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Role of Abiotic Factors on the Development and Life History of the Black Soldier Fly, Hermetia illucens (L.) (Diptera: Stratiomyidae)

Leslie Holmes
University of Windsor

Role of Abiotic Factors on the Development and Life History of the Black Soldier Fly, *Hermetia illucens* (L.) (Diptera: Stratiomyidae)

By

Leslie Holmes

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ABSTRACT

Hermetia illucens (L.) (Diptera: Stratiomyidae) has been found to successfully reduce manure in confined animal feeding operations of poultry, swine and bovine. In equatorial climates, *H. illucens* is active year round, however, in more temperate climates they are only active during the warmer months of the year. Since insect development is greatly influenced by the ambient environment including pupation substrate, relative humidity, daylength and temperature, it is necessary to explore the effects these abiotic factors have on black soldier fly development when proposing to utilize a species that is not active throughout the year in temperate climates for a year-round waste management process. The objectives for this study include determining the abiotic factors limiting *H. illucens* egg eclosion and adult emergence. The results of this study indicate pupation substrate facilitated or impeded development depending on substrate type as well development improved with increasing temperature, relative humidity and hours of daylight.

There is nothing like looking, if you want to find something. You certainly usually find something, if you look, but it is not always quite the something you were after.

J.R.R. Tolkien

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CHAPTER I
REVIEW OF THE LITTERATURE

Waste management is a worldwide philosophy adopted by most industrialized nations to minimize waste and recover materials through the use of waste prevention, landfills and incineration (Sakai et al. 1996). The public is becoming more aware of our environment and the stress mankind has bestowed upon it, forcing industry to appear more environmentally friendly by optimizing product quality and durability while reducing consumption costs and minimizing the use of raw materials (Sakai et al. 1996). However, despite our efforts in improving waste management such as adopting recycling procedures, development of biological treatment technologies to reduce organic waste and incineration, costs and further environmental damage due to emissions are becoming a prominent concern with the ever-growing municipal solid waste accumulations (Sakai et al. 1996).

Aside from municipal solid waste accumulations, organic wastes, including animal manures, crop residues, food processing wastes, municipal biosolids and some wastes from industry such as those by-products typically from agriculture, are becoming of great concern (Westerman and Bicudo 2005). With the combination of municipal solid waste and organic waste accumulating worldwide as the world's population grows, residents are becoming increasingly concerned with not just the accumulated waste, but also the resulting greenhouse gas emissions they inevitably emit (Neitzert and Steenhof 2008). Greenhouse gas emissions are comprised mostly of carbon dioxide, methane and nitrous oxide. Carbon dioxide emissions are attributed to fossil fuel combustion, deforestation and industrial processes. Methane emissions are expedited by livestock production, rice cultivation, biomass burning, landfills and coal mining, whereas nitrous oxide emissions are accelerated by fertilization in agriculture and the combustion of fossil fuels (Neitzert and Steenhof, 2008).

Similar to the waste management philosophy of reducing/preventing waste, environmentalists' solutions to accumulated organic waste is to use the waste as a resource, such as fertilizer, soil amendment, energy recovery (heat, biofuels, and electricity) and chemical production instead of simply discarding it, creating higher

emissions (Westerman and Bicudo 2005). In addition, increases in regulations governing waste reduction procedures with an emphasis on reducing emissions has resulted in considering alternative treatment strategies such as vermicomposting, utilizing earthworms and anaerobic and aerobic digestion of organic waste (Westerman and Bicudo 2005). Despite these efforts, redirecting organic waste for the use in agriculture is producing equivalent greenhouse gas emissions and becoming a concern in Canada (Neitzert and Steenhof 2008). Canada's greenhouse gas emissions from animal production, crop production and on-farm fuel use accounts for 61%, 29% and 10% of agricultural greenhouse gas emissions, respectively (Neitzert and Steenhof 2008). Since 1990, agricultural animal production has resulted in an 80% increase in greenhouse gas emissions (Neitzert and Steenhof, 2008). Agriculture has always been a necessity for human survival, and as the world population increases, agriculture also increases. However, with agriculture increasing worldwide, the greenhouse gas emissions, particularly from animal production, have increased proportionately as well. Ecologists have struggled with developing methods to sustain the levels of agriculture required for large human populations and yet still protect the environment.

