BACTERIAL INFLUENCES ON THE NUTRITIONAL ECOLOGY OF *LUCILIA SERICATA* (DIPTERA: CALLIPHORIDAE)

A Thesis

by

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ABSTRACT

Nutritional ecology provides a novel framework linking biotic factors, such as life-history traits of an animal, to abiotic factors, such as nutrients. Carrion serves as a nutrient-rich and ephemeral environment attracting a host of consumers ranging from microbes to mammals. These diversified consumers accommodate complex ecological relationships. My study primary focuses on bacteria as related to the nutritional ecology of the blow fly, *Lucilia sericata* (Diptera: Calliphoridae). Four objectives were investigated: 1) to develop a proper sterile diet for examining the bacterial dimension of nutritional ecology framework as related to *L. sericata* development. 2) to measure the impact of protein: lipid on the life-history traits of *L. sericata*. 3) to quantify the alternation of life-history traits of *L. sericata* due to the interactions of exogenous bacteria (Proteus mirabilis and Salmonella) and diet (protein: lipid ratios). 4) investigate the impact of these interactions on associated bacterial communities.

I compared the life-history traits of *L. sericata* developing on six sterilized diets. Overall, the liver-based diets (decomposed and powdered) resulted in similar *L. sericata* development as those reared on the fresh beef liver. Larvae reared on blood agar resulted in a significantly (increased 20.56% ± 8.09%) greater pupation rates than those raised on the decomposed and powdered beef liver diets. Pupae from larvae fed the fresh beef liver were significantly larger (6.27 ± 1.01 mg, 4.05 ± 0.94 mg greater, respectively) than those reared on the blood agar diet, decomposed beef liver, and powdered beef liver diets. However, none of these diets were appropriate for my following objectives,

because the macronutrients are not adjustable. Therefore, a modified herbivore insect diet was used for the remaining objectives.

Through studies with the sterile herbivore diet, I demonstrated increasing protein: lipid ratio leads to an acceleration of larval development as well as a greater survivorship of *L. sericata*. Moreover, the introduction of exogenous bacteria altered the responses of *L. sericata* to the dietary impacts, like the extended duration of each developmental stage. I hypothesized that the alternative in the life-history trait of *L. sericata* could cause the accumulation of the protein in the diet treatments and the cross-talk with the exogenous bacterial treatment.

Furthermore, to examine the cross-talk with the exogenous bacteria and *L. sericata*, the 16s rRNA gene amplicon analysis was employed for investigating the bacterial community of L. sericata. According to the result, the diet treatment was unable to alter the bacterial community of *L. sericata*. Regardless of diet impacts on the development of *L. sericata*, *L. sericata* was successfully regulated itself and surrounding bacterial community. However, the regulation mechanism was disrupted by introducing the exogenous bacteria. I also determined the exogenous bacterial treatment did not impact the bacterial community of *L. sericata* at later development stages, including the pupal and adult. There has yet to be a study investigating the bacterial dimension of nutritional ecology in the carrion system. Therefore, my study contributes to an understudied area of the carrion ecology.

DEDICATION

This thesis is dedicated to Mom and Dad. I love you both.

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First, I would like to acknowledge my mom, dad, and my fiancée. I never could have accomplished so much throughout my life without your support. Thank you all for everything.

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Dr. Jeff Tomberlin contributed to direct the goals of the research as well as the supporting of the experiment.

Dr. Tawni Crippen contributed to the experiment design as well as the supporting of the experiment.

Dr. Aaron Tarone contributed the advice and suggestion of the experiment design as well as the partial preliminary experiment.

Dr. Spencer Behmer provided the diet formula in the 3rd chapter as well as the partial preliminary experiment.

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CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

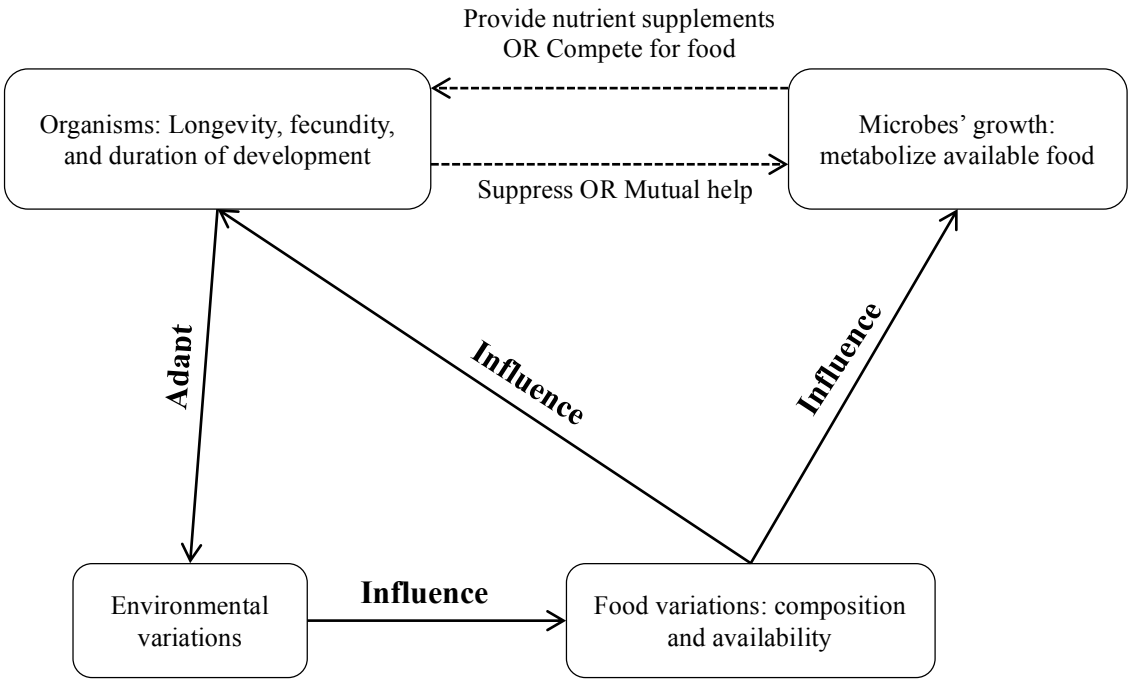
Nutritional ecology fundamentally integrates a broad spectrum of sciences into a framework allowing researchers to investigate the roles that nutrients play in mediating various responses of a consumer ranging from its behavior to morphology (Raubenheimer and Boggs 2009). Furthermore, on a basic level, nutritional ecology explains ecological phenomena allowing for a greater understanding of how organisms adapt (evolve) to shifts in their environment (Parker et al 2009, Raubenheimer et al 2009). Such results are often applied in areas such as conservation and wildlife management (Parker et al 2009, Raubenheimer et al 2009).

Most nutritional ecology research in entomology has focused on herbivores (in Figure 1). For example, Boggs (2009), using the *Drosophila melanogaster* (Diptera: Drosophilidae) model, summarized a framework to understand the relationship of nutrient acquisition and allocation with foraging and life-history traits in insects under benign and stressful environments. This area of research has expanded to include other trophic levels in order to explain ecological phenomena beyond the herbivore and the plant it consumes. Douglas (2009) indicated that microbial symbionts widely distributed in herbivorous insects enhance subsequent plant utilization.

Little is known about the nutritional ecology associated with the vertebrate carrion system. Previous works has explored the growth rates of carrion-associated fly

larvae on different organs or tissues (Clark et al 2006, Kaneshrajah and Turner 2004) For instance, the larvae of *Calliphora vicina* R. -D. (Diptera: Calliphoridae) developed two days faster on pig lung, kidney, heart or brain than on pig liver (Kaneshrajah and Turner 2004). *Lucilia sericata* Meigen (Diptera: Calliphoridae) also grew faster and produced larger adults when cultured on pig rather than cow tissue, and when cultured on lung and heart in comparison with liver (Clark et al 2006). These data imply that variation in development experienced by these arthropods could be due to nutritional differences between the tissue types provided as the larval resource. For example, the nutrient composition varies across tissue types. Pig lung contains 0.00% carbohydrates and 14.08% proteins, while pig liver contains 2.47% carbohydrates and 21.39% proteins (information from U. S. Department of Agriculture Nutrients Database http://ndb.nal.usda.gov). However, this research focused on the interactions between tissue type and development rate of the flies (strictly forensic application) but failed to discuss the nutritional component and its relevance to the data produced (ecological significance).

Figure 1 | Concept of Organisms-Microbes interaction based on food variations



Vertebrate carrion is an ephemeral and nutrient-rich resource. It plays a critical role in its surrounding ecological system by introducing nutrient-rich materials, which influence the larger scale community structure and biodiversity. For example, in forest ecosystems, herbaceous species usually dominate the landscape. They act as ecological filters reducing the number of seeds from trees from reaching the forest floor and germinating (Bump et al 2009, Coomes et al 2005, George and Bazzaz 1999a, George and Bazzaz 1999b, Gilliam 2007, M Dearden and A Wardle 2008). However, the introduction of vertebrate carrion into an ecosystem creates a nutrients-rich patch and further attracts and aggregates the arthropod community of the forest. For example, an estimated 522 arthropod species feed directly on the baby pig (*Sus scrofa Linnaeus*) carrions source or indirectly on organisms feeding on the carrion (Payne 1965). The

microbial biomass of soil beneath a rat carcass increased by 400% (Carter et al 2008).

The framework governing research within the vertebrate carrion system usually involves three trophic levels. These levels are the scavenger-large animals, consumers-arthropods, and decomposer-microorganisms. In some terrestrial ecosystem, the vertebrate scavengers are the secondary consumer, arriving just after arthropods (Wilson and Wolkovich 2011). Although scavengers, such as raccoons and vultures, feeding on carrion accelerates the degradation process, they usually serve as unpredictable opportunists, unlike arthropods and bacteria that heavily rely on vertebrate carrion for survival (Campobasso et al 2001, DeVault et al 2004).

Arthropods play a dominant role in the degradation of vertebrate carrion in terrestrial habitats. The succession of arthropods on carrion has been well studied (Benecke and Leclercq 1999, Mégnin 1894, Motter 1898, Payne 1965, Sorg and Haglund 1996). Two arthropod orders, Diptera and Coleoptera, are the dominate decomposers of carrion, while other orders, such as Lepidoptera, Hymenoptera, are secondary consumers and tend to be generalists (Campobasso et al 2001).

Many species associated with carrion are temporally driven (i.e., activity on carrion dependent on the level of its degradation). Their occurrence on carrion is fairly predictable with regards to the amount of time to pass after death of the animal and the arrival and utilization of the resource by a particular arthropod. Diptera, particularly larvae of Calliphoridae and Sarcophagidae, usually inhabit carrion during the initial period following the death of an animal, when large quantities of soft tissue are present. *Lucilia sericata* Meigen (Diptera: Calliphoridae) can locate and colonize a fresh exposed

carrion within 2-3 hours after its death (Campobasso et al 2001) while *Cochliomyia macellaria* Fabricius (Diptera: Calliphoridae) is even quicker and detected an accessible carrion in 10 minutes (Payne 1965). Coleoptera, including Silphidae and Dermestidae, are colonizers of carrion in the later stages of decomposition when less soft tissue is on the carrion (Campobasso et al 2001).

Microbes play an essential part in the putrefaction of carrion. They are known to initiate this process through the rendering of complex organic macromolecules, such as proteins, carbohydrates, and lipids into micromolecules, such as amino acids, water, fatty acids, while emitting gases and volatiles (Coe 1993, Spitz and Spitz 2006). Although autolysis of individual cells can affect the process of putrefaction, exogenous and endogenous microbes are more efficient at degrading the carcass than just autolysis (Macchiarelli and Feola 1995). Exogenous microbial factors are mainly aerobic, including mostly airborne bacteria such as *Staphylococcus* species. Endogenous microbes are anaerobic bacteria and are typically located in the carrion's intestine (Spitz and Spitz 2006). Gases and volatiles, including ammonia, hydrogen sulphide, methane, nitrogen, lactic acid, and phenol, are formed and released as a part of microbial destruction of organic matters. These volatiles can attract arthropods to carrion and serve as an oviposition cue (Campobasso et al 2001, Ma et al 2012). Bacteria can also elevate temperatures of microclimates in and on carrion and assist in attracting more insects to oviposit on carrion (Nuorteva 1959, Rodriguez and Bass 1985). In addition, bacteria help larvae to suppress detrimental fungi on the larval food or the bacteria serve as an oviposition cue to affect behavior of gravid females, such as *Klebsiella oxytoca* with

Musca domestica (Diptera: Muscidae) (Lam et al 2007, Lam et al 2009b).

An increasing number of studies indicate that an animal's microbiota significantly influences their host range (Adams and Douglas 1997, Bäckhed et al 2007, Douglas 1998, Douglas 2009, Peterson et al 2009, Raubenheimer and Boggs 2009, Stappenbeck et al 2002, Turnbaugh et al 2006, Turnbaugh et al 2007). For example, aphids (Homoptera: Aphidae) usually feed on phloem sap, which is known to have a low concentration of essential amino acids. Aphids therefore rely on endosymbiotic gut bacteria to provide those required amino acids (Adams and Douglas 1997, Douglas 1998). Germ-free mice also required higher calorie diet compared to mice with microbiota (Bäckhed et al 2007, Stappenbeck et al 2002). In addition, gut microbiota of humans is an important factor that contributes human obesity (Turnbaugh et al 2006). Thus, research and exploration of the nutritional ecology of microbial roles in carrion-associated flies are indeed and necessary.

Research interests

Microbes and arthropods dominate the consumption of carrion. This ephemeral resource impacts the surrounding environment by providing nutrients aiding in the proliferation of microbes and invertebrates. There is a wealth of research focused on insect succession on carrion and how environmental variables affect community and species level succession patterns (Greenberg 1991, Payne 1965, Tomberlin et al 2011). However, the nature of the relationship and interactions between Diptera and bacteria are still poorly understood (Tomberlin et al 2017). Outside of research examining the anti-bacterial capabilities of some Diptera (Davydov 2011, Parnés and Lagan 2007, Sherman

et al 2000), little else is known.

We now know that volatile organic compounds associated with a *Proteus mirabilis* (a commensal of *L. sericata*) behavior, which is quorum sensing regulated, serve as a mechanism governing fly attraction and colonization. This suggests that the fly is able to "listen" to bacterial decision-making using the signaling information to make decisions regarding the use of a resource (Ma et al 2012). However, it is not known if this response is density dependent. Bacteria at select densities might be favorable for blow fly larval development while other densities might be detrimental. For example, oviposition-inducing strains of bacteria were isolated from the surface of *M. domestica* L. (Diptera: Muscidae) eggs, and those bacteria at 5×10^4-10^5 cfu/mL (Colony-forming unit per milliliter) larval diet resulted in greater survivorship of *M. domestica* larvae than those bacteria at 10^2-10^5 CFU (Lam et al 2009a).

Bacterial roles with blow fly larvae could also shift depending on nutrients present within the resource. As with aphids, these bacteria could provide essential nutrients absent or in low quantity in the larval resource. Nutritional contributions can have varied effects, including increased survival on suboptimal diets, better digestive efficiency, provision of vitamins, and additional digestive enzymes, like aphid's endosymbiotic gut bacteria provide aphid essential amino acids when feed on low nutrients plants (Adams and Douglas 1997, Dillon and Dillon 2004, Douglas 1998). Another effect of symbiotic insect-microbe interactions could be improved resistance of the insect host to pathogens (Dillon and Charnley 2002). Desert locust *Schistocerca gregaria* Forsskål (Orthoptera: acrididae) are known to have increased pathogen

resistance due to gut bacteria to form the colonization resisitance (Dillon and Charnley 2002). One possible mechanism for this is a commonly gut bacteria *Pantoea agglomerans* with locusts resulted in antimicrobial agents production to prevent germination and infection of fungal entomopathogens (Dillon and Charnley 2002).

As R. J. Dillon and V. M. Dillon said (2004), "Insect feeding on plants or animals must first negotiate the extensive microbiota on the surface of the host and any metabolic products released by that community. Conversely, the host is also exposed to microbial products released from the insect feces and from the regurgitant of the insect." They suggest there is an interaction loop between microbes and insects based on carrion nutrients beyond attraction and repletion. Therefore, the goal of this work is try to understand the nutritional facts behind insect behavior reaction interacting with microbes, which explain the phenomenon of bacterial fly attraction. The objectives of this research are described in the following paragraphs.

Focus 1: formulation of artificial diet

Goal. Develop a larval diet for testing interaction between *L. sericata* and different bacteria.

Expected results. The development of a sterile diet that will suit for larvae of *L. sericata* development as well as select microbes growth.

Rationale. The goal for this objective is to develop an artificial diet for *L. sericata,* which will serve as a model organism. This fly species was selected as a model for three reasons. First, *L. sericata* is cosmopolitan and they are known to be primary colonizers of vertebrate carrion (Campobasso et al 2001). Second, there is ample

literature related to the antimicrobial nature of *L. sericata*. Moreover, some of this work has generated valuable insight by culturing both bacteria and *L. sericata* concurrently (Barnes and Gennard 2011). Third, the current established colony in lab that has been used in the past to generate a transcriptome as well as conduct research examining microbe-insect interactions (Ma et al 2012, Sze et al 2012, Tomberlin et al 2011).

The artificial diet can be easily modified allowing for shifts in protein and lipids allowing for a geometric framework with regards to nutrition to be developed as described Simpson and Raubenheimer (2012). Though the classical nutrition framework diets are altered by shifting proteins and carbohydrates ratio such as exemplified in Simpson and Raubenheimer's framework model, this diet will be altered by changing protein: lipid ratio. Lipids have been chosen instead of carbohydrates for the following reasons: First, Simpson and Raubenheimer's framework model was developed for insect herbivores, but *L. sericata* is a carrion associated species. Furthermore, *L. sericata* can complete its life cycle on beef and pork lung, neither of which contains carbohydrates (Clark et al 2006). Second, the lipids content of *L. sericata* changes remarkably during its immature development (Yuill and Craig 1937).

Experiment design. Diet development is based upon quantified measures of the nutrient content of raw beef liver (information from USDA http://ndb.nal.usda.gov). The diet medium will include the following components: proteins, lipids, minerals, and vitamins. Important characteristics of the diet include: 1) that it be autoclavable; 2) that the formulation of artificial diet includes raw beef liver; 3) that it be possible to alter the ratio of proteins to lipids by adding supplements, which is to build a framework of

nutrition (Simpson and Raubenheimer 2012). The framework in here will include lipids concentration as Y-axis while proteins concentration as X-axis, and this coordinate frame presents different proteins: lipids ratio diets to further compare *L. sericata* performances. And 4) the contents of minerals and vitamins be maintainable at fixed level, identical to that of raw beef liver, by adding different stock solutions (Dr. J Tomberlin lab unpublished data). A chemically defined diet medium that is equivalent to raw beef liver in protein: lipid ratio and vitamin and mineral content will the control diet.

The effects of the diet on bacteria will be measured only by quantifying bacterial growth. One bacterium will be tested, *Proteus mirabilis*. *P. mirabilis* has been demonstrated to produce a strong attraction response in *L. sericata* adults when exhibiting the quorum-sensing behavior of swarming. Furthermore, this bacterium is found inside the salivary gland of 3rd instar *L. sericata* larvae (Ma et al 2012).

Focus 2: effect of altered protein: lipid ratio on *Lucilia sericata* growth and development.

Null hypothesis: shifting the protein: lipid ratio of the diet will have no effect on the life table of *L. sericata*. The life table of *L. sericata* includes duration of larval stage, pupae stage, longevity, and fecundity.

Alternative hypothesis: shifting the protein: lipid ratio of the diet will affect *L. sericata*. The growth of bacteria is based on its growth curve, which its generation time may extend or shorten.

Expected results. Two suboptimal diets for *L. sericata* have been developed, including one of high protein content with low lipids content, and one of low proteins

content with high lipids content.

Rationale. The purpose of this objective is to create a nutrient shortage stress on *L. sericata* by adjusting ratio of proteins: lipids for further bacteria-*L. sericata* interaction testing. According to the Simpson and Raubenheimer's framework, the optimal diet achieves an intake target which is defined as the point of nutrient intake that is most beneficial for the animal fitness (Sherman and Tran 1995). Due to the wide use of raw beef liver for *L. sericata* feed, it assumes that the raw beef liver indicates optimal fitness and performances of *L. sericata*. Moreover, if the intake target cannot be achieved, *L. sericata* will compromise its intake target as close as possible, for example, eating less diet or prolonging its development duration.

Experiment design. The ratio of proteins: lipids of shifting diet will be 5:1, 1:1, and 1:2 at 30% total macronutrient concentration. These ratios base on beef tissue nutrient value. For example, the ratio 5:1 represents beef liver, ratio 1:1 represents beef brain, and ratio 1:2 represents beef thymus. Life table of *L. sericata* will be examined independently on these alternative suboptimal diets.

