Winter 12-14-2018

Black Soldier Fly Larvae as Value-Added Feed for Aquaculture in Maine

Joshua Villazana
University of Maine, joshua.villazana@gmail.com

BLACK SOLDIER FLY LARVAE AS VALUE-ADDED FEED

FOR AQUACULTURE IN MAINE

By

Joshua Villazana

B.A. University of Texas at Brownsville, 2013

A THESIS

Submitted in Partial Fulfillment of the

Requirements for the Degree of

Master of Science

(in Entomology)

The Graduate School

The University of Maine

December 2018

Advisory Committee:

Andrei Alyokhin, Professor of Applied Entomology, Advisor

Allison Gardner, Assistant Professor of Arthropod Vector Biology

Edward Bernard, Lecturer of Microbiology

BLACK SOLDIER FLY LARVAE AS VALUE-ADDED FEED FOR AQUACULTURE IN MAINE

By

Joshua Villazana

Thesis Advisor: Dr. Andrei Alyokhin

An Abstract of the Thesis Presented
in Partial Fulfillment of the Requirements for the
Degree of Master of Science
(in Entomology)

December 2018

Black soldier flies (BSF), *Hermetia illucens* (Linnaeus) (Diptera: Stratiomyidae) consume decaying organic waste as larvae (BSFL) and can be used for recycling a variety of biogenic wastes. BSFL can also be processed into value-added animal feeds, including those used in aquaculture. An overarching goal of this project was to obtain additional insights into BSF biology to improve their rearing and handling in future mass-production facilities serving Maine aquaculture.

We tested BSFL growth in the laboratory on seven seafood wastes from Maine fish processing facilities. Substrates potentially suitable for BSFL rearing included finfish trimmings, wet sea cucumber, dry quahog, and sea urchin. Crab meal produced large larvae in short time, but larval mortality was high. There were dramatic differences in seemingly similar materials, such as wet and dry sea cucumber or wet and dry quahog, highlighting the importance of thorough testing of specific substrates instead of extrapolating from published data.

We investigated antixenotic and antibiotic effects of finfish substrate inhabited by BSFL, which are important for hygiene in mass-rearing facilities. Green blow flies, *Lucilia sericata* (Meigen) (Diptera: Calliphoridae), were reluctant to colonize substrates previously inhabited by BSFL even after the latter were sifted out. When released into BSFL-containing substrates, the majority of *L. sericata* larvae emigrated. There was significantly lower enzymatic activity as measured by fluorescein diacetate in substrate inhabited by BSFL than in the control. Subsequent plating and enumeration of colony forming units attributed that to decline in bacterial abundance. Fungal abundance were not significantly different from the control when BSFL were present but increased after their removal. BSFL also altered the taxonomic composition of microbial communities in finfish substrate, as estimated by a diversity assay using bTEFAP® illumina sequencing with 20k reads of fungal ITS and 16s primers.

Migration of BSF prepupae is commonly exploited for self-harvesting biomass in commercial rearing facilities. We tested if harvesting larvae prior to the prepupal stage can produce larger larvae than those harvested as prepupae. Our results indicated that larvae were at their highest weight immediately before transition into prepupae. Therefore, the late larval instar before reaching prepupae can be considered as the optimal harvest period for larval biomass.

BSF are normally found in subtropical and warm temperate regions. Potentially, cold temperatures can also be used to manipulate the rate of BSFL development, which may be needed for obtaining certain life stages in mass-production facilities. We determined that BSFL were highly susceptible to freezing. Chilling at above-freezing temperature also had a significant negative effect. However, it was much smaller, especially for fifth instars. We do not anticipate that BSF will become an invasive species in Maine. Low temperatures may be used to manipulate development of the late instars, but at a cost of higher mortality.

ACKNOWLEDGEMENTS

I would first like to thank my advisor Andrei Alyokhin. The door to Andrei's office was always open whenever I ran into a trouble spot or had a question about my research or writing. He steered me in the right the direction whenever I needed it. Without him, this project would not have been possible. I would also like to thank my committee who were involved in this research project: Edward Bernard, Allison Gardner, and Eleanor Groden. Without their passionate participation and input, this project would not have been successfully conducted.

I am grateful for the many people who provided technical assistance in the project. Thank you to: Hanna Anderson, Alex Baron, Dylan Cunningham, Douglas Everett, Jason Rose, Anna Landry, Merab Apkhazava, Sean Driscoll, Segolene Podvin, and Mellissa Smith. Thank you to Patrick Erbland for allowing me to consult you on the parts of the project dealing with engineering problems and his late nights spent with the GC-SM data, Brad Libby for helping keep a safe and sterile environment in the greenhouse and Scot E. Dowd for being available to answer my questions about the sequence data his company provided. Thanks to all the Maine Entomology Society members for always asking excellent questions about my research and feedback on my project.

Finally, I must express my very profound gratitude to my mom for her unconditional support and my spouse, Liang Chen for providing me with unfailing support and continuous encouragement throughout my years of study and through the process of researching and writing this thesis. As well as my wonderful lab mates, Jonas Insinga and Andy Galimberti and fellow graduate students Chase Gagne and Karla Boyd. This accomplishment would not have been possible without them. Thank you.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

BSF – black soldier fly

BSFL – black soldier fly larvae

SBSF – sieved black soldier fly

FDA – fluorescein diacetate

MSW – municipal solid waste

NOAA – National Oceanic and Atmospheric Administration

ALA – alpha-linolenic acid

EPA – eicosapentaenoic acid

DHA – docosahexaenoic acid

EFA – essential fatty acids

DMR – Maine Department of Marine Resources

HSD – honestly significant difference

TSA – tryptic soy agar

PDA – potato dextrose agar

CFU – colony forming units

OTU – operational taxonomic units

CHAPTER 1

THE USE OF BLACK SOLDIER FLY LARVAE AS A BENEFICIAL RECYCLER OF ORGANIC WASTES

1.1 Waste management challenge

Sustainable waste management is one of the most important issues facing highly industrialized modern-day societies. Americans generated approximately 258 million tons of post-consumer municipal solid waste (MSW) in 2014 (US EPA 2016). Current efforts to improve environmental impact of waste management practices involve reduction, reuse, recycling and composting. However, landfilling and incineration are still the most common method for final disposal of solid wastes (US EPA 2016). The increasing concerns about sustainability of burying waste in landfills have caused a shift in public opinion towards preference for alternative methods. There are programs to reduce MSW, such as recycling and the reuse of resources, as well as organic composting. However, it is not simple, especially when animal protein wastes are used (Arvanitoyannis and Kassaveti 2008).

Additionally, large amounts of pre-consumer organic waste originate from over production, shelf life expiration, trim waste, and, spoilage. Each year, between 56 and 72 million tons of food are not consumed throughout food supply chain in the United States (Gunders 2017). Food can be lost on farms, during distribution, storage, in retail stores, food service operations, and in households. It has been estimated that as much as 25 percent of the world's food is lost post-harvest due to microbial spoilage, while 40 percent of America's food is wasted due to inefficient production and careless consumer habits (Nellemann et al. 2009, Gunders 2017). Pre-consumer waste is generated in restaurants, during food processing, or manufacturing, which includes everything from canning, freezing, drying, and precutting fruits and vegetables to

making cheese, soup, or frozen meals. It is estimated that 0.9 million tons of food waste is generated at this stage, not including food waste going to animal feed, compost, or otherwise recycled (Gunders 2017).

Manure waste produced by livestock is another challenge to be faced within the food processing supply chain. More than 335 million tons of dry matter waste is produced annually on farms in the United States, representing almost a third of the total municipal and industrial waste produced every year (USDA 2005). Depending on their type and size, farms where livestock are kept and raised in confined space can produce between 2,800 tons and 1.6 million tons of manure annually (GAO 2008). It is estimated that in the United States, livestock produce between three and twenty times more manure than people, amounting to as much as 1.2 – 1.37 billion tons of waste (US EPA 2005). The environmental protection agency does not require that livestock waste be treated like human waste through sewage treatment plants. This is because it can be utilized as fertilizers in the farming industry. However, excessive amounts of manure may become unmanageable and cause environmental and health risks (Hribar 2010).

In addition to livestock waste, approximately 1.04 million tons of marine waste is generated each year in the United States and 1.5×10^5 tons are lost in distribution and retail, including 2.6×10^5 tons of bycatch that are lost when commercial fishers catch the wrong species of fish and then discard them back into the ocean (Love et al. 2015). A considerable amount of waste generated while processing farmed fish are discarded each year, often by being disposed of in the ocean (Ramakrishnan 2013). In 2004, a seafood processing waste survey was conducted to estimate the volume of shellfish and finfish waste that may require ocean disposal in the UK in the future. It was estimated that there is a potential for 45,238 tons per year of shellfish waste and 26,214 tons per year of finfish waste to be disposed of at sea (Large 2004, Mazik et al. 2005).

The breaking down of marine waste in the oceans cause aerobic bacteria to reduce the amount of dissolved oxygen in the water, as well as to increase nitrogen, phosphorus, and ammonia levels. It also results in unstable pH levels that may be unsuitable for fish habitats. Limited oxygen will cause anaerobic conditions that can lead to the release of greenhouse gases such as carbon dioxide and methane (Tchoukanova et al. 2012). The International Labor Organization stated that the trend of processing more fish products within the supply chain has created an increase of fish offal and other by-products, that may constitute up to 70 percent of fish and shellfish after industrial processing that is discarded. The remaining 30 percent of by-products are utilized for food products for human consumption such as fish sausages, cakes, gelatin and sauces (Food and Agriculture Organization of the United Nations (FAO) 2016). Some are used in feeds formulated for aquaculture, livestock, and pet food. In the last two decades, the use of fish by-products has gained attention because they can represent a significant additional source of nutrition. However, there is still concern due to low consumer acceptance and sanitary regulation restrictions for their use (FAO 2004).

1.2. The importance of aquaculture

The National Oceanic and Atmospheric Administration (NOAA) reported that U.S. aquatic farmers produced 2,800 tons of seafood in 2015 valued at $1.39 billion (Fisheries of the United States (FUS) 2015). Worldwide, aquaculture produces 45% of all seafood consumed, and the production is expected to rise to 75% in the next 20 years (Papadoyianis 2007). Wild caught fish used for fish meal will not be able to sustain aquaculture industries, and its continuous harvest could lead to detrimental ecological consequences. The Food and Agriculture Organization of the United Nations (2016) estimated that 31.4% of assessed fish stocks were harvested at a biologically unsustainable level, resulting in a serious global problem of

overfishing. Total world fishery production (capture plus aquaculture) is projected to expand over the next eight years, reaching 196 million tons in 2025. The share of aquaculture in total fishery production will grow from ca. 44% in 2013–15 to over 50% in 2021. In 2025, this share will reach 52% (Food and Agriculture Organization of the United Nations (FAO) 2016). These projections indicate that aquaculture will be the main source of fish production, while the relative importance of harvesting the already depleted wild stocks will progressively decline.

1.3. Susceptibility of aquafeed production

Currently, 25% of the global fish production is used to produce fish meal and fish oil, even though the world fish stocks are in decline (Papadoyianis 2007). Fishmeal and fish oil are highly valued products for human consumption and are an important source of revenue for some countries (Food and Agriculture Organization of the United Nations (FAO) 2016). They are considered to be the most nutritious and digestible components for farmed fish feeds (Food and Agriculture Organization of the United Nations (FAO) 2016). As such, they are essential for supporting continuously increasing aquaculture production (Food and Agriculture Organization of the United Nations (FAO) 2016). However, both are largely made from wild-caught fish. With the wild fish stocks already declining due to overfishing, the amount of fishmeal and fish oil available for processing into feed for aquaculture has shown a declining trend. A variety of fish species are used for fishmeal and fish-oil production, with Peruvian anchoveta, *Engraulis ringens* (Jenyns), being one of the most important. Due to reduced catches of *E. ringens*, world fishmeal production was 15.8 million tons in 2014, whereas it was over 146 million tons in the 1960s. This has led to implementation of some conservation measures, such as the use of fish by-products for fish meal that were previously discarded. Usage of feeds that contain fish by-product ingredients are used on specific stages of production, such as in hatcheries and for brood

stock (Food and Agriculture Organization of the United Nations (FAO) 2016). While this is a welcome development, these measures are not likely to fully compensate for declining availability of fish meal and fish oil. Therefore, finding feasible alternatives for these products is important for supporting sustainable growth of aquaculture industry.

1.4. Black soldier fly

1.4.1. Biology

Black soldier fly (BSF), *Hermetia illucens* (Linnaeus) (Diptera: Stratiomyidae), is a common species that is not considered a pest. Its distribution ranges throughout the Western Hemisphere, including the continental United States (Oliveira et al. 2015). BSF have four life stages: egg, larvae, pupae, and adult. The final instar is known as the prepupae, a non-feeding migratory stage that searches for a pupation site. The adult fly may survive on the stored fat obtained during feeding at the larval stage (Myers et al. 2008), but a recent study also reported adult feeding on a sugar solution (Richard-Giroux and Spindola 2017). In the absence of feeding, the adult dies when the stored fat depletes (Myers et al. 2008). The larvae are saprophytic feeders and can consume a variety of organic substrates, including feces, rotting and fresh fruit, animal flesh, food waste, and even cellulose (Sheppard et al. 2002, Tomberlin et al. 2002, Holmes 2010, Nguyen 2010).

BSF have approximately a 40-day life cycle from egg to adult, depending on the environmental conditions and the available diet. The eggs incubate for about three days. Upon hatching, neonates immediately search for a suitable food source. The larval stage consists of six instars and lasts for 14−22 days. Rate of consumption depend on larval size and the type of food being consumed (Diener et al. 2009, Nguyen 2010). Adults have an average lifespan of ten days. Mating starts two days after adults emerge (Tomberlin and Sheppard 2002).

Optimal moisture of larval substrates ranges from 60% - 90% (Myers et al. 2008). Feeding larvae produce metabolic heat, allowing them to develop in temperatures lower than their preferred range, albeit at lower rates (Tomberlin et al. 2009). Moisture content is important for growth. Outside of an optimal range, the colony will exhibit little growth, convert waste inefficiently, or prematurely migrate to another food source (Fatchurochim et al. 1989). As a result, manipulation of the moisture preference can be used as a tool in a mass rearing facility to control direction and development of larvae.

BSF are generally considered to be warm-climate species preferring the areas with subtropical and warm temperate climates. Observations submitted by citizen scientists and records of preserved specimens available on iNaturalist.org have provided year-round data from around the world between 1913 – 2018 of BSF at all life stages with images included. The iNaturalist recorded distribution shows a northern most range limit reaching up to Washington State in the United States (Figure 1.1), and with the most southern range in New Zealand (iNaturalist 2018). In New England, the most northern range reaches Massachusetts. Temperatures optimal for their development range from 27°C - 33°C (Sheppard et al. 2002). Higher temperatures have a detrimental effect on this species. Little is currently known about its response to lower temperatures (Tomberlin et al. 2009).

When approaching pupation, larvae stop feeding and enter a migratory stage looking for a dark and dry place to continue their life cycle (Craig Sheppard et al. 1994). They change color from creamy white to dark brown or black and are commonly referred to as prepupae. The duration of the prepupal stage varies, and may depend, in part, on the ability to find a suitable place to pupate. The prepupae prefer dry pupation substrates but require ambient humidity levels around 60% to emerge as adults (Sheppard et al. 2002, Holmes 2010).

BSF can pupate without medium but will have a higher mortality as pupae (Holmes 2010). The optimal medium depth for pupation is 15 – 20 cm. Depending on temperature and humidity, pupation can last 5 – 7 days. In mass rearing conditions, migration behavior can be exploited to self-harvest larval biomass by directing emigrating larvae into a collection tray for processing or incubation for re-introduction into a colony (Holmes 2010).

Ambient light levels are important factors for mating to occur. If indoors, lighting with irradiance of over 200 micromoles /m^2/s is optimal (Sheppard et al. 2002). Mating occurs best with full natural sunlight; therefore, the use of artificial lights should be supplemental when there is little natural sunlight. Mating begins with aerial coercion stimulated by light, which then leads to copulation (Furman et al. 1959). Although observations made by undergraduate student workers in a BSF colony reared in a greenhouse in the University of Maine (Personal Communication 2016) suggest that mating can occur on the ledges of the cage, published studies indicate aerial coercion is an important process to ensure mating (Tomberlin et al. 2002). Therefore, rearing facilities should have enough space for adults to fly and land to mate.

Egg-laying occurs approximately two days after mating. Females normally lay eggs in a dry area near the food source. Detecting pungent chemicals from waste is thought to be the mechanism for this action (Sheppard et al. 2002). Egg laying sites should be maintained at 27°C and relative humidity of 60% or higher because successful hatching has been observed at these conditions (Sheppard et al. 2002, Holmes 2010). Eggs can be removed from food source and placed in similar conditions in a separate container with food available.

1.4.2. Uses for larvae & prepupae

Black soldier fly larvae (BSFL) have been utilized in agricultural settings to remediate manure from swine, bovine, and poultry operations in areas that sustain BSF year-round through

consumption of manure in livestock housing (Gayatri and Madhuri 2013). Another benefit the BSFL can provide is harvestable biomass consisting of their nutrient-rich bodies. They contain useful compounds that have commercial value, being comprised of approximately 40% protein and 30% fat (Sheppard 1993, Craig Sheppard et al. 1994, Newton et al. 2004). Depending on what they eat, the larvae may also acquire essential amino acids and fatty acids that are important for a value-added livestock and fish feed (Barroso et al. 2017).

Considerable amount of research was dedicated to finding BSFL uses for livestock feed (Newton et al. 2004, Oyegoke et al. 2006). Overall, BSFL show a good potential for being incorporated into animal feeds. For instance, Newton et al. (1977) evaluated the palatability of dried BSFL to pigs. The meal composed of 33% of diet with addition to soy and compared to 100% soy meal. They determined that the apparent digestibility of dry matter for pigs that were fed dry BSFL meal diet was significantly lower than for the pigs that were fed soybean meal diet. Additionally, a choice experiment was conducted between the two diets and the pigs did not discriminate against a diet containing BSFL meal. Another study by Al-Qazzaz et al. (2016) was conducted to evaluate the effects of using BSFL as a source of added protein in layer hen basal diets. Daily egg production, egg appearance, texture, taste, brightness of the egg yolk color, and the acceptance by a panel of thirty judges conducting sensory evaluations were improved when hens were fed an increasing level of BSFL in their basal diets. Feed intake, weight gain, egg protein quality, and hatchability were not affected by BSFL when compared to the basal diet. However, the shell thickness and shell weight decreased with increasing levels of BSFL in the diet, possibly due to the calcium content of BSFL when compared to the basal diet (Al-Qazzaz et al. 2016).

BSFL also has promise as feed in aquaculture systems. Bondari and Sheppard (1981) found no differences in the body weight and total length of channel catfish, *Ictalurus punctatus* (Rafinesque) and tilapia, *Oreochromis* sp. (Günther) fed on a 100% BSFL diet and a mixed diet of 50% BSFL : 50% commercial feed compared to high-(45%) and low-(30%) protein commercial diets. Similarly, the replacement of 25% of the fishmeal and 38% of the fish oil components of a commercial diet had no effect on the feed conversion ratio in rainbow trout, *Oncorhynchus mykiss* (Walbaum), (St-Hilaire, Cranfill, et al. 2007). However, in the experiments by Sealey et al. (2011) and Sánchez-Muros et al. (Sánchez-Muros et al. 2014) the effects of replacing 25% and 50% of the fishmeal on the growth of *O. mykiss* depended on whether BSFL meal was enriched with fish offal. The growth of fish that were fed offal-enriched BSFL diets was not significantly different from those fish that were fed the fishmeal-based control diet, whereas the growth of fish that were fed BSFL-only diets was significantly reduced compared with the control diet.

An important factor responsible for aquafeed performance is their fatty acid composition, in particular omega-3 fatty acids. There are three main omega-3 fatty acids: alpha-linolenic acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA). ALA is found mainly in oils of plant origin, such as flaxseed, soybean, and canola, whereas DHA and EPA are found in seafoods (Glencross 2009).

Essential fatty acids (EFA) are not produced by an organism's body. Therefore, they must be obtained from another source. ALA is an EFA, which can then be converted into EPA and then to DHA by all vertebrates. Therefore, getting EPA and DHA from foods or supplements is the only way to increase levels of these omega-3 fatty acids in humans (NIH 2018). Omega-3s also play an important role in the cell membrane function. Concentrations of certain fatty acids,

such as DHA, are especially high in retina, neural, and sperm cells in many animals, especially fish (Mourente and Tocher 1992, Sargent et al. 1993, Masuda 2003). The molecular structure of DHA also provides strong and flexible support for the conformational transitions in cell types that are subject to rapid and repeated membrane reorganization, which is particularly true in the brain (Glencross 2009). Omega-3s have many functions related to heart, blood vessels, lungs, immune system, and endocrine system health and serve as a store of energy (NIH 2018).

Many studies with different fish species (Castell et al. 1972, Hardy et al. 1987, Santha and Gatlin 1991, Kalogeropoulos et al. 1992, Kennish et al. 1992, Ruyter et al. 2000) have shown that the fatty acid composition of body lipids closely follows that of the diet provided. When a fish is fed a diet poor in EFA, there are health risks involved with EFA deficiency (Glencross 2009). For example, erosion of the caudal fin, myocarditis (inflammation of the heart muscle), and shock syndrome were observed in an EFA-deficient rainbow trout (*O. mykiss*) (Castell et al. 1972). Other deleterious effects included a fainting reaction when handled and increased sensitivity to stressful situations (Castell et al. 1972, Millikin 1982, Watanabe 1982). Slow or stunted growth and increased mortality were also reported (Castell et al. 1972, Hardy et al. 1987, Santha and Gatlin 1991, Kalogeropoulos et al. 1992, Kennish et al. 1992, Ruyter et al. 2000).

Another aspect of BSFL biology that can benefit mass-rearing operations are antimicrobial secretions they produce that are left in the frass while they feed. For example, *Escherichia coli* (Migula) and *Salmonella enterica* serovar Enteritidis (Kauffmann & Edwards) were inactivated by BSF larvae in chicken manure up to six days (Erickson et al. 2004). Similarly, a study conducted by Choi et. al. (2012) demonstrated that BSFL have antibacterial activity which strongly inhibits the growth of *Klebsiella pneumoniae* (Schroeter), *Neisseria*

gonorrhoeae (Zopf) and *Shigella sonneio* (Levine). However, antibacterial effects in that study were not induced in Gram-positive bacteria. It was also reported lower concentrations of *Salmonella* spp. and several virus species in a mixture of pig manure, dog food, and human feces processed by BSF larvae (Lalander et al. 2014).

BSF frass can also be used to repel pest insects. Oviposition by house fly, *Musca domestica* (Linnaeus) in poultry manure was inhibited when BSFL were present in high densities (Bradley and Sheppard 1984). However, little is known about mechanisms of this phenomenon in Diptera, or about its range against different taxonomic groups of filth-dwelling insect pests. Deterrent effects of larval frass have been observed in phytophagous and entomophagous insects in the order Lepidoptera and Coleoptera. This includes: the yellow cutworm *Agrotis segetum* (Hufnagel), pineapple borer *Thecla basilides* (Geyer), egyptian cotton leaf worm *Spodoptera littoralis* (Boisduval), fall armyworm *Spodoptera frugiperda* (J.E. Smith), cabbage looper *Trichoplusia ni* (Hubner), European corn borer *Ostrinia nubilalis* (Hubner), Japanese pine sawyer *Monochamus alternatus* (Hope), Asian ladybeetle *Harmonia axyridis* (Pallas) and the turtle vein lady beetle *Propylea japonica* (Mulsant) (Ditrick et al. 1983, Renwick and Radke 1985, Williams et al. 1986, Klein et al. 1990, Anderson et al. 1993, Anderson and Löfqvist 1996, Rhainds et al. 1996, Anbutsu and Togashi 2002, Agarwala et al. 2003). In many insect species, chemicals in larval frass may deter oviposition of conspecific females to avoid competition among larvae for food on the host (Li and Ishikawa 2004). In all species listed above, it was demonstrated that larval frass decreased oviposition of conspecific females significantly, with the highest reduction rate of in *A. segetum* (Anderson and Löfqvist 1996) and *O. nubilalis* (Ditrick et al. 1983). Deterrence of heterospecific insects was also demonstrated for females of both *H. axyridis* and *P. japonica.* They exhibited similar behavior in response to water extracts of their

own feces. For *P. japonica*, the deterrence of heterospecific feces was greater than that of conspecific feces. This demonstrates that feces of ladybirds contain odors that have the potential to deter the feeding and oviposition activities of conspecific as well as heterospecific ladybirds that may allow these insects to avoid predation risk (Agarwala et al. 2003).

BSFL may also be used as a vermicomposting agent. Vermicomposting is a popular approach to converting solid organic wastes into a useful soil amendment. It is commonly described with the use of frass from earthworms as a soil amendment; therefore, it may be feasible to replicate vermireactors with BSFL. Vermicomposting was originally defined as a biological process that involves the oxidation and stabilization of organic wastes through the joint effort of invertebrates, such as earthworms, and microorganisms (Domínguez et al. 2004, Westerman and Bicudo 2005, Yadav and Garg 2011), and turns waste into a valuable soil amendment, known as vermicompost, together with valuable worm byproducts. The ability of vermicomposting to improve biochemical features, reserve nutrients, reduce pathogens, and reduce odor emission, has allowed it to be used to process various types of wastes, including animal manure, food-processing waste, municipal sludge, and even industrial waste (Boushy 1991, Marchaim et al. 2003, Bai et al. 2007, Diener et al. 2009, Yadav and Garg 2011).

Similar to BSFL, earthworms play a role in the decomposition of organic waste and biodegradation of cellulosic and proteinaceous materials in organic waste due to the presence of a complex of enzymes in the gut of the earthworms, such as proteases, lipases, amylases, cellulases, and chitinases (Kim et al. 2011), as well as the microorganism community in the intestinal tract (Aira et al. 2007, Monroy et al. 2009, Gómez-Brandón et al. 2011, Jeon et al. 2011). The process of vermicomposting also regulates the dynamic curves of the enzymatic activity of b-glucosidase, cellulases, proteases, and phosphatases in the waste or vermicompost

(Aira et al. 2007, Gómez-Brandón et al. 2011), which directly determines the biodegradation of organic carbon, nitrogenous organic compounds, and phospholipids in waste. Moreover, earthworms also excrete large amounts of casts containing more soluble and available nutrients, such as nitrogen, phosphorus, potassium, and calcium, compared to those present in raw manure (Bai et al. 2007, Knapp et al. 2009). Microbial communities have been studied to elucidate the microbial functioning mechanisms of earthworm vermireactors, which may control the pace of the vermicomposting process (Yasir et al. 2009, Zhao et al. 2010, Jeon et al. 2011). However, the link between biochemical features and microbial functioning has not been evaluated with the use of BSFL consuming marine waste.

1.4.3. Safety considerations

BSF does not vector disease or become a nuisance to humans (Furman et al. 1959), probably because adult flies use energy accumulated at a larval stage and do not require additional food (Furman et al. 1959, Sheppard et al. 2002). Furthermore, unlike many other flies that are saprophagous at a larval stage, BSF adults lay their eggs not directly onto decaying substrates, but adjacent to them. As a result, their contact with potentially pathogenic bacteria is more limited (Furman et al. 1959).