Another alarming contributor to greenhouse gas emissions in Canada are landfill gas emissions with respect to solid waste (Mohareb, Warith and Diaz 2008). Several waste management programs are currently in use to reduce landfill gas emissions, including source reduction, recycling, composting and anaerobic digestion programs. Although these methods have been successful in reducing landfill gas emissions, such methods can be very costly in large scale operations (Mohareb et al. 2008). Mohareb et al. (2008) performed life-cycle assessment modelling on the greenhouse gas emissions in Ottawa, Ontario from its municipal solid waste. They concluded reducing production emissions by using recycled materials instead of new materials in production processes had the greatest impact on reducing greenhouse gas emissions. Incineration, composting and anaerobic digestion respectively were the following categories in descending order impacting greenhouse gas emissions (Mohareb et al. 2008). Such methods had an exceptional impact on current diversion of food wastes, however, incinerating wastes and recycling operations currently in place will experience increased operations costs with greater community participation. Thus, improving the cost effectiveness of the system by

implementing an ecological system that is equipped to manage municipal solid waste diverted to landfills is a priority.

More recently, black soldier flies, *Hermetia illucens* (L.) (Diptera: Stratiomyidae), have been studied in North America and parts of Asia demonstrating their potential use in waste management systems, including large confined animal feeding operations (Sheppard et al. 2002) and bio-conversion in food supplementation (Booram et al. 1977). The black soldier fly is a wasp-like fly distributed worldwide prominently in equatorial tropics (Brammer and von Dohlen 2007). In the southern United States, *H. illucens* have three generations per year; April to November (Sheppard et al. 2002). Black soldier flies usually acclimate to tropical, subtropical and temperate climates, however only during the spring and summer months (April-October). Conversely, it is not yet clear if *H. illucens* enter diapause in environments unbefitting development and overwinter in these temperate zones, disperse to warmer climates, or simply die off.

The black soldier fly develops in decomposing organic material, including decayed fruits and vegetables, detritus and animal waste (Tomberlin, Sheppard and Joyce 2002; Bondari and Sheppard 1987). Females oviposit approximately 320-620 eggs in dry crevices near decaying organic waste (Tomberlin et al. 2002). Studies in the southeastern United States determined *H. illucens* larvae reduce manure by 50% in large confined animal feeding operations, such as swine and poultry (Sheppard et al. 2002). Post-feeding larvae fed animal wastes, such as poultry and swine manure, are composed of approximately 40% protein and 30% fat (Newton et al. 1977), which is a suitable diet for fish (St-Hilaire et al. 2007), as an additive in poultry feed (Hale 1973) and swine feed (Newton et al. 1975). Furthermore, *H. illucens* larvae consume and digest microorganisms, including *Escherichia coli*, thereby potentially reducing harmful bacteria easily transmitted by the house fly (Erickson et al. 2004, Liu et al. 2008). Furthermore, *H. illucens* consumes landfill-type wastes (Diener, Zurbrugg and Tockner 2009; Myers et al. 2008; St-Hilaire et al. 2007; Sheppard et al. 1994; Larde 1990; Bondari and Sheppard 1987; Booram et al. 1977) including carrion (Tomberlin, Sheppard and Joyce 2005, Watson et al. 2000).

Black soldier fly larval development from egg eclosion to the post-feeding larval stage of development takes 19-20d and 17-18d at 27°C and 30°C respectively, with

females generally spending more time feeding than males (Tomberlin, Adler and Myers 2009). Black soldier flies do not feed as adults, using energy reserves stored during the feeding larval stages of development for adult fitness and longevity, however, adult longevity is increased when provided water (Tomberlin et al. 2002). It has also been demonstrated that adults require sunlight stimulation to successfully reproduce (Tomberlin and Sheppard 2002; Zhang et al. unpublished). Aside from temperature, the abiotic and biotic factors affecting *H. illucens* development are not well understood.

Based on the aforementioned studies, employing a waste management system in southern Ontario using black soldier flies to consume organic waste such as animal manure should reduce wastes faster and reduce greenhouse gas emissions, since animal waste production is a large contributor to the agricultural emissions of greenhouse gases (Neitzert and Steenhof 2008). However, the black soldier fly is sensitive to its environment and in ambient conditions, northern latitudes (including southern Ontario), are not conducive for maintaining an active colony on a year-round basis without environmental manipulation. Preliminary observations indicate black soldier flies are sensitive to abiotic factors including temperature, relative humidity and daylength (personal observation). Initial rearing attempts in the fall and winter of 2008 in Windsor, Ontario were unsuccessful, presumably due to low temperatures and low humidities and potentially the influence of shorter daylengths throughout the fall and winter. Eggs and pupae appeared desiccated and did not eclose. Mating was not observed and therefore eggs could not be produced. Thus, it is important to understand the effects these abiotic factors have on *H. illucens* development and their lower developmental thresholds, such that an artificial environment befitting growth and development can be employed during times of the year when *H. illucens* would not normally be active.