Focus 3: effect of nutritional background on the interaction of exogenous bacteria and *L. sericata*

Null hypothesis: There is no interaction of *P. mirabilis* or *Salmonella* with *L. sericata* on optimal or suboptimal diets.

Alternative hypothesis: At least one bacterium has interaction with *L. sericata* on optimal or suboptimal diets.

Expected results. 1) Concurrent culturing of *P. mirabilis* or *Salmonella* with *L.*

sericata will result in significant differences in larval development when compared with mono-feeding of *L. sericata* on the diets; 2) *L. sericata* positive or negative influences *P. mirabilis* or *Salmonella* growth; 3) The effect of culturing the bacteria and *L. sericata* will describe the ecological relationship, be it inter-kingdom competition, mutualism, or commensalism.

Rationale. There are two variables, which will be tested and determined in this objective. The first is to determine effective concentrations of *P. mirabilis* or *Salmonella* that are able to induce interaction with *L. sericata*. The interaction in here indicates at certain concentrations of *P. mirabilis* or *Salmonella* coating on the diet may change *L. sericata*'s life table significantly. Second, different artificial diets-the optimal diet, high protein/low lipid suboptimal diet, and low protein/high lipids suboptimal diet will also be tested. Different types of artificial diets could contribute to differentiating the performance of *L. sericata* when co-cultured with bacteria, including the optimal diet and suboptimal diets. Different effective concentrations of bacteria added to these diets may impact *L. sericata* development. One potential observation is that the bacteria (*P. mirabilis* or *Salmonella*) may act as a competitor for nutrient uptake and have a negative impact on *L. sericata* on the optimal diet such as significant change on its life table. However, on the suboptimal diets, the bacteria and *L. sericata* become mutually beneficial as the bacteria assist *L. sericata* to consume the over-dose proteins or lipids to help *L. sericata* to reach its intake target. Therefore, it is vital to identify the combination of factors, which mediate these insect-microbe interactions, including effective concentrations of bacteria, artificial diets of different nutrients concentrations.

Experiment design. The interaction between *L. sericata* and *P. mirabilis* or *Salmonella* will be tested in this objective. The life table of *L. sericata* and growth curve of *P. mirabilis* will record by rising *L. sericata* with *P. mirabilis* concurrently on different diet, including optimal diet and suboptimal diets.

Research significance and future application

This research will be the first to quantify the interaction between bacteria and *L. sericata*. A low-cost and achievable artificial diet for *L. sericata* also will be provided. As described, *L. sericata* is a species of forensic and medical importance. In forensic science, this work will help us to increase the accuracy of minimum postmortem interval (Tomberlin et al 2011) estimations by allowing us to include a microbe-flies interaction factor instead of data from each group developed in isolation. Further, in medical science, larvae of *L. sericata* appear to be the most suitable species for maggot therapy. This quantified interaction model will assist researchers studying antimicrobial treasure of *L. sericata*.

CHAPTER II

EVALUATION OF STERILIZED ARTIFICIAL DIETS FOR MASS-REARING THE GREEN BOTTLE FLY, *LUCILIA SERICATA* (DIPTERA: CALLIPHORIDAE)

Introduction

Maggot debridement therapy (MDT) can be used to treat chronically infected wounds. This practice was well-established in Western medicine during World War I (Baer 1931); however, it was abandoned due to an increase in effective antibiotic use following the discovery of penicillin (Davydov 2011, Sherman et al 2000). Recently however, due to increases in antibiotic resistance in microbes, MDT has experienced a resurgence due its ability to kill such microbes. In 2004, the Federal Drug Administration approved MDT as a medical device in the United States for treating necrotic wounds and ulcers (Andersen et al 2010). MDT has even been demonstrated to be effective against one of the most challenging multidrug-resistant organisms to treat because of its prevalence to be easily spread within the health care-associated settings, methicillin-resistant *Staphylococcus aureus* (MRSA) (Jarvis et al 2012, Parnés and Lagan 2007). The primarily species used for MDT are from the blow fly family (Diptera: Calliphoridae. Examples include *Calliphora vicina* (Robineau-Desvoidy) (Teich and Myers 1986), *Lucilia caesar* (Linnaeus) (McLellan 1932), *Lucilia cuprina* (Wiedemann) (Fine and Alexander 1934), *Lucilia illustris* (Meigen) (Leclercq 1990), *Phormia regina* (Meigen) (Baer 1931), and *Lucilia sericata* (Meigen) (Baer 1931). *L. sericata* is the most

commonly used to reduce necrotic tissue and infection in chronic wounds (Weil et al 1933).

Some bacteria can be eliminated through larval ingestion and by the production of antimicrobials in the excretion/secretions (ES) of indigenous bacteria, such as *P. mirabilis* (Greenberg 1968, Jaklič et al 2008, Lerch et al 2004, Mumcuoglu et al 2001, Sherman et al 2000). Presently, research on MDT has focused on the isolation and identification of those antimicrobial factors. Thomas et al. (1999) indicated ES from *L. sericata* larvae is more effective at killing Gram-positive bacteria (i.e., *Pseudomonas* sp. and *Staphylococcus aureus*), but less effective in eliminating Gram-negative bacteria (i.e. *Escherichia coli* and *Proteus* sp.). Initially three sizes of bioactive molecules (<0.5 kDa, 0.5-10 and >10 kDa) were identified from the ES of larval *L. sericata* (Bexfield et al 2004, Bexfield et al 2008, Huberman et al 2007). Then Cerovský et al. (2010) successfully isolated Lucifensin® from the larvae, which expressed activity against wound bacteria, even MRSA infections.

In order to continue further exploration of the interactions between pathogenic bacteria and *L. sericata* larvae, a sterile rearing substrate is needed to determine if the antibacterial effect is directly from the larvae or from bacteria residing in and on the larvae. It is not known if variation in the diversity of microbes present during rearing of the larvae could impact the efficacy of the MDT treatment. Despite the enormous number of publications on MDT antimicrobial component isolation, the sterility of the larvae themselves and their rearing substrates are often not validated (Barlow and Kollberg 1971, Daeschlein et al 2006, Daniels et al 1991, Lerch et al 2004, Sherman and

Tran 1995, Tachibana and Numata 2001). Combined with a lack of information about the composition of the diets makes study comparisons difficult (Andersen et al 2010, Barnes and Gennard 2013, Daeschlein et al 2006, Jaklič et al 2008, Kerridge et al 2005, Mumcuoglu et al 2001).

The objective of this research was to evaluate six artificial sterile diets for sustainable production of *L. sericata*. We also explored the impact of larval density on development and survivorship on the diets, as previous studies indicate larval density is a critical factor for insect survivorship (Dos Reis et al 1999, Turner and Howard 1992). Such information could provide a standardized method for future MDT studies.

Materials and methods

Fly source and eggs collection. A *L. sericata* colony initiated from specimens collected from Davis, CA, USA, in 2006 (Tarone and Foran 2008) was used. The colony was maintained in a BudDorm® (DP1000, 30 × 30 ×30 cm, MegaViewScience, Taichung, Taiwan, China) cage at 27˚C, 70%, 14:10 L:D. For each experiment, eggs were collected by placing ~30 g fresh beef liver inside a 50 mL beaker in the cage for 3 h. Eggs were transferred aseptically to a sterile petri dish covered with Kimwipes® (Kimberly-Clark Corp., Irving, TX, USA) soaked with deionized water and maintained in a Percival® I-36VL growth chamber set at 27˚C, 70% RH, 14:10 L:D. Eggs were monitored hourly for hatch after the initial 8 h. Resulting larvae were used in the subsequent experiments.

Dietary treatments. Six dietary treatments were tested against a fresh beef liver, our standard larval colony maintenance diet (Tarone and Foran 2008). These diets were;

1) a blood agar, 2) a decomposed beef liver, 3) a powdered beef liver, 4) a powdered fish, 5) a milk-based, and 6) a chemically-defined diet (see Tables 1 and 2 for detailed formulas). The powdered beef liver diet and powdered fish diet were included as they were nutritionally similar to beef liver, a common material used to raise blow flies (Tarone and Foran 2008), and are commercially available. Fresh beef liver was obtained from Rosenthal Meat Science and Technology Center, Texas A&M University (College Station, TX, USA) served as the control for comparisons. The powdered beef liver diet was prepared from freeze-dried beef liver (Simply Nourish™, Phoenix, AZ, USA). The formulation of the decomposed beef liver diet followed the description from Sherman and Tran (2008). The powdered fish diet was made from dehydrated fish powder (CarlPool products, Gladewater, TX, USA). The formulation of the blood agar diet followed that described by Daniels et al. (1991). The formulation of the milk-based diet followed that described by Tachibana and Numata (2001). The formulation of the chemically-defined diet followed that described by Barlow and Kollberg (1971). Except for the fresh beef liver control, all other diets were autoclaved for sterilization at 121˚C, 20 min. After being autoclaved, each diet was distributed into 33 mL sterilized medical cups and lids (Dart® Container Corp., Mason, MI, USA), which contained 20 g of each assigned diet. All the dietary treatments were stored at 4˚C until use, except for the fresh beef liver, which was stored at -20˚C.

Experiment design and data collection. Three densities of larvae on the dietary treatments were tested in two trials; each consisting of five technical replicates. Density treatments included 10, 20 and 40 larvae/20 g diet; equaling 2, 1 and 0.5 g diet per larva.

Newly hatched first instars were transferred to replicate cups covered with a breathable lid via a 12 mm diameter hole punched in the center. A sterilized cotton ball was inserted into the hole and served as a means to regulate ventilation. During larval development, deionized, sterile water was provided *ad libitum* at 12 h intervals by soaking the cotton ball. Once the post-feeding stage was observed for a given replicate, it was transferred into a 946 mL Mason jar (Ball®, Daleville, IN, USA), which was covered with a double layer Wypall-wipes (Kimberly-Clark Corp., Irving, TX, USA) held in place with a rubber band. These replicates were observed at 24 h interval for larval pupation. All pupae were collected and the percent pupation per replicate and individual pupal weight was recorded. Individual pupae were transferred into a new medical cup with 10 g 1:1 sugar-sand mix (Quikrete® Play Sand; Great Value® Pure Cane Sugar) in order to provide a carbohydrate resource and pupation substrate. Such an approach allowed for adult longevity to be based on fat reserves acquired during larval development. Pupae in cups were observed at 24 h intervals for adult emergence and resulting longevity.

Table 1 | Diet composition of Fresh beef liver, Powdered beef liver diet, Decomposed beef liver diet, Powdered fish diet, Blood agar diet, and Milk based diet (per 100 g diet) used for rearing *Lucilia sericata* at different densities on different diets at 27˚C, 70%, 14:10 L:D

Ingredients	**Control**	**Diets**				
	Fresh beef liver	Powdered beef liver	Decomposed beef liver	Powdered fish	Blood agar	Milk based
Beef Liver (g)						
Fresh	100					
Powdered		30.77				
Decomposed			100			
Fishmeal (g)						
Powdered				32.25		
Horse Blood (mL)						
Defibrinated					23.81	
Whole Milk (g)						
Powdered						5
Blood agar base (g)					3.64	
Brewer's yeast (g)					4.57	5
Wheat germ (g)						5
Agar-agar (g)		1.18	2.7	1.18		2
Deionized water (mL)		100	100	100	91.43	100

Table 2 | Chemical defined diet composition (milligram per 100 g diet) used for rearing *Lucilia sericata* at different densities on different diets at 27˚C, 70% RH, 14:10 L:D

Amino acids		Salts		Lipids	
Glycine	270.00	$CaCl_2$	11.00	Cholesterol	125.00
L-Alanine	257.00	$CoCl_2*6H_2O$	1.70	Linoleic acid	75.00
L-Arginine	211.00	$CuSO_4*5H_2O$	2.00	Oleic acid	240.00
L-Aspartic acid	7.60	$FeCl_3$	3.90	Palmitic acid	110.00
L-Cysteine HCl	71.00	$K_2HPO_4*3H_2O$	241.60	Stearic acid	50.00
L-Glutamic acid	27.30	KOH	576.80	α-Linolenic acid	25.00
L-Histidine	120.00	$MgSO_4$	23.18	Others	
L-Isoleucine	304.00	$Na2HPO_4$	33.84	Agar	1500.00
L-Leucine	55.90	$ZnCl_2$	2.50	D-Glucose	1250.00
L-Lysine HCl	252.00	Vitamins		Ribonucleic acid	188.00
L-Methionine	188.00	Biotin	7.00		
L-Phenylalanine	279.00	Ca pantothenate	113.00		
L-Proline	399.00	Choline Chloride	523.00		
L-Serine	351.00	Folic acid	89.00		
L-Threonine	188.00	*myo*-Inositol	1490.00		
L-Tryptophane	94.00	Nicotinic acid	81.00		
L-Tyrosine	8.20	Pyridoxine HCl	225.00		
L-Valine	328.00	Riboflavin	41.00		
trans-4-Hydroxyproline	96.00	Thiamine HCl	33.00		
γ-Aminobutyric acid	2.26				

Statistical analysis. Since no larvae survived on the powdered fish diet, milk-based diet, and chemical-defined diet, they were excluded from the analysis. Initially, multivariate linear regressions were performed for multivariate model independent variables backward selection with Pillai's trace test from Multivariate Analysis of Variance (MANOVA) by car package (v2.0-25) in R (v3.2.1). Results from the MANOVA indicated no significant effects on larval density treatments, as well as no significant differences between biological replicates. Therefore, these biological replicates were combined for further analysis. Hence, Canonical Discriminant Analysis (CDA) was applied for subsequent analysis. CDA defined pupal weight, pupation percentage, eclosion percentage, and adult lifespan as dependent matrices, and diet treatments as independent matrices. These analyses were conduced using candisc package (v0.6-7), and plotted using ggplot2 package (v1.0.1) in R (v3.2.1). Additionally, Mahalanobis squared distance, D_M^2, associated with F test between diet treatments, was calculated using SAS (University Edition 3.3, SAS Institute, Inc., Cary, NC) CANDISC procedure with distance option, which measured and compared all pairwise distances between the centroids of diet treatments for clustering. The dependent variables with largest influences in CDA were picked for post hoc analysis through pairwise t-test with Bonferrnoi correction by lsmeans package (v2.18) in R (v3.2.1).

Results

Multivariate model selection. Pupal weight, pupation percentage, eclosion percentage, and adult lifespan were used in the model (Table 3). While larval density had a marginally significant influence on pupal weight, pupation percentage, eclosion

percentage, and adult lifespan ($F_{8, 78} = 1.877$, $P = 0.076$,) compared to diet effects, the backward multivariate model selection indicated diet ($F_{12, 126} = 3.547$, $P < 0.001$) as the only significant factor in the model (Table 4). Using pupal weight, pupation percentage, eclosion percentage, and adult lifespan, univariate regressions were performed for further confirmation of larval density effects. While the results were consistent for three of the four variables, eclosion percentage was significantly ($F_{2, 44} = 3.280$, $P = 0.047$) impacted by larval density. In order to explore the biological impacts of larval density on eclosion percentage, pairwise t-tests have performed. However, pairwise t-tests did not indicate any significant differences. A density of 40 larvae/20 g eclosed more larvae than a density of 10 larvae/20 g, but was only marginally significant (mean difference = -17.19 ± 7.3%, with $t_{44} = -2.353$, $P = 0.069$).

Diet treatment overall effects. Diet significantly ($F_{12, 126} = 3.547$, $P = 0.0001$) impacted pupal weight, pupation percentage, and eclosion percentage. Based on CDA, diet treatments divided into three discreet clusters: Cluster 1=Fresh beef liver; Cluster 2=Decomposed beef liver diet and Powdered beef liver diet; Cluster 3=Blood agar diet (Figure 2). Table 5 provides the specific dependent parameters contributing to the discrimination between clusters. Both canonical axes were significantly correlated with original dependent variables, and the 1st and 2nd canonical axes explained 93.43% variance of the data. The clustering between diets was further confirmed with the Mahalanobis distance criterion, D_M^2, in Table 6. Pupal weight, pupation percentage, and eclosion percentage were selected for downstream post hoc analysis, because of their higher weight in explaining the variances (underlined in Table 5).

Diet treatment effects on pupal weight. Mean pupal weight by treatment distinguished Cluster 1 from Clusters 2 and 3 (Figure 3). Mean pupal weight from Cluster 1 was 6.268 ± 1.008 mg more than Cluster 2 ($P < 0.001$, $t_{44} = 6.216$), and 4.049 ± 0.940 mg more than those in Cluster 3 ($P < 0.001$, $t_{44} = 4.309$). However, pupal weight associated with Cluster 2 was marginally significantly ($P = 0.068$, $t_{44} = -2.361$) less (-2.219 ± 0.940 mg) than those associated with Cluster 3.

Table 3 | Mean ± SE (n = 10) of different life-history traits of *Lucilia sericata* reared at different densities on different diets at 27˚C, 70% RH, 14:10 L:D

Diet Treatment	Density Treatment (larvae per 20 g diet)	Pupal weight (mg ± SE)	% Pupation (% ± SE)	% Eclosion (% ± SE)	Lifespan (day ± SE)
Fresh beef liver	10	23.03 ± 1.34	69.55 ± 5.40	64.98 ± 5.76	7.32 ± 0.89
Fresh beef liver	20	23.33 ± 1.33	61.52 ± 5.75	62.75 ± 5.90	6.89 ± 0.48
Fresh beef liver	40	23.99 ± 1.12	66.30 ± 6.00	77.10 ± 3.21	7.20 ± 0.41
Blood agar diet	10	21.60 ± 1.34	57.08 ± 6.02	58.59 ± 7.86	6.70 ± 0.73
Blood agar diet	20	20.98 ± 1.96	64.78 ± 6.96	58.65 ± 8.32	6.19 ± 0.64
Blood agar diet	40	20.57 ± 1.65	69.89 ± 6.98	65.47 ± 8.43	5.92 ± 0.29
Decomposed beef liver diet	10	22.34 ± 1.76	55.56 ± 7.87	74.91 ± 7.70	6.06 ± 0.72
Decomposed beef liver diet	20	21.89 ± 1.39	62.50 ± 5.18	69.13 ± 7.35	6.10 ± 0.59
Decomposed beef liver diet	40	21.46 ± 1.13	77.80 ± 3.68	76.19 ± 4.59	6.66 ± 0.25
Powdered beef liver diet	10	23.23 ± 2.70	42.00 ± 7.93	44.90 ± 15.51	6.21 ± 1.50
Powdered beef liver diet	20	23.15 ± 1.72	42.95 ± 5.21	62.50 ± 7.44	6.15 ± 0.64
Powdered beef liver diet	40	23.99 ± 1.68	60.83 ± 6.10	66.55 ± 5.59	7.43 ± 0.65

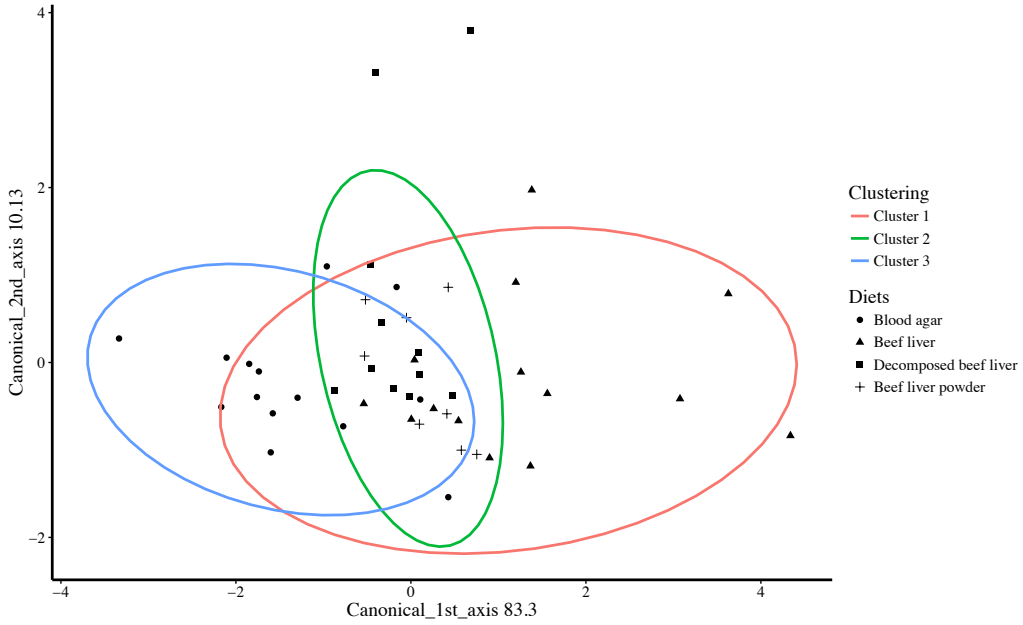
Table 4 | Model parameters table for model selection for determining critical factors for different life-history traits of *Lucilia sericata* reared at different densities on different diets at 27˚C, 70% RH, 14:10 L:D

Models	Explanatory variables
Full model	(Intercept) + (Diet treatment) * (Density treatment) * (Trial)
Reduced model 1	(Intercept) + (Diet treatment) * (Density treatment)
Reduced model 2	(Intercept) + (Diet treatment) + (Density treatment)
Treatment only model	(Intercept) + (Diet treatment)
Naïve model	(Intercept)

Models	Reduced model 1	Reduced model 2	Treatment only model §	Naïve model
Full model	$F_{32, 108} = 0.995$; $P = 0.487$	$F_{56, 108} = 0.951$; $P = 0.576$	$F_{64, 108} = 1.002$; $P = 0.489$	$F_{76, 108} = 1.458$; $P = 0.036$
Reduced model 1		$F_{24, 140} = 0.864$; $P = 0.649$	$F_{32, 140} = 1.098$; $P = 0.345$	$F_{44, 140} = 1.727$; $P = 0.009$
Reduced model 2			$F_{8, 78} = 1.877$; $P = 0.076$	$F_{20, 164} = 2.839$; $P < 0.001$
Treatment only model				$F_{12, 126} = 3.547$; $P < 0.001$

All models response variables were: Pupal weight, Pupation percentage, Eclosion percentage, Adult lifespan.
The interaction terms in each model included all lower order interactions.
§ Final model being selected for further canonical discriminant analysis

Figure 2 | Canonical discriminant analysis plot of diet treatments used for *Lucilia sericata* reared on different diets at 27˚C, 70% RH, 14:10 L:D



Canonical discriminant analysis plot showing the four diet treatments separated into three discreet clusters: Cluster 1 = Fresh beef liver (As red dot label, with 95% confident ellipse); Cluster 2 = Decomposed beef liver diet and Powdered beef liver diet (As green triangle label, with 95% confident ellipse); Cluster 3 = Blood agar diet (As blue square label, with 95% confident ellipse) with the shifts between the clusters along Canonical 1st axis.