Although uncommon, there were several reported cases of accidental intestinal myiasis caused by BSFL in humans (Meleney and Harwood 1935, Lee et al. 1995). Myiasis is a parasitic infestation of vertebrate hosts by fly larvae that consume their tissues. There are three different types of myiasis: obligatory, facultative, and accidental. Obligatory myiasis is caused by species such as bot flies (e.g., *Dermatobia hominis* (Linnaeus Jr.)) that require a vertebrate host for development. Facultative myasis occurs when species such as blow flies (e.g., *Calliphora vicina* (Robineau-Desvoidy)) that normally develop on carrion invade living hosts, often through

wound openings. In this case, larvae of the same species can be free living or parasitic (Catts and Mullen 2002). Accidental myiasis occurs when fly larvae or eggs contaminate food or other items and are consequently ingested by a vertebrate host (Catts and Mullen 2002). Species involved in accidental myiasis, including BSF, are not normally parasitic, but at some of them can become such in order to survive. Known cases of BSFL myiasis occurred in poverty-stricken tropical areas, where access to clean water for washing was not readily available (Yang 2014). Currently, there have been no reports of myiasis in waste management facilities that use BSF. Overall, in the presence of proper sanitation efforts the risk of BSFL myiasis appears to be low.

Spread of invasive insect pest species that can be detrimental to the environment and economy is a serious worldwide concern. For example, one Dipteran species native to Asia, the spotted wing drosophila, *Drosophilia suzukii* (Matsumura), is listed as serious invasive pest in Maine, (DACF 2018). Large populations of this insect can severely damage healthy fruit such as blueberries and reduce blueberry yields for farmers. BSF has a global distribution and is generally considered beneficial. Still, if BSF can establish populations in Maine, where it currently does not reside, local saprophagous Diptera may be negatively affected through competition. However, this species appears to be prevalent mostly in tropical and subtropical zones. Therefore, its invasion is likely to be impeded by harsh winters in Maine.

1.5. The importance of present study

The need for more sustainable alternative sources of high protein feed, together with the interesting opportunities that insects offer in this regard, are driving research to mass produce these organisms at the industrial scale (Rumpold and Schlüter 2013). The black soldier fly is one of the most promising insects used for this. Its larvae are capable of bio converting a wide range of organic substrates to turn them into high-quality protein to be used as feed, while other

compounds such as oils derived from fats and chitin can be used as a raw material in chemical industries (Diener et al. 2009, Maurer et al. 2015). It may no longer be a question of whether an entire food value chain will arise for mass rearing of BSF, but how fast it will be installed. It is only a matter of time before the use of insects as feed booms into a stable industry. Therefore, it is important to gather a similar level of in-depth knowledge on their biology that is available for other farmed animals to support this novel industry.

Technological and conceptual advances have set a standard for the investigations of how insects interact with bacteria and other microbes. Pairing studies with behavior and physiology could potentially deepen our understanding of how BSFL interact with bacterial symbionts if present. Due to their close association with decomposing organic matter, BSFL also have close associations with numerous bacteria that thrive in decaying environments (Wang and Shelomi 2017). Literature has shown that bacteria are the initial colonizers of such material, and their degradation of these materials results in the production of volatile organic compounds that attract pest flies such as blow flies to colonize the detritus (Tomberlin et al. 2017). Applications of pairing studies with behavior and physiology represent the start of this exciting area of research and the establishment of BSFL interactions with bacteria as a model system for studying the effects and impacts of microbial and insect interactions and the role of interkingdom communication. In future studies, the utilization of microbial communities could be used to enhance production of insect biomass and the application of BSF associated microbial communities to find new biotechnological tools.

Currently, the economic benefits of conventional composting are often marginal and sometimes negative if no financial subsidy is received from the government (Westerman and Bicudo 2005). Due to the remarkable capacity of waste reduction and stabilization as well as the

considerable economic gain, the advantages of BSFL vermicomposting will be more prominent in land restricted and over populated developing countries, referenced on the operational requirement (Westerman and Bicudo 2005, Yadav and Garg 2011). The application of BSFL vermicomposting for marine waste treatment and the establishment of the corresponding value-added waste to the economy may help benefit ecosystem stability and the sustainable development of the insect mass production industry.

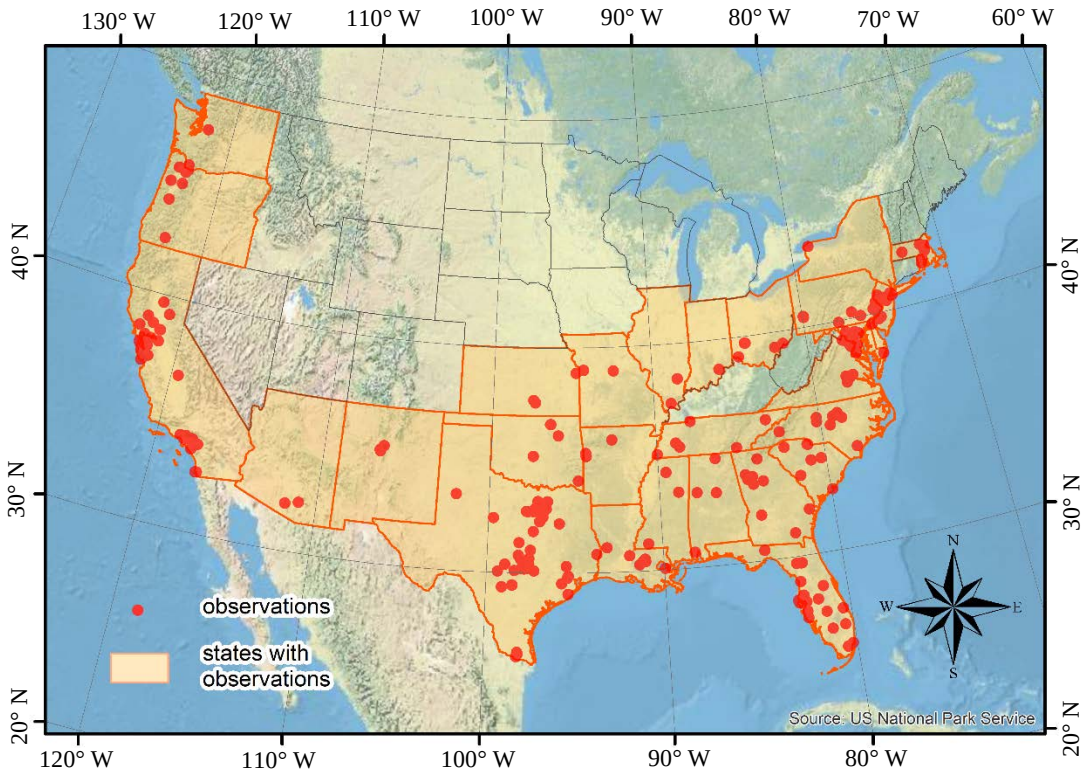

Figure 1.1. Distribution of *Hermetia illucens* in the United States recorded as observations submitted by citizen scientists and records of preserved specimens from iNaturalist.org from 1913 – 2018 at all life stages.

CHAPTER 2

BLACK SOLDIER FLY LARVAE DEVELOPMENT ON MARINE WASTES

2.1. Introduction

Black soldier fly, *Hermetia illucens* (Linnaeus) (Diptera: Stratiomyidae) (BSF) is a beneficial fly species that can be used for remediating biological waste. Their larvae (BSFL) are rich in nutrients, grow rapidly, convert a variety of decaying organic wastes to vermicompost while suppressing human pathogens and pests, and contain a number of potentially valuable biologically active compounds (Choi et al. 2012a). Therefore, using the BSFL as a replacement ingredient for fish meal in aquafeed formulations may benefit the environment by reducing the amount of wild-caught forage fish currently used for fish meal production in aquaculture.

In the United States, in 2015, freshwater and marine aquaculture production was around 2.84×10^5 tons, with a value of $1.4 billion. That was an increase of 8.8×10^3 tons worth $61.5 million from 2014 (Fisheries of the United States (FUS) 2015). Fish meal is an important component of most aquafeed formulations. Therefore, aquaculture itself can be considered a major consumer of fish protein. In fact, consumption by aquaculture accounts for 60 to 70% of the annual production of fish meal (Rust et al. 2011). It was reported that domestic production of fish and shellfish meal in the U.S. was 2.53×10^5 tons valued at $307.7 million (Fisheries of the United States (FUS) 2015).

There are several species of forage fish commonly harvested for the use of fish meal such as: Peruvian anchoveta *Engraulis ringens* (Jenyns), Pacific sardine *Sardinops sagax* (Jenyns), Chilean jack mackerel *Trachurus murphyi* (Nichols), and Atlantic herring *Clupea harengus* (Linnaeus) (Cashion et al. 2017). Along the trophic pyramid, these fish play a vital role in marine ecosystems by relocating energy from low to high trophic-level species, which includes commercially available fish with high economic value such as salmons, cods and tunas

(Cashion et al. 2017). The importance of keeping sustainable forage fish populations is at an all-time high for functioning ecosystems. Thus, the forage fish wild-caught for fishmeal production may represent a loss in higher trophic-level species, and a less valuable ecosystem service since commercial fish that consume forage fish are worth more (Pikitch et al. 2014).

Harvesting high amounts of forage fish to produce fishmeal when wild fish stocks are decreasing is not an economically and environmentally sustainable approach. Therefore, adopting an alternative aquafeed production technology that relies on the biological conversion of organic wastes using BSFL could potentially reduce overharvesting of wild-caught forage fish. When compared to other insects, BSFL has higher ash and crude protein contents. Additionally, its amino acid composition is similar to fish meal (Barroso et al. 2014). Since the 1970's, there has been an interest in utilizing BSFL for feed in aquaculture (Bondari and Sheppard 1981), as well as in its use as value added supplement in livestock feed (Newton et al. 1977). More recent research confirmed the high potential of using BSFL for aquafeed (St-Hilaire, Cranfill, et al. 2007, Sealey et al. 2011). Furthermore, a sensory evaluation of rainbow trout, *Oncorhynchus mykiss* (Walbaum) and BSFL showed that the fish consuming experimental feed formulation containing BSFL had a milder and fresher taste when compared to the fish consuming commercial fish meal formulation containing anchovy, corn, and soybean meal, fish oil, and vitamin C (Sealey et al. 2011). It is also known that BSFL can intake essential fatty acids that are important for fish development (St-Hilaire 2007, Wang and Shelomi 2017).

There are several commercial facilities that mass produce BSF worldwide (Dossey et al. 2016). They use a variety of wastes and several of them generate output on the scale of tons of larvae per day. The number of BSFL facilities continue to rise with the ongoing efforts of bio converting waste into valuable products.

As of 2016, Maine ranked second in the top five states by value of landings, which reached $633.6 million according to the (Fisheries of the United States (FUS) 2015). Landings represent a report of the total quantity or poundage of all marine species harvested, brought to shore, and sold to another person or party for direct human consumption; this includes anything received from a fisherman whether it is discarded or not sold. The Maine Department of Marine Resources (DMR) reported that $569 million worth of commercial Maine landings occurred as of February 12, 2018, amounting to $1.15x10^5$ tons. Of those commercial landings, over $17 million were Atlantic herring *C. harengus,* for a total of $3.0x10^4$ tons (Watts 2018).

Maine's aquaculture industry is the largest in the United States and is rapidly growing. It will greatly benefit from a BSFL commercial operation that utilizes marine waste from fish processing facilities. Therefore, the aim of our study was to identify the best fish waste available in Maine that can optimize bioconversion rates through BSFL feeding.

2.2. Materials and methods

2.2.1. Marine wastes tested

Seven sea food wastes provided by fish processing facilities were tested under laboratory conditions. Fresh finfish trimmings (viscera, skins and trim) of various consumer species were gathered at Harborfish Market, Portland, ME. That material comprises 25-30% of the whole fish and did not include the muscle fillet, head, bone, and scales. The intended use of this material in New England is for the pet food market. Dry and wet clam viscera containing quahog, *Mercenaria mercenaria* (Linnaeus), was gathered from Sea Watch Int'l., New Bedford, MA, which is the largest clam processor in the country. Technological process to dry clam viscera involves pressing the wet version to 35-40% moisture, which increases the crude protein content (Wohlt et al. 1994). Currently, this waste is being sent to compost facilities in Rhode Island and

Maine. Sea cucumber, *Cucumaria frondosa* (Gunnerus), residuals were gathered from ISF Trading, Portland, ME, that currently sells sea cucumber as a dried ingredient into the pet and human nutraceutical market. The wet and dry sea cucumber residuals used in our study consisted of everything but the tentacles and muscles from the body wall (Feindel et al. 2011). Sea urchin, *Strongylocentrotus droebachiensis* (Muller), residuals were also gathered from ISF Trading, which discards them in a Portland, ME, area compost. Crab meal was obtained from Ocean Organics, Waldoboro, ME.

2.2.2. Colony maintenance of black soldier flies

The black soldier fly breeding colony was first purchased on May 2016 from Symton Black Soldier Fly Solutions (College Station, TX). The colony arrived in a plastic container with a total of 20,000 immature larvae in coconut coir substrate. The 20,000 immature larvae were distributed into two separate 20 by 33 by 38 cm plastic dish pans (United Solutions, Leominster, MA). The two dish pans were then placed side by side in a transparent 89.9 by 42.5 by 14.9 cm plastic bin (Sterlite, Townend, MA). The lid of the plastic bin had two 12.7 by 12.7 cm squares cut out and covered with aluminum mesh for ventilation. Larvae were fed with commercially available non-medicated chicken feed (Home Fresh® Extra Egg, Blue Seal, Lawrence, MA) mixed with tap water to achieve 60% (w : w) moisture contents of the resulting substrate (Fatchurochim et al. 1989). Larvae were reared in laboratory with ambient temperature at 261°C (range: 24 - 27.7°C) and 508% (range: 42.5 - 69%) relative humidity.

Once pupation occurred, pupae and substrate were transferred into a separate 89.9 by 42.5 by 14.9 cm plastic bin and placed in a 2 by 2 by 4-m custom-made wooden frame screen cage in the greenhouse with ambient temperature at 261°C (range: 24°C - 30°C) and 60% (range:

42.5% - 69%) relative humidity. All temperature readings were recorded with an EL-USB-2-LCD USB Humidity Data Logger w/ LCD Display (Lascar, Erie, PA).

Emerging adults were continuously provided with water by spraying a 17 cm by 17 cm by 17 cm custom-made screen mesh cube from inside with a 0.8 GPH, 6.3 mm hose barb with fogging nozzle (Home Depot, Bangor, ME) equipped with a brass water pressure reducing valve (Watts Water Technologies, North Andover, MA). Two days after emergence, when females were ready to lay eggs, a 21.8 by 16.2 by 13.2 cm (GladWare Oakland, CA), container with 50 ml of moist chicken feed was placed inside the cage. Three bundles of three dry 12.7 by 5 cm corrugated cardboard strips with 5 mm flutes were suspended above the container on a skewer. The strips served as an oviposition substrate for gravid females.

Every five days, eggs were harvested by removing cardboard strips filled with egg masses and replacing them with new ones in the cage. The cardboard strip with eggs was then placed in a 21.8 by 16.2 by 13.2 cm, 1892 ml GladWare container with 50 ml of chicken feed and 30 ml of water and moved to the lab. Once eggs hatched, moist chicken feed was provided *ad libitum* as described above.

2.2.3. Larval development on sea food wastes

Experiment was conducted in 21.8 by 16.2 by 13.2 cm GladWare containers with a spout and ventilated lid covered with screen mesh. Seven sea food wastes (see above) were tested: dry sea cucumber, wet sea cucumber, crab meal, finfish, dry quahog, wet quahog, and sea urchin. All wastes were dried in a soil-drying room overnight at 76°C. After that, 25 g of dry substrate, 25 g of blended sawdust, and 75 ml of water were homogenized and placed into each container. Additional 25 g of dry substrate was added once a week, and additional 25 g of blended sawdust was added once a month until the end of the experiment. Every week, a 5-g sample of the

substrate was removed and placed in the soil drying room as described above. Water content of a sample was determined as the difference between its weight before and after drying. Water was added to the substrate as needed, allowing to keep moisture levels between 60-70% (w : w) throughout the experiment (Fatchurochim et al., 1989). A total of 25 g of saw dust was added to the substrates to maintain their porosity. Chicken feed (see above) was used as a reference treatment. Once substrates were prepared, 29.1 mg of eggs was placed on top of a wax paper on their surfaces. After one week from the time of hatching, larvae were large enough to measure their weight with analytical scale (Ohause Co., Pine Brook, NJ). The measurements were taken every five days until all larvae pupated. Feeding substrate prepared as described above was added once a week. Larvae were maintained at the ambient temperature of 26±1°C and 50±8% humidity. The spout was plugged with a rubber cork on the inside to prevent escape of immature larvae. Once prepupae were noticed, the cork was removed. A 120 ml plastic cup filled with vermiculite was placed beneath the spout to allow migrating prepupae to fall in. The numbers of emigrating pupae were counted once a week.

To analyze differences in survival, mean and maximum weights of larvae associated with different marine wastes, and durations of development to prepupae, ANOVAs followed by a Tukey's honestly significant difference (HSD) tests were conducted using SAS (PROC MIXED, SAS Institute 2017). Data normality was tested prior to the analyses using Wilk-Shapiro test at $P<0.01$ (PROC UNIVARIATE, SAS Institute 2017). Non-normal data were transformed using rank transformations (PROC RANK, SAS Institute 2017; (Conover and Iman 1981).

2.3. Results

2.3.1. Larval development on sea food wastes

There was a significant difference in the number of prepupae emigrating from containers with different sea food wastes (d.f.=7,29, F=29.70, P<0.0001). Feeding on wet sea cucumber and finfish trimmings resulted in the best survival of black soldier fly larvae to prepupal stage compared to other treatments. Virtually no larvae survived on dry sea cucumber. Other substrates were somewhat in between the two extremes (Figure 2.1).

Diet also had a significant effect on the mean (d.f.=7,29, F=5.25, P=0.0006) and maximum larval weights (d.f.=7,29, F=4.46, P=0.0018). Despite relatively low survival on crab meal and chicken feed compared to wet sea cucumber and finfish trimmings, larval weights were fairly similar on the four substrates (Figure 2.2 A). Furthermore, maximum larval weights were similar for all the treatments except for dry sea cucumber and wet quahog (Figure 2.2 B).

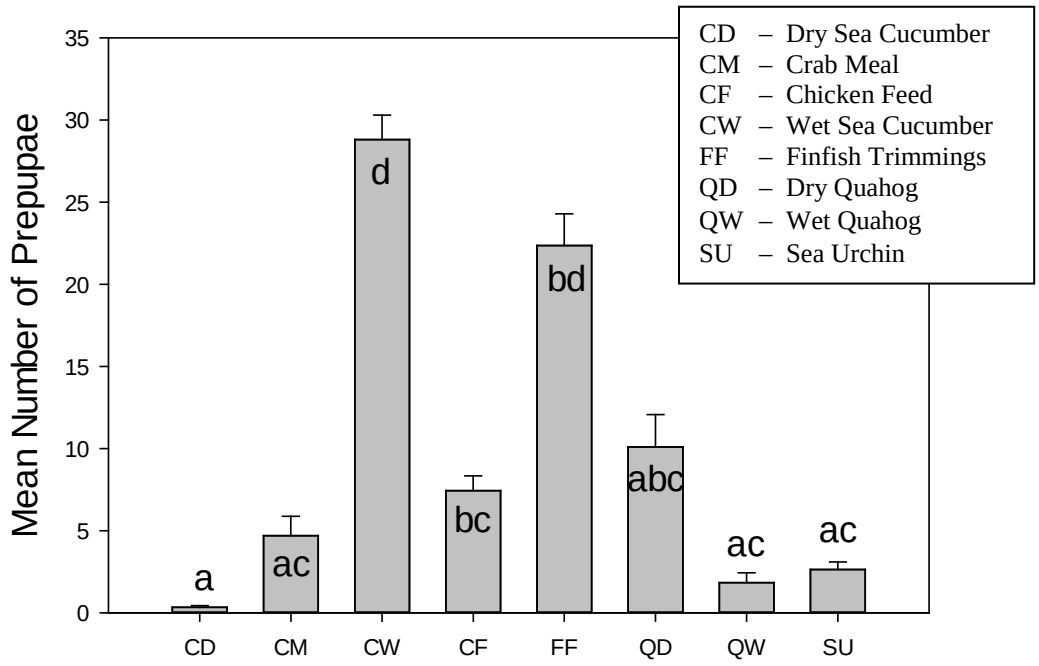

Figure 2.1. Emigration of black soldier fly prepupae that completed their development on different marine wastes. CD – dry sea cucumber, CM – crab meal, CW – wet sea cucumber, CF – chicken feed (control), FF – finfish trimmings, QD – dry quahog, QW – wet quahog, SU – sea urchin. Bars followed by the same letter are not significantly different from each other.

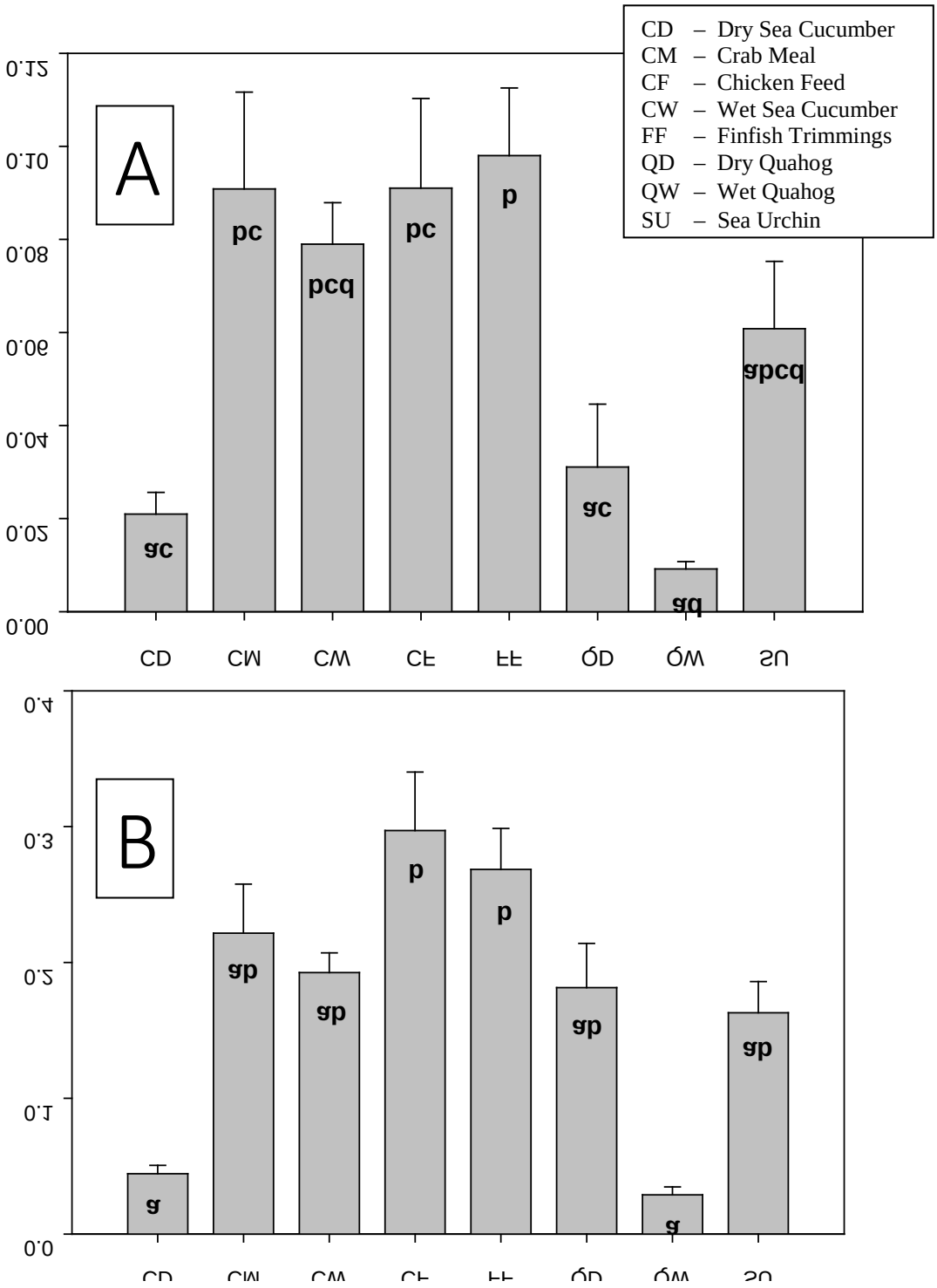

Figure 2.2. Weights of black soldier fly larvae that completed their development to prepupae on different marine wastes (A – mean; B – maximum). CD – dry sea cucumber, CM – crab meal, CW – wet sea cucumber, CF – chicken feed (control), FF – finfish trimmings, QD – dry quahog, QW – wet quahog, SU – sea urchin. Bars followed by the same letter are not significantly different from each other.

The amounts of time necessary for reaching prepupal stage were different among the treatments (d.f.=7,26, F=6.23, P=0.0002). The larvae feeding on dry sea cucumber and crab meal developed faster than the larvae feeding on wet sea cucumber, finfish trimmings, and sea urchin (Figure. 2.3). There was no difference among other treatments.

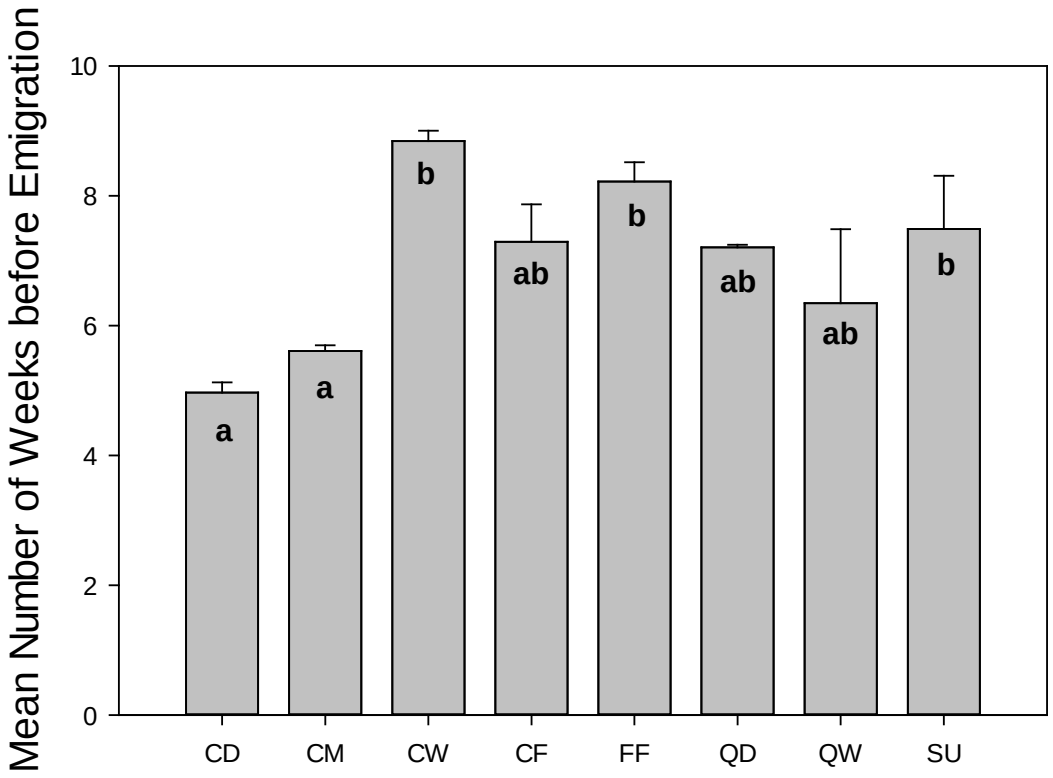

Figure 2.3. Time between release of black soldier fly larvae into containers with different marine wastes and their emigration in search of pupation habitats. CD – dry sea cucumber, CM – crab meal, CW – wet sea cucumber, CF – chicken feed (control), FF – finfish trimmings, QD – dry quahog, QW – wet quahog, SU – sea urchin. Bars followed by the same letter are not significantly different from each other.

