within the Journal, on its own, or with other related material throughout the world, in printed, electronic or any other format or medium whether now known or hereafter devised;
- to make translations and abstracts of the Article and to distribute them to the public;
- to authorize or grant licences to third parties to do any of the above;
- to deposit copies of the Article in online archives maintained by OUP or by third parties authorized by OUP

You authorize us to act on your behalf to defend the copyright in the Article if anyone should infringe it and to register the copyright of the Article in the US and other countries, if necessary. In the case of a multi authored article, you confirm that you are authorized by your co-authors to enter the licence on their behalf.

You confirm to OUP that the Article:

- is your original work;
- has not previously been published (in print or electronic format), is not currently under consideration by another journal, or if it has already been submitted to other journal, it will be immediately withdrawn;
- will not be submitted for publication to any other journal following acceptance in the above Journal; and
- will be first published by OUP

You warrant to OUP that:

- no part of the Article is copied from any other work,
- you have obtained ALL the permissions required (for print and electronic use) for any material you have used from other copyrighted publications in the Article; and
- you have exercised reasonable care to ensure that the Article is accurate and does not contain anything which is libellous, or obscene, or infringes on anyone's copyright, right of privacy, or other rights

RETAINED RIGHTS

Abstract and Citation information - Authors may reuse the Abstract and Citation information (e.g. Title, Author name, Publication dates) of their article anywhere at any time including social media such as Facebook, blogs and Twitter, providing that where possible a link is included back to the article on the OUP site. Preferably the link should be, or include, the Digital Object Identifier (DOI) which can be found in the Citation information about your article online.

Author's Original Version - Authors may reuse the Author's Original Version (AOV) anywhere at any time, providing that once the article is accepted they provide a statement of acknowledgement, and that once the article has been published this acknowledgement is updated to provide details such as the volume and issue number, the DOI, and a link to the published article on the journal's website.

Accepted Manuscript - On first online publication authors may immediately upload their Accepted Manuscript (AM) to their own personal webpage, or their institutional repository on the proviso that it is not made publically available until **12 months** after the online date of publication. After the embargo period, authors may make their AM publicly available through their institutional repository or other non-commercial repositories PROVIDED THAT (1) you include a link (URL) to the VERSION OF RECORD on the journal's website; and (2) the Journal is attributed as the original place of publication with the correct citation details given. AM may not be uploaded to commercial websites or repositories, unless the website or repository has signed a licensing agreement with OUP.

Version of Record - The Version of Record (VOR) as it appears in the journal following copyediting and proof correction may not be deposited by authors in institutional repositories or posted to third party websites unless the repository or website has signed an agreement with OUP allowing posting.

Free link to published article - On publication, authors will be sent an online offprint link allowing access to their article on the OUP website without subscription. This link may be shared directly with interested colleagues, but is not intended for mass distribution through, repositories, or social media. If you wish to share links or publicize your article we would ask that you instead distribute a link to the abstract of the article.

Funding bodies - Please be aware that you are responsible for all funding agency compliance and the accuracy of information you provide in relation to your article. OUP and/or the controlling Learned Society shall not be responsible for checking that funding agency requirements have been complied with.

Educational use - You may use the Article within your employer's institution or company for educational or research purposes only, including use in course-packs, as long as: (1) you do not use it for commercial purposes or re-distribution outside of the institution/company; (2) you acknowledge the Journal as the original place of publication with the correct citation details given.

Further information - details of OUP's publication rights policies, including author rights can be found at https://academic.oup.com/journals/pages/access_purchase/rights_and_permissions/publication_rights

zhy.liu@hotmail.com signed this licence on 2021-11-04 00:58:38 GMT.