Table 5 | Standardized canonical coefficients for different life-history traits of *Lucilia sericata* reared at different densities on different diets at 27˚C, 70% RH, 14:10 L:D

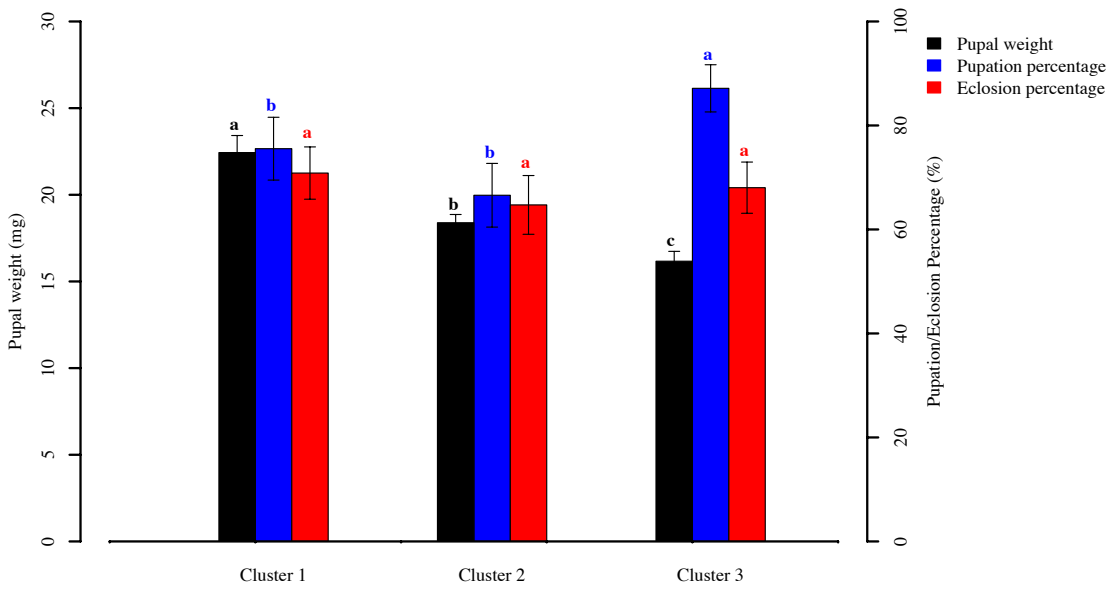
Parameters	Canonical Variables	
	1	2
Pupal weight (mg)	1.078	-0.209
Pupation percentage (%)	-0.402	-0.860
Eclosion percentage (%)	-0.285	0.531
Adult lifespan (d)	-0.118	-0.164
Canonical correlation	0.738	0.356
Approximate F value	$F_{9, 99} = 5.726$	$F_{4, 84} = 2.510$
P level of significance	<0.001	0.048
Variance accounted for (%)	83.300	10.130

The parameters with the largest influence for with significant canonical variety are underlined

Table 6 | Clustering based on Mahalanobis's Distance (squared distance, DM) for summary of different life-history traits of *Lucilia sericata* reared on different diets at 27˚C, 70% RH, 14:10 L:D

Diet Treatments		Blood agar diet	Decomposed beef liver diet	Powdered beef liver diet	Clustering
Fresh beef liver	D_M^2	7.254	2.829	2.745	
	$F_{4, 41}$	11.829	4.060	3.503	**Cluster 1**
	P-value	<0.001	0.007	0.015	
Decomposed beef liver diet	D_M^2			1.348	
	$F_{4, 41}$			1.555	
	P-value			0.205	**Cluster 2**
Powdered beef liver diet	D_M^2			0.000	
	$F_{4, 41}$			0.000	
	P-value			1.000	
Blood agar diet	D_M^2		2.360	2.994	
	$F_{4, 41}$		3.386	3.820	**Cluster 3**
	P-value		0.018	0.010	

Figure 3| Mean values for pupal weight, pupation percentage, and eclosion percentage for *Lucilia sericata* reared on different diets at 27˚C, 70% RH, 14:10 L:D



The mean value of pupal weight (mg) from Cluster 1 is significantly greater than Cluster 2 ($t_{44} = 4.309$, $P < 0.001$) as well as Cluster 3 ($t_{44} = 6.216$, $P < 0.001$). And the mean value of pupation percentage from Cluster 3 is significantly higher than Cluster 2 ($t_{44} = 2.542$, $P = 0.044$)

Discussion

Our results indicate decomposed liver, powdered liver, and blood-based diets are suitable sterilized diets for rearing and maintaining *L. sericata* in colony. These three diets have similar nutritional values to fresh beef liver (20.36% protein, 3.63% lipid, and 3.89% carbohydrate). Moreover, the habitat for larval *L. sericata* is vertebrate carrion, which probably explains why larvae did so well on the previously mentioned diets (Smith and Wall 1997).

However, results for some diets in our study differed from those previously published. For example, a milk-based diet was suggested to produce 81% survivorship to adult stage (Tachibana and Numata 2001), and a chemically defined diet to produce 68% survivorship to pupal or 3rd instar stages (Barlow and Kollberg 1971). In our study, those two diet treatments did not support good larval survivorship. The heat sterilization processes may have contributed to reductions in survivorship due to nutrient structural modification or loss of individual nutritive components. For example, autoclaving for 20 min at 15 PSI to sterilized diets can significantly reduced (22.56%) free amino acids in powdered infant formula (Cohen 2015, McCollum and Davis 1915, Yeung et al 2006), and such nutrient loss may have led to the survivorship difference in our study. Moreover, regarding the powdered fishmeal diet, the higher lipid content (9.08% dry weight in fishmeal verses 3.63% in beef liver) in fish may have affected survivability by *L. sericata* (U.S. Department of Agriculture Nutrients Database http://ndb.nal.usda.gov). For instance, increased dietary fat content resulted in reduced development time of the 3rd instar in the blow fly, *Chrysomya megacephala* (Fabricius) (Diptera: Calliphoridae), (Li et al 2014). Li et al. (2014) increased dietary fat from 0% to 80%, which resulted in a shorter development time by 21 h, but increased larval mortality from 5.5% to 58.3%.

Pupal weight is a critical factor implicated in determining the survivorship of insects (Davidowitz et al 2003). Pupal weight of *L. sericata* in this research was useful in differentiating the impact of diets on development with Cluster 1 (fresh beef liver) diet producing the largest pupae, Cluster 3 (blood agar diet) producing the smallest pupae, and Cluster 2 (decomposed beef liver diet and powdered beef liver diet) producing

intermediate sized pupae. Previous research with other species demonstrated the importance of pupal weight as a measure of colony fitness. For example, the primary screwworm *Cochliomyia hominivorax* (Conquerel) (Diptera: Calliphoridae) pupated successfully only 3.9% of the time when the larval weight ranged between 21–25 mg, but had 100% successful pupation when larvae weighed 56–60 mg (Hightower et al 1972).

The nutritional composition of the diet interacting with indigenous microbial populations in the larval gut may contribute to the variation of the traits (pupal weight, pupation percentage, and eclosion percentage) and could explain some of the differences observed in the percentage of successful pupation (see Table 1 and 2; U.S. Department of Agriculture Nutrients Database http://ndb.nal.usda.gov). Moreover, these microbial populations brought by the larvae that can subsequently colonize the different diets could also affect larval development and survivorship. Microbes can produce toxins or convert food into unpalatable resources for other competitors (i.e., blow fly larvae), and hence the nutrient composition is shifted (Janzen 1977). For example, *Proteus mirabilis*, an opportunistic pathogen, produces "mirabilicide" antimicrobials suspected to suppress Gram-positive and Gram-negative bacteria that are harmful to blow fly larvae (Greenberg 1968). Alternatively, some microbes are required for *L. sericata* survival; for instance, the same *Proteus mirabilis* can synthetize methionine, which is beneficial for metamorphism (Grabow and Smit 1967, Levenbook and Dinamarca 1966). However, an acidic environment can suppress *P. mirabilis* growth (Garrity et al 2006), and the decomposed beef liver diet is an acidic (pH=3) environment.

Although this research indicated there are not significant density effects, this may be due to the conservative statistical methods utilized for analysis and the limited sample size (Picheny et al 2010). But there is a trend of better and more stable survivorship of *L. sericata* at the highest larval density (40/g diet). Larval aggregation is beneficial for predigesting food sources by larvae as it concentrates larval secretions, proteolytic enzymes and metabolic heat, and thus the efficiency of their feeding behavior (Dos Reis et al 1999, Turner and Howard 1992). Therefore, the highest larval density diet treatment allowed for larval aggregation, and increased survivorship through eclosion. Whereas at low densities, the overproduction of larval secretions required to process the same food source may be too costly for the few larvae present and thus diminish the individual's chances of reaching an adult stage. This would explain the lower eclosion percentage associated with lower density rearing. Unfortunately, many maggot secretion studies focus on antimicrobial action, thus the relationship of larval secretions to larval density and the success of the individual life cycle remains understudied.

Each diet had advantages and drawbacks with regards to preparation and utilization. A decomposed liver diet can challenge the olfactory senses due to its foul odor, and preparation of the diet is time consuming (requires 5 d to age), but the material is widely available at a low cost. In contrast, the blood-based diet is more suitable choice for laboratory research since it can be prepared within 3 h, but the blood needs to be preserved in a freezer. Thus, the powdered liver diet could be a better choice since the freeze-dried liver can stored at ambient temperatures for longer periods than blood or fresh liver; however, the availability of the freeze-drying equipment for preprocessing

could be a limiting factor.

In future studies, interactions between bacteria and larvae on sterile diets should be taken into consideration. As previously indicated, some bacteria, such as *P. mirabilis*, could be essential for successful larval development and such information could prove critical for MDT, especially if there is a true commensal relationship. The data generated from this study in conjunction with methods for sterilizing fly eggs developed by Brundage et al. (2016) could lead to advancements in the sterile process for fly production subsequent to use in MDT.

CHAPTER III

BACTERIAL INFLUENCES ON THE NUTRITIONAL ECOLOGY OF *LUCILIA SERICATA* (DIPTERA: CALLIPHORIDAE)

Introduction

Nutritional ecology fundamentally integrates a broad spectrum of sciences into a framework allowing researchers to investigate the roles that nutrients play in mediating various responses of a consumer ranging from its behavior to physiology (Raubenheimer and Boggs 2009). Nutritional ecology also explains ecological phenomena allowing for a greater understanding of how organisms adapt (evolve) to shifts in their environment (Parker et al 2009, Raubenheimer et al 2009). Insects as the most diversified group, their diets are remarkable diversified, such as nectar feeders, blood feeders, sarcophagus feeders, and etc (Triplehorn et al 2005). Under the nutritional ecology framework, the diet alone is effectively impacted the development of insects. For example, the imbalance of amino acid drives the dietary restriction effects in terms of the trade-off between lifespan and fecundity of *Drosophila* (Grandison et al 2009). However, in order to adapt to the diversified diets, bacterial dimension of nutritional ecology plays an important role for nutrients acquisition of insects (Douglas 2009). For instance, aphids (Homoptera: Aphidae) usually feed on phloem sap, which is known to have a low concentration of essential amino acids. Aphids therefore rely on endosymbiotic gut bacteria to provide those required amino acids (Adams and Douglas 1997, Douglas

1998). Moreover, vertebrate carrion is an ephemeral and nutrient-rich resource, and attract a host of consumers (Benbow et al 2015). For example, an estimated 522 arthropod species feed directly on the baby pig (*Sus scrofa Linnaeus*) carrions source or indirectly on organisms feeding on the carrion (Payne 1965). The microbial biomass of soil beneath a rat carcass increased by 400% (Carter et al. 2008). Thus, the vertebrate carrion system may provide an 'arena' for a host of interactions between arthropods and bacteria.

However, little is known about the nutritional ecology associated with the vertebrate carrion system. Previous works has explored the growth rates of carrion-associated fly larvae on different organs or tissues with various moisture effects (Bernhardt et al 2016, Clark et al 2006, Kaneshrajah and Turner 2004, Tarone and Foran 2006). For instance, the larvae of *Calliphora vicina* R. -D. (Diptera: Calliphoridae) developed two days faster on pig lung, kidney, heart or brain than on pig liver (Kaneshrajah and Turner 2004). *Lucilia sericata* Meigen (Diptera: Calliphoridae) also grew faster and produced larger adults when cultured on pig rather than cow tissue, and when cultured on lung and heart in comparison with liver (Clark et al 2006). These data imply that variation in development experienced by these arthropods could be due to nutritional differences between the tissue types provided as the larval resource. For example, the nutrient composition varies across tissue types. Pig lung contains 0.00% carbohydrates and 14.08% proteins, while pig liver contains 2.47% carbohydrates and 21.39% proteins (information from U. S. Department of Agriculture Nutrients Database http://ndb.nal.usda.gov). However, this research focused on the interactions between

tissue type and development rate of the flies (strictly forensic application) but failed to discuss the nutritional component and its relevance to the data produced (ecological significance).

We now know that volatile organic compounds associated with a *Proteus mirabilis* (a commensal bacterium of *L. sericata*) behavior, which is quorum sensing regulated, serve as a mechanism governing fly attraction and colonization (Ma et al 2012). Subsequent research hypothesized flies were tapping into 'public information' of the bacteria as a means to determine quality of the potential larval resource (Liu et al 2016). Such interactions are beneficial to the bacteria as they could be dispersed by the fly to new resources (Nayduch and Burrus 2017). Moreover, a selected bacterium at a given concentration might be favorable for blow fly larval development while other species and concentration might be detrimental. For example, oviposition-inducing strains of bacteria were isolated from the surface of *Musca domestica* L. (Diptera: Muscidae) eggs, and those bacteria at 5×104-105 cfu/mL (colony-forming unit per milliliter) larval diet resulted in greater survivorship of *M. domestica* larvae than those bacteria at 102-105 cfu/mL (Lam et al 2009a).

In order to investigate the effects of bacterial dimension of nutritional ecology in vertebrate carrion system, we selected *L. sericata* as a model organism to accomplish the following goals: (1) the nutritional effects on the development of *L. sericata*; (2) the nutritional impacts on the bacterial community of *L. sericata*; (3) the perturbation (i.e., introduced an exogenous bacteria) of nutritional effects on the development and bacterial community of *L. sericata*.

Material and method

Fly source and eggs collection. A *L. sericata* colony initiated from specimens collected from Davis, CA, USA, in 2006 (Tarone and Foran 2006) was used. The colony was maintained in a BudDorm® (DP1000, 30 × 30 ×30 cm, MegaViewScience, Taichung, Taiwan, China) cage at 27˚C, 70% RH, 14:10 L: D. For each experiment, eggs were collected by placing ~30 g fresh beef liver inside a 50 mL glass beaker in the cage for 3 h. Eggs were transferred aseptically to a sterile petri dish covered with Kimwipes® (Kimberly-Clark Corp., Irving, TX, USA) soaked with deionized water and maintained in a Percival® I-36VL growth chamber set at 27˚C, 70% RH, 14:10 L: D. Eggs were monitored hourly for hatch after the initial 8 h. Resulting larvae were used in the experiments.

Dietary treatments. Three sterilized dietary treatments with different Protein:Lipid percentage were tested. These diets were; 1) 23% protein, 7% lipid, 2) 25% protein, 5% lipid, 3) 27% protein, 3% lipid (detailed composition see **Table 7**). The diet with 25% protein and 5% lipid was selected as it is similar in composition for these nutrients in fresh beef liver, a common material used to raise blow fly (Tarone and Foran 2008). According to preliminary result, *L. sericata* is unable to complete development on a diet with lipid concentration greater than 7%. The 27% protein and 3% lipid diet was selected as a means to test an inverse symmetric shift in protein and lipid percentages. All dietary treatments were sterilized in an autoclave at 121˚C, 20 min prior to use. After being autoclaved, each diet was individually partitioned at 20 g allotments in 33 mL sterilized medical cups (cups: Dart® 125PCG, lids: Dart® 125PCL25; Dart Container

Corp., Mason, MI, USA). All dietary treatments were stored at 4˚C until inoculated with assigned bacterial treatments.

Bacterial treatments. Four bacterial treatments were tested. These treatments were: 1) PBS (Phosphate Buffered Saline) as a control; 2) 10^3 cfu/gram *Proteus mirabilis*; 3) 10^7 cfu/gram *Proteus mirabilis*; 4) 10^7 cfu/gram *Salmonella*. *P. mirabilis* is known to play an important role in *L. sericata* larval and adult ecology (e.g., larval development and adult attraction) (Ma et al 2012). *Salmonella* was selected, as it has been associated with *L. sericata* in the past (Singh et al 2015).

Experiment design and data collection. Previous research indicated sampling larvae from a population resulting in reduced density impacted corresponding life-history traits. In order to avoid this impact, replicates were randomly assigned either to blow fly life-history trait or bacterial community assessment. Replicates assigned for assessing life-history traits *L. sericata* were monitored until all resulting adults died; thus, contact was reduced. Replicates of each treatment assigned for bacterial community assessment were destructively sampled at select time points (**Table 8**). The combination of four bacterial treatments and three dietary treatments were tested (**Table 8**). Three biological replicates with each consisting of three technical replicates were used. Twenty newly hatched first instars were transferred to each replicate. Cups (replicates) containing larvae were covered with a breathable lid with a 12 mm diameter hole punched in the center. A sterilized cotton ball was inserted into the hole and served as a means to regulate ventilation. During larval development, deionized, sterile water was provided *ad libitum* at 12 h intervals. Post-feeding larvae within a given replicate

were transferred into a sterilized 1 L Mason jar (Ball® 1440096254, Daleville, IN, USA), which was covered with a double layer sterilized Wypall-wipes (Kimberly-Clark Corp., Irving, TX, USA) held in place with a rubber band. These replicates were observed at 8 h interval until pupation, and the larval development duration recorded. All pupae were collected and pupation proportion per replicate and individual pupal weight recorded. Individual pupae were transferred into a sterilized medical cup with 10 g sterilized sugar-sand mix (sugar: sand = 1:1, Quikrete® Play Sand; Great Value® Pure Cane Sugar) in order to provide a carbohydrate resource and pupation substrate. Such an approach allowed for adult longevity to be based on fat reserves acquired during larval development. Pupae in cups were observed at 8 h interval for adult emergence, pupal duration, and longevity.

Amplicon data collection. From each replicate, 3-d-old larvae, pupae, and adults were sampled and stored at -20˚C for further Amplicon sequencing. DNA extraction on three larvae/pupae/adults were accomplished with the PowerLyzer® PowerSoil® DNA Isolation Kit (MO BIO LABORATORIES, Inc., Carlsbad, CA). Amplicon (V4 region, 515f/806r) sequencing was done with the Illumina MiSeq (v2 500 cycle regent cartridge) plotfrom with PE250bp at MSU RTSF Genomics Core.

Statistical analysis

Life-history trait data has been analyzed in JMP (v12.2.0). Analysis of Variance (ANOVA) and Tukey HSD ($P < 0.05$) were applied for analyzing pupation proportion, eclosion proportion, and pupal weight. The semi-parametric proportional hazard model and the Kaplan-Meier survival analysis were used for analyzing larval duration, pupal

duration, and adult longevity.