2.4. Discussion

BSFL are saprophagous and can colonize a wide variety of organic materials in their natural habitat ranging from decaying human and pig cadavers (Tomberlin et al. 2005, Martínez-Sánchez et al. 2011), to decaying vegetables (Banks et al. 2014). Therefore, their ability to successfully utilize some of the marine wastes is not surprising. However, even highly

omnivorous species have certain restrictions on their diets. For instance, some digestive limitations of BSF include organic matter high in lignin or cellulose, such as rice straw, that are also considered low quality as livestock feed (Zheng et al. 2012, Manurung et al. 2016).

In our study, the highest mean number of prepupae were found when developing on wet sea cucumber and finfish trimmings. The next highest was dry quahog, followed by chicken feed. All others resulted in relatively low survival, with the highest mortality found for dry sea cucumber. Mean weight of developing larvae was also strongly affected by sea food waste fed, although not all treatments causing high larval mortality resulted in small larvae. Finfish trimmings and chicken feed produced the largest larvae, followed by crab meal, then by wet sea cucumber, and then by sea urchin. All other wastes resulted in relatively small larvae. Developing on dry sea cucumber and crab meal led to the shortest time period before emigration of prepupae.

BSFL survival and weight gain was dramatically different on wet versus dry sea cucumber, with the latter being largely unsuitable for growing larvae. The reverse was true for quahog, with significantly better growth on dry residues compared to wet residues. Processing leads to significant changes in the composition of both quahog (Wohlt et al. 1994) and sea cucumber (Zhong et al. 2007), which was likely responsible for the observed effects. It is possible that technological process that produced dry sea cucumbers extracted some nutrients essential for BSFL development or compromised the suitability of this substrate in some other way. If sea cucumber waste is to be used for BSFL commercial operations, it would be advisable to refrain from using dried versions or combine them with other waste material. Furthermore, sea cucumber byproducts are sold as nutritional supplements for arthritis in humans and pets. Maine

DMR has increased regulations on harvest size due to declining populations of *C. frondosa*, and their prices are also relatively high (Feindel et al. 2011). Therefore, the likelihood of using sea cucumber waste for commercial BSFL operations seems to be low for economic reasons.

Finfish trimmings produced the largest larvae and resulted in high survivorship compared to the other experimental wastes. Similarly, when larvae were fed a diet of cow manure and a mix of fish offal with cow manure, the weight of the prepupae fed fish offal were larger (St-Hilaire, Cranfill, et al. 2007). Unfortunately, finfish trimmings are used extensively by pet food industry. Therefore, they are relatively expensive. However, an underutilized finfish trimming waste streams may be an option for BSFL mass production facilities.

Crab meal normally contains ca. 32% protein and 41% ash and is traditionally marketed as an additive to livestock feeds as a protein source. However, due to the low protein digestibility when fed to livestock, it has a minor feed value and is better suited for crustacean or mollusk diets (Hertrampf and Piedad-Pascual 2000, Stewart and Noyes-Hull 2010). It is also susceptible to contamination by salmonella and other harmful microbes that produce toxins, which makes processing it a health hazard (Hertrampf and Piedad-Pascual 2000). Other uses include fertilizers due to its high chitosan content.

Our results show that BSFL had the ability to attain a relatively high mean weight in a relatively short period of time when fed crab meal, comparable to those attained when feeding on wet sea cucumber, finfish trimmings, and chicken feed. However, their survival was very poor compared to the other marine wastes except dry sea cucumber, wet quahog, and sea urchin. Apart from being potentially nutritionally inadequate, crab meal had fine powder consistency that may have made it difficult for BSFL to transpire. Observations during experiment indicated that crab meal would stick to the larvae, which may have clogged their spiracles or prevented

proper molting. Since crab meal is mostly composed of chitin, it could have also promoted microbial populations that were detrimental to BSFL. Products containing blue crab scrap, have been shown to suppress soil dwelling plant-parasitic nematode populations (Rich and Hodge 1993). The mode of action of chitin on nematodes has been hypothesized to be the production of ammoniacal nitrogen and enhanced chitinolytic fungal activity (Godoy et al. 1983, Rodriguez-Kabana et al. 1987, Rodriguez-Kabana and Morgan-Jones 1988). Knowing mechanisms responsible for negative effects of crab meal on BSFL may allow finding ways of using this byproduct for BSFL rearing, as it allows surviving larvae to grow to full size.

Chicken feed commonly serves as a standard control diet in BSFL studies (Nguyen et al. 2015). However, larval survival on this substrate recorded in our study was not very high compared to other treatments. The feed formulation used in our experiment was mostly plant-based in origin. Although BSFL are capable of developing on plant materials (Nguyen et al. 2015, Barragan-Fonseca et al. 2017, Tinder et al. 2017), it is possible that the chicken feed had smaller amounts of certain nutrients compared to the seafood wastes. In particular, high protein contents, typical for animal carcasses such as sea food wastes tested in this study, have been shown to be beneficial for BSFL (Cammack and Tomberlin 2017, Tinder et al. 2017).

Lower survival did not necessarily correlate with smaller size of surviving larvae. It is likely that we observed a density-dependent response when lower nutritional qualities were compensated for by lower larval densities due to the die-off of less competitive individuals. For saprophagous dipteran species, the short-lived immature stages determine the fitness of adults. Second to early third instar larvae commonly experience crowding that usually results in exploitative competition for resources when the larvae need to acquire food necessary for successful pupation (Levot et al. 1979, Mueller 1988). If the ageing substrate is losing nutritional

value over time, and the feeding rate of BSFL is highest in the later instars, it is likely that the older larvae will need to consume more low nutrition feed (Banks et al. 2014). Release from competition may allow survival on otherwise unfavorable diets.

Other species of saprophagous insects are capable of subsisting on non-preferred foods when preferred foods are not available. For example, *Chrysomya rufifacies* (Macquart) larvae shifted from saprophagy to predation and cannibalism in instances where food was limited (Goodbrod and Goff 1990). Similarly, dung beetle *Deltochilum verruciferum* (Felsche) had a strong preference to feeding on carrion and excrement, but could utilize decomposing fruits, and even seeds, if preferred resources were scarce (Salomão et al. 2018). BSF may have the potential to do the same and adapt to available low-quality substrates.

Faster time of development in fly larvae may also help surviving in nutrient-poor environments. Individuals that pupate early, before reaching their optimal sizes, are likely to have increased energy costs for flight and decreased fecundity as consequences of their smaller adult sizes. However, these are smaller fitness penalties than the increased risk of mortality in the larval or pupal stages (Williams and Richardson 1983). Indeed, the fastest-developing BSFL fed on dry sea cucumber in our study also had the smallest size. Their mortality was also the highest, although at least some larvae survived to prepupal stage. Interestingly, BSFL also developed quickly on crab meal, which was not a favorable substrate based on their mortality. However, there was no decrease in the size of survivors. As discussed above, it is possible that high BSFL mortality on crab meal was caused by its physical properties or by the activity of chitinolytic microorganisms rather than by its nutrient deficiencies.

Our results expand the list of wastes potentially available for BSFL rearing to include wet sea cucumber, dry quahog, sea urchin, and, possibly, crab meal. They also highlight the importance of thorough testing of specific substrates, as there were dramatic differences in seemingly similar materials, such as wet and dry sea cucumber or wet and dry quahog. Currently, fish meal and fish oil are primarily made with small pelagic fish and trimmings of processed fish from wild-caught and aquaculture sources. Due to low fish stocks, the use of by-products that were previously discarded are being incorporated into feeds (Food and Agriculture Organization of the United Nations (FAO) 2016). At the same time, approximately 1.04 million tons of marine waste is generated each year in the United States alone (Love et al. 2015). Therefore, diverting the seafood waste stream for the use of BSFL can help with more sustainable practices, especially if various types of waste are used, and not only finfish trimmings.

CHAPTER 3

ANTIBIOTIC AND ANTIXENOTIC PROPERTIES OF SUBSTRATES INHABITED BY BLACK SOLDIER FLY LARVAE

3.1. Introduction

Many studies on black soldier fly (BSF), *Hermetia illucens* (Linnaeus) (Diptera: Stratiomyidae) have been conducted on the development, bioconversion of waste materials, and nutritional value, but less attention has been paid to the associated microbial communities that occupy the same environment (De Smet et al. 2018). The few existing studies focused mostly on internal symbionts within their digestive system. Eight-day old BSF larvae (BSFL) reared on restaurant food waste, cooked rice, and vegetable-based calf forage had different microbial communities within their guts. Bacterial phyla were more diverse in larvae fed on food waste than on cooked rice and calf forage, partly due to carbohydrates being the main source of nutrients Degradation of substrates by the intestinal bacteria of BSFL played an important role in reduction of waste material that produce beneficial probiotic compounds. That confirmed the likely effect of BSFL presence on microbes that inhabit their feeding substrates (Jeon et al. 2011). There was also a significant change in microbial communities when DNA was extracted from whole specimens at various life stages after larvae were fed Gainesville diet (corn mean, alfalfa meal, wheat bran, and water) inhabited by the first vs. the second generations of BSFL. That demonstrated the capability of particular bacteria to be kept throughout life stages (Zheng et al. 2013).

Fungal communities of the BSFL guts reared on chicken feed for 17, 14, and 21 days followed by vegetable waste for 4 and 7 days were assessed (Boccazzi et al. 2017). Larval guts from eight surface-sterilized larvae per trial period were dissected. The fungal communities observed by OTU composition presence or absence within samples were influenced by substrate

variations similar to the bacterial communities described above. There was more fungal diversity estimated with diversity indices: Shannon *H* diversity, Pielou's *J* evenness indices and Chao-1 when larvae fed on different wastes (Boccazzi et al. 2017).

BSFL are known to feed on a wide variety of organic material at different stages of decay (Tomberlin et al. 2005, Martínez-Sánchez et al. 2011, Banks et al. 2014). In a mass rearing operation using BSFL to consume marine waste from fish processing facilities, other Dipteran species may be attracted to the same marine waste material during transport or in storage units prior to being fed to BSFL. Contamination by wild pest fly larvae may affect the development of BSFL or decrease the quality of harvested biomass. The blow fly *Lucilia sericata* (Meigen) (Diptera: Calliphoridae), is commonly the first species to be attracted to decaying matter (Byrd and Castner 2009, Australian Museum 2015). This can be due to the volatile organic compounds produced during the break down of organic material by microorganisms being attractive to *L. sericata* (Hammack 1991). Adult Calliphoridae are a potential health risk because they can be carriers of human and animal pathogens (Pava-Ripoll et al. 2012).

Sterilizing the substrate to eliminate biological contamination prior to feeding it to BSFL may not result in optimal BSFL development. In commercial rearing facilities, sterilized substrates need to be inoculated with a small amount of untreated substrate in order to obtain proper BSFL performance (De Smet et al. 2018). Fortunately, decaying substrates inhabited by BSFL appear to naturally acquire antibiotic and antixenotic properties against a range of micro- and macroorganisms. If larvae do become contaminated, feeding becomes minimal or nonexistent, reducing larval mass. These negative effects were reported for *Escherichia coli* (Migula) and *Salmonella enterica* serovar Enteritidis (Kauffmann & Edwards) (Erickson et al. 2004, Lalander et al. 2015), *Klebsiella pneumoniae* (Schroeter), *Neisseria gonorrhoeae* (Zopf)

and *Shigella sonneio* (Levine) Choi et. al. (2012), and several virus species (Lalander et al. 2015). Furthermore, oviposition by house fly, *Musca domestica* (Linnaeus) in poultry manure was inhibited when BSFL were present in high densities (Bradley and Sheppard 1983).

Our results on the development of BSFL demonstrated that they perform well when developing in finfish substrate, compared to six other marine wastes (see previous chapter). Preliminary observations made while rearing BSFL in the laboratory and greenhouse showed that *Calliphora vomitoria* (Linnaeus), *Lucilia coeruleiviridis* (Macquart), *Musca domestica* (Linnaeus), and *L. sericata* were attracted to the vicinity of finfish substrate but avoided ovipositing in the substrate presently or previously inhabited by BSFL. In the present study, we further investigated effects of BSFL on other saprotrophic organisms. We hypothesized that BSFL presence will serve as a deterrent to its colonization by blow flies and alter microbial communities associated with decaying finfish substrate.

3.2. Materials and methods

3.2.1. Blow fly origins

Wild flies were collected on sunny days with no cloud cover by leaving 200 g of beef liver in small white Styrofoam bowl outside a greenhouse on the University of Maine campus in Orono. Liver was left uncovered for at least six hours. It was then removed and placed in the laboratory to inspect for eggs. Hatching larvae were reared in Styrofoam bowls on beef liver. Water was added to the bowls to prevent liver from drying. The bowls with larvae were maintained in a 36 by 20 by 38 cm plastic storage box (Sterlite, Townend, MA) containing 20 ml of vermiculite. After larvae completed their development, they emigrated from the bowls and pupated in vermiculite. Pupae were transferred into a 120 ml plastic cups with vermiculite. Cups were covered with a mesh screen to prevent escape of any emerging flies.

After six to seven days, the adult flies emerged. All flies were identified using two taxonomic keys (Triplehorn et al. 2005, Marshall, S.A., Whitworth, T. and Roscoe 2011). *Lucilia sericata* adults were isolated for further rearing.

3.2.2. Blow fly rearing

Adult blow flies were kept in a 81 by 102 by 114 cm custom-made wooden frame screen cage in the greenhouse with ambient temperature at 261°C (mean standard deviation; range: 24°C - 30°C) and 60% (42.5% - 69%) relative humidity. They were fed *ad libitum* by adding 80 ml of granulated sugar, 100 g of fresh beef liver, and 120 ml of clean tap water to the cage as needed. The food and water were placed under a cardboard shelter to avoid direct sunlight. Larvae were reared in the same manner as described above for wild colony founders, but in the greenhouse in a 30 cm white parasitoid-resistant popup rearing and observation cage with vinyl window (BioQuip, Rancho Dominguez, CA) to prevent parasitoids and other pest flies from contaminating the colony.

3.2.3. Black soldier fly origins and rearing

The black soldier fly breeding colony was originally obtained from Symton Black Soldier Fly Solutions (College Station, TX). It was maintained in our laboratory following the procedures described in Chapter 2. Third instars were used to create experimental treatments described below.

3.2.4. Substrate preparation

Substrate for fly oviposition and development used in the present study consisted of 100 g of Pacific whiting, *Merluccius productus* (Ayres), fillets (The Fishin' Company, Munhall, PA) and 10 g of pine shavings (AWF Pets Pick, Columbia, MD) mixed with tap water to achieve 60% moisture content (w : w). The Pacific whiting filets were purchased frozen and processed in

laboratory by blending them at room temperature, then evenly spreading over a large tray, and placing in a soil drying room at 76°C for 24 hours. The dried filets were re-blended, weighed with an analytical scale (Ohause Co., Pine Brook, NJ), and mixed with pine shaving and water as described above. After that, prepared substrate was added to a 22 by 16 by 13 cm, 1892 ml GladWare container (GladWare, Oakland, CA) with a lid that had a 10 by 5 cm opening covered with an antivirus insect mesh (BioQuip Products, Rancho Dominguez, California). All treatments were maintained in the laboratory on a shelf under ambient temperature at 261°C (range: 24 - 27.7°C) and 508% (range: 42.5 - 69%) relative humidity. The shelf was covered by a black plastic film to prevent desiccation.

3.2.5. Experimental treatments

Unless specified otherwise, the following three treatments with five replications were tested in the present study: 1) substrate with BSFL feeding for two weeks and still present at the time of the experiment, 2) substrate with BSFL removed after one week of feeding, and 3) substrate with no BSFL that served as control. All samples were mixed gently for approximately ten seconds prior to removing for microbial community analysis. Baseline microbial activity and microbial community composition at the beginning of the experiment before BSFL introduction into experimental containers was determined by taking samples from each replicate of the control substrate only.

3.2.6. Substrate colonization

We tested colonization by *L. sericata* of the following: 1) substrate with BSFL feeding for two weeks and still present at the time of the experiment, 2) substrate with BSFL removed after one week of feeding, and 3) substrate with no BSFL to serve as our control. A total of five replicates were set up for each treatment. Treatments with BSFL were seeded with 50 third

instars. All treatments were maintained for two weeks before exposure to *L. sericata* oviposition in a greenhouse under ambient temperature at 26±1°C (range: 24°C - 30°C) and 60±6% (range: 42.5% - 69%) relative humidity. For the duration of the experiment, 20 mL of tap water was added daily to all boxes to prevent desiccation. Trials were conducted in a greenhouse by introducing 25 gravid *L. sericata* to a 2 by 2 by 4 m wooden screened cage with six uncovered GladWare containers arranged following randomized complete block design for 24 hours. Twenty-five newly separated gravid *L. sericata* were used in every replication. After 24 hours, the GladWare containers were covered with screened lids, removed from cages, and maintained in the same greenhouse with daily additions of 20 mL of tap water until all *L. sericata* had pupated. The number of pupae were then counted.

3.2.7. Blow fly larvae emigration

This experiment was limited to two treatments, substrate with no BSFL and substrate that was fed on by BSFL for two weeks. A total of five replications were set up for each treatment. One hundred larvae were placed into each of the ten treatment boxes made of 22 by 16 by 13 cm, 1892 ml GladWare containers (GladWare, Oakland, CA) without the lids. Boxes were individually placed into a 36 by 20 by 38 cm plastic storage box (Sterlite, Townend, MA) to collect emigrating larvae. Each treatment box was then individually placed into a 30 cm white parasitoid-resistant popup rearing and observation cage with vinyl window (BioQuip, Rancho Dominguez, CA) in the laboratory with ambient temperature at 261°C (range: 24 - 27.7°C) and 508% (range: 42.5 - 69%) relative humidity. The number of larvae that emigrated from the treatment boxes were counted after 24 hours.

3.2.8. Determination of microbial activity using fluorescein diacetate

Substrate samples were taken after one week immediately following BSFL removal from the containers assigned to Treatment 2, and at the end of the experiment. Following Schumacher et al. (2015), chemical reagents including potassium phosphate buffer (60 mM, pH 7.6), fluorescein diacetate (FDA) stock solution (1000 μgmL^{-1}), and 0.2 ml chloroform were used. A total of 0.5 g of substrate was taken from each box and transferred into a 100-mL Erlenmeyer flask and mixed with 15 mL of 60mM potassium phosphate buffer (pH 7.6). Then, 0.2 mL of FDA stock solution (1000 $\mu gmL^{-1}1$) was added to the Erlenmeyer flask to initiate the hydrolyzing reaction. Control substrate samples were also prepared without adding FDA stock solution. All these samples with FDA and controls in Erlenmeyer flasks were closed with stoppers and shaken by hand for few minutes. These Erlenmeyer flasks of six replications were placed in an incubator with orbital shaker (with 100 rpm) at 30 °C for 20 min. After incubation, 15 mL of chloroform were immediately added to the flask to stop the hydrolyzing of FDA by enzymes released by microbial functional groups in the substrate. The contents of Erlenmeyer flasks were then thoroughly shaken before transferring into 50-mL centrifuge tubes. Those tubes with samples were centrifuged at 2000 rpm for 5 minutes using an Eppendorf Centrifuge 5810 R (Fisher Scientific, Ottawa, Ontario). The clear supernatant solutions were filtered, and the yellow color intensity measured as absorbance at 490 nm using a UV-Vis spectrophotometer (Spectronic Instruments Genesys 20, Thermo Scientific Waltham, MA). The concentration of fluorescein in supernatant solutions of samples was determined using a calibration curve of 0–5 μgmL^{-1} FDA standards.

3.2.9. Determination of microbial activity using dilution plating

To supplement the FDA analysis, estimates of populations of total culturable bacteria and fungi in black soldier fly substrates were assayed by dilution plating and subsequent counting of the number of colonies growing in Petri dishes. Samples were taken at the end of the experiment from the same containers as for the FDA analysis described above. For logistical reasons, the number of replications for all treatments was reduced to three by randomly omitting two out of five containers.

Three separate 10 g subsamples (technical replicates) were then taken from each sample, independently processed, and plated as follows. Each 10 g subsample was added to 90 milliliters of 0.2% sterile water agar, stirred on magnetic stir plates for 5 min, and subsequently serially diluted onto 1/10-strength tryptic soy agar (TSA) for bacterial counts (dilutions of 10^7 and 10^8) and potato dextrose agar (PDA) containing 50 milligrams of chlortetracycline and 1 mL/L of tergitol for fungal counts (dilutions of 10^4 and 10^5) (Larkin 1993). Each dilution of each technical replicate was plated in duplicate. Bacterial plates were kept at 28°C for 3 days in a biological incubator (Percival Scientific, Peabody, MA) and fungal plates were incubated at 22°C for 7 days in a closed cardboard box in the laboratory before colonies were enumerated. All microbial counts were recorded as the number of colony forming units (CFUs) per gram of substrate. An additional 10 g subsample of the substrate was removed from each technical replicate onto a 42 ml fluted aluminum weighing dish (Fisher Scientific, Ottawa, Ontario) and placed in a soil drying room at 76°C for 24 hours. The wet weight and dry weights were measured to account for the substrate moisture concentrations of each replicate. Drying the substrate was necessary to calculate the number of microbes per gram of dry substrate to correct for variation in the number of microbes at different moisture levels (Bernard 2012).

3.2.10. DNA sequencing

Taxonomic composition of microbial communities was estimated by sequencing DNA extracted from the experimental substrates. On the seventh day, when BSFL were removed from the containers assigned to Treatment 2, and at the end of the experiment on the fourteenth day, samples were taken from each container. The extracted DNA was pooled for all replicates, producing composite samples for each of the treatments.

DNA was extracted following manufacturer's recommendation from 0.25 g of the substrate sample using MOBIO Laboratories, Inc, PowerLyzer® PowerSoil® DNA Isolation Kit (QIAGEN, Germantown, MD). A total of 750 μl of bead solution was added to a 0.1 mm glass bead tube and gently vortexed. Next, 60 μl of Solution C1 was added and the tube was inverted several times. The glass bead tubes were placed horizontally on a Vortex Genie 2 (Scientific Industries, Inc., Bohemia, NY), secured on a flat-bed vortex pad with tape, and vortexed at maximum speed for 10 minutes. The bead tubes were placed in an Eppendorf centrifuge 5424 (Fisher Scientific, Ottawa, Ontario) at 10,000 x g and spun for 30 seconds at room temperature. The supernatant was transferred to a clean 2 ml collection tube and 200 μl of solution C3 was and vortexed briefly. After vortexing, it was incubated at 4°C for 5 minutes and then centrifuged for 1 minute at room temperature at 10,000 x g. Avoiding the pellet, up to 750 μl of the supernatant was placed into a clean 2 ml collection tube. Subsequently, 1200 μl of solution C4 was added to the supernatant and vortexed for 5 seconds. After that, approximately 675 μl were loaded onto a spin filter and centrifuged it at 10,000 x g for 1 minute at room temperature. The flow through was discarded, an additional 675 μl of supernatant were added to the spin filter, and centrifuged at 10,000 x g for 1 minute at room temperature. We loaded the remaining supernatant onto the spin filter and centrifuged it at 10,000 x g for 1 minute at room temperature.

A total of 500 µl of solution C5 was added and centrifuged at room temperature for 30 seconds at 10,000 x g. The flow thorough was discarded and centrifuged again at room temperature at 10,000 x g. The spin filter was placed in a clean 2 ml collection tube. Next, 100 µl of Solution C6 was added to the center of the white filter membrane and centrifuged at room temperature for 30 seconds at 10,000 x g. Finally, the spin filter was discarded, and the DNA was placed in a freezer at -12°C. DNA was sent to Molecular Research (MRDNA) Company (Shallowater, TX), where a diversity assay was conducted using bTEFAP® illumina sequencing with 20k reads using the ITS primers, ITS1F-Bt1 (5′-CTTGGTCATTTAGAGGAAGTAA-3′) and ITS2R (5′-GCTGCGTTCTTCATCGATGC-3′) and 16s primers, 515F (5′- GTGCCAGCMGCCGC GGTAA -3′) and 806R (5′- GGACTACHVGGGTWTCTAAT -3′).

3.2.11. Data analyses

To calculate the proportion of boxes in each treatment colonized by *L. sericata* pupae, a Chi-square test was conducted. For the number of pupae in colonized substrates for each treatment, an ANOVA. A T-test was conducted for the number of *L. sericata* larvae remaining in two treatments.

The number of microorganisms in a substrate sample was estimated by counting the number of CFUs at dilutions of 10^7, 10^8 for bacteria, and 10^4, 10^5 for fungi. The higher dilution counts for example (10^8), were multiplied by a dilution factor of 10 and averaged with the lower dilution counts (10^7) for both fungi and bacteria. Number of colonies as CFU per gram of wet substrate values were weight corrected to represent the number of colonies as CFU per gram of dry substrate. That was done by dividing dry weight by wet weight to obtain a weight correction factor, and then multiplying the number of CFUs per gram of wet substrate by that weight correction factor. FDA values were converted to fluorescein concentration (µg/ml) and were

weight corrected in the same way. One-way ANOVAs were conducted to compare weight-corrected numbers of CFUs among the treatments. Mean separation was carried out with Tukey's honestly significant difference (HSD) tests ($P < 0.05$). Repeated measures ANOVA was used to analyze the FDA data. When the interaction between treatment and time was not significant, a Tukey HSD was conducted on the overall means ($P < 0.05$). All analyses were performed using the Statistical Analysis Systems R software (R free software, Cary, North Carolina). Normality was tested using Shapiro–Wilk test, and skewed data was transformed by cube root transformation.

Operational taxonomic units (OTUs) assigned to the same genus and species were merged into a single composite taxonomic unit, with the number of reads added together (Soh et al. 2013, He et al. 2015, Tang et al. 2015)**.** After that, genera and species were arranged in a descending order based on the number of reads. Subsequent discussion was limited to the top 20 taxonomic units. Venn diagrams were produced using Venny software package (Oliveros 2015) to identify taxa unique to each treatment.

3.3. Results

3.3.1. Substrate colonization

Results suggested that introducing BSFL into the substrate resulted in approximately a ten-fold decrease in the number of encountered *L. sericata* pupae. The difference among the treatments was marginally significant (df=2,27, F=3.03, P=0.0651) (Figure 3.1). The proportion of boxes being colonized by blowfly pupae in each treatment was 40% (control), 50% (BSF), and 30% (SBSF). The Chi-square test showed that they were not significantly different among the treatments (χ^2=0.83, P=0.6592).

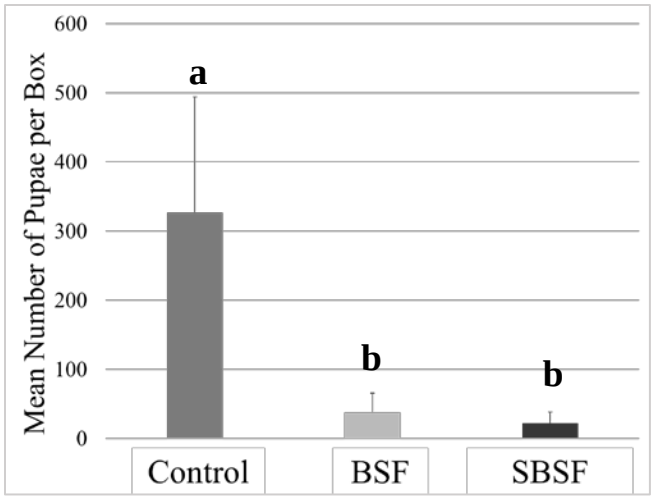

Figure 3.1. Mean number of green blow fly *Lucilia sericata* pupae in colonized substrate. Means followed by the same letter were not significantly different from each other (HSD test, P > 0.05). Experimental treatments are: Control (finfish substrate without BSFL), BSF (finfish substrate with BSFL feeding for fourteen days), and SBSF (finfish substrate with BSFL feeding for seven days and then removed).

3.3.2. Blow fly larvae emigration

For the short-term behavioral response, no *L. sericata* larvae were found outside of substrate that did not have any BSFL. All 100 initially released larvae were recovered from each of the test arenas. In the same time, less than one-third of blow fly larvae remained within the substrate inhabited by black soldier fly larvae. The difference in the number of emigrating larvae between the treatments was highly significant (df=4, T=12.66, P=0.0002) (figure 3.2).

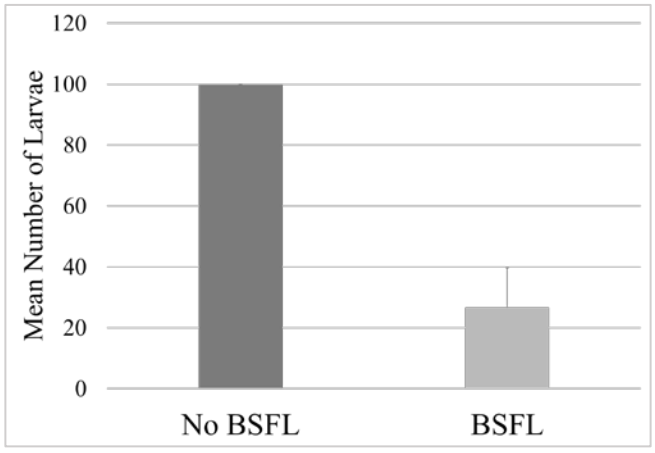

Figure 3.2. Mean number of green blow fly *Lucilia sericata* larvae remaining in substrate with and without black soldier fly larvae (BSFL).

3.3.3. Determination of microbial activity using fluorescein diacetate

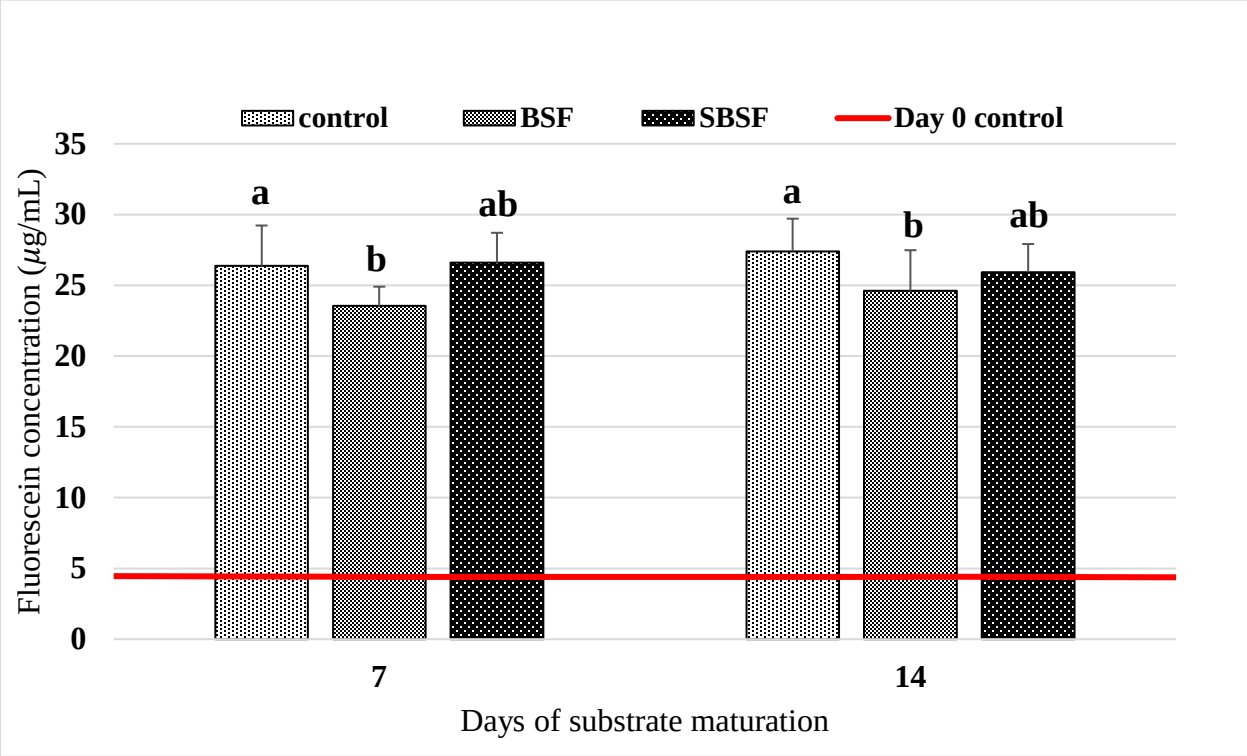

Figure 3.3. The fluorescein concentration indicates the amount of esterase activity on day 0, 7 and 14 when black solider fly larvae fed during experimental treatments: Control (finfish substrate without BSFL), BSF (finfish substrate with BSFL feeding for fourteen days), and SBSF (finfish substrate with BSFL feeding for seven days and then removed). Horizontal line shows baseline concentration measured at the Control samples in the beginning of the experiment. Bars followed by the same letter are not significantly different from each other.

The fluorescein concentration was significantly different among the treatments (df= 2,12, F= 3.882, P= 0.050). Therefore, the esterase activity was significantly influenced by the different treatments. Meanwhile, there was no significant time effect. In other words, the treatments did not show different esterase activity over time (df= 1, F= 0.333, P= 0.575). The treatment by time interaction was not significant (df= 2, F= 0.501, P= 0.618). Therefore, differences among the treatments were consistent throughout the experiment. Tukey HSD test conducted on the overall means indicated that the control and BSF treatment were significantly different from each other, but not from the SBSF treatment (Figure 3.3).

3.3.4. Determination of microbial activity using dilution plating

3.3.4.1 Fungi

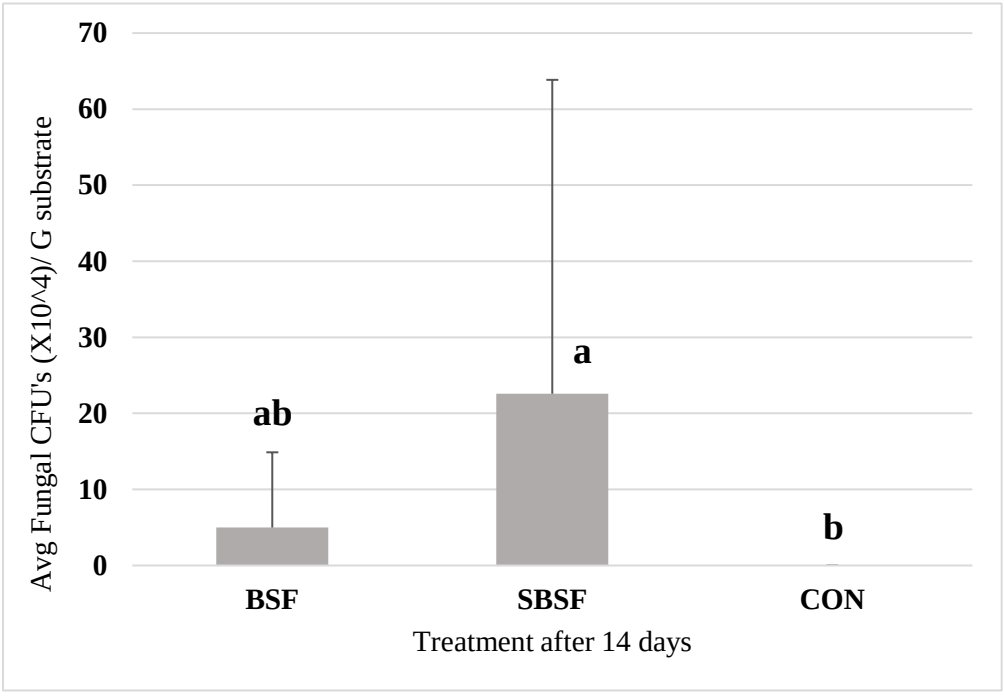

Figure 3.4. Average number of fungal colony forming units (CFUs) observed for experimental treatments: Control (finfish substrate without BSFL), BSF (finfish substrate with BSFL feeding for fourteen days), and SBSF (finfish substrate with BSFL feeding for seven days and then removed). Average number of CFUs were calculated with the concentration of 10^4 CFU per gram of substrate multiplied by 10 (dilution factor), averaged with the concentration of 10^5 CFU per gram of substrate, and dry weight corrected. Bars followed by the same letter are not significantly different from each other.

The average number of fungal colony forming units was significantly influenced by the treatment (df= 2,51, F=4.335, P=0.01). The Tukey HSD test suggested that the SBSF treatment had significantly more fungi than control, but there was no difference between the control and BSF treatment. No significant difference was found between the BSF and SBSF treatment (Figure 3.4).

3.3.4.2. Bacteria

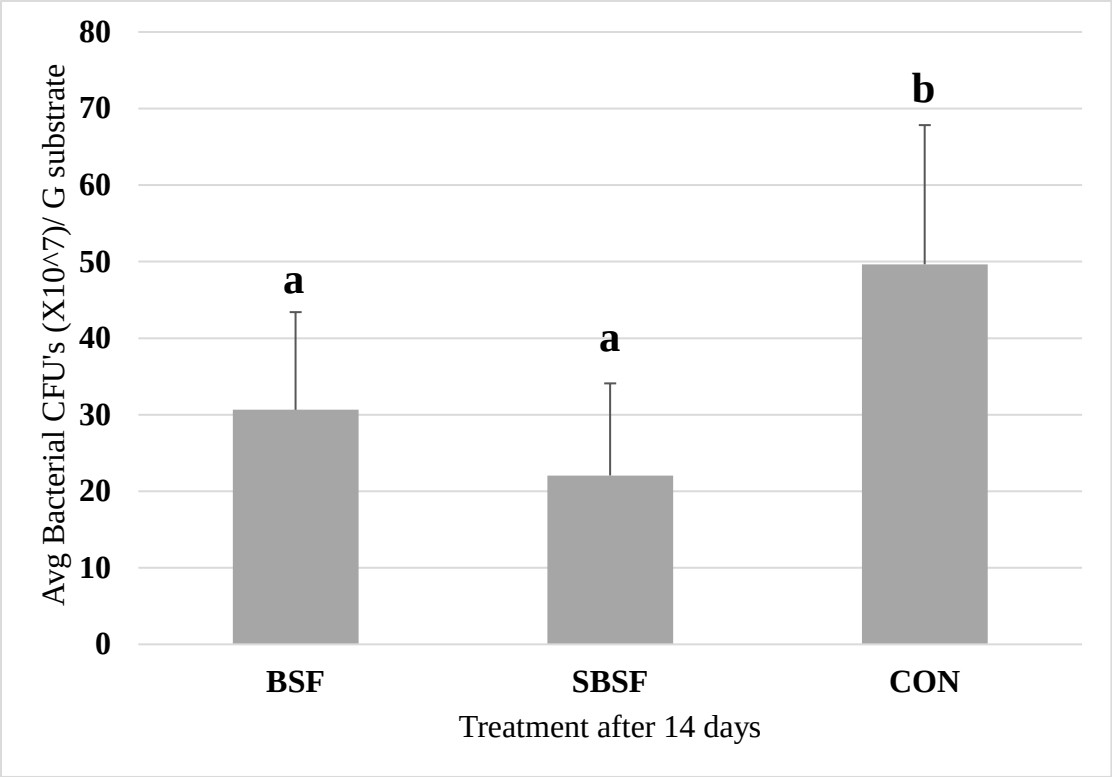

Figure 3.5. Average number of bacterial colony forming units (CFUs) observed for experimental treatments: Control (finfish substrate without BSFL), BSF (finfish substrate with BSFL feeding for fourteen days), and SBSF (finfish substrate with BSFL feeding for seven days and then removed). Average number of CFU's were calculated with the concentration of 10^7 CFU per gram of substrate multiplied by 10 (dilution factor), averaged with the concentration of 10^8 CFU per gram of substrate and dry weight corrected. Bars followed by the same letter are not significantly different from each other.

The average number of bacterial CFUs was significantly influenced by the treatment (df= 2,51, F=16.87, P=0.0001). Both BSF and SBSF treatments had significantly fewer bacteria than the control, but they were not different from each other (Tukey HSD test, P<0.05) (Figure 3.5).

3.3.5. Taxonomic composition of microbial communities

3.3.5.1. Fungi

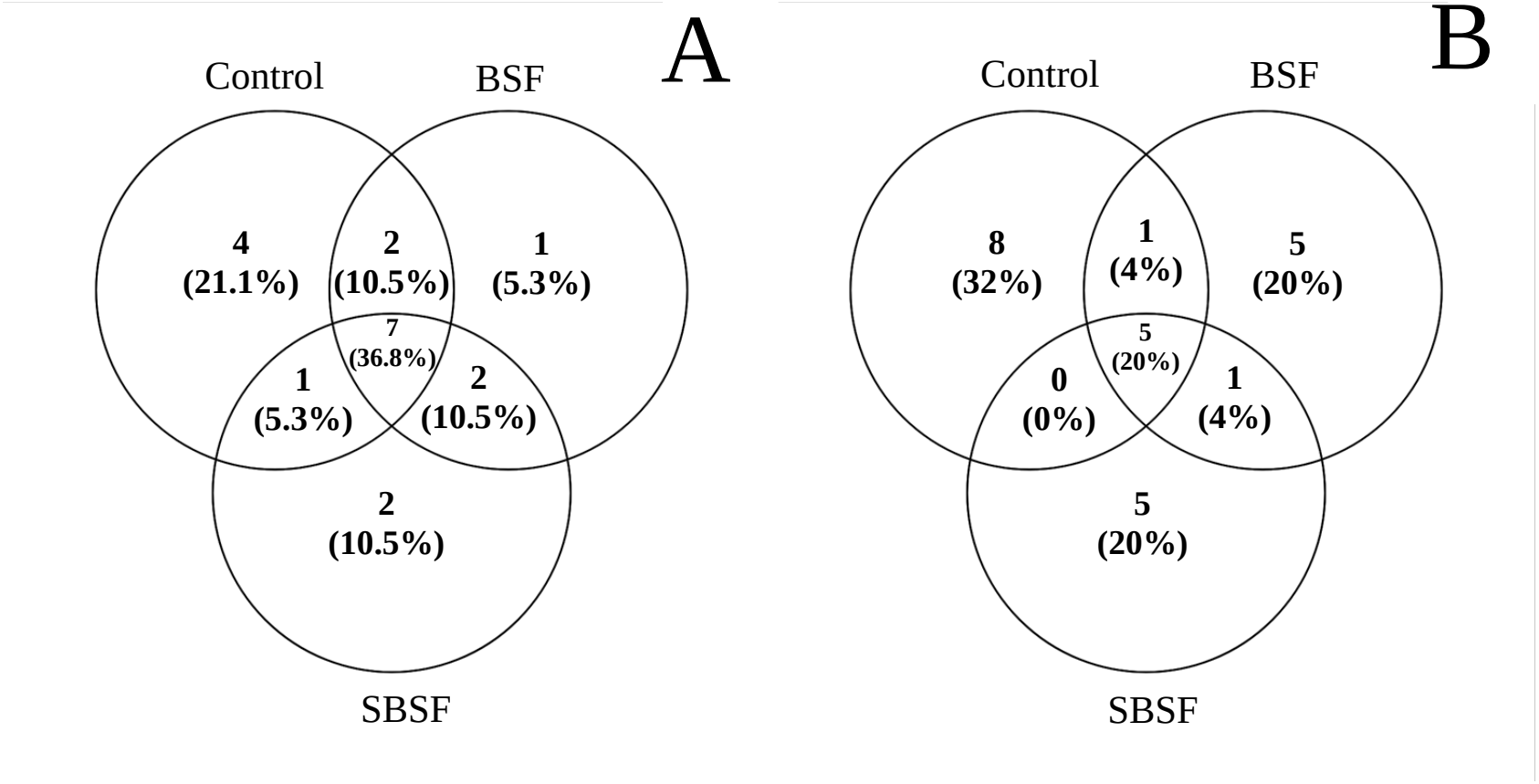

Figure 3.6. Similarities between fungal taxa observed on day 7 (A) and day 14 (B) for experimental treatments: Control (finfish substrate without BSFL), BSF (finfish substrate with BSFL feeding for fourteen days), and SBSF (finfish substrate with BSFL feeding for seven days and then removed).

Presence of BSFL affected fungal composition of substrates, although there were similarities among the treatments. On Day 7, 21.1% of fungal taxa were unique in the control, while 36.8% were ubiquitous (Figure 3.6 A). On Day 14, the number of taxa unique to the control increased to 32%, while the number of ubiquitous taxa decreased to 20% (Figure 3.6 B).

The top five fungal families observed across all treatments for Days 7 and 14 were: Aspergillaceae, Debaryomycetaceae, Lichtheimiaceae, Liposcelidae, Microascales, Pichiaceae, Rhizopodaceae, Saccharomycetales, Taphrinaceae, and Tremellales (Table 3.1).

Lichtheimiaceae, Taphrinaceae and Rhizopodaceae were only observed in the Control on Days 7 and 14, Microascales – in the BSF treatment on Day 14, and Liposcelidae – in the SBSF treatment on Day 14. All other families were more consistent across the treatments and days, although their relative abundances varied (Table 3.1).

Aspergillus oryzae, Candida rugosa, and *Trichosporon asahii* were observed in all the treatments. *Lichtheimia corymbifera, Penicillium citrinum, Penicillium griseofulvum, Rhizopus oryzae*, *Saccharomycopsis fibuligera* and *Taphrina johansonii* were unique to the Control, while *Scopulariopsis* sp. was unique in the BSF treatment and *Liposcelis* decolor in SBSF. *Pichia kudriavzevii*, and *Candida tropicalis* were both found in BSF and SBSF treatments, while *Aspergillus ochraceus* was found in the Control and SBSF treatment (Table 3.1).

Species composition was generally consistent throughout the experiment, with a few exceptions (Table 3.1). *Malassezia restricta* was the most abundant fungus in fresh substrate without larvae. *Aspergillus oryzae* became prominent on subsequent sampling dates regardless of treatment. *Scopulariopsis* sp., was rare on earlier dates, but became the most dominant species in the BSF treatment on Day 14. Somewhat similarly, *Liposcelis decolor* became the most abundant fungus in the SBSF treatment on Day 14, while being completely absent from all other samples.

3.3.5.2. Bacteria

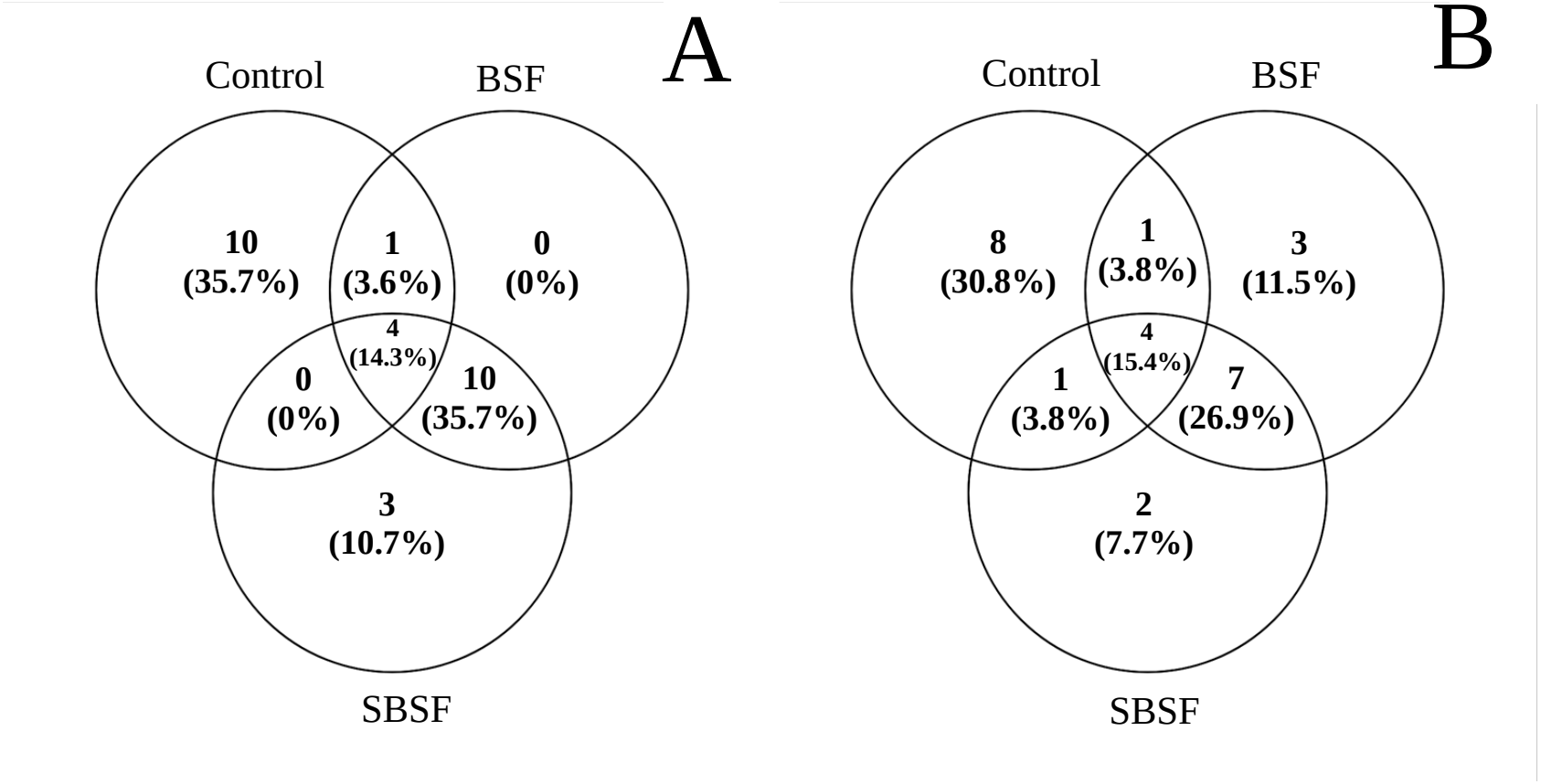

Figure 3.7. Similarities between bacterial taxa observed on day 7 (A) and day 14 (B) for experimental treatments: Control (finfish substrate without BSFL), BSF (finfish substrate with BSFL feeding for fourteen days), and SBSF (finfish substrate with BSFL feeding for seven days and then removed).

There was a considerable difference between the control and the two treatments that contained soldier fly larvae. On Day 7, 35.7% of taxa were unique to the control, and 35.7% of taxa were shared between BSF and SBSF, but absent from the control. 14.3% of taxa were ubiquitous among the treatments, and only 3.6% of taxa was shared between the control and the BSF (Figure 3.7 A). Similarities increased on Day 14, but not by much (Figure 3.7 B).

The top five bacterial families observed across all treatments for Days 7 and 14 were: Bacillaceae, Brevibacteriaceae, Corynebacteriaceae, Micrococcaceae, Planococcaceae, and Staphylococcaceae (Table 3.2).

For the Control treatment, Staphylococcaceae and Micrococcaceae had the highest number of reads on Days 7 and 14. On Day **7,** Planococcaceae first appeared in the BSF treatment and became relatively abundant on Day 14 in all the treatments. All other families remained the same, but their relative abundance changed. In the BSF treatment, all families remained the same between Days 7 and 14. More Planococcaceae were observed on Day 14 while on Day 7 in the BSF and SBSF treatments Staphylococcaceae had the highest number of reads. In the SBSF treatment on Day 7 all families remained the same as in the BSF treatment. On Day 14 Enterobacteriaceae first appeared replacing Planococcaceae while all other families remained the same, but their relative abundance changed.

Some bacterial species were unique to a particular treatment, while others appeared in all treatments. *Bacillus szutsauensis, Corynebacterium variabile, Pantoea agglomerans, Shigella sonnei,* and *Vagococcus lutrae* were unique to the control samples, *Bacillus* sp., *Nosocomiicoccus ampullae*, and *Ornithinibacillus* sp. were unique to the BSF treatment, and *Proteus myxofaciens* and *Providencia rustigianii* were unique to the SBSF treatment.

Species composition was generally consistent among the days of the experiment, with a few notable exceptions (Table 3.2). *Bacillus cytotoxicus* was the most abundant bacteria in the fresh substrate without any larvae added. *Staphylococcus sciuri* was prominent on Day 7 for all treatments while *Sporosarcina* sp. had a relative low abundance. On Day 14 *Sporosarcina* sp. replaced *Staphylococcus sciuri* in the BSF and SBSF treatment. In the control treatment for both days, *Staphylococcus epidermidis* and *Staphylococcus cohnii* were not present in the BSF and SBSF treatment including the unique species above. Species that were only observed in the BSF and SBSF treatment on Days 7 and 14 were: *Bacillus cytotoxicus*, *Brachybacterium paraconglomeratum, Brevibacterium albus, Brevibacterium luteolum, Brevibacterium picturae, Corynebacterium* sp., *Jeotgalicoccus halotolerans, Natronobacillus azotifigens,* and *Sporosarcina soli.* On Day 14, some species that first appeared and were absent from all other samples were: *Bacillus* sp., *Nosocomiicoccus ampullae,* and *Ornithinibacillus* sp. in the BSF treatment while in the SBSF treatment, *Proteus myxofaciens* and *Providencia rustigianii*.

Table 3.1. Top 20 most abundant fungal species based on sequencing analyses of substrates with and without black soldier fly larvae for experimental treatments: Control (finfish substrate without BSFL), BSF (finfish substrate with BSFL feeding for fourteen days), and SBSF (finfish substrate with BSFL feeding for seven days and then removed).