Raw sequencing data were processed through the MICCA (v1.5.0) (Albanese et al 2015). The (Operational Taxonomy Unit) OTU was picked by VSERCH (v2.3.4) with green gene database (v2013.05) (DeSantis et al 2006, Rognes et al 2016). The Bergey's bacterial taxonomy assigned to each OTU through the RDP classifier (v2.11) (Wang et al 2007). Due to bacterial treatments, higher-level taxonomic resolution was not possible. Five samples were removed due to their low count at genus level (less than 1,000). In addition, the total sum normalization was applied to the fly sequencing data and diet residual data, respectively. Rare taxa were defined as those with counts less than 0.01% of the total abundance.

The further bacterial community analyses were conducted in data were in R (v3.3.2). In order to determine the richness and evenness of bacterial community, the alpha diversity (Shannon index) and the beta diversity (Bray-Curtis) calculated on the bacterial community data (Beals 1984, Shannon 2001). Furthermore, the Permutation Multivariate Analysis of Variance (PerMANOVA) applied for comparing the beta diversity among treatments (Legendre and Anderson 1999). The non-Metric Multi-Dimensional Scaling (nMDS) provided the dimension-reduction visualization of the beta diversity (Constantine and Gower 1978). In addition, the Bonferroni-corrected Multi-Response Permutation Procedure (MRPP) employed for the post-hoc analyses of PerMANOVA (Jammalamadaka 2003). The Bonferroni-corrected Indicator Species Analysis (ISA) complemented the MRPP by assigning representative indicator species (Dufrene and Legendre 1997).

Table 7 | The composition of diet treatments (100g) for investigating the interaction effects of nutrients and exogenous bacteria on *Lucilia sericata* reared at 27˚C, 70% RH, 14:10 L:D

Source	Ingredient	Unit	23% Protein, 7% Lipid	25% Protein, 5% Lipid	27% Protein, 3% Lipid
Protein source	Casein	g	13.8	15	16.2
	Peptone	g	4.6	5	5.4
	Albumen	g	4.6	5	5.4
Cholesterol source	Cholesterol	mg	550	550	550
Lipid source	Linoleic acid	mL	7.7	5.5	3.3
Mineral source	Wesson's salt	g	2.5	2.5	2.5
Carbohydrate source	Sucrose	g	3.5	3.5	3.5
	Dextrin	g	3.5	3.5	3.5
Vitamin source	Ascorbate acid	mg	275	275	275
	Thiamine	mg	0.42	0.42	0.42
	Riboflavin	mg	0.42	0.42	0.42
	Nicotinic acid	mg	1.67	1.67	1.67
	Pyridoxine	mg	0.42	0.42	0.42
	Folic acid	mg	0.42	0.42	0.42
	myo-inositol	mg	4.17	4.17	4.17
	Ca panththenate	mg	0.83	0.83	0.83
	4-aminobenzoic acid	mg	0.42	0.42	0.42
	Choline	mg	20.83	20.83	20.83
	Biotin	mg	0.02	0.02	0.02
	Agar-agar	g	3.5	3.5	3.5
	Deionized H_2O	mL	70	70	70

Table 8 | The treatments and sampling time point for investigating the interaction effects of nutrients and exogenous bacteria on *Lucilia sericata* reared at 27˚C, 70% RH, 14:10 L:D; The experiment repeat the entire table 3 times (3 trials).

Diet Treatments		27% Protein 3% Lipid		25% Protein 5% Lipid		23% Protein 7% Lipid	
	Purpose	Life-history Trait	Bacterial Community at each stage*	Life-history Trait	Bacterial Community at each stage	Life-history Trait	Bacterial Community at each stage
Bacterial Treatments	Phosphate Buffered Saline (PBS)	3 Reps	1 Reps	3 Reps	1 Reps	3 Reps	1 Reps
	Low *Proteus mirabilis* (10^3 cfu/g)	3 Reps	1 Reps	3 Reps	1 Reps	3 Reps	1 Reps
	High *Proteus mirabilis* (10^3 cfu/g)	3 Reps	1 Reps	3 Reps	1 Reps	3 Reps	1 Reps
	High *Salmonella* (10^3 cfu/g)	3 Reps	1 Reps	3 Reps	1 Reps	3 Reps	1 Reps

*, the bacterial community sample was collected at each development stage: 3-day old larvae, 3-day old pupae, 3-day old adult.

Results: life-history trait data

Larval duration of *L. sericata*. Diet ($P < 0.001$, $F_{2,96} = 18.938$) and bacterial treatments ($P < 0.001$, $F_{3,96} = 23.755$) significantly impacted larval duration (Table 9). No interaction effects were significant ($P > 0.05$). Moreover, there is an approximate 6% reduction in larval duration as the protein: lipid ratio increased (Table 10, Figure 4). In contrast, there is an approximate 5% increase in larval duration as concentration of *P. mirabilis* increased or if *Salmonella* introduced (Table 11, Figure 5).

Table 9 | ANOVA on larval duration of *Lucilia sericata* reared on combinations of three diets and two exogenous bacteria (*Proteus mirabilis* and *Salmonella*) in an incubation room set at 27˚C, 70% RH, 14:10 L:D, n=9

Factor	DF	SumSq	F	P
Diet treatment	2	18.938	68.102	**<0.001**
Bacterial treatment	3	9.909	23.755	**<0.001**
Diet treatment: Bacterial treatment	6	0.441	0.528	0.786
Residual	96	13.348		

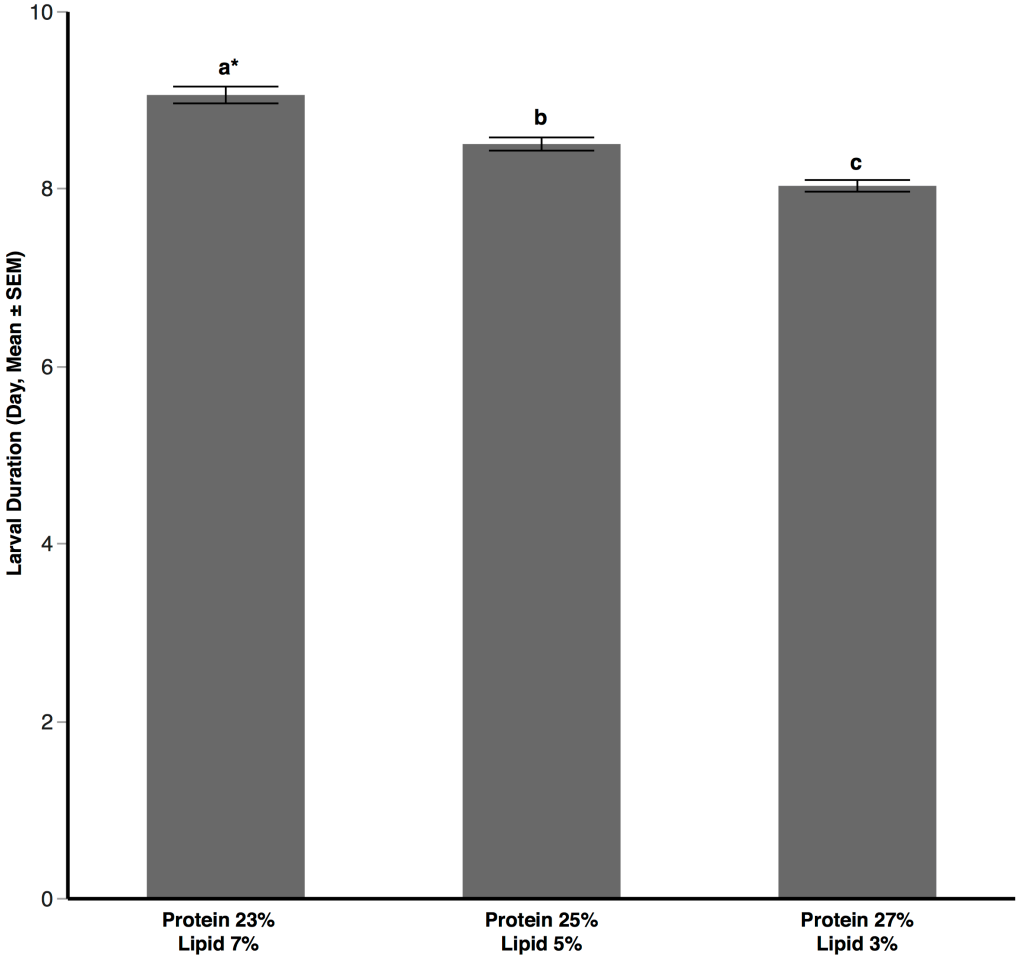
Table 10 | Tukey HSD ($P < 0.05$) results of larval duration of *Lucilia sericata* reared on three diets in an incubation room set at 27˚C, 70% RH, 14:10 L:D, n=9

Treatment	Mean ± SEM (day)	Letter Report
23%[Protein],7%[Lipid]	9.063 (± 0.006)	A
25%[Protein],5%[Lipid]	8.510 (± 0.006)	B
27%[Protein],3%[Lipid]	8.039 (± 0.006)	C

Table 11 | Tukey HSD ($P < 0.05$) results of larval duration of *Lucilia sericata* reared on combinations of three diets and two exogenous bacteria (*Proteus mirabilis* and *Salmonella*) at different densities in an incubation room set at 27˚C, 70% RH, 14:10 L:D, n=9

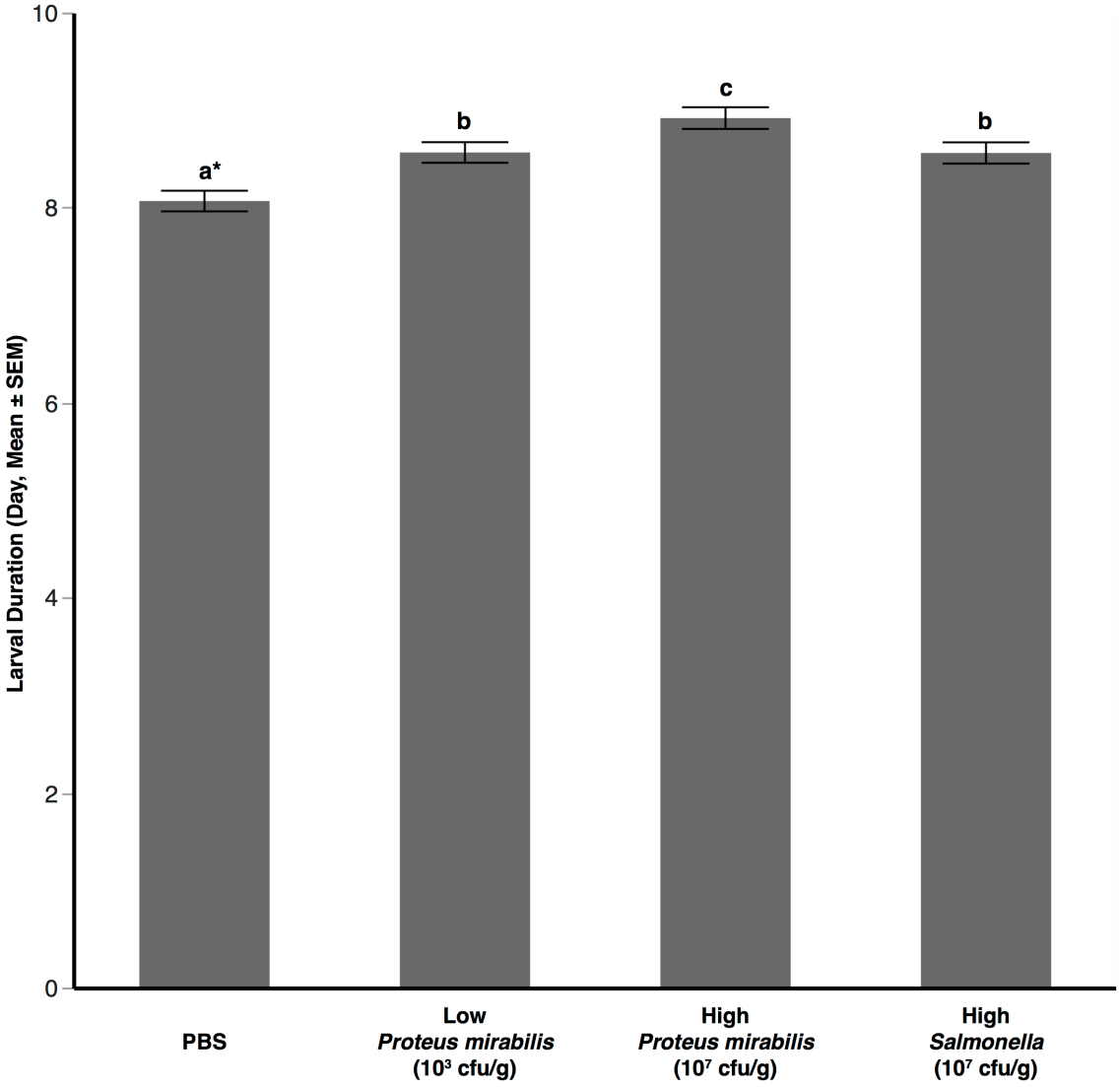
Treatment	Mean ± SEM (day)	Letter Report
PBS	8.077 (± 0.072)	A
P. mirabilis (10^3 cfu/g)	8.575 (±0.072)	B
P. mirabilis (10^7 cfu/g)	8.928 (±0.072)	C
Salmonella (10^7 cfu/g)	8.570 (± 0.072)	B

Figure 4 | Effects of diet on the larval duration ± SEM of *Lucilia sericata* reared on the treatments with three diets in an incubation room set at 27˚C, 70% RH, 14:10 L:D, n=9



*Treatments with different lowercase letter indicates significant ($P < 0.05$) difference.

Figure 5 | Effects of exogenous bacteria (*Proteus mirabilis* and *Salmonella*) on larval duration ± SEM of *Lucilia sericata* reared on combinations of three diets and two exogenous bacteria (*Proteus mirabilis* and *Salmonella*) in an incubation room set at 27˚C, 70% RH, 14:10 L:D, n=9



*Treatments with different lowercase letter indicates significant ($P < 0.05$) difference.

Pupal weight of *L. sericata*. Diet ($P < 0.001$, $F_{2,96} = 28.453$) and bacterial treatments ($P < 0.001$, $F_{3,96} = 25.140$) significantly impacted larval duration (Table 12).

A significant interaction between diet and bacterial treatments (P = 0.009, $F_{6,96}$ = 112.378) was determined for pupal weight (Table 12). Larvae exposed to high and moderate protein: lipid diets in conjunction with the high concentrations of *P. mirabilis* were 15% smaller than the PBS controls or the other bacterial treatments (Table 13, Figure 6). This effect was lower (5%) when larvae were provided the low protein diet, high *P. mirabilis*, or *Salmonella* inoculums.

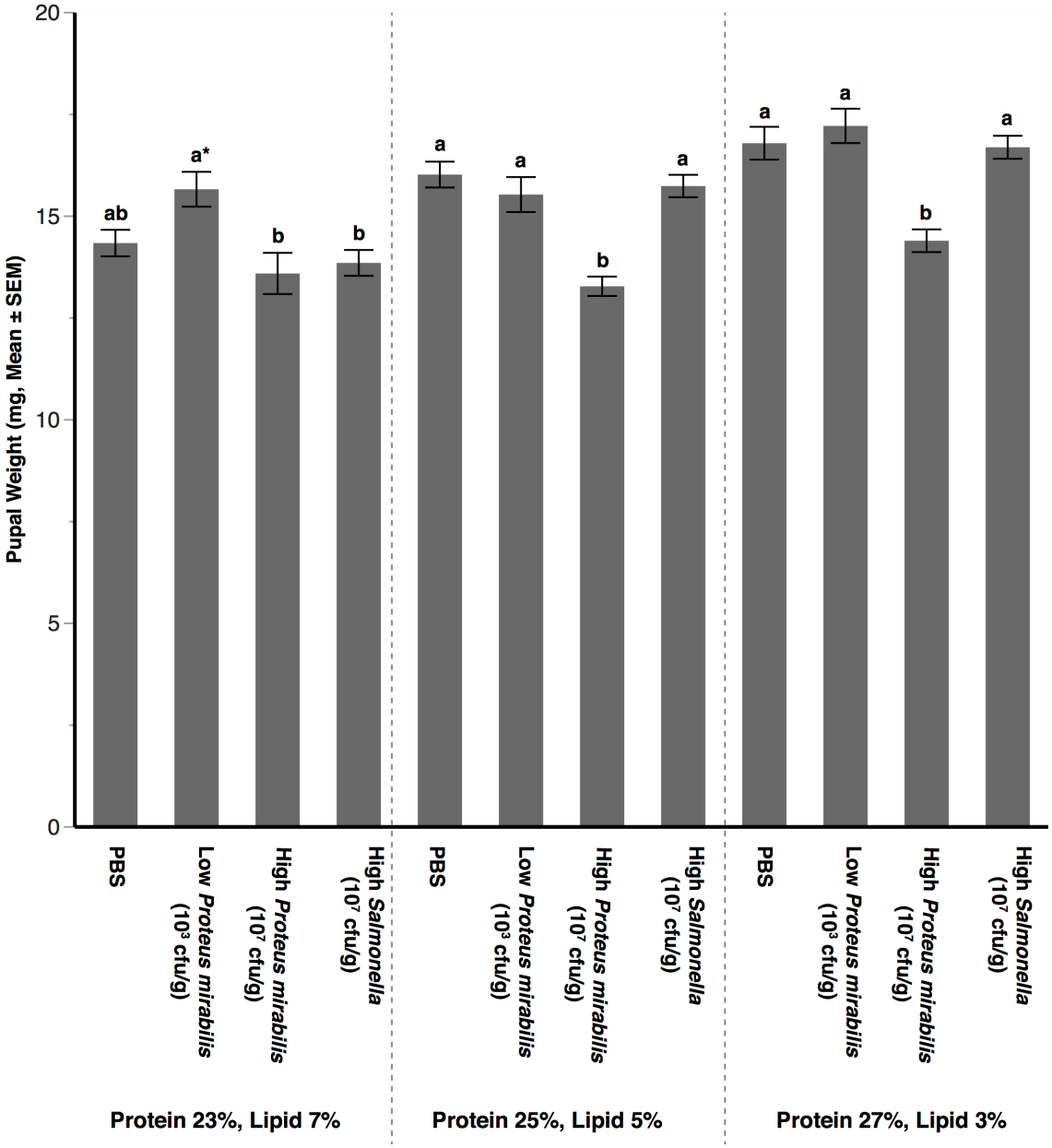
Table 12 | ANOVA of pupal weight of *Lucilia sericata* on combinations of three diets and two exogenous bacteria (*Proteus mirabilis* and *Salmonella*) an incubation room set at 27˚C, 70% RH, 14:10 L:D, n=9

Factor	DF	SumSq	F	P
Diet treatment	2	66.615	28.453	**<0.001**
Bacterial treatment	3	88.287	25.140	**<0.001**
Diet treatment: Bacterial treatment	6	21.141	3.010	**0.009**
Residual	96	112.378		

Table 13 | Tukey HSD ($P < 0.05$) results of pupal weight ± SEM of *Lucilia sericata* reared on combinations of three diets and two exogenous bacteria (*Proteus mirabilis* and *Salmonella*) an incubation room set at 27˚C, 70% RH, 14:10 L:D, n=9

Diet Treatment	Bacterial Treatment	Mean ± SEM (mg)	Letter Report
23%[Protein], 7%[Lipid]	PBS	14.341 (± 0.402)	AB
	P. mirabilis (10^3 cfu/g)	15.663 (± 0.402)	A
	P. mirabilis (10^7 cfu/g)	13.594 (± 0.402)	B
	Salmonella (10^7 cfu/g)	13.854 (± 0.402)	B
25%[Protein], 5%[Lipid]	PBS	16.023 (± 0.323)	A
	P. mirabilis (10^3 cfu/g)	15.533 (± 0.323)	A
	P. mirabilis (10^7 cfu/g)	13.279 (± 0.323)	B
	Salmonella (10^7 cfu/g)	15.742 (± 0.323)	A
27%[Protein], 3%[Lipid]	PBS	16.794 (± 0.353)	A
	P. mirabilis (10^3 cfu/g)	17.219 (± 0.353)	A
	P. mirabilis (10^7 cfu/g)	14.398 (± 0.353)	B
	Salmonella (10^7 cfu/g)	16.694 (± 0.353)	A

Figure 6 | Effects of diet and exogenous bacteria on pupal weight ± SEM of *Lucilia sericata* reared on combinations of three diets and two exogenous bacteria (*Proteus mirabilis* and *Salmonella*) in an incubation room set at 27˚C, 70% RH, 14:10 L:D, n=9



*Treatments with different lowercase letter indicates significant ($P < 0.05$) difference.