	FUNGI								
	DAY 0			DAY 7			DAY 14		
Treatment	**# of Reads**	**Species (Family[1])**		**# of Reads**	**Species (Family[1])**		**# of Reads**	**Species (Family[1])**	
Control	16556	*Malassezia restricta*	(M1)	45603	*Aspergillus oryzae*	(A2)	11388	*Aspergillus oryzae*	(A2)
	10497	*Saccharomycopsis fibuligera*	(S3)	14748	*Lichtheimia corymbifera*	(L1)	7646	*Penicillium citrinum*	(A2)
	10117	*Trichaptum abietinum*	(P4)	4887	*Aspergillus ochraceus*	(A2)	2404	*Trichosporon asahii*	(T3)
	9933	*Cyberlindnera fabianii*	(P1)	3080	*Penicillium griseofulvum*	(A2)	1570	*Candida rugosa*	(S2)
	6394	*Saccharomyces bayanus*	(S1)	2158	*Trichosporon asahii*	(T3)	1126	*Taphrina johansonii*	(T1)
	6325	*Candida tropicalis*	(D)	2084	*Rhizopus oryzae*	(R)	610	*Saccharomycopsis fibuligera*	(S3)
	3342	*Cladosporium cladosporioides*	(C1)	1674	*Penicillium citrinum*	(A2)	514	*Penicillium griseofulvum*	(A2)
	2704	*Kodamaea ohmeri*	(M2)	1612	*Candida rugosa*	(S2)	309	*Lichtheimia corymbifera*	(L1)
	1860	*Perenniporia subacida*	(P4)	1033	*Rhizopus stolonifer*	(R)	154	*Aspergillus ochraceus*	(T3)
	1532	*Cladosporium* sp.	(C1)	1036	*Mucor plumbeus*	(M5)	146	*Scopulariopsis* sp.	(M4)
	1517	*Malassezia globosa*	(M1)	590	*Saccharomycopsis fibuligera*	(S3)	117	*Candida tropicalis*	(D)
	1328	*Geomyces pannorum*	(P6)	564	*Aspergillus* sp.	(A2)	111	*Hymenochaete spreta*	(H2)
	1315	*Clavispora lusitaniae*	(M2)	387	*Scopulariopsis* sp.	(M4)	98	*Lotharella globosa*	(E)
	1156	*Phialophora* sp.	(H1)	359	*Lichtheimia ramosa*	(L1)	88	*Clavispora lusitaniae*	(M2)
	931	*Aspergillus oryzae*	(A2)	235	*Eurotium* sp.	(A2)			
	891	*Rhizomucor pusillus*	(L1)						
	754	*Rhodosporidium sphaerocarpum*	(S4)						
BSF				6930	*Trichosporon asahii*	(T3)	11057	*Scopulariopsis* sp.	(M4)
				3823	*Aspergillus oryzae*	(A2)	5005	*Aspergillus oryzae*	(A2)
				1713	*Candida rugosa*	(S2)	4570	*Trichosporon asahii*	(T3)
				1455	*Pichia kudriavzevii*	(P3)	1203	*Pichia kudriavzevii*	(P3)
				493	*Candida tropicalis*	(D)	1011	*Candida rugosa*	(S2)
				401	*Rhizopus oryzae*	(R)	711	*Candida tropicalis*	(D)
				305	*Scopulariopsis* sp.	(M4)	617	*Kernia pachypleura*	(M3)
				234	*Aspergillus ochraceus*	(A2)	347	*Ceriporia purpurea*	(P2)
				158	*Lichtheimia corymbifera*	(L1)	167	*Hydnellum scrobiculatum*	(T2)
				98	*Penicillium citrinum*	(A2)	139	*Lichtheimia corymbifera*	(L1)
				82	*Saccharomycopsis fibuligera*	(S3)	122	*Mycena maurella*	(T4)

Table 3.1 Continued.

	57	*Kernia pachypleura*	(M3)	120	*Phlebia lindtneri*	(C2)
	9309	*Aspergillus oryzae*	(A2)	27638	*Liposcelis decolor*	(L2)
	6158	*Trichosporon asahii*	(T3)	4911	*Trichosporon asahii*	(T3)
	2307	*Candida rugosa*	(S2)	4674	*Aspergillus oryzae*	(A2)
	1165	*Candida tropicalis*	(D)	1821	*Candida tropicalis*	(D)
	1092	*Aspergillus ochraceus*	(A2)	1818	*Candida rugosa*	(S2)
SBSF	705	*Pichia kudriavzevii*	(P3)	1664	*Pichia kudriavzevii*	(P3)
	342	*Saccharomycopsis fibuligera*	(S3)	574	*Cyberlindnera fabianii*	(P1)
	258	*Lichtheimia corymbifera*	(L1)	506	*Amanita muscaria*	(A1)
	209	*Kodamaea ohmeri*	(M2)	263	*Malassezia restricta*	(M1)
	131	*Penicillium griseofulvum*	(A2)	208	*Scopulariopsis* sp.	(M4)
	94	*Clavispora lusitaniae*	(M2)	208	*Steccherinum* sp.	(P5)
	92	*Penicillium citrinum*	(A2)			

[1](A1) – Amanitaceae, (A2) – Aspergillaceae, (C1) – Cladosporiaceae, (C2) – Corticiaceae, (D) – Debaryomycetaceae, (E) – Eukaryota, (H1) – Herpotrichiellaceae, (H2) – Hymenochaetaceae, (L1) – Lichtheimiaceae, (L2) – Liposcelidae, (M1) – Malasseziaceae, (M2) – Metschnikowiaceae, (M3) – Microascaceae, (M4) – Microascales, (M5) – Mucoraceae, (P1) – Phaffomycetaceae, (P2) – Phanerochaetaceae, (P3) – Pichiaceae, (P4) – Polyporaceae, (P5) – Polyporales, (P6) – Pseudeurotiaceae, (P6) – Rhizopodaceae, (S1) – Saccharomycetaceae, (S2) – Saccharomycetales, (S3) – Saccharomycopsidaceae, (S4) – Sporidiobolales, (T1) – Taphrinaceae, (T2) – Thelephoraceae, (T3) – Tremellales, (T4) – Tricholomataceae

Table 3.2. Top 20 most abundant bacterial species based on sequencing analyses of substrates with and without black soldier fly larvae for experimental treatments: Control (finfish substrate without BSFL), BSF (finfish substrate with BSFL feeding for fourteen days), and SBSF (finfish substrate with BSFL feeding for seven days and then removed).

	BACTERIA								
	DAY 0			DAY 7			DAY 14		
Treatment	**# of Reads**	**Species (Family[1])**		**# of Reads**	**Species (Family[1])**		**# of Reads**	**Species (Family[1])**	
Control	95606	*Bacillus cytotoxicus*	(B1)	168310	*Staphylococcus sciuri*	(S)	37404	*Staphylococcus sciuri*	(S)
	15619	*Bacillus szutsauensis*	(B2)	15336	*Staphylococcus epidermidis*	(S)	20490	*Arthrobacter creatinolyticus*	(M1)
	7434	*Bacillus subtilis*	(B1)	10109	*Staphylococcus cohnii*	(S)	17665	*Sporosarcina* sp.	(P2)
	3209	*Staphylococcus sciuri*	(S)	5285	*Arthrobacter creatinolyticus*	(M1)	6119	*Staphylococcus epidermidis*	(S)
	2042	*Macrococcus equipercicus*	(S)	2781	*Staphylococcus saprophyticu*	(S)	5187	*Corynebacterium brevibacterium stationis*	(C)
	1131	*Weissella paramesenteroides*	(S)	1676	*Corynebacterium spheniscorum*	(C)	4392	*Sporosarcina pasteurii*	(P2)
	760	*Pantoea agglomerans*	(L)	1405	*Bacillus cereus subsp.*Cytotoxis	(B1)	3716	*Staphylococcus cohnii*	(S)
	697	*Sphingobacterium* sp.	(E1)	1305	*Corynebacterium brevibacterium* stationis	(C)	3427	*Gracilibacillus bigeumensis*	(B1)
	530	*Planococcus* spp.	(P2)	701	*Pantoea agglomerans*	(E1)	3288	*Cosenzaea proteus* myxofaciens	(E1)
	401	*Sporosarcina* sp.	(P2)	623	*Shigella sonnei*	(E1)	2760	*Vagococcus lutrae*	(E2)
	379	*Bacillus acidicola*	(B1)	618	*Corynebacterium variabile*	(C)	1370	*Atopostipes* spp.	(C)
	377	*Bacillus funiculus*	(B1)	561	*Staphylococcus* spp.	(S)	1254	*Bacillus cereus subsp.* Cytotoxis	(B1)
	323	*Ochrobactrum pseudintermedium*	(B4)	473	*Sporosarcina* sp.	(P2)	930	*Bacillus acidicola*	(B1)
	319	*Bacillus fastidiosus*	(B1)	457	*Bacillus szutsauensis*	(B2)	636	*Corynebacterium spheniscorum*	(C)
	299	*Phyllobacterium trifolii*	(P1)						
	292	*Bacillus sp.*	(B1)						
	235	*Shigella sonnei*	(E1)						
	221	*Corynebacterium brevibacterium* stationis	(C)						
				91568	*Staphylococcus sciuri*	(S)	30686	*Sporosarcina* sp.	(P2)
				29466	*Corynebacterium brevibacterium* stationis	(C)	18696	*Staphylococcus sciuri*	(S)
				6572	*Jeotgalicoccus halotolerans*	(S)	15188	*Corynebacterium* sp.	(C)
				3950	*Brevibacterium picturae*	(B3)	12356	*Sporosarcina pasteurii*	(P2)

Table 3.2 Continued.

BSF	2103	*Bacillus cereus subsp.* Cytotoxis	(B1)	11088	*Jeotgalicoccus halotolerans*	(S)
	2055	*Brevibacterium albus*	(B3)	7275	*Gracilibacillus bigeumensis*	(B1)
	671	*Staphylococcus* spp.	(S)	1974	*Brevibacterium picturae*	(B3)
	663	*Brevibacterium luteolum*	(B3)	1945	*Bacillus* sp.	(B1)
	524	*Sporosarcina* sp.	(P2)	1852	*Nosocomiicoccus ampullae*	(S)
	500	*Arthrobacter creatinolyticus*	(M1)	1105	*Arthrobacter creatinolyticus*	(M1)
	476	*Staphylococcus saprophyticus*	(S)	1094	*Bacillus cytotoxicus*	(B1)
	448	*Gracilibacillus bigeumensis*	(B1)	832	*Sporosarcina soli*	(P2)
	430	*Brachybacterium paraconglomeratum*	(D)	827	*Cosenzaea proteus* myxofaciens	(E1)
	417	*Cosenzaea proteus myxofaciens*	(E1)	777	*Natronobacillus azotifigens*	(B1)
	342	*Corynebacterium spheniscorum*	(E1)	711	*Ornithinibacillus* sp.	(B1)
SBSF	67788	*Staphylococcus sciuri*	(S)	48371	*Sporosarcina* sp.	(P2)
	20896	*Corynebacterium brevibacterium* stationis	(C)	24640	*Gracilibacillus bigeumensis*	(B1)
	15555	*Jeotgalicoccus halotolerans*	(S)	14558	*Jeotgalicoccus halotolerans*	(S)
	2418	*Bacillus cereus subsp.* Cytotoxis	(B1)	12302	*Staphylococcus sciuri*	(S)
	1920	*Brevibacterium picturae*	(B3)	10405	*Corynebacterium* sp.	(C)
	1331	*Sporosarcina pasteurii*	(P2)	4273	*Sporosarcina pasteurii*	(P2)
	1305	*Sporosarcina sp*	(P2)	1555	*Proteus myxofaciens*	(E1)
	1210	*Brevibacterium albus*	(B3)	1243	*Providencia rustigianii*	(E1)
	1090	*Staphylococcus* spp.	(S)	1054	*Arthrobacter creatinolyticus*	(M1)
	783	*Gracilibacillus bigeumensis*	(B1)	965	*Sporosarcina soli*	(P2)
	640	*Arthrobacter creatinolyticus*	(M1)	902	*Bacillus cytotoxicus*	(B1)
	461	*Cosenzaea proteus* myxofaciens	(E1)	824	*Natronobacillus azotifigens*	(B1)
	404	*Staphylococcus saprophyticus*	(S)	592	*Brevibacterium picturae*	(B3)
	308	*Brachybacterium paraconglomeratum*	(D)	582	*Atopostipes* spp.	(C)
	283	*Bacillus acidicola*	(B1)			
	277	*Brevibacterium luteolum*	(B3)			
	274	*Corynebacterium spheniscorum*	(C)			

[1](B1) – Bacillaceae, (B2) – Bacillidae, (B3) – Brevibacteriaceae, (B4) – Brucellaceae, (C) – Carnobacteriaceae, (D) – Dermabacteraceae, (E1) – Enterobacteriaceae, (E2) – Enterococcaceae, (L) – Leuconostocaceae, (M1) – Micrococcaceae, (P1) – Phyllobacteriaceae, (P2) – Planococcaceae, (S) - Staphylococcacea

3.4. Discussion

Our results demonstrated that BSFL make substrates less suitable for *L. sericata* larvae. In an earlier study, there was a 94-100% reduction of *M. domestica* production and a 97% reduction in oviposition when BSFL fed on poultry manure in the laboratory (Bradley and Sheppard 1984). However, *M. domestica* readily colonized substrates previously inhabited by BSFL, which was different from our study. Also, Bradley and Sheppard (1984) hypothesized that severely reduced number of *M. domestica* larvae and pupae in BSFL-inhabited substrate was caused by oviposition-deterring allomone produced by BSFL. Although reduced oviposition cannot be ruled out for *L. sericata*, rapid emigration of its larvae from the BSFL-infested substrate showed the existence of an alternative antixenotic mechanism for this species. Considering there is evidence that blow flies are capable of developing on substrate at different stages of decay and also produce antimicrobial peptides (Tomberlin et al. 2017) just as BSFL (Park et al. 2015, Elhag et al. 2017), it is interesting why *L. sericata* is less competitive compared to BSF.

Antagonism with *L. sericata* suggests that BSFL not only have the potential to be used a value-added feed but have the capability of deterring pest flies with their presence. From a veterinary perspective, BSFL could discourage blow flies where livestock operations contain excess manure and injured livestock that attract Calliphoridae flies that cause myiasis (Tomberlin et al. 2017). BSFL could consume the waste while simultaneously preventing pestiferous flies from ovipositing.

Possible explanations of the observed effect include depletion of nutrients due to consumption, repellant chemicals produced by BSFL either through their frass or salivary glands, or alteration of microbial biota by BSFL that are not favorable for blow fly larvae (Bradley and

Sheppard 1984, Zurek and Nayduch 2016). It is also possible that they alter the chemical composition of their substrates. When reared on decaying vegetal and food scrap waste, BSFL increased nitrogen mineralization, a process where organic nitrogen is converted to an inorganic form that is utilized by plants, by up regulating the concentration of ammonium in the leachate produced (Pepper et al. 2015). They also facilitated recovery of nitrate present in the leachate via nitrate/nitrite ammonification. Therefore, the nitrogen present in the BSFL leachate could be used as fertilizer for crops (Green and Popa 2012). Excess inorganic nitrogen is not an ideal environment for saprophages like *L. sericata* (Brown 1938).

Secretions produced by BSFL can also contain antimicrobial peptides that reduce microbial numbers. Park et al. (2014) demonstrated that BSFL suppressed *Escherichia coli, Bacillus subtilis* and methicillin resistant *Staphylococcus aureus*. Choi et al. (2012) reported that BSFL had Gram-negative specific antibacterial activities including *Klebsiella pneumonia, Neisseria gonorrhoeae,* and *Shigella sonnei*. A molecule responsible for reductive activity against Gram-positive bacteria was identified as a defensin peptide, which is in the first line of host defense against pathogenic infections (Tu et al. 2015).

In our study, microbial activity measured by FDA was significantly lower in the substrate inhabited by BSFL than in the control substrate both on Day 7 and Day 14 of the experiment (Figure. 3.3). Results were inconclusive for the substrate from which BSFL were removed after seven days of feeding, which was different neither from control nor from the substrate still inhabited by BSFL (Figure. 3.3). Plating data revealed increase in fungal abundance in the substrate from which BSFL were removed (Figure. 3.4) but decline in bacterial abundance in both BSFL treatments (Figure. 3.5). Thus, overall decline in microbial activity detected by the FDA analyses was most likely driven by decline in the number of bacteria.

Several odors produced by bacteria attract different species of Calliphoridae species and have been described in numerous studies because of their veterinary importance. Indole produced by *E. coli*, lactic acid produced by *Lactobacillus acidophilus*, ammonia and putrescine produced by *Proteus mirabilis* attract *L. sericata, L. cuprina* (Wiedemann), and *Calliphora erythrocephala* (Meigen). *Cochliomyia* spp. (Townsend) and *Chrysomya* spp. (Robineau-Desvoidy) are attracted by *p*-Cresol produced by *Lactobacillus* spp., butyric acid produced by pig cecal bacteria, and phenol produced by marine bacteria (Hepburn and Nolte 1943, Cragg 1950, Urech et al. 2004, Ma et al. 2012). The majority of saprophytic bacteria associated with *L. sericata* and *L. cuprina* that are found in organic matter they consume belong to the phyla Proteobacteria, Firmicutes, and Bacteroidetes. *P. mirabilis* has been shown to induce a greater incidence of oviposition by *L. sericata* (Ma et al. 2012). Therefore, it is possible that altered microbial communities in substrates inhabited by BSFL could have been at least partially responsible for their lower suitability to *L. sericata.*

DNA sequencing analyses revealed diverse and dynamic microbial communities that were different among the treatments and between the days of sampling. Larger, replicated studies are necessary to elucidate their ecology and relationships to BSFL . It is worth noting, however, that some species were present in the control samples, but absent from both substrates containing BSFL. It is possible that those were directly or indirectly suppressed by BSFL or were gut associated microbes that facilitate with feeding. Many of those are common and may be economically significant.

The fungal species observed in the fin fish substrate that were unique to the control treatment but absent from the BSF and SBSF treatments were found to be both pathogenic and saprophytic. *Penicillium citrinum* is a commonly occurring filamentous fungus with a worldwide

distribution. It can be found in various substrates such as soil, cereals, spices and indoor environments. *P. citrinum* produces a mycotoxin citrinin, classified as a nephrotoxin, a substance that inhibits cells within animal kidneys if ingested (Houbraken et al. 2010). Even though *P. citrinum* is recognized to be ubiquitous in the environment, in rare cases it has been reported to cause human infection such as: keratitis, inflammation of the cornea, urinary tract infection, and pneumonia (Mok et al. 1997).

Penicillium griseofulvum is a blue mold, associated with stored apples, corn, wheat, barley, flour, walnuts, and meat products. It is considered an important postharvest disease of pome fruit. It can produce both detrimental and beneficial secondary metabolites such as patulin, a mycotoxin that can cause acute toxicity when consumed by animals and humans. Recently it has the potential to be used in cancer chemotherapy and suppress the hepatitis C virus *in vitro* (Banani et al. 2016).

Rhizopus oryzae is a saprotroph found in soil, dung, and rotting vegetation. *R. oryzae* have been used for centuries as fermented food starters in tempeh and other Asian foods. Species in this group have been known to act as opportunistic, invasive animal and human pathogens that cause deadly infections in immunocompromised individuals (Gryganskyi et al. 2010).

Saccharomycopsis fibuligera is found worldwide in high starch substrates such as cereal-based fermented foods and beverages (Cronk et al. 1977). It has also been used to break down potato processing wastes to produce yeast cells for cattle feed (Lachance and Kurtzman 2011).

Taphrina johansonii are parasitic yeast-like fungi that cause yellow swelling of carpels, known as aspen tongue, on the common aspen *Populus tremula* (Linnaeus), where they are normally found (Petrýdesová et al. 2013).

Lichtheimia corymbifera is a thermophilic mold found in soil, decaying plants and food with a worldwide distribution. It has been reported to be responsible for 5% of zygomycosis, an infection caused by fungi in the zygomycota phylum that occur on the face or nose and mouth cavity on humans and animals (Garcia-Hermoso et al. 2009, LIFE 2018).

The bacterial species observed in the finfish substrate that were unique to the control treatment but absent from BSF and SBSF treatments were mostly saprophytic. Bacillus, a genus of gram-positive, rod-shaped bacteria in the phylum Firmicutes and are ubiquitous in nature. They can be obligate aerobes or facultative anaerobes. Depending on the environment, they can produce endospores and remain dormant till conditions are habitable (Pepper et al. 2015). The species *Bacillus szutsauensis* is reported as a dominant species found in the gut microbiota of the Asian fish *Labeo rohita* (Hamilton), commonly used in aquaculture (Ghori et al. 2018)

The genus Corynebacterium includes a diverse collection of pathogenic and non-pathogenic species, that have been found in a variety of habitats such as soil, plant material, waste water, and dairy products. *Corynebacterium variabile* is non-pathogenic, Gram-positive, and are nonmotile rods part of a complex microflora on the surface of smear-ripened cheeses that contribute to the development of flavor and texture during cheese ripening (Schröder et al. 2011).

Pantoea agglomerans, is a ubiquitous bacterium associated with plants as a symbiont or mutualist, and is found in animal or human feces. *P. agglomerans* is normally not pathogenic but can cause opportunistic human infections produced by wounds from vegetation. It has also been identified to a cause plant diseases in a variety of cultivable plants, animal diseases, and hospital-acquired infections on immunocompromised individuals (Dutkiewicz et al. 2016).

Shigella sp. are Gram-negative, nonmotile and non-spore-forming rod-like bacteria that can grow in aerobic and anaerobic conditions. *Shigella sonnei* can cause a diarrheal disease when ingested by humans called shigellosis, and is the most common species in the United States to cause this (Scallan et al. 2011, Centers for Disease Control and Prevention 2017).

Vagococcus lutrae is a Gram-positive single celled, non-spore-forming motile, nonpathogenic bacterium that is considered a rare bacterial species. It was described when isolated from the common otter's blood, liver, lungs and spleen, and has also been isolated from the intestine of the largemouth bass. The first human skin infection case was recently reported as a food-mediated acquisition caused by poor hygiene (Lawson et al. 1999, Garcia et al. 2016).

Overall, our results confirm that BSFL have antibiotic properties affecting other organisms. These properties may have important implications for pest management in a variety of situations that involve decaying materials. Furthermore, identifying individual factors responsible for the observed antibiosis may provide new biologically active ingredients for pharmaceutical and pest control industries.

CHAPTER 4

TEMPORARY DYNAMICS OF WEIGHT GAIN BY BLACK SOLDIER FLY LARVAE

4.1. Introduction

Black soldier fly, *Hermetia illucens* (Linnaeus) (Diptera: Stratiomyidae) (BSF) is a beneficial fly species that can be used for remediating biological waste. Their larvae (BSFL) are saprophagous and can colonize a wide variety of organic material at different stages of decay (Tomberlin et al. 2005, Martínez-Sánchez et al. 2011, Banks et al. 2014). Recent interest to use BSFL for waste management has been drawn from the increase in the amounts of livestock manure, pre- and post-consumer municipal solid waste, and the increased demand for high quality fish meal, all of which has led to environmental and health risks (USDA 2005, Nellemann et al. 2009, Hribar 2010, Love et al. 2015, Gunders 2017). BSFL show a great promise for mass-rearing operations on both small and large scale because they are rich in nutrients, grow at a fast rate, convert organic wastes to vermicompost while suppressing human pathogens and pests, and contain a number of potentially valuable biologically active compounds (Choi et al. 2012a). The prepupal stage contains between 42 – 45% protein and 31 – 35% fat (Newton et al. 1977, Sheppard 1993, Craig Sheppard et al. 1994); therefore, they can be converted into beneficial end products such as feed, oil, and fertilizer (Čičková et al. 2015, Henry et al. 2015, Barroso et al. 2017, Wang and Shelomi 2017). They can provide a suitable replacement for conventional fat and protein sources and can be fed to animals such as poultry (Gayatri and Madhuri 2013), swine (Newton et al. 1977), catfish, tilapia (Bondari and Sheppard 1981), and rainbow trout (St-Hilaire, Sheppard, et al. 2007).

When larvae of Dipteran species enter a post-feeding stage known as the prepupal phase of their development, they emigrate from their feeding substrate and search for suitable substrate for pupation to protect themselves from predation and desiccation (Lima et al. 2009). BSF prepupae have slight morphological changes compared to larvae in the feeding stage. Their bodies change color from cream white to a black color and their labrum acquires a downward bend that acts as a hook to assist with pulling them to a suitable pupation site (Schremmer 1986, Diener et al. 2011). The emigration of prepupae away from feeding substrate can be initiated by abiotic environmental cues including temperature, photo intensity, and soil moisture. These factors will encourage the prepupae to migrate toward cool, dark, and dry substrates (Gomes et al. 2006). Moving BSF prepupae are capable of climbing slopes of 40° when dry. Emigrant behavior makes them easy to direct for harvesting, with no mechanics needed to remove the prepupae from their food source (Craig Sheppard et al. 1994). Such an approach is commonly referred to as self-harvesting. In facilities designed for waste management that utilize BSFL and collect emigrating prepupae destined for animal feed, larvae climb up a ramp out of a rimmed container to eventually end in a collection chamber attached to the end of the ramp (Newton et al. 2004, Diener et al. 2011).

Most previous studies focused on the weight of BSF prepupae as a target stage to be used for harvest. It has been suggested that they are at their maximum size, exhibiting large protein and fat contents to sustain them through adulthood (Newton et al. 1977). It has also been shown that larval growth in medically important dipteran species, such as blow flies (Diptera: Calliphoridae) also exhibit a pattern of rapid increase in length followed by a decrease once they reached pupation (Donovan et al. 2006). However, Banks et al. (2014) recently demonstrated

that BSFL fed on fresh human feces reached their highest mean weight two days before reaching the prepupae stage. A similar decrease in weight in the migratory post-feeding stage was seen in other fly species, especially for prepupae that traveled longer distances (Goodbrod and Goff 1990, Gomes et al. 2006).

Maine can greatly benefit from a BSFL commercial operation that utilizes live-stock manure, marine, pre- and post-consumer waste. With the increased interest in using BSFL for feed, there is a competitive pressure to maximize yield in mass-rearing facilities. Therefore, this study aims to understand if harvesting larvae prior to the prepupae stage can produce larger larvae than those harvested as prepupae to benefit BSF commercial rearing operations in a climate-controlled facility.

4.2. Materials and methods

4.2.2. Colony maintenance of black soldier flies

The black soldier fly breeding colony was first purchased May 2016 from Symton Black Soldier Fly Solutions (College Station, TX). The colony arrived in a plastic container with a total of 20,000 immature larvae in coconut coir substrate. Once in our laboratory, the colony was maintained as described in Chapter 2.

4.2.3. Larval growth curve

One egg mass was placed in a Petri dish with a 2.5 cm diameter hole covered with a black cloth for ventilation containing a moist 2.5 by 2.5 cm square sponge, wrapped in parafilm. The dishes were placed in a tray with a paper towel saturated with water to prevent egg desiccation. The tray was maintained inside environmental chamber (Percieval Scientific, Perry, Iowa) at 28±1ºC and 18L : 6D photoperiod. Once the eggs hatched, the larvae were fed *ad libitum* with moist chicken feed. Daily, their lengths were measured with digital calipers

(General Tools and Instruments, New York, NY), and their weights were measured with an analytical scale (Ohause Co., Pine Brook, NJ). Water was added to the sponge as needed. The experiment was replicated ten times.

Visual examination of the data suggested that a period of weight gain by developing larvae is eventually replaced by a period of weigh decline (Figure 4.1). To confirm the existence of such a trend, the data were analyzed by fitting peak functions using TableCurve 2D software package (SYSTAT, 2002), with the best function selected based on r^2 values (Golubev 2010).

4.2.4. Biomass of larvae

Experiment was conducted in ten 21.8 by 16.2 by 13.2 cm ventilated GladWare boxes. Two hundred larvae (a mix of second and early third instars) were placed into each container. All larvae were fed *ad libitum* with non-medicated chicken feed (see above) with tap water to achieve 60% (w : w) moisture contents of the resulting substrate (Fatchurochim et al. 1989). The boxes were maintained on shelves in laboratory with ambient temperature at 26±1°C (range: 24 - 27.7°C) and 50±8% (range: 42.5 - 69%) relative humidity.

Biomass accumulation was measured by weighing a pooled sample of ten randomly selected larvae from each container using an analytical scale (Ohause Co., Pine Brook, NJ). The first measurement was done in the beginning of the experiment to get baseline information on larval size. After that, larval development was monitored daily by visual observations. The second measurement was taken when the first darkened pre-pupa appeared. The third measurement was taken when approximately half of larvae become darkened pre-pupae. The final measurement was done by weighing a pooled sample of ten randomly selected pupae from each container. In addition, ten cream-colored larvae and ten grayish-brown prepupae were collected from each box and weighed at the time point when approximately half of the larvae

became pre-pupae. Data normality was tested using Wilk-Shapiro test (PROC UNIVARIATE, SAS Institute 2017). Non-normally distributed data were transformed using rank transformations (Conover and Iman, 1989). Changes in weight from early instars to pupae were analyzed using one-way repeated measure ANOVA (PROC MIXED, SAS Institute 2017). Differences in weight between larvae and prepupae were analyzed using Student's paired t-test (PROC TTEST, SAS Institute 2017).

4.3. Results

4.3.2. Larvae growth curve

BSFL underwent a period of rapid weight gain, followed by slow decline around the time when the first prepupae appeared (Figure 4.1).

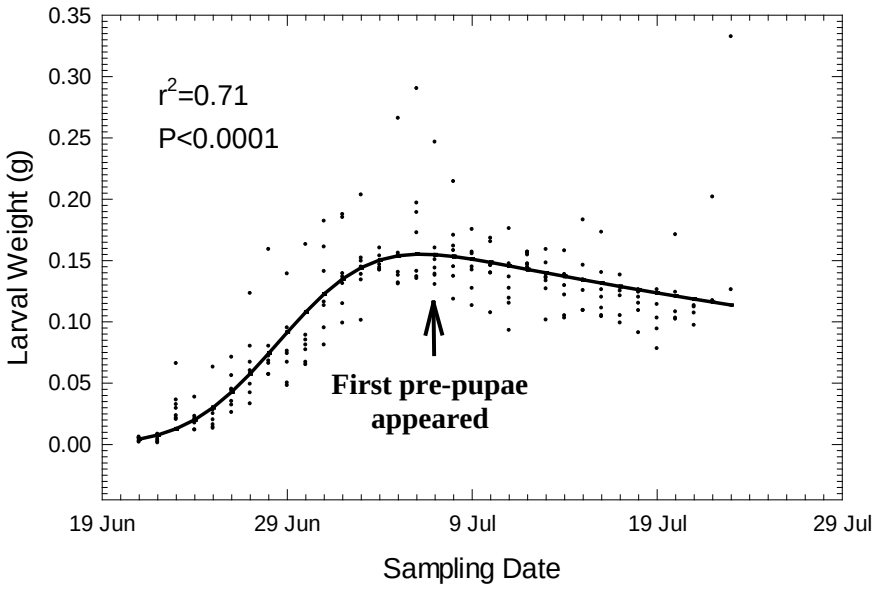

Figure 4.1. Weight gain of black soldier fly larvae. Trendline shows exponentially modified Gaussian function.

Exponentially modified Gaussian distribution (Equation 1) provided the best description of the increase in larval weights over the duration of experiment.

$$f(x; h, \mu, \sigma, \tau) = \frac{h\sigma}{\tau}\sqrt{\frac{\pi}{2}}\exp\left(\frac{1}{2}\left(\frac{\sigma}{\tau}\right)^2 - \frac{x-\mu}{\tau}\right)\operatorname{erfc}\left(\frac{1}{\sqrt{2}}\left(\frac{\sigma}{\tau} - \frac{x-\mu}{\sigma}\right)\right)$$

Erfc denotes the complementary error function, which is defined as

$$\operatorname{erfc}(z) = 1 - \operatorname{erf}(z)$$

$$= \frac{2}{\sqrt{\pi}}\int_x^\infty e^{-t^2}dt.$$

where h is the amplitude of the normal distribution, μ is the mean of the normal distribution, σ is the standard deviation of the normal distribution, and τ is the exponential decay parameter (τ = 1 / λ) (Equation 1).

Table 4.1. Model parameters

Parameter	Value	Std Error	t-value	95% Confidence Limits		P>|t|
h	0.913139	0.247831	3.684528	0.424571	1.401706	0.00029
μ	179.7844	0.415375	432.8247	178.9656	180.6032	0.00000
σ	3.94382	0.459635	8.580325	3.037705	4.849936	0.00000
τ	48.20502	12.33175	3.909017	23.89446	72.51558	0.00013

4.3.3. Biomass of larvae

There was a significant difference in weight among the different life stages (d.f.=3, 36, F=55.34, P < 0.0001). Larval mass increased rapidly between early-and late instars, but there was no significant weight change between the time when the first prepupae was detected and the time when approximately half of the larvae entered prepupal stage. Pupae were significantly lighter than late instars (Figure 4.2).

Larvae weighed significantly more than prepupae co-occurring with them at approximately 50 : 50 ratio (d.f.=9, t=-6.95, P < 0.0001). On average, the former weighed 0.2289 ± 0.0072 g (mean ± SE), while the latter weighed 0.1709 ± 0.0072 g.

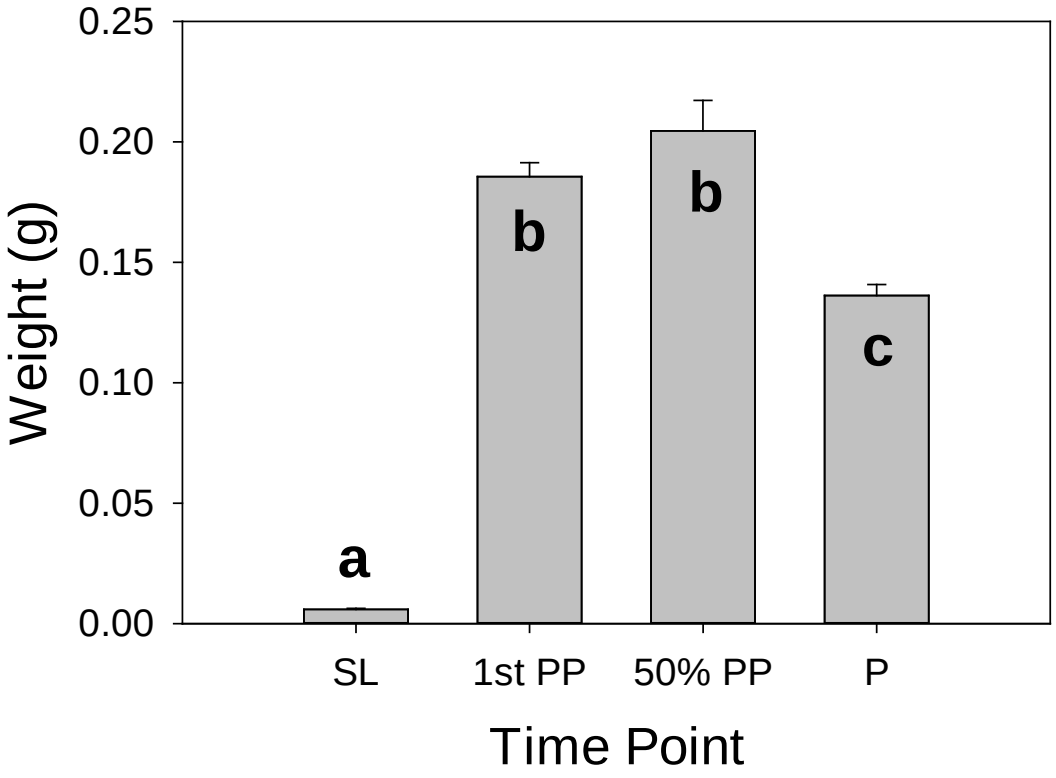

Figure 4.2. Weight of immature black soldier fly at different time points. SL – small larvae; 1st PP – appearance of the first prepupae; 50% PP – 50 : 50 larvae / prepupae mix; P – pupae. Error bars denote standard errors. Means followed by the same letters are not significantly different from each other (Tukey tests, P > 0.05).

4.4. Discussion

Our results indicate that maximum weight gain by BSFL was reached around the time when they were ready to become prepupae. This makes sense because at this point they stop feeding while remaining physically active and migrating in search of pupation substrates. Other dipteran species such the blow flies *Calliphora vicina* (Robineau-Desvoidy), *Chrysomya albiceps* (Wiedemann), *Lucilia cuprina* (Wiedemann), *Chrysomya megacephala* (Fabricius), and

Chrysomya rufifacies (Macquart) also display a migratory behavior during the post feeding phase. Similar to our findings, the highest weight in those species was observed in post-feeding larvae, which then decreased in size when crawling or burrowing (Goodbrod and Goff 1990, Donovan et al. 2006, Gomes et al. 2006).

Although mathematically complicated, exponentially modified Gaussian distribution describes a fairly straightforward process of rapid initial growth of a measured response, followed by its stabilization and slight decline. It has been successfully used to describe a number of biological processes, including cell proliferation and differentiation (Golubev 2010), cell population dynamics (Tyson et al. 2012), latency and interresponse time in free recall of previously memorized items (Rohrer and Wixted 1994), and response times in visual search (Palmer et al. 2011). Knowing mathematical relationship between time and weight may provide an opportunity for predicting larval size at given points in time. However, BSFL development varies depending on diet, feeding rate, temperature and humidity (Tomberlin et al. 2002, 2009, Diener et al. 2009, 2011, Holmes et al. 2012). Therefore, certain caution needs to be exercised when extrapolating results obtained under different sets of conditions.

In this study, BSFL were significantly heavier compared to prepupae inhabiting the same substrate at the same time. Therefore, there was no evidence that heavier larvae were the first ones to pupate. Instead, weight losses were likely to be driven by metabolic processes linked to transition to adulthood.

Our findings are consistent with those of Liu et al. (2017), who presented the nutritional composition of BSF at different phases of its life span. In that study, there was also a trend towards a rapid increase in crude protein, crude fat, and dry mass from egg to late instars and

early prepupae, followed by a decline from late prepupae to adults. Early prepupae also had significantly less vitamin E, sodium, iron, and zinc compared to late feeding instars, but more phosphorus (Liu et al. 2017).

Based on the larval growth curve, the optimal harvest period of larval biomass is during the time immediately before they turn into prepupae and start searching for pupation habitats. However, there could be other considerations in designing harvesting approaches. If most prepupae emigrate from their feeding substrates, which we do not know at this point, self-harvesting eliminates necessity for sifting them out. This may result in savings on equipment and labor. Also, BSF pupae float in water (Alyokhin, unpublished). Therefore, flotation may be a viable option for their separation from feeding substrates. Further investigations are needed to better understand these issues. Although migration behavior can be exploited to self-harvest BSFL for processing or for incubation and re-introduction into a colony (Holmes 2010), convenience of such an approach needs to be carefully weighed against biomass losses linked to cessation of feeding and spending accumulated energy reserves on movement.

CHAPTER 5

COLD TOLERANCE OF BLACK SOLDIER FLY LARVAE

5.1. Introduction

The black soldier fly (BSF), *Hermetia illucens* (Linnaeus) (Diptera: Stratiomyidae) has been shown as a valuable source of feed, oil, and fertilizer in a variety of large and small-scale livestock operations (Čičková et al. 2015, Henry et al. 2015, Barroso et al. 2017, Wang and Shelomi 2017), especially in areas where black soldier fly larvae (BSFL) can develop in a suitable habitat year-round. BSF are generally considered to be warm-climate species preferring areas with subtropical and warm temperatures. They are common to North and South America and the Australasian region (Oliveira et al. 2015 Üstüner et al. 2003). In the southeastern U.S., BSF have three generations per year. Temperatures for optimal development in the laboratory and in the wild range from 27°C – 33°C (Sheppard et al. 2002). The distribution compiled based on observations submitted by citizen scientists and records of preserved specimens available on iNaturalist.org between 1913 – 2018 shows a northern most BSF range limit reaching up to Washington State in the United States (Figure 1.1). In New England, the most northern range reaches Massachusetts (iNaturalist 2018).

Even though BSF has a global distribution and reaches across southern and central U.S., there is apprehension toward BSF becoming a harmful invasive species in Maine. BSF are not currently recorded as occurring naturally in Maine, which is a significant issue because there is interest in utilizing BSFL in Maine for marine waste processing and aquafeed production. Mass rearing companies from Georgia, Ohio, and Texas currently distribute live BSFL throughout the U.S. Therefore, there is considerable probability of this species' establishment beyond its native range. However, low winter temperatures may set a northern limit to BSF range.

Better understanding of BSF response to low temperatures may also have important implications for its rearing on a commercial scale. Mass production of insects demands optimization of the efficacy and decreasing the costs of their rearing. This includes preservation of live beneficial insects for shipping and storage (Ortiz et al. 2016). Cold treatments can help extend their shelf life. These include cryopreservation, induction and maintenance of diapause, and slowing down insect metabolism by placing them under temperatures close to lower developmental thresholds.

Cryopreservation is a method of preservation accomplished through chemical/physical manipulation prior to placing the insects in liquid nitrogen storage (Leopold 2005). Currently there are eight dipteran species of importance in forensic sciences, medicine, and agriculture that have been successfully cryopreserved as late stage embryos: *Musca domestica* (Linnaeus), *Cochliomyia hominivorax* (Coquerel), *Ceratitis capitate* (Wiedemann), *Anastrepha suspensa* (Leow), *A. ludens* (Leow), and *Lucilia sericata* (Meigen) (Leopold 2005, Rajamohan et al. 2014). However, cryopreservation is a complicated process that requires specialized skills and equipment. Therefore, it is unlikely to be widely adopted in small- and medium-scale BSF production, especially in developing countries.

Diapause is elicited through management of environmental factors such as photoperiod, temperature, and moisture (Leopold 2005). It has been observed in another soldier fly species, *Ptecticus flavifemoratus* (Rozkosny) (Diptera: Stratiomyidae) (Rozkošný and Kovac 1998). However, we are not aware of any reports on diapause in the BSF. Because insects are poikilothermic organisms, development of non-diapausing individuals can also be slowed down by low temperatures. This can be used for short-term storage, such as during shipping to

different locations, and for synchronization of development in rearing operations. In laboratory colonies used for research purposes, this has been successfully carried out with the Colorado potato beetle *Leptinotarsa decemlineata* (Say), a predatory midge *Aphidoletes aphidimyza* (Rondani), and the predatory mite *Phytoseiulus persimilis* (Evans) (Boiteau and Alford 1983, Kostal and Havelka 2001, Luczynski et al. 2008). Therefore, there is a possibility of using this approach on a larger scale in mass-rearing of BSF.

Development of BSF within the optimal developmental temperature thresholds is relatively well understood. An investigation on the life history traits of BSFL reared at three different temperatures was conducted to examine the relation of immature development features to adult fitness characters. Results demonstrated that adults reared at 27°C weigh 5% more and live roughly 10% longer than those reared at 30°C. The authors suggested that longer larval development might allow more energy reserves to accumulate and produce more fitness tradeoffs such as having the ability to withstand lower temperatures (Tomberlin et al. 2009). A recent study analyzed whether growth rates of BSF at varying temperatures differed between larvae fed on a grain-based diet and a vertebrate tissue diet. BSFL reared on pork required more degree hours to complete larval development than those reared on the beef and grain-based diets. Larvae reared at 27.6°C and 32.2°C required more degree hours to complete development and had a final larval weight 30% greater than larvae reared at 24.9°C (Harnden and Tomberlin 2016).

Less attention was paid to the effects of low temperatures on BSF survival and development. Furman et al. (1959) reported that BSFL were killed by 30 min exposure to -2°C, but most of them survived 24 h exposure. However, those authors did not provide any data to support their claims. Holmes et al. (2016) determined the lower temperature developmental threshold of BSF immature stages. Mean development time from egg to adult at 19°C was 72

days while BSF failed to survive at lower temperatures (Holmes et al. 2016). Another study examined the cold tolerance of different developmental stages using the supercooling point and lethal time at 5°C as parameters. However, mortality rates following short-term exposure to temperatures approaching supercooling points was not measured. Lethal time experiments at 5°C indicated that prepupae and pupae were the most cold-tolerant life stages. Still, they died after a few weeks, suggesting they are susceptible to chilling injury. The authors suggested that differences in supercooling points of young versus older larvae may be related to their body size, where smaller size generally have a lower supercooling points (Spranghers et al. 2017).

In the present study, we further investigated BSF response to low temperatures. Our objectives were to measure mortality of different life stages exposed to suboptimal temperatures for different periods of time and to determine whether experiencing cold temperatures as larvae affects the adult stage.

5.2. Materials and methods

5.2.1. Insect origins

Black soldier fly larvae (BSFL) to start our colony were initially purchased from Symton BSF Company (College Station, Texas) and reared to adulthood as described below. Eggs produced by our colony were harvested from a 2 by 2 by 4-m custom-made wooden frame screen cage kept in the University of Maine greenhouse under ambient temperature at 26±1°C (mean±SE; range: 24°C - 30°C) and 60±6% (42.5% - 69%) relative humidity. Eggs were incubated in the laboratory at 26±1°C (24 - 27.7°C) and 50±8% (42.5 - 69%) relative humidity in a 21.8 by 16.2 by 13.2 cm, 1892 ml GladWare container (GladWare, Oakland, CA). Hatched larvae were fed ad libitum in 50 ml increments with non-medicated chicken feed (Home Fresh® Extra Egg, Blue Seal, Lawrence, MA) mixed with tap water to achieve 60% moisture content (w : w). After the

total volume of larval mass and substrate became greater than the volume of the container, they were placed in 20 by 33 by 38 cm plastic dish pans (United Solutions, Leominster, MA) where they remained till they were removed for experimental trials or pupated. Every two to three days, 100 – 300 ml of chicken feed mixed with tap water to achieve 60% moisture content (w : w) was added along with 250 – 500 ml of pine shavings (AWF Pets Pick, Columbia, MD) to improve aeration and prevent accumulation of free liquids. Once BSFL reached desired life stage, they were removed individually from the colony, and placed into respective boxes for experimental trials. If larvae were not used, they pupated and were transferred to the greenhouse and placed in rearing cages to become reproductive adults.

5.2.2. Cold treatments

Different black soldier fly immature life stages were exposed to cold temperatures by being stored in a residential Frigidaire refrigerator - freezer (WCI Canada Inc., Augusta, GA). Cold tolerance was tested by keeping them at 4°C for 0 hrs, 24 hrs, 48 hrs, and 72 hrs. Freeze tolerance was tested by keeping them at -12°C for 0 min, 10 min, 30 min, and 60 min. The chosen freezing temperature was within the range of supercooling points reported by (Spranghers et al. 2017). All experiments were replicated five times, except the experiment on cold tolerance of eggs that was replicated ten times.

5.2.3. Survival of eggs at 4°C

To determine whether eggs could survive cold temperatures, 29.1 mg of an egg mass, which is equivalent to ca. 1,000 eggs (Booth 1983), were placed in a 120 ml plastic cup lined with a 2.5 cm layer of folded paper towel saturated with water. Eggs were situated on top of a 2.5 by 2.5 cm strip of wax paper. Cheese cloth was secured on top of each cup with a rubber band.

Eggs were stored in a refrigerator at 4°C as described above. Once removed, cups were placed in laboratory at 26±1°C (24 - 27.7°C) and 50±8% (42.5 - 69%) relative humidity for the eggs to hatch. The number of hatching first instars was counted under dissecting scope.

5.2.4. Survival of eggs

To determine whether eggs were viable following exposure to cold treatments, 29.1 mg of eggs were placed in a 120 ml plastic cup lined with a 2.5 cm layer of folded paper towel saturated with water and sealed with a plastic lid. Eggs were treated as described above. Once removed from the refrigerator - freezer, cups were placed in laboratory at 26±1°C (24 - 27.7°C) and 50±8% (42.5 - 69%) relative humidity for one week to allow the eggs to hatch. At the end of the week, all replicates were frozen to halt development and preserve the condition of the larvae. The frozen neonates were not removed from the freezer until counting to reduce the risk of decay or fungal growth.

Neonates hatching from the eggs exposed to 4°C were counted under dissecting scope. That approach proved to be very labor-consuming. Therefore, the neonates hatching from the eggs exposed to -12°C were scanned using an Epson Expression 1680 Flatbed Scanner (Epson America Inc., Long Beach, CA). Prior to scanning, each cup of neonates was placed onto a clean Pyrex glass petri dish 20 mm by 150 mm (Corning Inc., Corning, NY) for separation from paper towel bits. Neonates stuck to the sides of the cup were also removed and placed in the clean petri dish at this time. They were then poured out, one petri dish at a time onto the scanner, which was cleaned with Sprayway (Sprayway Inc., Downers Grove, IL) and a Kimwipe (VWR, Radnor, PA) between scanning larvae from each petri dish. Each scanned image was imported into ImageJ software package (NIH Image, Bethesda, MD) for automatic counting. Each image was converted into binary black and white format. Larval size was set to range between 15-200

pixels. Watershed function was used to recognize potentially overlapping larvae as separate and count them as two instead of one. The output file was then saved, and the results of processing all images were compiled into one Excel spreadsheet.

5.2.5. Survival of other immature stages

Second, 3rd, and 5th instar larvae, as well as pupae, were tested for cold and freeze tolerance. For every time duration, 100 larvae or pupae were tested. They were extracted and individually counted from the breeding colony and placed in a 10 by 12.7 by 5 cm plastic tray with 50 ml of vermiculite. To prevent escape, a lid with a 4 by 2 in opening covered with an antivirus insect mesh (BioQuip Products, Rancho Dominguez, California) was secured, which also provided ventilation. Once removed from the refrigerator – freezer, the larvae were placed in the laboratory for one hour to allow them to readjust to ambient room temperature. Pupae were maintained in the laboratory until adult emergence. The number of live and dead immature stages were then counted. All trials were kept in a small room in the lab with ambient air temperature at 27±6.2°C (21 - 28°C) and 39.3±6.9% (30% - 56.5) relative humidity. All temperature readings were recorded with an EL-USB-2-LCD USB Humidity Data Logger w/ LCD Display (Lascar, Erie, PA).

5.2.6. Size of adults surviving exposure to cold temperatures as immatures.

Fifth instars surviving the cold treatments were maintained in the laboratory and observed daily until no more adults emerged. The number of successfully emerging adults and dead pupae from which adults failed to emerge were counted. The adult length from head to wing tip was measured with digital calipers (General Tools and Instruments, New York, NY).

5.2.7. Statistical analysis

Survival of immature black soldier flies at different temperatures was analyzed by one-way ANOVA (R version 3.4.1, The R Foundation for Statistical Computing). Data were converted to proportions and then the mean percent and standard deviations were calculated. The normality of the data was tested using the Shapiro-Wilk normality test. If data were not normal, it was arcsine transformed. To test if means were significantly different among treatments, a Tukey's Honest Significant Difference (HSD) test was conducted. With a few exceptions, all analyzed data were not normally distributed even after arcsine transformation. However, W values substantially increased following the transformations. Therefore, we considered ANOVAs performed on the transformed data to be sufficiently robust to detect differences among the treatments.

5.3. Results

5.3.1. Effects of exposure at 4°C

All black soldier fly life stages tested in this study suffered significantly higher mortality after being exposed to cold treatments (Table 5.1). Severity of the impact depended on the duration of the exposure. Larvae were more tolerant of chilling than eggs, with higher mortality usually detected only after spending 72 h in the refrigerator. The fifth instars were somewhat of an exception, with statistically significant difference of mortality was detected only after 24 h of exposure to cold temperature. However, their mortality was generally low, with close to 92% of larvae surviving even after 72 h in the refrigerator. Adults emerging from the chilled fifth instars were significantly smaller compared to the control adults. Reduction in size followed the length of exposure to cold temperature.

Table 5.1. Percent survival and adult length (mean ± SE) of the black soldier flies exposed to 4°C. Means in the same rows followed by the same letters are not different from each other (Tukey tests, P < 0.05).

	Duration of Exposure at 4°C						
Life stage	0 h	24 h	48 h	72 h	df	*F*	*P*
Eggs	33.2 ± 19.4a	8.2 ± 9.6b	0.93 ±0.4c	1.66 ± 1.4c	3, 36	31.1	<0.001
2nd instars	96.6 ± 2.2a	86.6 ± 10.6a	94.4 ± 5.5a	2.0 ± 2.5b	3, 16	118	<0.001
3rd instars	98.6 ± 1.7a	89.0 ± 5.0a	94.0 ± 11.8a	81.3 ± 13.3b	3, 16	4.26	0.021
5th instars	99.2 ± 0.83a	94.0 ± 2.54b	93.2 ± 2.58b	91.8 ± 6.8b	3, 16	6.34	0.004
Pupae	80.8 ± 3.2a	50.2 ± 7.8b	45.8 ± 7.7b	22.8 ± 6.6c	3, 16	65.4	<0.001
Adult[a] length, mm	15.3 ± 0.6a	13.2 ± 0.5b	11.6 ± 0.4b	6.0 ± 0.2c	3, 16	101	0.001

[a] Flies eclosing from pupae that were exposed to 4°C as fifth instars.

5.3.2. Effects of exposure at -12°C.

Similar to the effects of chilling, exposure to -12°C had a pronounced negative effect on survival of black soldier fly eggs and larvae (Table 5.2). Longer periods of exposure resulted in higher mortality. To the contrary, among the fifth instars that were alive after being removed from the freezer, more insects survived to adulthood after 30 min of exposure (Table 5.3). Their size was also larger compared to other treatments (Table 5.2). In the same time, none of the fifth instars kept at -12°C for 60 min developed to adulthood.

Table 5.2. Percent survival and adult length (mean ± SE) of the black soldier flies exposed to -12°C. Means in same rows followed by the same letters are not different from each other (Tukey tests, P < 0.05).

	Duration of Exposure at -12°C						
Life stage	0 min	10 min	30 min	60 min	df	*F*	*P*
Eggs	17.7 ± 13.4a	3.6 ± 2.2b	2.4 ± 1.4b	2.1 ± 1.6b	3, 16	8.69	0.001
2nd instars	94.0 ± 2.4a	93.8 ± 9.0a	6.6 ± 4.1b	0.6 ± 1.34c	3, 16	203	<0.0001
3rd instars	97.6 ± 1.8a	91.8 ± 3.4a	89.2 ± 7.2b	0.6 ± 1.3c	3,16	280	<0.0001
5th instars	97.9 ± 2.0a	99.2 ± 1.1a	18.2 ± 9.4b	0.8 ± 1.8c	3, 16	284	<0.0001
Pupae	94.6 ± 0.9a	86.0 ± 11.2a	48.0 ± 23.5b	0.0 ± 0.0c	3, 16	81.6	<0.0001
Adult[a] length, mm	5.3 ± 0.5a	5.2 ± 0.4a	6.7 ± 0.2b	N/A	2, 12	20.2	<0.0001

[a] Flies eclosing from pupae that were exposed to -12°C as fifth instars.

Table 5.3. Adult black soldier fly survivorship (mean ± SE) indicated by eclosing from pupae that were exposed -12°C and 4°C as fifth instars. Means in same rows followed by the same letters are not different from each other (Tukey tests, $P < 0.05$).