Pupation proportion of *L. sericata*. Diet treatments ($P < 0.001$, $F_{2,96} = 10.357$) significantly impacted pupation proportion, while bacterial treatments were not ($P > 0.05$) (Table 14). No interaction effects were significant ($P > 0.05$). An approximate 12% increase in pupation proportion was determined from the lowest and moderate protein: lipid ratios to the highest ratio (Table 15, Figure 7).

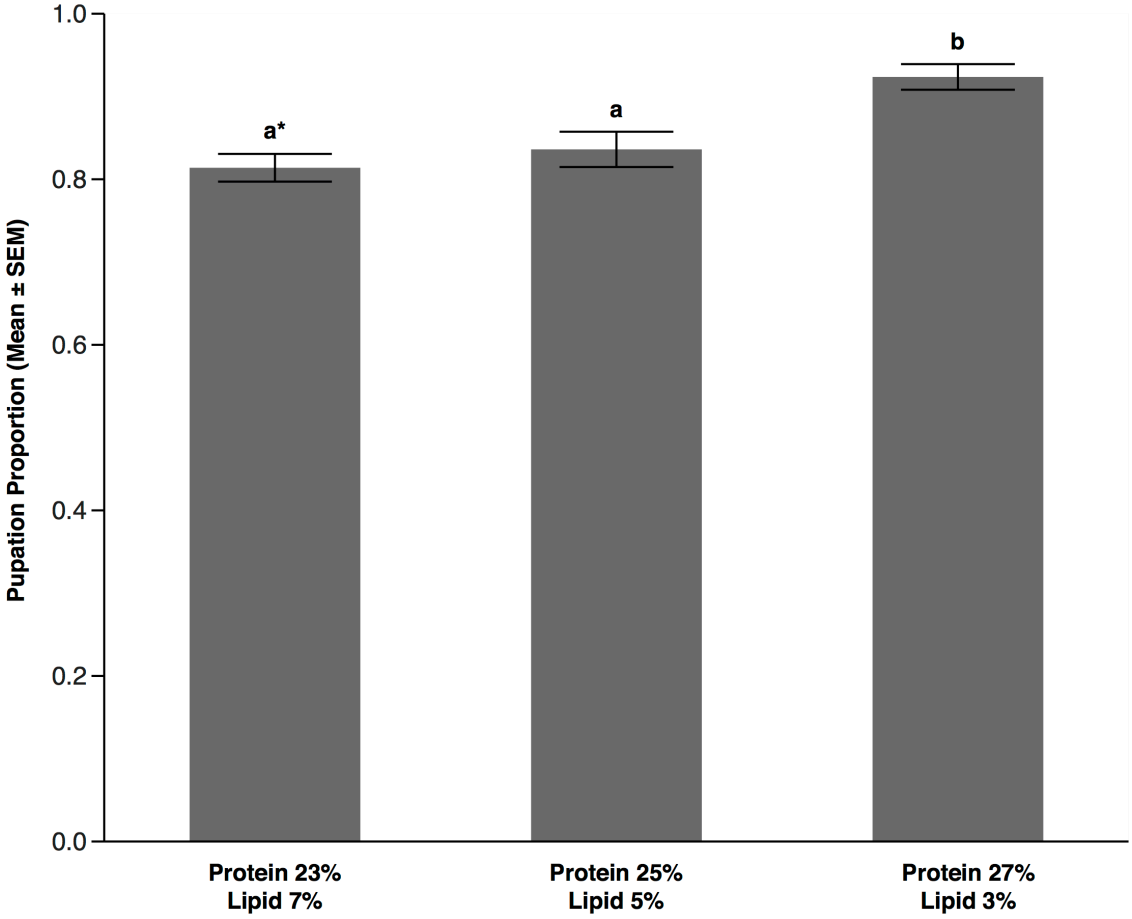
Table 14 | ANOVA of pupation proportion of *Lucilia sericata* reared on combinations of three diets and two exogenous bacteria (*Proteus mirabilis* and *Salmonella*) in an incubation room set at 27˚C, 70% RH , 14:10 L:D, n=9

Factor	DF	SumSq	F	P
Diet treatment	2	0.242	10.357	**<0.001**
Bacterial treatment	3	0.050	1.422	0.241
Diet treatment: Bacterial treatment	6	0.056	0.796	0.575
Residual	96	1.123		

Table 15 | Tukey HSD ($P < 0.05$) results of pupal proportion of *Lucilia sericata* reared on combinations of three diets and two exogenous bacteria (*Proteus mirabilis* and *Salmonella*) in an incubation room set at 27˚C, 70% RH , 14:10 L:D, n=9

Treatment	Mean ± SEM	Letter Report
23%[Protein],7%[Lipid]	0.814 (± 0.018)	A
25%[Protein],5%[Lipid]	0.836 (± 0.018)	A
27%[Protein],3%[Lipid]	0.924 (± 0.018)	B

Figure 7 | Effects of diet on pupation proportion ± SEM of *Lucilia sericata* reared on combinations of three diets and two exogenous bacteria (*Proteus mirabilis* and *Salmonella*) an incubation room set at 27˚C, 70% RH, 14:10 L:D, n=9



*Treatments with different lowercase letter indicates significant ($P < 0.05$) difference.

Pupal duration of *L. sericata*. Diet did not significantly ($P > 0.05$) impact pupal duration. However, bacterial treatment significantly ($P < 0.001$, $F_{3,96} = 13.656$) (Table 16) impacted pupal duration. No interaction effects were significant ($P > 0.05$). Pupal duration of those larvae exposed to the bacterial treatments was 6% than for those in the PBS control (Table 17, Figure 8).

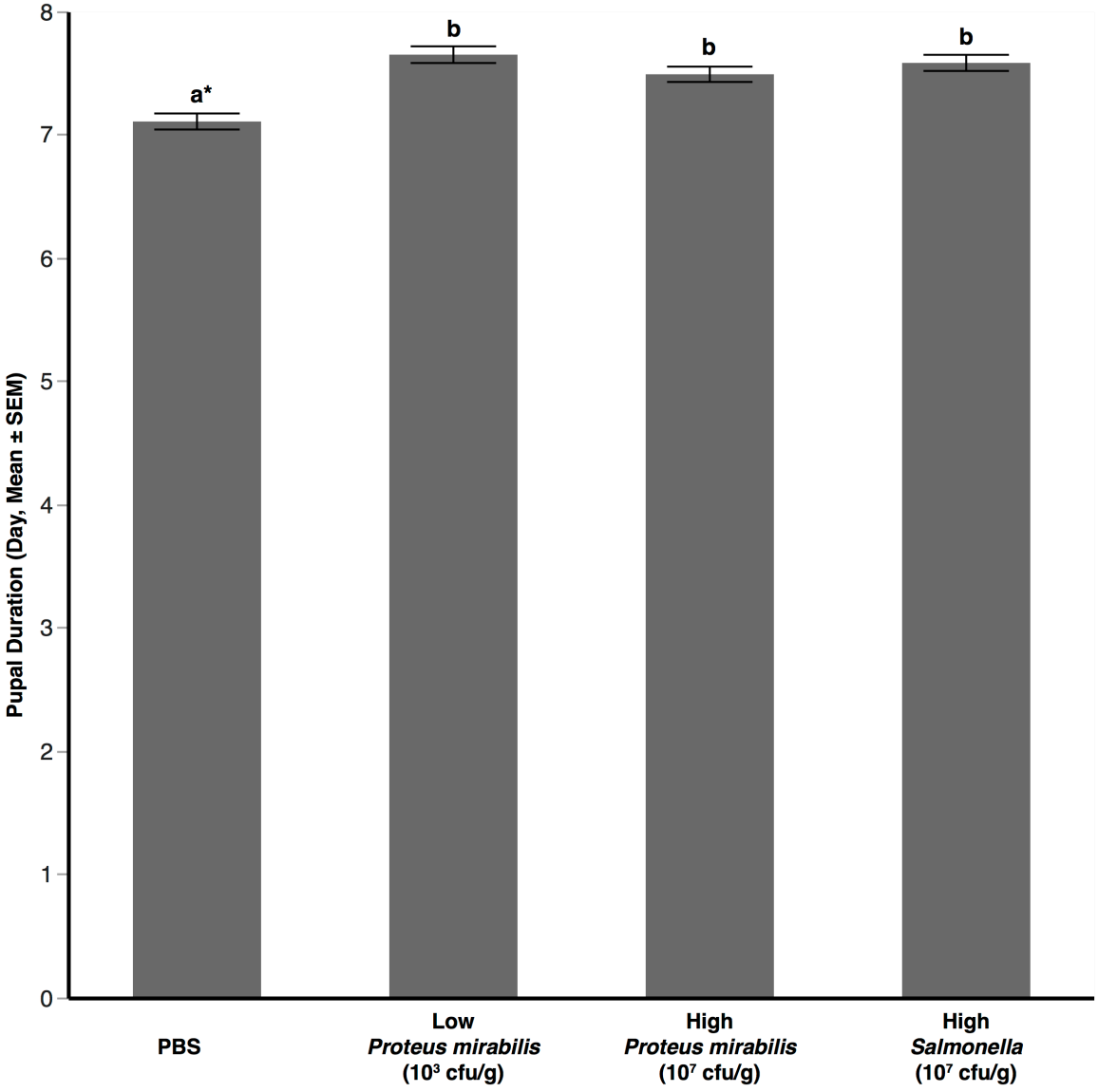
Table 16 | ANOVA on diet and bacterial treatments effects on pupal duration of *Lucilia sericata* reared on combinations of three diets and two exogenous bacteria (*Proteus mirabilis* and *Salmonella*) in an incubation room set at 27˚C, 70% RH, 14:10 L:D, n=9

Factor	DF	SumSq	F	P
Diet treatment	2	0.072	0.309	0.735
Bacterial treatment	3	4.759	13.656	**<0.001**
Diet treatment: Bacterial treatment	6	0.683	0.980	0.443
Residual	96	11.152		

Table 17 | Tukey HSD ($P < 0.05$) results of pupal duration of *Lucilia sericata* reared on combinations of three diets and two exogenous bacteria (*Proteus mirabilis* and *Salmonella*) in an incubation room set at 27˚C, 70% RH , 14:10 L:D, n=9

Treatment	Mean ± SEM (day)	Letter Report
PBS	7.114 (± 0.066)	A
P. mirabilis (10^3 cfu/g)	7.656 (± 0.066)	B
P. mirabilis (10^7 cfu/g)	7.497 (± 0.066)	B
Salmonella (10^7 cfu/g)	7.589 (± 0.066)	B

Figure 8 | Effects of exogenous bacteria on the pupal duration ± SEM of *Lucilia sericata* reared on combinations of three diets and two exogenous bacteria (*Proteus mirabilis* and *Salmonella*) in an incubation room set at 27˚C, 70% RH, 14:10 L:D, n=9



*Treatments with different lowercase letter indicates significant ($P < 0.05$) difference.

Eclosion proportion of *L. sericata.* Diet ($P < 0.001$, $F_{2,96} = 15.324$) and bacterial treatment ($P = 0.003$, $F_{3,96} = 0.003$) significantly impacted eclosion proportion (Table 18). No interaction effects were significant ($P > 0.05$). Larvae reared on the diet with the lowest protein experienced 18.5% greater eclosion than those raised on the lowest protein: lipid ratio treatments (Table 19, Figure 9). Additionally, when considering the bacterial treatments, eclosion proportion decreased approximately 9% from the PBS and low dosage *P. mirabilis* treatments to the high dosage *P. mirabilis* and *Salmonella* treatments (Table 20, Figure 10).

Table 18 | ANOVA on diet and exogenous bacterial treatments effects on eclosion proportion of *Lucilia sericata* reared on combinations of three diets and two exogenous bacteria (*Proteus mirabilis* and *Salmonella*) in an incubation room set at 27˚C, 70% RH, 14:10 L:D, n=9

Factor	DF	SumSq	F	P
Diet treatment	2	0.416	15.324	**<0.001**
Bacterial treatment	3	0.203	4.972	**0.003**
Diet treatment: Bacterial treatment	6	0.146	1.794	0.108
Residual	96	1.304		

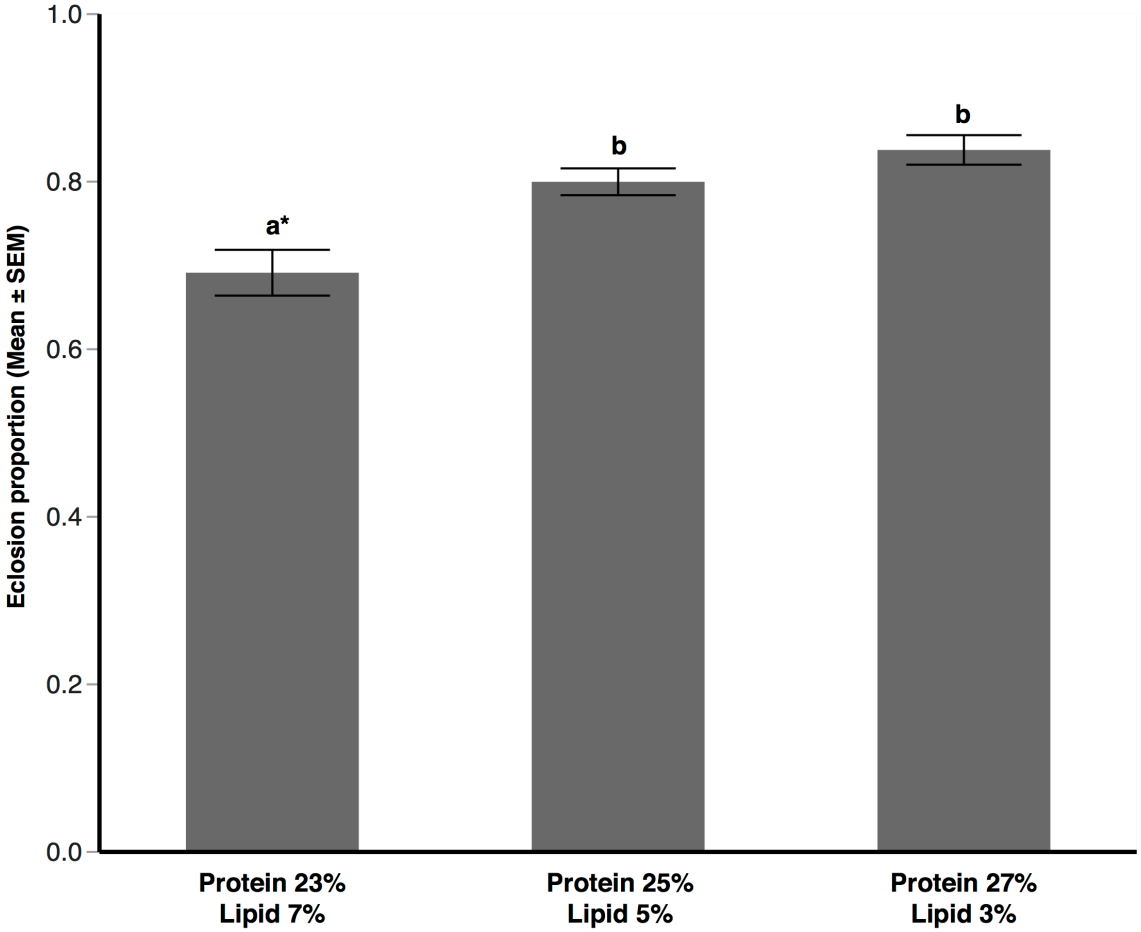
Table 19 | Tukey HSD ($P < 0.05$) results of eclosion proportion of *Lucilia sericata* reared on combinations of three diets and two exogenous bacteria in an incubation room set at 27˚C, 70% RH, 14:10 L:D, n=9

Treatment	Mean ± SEM	Letter Report
23%[Protein],7%[Lipid]	0.691 (± 0.019)	A
25%[Protein],5%[Lipid]	0.800 (± 0.019)	B
27%[Protein],3%[Lipid]	0.838 (± 0.019)	B

Table 20 | Tukey HSD ($P < 0.05$) results of eclosion proportion of *Lucilia sericata* reared on combinations of three diets and two exogenous bacteria in an incubation room set at 27˚C, 70% RH, 14:10 L:D, n=9

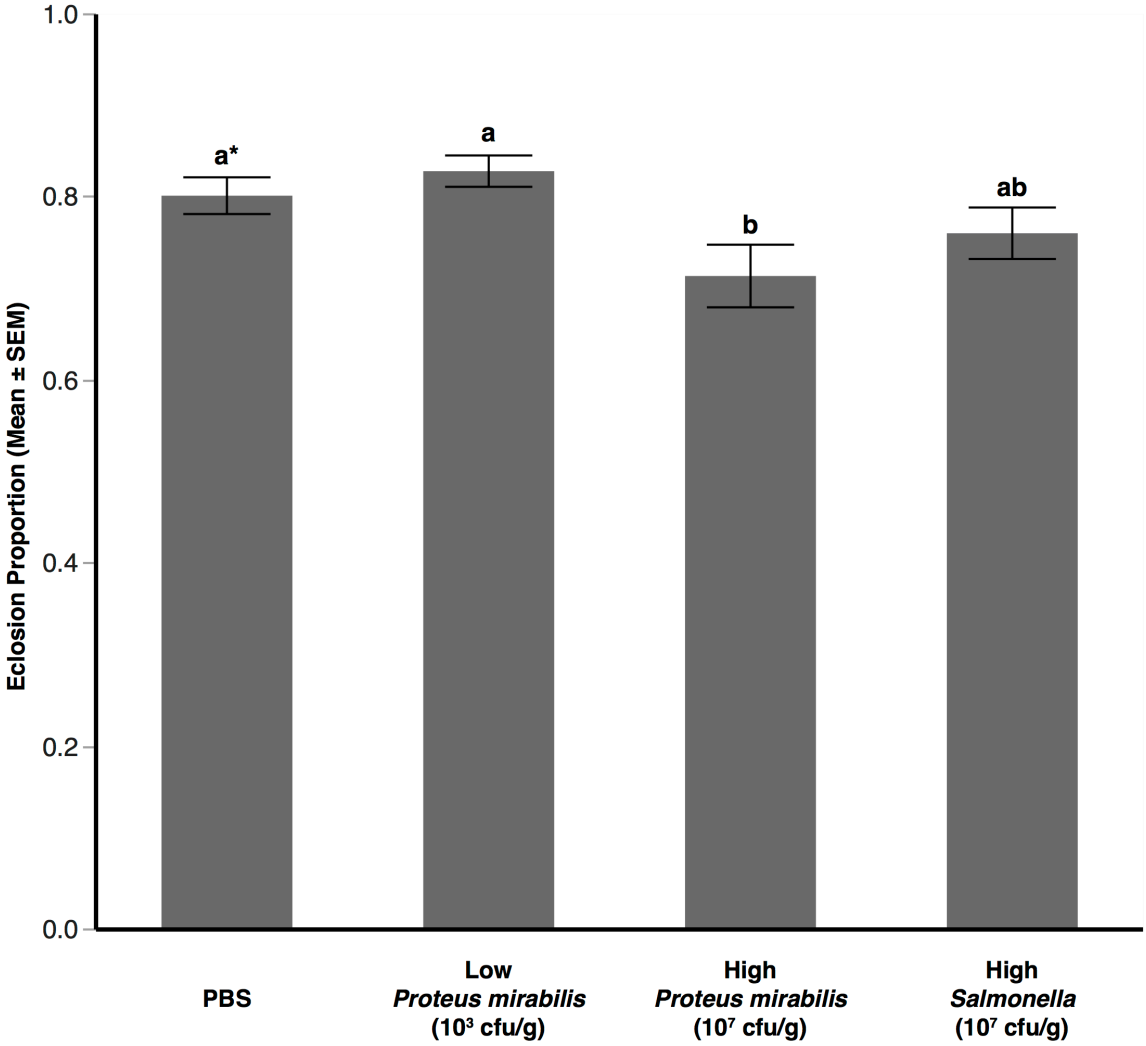
Treatment	Mean ± SEM	Letter Report
PBS	0.802 (± 0.022)	A
P. mirabilis (10^3 cfu/g)	0.829 (± 0.022)	A
P. mirabilis (10^7 cfu/g)	0.714 (± 0.022)	B
Salmonella (10^7 cfu/g)	0.761 (± 0.022)	AB

Figure 9 | Effects of diet on the eclosion proportion ± SEM of *Lucilia sericata* reared on combinations of three diets and two exogenous bacteria (*Proteus mirabilis* and *Salmonella*) in an incubation room set at 27˚C, 70% RH, 14:10 L:D, n=9



*Treatments with different lowercase letter indicates significant ($P < 0.05$) difference.

Figure 10 | Effects of exogenous bacteria on the eclosion proportion ± SEM of *Lucilia sericata* reared on combinations of three diets and two exogenous bacteria (*Proteus mirabilis* and *Salmonella*) in an incubation room set at 27˚C, 70% RH, 14:10 L:D, n=9



*Treatments with different lowercase letter indicates significant ($P < 0.05$) difference.