Adult survivorship			
4°C		-12°C	
0 min	100 ± 0a	0 h	97.2 ± 0.4a
10 min	84.8 ± 1.2b	24 h	98.8 ± 0.4a
30 min	74.0 ± 0c	48 h	99.4 ± 0.5b
60 min	41.8 ± 1.8d	72 h	N/A
df	3, 16	df	2, 12
F	3850	*F*	27.7
P	<0.001	*P*	<0.001

5.4. Discussion

Our results confirm negative effect of low temperatures on BSF. This is not surprising, because BSF naturally inhabit subtropical and temperate regions of the Americas between 45° N and 40° S, which are characterized by mild winters (Üstüner et al. 2003). Generally, insects exhibit freeze-tolerant or freeze avoidance behaviors to withstand cold temperatures. Freeze-tolerant species synthesize ice-forming agents to allow extracellular freezing, while freeze-avoiding species remove all potential ice-forming material, such as gut content and produce antifreeze agents (Bale and Hayward 2010). For most insects, prolonged exposure even to above-zero cold temperatures result in increased mortality due to the accumulation of chilling injury. The associated thermotropic damage to the cell membranes may lead to metabolic imbalances, such as loss of cell turgor, leakage of cytoplasmic solutes, disturbance in ion homeostasis, lack of energy, or cell autolysis and death, and ultimately result in death of an affected organism (Lee and Roh 2010, Kostal et al. 2004, 2006, Spranghers, Noyez, Schildermans, and De Clercq 2017). These imbalances can lead to the accumulation of a toxic metabolites, implying that low

temperatures can inhibit enzymes or a metabolic pathways (Lyons and Raison 1970, Renault et al. 2002). It is reasonable to suggest that the effects observed in our experiments had similar underlying mechanisms.

Survivorship of BSFL and eggs decreased when exposed to above-freezing cold temperatures at 4°C longer than 72 hours, more so in younger life stages. Adults developing from the chilled fifth instars were smaller compared to the control adults. This may have been stemming from higher survival of smaller larvae in above-freezing cold temperatures because smaller insects have a lower supercooling points making them less susceptible to chill damage. For instance, Collembola and mites commonly have supercooling points of -20°C or lower, while large insects frequently have supercooling points of -15°C or higher (Johnston and Lee 1990). Alternatively, it is possible that repairing chilling damage incurred energetic costs that prevented developing larvae from growing to their full sizes.

Smaller adult size may provide an advantage when reproductive success is dependent on completing life cycle in a short period of time in a highly seasonal environment. Larger insect size tends to correlate with a longer development time in many insect species (Price et al. 2011). Tomberlin et al. (2009) described BSFL time of development as an excellent predictor of adult longevity, with longer-living adults originating from slower-developing larvae. Therefore, if breeding time is short, larger individuals are at a disadvantage. There may be selective pressure to reduce development time when exposed to above-freezing cold temperatures to prevent chill damage and increase reproductive success. However, there is also considerable evidence that larger insect females lay more eggs (Price et al. 2011). For example, *Drosophila melanogaster* (Meigen) (Roff 1981) and many species of mosquitoes (Gobbi et al. 2013) had higher reproductive output as female size increased. BSF females have also shown this relationship.

Larger ovaries and basal oocytes were found in females with large wing and large body size than small females (Gobbi et al. 2013). Therefore, there is likely to be a trade-off if the size is decreased to survive low temperatures.

When exposed for 60 minutes to freezing temperatures at -12°C, all life stages suffered heavy mortality. Unlike results obtained for 4°C, fifth instars that survived the cold treatments developed into larger adults. Apparently, physiological processes responsible for larval survivorship were different between above and below-freezing temperatures. It is possible that certain characteristics of larger larvae, such as smaller surface-to-volume ratio or higher fat reserves, allowed for their better survivorship and subsequent development into larger adults. However, we did not measure the weight of surviving larvae, while Spranghers et al. (2017) found no significant relationship between supercooling points and body weights within different life stages. It is also possible that exposure to a short-term cold shock had a hormetic effect, increasing general fitness of survivors (Calabrese 2009). Since all individuals, irrespective of their developmental stage, eventually died after 60 minutes in freezing temperatures, BSF may be a freeze-intolerant species. Majority of freeze-intolerant insects die before a super cooling point is reached as a result of cumulative chilling injury (Spranghers et al. 2017).

Earlier instars were generally more susceptible to cold treatments compared to later instars. This is similar to the results reported by Spranghers et al. (2017), who found significantly shorter lethal times for early instars than to late instars. Interestingly, supercooling points in that study were lower for early instars. This may be due to differences in gut content. When few items are present in the gut, there are also fewer potential ice-forming particles present. This can explain lower mortality (Spranghers et al. 2017).

Pupae tested in our experiments showed a stronger response to cold injury compared to larvae, both when exposed to 4°C and -12°C. This is different from Spranghers et al. (2017), who reported prepupae and pupae to be the most cold-tolerant BSF stadia. Further investigations are needed to explain the observed discrepancy. It is possible that it was caused by genetic differences between BSF strains, differences in pupal maturity, or some other unknown factor(s).

An important consideration when discussing BSF ability to withstand cold temperatures is its ability to enter a diapause. Unfortunately, little is currently known about diapause in this species. Rozkošný and Kovac (1998) reported that *Ptecticus flavifemoratus* (Rozkosny) (Diptera: Stratiomyidae) is capable of entering diapause. To the best of our knowledge, no such information exists for the BSF. Field observations at Buenos Aires, Argentina reported adult emergence from spring through the summer and early fall months with larvae spending the winter in quiescence (Furman et al. 1959). In 1958, BSFL were used to reduce poultry manure found beneath cages in open housing in California. Observations demonstrated that BSF adults and larvae can be found throughout the year in poultry manure, while adult emergence from pupae was delayed until the warm, spring weather (Furman et al. 1959). However, the authors did not investigate whether the observed quiescence was a true diapause, or a slow-down in metabolic rates caused by low temperatures.

Another factor that could influence the cold tolerance of BSFL is the diet on which it is reared (Harnden and Tomberlin 2016). A diet rich in certain nutrients may be able to provide the insect with components that promote its cold hardiness (Spranghers et al. 2017). Amino acids such as proline or the sugar trehalose, could assist with preserving the cell structure by binding to cell membranes and prevent water molecules from binding (Doucet et al. 2009). Prepupae require a suitable substrate for pupation and a significant part of their life in the wild takes place

in the upper soil layer (Holmes et al. 2013). Moreover, Harnden and Tomberlin (2016) found that grain-fed larvae were usually smaller and weighed less than those fed pork or beef diets. In our study, larvae were reared on vegetable-based chicken feed, which may limit their size and effect their cold tolerance because of less nutrient available from their diet.

The majority of BSF populations in the U.S. and other countries are associated with animal production facilities (Newton et al. 2004). Therefore, during cold weather BSFL may avoid low temperatures by overwintering indoors in pig or poultry stables, where relatively high temperatures are maintained year-round. Observations made in open poultry housing manure management studies using BSFL, demonstrated it was possible for BSFL to remain active year-round (Furman et al. 1959). However, adult BSF are often reluctant to enter enclosed structures, including modern environmental animal housing, where they rarely colonize manure in these situations (Sheppard et al. 2002). There is also potential for BSFL or pupae to survive during northern U.S. winters by inhabiting specific hibernacula like manure, organic waste, or compost heaps where temperature remains higher than in the surrounding environment.
Nevertheless, our results appear to confirm the findings by (Spranghers et al. 2017) that BSF do not survive well at low temperatures. Therefore, it is unlikely to permanently inhabit the regions with cooler temperate climate, such as Maine. This is also confirmed by their apparent current absence from Maine, even though there are no geographic barriers limiting their northward expansion from Massachusetts.

Using above-freezing cold temperatures for life cycle synchronization or to improve shipping and storage of live flies may have some use for late-instar BSF, but not for earlier instars, eggs, or pupae (Table 1). However, a caution needs to be exercised while using this approach, especially for time periods longer than 48 h. Although their survivorship was fairly

high, chilled larvae still suffered higher mortality compared to the controlled larvae. Furthermore, negative effects of chilling fifth instars were carried over to an adult stage. The use of chilling is better suited for diapausing insects (Rajamohan et al. 2014). Freezing does not appear to be a viable option for manipulating BSFL development when maintaining live stock is necessary, but can be used for their slaughter and storage.

BIBLIOGRAPHY

Agarwala, B. K., P. Bardhanroy, H. Yasuda, and T. Takizawa. **2003**. Effects of conspecific and heterospecific competitors on feeding and oviposition of a predatory ladybird: A laboratory study. Entomol. Exp. Appl. 106: 219–226.

Aira, M., F. Monroy, and J. Domínguez. **2007**. Microbial biomass governs enzyme activity decay during aging of worm-worked substrates through vermicomposting. J. Environ. Qual. 36: 448–552.

Al-Qazzaz, M. F. A., D. Ismail, H. Akit, and L. H. Idris. **2016**. Effect of using insect larvae meal as a complete protein source on quality and productivity characteristics of laying hens. Rev. Bras. Zootec. 45: 518–523.

Anbutsu, H., and K. Togashi. **2002**. Oviposition deterrence associated with larval frass of the Japanese pine sawyer, Monochamus alternatus (Coleoptera: Cerambycidae). J. Insect Physiol. 48: 459–465.

Anderson, P., M. Hilker, B. S. Hansson, S. Bombosch, B. Klein, and H. Schildknecht. **1993**. Oviposition deterring components in larval frass of Spodoptera littoralis (Boisd.) (Lepidoptera: Noctuidae): A behavioural and electrophysiological evaluation. J. Insect Physiol. 39: 129–137.

Anderson, P., and J. Löfqvist. **1996**. Asymmetric oviposition behaviour and the influence of larval competition in the two Pyralid moths Ephestia kuehniella and Plodia interpunctella. Oikos. 76: 47–56.

Arvanitoyannis, I. S., and A. Kassaveti. **2008**. Fish industry waste: Treatments, environmental impacts, current and potential uses. Int. J. Food Sci. Technol. 43: 726–745.

Australian Museum. **2015**. Decomposition: fly life cycle and development times. (https://australianmuseum.net.au/decomposition-fly-life-cycles).

Bai, L., X. W. Li, L. Zhang, Y. C. Wang, and Y. Guo. **2007**. Nutrient status and energy transformation of swine manure under housefly larva bioconversion Chinese. J. Anim. Sci. 43: 59–63.

Bale, J. S., and S. A. L. Hayward. **2010**. Insect overwintering in a changing climate. J. Exp. Biol. 213: 980–994.

Banani, H., M. Marcet-houben, A. Ballester, P. Abbruscato, L. González-candelas, T. Gabaldón, and D. Spadaro. **2016**. Genome sequencing and secondary metabolism of the postharvest pathogen Penicillium griseofulvum. BMC Genomics. 17: 1–14.

Banks, I. J., W. T. Gibson, and M. M. Cameron. **2014**. Growth rates of black soldier fly larvae fed on fresh human faeces and their implication for improving sanitation. Trop. Med. Int. Heal. 19: 14–22.

Barragan-Fonseca, K. B., M. Dicke, and J. J. A. van Loon. **2017**. Nutritional value of the black soldier fly (Hermetia illucens L.) and its suitability as animal feed – a review. J. Insects as Food Feed. 3: 105–120.

Barroso, F. G., C. de Haro, M. J. Sánchez-Muros, E. Venegas, A. Martínez-Sánchez, and C. Pérez-Bañón. **2014**. The potential of various insect species for use as food for fish. Aquaculture. 422–423: 193–201.

Barroso, F. G., M. J. Sánchez-Muros, M. Segura, E. Morote, A. Torres, R. Ramos, and J. L. Guil. **2017**. Insects as food: Enrichment of larvae of Hermetia illucens with omega 3 fatty acids by means of dietary modifications. J. Food Compos. Anal. 62: 8–13.

Bernard, E. 2012. Regulation of virulence in Rhizoctonia solani: Global gene expression profile and impact of soil amendments on Rhizoctonia disease of potato. Electron. Theses Diss.

Boccazzi, I. V., M. Ottoboni, E. Martin, F. Comandatore, L. Vallone, T. Spranghers, M. Eeckhout, V. Mereghetti, L. Pinotti, and S. Epis. **2017**. A survey of the mycobiota associated with larvae of the black soldier fly (Hermetia illucens) reared for feed production. PLoS One. 12: 1–15.

Boiteau, G., and A. Alford. **1983**. Synchronization of Colorado potato beetle (Coleoptera: Chrysomelidae) emergence by temporary storage of eggs at low temperature. Can. Entomol. 115: 1233–1234.

Bondari, K., and D. C. Sheppard. **1981**. Soldier fly larvae as feed in commercial fish production. 24: 103–109.

Boushy, A. R. El. **1991**. House-fly pupae as poultry manure converters for animal feed: A review. Bioresour. Technol. 38: 45–49.

Bradley, S. W., and D. C. Sheppard. **1984**. House fly oviposition inhibition by larvae of Hermetia illucens, the black soldier fly. J. Chem. Ecol. 10: 854–859.

Brown, A. W. 1938. The nitrogen metabolism of an insect (Lucilia sericata Mg.): Ammonia and other metabolites. Biochem. J. 32: 903–12.

Byrd, J., and J. Castner. **2009**. Forensic entomology: The utility of arthropods in legal investigations, 2nd ed. CRC Press, Boca Raton, FL.

Calabrese, E. J. 2009. Getting the dose-response wrong: Why hormesis became marginalized and the threshold model accepted. Arch. Toxicol. 83: 227–247.

Cammack, J. A., and J. K. Tomberlin. **2017**. The impact of diet protein and carbohydrate on select life-history traits of the black soldier fly Hermetia illucens (L.) (Diptera: Stratiomyidae). Insects. 8: 56.

Cashion, T., F. Le Manach, D. Zeller, and D. Pauly. **2017**. Most fish destined for fishmeal production are food-grade fish. Fish Fish. 18: 837–844.

Castell, J. D., D. J. Lee, and R. O. Sinnhuber. **1972**. Essential fatty acids in the diet of rainbow trout (Salmo gairdneri): Lipid metabolism and fatty acid composition. J. Nutr. 102: 93–99.

Catts, E. P., and G. R. Mullen. **2002**. 16 - MYIASIS (Muscoidea, Oestroidea), pp. 317–348. *In* MULLEN, G., DURDEN, L.B.T.-M. and V.E. (eds.), Med. Vet. Entomol. Academic Press, San Diego.

Centers for Disease Control and Prevention. **2017**. Questions & Answers | Shigella – Shigellosis | CDC. (https://www.cdc.gov/shigella/general-information.html).

Choi, W.-H., J.-H. Yun, J.-P. Chu, and K.-B. Chu. **2012a**. Antibacterial effect of extracts of Hermetia illucens (Diptera: Stratiomyidae) larvae against Gram-negative bacteria. Entomol. Res. 42: 219–226.

Choi, W.-H., J.-H. Yun, J.-P. Chu, and K.-B. Chu. **2012b**. Antibacterial effect of extracts of Hermetia illucens (Diptera: Stratiomyidae) larvae against Gram-negative bacteria. Entomol. Res.

Čičková, H., G. L. Newton, R. C. Lacy, and M. Kozánek. **2015**. The use of fly larvae for organic waste treatment. Waste Manag. 35: 68–80.

Conover, W. J., and R. L. Iman. **1981**. Rank transformations as a bridge between parametric and nonparametric statistics. Am. Stat. 35: 124–129.

Cragg, J. B. 1950. The reactions of Lucilia sericata (Mg.) to various substances placed on sheep. Parasitology. 40: 179–186.

Craig Sheppard, D., G. Larry Newton, S. A. Thompson, and S. Savage. **1994**. A value added manure management system using the black soldier fly. Bioresour. Technol. 50: 275–279.

Cronk, K. H., T. C. Steinkraus, L. R. Hackler, and L. R. Maitick. **1977**. Indonesian tape ketan fermentation. Appl. Environ. Microbiol. 33: 1067–1073.

DACF. **2018**. Invasive Pests. Dep. Agric. Conserv. For. (https://www.maine.gov/dacf/php/gotpests/invasive-pests.htm).

Diener, S., C. Zurbrügg, F. R. Gutiérrez, D. H. Nguyen, A. Morel, T. Koottatep, and K. Tockner. **2011**. Black soldier fly larvae for organic waste treatment – prospects and constraints. Proc. WasteSafe 2011 – 2nd Int. Conf. Solid Waste Manag. Dev. Ctries. 52: 978–984.

Diener, S., C. Zurbrügg, and K. Tockner. **2009**. Conversion of organic material by black soldier fly larvae: establishing optimal feeding rates. Waste Manag. Res. 27: 603–610.

Ditrick, L. E., R. L. Jones, and H. C. Chiang. **1983**. An oviposition deterrent for the European corn borer, Ostrinia nubilalis (Lepidoptera: Pyralidae), extracted from larval frass. J. Insect Physiol.

Domínguez, J., P. J. Bohlen, and R. W. Parmelee. **2004**. Earthworms Increase Nitrogen Leaching to Greater Soil Depths in Row Crop Agroecosystems. Ecosystems. 7: 672–685.

Donovan, S. E., M. J. R. Hall, B. D. Turner, and C. B. Moncrieff. **2006**. Larval growth rates of the blowfly, Calliphora vicina, over a range of temperatures. Med. Vet. Entomol. 20: 106–114.

Dossey, A. T., J. T. Tatum, and W. L. McGill. **2016**. Modern Insect-Based Food Industry: Current Status, Insect Processing Technology, and Recommendations Moving Forward, Insects as Sustain. Food Ingredients. Elsevier Inc.

Doucet, D., V. K. Walker, and W. Qin. **2009**. The bugs that came in from the cold: Molecular adaptations to low temperatures in insects. Cell. Mol. Life Sci. 66: 1404–1418.

Dutkiewicz, J., B. Mackiewicz, M. K. Lemieszek, M. Golec, and J. Milanowski. **2016**. Pantoea agglomerans: A mysterious bacterium of evil and good. Part III. Deleterious effects: Infections of humans, animals and plants. Ann. Agric. Environ. Med. 23: 197–205.

Elhag, O., D. Zhou, Q. Song, A. A. Soomro, M. Cai, L. Zheng, Z. Yu, and J. Zhang. **2017**. Screening, expression, purification and functional characterization of novel antimicrobial peptide genes from hermetia illucens (L.). PLoS One. 12: 1–15.

Erickson, M. C., M. Islam, C. Sheppard, J. Liao, and M. P. Doyle. **2004**. Reduction of Escherichia coli O157:H7 and Salmonella enterica serovar enteritidis in chicken manure by larvae of the black soldier fly. J. Food Prot. 67: 685–690.

FAO. **2004**. The state of world fisheries and aquaculture. State World Fish. Aquac. 2004. 1–161.

Fatchurochim, S., C. J. Gden, and R. C. Axtell. **1989**. Filth fly (diptera) oviposition and larval development in poultry manure of various moisture levels. J. Entomol. Sci. 24: 244–231.

Feindel, S., T. Bennett, and K. Kanwit. **2011**. The Maine sea cucumber (Cucumaria frondosa) fishery. (https://www.maine.gov/dmr/science-research/species/cukes/documents/feindeletal2011.pdf).

Fisheries of the United States (FUS). **2015**. A Statistical Snapshot of 2015 Fish Landings. (https://www.st.nmfs.noaa.gov/Assets/commercial/fus/fus15/documents/FUS2015 Fact Sheet.pdf).

Food and Agriculture Organization of the United Nations (FAO). **2016**. Contributing to food security and nutrition for all. State World Fish. Aquac. 1–24.

Furman, D. P., R. D. Young, and E. P. Catts. **1959**. Hermetia illucens (Linnaeus) as a Factor in the Natural Control of Musca domestica Linnaeus. J. Econ. Entomol. 52: 917–921.

GAO. **2008**. Concentrated animal feeding operations: EPA needs more information and a clearly defined strategy to protect air and water quality from pollutants of concern.

Garcia-Hermoso, D., D. Hoinard, J. C. Gantier, F. Grenouillet, F. Dromer, and E. Dannaoui. **2009**. Molecular and phenotypic evaluation of Lichtheimia corymbifera (formerly Absidia corymbifera) complex isolates associated with human mucormycosis: Rehabilitation of L. ramosa. J. Clin. Microbiol. 47: 3862–3870.

Garcia, V., C. Abat, and J. M. Rolain. **2016**. Report of the first Vagococcus lutrae human infection, Marseille, France. New Microbes New Infect. 9: 56–57.

Gayatri, G. R., and P. K. Madhuri. **2013**. Occurrence of black soldier fly Hermetia illucens (Diptera: Stratiomyidae) in biocompost. Res. J. Recent Sci. 2: 65–66.

Ghori, I., M. Tabassum, T. Ahmad, A. Zuberi, and M. Imran. **2018**. Geotrichum candidum Enhanced the Enterococcus faecium impact in improving physiology, and health of Labeo rohita (Hamilton, 1822) by modulating gut microbiome under mimic aquaculture conditions. urkish J. Fish. Aquat. Sci. 18: 1255–1267.

Glencross, B. D. 2009. Exploring the nutritional demand for essential fatty acids by aquaculture species. Rev. Aquac. 1: 71–124.

Gobbi, P., A. Martínez-Sánchez, and S. Rojo. **2013**. The effects of larval diet on adult life-history traits of the black soldier fly, Hermetia illucens (Diptera: Stratiomyidae). Eur. J. Entomol. 110: 461–468.

Godoy, G., R. Rodriguez-Kabana, R. A. Shelby, and G. Morgan-Jones. **1983**. Chitin amendments for control of Meloidogyne arenaria in infested soil. II. effects on microbial population. Nematropica. 13: 63–74.

Golubev, A. 2010. Exponentially modified Gaussian (EMG) relevance to distributions related to cell proliferation and differentiation. J. Theor. Biol. 262: 257–266.

Gomes, L., W. A. C. Godoy, and C. J. Von Zuben. **2006**. A review of postfeeding larval dispersal in blowflies: Implications for forensic entomology. Naturwissenschaften. 93: 207–215.

Gómez-Brandón, M., M. Aira, M. Lores, and J. Domínguez. **2011**. Changes in microbial community structure and function during vermicomposting of pig slurry. Bioresour. Technol. 102: 4171–4178.

Goodbrod, J. R., and M. L. E. E. Goff. **1990**. Effects of larval population density on rates of development and interactions between two species of Chrysomya (Diptera : Calliphoridae) in laboratory culture. J. Med. Entomol. 27: 338–343.

Green, T. R., and R. Popa. **2012**. Enhanced ammonia content in compost leachate processed by black soldier fly larvae. Appl. Biochem. Biotechnol. 166: 1381–1387.

Gryganskyi, A. P., S. C. Lee, A. P. Litvintseva, M. E. Smith, G. Bonito, T. M. Porter, I. M. Anishchenko, J. Heitman, and R. Vilgalys. **2010**. Structure, function, and phylogeny of the mating locus in the Rhizopus oryzae complex. 5: 1–12.

Gunders, D. 2017. Wasted: How America is losing up to 40 percent of its food from farm to fork to landfill. Natl. Resour. Def. Counc. 2: 1–58.

Hammack, L. 1991. Oviposition by screwworm flies (Diptera : Calliphoridae) on contact with host fluids. J. Econ. Entomol. 84: 185–190.

Hardy, J., S. Kiesser, L. Antrim, A. Stubin, R. Kocan, and J. Strand. **1987**. The sea-surface microlayer of puget sound: Part I. Toxic effects on fish eggs and larvae. Mar. Environ. Res. 23: 227–249.

Harnden, L. M., and J. K. Tomberlin. **2016**. Effects of temperature and diet on black soldier fly, Hermetia illucens (L.) (Diptera: Stratiomyidae), development. Forensic Sci. Int. 266: 109–116.

He, Y., J. G. Caporaso, X.-T. Jiang, H.-F. Sheng, S. M. Huse, J. R. Rideout, R. C. Edgar, E. Kopylova, W. A. Walters, R. Knight, and H.-W. Zhou. **2015**. Stability of operational taxonomic units: An important but neglected property for analyzing microbial diversity. Microbiome. 3: 34.

Henry, M., L. Gasco, G. Piccolo, and E. Fountoulaki. **2015**. Review on the use of insects in the diet of farmed fish: Past and future. Anim. Feed Sci. Technol. 203: 1–22.

Hepburn, G., and M. Nolte. **1943**. Sheep blowfly research III. studies on the olfactory reactions of sheep blowflies. Onderstepoort J. Vet. Sci. Anim. Ind. 18: 27–48.

Hertrampf, J. W., and F. Piedad-Pascual. **2000**. Handbook on Ingredients for Aquaculture Feeds. Kluwer Academic Publishers, London.

Holmes, L. 2010. Role of abiotic factors on the development and life history of the black soldier fly, Hermetia illucens(L.)(Diptera: Stratiomyidae). Electron. Theses Diss.

Holmes, L. A., S. L. Vanlaerhoven, and J. K. Tomberlin. **2012**. Relative humidity effects on the life history of Hermetia illucens (Diptera: Stratiomyidae). Physiol. Ecol. 41: 971–978.

Holmes, L. A., S. L. Vanlaerhoven, and J. K. Tomberlin. **2013**. Substrate effects on pupation and adult emergence of Hermetia illucens (Diptera: Stratiomyidae). Physiol. Ecol. 42: 370–374.

Holmes, L. A., S. L. VanLaerhoven, and J. K. Tomberlin. **2016**. Lower temperature threshold of black soldier fly (Diptera: Stratiomyidae) development. J. Insects as Food Feed. 2: 255–262.

Houbraken, J. A. M. P., J. C. Frisvad, and R. A. Samson. **2010**. Taxonomy of Penicillium citrinum and related species. Fungal Divers. 117–133.

Hribar, C. 2010. Understanding concentrated animal feeding operations and their impact on communities. Natl. Assoc. Local Boards Heal. 1–30.

ILO. **2018**. International Labour Organization. (https://www.ilo.org/global/lang--en/index.htm).

iNaturalist. **2018**. Black Soldier Fly (Hermetia illucens). (https://www.inaturalist.org/taxa/82177-Hermetia-illucens).

Jeon, H., S. Park, J. Choi, G. Jeong, S.-B. Lee, Y. Choi, and S.-J. Lee. **2011**. The intestinal bacterial community in the food waste-reducing larvae of Hermetia illucens. Curr. Microbiol. 62: 1390–1399.

Johnston, S. L., and R. E. Lee. **1990**. Regulation of supercooling and nucleation in a freeze intolerant beetle (Tenebrio molitor). Cryobiology. 27: 562–568.

Kalogeropoulos, N., M. N. Alexis, and R. J. Henderson. **1992**. Effects of dietary soybean and cod-liver oil levels on growth and body composition of gilthead bream (Sparus aurata). Aquaculture. 104: 293–308.

Kennish, J. M., J. L. Sharp-Dahl, K. A. Chambers, F. Thrower, and S. D. Rice. **1992**. The effect of a herring diet on lipid composition, fatty acid composition, and cholesterol levels in the muscle tissue of pen-reared chinook salmon (Oncorhynchus tshawytscha). Aquaculture. 108: 309–322.

Kim, W., S. Bae, K. Park, S. Lee, Y. Choi, S. Han, and Y. Koh. **2011**. Biochemical characterization of digestive enzymes in the black soldier fly, Hermetia illucens (Diptera: Stratiomyidae). J. Asia. Pac. Entomol. 14: 11–14.

Klein, B., H. Schildknecht, M. Hilker, and S. Bombosch. **1990**. Oviposition Deterrent from Larval Frass of Spodoptera littoralis (Boisd.). Zeitschrift für Naturforsch. C. 45: 895–901.

Knapp, B. A., S. M. Podmirseg, J. Seeber, E. Meyer, and H. Insam. **2009**. Diet-related composition of the gut microbiota of Lumbricus rubellus as revealed by a molecular fingerprinting technique and cloning. Soil Biol. Biochem. 41: 2299–2307.

Kostal, V., and J. Havelka. **2001**. Low Temperature Storage of Larvae and Synchronization of Adult Emergence in the Predatory Midge Aphidoletes aphidimyza. Cryobiology. 42: 112–120.