Adult duration of *L. sericata*. Diet ($P < 0.001$, $F_{2,96} = 9.765$) and bacterial treatments ($P < 0.001$, $F_{3,96} = 10.301$) significantly impacted larval duration (Table 21). A significant ($P = 0.009$, $F_{6,96} = 3.016$) interaction effect between diet and bacterial

treatments on adult duration was determined (Table 21). For those larvae provided the lowest protein: lipid ratio diet and the high *P. mirabilis* treatment, adults lived an average of 15% longer than those provided the other diet/bacteria combinations, including the PBS (Table 22, Figure 11). However, it should be noted, with the highest protein: lipid ratio diet, the *Salmonella* treatment, resulted in adults lived 20% longer than those provided the other diet/bacteria combinations, including the PBS (Table 22, Figure 11).

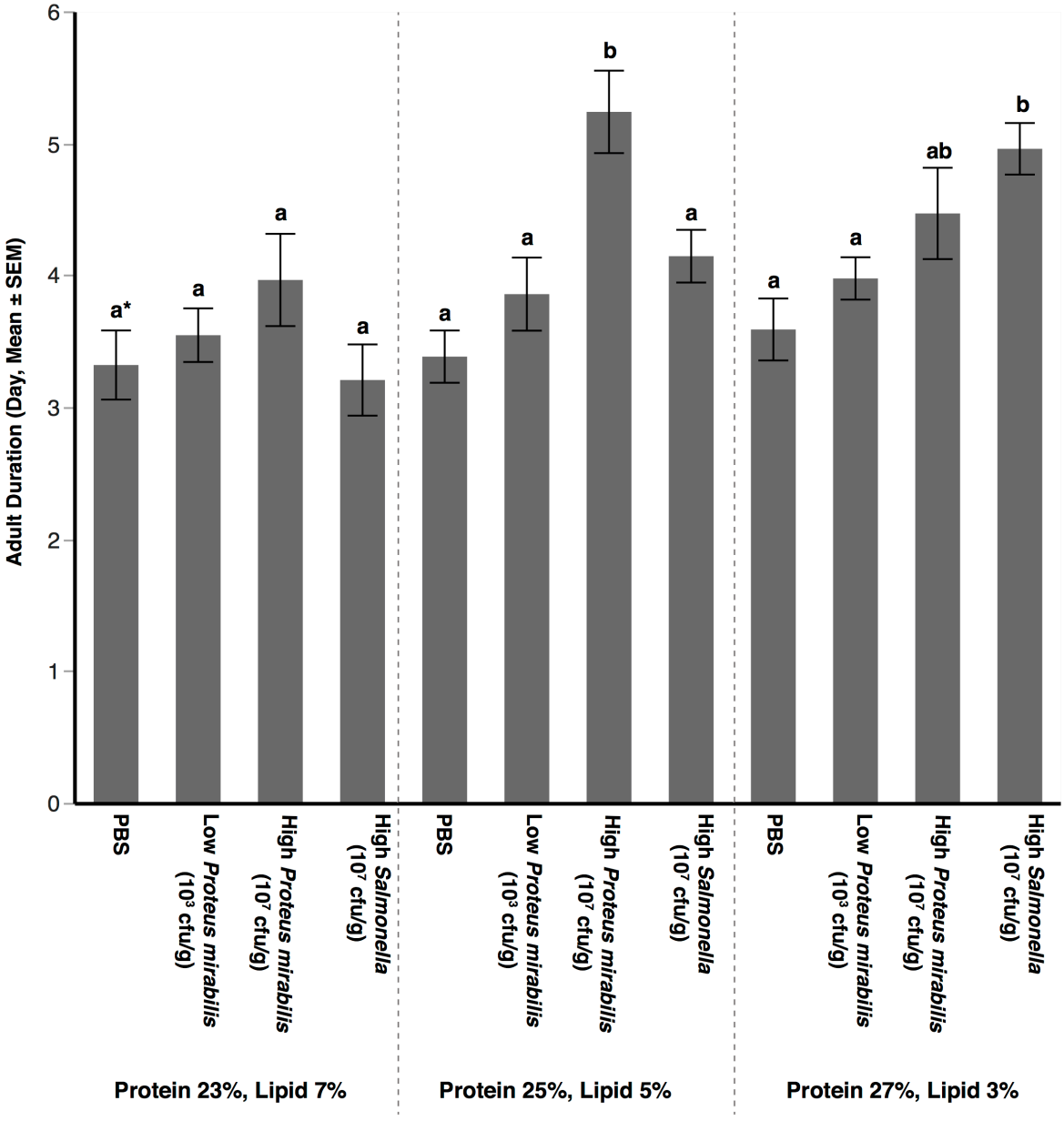
Table 21 | ANOVA of adult duration of *Lucilia sericata* reared reared on combinations of three diets and two exogenous bacteria (*Proteus mirabilis* and *Salmonella*) in an incubation room set at 27˚C, 70% RH, 14:10 L:D, n=9

Factor	DF	SumSq	F	P
Diet treatment	2	11.682	9.765	**<0.001**
Bacterial treatment	3	18.483	10.301	**<0.001**
Diet treatment: Bacterial treatment	6	10.822	3.016	**0.009**
Residual	96	57.420		

Table 22 | Tukey HSD ($P < 0.05$) of adult duration ± SEM of *Lucilia sericata* reared on combinations of three diets and two exogenous bacteria (*Proteus mirabilis* and *Salmonella*) in an incubation room set at 27˚C, 70% RH, 14:10 L:D, n=9

Diet Treatment	Bacterial Treatment	Mean ± SEM (day)	Letter Report
23%[Protein], 7%[Lipid]	PBS	3.326 (± 0.252)	A
	P. mirabilis (10^3 cfu/g)	3.552 (± 0.252)	A
	P. mirabilis (10^7 cfu/g)	3.971 (± 0.252)	A
	Salmonella (10^7 cfu/g)	3.212 (± 0.252)	A
25%[Protein], 5%[Lipid]	PBS	3.390 (± 0.258)	A
	P. mirabilis (10^3 cfu/g)	3.863 (± 0.258)	A
	P. mirabilis (10^7 cfu/g)	5.245 (± 0.258)	B
	Salmonella (10^7 cfu/g)	4.151 (± 0.258)	A
27%[Protein], 3%[Lipid]	PBS	3.596 (± 0.244)	A
	P. mirabilis (10^3 cfu/g)	3.982 (± 0.244)	A
	P. mirabilis (10^7 cfu/g)	4.475 (± 0.244)	AB
	Salmonella (10^7 cfu/g)	4.965 (± 0.244)	B

Figure 11 | Effects of diet and exogenous bacteria on the adult duration ± SEM of *Lucilia sericata* reared on combinations of three diets and two exogenous bacteria (*Proteus mirabilis* and *Salmonella*) in an incubation room set at 27˚C, 70% RH, 14:10 L:D, n=9



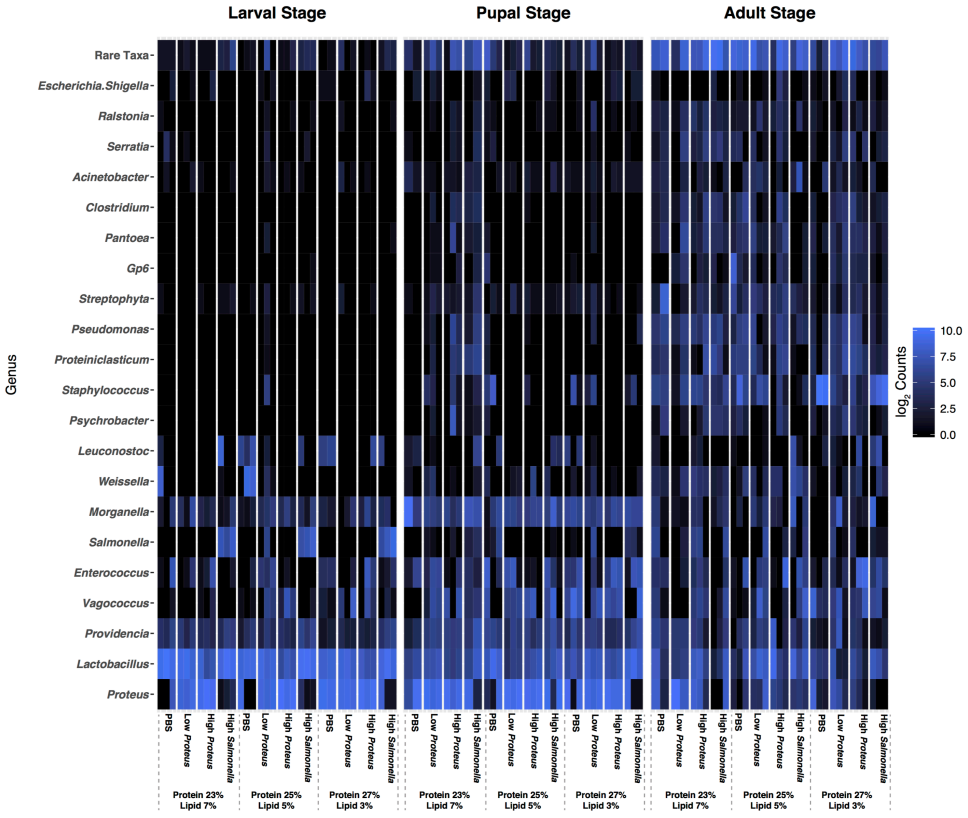
Results: bacterial community data

Bacterial community diversity indices across development stage of fly. A heatmap for genera abundance at each developmental stage of *L. sericata* is presented in Figure 12. Shannon diversity was only significantly ($P < 0.001$, $F_{2,67} = 28.52$) different across development stages (Figure 13) with an approximately 45% increase in diversity from larva to adult.

The nMDS plot (dimension=3) of the impact of diet and bacteria treatments on beta diversity across development stages is presented in Figure 14. The nMDS plot had an overall stress of 0.1047, with 49.98% beta diversity explained by x-axis, and 32.57% beta diversity explained by y-axis.

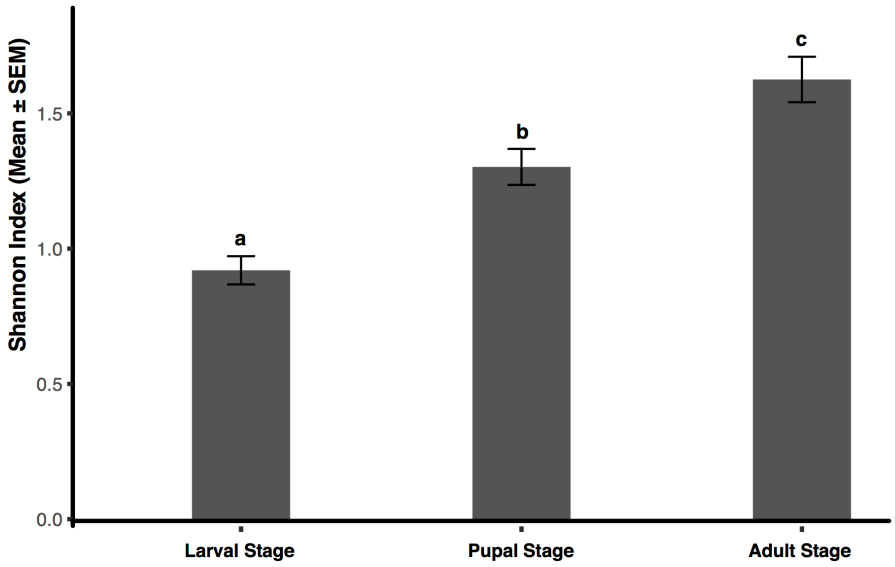
For the PerMANOVA results, significant two-way interactions between development stage and diet ($P = 0.014$, pseudo-$F_{4,67} = 1.973$) as well as development stage and bacterial treatment ($P = 0.001$, pseudo-$F_{6,67} = 2.454$) on fly bacterial communities were determined (Table 23). Due to the complexity of the results from the additional analyses (PerMANOVA by stage, MRPP and ISA), they have been broken down and are presented by development stage and are presented below.

Figure 12 | Heatmap of bacterial genera demonstrating diet and exogenous bacterial treatment effects on development stage of *Lucilia sericata* reared on combinations of three diets and two exogenous bacteria (*Proteus mirabilis* and *Salmonella*) in an incubation room set at 27˚C, 70% RH, 14:10 L:D, n=3



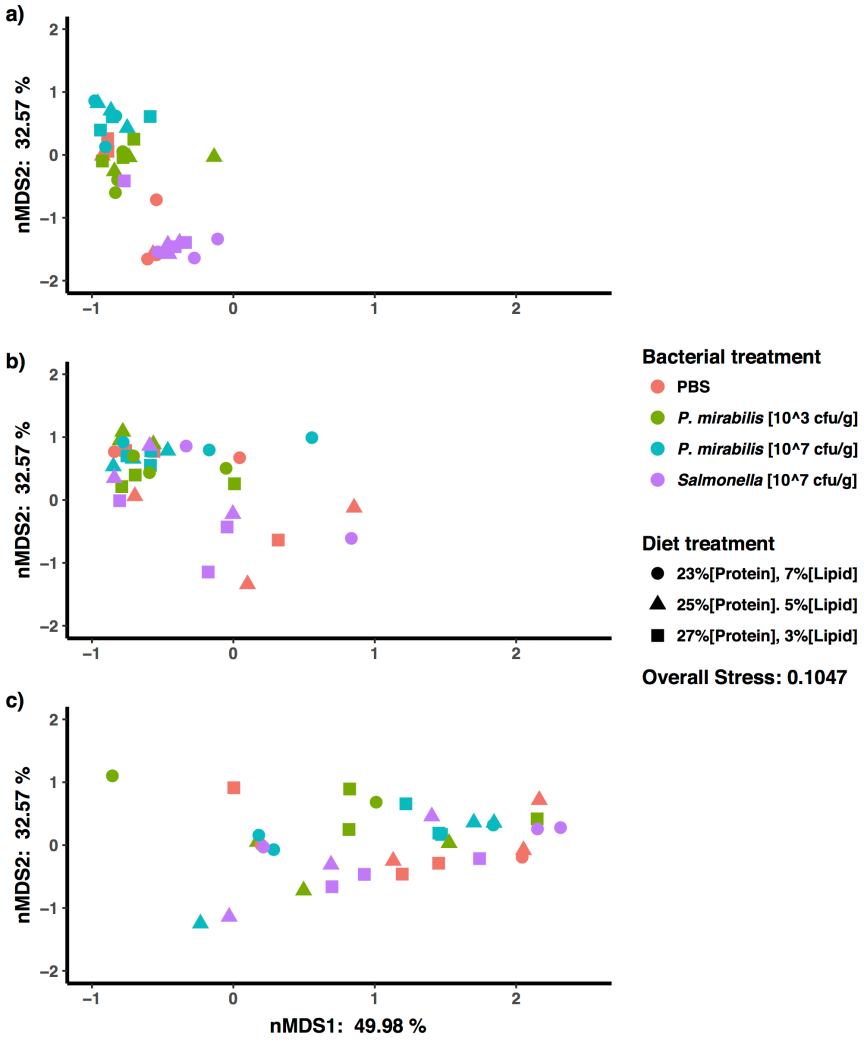
A $\log_2(x+1)$ transformation applied on the count table. The lighter blue the color is, the higher the abundance of the genus.

Figure 13 | Shannon diversity index by averaging the bacterial and diet effects at each development stage of *Lucilia sericata* reared on combinations of three diets and two exogenous bacteria (*Proteus mirabilis* and *Salmonella*) in an incubation room set at 27˚C, 70% RH, 14:10 L:D, n=9



The diversity of bacterial community has significantly increased as the development stage progressing ($P < 0.001$, $F_{2,67} = 28.52$). The small case letter indicates significant difference. The Shannon diversity index (Mean ± SEM) of larval, pupal, and adult stages are 0.920 ± 0.052, 1.302 ± 0.067, and 1.625 ± 0.084, respectively.

Figure 14 | nMDS plots on normalized count data by diet and bacterial effects on development stage of *Lucilia sericata* reared on combinations of three diets and two exogenous bacteria (*Proteus mirabilis* and *Salmonella*) in an incubation room set at 27˚C, 70% RH, 14:10 L:D, n=3



(a) Larval stage, **(b)** Pupal stage, and **(c)** Adult stage. The overall stress of the nMDS simulation with (dimension = 3) is 0.1047. Moreover, the nMDS x-axis explained 49.98% of the total beta diversity. And the nMDS y-axis explained 32.57% of the total beta diversity.

Table 23 | PerMANOVA of normalized bacterial community of each development stage of *Lucilia sericata* reared on combinations of three diets and two exogenous bacteria (*Proteus mirabilis* and *Salmonella*) in an incubation room set at 27˚C, 70% RH, 14:10 L:D, n=9

Factor	DF	SumSq	Pseudo-F	*P*
Diet treatment	2	0.521	2.131	**0.035**
Bacterial treatment	3	2.714	7.399	**0.001**
Development stage	2	7.288	29.808	**0.001**
Diet treatment: Bacterial treatment	6	1.113	1.518	0.052
Development stage: Diet treatment	4	0.965	1.973	**0.014**
Development stage: Bacterial treatment	6	1.800	2.454	**0.001**
Development stage: Diet treatment: Bacterial treatment	12	1.649	1.124	0.270
Residual	67	8.191		

Treatment effects on larval bacterial community. The nMDS plot (dimension=3) allow for visualizing the beta diversity across diet and bacterial treatments for the larval stage (Figure 15). The nMDS plot has an overall stress of 0.0453, with 78.15% beta diversity explained by x-axis, and 12.22% beta diversity explained by y-axis.

PerMANOVA indicated diet treatment ($P = 0.031$, pseudo-$F_{2,23} = 0.295$) and the bacterial treatment ($P = 0.001$, pseudo-$F_{3,23} = 2.852$) were impacted bacterial community structure associated with the larval stage (Table 24). Not interaction effect was significant ($P > 0.05$, Table 14). Based on pairwise MRPP analyses bacterial community associated with the larval stage, while not significant with regards to diet (Table 25), were significant for bacterial treatments (Table 26). ISA indicated *Lactobacillus* (43.49% of the total bacterial composition on diet residuals), *Proteus* (36.96% of the total bacterial composition of the larvae), *Salmonella* (5.36% of the total bacterial

composition of the larvae), *Providencia* (1.92% of the total bacterial composition of the larvae) as well as the rare taxa (Table 27) were the major taxa driving these differences.

Figure 15 | nMDS plots on normalized count data of diet treatment and exogenous bacterial treatment effects on larval stage of *Lucilia sericata* reared on combinations of three diets and two exogenous bacteria (*Proteus mirabilis* and *Salmonella*) in an incubation room set at 27˚C, 70% RH, 14:10 L:D, n=3

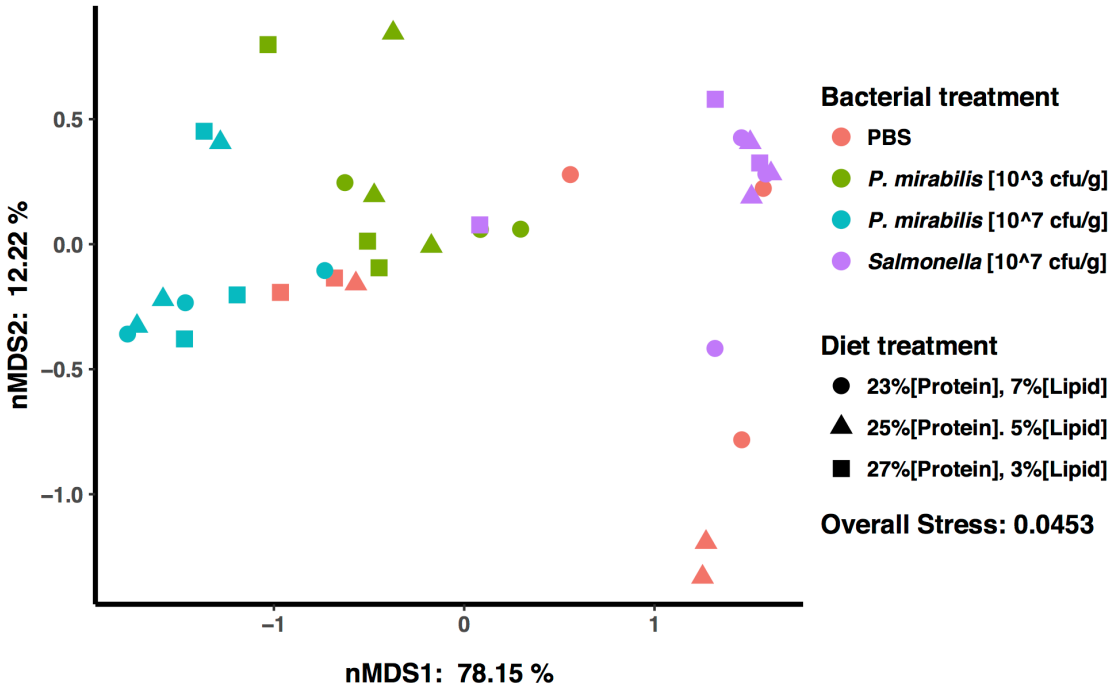


Table 24 | PerMANOVA of normalized bacterial community of larval stage of *Lucilia sericata* reared on combinations of three diets and two exogenous bacteria (*Proteus mirabilis* and *Salmonella*) in an incubation room set at 27˚C, 70% RH, 14:10 L:D, n=3

Factor	DF	SumSq	Pseudo-F	*P*
Diet treatment	2	0.295	3.193	**0.031**
Bacterial treatment	3	2.852	20.615	**0.001**
Diet treatment: Bacterial treatment	6	0.402	1.452	0.200
Residual	23	1.061		

Table 25 | Multi-Response Permutation Procedure (MRPP) of normalized bacterial community of larval stage of *Lucilia sericata* reared on combinations of three diets and two exogenous bacteria (*Proteus mirabilis* and *Salmonella*) in an incubation room set at 27˚C, 70% RH, 14:10 L:D, n=3

Diet treatment	-Diet treatment	delta	*P*	*P**
23%[Protein],7%[Lipid]	25%[Protein],5%[Lipid]	0.498	0.869	1.000
23%[Protein],7%[Lipid]	27%[Protein],3%[Lipid]	0.442	0.107	0.321
25%[Protein],5%[Lipid]	23%[Protein],7%[Lipid]	0.457	0.386	1.000

*, the Bonferroni corrected *P*-value

Table 26 | Multi-Response Permutation Procedure (MRPP) of normalized bacterial community of larval stage of *Lucilia sericata* reared on combinations of three diets and two exogenous bacteria (*Proteus mirabilis* and *Salmonella*) in an incubation room set at 27˚C, 70% RH, 14:10 L:D, n=3

Bacterial treatment	-Bacterial treatment	delta	*P*	*P**
PBS	*P. mirabilis* (10^3 cfu/g)	0.331	0.018	0.108
PBS	*P. mirabilis* (10^7 cfu/g)	0.332	0.001	**0.006**
PBS	*Salmonella* (10^7 cfu/g)	0.368	0.004	**0.024**
P. mirabilis (10^3 cfu/g)	*P. mirabilis* (10^7 cfu/g)	0.217	0.001	**0.006**
P. mirabilis (10^3 cfu/g)	*Salmonella* (10^7 cfu/g)	0.251	0.001	**0.006**
P. mirabilis (10^7 cfu/g)	*Salmonella* (10^7 cfu/g)	0.252	0.001	**0.006**

*, the Bonferroni corrected *P*-value

Table 27 | Indicator Species Analyses (ISA) of normalized bacterial community of larval stage of *Lucilia sericata* reared on combinations of three diets and two exogenous bacteria (*Proteus mirabilis* and *Salmonella*) in an incubation room set at 27˚C, 70% RH, 14:10 L:D, n=3

Comparisons	Treatments	ISA	STAT	*P*	*P**
Comparison 1	PBS	***Lactobacillus***	0.872	0.005	**0.025**
		Leuconostoc	0.771	0.020	0.100
	P. mirabilis (10^7 cfu/g)	***Proteus***	0.864	0.010	**0.050**
Comparison 2	PBS	NA			
	Salmonella (10^7 cfu/g)	***Salmonella***	1.000	0.005	**0.025**
		Providencia	0.839	0.025	0.125
		Rare taxa	0.959	0.005	**0.025**
Comparison 3	*P. mirabilis* (10^3 cfu/g)	***Lactobacillus***	0.849	0.005	**0.025**
	P. mirabilis (10^7 cfu/g)	***Proteus***	0.783	0.005	**0.025**
Comparison 4	*P. mirabilis* (10^3 cfu/g)	***Proteus***	0.953	0.005	**0.025**
	Salmonella (10^7 cfu/g)	***Salmonella***	0.986	0.005	**0.025**
		Providencia	0.853	0.005	**0.025**
		Lactobacillus	0.774	0.020	0.100
Comparison 5	*P. mirabilis* (10^7 cfu/g)	***Proteus***	0.969	0.005	**0.025**
		Vagococcus	0.868	0.020	0.100
	Salmonella (10^7 cfu/g)	***Salmonella***	1.000	0.005	**0.025**
		Lactobacillus	0.891	0.005	**0.025**
		Providencia	0.890	0.005	**0.025**
		Rare taxa	0.946	0.005	**0.025**

*, the Bonferroni corrected *P*-value

Treatment effects on pupal bacterial community. The nMDS plot (dimension=3) allow for visualizing the beta diversity across diet and bacterial treatments for the pupal stage (Figure 16). The nMDS plot has an overall stress of 0.0808, with 57.79% beta diversity explained by x-axis, and 23.16% beta diversity explained by y-axis.

PerMANOVA indicated bacterial treatment is impacted bacterial community structure associated with the pupal stage ($P = 0.037$, pseudo-$F_{3,22} = 2.180$), while the diet treatment is not ($P > 0.05$, Table 28). Not interaction effect was significant ($P > 0.05$, Table 28). Based on pairwise MRPP analyses, the highest *P. mirabilis* and the *Salmonella* treatment (Table 29) were significant ($P = 0.048$, delta = 0.426) drivers of these differences. ISA indicated *Proteus* (46.77% of the total bacterial composition of the pupae), *Lactobacillus* (16.08% of the total bacterial composition of the pupae), *Morganella* (8.62% of the total bacterial composition of the larvae), *Providencia* (3.96% of the total bacterial composition of the larvae), and *Salmonella* (0.59% of the total bacterial composition of the pupae) were the primary genera driving these differences (Table 30).

Figure 16 | nMDS plots on normalized count data of diet treatment and exogenous bacterial treatment effects on pupal stage of *Lucilia sericata* reared on combinations of three diets and two exogenous bacteria (*Proteus mirabilis* and *Salmonella*) in an incubation room set at 27˚C, 70% RH, 14:10 L:D, n=3

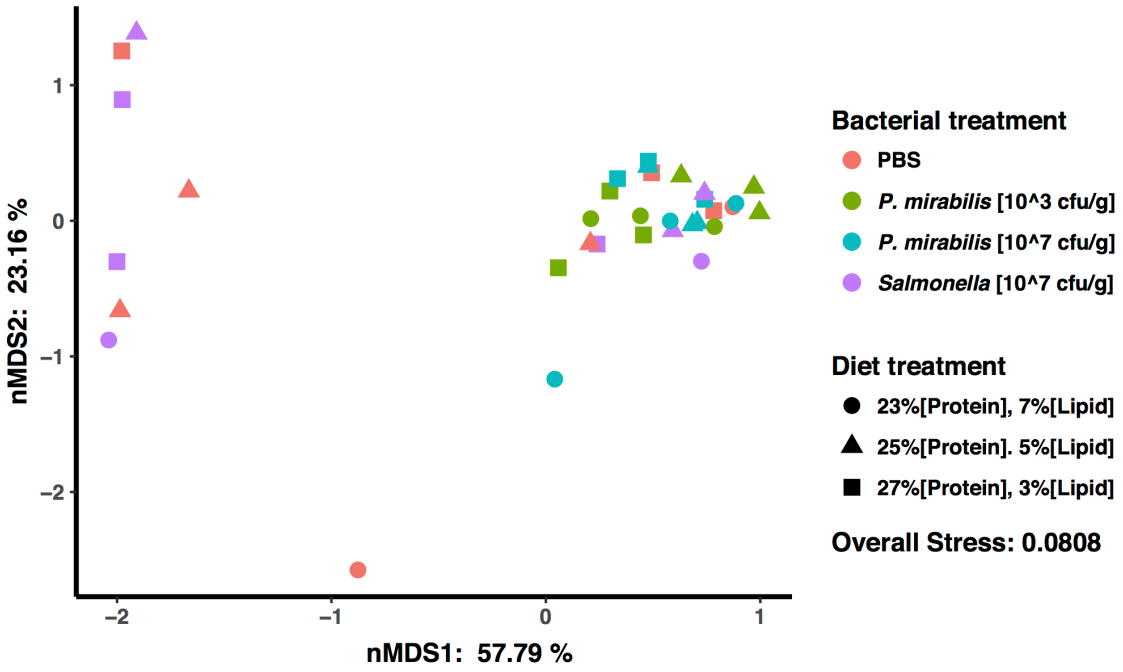


Table 28 | PerMANOVA of normalized bacterial community of pupal stage of *Lucilia sericata* reared on combinations of three diets and two exogenous bacteria in an incubation room set at 27˚C, 70% RH, 14:10 L:D, n=3

Factor	DF	SumSq	Pseudo-F	*P*
Diet treatment	2	0.318	1.319	0.260
Bacterial treatment	3	0.788	2.180	**0.037**
Diet treatment: Bacterial treatment	6	0.864	1.194	0.247
Residual	22	2.652		

Table 29 | Multi-Response Permutation Procedure (MRPP) of normalized bacterial community of pupal stage of *Lucilia sericata* reared on combinations of three diets and two exogenous bacteria (*Proteus mirabilis* and *Salmonella*) in an incubation room set at 27˚C, 70% RH, 14:10 L:D, n=3

Bacterial treatment	-Bacterial treatment	delta	*P*	*P**
PBS	*P. mirabilis* (10^3 cfu/g)	0.462	0.085	0.510
PBS	*P. mirabilis* (10^7 cfu/g)	0.468	0.066	0.396
PBS	*Salmonella* (10^7 cfu/g)	0.620	0.951	1.000
P. mirabilis (10^3 cfu/g)	*P. mirabilis* (10^7 cfu/g)	0.288	0.711	1.000
P. mirabilis (10^3 cfu/g)	*Salmonella* (10^7 cfu/g)	0.420	0.028	0.168
P. mirabilis (10^7 cfu/g)	*Salmonella* (10^7 cfu/g)	0.426	0.008	**0.048**

*, the Bonferroni corrected *P*-value

Table 30 | Indicator Species Analyses (ISA) of normalized bacterial community of pupal stage of *Lucilia sericata* reared on combinations of three diets and two exogenous bacteria (*Proteus mirabilis* and *Salmonella*) in an incubation room set at 27˚C, 70% RH, 14:10 L:D, n=3

Comparisons	Treatments	ISA	STAT	*P*	*P**
Comparison 1	*P. mirabilis* (10^7 cfu/g)	***Proteus***	0.812	0.040	**0.080**
	Salmonella (10^7 cfu/g)	***Salmonella***	0.846	0.010	**0.020**
		Lactobacillus	0.856	0.020	**0.040**
		Providencia	0.851	0.020	**0.040**
		Morganella	0.839	0.025	**0.050**

*, the Bonferroni corrected *P*-value

Treatment effects on adult bacterial community. The nMDS plot (dimension=3) allow for visualizing the beta diversity across diet and bacterial treatments for the pupal stage (Figure 17). The nMDS plot has an overall stress of 0.0985, with 45.01% beta diversity explained by x-axis, and 32.68% beta diversity explained by y-axis.

PerMANOVA indicated diet treatment (P = 0.016, pseudo-$F_{2,22}$ = 2.112) is significantly impacted bacterial community structure associated with the adult stage, while the bacterial treatment is not (P > 0.05, Table 31). Not interaction effect was significant (P > 0.05, Table 29). However, Pairwise MRPP applied for comparing diet effects on bacterial communities associated with resulting adults were not significantly different (Table 32).

Figure 17 | nMDS plots on normalized bacterial community on adult stage of *Lucilia sericata* reared on combinations of three diets and two exogenous bacteria (*Proteus mirabilis* and *Salmonella*) in an incubation room set at 27˚C, 70% RH, 14:10 L:D, n=3

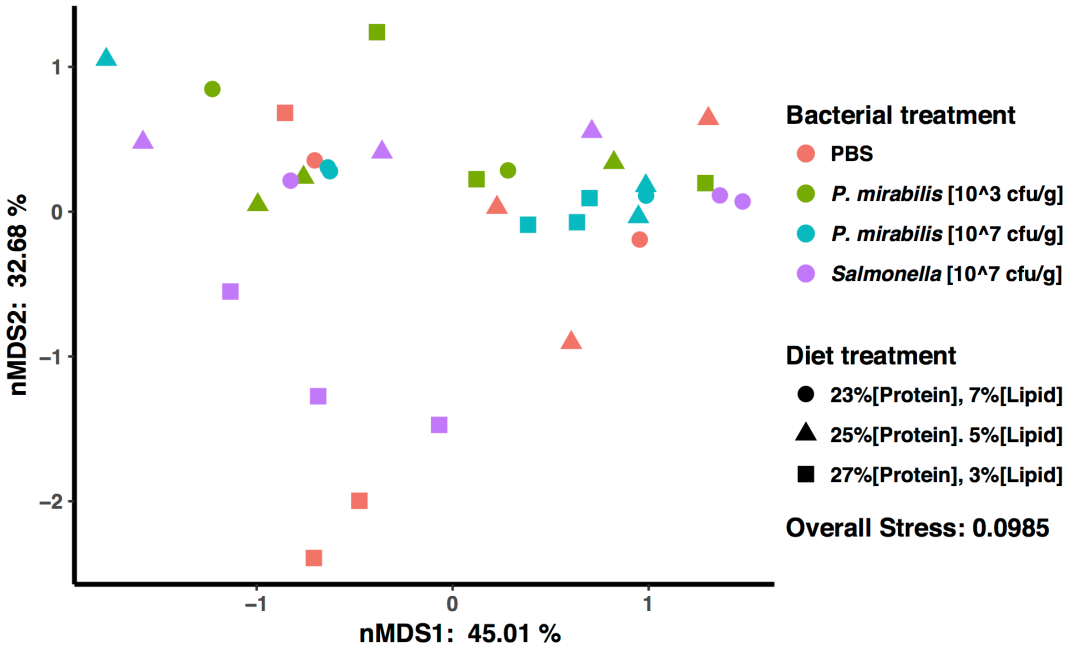


Table 31 | PerMANOVA of normalized bacterial community of adult stage of *Lucilia sericata* reared on combinations of three diets and two exogenous bacteria (*Proteus mirabilis* and *Salmonella*) densities in an incubation room set at 27˚C, 70% RH, 14:10 L:D, n=3

Factor	DF	SumSq	Pseudo-F	*P*
Diet treatment	2	0.860	2.112	**0.016**
Bacterial treatment	3	0.773	1.265	0.230
Diet treatment: Bacterial treatment	6	1.488	1.218	0.207
Residual	22	4.478		

Table 32 | Multi-Response Permutation Procedure (MRPP) of normalized bacterial community of adult stage of *Lucilia sericata* reared on combinations of three diets and two exogenous bacteria (*Proteus mirabilis* and *Salmonella*) in an incubation room set at 27˚C, 70% RH, 14:10 L:D, n=3

Diet treatment	-Diet treatment	delta	*P*	*P**
23%[Protein],7%[Lipid]	25%[Protein],5%[Lipid]	0.656	0.153	0.459
23%[Protein],7%[Lipid]	27%[Protein],3%[Lipid]	0.626	0.031	0.093
25%[Protein],5%[Lipid]	23%[Protein],7%[Lipid]	0.586	0.136	0.408

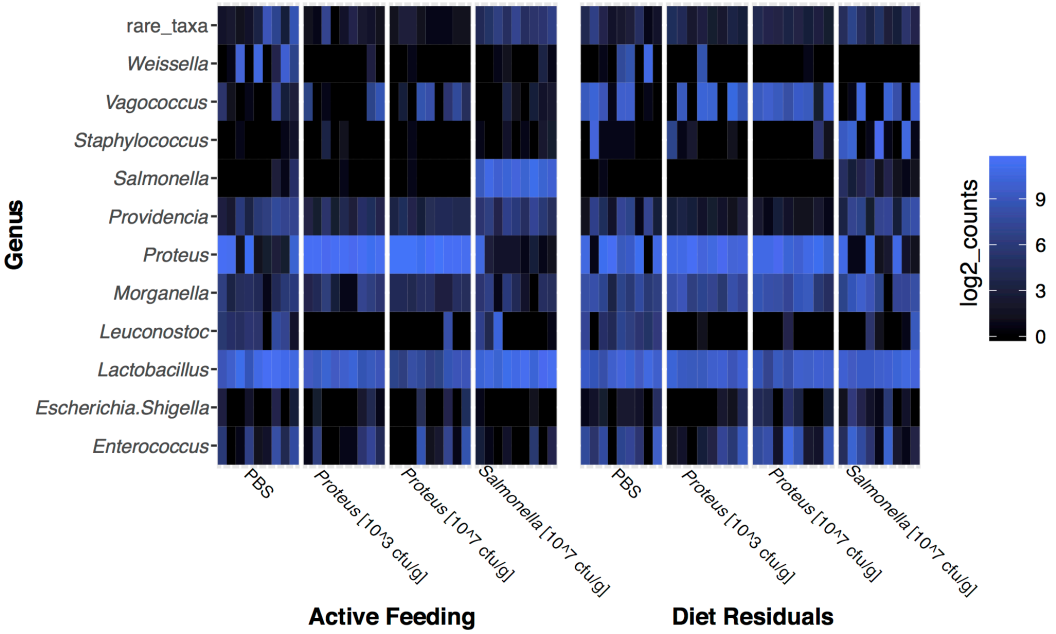
*, the Bonferroni corrected *P*-value

Bacterial community diversity indices across feeding stage on diet residual. A heatmap for genera abundance between active-feeding-stage diet residual and post-feeding-stage diet residual (Figure 18). The nMDS (dimension=3) has overall stress at 0.0672 with 65.32% and 22.81% beta diversity on x-axis and y-axis, respectively (Figure 19).

PerMANOVA further indicated bacterial treatments in conjunction with life stage of the blow fly significantly impacted (P = 0.001, pseudo-$F_{3,46}$ = 1.045) the bacterial community associated with the residual diet (Table 33). Pairwise MRPP indicate only during the active feeding stage, all the bacterial treatments were significant different

from each other (6 pairwise comparison, see Table 34 for details). Further for the diet residual (after active feeding stage), both the highest bacterial treatment in *P. mirabilis* and the *Salmonella* treatments were the primary drivers (P = 0.042, delta = 0.447, see Table 35 for details) of these differences. ISA indicate *Proteus* (43.67% of the total bacterial composition on diet residuals), *Lactobacillus* (27.33% of the total bacterial composition on diet residuals), *Salmonella* (4.53% of the total bacterial composition on diet residuals), *Leuconostoc* (1.40% of the total bacterial composition on diet residuals), *Providencia* (1.29% of the total bacterial composition on diet residuals), as well as the rare taxa with abundance less than 0.01% of the community (Table 36 and 37).

Figure 18 | Heatmap of bacterial genera in residual diet after being fed to *Lucilia sericata* larvae in an incubation room set at 27˚C, 70% RH, 14:10 L:D, n=9



A $\log_2(x+1)$ transformation applied on the count table. The lighter blue the color is, the higher the abundance of the genus.