Kostal, V., J. Vambera, and J. Bastl. **2004**. On the nature of pre-freeze mortality in insects : water balance , ion homeostasis and energy charge in the adults of Pyrrhocoris apterus. 1509–1521.

Kostal, V., M. Yanagimoto, and J. Bastl. **2006**. Chilling-injury and disturbance of ion homeostasis in the coxal muscle of the tropical cockroach (Nauphoeta cinerea). 143: 171–179.

Lachance, M.-A., and C. P. Kurtzman. **2011**. Chapter 36 - Kodamaea Y. Yamada, T. Suzuki, Matsuda & Mikata emend. Rosa, Lachance, Starmer, Barker, Bowles & Schlag-Edler (1999), pp. 483–490. *In* Kurtzman, C.P., Fell, J.W., Boekhout, T. (eds.), Yeast (Fifth Ed. Elsevier, London.

Lalander, C. H., J. Fidjeland, S. Diener, S. Eriksson, and B. Vinnerås. **2014**. High waste-to-biomass conversion and efficient Salmonella spp. reduction using black soldier fly for waste recycling. Agron. Sustain. Dev. 35: 261–271.

Large, M. 2004. Seafish Waste Survey 2004.

Larkin, R. P. 1993. Ecology of Fusarium oxysporum f. sp. niveum in Soils Suppressive and Conducive to Fusarium Wilt of Watermelon. Phytopathology. 83: 1105–1116.

Lawson, P. A., G. Foster, E. Falsen, M. Ohlen, and M. D. Collins. **1999**. Vagococcus lutrae sp. nov., isolated from the common otter (Lutra lutra). Int. J. Syst. Bacteriol. 1251–1254.

Lee, H. L., P. Chandrawathani, W. Y. Wong, S. Tharam, and W. Y. Lim. **1995**. A case of human enteric myiasis due to larvae of Hermetia illucens (Family: Stratiomyiadae): first report in Malaysia. Malays. J. Pathol. 17: 109–11.

Lee, K. P., and C. Roh. **2010**. Temperature-by-nutrient interactions affecting growth rate in an insect ectotherm. Entomol. Exp. Appl. 136: 151–163.

Levot, G. W., K. R. Brown, and E. Shipp. **1979**. Larval growth of some calliphorid and sarcophagid Diptera. Bull. Entomol. Res. 69: 469–475.

Li, G., and Y. Ishikawa. **2004**. Oviposition deterrents in larval frass of four Ostrinia species fed on an artificial diet. J. Chem. Ecol. 30: 1445–1456.

LIFE. **2018**. Life: Leading International Fungal Education. (http://www.life-worldwide.org/fungal-diseases/lichtheimia-corymbifera/).

Lima, E. A. B. F., C. P. Ferreira, A. M. Bernardes, and W. A. C. Godoy. **2009**. Neighborhood interactions and larval dispersal behavior in blowflies. J. Insect Behav. 22: 245–255.

Liu, X., X. Chen, H. Wang, Q. Yang, K. Rehman, W. Li, and M. Cai. **2017**. Dynamic changes of nutrient composition throughout the entire life cycle of black soldier fly. PLoS One. 12: 1–21.

Love, D. C., J. P. Fry, M. C. Milli, and R. A. Neff. **2015**. Wasted seafood in the United States: Quantifying loss from production to consumption and moving toward solutions. Glob. Environ. Chang. 35: 116–124.

Luczynski, A., J. P. Nyrop, and A. Shi. **2008**. Pattern of female reproductive age classes in mass-reared populations of Phytoseiulus persimilis (Acari : Phytoseiidae) and its influence on population characteristics and quality of predators following cold storage. Biol. Control. 47: 159–166.

Lyons, J. M., and J. K. Raison. **1970**. Oxidative activity of mitochondria isolated from plant tissues sensitive and resistant to chilling injury. Plant physiol. 45: 386–389.

Ma, Q., A. Fonseca, W. Liu, A. T. Fields, M. L. Pimsler, A. F. Spindola, A. M. Tarone, T. L. Crippen, J. K. Tomberlin, and T. K. Wood. **2012**. Proteus mirabilis interkingdom swarming signals attract blow flies. ISME J. 6: 1356–1366.

Manurung, R., A. Supriatna, and R. R. Esyanthi. **2016**. Bioconversion of rice straw waste by black soldier fly larvae (Hermetia illucens L.): Optimal feed rate for biomass production. J. Entomol. Zool. Stud. 4: 1036–1041.

Marchaim, U., A. Gelman, and Y. Braverman. **2003**. Reducing waste contamination from animal-processing plants by anaerobic thermophilic fermentation and by flesh fly digestion. Appl. Biochem. Biotechnol. 109: 107–15.

Marshall, S.A., Whitworth, T. and Roscoe, L. 2011. Blow flies (Diptera; Calliphoridae) of eastern Canada with a key to Calliphoridae subfamilies and genera of eastern North America, and a key to the eastern Canadian species of Calliphorinae, Luciliinae and Chrysomyiinae. Canadian Journal of Arthropod Identification No 11. (http://cjai.biologicalsurvey.ca/mwr_11/mwr_11.html).

Martínez-Sánchez, A., C. Magaña, M. Saloña, and S. Rojo. **2011**. First record of Hermetia illucens (Diptera: Stratiomyidae) on human corpses in Iberian Peninsula. Forensic Sci. Int. 206: e76–e78.

Masuda, R. 2003. The critical role of docosahexaenoic acid in marine and terrestrial ecosystems: from bacteria to human behavior. Big Fish Bang. 249–256.

Maurer, V., M. Holinger, Z. Amsler, B. Früh, J. Wohlfahrt, A. Stamer, and F. Leiber. **2015**. Replacement of soybean cake by Hermetia illucens meal in diets for layers. J. Insects as Food Feed. 2: 83–90.

Mazik, K., D. Burdon, and M. Elliot. **2005**. Seafood-waste disposal at sea – a scientific review, Inst. Estuar. Coast. Stud.

Meleney, H. E., and P. D. Harwood. **1935**. Human Intestinal Mylasis due to the Larvae of the Soldier Fly, Hermetia illucens Linné (Diptera, Stratiomyidae). Am. J. Trop. Med. Hyg. s1-15: 45–49.

Millikin, M. R. 1982. Effects of Dietary Protein Concentration on Growth, Feed Efficiency, and Body Composition of Age-0 Striped Bass. Trans. Am. Fish. Soc. 111: 373–378.

Mok, T., A. P. Koehler, M. Y. Yu, D. H. Ellis, and P. J. Johnson. **1997**. Fatal Penicillium citrinum Pneumonia with Pericarditis in a Patient with Acute Leukemia. 35: 2654–2656.

Monroy, F., M. Aira, and J. Domínguez. **2009**. Reduction of total coliform numbers during vermicomposting is caused by short-term direct effects of earthworms on microorganisms and depends on the dose of application of pig slurry. Sci. Total Environ. 407: 5411–5416.

Mourente, G., and D. R. Tocher. **1992**. Lipid class and fatty acid composition of brain lipids from Atlantic herring (Clupea harengus) at different stages of development. Mar. Biol. 112: 553–558.

Mueller, L. D. 1988. Density-dependent population growth and natural selection in food-limited environments. Am. Nat. 132: 786–809.

Myers, H. M., J. K. Tomberlin, B. D. Lambert, and D. Kattes. **2008**. Development of black soldier fly (Diptera: Stratiomyidae) larvae fed dairy manure. Environ. Entomol. 37: 11–15.

Nellemann, C., M. MacDevette, T. Manders, B. Eickhout, B. Svihus, A. G. Prins, and B. P. Kaltenborn. **2009**. The environmental food crisis – The environment's role in averting future food crises. United Nations Environ. Program. 78: 1–101.

Newton, G. L., C. V Booram, R. W. Barker, and O. M. Hale. **1977**. Dried Hermetia illucens larvae meal as a supplement for swine. J. Anim. Sci. 44: 395–400.

Newton, G. L., D. C. Sheppard, D. W. Watson, G. J. Burtle, C. R. Dove, J. K. Tomberlin, and E. E. Thelen. **2004**. The black soldier fly, Hermetia illucens, as a manure management/resource recovery tool. 57: 275–296.

Nguyen, T. 2010. Influence and diet on black soldier fly (Hermetia illucens Linnaeus) (Diptera: Stratiomyidae) life history traits. Masters Thesis Univ. Wind.

Nguyen, T. T. X., J. K. Tomberlin, and S. Vanlaerhoven. **2015**. Ability of black soldier fly (Diptera: Stratiomyidae) larvae to recycle food waste. Environ. Entomol. 44: 406–410.

NIH. **2018**. Omega-3 Supplements: In Depth. Natl. Cent. Complement. Integr. Heal. (https://ods.od.nih.gov/factsheets/Omega3FattyAcids-Consumer/).

Oliveira, F., K. Doelle, R. List, and J. R. O. Reilly. **2015**. Assessment of Diptera: Stratiomyidae, genus Hermetia illucens (L., 1758) using electron microscopy. J. Entomol. Zool. Stud. 3: 147–152.

Oliveros, J. C. 2015. Venny. An interactive tool for comparing lists with Venn's diagrams. (http://bioinfogp.cnb.csic.es/tools/venny/).

Oyegoke, O., A. Akintola, and J. O. Fasoranti. **2006**. Dietary potentials of the edible larvae of Cirina forda (westwood) as a poultry feed. African J. Biotechnol. 5: 1799–1802.

Palmer, E. M., T. S. Horowitz, A. Torralba, and J. M. Wolfe. **2011**. What are the shapes of response time distributions in visual search? J. Exp. Psychol. Hum. Percept. Perform. 37: 58–71.

Papadoyianis, E. D. 2007. Insects offer a promising solution to the protein bottleneck. Feed Technol. Updat. Solut. Glob. Feed Ind. 2: 1–37.

Park, S. I., J. W. Kim, and S. M. Yoe. **2015**. Purification and characterization of a novel antibacterial peptide from black soldier fly (Hermetia illucens) larvae. Dev. Comp. Immunol. 52: 98–106.

Pava-Ripoll, M., R. E. G. Pearson, A. K. Miller, and G. C. Ziobro. **2012**. Prevalence and relative risk of Cronobacter spp., Salmonella spp., and Listeria monocytogenes associated with the body surfaces and guts of individual filth flies. Appl. Environ. Microbiol. 78: 7891–7902.

Pepper, I. L., C. P. Gerba, and T. J. Gentry. **2015**. Environmental Microbiology, 3rd ed. Academic Press, San Diego.

Personal Communication. **2016**. UMaine undergraduate workers.

Petrýdesová, J., K. Bacigálová, and P. Sulo. **2013**. The reassignment of three "lost" Taphrina species (Taphrina bullata, Taphrina insititiae and Taphrina rhizophora) supported by the divergence of nuclear and mitochondrial DNA. Int. J. Syst. Evol. Microbiol. 63: 3091–3098.

Pikitch, E. K., K. J. Rountos, T. E. Essington, C. Santora, D. Pauly, R. Watson, U. R. Sumaila, P. D. Boersma, I. L. Boyd, D. O. Conover, P. Cury, S. S. Heppell, E. D. Houde, M. Mangel, É. Plagányi, K. Sainsbury, R. S. Steneck, T. M. Geers, N. Gownaris, and S. B. Munch. **2014**. The global contribution of forage fish to marine fisheries and ecosystems. Fish Fish. 15: 43–64.

Price, P. W., R. F. Denno, M. D. Eubanks, D. L. Finke, and I. Kaplan. **2011**. Insect Ecology: Behavior, Populations and Communities. Cambridge University Press.

Rajamohan, A., J. P. Rinehart, and R. a Leopold. **2014**. Cryopreservation of embryos of Lucilia sericata (Diptera: Calliphoridae). J. Med. Entomol. 51: 360–7.

Ramakrishnan, V. 2013. Fish processing wastes as a potential source of proteins, amino acids and oils: A critical review. J. Microb. Biochem. Technol. 2: 107–129.

Renault, D., C. Salin, G. Vannier, and P. Vernon. **2002**. Survival at low temperatures in insects: What is the ecological significance of the supercooling point? CryoLetters. 23: 217–228.

Renwick, J. A. A., and C. D. Radke. **1985**. Constituents of host- and non-host plants deterring oviposition by the cabbage butterfly, Pieris rapae. Entomol. Exp. Appl. 39: 21–26.

Rhainds, M., G. Gries, and J. L. Morales. **1996**. Oviposition deterrency in pineapple borer females, Thecia basilides (Lepidoptera: Lycaenidae). Ecol. Entomol. 21: 105–106.

Rich, J. R., and C. H. Hodge. **1993**. Utilization of blue crab scrap compost to suppress Meloidogyne javanica on tomato. Nematropica. 23: 1–5.

Richard-Giroux, J., and A. Spindola. **2017**. Impact of sucrose on black soldier fly longevity and egg production. *In* Present. Annu. Meet. Entomol. Soc. Am.

Rodriguez-Kabana, R., and G. Morgan-Jones. **1988**. Potential for nematode control by mycofloras endemic in the tropics. J. Nematol. 20: 191–203.

Rodriguez-kabana, R., G. Morgan-jones, and I. Chet. **1987**. Biological control of nematodes: Soil amendments and microbial antagonists. Plant Soil. 100: 237–247.

Roff, D. 1981. On Being the Right Size. Am. Nat. 118: 405–422.

Rohrer, D., and J. T. Wixted. **1994**. An analysis of latency and interresponse time in free recall. 22: 511–524.

Rozkošný, R., and D. Kovac. **1998**. Descriptions of bamboo-inhabiting larvae and puparia of Oriental soldier flies Ptecticus brunettii and P-flavifemoratus (Diptera : Stratiomyidae : Sarginae) with observations on their biology. Eur. J. Entomol. 95: 65–86.

Rumpold, B. A., and O. K. Schlüter. **2013**. Potential and challenges of insects as an innovative source for food and feed production. Innov. Food Sci. Emerg. Technol. 17: 1–11.

Rust, M. B., F. T. Barrows, R. W. Hardy, A. Lazur, K. Naughten, and J. Silverstein. **2011**. The Future of Aquafeeds. NOAA Tech. Memo. NMFS F/SPO-124. NOAA/USDA: 93.

Ruyter, ROsjO, Einen, and Thomassen. **2000**. Essential fatty acids in Atlantic salmon: time course of changes in fatty acid composition of liver, blood and carcass induced by a diet deficient in n-3 and n-6 fatty acids. Aquac. Nutr. 6: 109–117.

Salomão, R., A. Maia, B. Bezerra, and L. Iannuzzi. **2018**. Attractiveness of different food resources to dung beetles (Coleoptera: Scarabaeidae) of a dry tropical area. Neotrop. Entomol. 47: 69–78.

Sánchez-Muros, M. J., F. G. Barroso, and F. Manzano-Agugliaro. **2014**. Insect meal as renewable source of food for animal feeding: A review. J. Clean. Prod. 65: 16–27.

Santha, C. R., and D. M. Gatlin. **1991**. Growth response and fatty acid composition of channel catfish fry fed practical diets supplemented with menhaden fish oil. Progress. Fish-Culturist. 53: 135–140.

Sargent, J. R., J. G. Bell, M. Bell, R. J. Henderson, and D. R. Tocher. **1993**. The metabolism of phospholipids and polyunsaturated fatty acids in fish. Coast. Estuar. Stud. 43: 103–124.

Scallan, E., R. M. Hoekstra, F. J. Angulo, R. V. Tauxe, M. A. Widdowson, S. L. Roy, J. L. Jones, and P. M. Griffin. **2011**. Foodborne illness acquired in the United States-Major pathogens. Emerg. Infect. Dis. 17: 7–15.

Schremmer, F. 1986. The polymetabol development of the soldier fly larva Hermetia illucens. - A contribution to the Metamorphosis of the Stratiomyidae. Ann. des Naturhistorischen Museums Wien. Serie B,: 405–429.

Schröder, J., I. Maus, E. Trost, and A. Tauch. **2011**. Complete genome sequence of Corynebacterium variabile DSM 44702 isolated from the surface of smear-ripened cheeses and insights into cheese ripening and flavor generation. BMC Genomics. 12: 2–23.

Schumacher, T. E., A. Eynard, and R. Chintala. **2015**. Rapid cost-effective analysis of microbial activity in soils using modified fluorescein diacetate method. Environ. Sci. Pollut. Res. 22: 4759–4762.

Sealey, W. M., T. G. Gaylord, F. T. Barrows, J. K. Tomberlin, M. A. McGuire, C. Ross, and S. St-Hilaire. **2011**. Sensory Analysis of Rainbow Trout, Oncorhynchus mykiss, Fed Enriched Black Soldier Fly Prepupae, Hermetia illucens. J. World Aquac. Soc. 42: 34–45.

Sheppard, C. D. 1993. Using soldier flies as a manure management tool for volume reduction, house fly control and feedstuff production. Sustain. Agric. Res. Educ. 1–12.

Sheppard, D. C., J. K. Tomberlin, J. A. Joyce, B. C. Kiser, and S. M. Sumner. **2002**. Rearing methods for the black soldier fly (Diptera : Stratiomyidae). J. Med. Entomol. 39: 695–698.

De Smet, J., E. Wynants, P. Cos, and L. Van Campenhout. **2018**. Microbial community dynamics during rearing of black soldier fly larvae (Hermetia illucens) and impact on exploitation potential. Appl. Environ. Microbiol. 1–17.

Soh, J., X. Dong, S. M. Caffrey, G. Voordouw, and C. W. Sensen. **2013**. Phoenix 2: A locally installable large-scale 16S rRNA gene sequence analysis pipeline with Web interface. J. Biotechnol. 167: 393–403.

SoonIk, P., C. ByungSoo, and Y. SungMoon. **2014**. Detection of antimicrobial substances from larvae of the black soldier fly, Hermetia illucens (Diptera: Stratiomyidae). Entomol. Res. 44: 58–64.

Spranghers, T., A. Noyez, K. Schildermans, and P. De Clercq. **2017**. Cold Hardiness of the Black Soldier Fly (Diptera: Stratiomyidae). J. Econ. Entomol. 110: 1501–1507.

St-Hilaire, S. 2007. Fish Offal Recycling by the Black Soldier Fly Produces a Foodstuff High in Omega-3 Fatty Acids. 38: 309–313.

St-Hilaire, S., K. Cranfill, M. A. McGuire, E. E. Mosley, J. K. Tomberlin, L. Newton, W. Sealey, C. Sheppard, and S. Irving. **2007**. Fish Offal Recycling by the Black Soldier Fly Produces a Foodstuff High in Omega-3 Fatty Acids. J. World Aquac. Soc. 38: 309–313.

St-Hilaire, S., C. Sheppard, J. K. Tomberlin, S. Irving, L. Newton, M. A. McGuire, E. E. Mosley, R. W. Hardy, and W. Sealey. **2007**. Fly prepupae as a feedstuff for rainbow trout, Oncorhynchus mykiss. J. World Aquac. Soc. 38: 59–67.

Stewart, G., and G. Noyes-Hull. **2010**. Feasibility of producing value added products from snow crab processing waste in Cape Breton, Nova Scotia. Nov. Scotia Dep. Fish. Aquac. 1–44.

Tang, M., C. J. Hardman, Y. Ji, G. Meng, S. Liu, M. Tan, S. Yang, E. D. Moss, J. Wang, C. Yang, C. Bruce, T. Nevard, S. G. Potts, X. Zhou, and D. W. Yu. **2015**. High-throughput monitoring of wild bee diversity and abundance via mitogenomics. Methods Ecol. Evol. 6: 1034–1043.

Tchoukanova, N., M. Gonzalez, and S. Poirier. **2012**. Best Management Practices: Marine Products Processing. Fish. Ocean. Canada. (http://www.glf.dfo-mpo.gc.ca/folios/00155/docs/bmp-eng.pdf).

Tinder, A. C., R. T. Puckett, N. D. Turner, J. A. Cammack, and J. K. Tomberlin. **2017**. Bioconversion of sorghum and cowpea by black soldier fly (Hermetia illucens (L.)) larvae for alternative protein production. J. Insects as Food Feed. 3: 121–130.

Tomberlin, J. K., P. H. Adler, and H. M. Myers. **2009**. Development of the black soldier fly (Diptera: Stratiomyidae) in relation to temperature. Environ. Entomol. 38: 930–9344.

Tomberlin, J. K., T. L. Crippen, A. M. Tarone, M. F. B. Chaudhury, B. Singh, J. A. Cammack, and R. P. Meisel. **2017**. A review of bacterial interactions with blow flies (Diptera: Calliphoridae) of medical, veterinary, and forensic importance. Ann. Entomol. Soc. Am. 110: 19–36.

Tomberlin, J. K., and D. C. Sheppard. **2002**. Factors influencing mating and oviposition of black soldier flies (Diptera: Stratiomyidae) in a colony. J. Entomol. Sci. 37: 345–352.

Tomberlin, J. K., D. C. Sheppard, and J. A. Joyce. **2002**. Selected life-history traits of black soldier flies (Diptera: Stratiomyidae) reared on three artificial diets. Ann. Entomol. Soc. Am. 95: 379–386.

Tomberlin, J. K., D. C. Sheppard, and J. A. Joyce. **2005**. Black soldier fly (Diptera: Stratiomyidae) colonization of pig carrion in south Georgia. J. Forensic Sci. 50: 152–153.

Triplehorn, C. A., N. F. Johnson, and D. J. Borror. **2005**. Borror and DeLong's Introduction to the Study of Insects, 7th ed. Thompson Brooks/Cole, Minnesota.

Tu, J., D. Li, Q. Li, L. Zhang, Q. Zhu, U. Gaur, X. Fan, H. Xu, Y. Yao, X. Zhao, and M. Yang. **2015**. Molecular evolutionary analysis of β-defensin peptides in vertebrates. Evol. Bioinforma. 11: 105–114.

Tyson, D. R., S. P. Garbett, P. L. Frick, and V. Quaranta. **2012**. Fractional proliferation: a method to deconvolve cell population dynamics from single-cell data. Nat. Methods. 9: 923–928.

Urech, R., P. E. Green, M. J. Rice, G. W. Brown, F. Duncalfe, and P. Webb. **2004**. Composition of chemical attractants affects trap catches of the australian sheep blowfly, Lucilia cuprina, and other blowflies. J. Chem. Ecol. 30: 851–866.

US EPA. **2005**. Municipal solid waste generation, recycling, and disposal in the United States: Facts and figures for 2005. US Environ. Prot. Agency. 1–165.

USDA. **2005**. FY-2005 annual report manure and byproduct.

USEPA, U. S. E. P. A. 2016. Advancing sustainable materials management: 2014 Fact Sheet. United States Environ. Prot. Agency, Off. L. Emerg. Manag. Washington, DC 20460. 22.

Üstüner, T., A. Hasbenlİ, and R. Rozkošný. **2003**. The first record of Hermetia illucens (Linnaeus, 1758) (Diptera, Stratiomyidae) from the Near East. Stud. Dipterologica. 10: 181–185.

Wang, Y.-S., and M. Shelomi. **2017**. Review of black soldier fly (Hermetia illucens) as animal feed and human food. Foods. 6: 1–23.

Watanabe, T. 1982. Lipid nutrition in fish. Comp. Biochem. Physiol. Part B Comp. Biochem. 73: 3–15.

Watts, R. 2018. Commericial Fishing Landings Data: Maine Department of Marine Resources. (https://www.maine.gov/dmr/commercial-fishing/landings/index.html).

Westerman, P. W., and J. R. Bicudo. **2005**. Management considerations for organic waste use in agriculture. Bioresour. Technol. 96: 215–221.

Williams, A. L., E. R. Mitchell, R. R. Heath, and C. S. Barfield. **1986**. Oviposition deterrents for Fall Armyworm (Lepidoptera: Noctuidae) from larval frass, corn leaves, and artificial Diet. Environ. Entomol. 15: 327–330.

Williams, H., and A. M. M. Richardson. **1983**. Life history responses to larval food shortages in four species of necrophagous flies (Diptera: Calliphoridae). Aust. J. Ecol. 8: 257–263.

Wohlt, J. E., J. Petro, G. M. Horton, R. L. Gilbreath, and S. M. Tweed. **1994**. Composition, preservation, and use of sea clam viscera as a protein supplement for growing pigs. J. Anim. Sci. 72: 546–553.

Yadav, A., and V. K. Garg. **2011**. Industrial wastes and sludges management by vermicomposting. Rev. Environ. Sci. Bio/Technology. 10: 243–276.

Yang, P. 2014. Two Records of Intestinal Myiasis Caused by Ornidia obesa and Hermetia illucens in Hawaii. Proc. Hawaiian Entomol. Soc. 46: 29.

Yasir, M., Z. Aslam, S. W. Kim, S.-W. Lee, C. O. Jeon, and Y. R. Chung. **2009**. Bacterial community composition and chitinase gene diversity of vermicompost with antifungal activity. Bioresour. Technol. 100: 4396–4403.

Zhao, L., Y. Wang, J. Yang, M. Xing, X. Li, D. Yi, and D. Deng. **2010**. Earthworm-microorganism interactions: a strategy to stabilize domestic wastewater sludge. Water Res. 44: 2572–2582.

Zheng, L., T. L. Crippen, B. Singh, A. M. Tarone, S. Dowd, Z. Yu, T. K. Wood, and J. K. Tomberlin. **2013**. A Survey of bacterial diversity from successive life stages of black soldier fly (Diptera: Stratiomyidae) by using 16S rDNA pyrosequencing. J. Med. Entomol. 50: 647–658.

Zheng, L., Y. Hou, W. Li, S. Yang, Q. Li, and Z. Yu. **2012**. Biodiesel production from rice straw and restaurant waste employing black soldier fly assisted by microbes. Energy. 47: 225–229.

Zhong, Y., M. A. Khan, and F. Shahidi. **2007**. Compositional characteristics and antioxidant properties of fresh and processed sea cucumber (Cucumaria frondosa). J. Agric. Food Chem. 55: 1188–1192.

Zurek, K., and D. Nayduch. **2016**. Bacterial associations across house fly life history: Evidence for transstadial carriage from managed manure. J. Insect Sci. 16: 14–17.

BIOGRAPHY OF THE AUTHOR

Joshua Villazana was born on March 7, 1991 in El Paso, TX. He grew up in Brownsville graduating from James Pace High School in 2009. Joshua earned a B.A. in Biology from the University of Texas at Brownsville (UTB) in 2013. He then attended an internship at Texas Agrilife Extension Services research station in Weslaco, TX where he researched the minute pirate bug and then transferred to Texas A&M University (TAMU) at College Station, TX where he studied for one year to acquire a minor in entomology. While attending TAMU, he worked in the entomology research collection and volunteered in Dr. Raul Medina's research lab assisting a graduate student studying the green stink bug. After graduating, Joshua worked as a lab technician at TAMU conducting toxicology research. Soon after, he was accepted for an internship at Disney World in Orlando, FL at The Land Pavilion in Epcot where he learned greenhouse integrated pest management. He then journeyed north to Virginia for a pest control company, Innovative Pest Management Co. He coordinated control of invasive vegetation at the Accotink Bay Wildlife Refuge in Fort Belvoir, VA, and museum pests at the National Gallery of Art, the Smithsonian Natural history Museum and American History Museum in Washington D.C. He also volunteered at the Smithsonian Natural history museum in the insect zoo in as an insect ambassador. During his time at the University of Maine he found an entomology club named The Maine Entomology Student Organization where he organized collecting trips and outreach events with staff, public, and students. He is also a member of the Maine Entomological Society and Entomology Society of America. Joshua is a candidate for the Master of Science degree in Entomology at the University of Maine in December 2018.