Figure 19 | nMDS plots of bacterial genera in residual diet after being fed to *Lucilia sericata* larvae in an incubation room set at 27˚C, 70% RH, 14:10 L:D, n=9

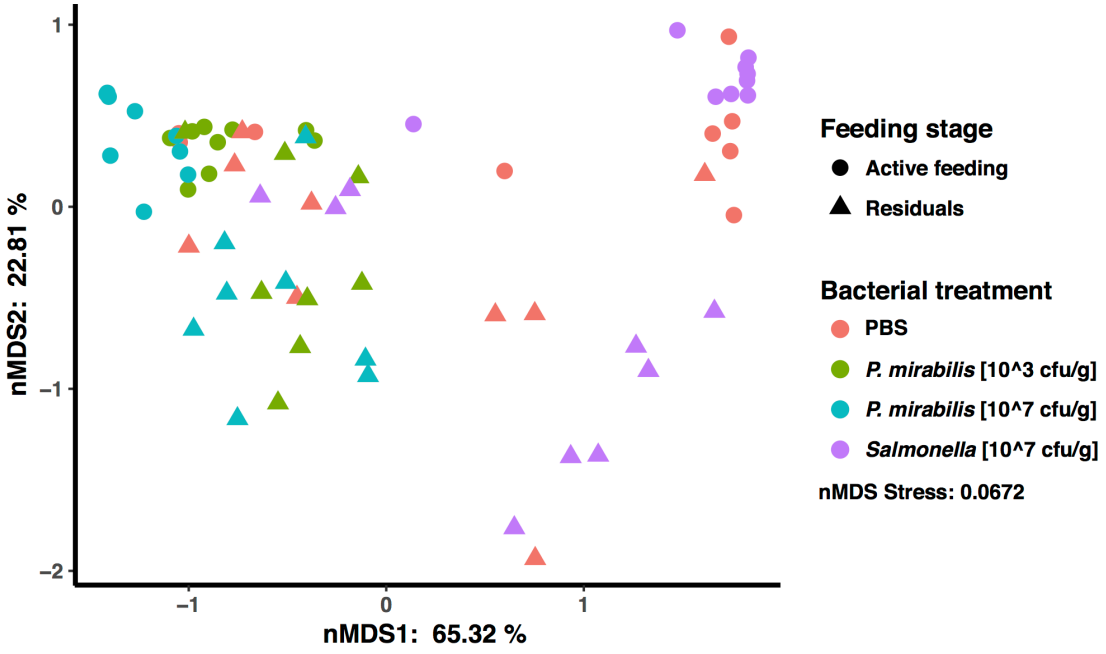


Table 33 | PerMANOVA of normalized bacterial community of diet residuals from *Lucilia sericata* post-feeding after being reared on combinations of three diets and two exogenous bacteria (*Proteus mirabilis* and *Salmonella*) in an incubation room set at 27˚C, 70% RH, 14:10 L:D, n=3

Factor	DF	SumSq	Pseudo-F	*P*
Diet treatment	2	0.354	1.909	0.072
Bacterial treatment	3	4.321	15.522	**0.001**
Type	1	1.318	14.209	**0.001**
Diet treatment: Bacterial treatment	6	0.320	0.575	0.896
Type: Diet treatment	2	0.091	0.492	0.808
Type: Bacterial treatment	3	1.045	3.753	**0.001**
Type: Diet treatment: Bacterial treatment	6	0.319	0.573	0.912
Residual	46	4.268		

Table 34 | Multi-Response Permutation Procedure (MRPP) of normalized bacterial community of the active-feeding stage of *Lucilia sericata* when being reared on combinations of three diets and two exogenous bacteria (*Proteus mirabilis* and *Salmonella*) in an incubation room set at 27˚C, 70% RH, 14:10 L:D, n=9

Bacterial treatment	-Bacterial treatment	delta	*P*	*P**
PBS	*P. mirabilis* (10^3 cfu/g)	0.330	0.004	**0.024**
PBS	*P. mirabilis* (10^7 cfu/g)	0.339	0.001	**0.006**
PBS	*Salmonella* (10^7 cfu/g)	0.409	0.001	**0.006**
P. mirabilis (10^3 cfu/g)	*P. mirabilis* (10^7 cfu/g)	0.134	0.004	**0.024**
P. mirabilis (10^3 cfu/g)	*Salmonella* (10^7 cfu/g)	0.205	0.001	**0.006**
P. mirabilis (10^7 cfu/g)	*Salmonella* (10^7 cfu/g)	0.214	0.001	**0.006**

*, the Bonferroni corrected *P*-value

Table 35 | Multi-Response Permutation Procedure (MRPP) of normalized bacterial community of the post-feeding stage of *Lucilia sericata* when being reared on combinations of three diets and two exogenous bacteria (*Proteus mirabilis* and *Salmonella*) in an incubation room set at 27˚C, 70% RH, 14:10 L:D, n=9

Bacterial treatment	-Bacterial treatment	delta	*P*	*P**
PBS	*P. mirabilis* (10^3 cfu/g)	0.413	0.324	1.000
PBS	*P. mirabilis* (10^7 cfu/g)	0.415	0.178	1.000
PBS	*Salmonella* (10^7 cfu/g)	0.539	0.204	1.000
P. mirabilis (10^3 cfu/g)	*P. mirabilis* (10^7 cfu/g)	0.307	0.692	1.000
P. mirabilis (10^3 cfu/g)	*Salmonella* (10^7 cfu/g)	0.445	0.010	0.060
P. mirabilis (10^7 cfu/g)	*Salmonella* (10^7 cfu/g)	0.447	0.007	**0.042**

*, the Bonferroni corrected *P*-value

Table 36 | Indicator Species Analysis (ISA) of normalized bacterial community of the active-feeding stage of *Lucilia sericata* when being reared on combinations of three diets and two exogenous bacteria (*Proteus mirabilis* and *Salmonella*) in an incubation room set at 27˚C, 70% RH, 14:10 L:D, n=9

Comparisons	Treatments	ISA	STAT	*P*	*P**
Comparison 1	PBS	***Leuconostoc***	0.943	0.005	**0.03**
		Providencia	0.905	0.020	0.12
		Lactobacillus	0.838	0.015	0.09
		Weissella	0.816	0.025	0.15
	P. mirabilis (10^3 cfu/g)	*Proteus*	0.848	0.010	0.06
Comparison 2	PBS	***Lactobacillus***	0.934	0.005	**0.03**
		Providencia	0.912	0.020	0.12
		Weissella	0.816	0.030	0.18
		Rare Taxa	0.989	0.025	0.15
	P. mirabilis (10^7 cfu/g)	***Proteus***	0.862	0.005	**0.03**
Comparison 3	PBS	*Enterococcus*	0.898	0.035	0.21
	Salmonella (10^7 cfu/g)	***Salmonella***	0.998	0.005	**0.03**
Comparison 4	*P. mirabilis* (10^3 cfu/g)	***Lactobacillus***	0.862	0.005	**0.03**
	P. mirabilis (10^7 cfu/g)	*Proteus*	0.730	0.040	0.24
Comparison 5	*P. mirabilis* (10^3 cfu/g)	***Proteus***	0.968	0.005	**0.03**
	Salmonella (10^7 cfu/g)	***Salmonella***	1.000	0.005	**0.03**
		Providencia	0.890	0.005	**0.03**
		Lactobacillus	0.849	0.005	**0.03**
Comparison 6	*P. mirabilis* (10^7 cfu/g)	***Proteus***	0.969	0.005	**0.03**
	Salmonella (10^7 cfu/g)	***Salmonella***	1.000	0.005	**0.03**
		Lactobacillus	0.939	0.005	**0.03**
		Providencia	0.898	0.005	**0.03**
		Rare taxa	0.976	0.005	**0.03**

*, the Bonferroni corrected *P*-value

Table 37 | Indicator Species Analysis (ISA) of normalized bacterial community of the post-feeding stage of *Lucilia sericata* when being reared on combinations of three diets and two exogenous bacteria (*Proteus mirabilis* and *Salmonella*) in an incubation room set at 27˚C, 70% RH, 14:10 L:D, n=3

Comparisons	Treatments	ISA	STAT	P	P*
Comparison 1	*P. mirabilis* (10^7 cfu/g)	*Proteus*	0.848	0.040	0.080
	Salmonella (10^7 cfu/g)	***Salmonella***	1.000	0.005	**0.010**
		Lactobacillus	0.790	0.010	**0.020**
		Providencia	0.989	0.005	**0.010**
		Staphylococcus	0.878	0.020	0.040
		Rare taxa	0.873	0.010	**0.020**

*, the Bonferroni corrected *P*-value

Discussion

Blow flies colonize a variety of decomposing vertebrate remains (Greenberg 1991). Such ability has resulted in tremendous evolutionary success for these species in many ecosystems (Benbow et al 2015). However, their ability to colonize, develop, and produce adults is highly dependent on the nutritional quality of the larval resource (Daniels et al 1991). Furthermore, recent publications indicate resource in combination with associated bacteria co-vary with regards to development responses by flies competing for these resources (Ferrandon et al 2007, Gerardo et al 2010, Gottar et al 2002).

Previous studies examined variation in *L. sericata* development in association with bacteria and diets, such as beef liver or a yeast-based diet; however, nutritional composition was lacking or coarsely described. Crooks *et al* (2016) determined bacterial effects on the development of *L. sericata* with a nutritional-undefined diet, while Singh *et al* (2015) indicated a similarity of bacterial community between *L. sericata* and a

single diet, beef liver. However, confounding factors in these studies prevented the investigation of specific factor effect, such as protein/lipid concentrations or variation in microbial communities. In order to conduct a more specific investigation into the factors driving development variation, the geometric framework in nutrition was implemented with the use of a sterile diet where macronutrients, such as carbohydrate, protein, and lipid amounts and types were defined (Simpson and Raubenheimer 2012). Doing so, allowed for a more refined consideration of the factors (e.g., diet and exogenous bacteria) driving the responses observed by blow fly larvae competing for ephemeral resources.

We demonstrate both factors (diet and exogenous bacteria) play a role in the manifestation of measured life-history traits (e.g., larval development and survival, pupal development and survival, and adult longevity). One notable observation of diet treatment effects is *L. sericata* had accelerated (~6%) larval development (Figure 4) as the protein: lipid ratio increased. Moreover, the higher the protein: lipid ratio, resulted in greater survivorship (~12%) to the pupal (Figure 7) and adult stages (Figure 9). One possible explanation (i.e., diet restriction hypothesis) is that there is a threshold in accumulation of protein acquisition for progressing from the larval to pupal stages, and lipids are less desirable (Eigenbrode and Espelie 1995). Hence, *L. sericata* larvae on the high protein: lipid ratio diet was able to accumulate adequate protein with little inhibitory effects due to lipids. For example, *L. sericata* failed to survive on the diet with lipid greater than 7% (preliminary studies). These data are supported by previous studies indicating blow flies demonstrate a high propensity to colonize high-protein resource,

such as carrion (Benbow et al 2015, Greenberg 1991, Villet 2011).

Explaining the impact of exogenous bacteria on the development of *L. sericata* is more complex as the role of microbes is highly dependent on the specific microbe being examined in conjunction with its physical attributes at the time of the study (e.g., concentration or development stage) (Janzen 1977). Such factors often translate into ecological function. In some instances, a microbe might be categorized as a mutualist, while at other concentrations the microbe could act as a commensal, pathogen, or competitor. For example, *Klebsiella oxytoca* produces a cue attracting (low concentration) or repelling (high concentration) *M. domestica* seeking oviposition sites. This mechanism is hypothesized as a nutrition resource protection behavior of *K. oxytoca*, and such mechanism may serve as an overpopulation cue for the gravid female (Lam et al 2007, Lam et al 2009b) Moreover, the impacts from the exogenous bacteria may depend on the stage of the host. For instance, the lifespan of the axenic adult *Drosophila* is enhanced by introducing exogenous bacteria during the first week after emergence, while the lifespan is reduced if the exogenous bacteria is introduced later than the first week (Brummel et al 2004). Furthermore, Liu (2016) demonstrated suppression of microbes (e.g., *P. mirabilis* through the use of antimicrobials) associated with *L. sericata* larvae resulted in their avoiding diets lacking essential amino acids (e.g., methionine); however, when microbes are not suppressed, the larvae would actively feed on these diets. With regards to *P. mirabilis*, it can synthetize methionine. These data suggest larvae often depend on select microbes for the production of essential nutrients possibly not present in a target resource. Such a relationship allows *L. sericata* to be

more plastic in terms of what it can utilize for larval development.

In our study, exogenous bacterial treatments impacted *L. sericata* throughout all development stages. More specifically, each stage experienced an extended duration (~6.67% in larval stage, ~7.14% in pupal stage, and 24.57% in adult stage) regardless of exogenous bacterial species and concentration (i.e., duration of larval, pupal, and adult stage, Figure 5, 8, 11). Possible mechanisms of extending the total lifespan are discussed in detail further down in the discussion. However, extending duration at different life stage may lead to different ecological implication. For example, a longer duration of larval stage could increase the probability of predation by competing blow fly larvae (Brundage et al 2014, Flores et al 2014) or increased risk of parasitism (Roberts 1933). Nonetheless, a longer duration of adult stage raise the chance of mating, and hereby, it leads to an increased reproduction.

These results are confounding when one considers *P. mirabilis* is considered a beneficial bacterium for *L. sericata* due to its ability to produce "mirabilicides" that suppress bacterial competitors and pathogens (Greenberg 1968) as well as reduce *Salmonella* (Greenberg 1965, Greenberg et al 1970). One potential hypothesis is *P. mirabilis* establishes a "cross-talk" with *L. sericata* in terms of metabolic pathway, and the metabolic molecule from *P. mirabilis* may extend lifespan of *L. sericata*. For example, cross-talk has been demonstrated between *Bacillus subtilis* and *Caenorhabditis elegans*. In this case, the bacterial-originated nitric oxide (NO) induced the activity of heat shock proteins (HSPs) in *C. elegans* resulting in increased host lifespan (Gusarov et al 2013). In our case, both *P. mirabilis* and *Salmonella* are Enterobacteriaceae, which are

capable of producing NO (Arkenberg et al 2011). While we recognize this as conjecture on our part, a similar model could exist in the system explored in this study. Thus, a further study at the molecular level is needed to determine if this phenomenon applies in this case.

Bacterial diversity associated with *L. sericata* increased as the insect progressed through its life cycle regardless of diet (Figure 12). For instance, the alpha diversity (represented by Shannon index) of bacterial community of pupal *L. sericata* was approximately 40% greater than larvae; and, diversity of the adult was 25% greater than that found with the pupa (Figure 13). Clearly, *L. sericata* regulates its bacterial community more during larval active feeding under controlled conditions. Because the environment (i.e., carrion) occupied by blow fly larvae is considered extreme (e.g., pathogenic microbes, unpredictable duration or occurrence, high competition), *L. sericata* larvae have evolved several low-cost mechanisms for eliminating surrounding and ingested bacteria, such as production of antimicrobials present in their excretion/secretions.

Even though *L. sericata* is able to rigorously regulate bacterial community as larvae, such capabilities were challenged when exogenous bacteria were introduced at high doses. For example, both *P. mirabilis* and *Salmonella* treatment at high dosage (10^7 cfu/g) reduced (8%) the survivorship of *L. sericata* to the adult stage (Table 20). Moreover, the bacterial community of *L. sericata* was significantly altered by the exogenous bacterial treatment at the high dosage (10^7 cfu/g for both *P. mirabilis* and *Salmonella* treatments), which could explain the decreased survivorship due to decreased

environmental stability for the larvae. However, what was interesting was the community composition converged across treatments when the adult stage was reach (Figure 12, Figure 14). For example, the exogenous bacterial treatment is divided into three groups by the community similarity (Table 26, Table 25): Group 1, PBS and Low dosage of *P. mirabilis* with indicator genus *Lactobacillus* (average indicator value: 0.861); Group 2, High dosage of *P. mirabilis* with indicator genus *Proteus* (average indicator value 0.872); Group 3, High dosage of *Salmonella* with indicator genera *Salmonella* (average indicator value 0.995), *Providencia* (average indicator value 0.872), and the collection of rare taxa (average indicator value 0.934). The grouping demonstrates high dosage of exogenous bacterial treatments interrupted the regulation of bacterial community at larval stage. *Lactobacillus* is commonly associated with *L. sericata* and their natural habitat (i.e., carrion) and suppresses harmful bacteria by creating an acidic environment (Singh et al 2015). In group 2, *Proteus*, which as previously mentioned is capable of suppressing competing bacteria (Greenberg 1968), was dominant. But, no single "bacteria" was determined in group 3; however, rare taxa were found to be an important indicator. Shifts between *P. mirabilis* and *Salmonella* populations within treatments could be due to *P. mirabilis* being known to suppress *Salmonella* in fly larvae (Greenberg 1970). Nonetheless, the groupings at larval stage are diminished when reach the adult stage (Table 31). Hereby, the bacterial community analysis supports previous statement of relaxing bacterial regulation as the development of *L. sericata* progressing.

According to the measurement of bacterial community on diet residuals, we

further demonstrate the regulation of the surrounding environment bacterial community by *L. sericata* (Figure 18, Figure 19). For instance, when larvae of *L. sericata* were actively feed on diets, the diets had dissimilar groupings and indicator genera (Table 34, Table 36). However, the diverged bacterial community on diets was converged as the larval regulation taking off from the diet (i.e., the bacterial community on diets after pupation).

In conclusion, this study provides a great of insight of exogenous-bacterial-influenced nutritional effects on *L. sericata* in terms of development and regulation of bacterial community. According to our results, there is a complex linkage between the bacterial community and development of *L. sericata*, and the outcome of such interactions is depended on the development stage of the insect and the concentration of the bacteria. The nutritional effects were present for all development stage of *L. sericata*, while the exogenous bacterial treatments confined the effects at larval stage. However, in order to further exploring the linkage, further studies are needed; specifically, the effects of diet and bacteria on surviving *L. sericata* fecundity as well as the molecular pathways (e.g., gene expression in the fly as well as the bacteria) responding to these factors.

CHAPTER IV

SUMMARY AND SUGGESTIONS FOR FUTURE RESEACH

In order to study the bacterial dimension of nutritional ecology as related to blow fly (Diptera: Calliphoridae) development, I initially focused on developing a sterile diet for the rearing *Lucilia sericata*, a common blow fly model. Doing so would provide a platform to explore questions on this topic. I evaluated several diets currently in the literature. These included powdered beef liver diet (See Chapter 2 for details of the ingredient), decomposed beef liver diet (Sherman and Tran 1995), powdered fish diet (See Chapter 2 for details of the ingredient), blood agar diet (Daniels et al 1991), chemical-defined diet (Barlow and Kollberg 1971), and milk based diet (Tachibana and Numata 2001). Through these efforts, I determined blood agar diet and decomposed beef liver diet produced similar life-history traits of *L. sericata* as the beef liver (control). Moreover, the evaluated diets can be autoclaved, so they are potential resources for use in studies requiring sterile maggots (Daniels et al 1991). However, those diets are not well defined in nutritional composition, and this limitation restricted my further studies in the framework of nutrition ecology (Raubenheimer and Boggs 2009, Raubenheimer et al 2009, Simpson and Raubenheimer 2012). In the end, I determined a diet commonly used in nutrition ecology studies with herbivores would be most appropriate for my future studies. However, the results of this study could be used in developing nations for rearing sterile larvae for medical purposes as they are inexpensive to prepare and most ingredients are readily available.

My second set of experiments explored the interactions of protein-lipid ratios in combination with the introduction of exogenous bacteria (*Proteus mirabilis* and *Salmonella*) on the development of *L. sericata* and its associated microbial community. I determined diet and exogenous bacteria did in fact impact the life-history traits of *L. sericata.* For example, the higher protein: lipid in the diet is (27% protein: 3% lipid, the greater survivorship to pupal and adult stage of *L. sericata* (~12% higher). In addition, introducing the exogenous bacteria has uniformly increased the lifespan of *L. sericata* at all development stages. Moreover, according to the bacteria community analyses, *L. sericata* is able to regulate itself and surrounding bacterial community under the alternation of the ingested nutrients (i.e., different protein: lipid ratio). However, introducing the exogenous bacteria to the diets disrupted regulation of the associated microbial community. As previously stated, studies, which examined the interaction effects of diet and exogenous bacteria in the vertebrate carrion system, did not provide a precise diet treatment in terms of nutritional composition (Barnes and Gennard 2011, Crooks et al 2016, Singh et al 2015). Thus, my study serves as a cornerstone for further precise exploration of the bacterial dimension in nutritional ecology discussed as below.

My study could serve as a foundation on which future research in nutrition ecology is built. For example, my study hypothesized that the activation of the Heat Shock Protein (HSP) pathway by Nitric Oxide (NO) may contribute to the extension of total lifespan for *L. sericata*. In the case of the bacteria used in my experiments, NO is a known metabolic product. Thus, it is possible this pathway also explains the extended life of flies in my experiments. However, in order to further investigate such effects in detail, a

molecular level research is needed, such as a total gene expression analysis of the bacterial community (Leimena et al 2013). Furthermore, a measurement on fecundity of *L. sericata* is necessary for studying the interaction between diet and exogenous bacteria, because fecundity is a presumptively ultimate goal of an organism.

However, there were limitations to this study. The study focused on only two macronutrients, protein and lipid; thus, interactions between nutrients, such as protein with carbohydrate, obviously were not investigated. Moreover, the study is unable to provide molecular insight of the macronutrients as they relate to insect physiology; such studies could provide greater understanding of the nutrient-host interaction. For example, the dietary-restriction on calorie usually lead to a trade-off between longevity and fecundity, and the mechanism has been explained by the imbalance diet of amino acids (Grandison et al 2009). Furthermore, my study restricted to the selected bacterial species at the given concentrations, and the selected bacteria are belonged to the Enterobacteriaceae. Nonetheless, the study neglected other bacteria that may lead to a different impact on the host (Moran and Bennett 2014).

My study may serve as a primer for further ecological studies: the nutrients-defined diets provides a basic foundation for studying the community succession in a finer scale, and the sterilized diets offer a platform for studying the bacterial-mediated completion of insect community in carrion system (Benbow et al 2015). Furthermore, the outcomes from my study can apply in forensic entomology. For example, different tissues were used in forensic entomology studies, such as beef liver, beef brain, or beef lung (Clark et al 2006). Hereby, the case study from a forensic investigation is able to

rely on those studies for estimating the minimum Postmortem Interval (PMI). Nonetheless, according to my study, it is certainly important to take into account the nutritional composition of decomposing remains as related to forensic entomology. In addition to the forensic entomology studies, my study can also contribute to the medical entomology studies, such as the maggot therapy, because the nutrients-defined sterilized diet is an ideal food source for understanding the elimination of bacteria in the maggot therapy (Daniels et al 1991, Sherman and Tran 1995).

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