Insects rely on abiotic factors to dictate certain life history parameters. Of particular interest are abiotic factors related to water loss and metabolic processes. Insects are ectotherms, relying on their external environment to regulate basic physiological functions including locomotion, development and reproduction (Deutsch et al. 2008). Relying completely on their ambient environment, insects acclimate to environments befitting their physiological structures (McGavin 2001). For example, bees and moths are required to ‘warm up’ their flight muscles before flight take off when temperatures are

cooler (McGavin 2001). An insects' performance is often described by a thermal performance curve with performance rising from a critical minimum temperature to an optimum temperature and dropping rapidly upon a critical maximum temperature (Deutsch et al. 2008). The minimum and maximum temperatures are termed developmental thresholds and when insects are faced with ambient environments beyond their developmental thresholds, development either slows or stops in what is called diapause or dormancy (Delobel 1983).

Diapause can occur during any stage of development (egg, larva, pupa, or adult), but is consistent and specific within a species (Bale and Hayward 2010). Depending on geographic location and latitude, diapause can have different triggers (Andrewartha 1952). For example, seed-sucking insects in Japan use either temperature, photoperiod or both as indicators of seasonal change inducing adult diapause during winters (Numata 2004). Insects adapted to temporal and arctic zones typically use photoperiodism as a cue that winter is on the horizon due to the varying degrees of daylength throughout the year, where as tropical insects near the equator tend to use temperature, population density and food quality as a cue, due to their fairly regular photoperiod all year round (Bale and Hayward 2010). Furthermore, diapause can be induced in the incumbent while exposed to diapausal triggers first-hand, or induced through maternal exposure (Shroyer and Craig 1980). In mosquitoes, the induction of egg diapause depends on the daylength experienced by the female parent. Interestingly, *Aedes triseriatus* (Say) (Diptera: Culicidae) can have diapause repeatedly induced and terminated in the same egg by exposing the egg already in diapause from the female parent to optimal photoperiods, thus terminating diapause, and then exposing the same egg to temperatures unbefitting development, reinstating diapause, until exposure to optimal temperatures (Shroyer and Craig 1980).

In hot environments, due to an insect's large surface to volume ratio and despite their waxy waterproof cuticle of armour, water loss can be quite extreme resulting in desiccation (McGavin 2001). Interestingly, Ramsay (1935) showed that the cockroach's, *Periplaneta americana* (L.) (Blattaria: Blattidae) cuticle layer of lipids undergoes a phase change at temperatures of 30ºC and above, allowing water to pass freely through the cuticle (Ramsay 1935). The transpiration rate of water through the cuticle has been

demonstrated to be species specific and species acclimated to moist soil environments tend to have higher rates of transpiration even at low temperatures than insects acclimated to more arid environments (Wigglesworth 1944). Furthermore, Wigglesworth (1944) determined that the lipid layer of cuticle impermeable to water is superficial and very thin, lying over the surface of the epicuticle. An exception to this discovery is *Calliphora erythrocephala* (Meigan) (Diptera: Calliphoridae), in which the permeability to water is not increased if this lipid layer on the surface of the puparium is broken down. Instead, the increased impermeability to water in the puparium is actually caused by the fragile cuticle of the true pupa within the puparium and the critical temperature of the pupa is much higher than the puparium (Wigglesworth 1944).

Since discovering the insects' physiology responsible for preventing desiccation, behavioural ecologists have been studying the behavioural mechanisms insects employ to prevent desiccation. These behaviours include, but are not limited to, oviposition strategies such as egg clustering versus single egg oviposition, and oviposition site selection, for example oviposition on the moist underside of a leaf or in a protected crevice near the food resource. Using these strategies, Lepidoptera can maintain adequate humidity necessary for growth and development (Clark and Faeth 1998; Courtney 1984; Stamp 1980). Furthermore, holometabolous insects spending a portion of their life cycle on or near their food resource prior to metamorphosis often rely on their pupation substrate for protection from predators, as well as the arid ambient climate (Tsitsipis and Papanicolaou 1979). In addition, arid periods of low relative humidity can cause dormancy in insects (Andrewartha 1952). Substrates vary chemically and physically, providing a more climate controlled environment, often with higher moisture levels, both of which can impact insect survival (Gomes et al. 2007). Thus abiotic factors potentially affecting development of holometabolous insects include, but are not limited to, temperature, relative humidity, daylength and pupation substrates.

Based on my aforementioned preliminary observations when rearing the black soldier fly in a greenhouse in southwestern Ontario during the winter months, the question remains: What effect do these abiotic factors have on black soldier fly development? Before approaching Canada's organic waste accumulations with the black soldier fly approach, it is important to first assess the impact these abiotic factors have on

black soldier fly development before establishing a waste management operation in a country where the natural abiotic factors are not conducive to year-round active black soldier fly development. It is imperative to determine the abiotic factors limiting egg eclosion and adult emergence, specifically, pupation substrate, relative humidity, daylength and temperature. The subsequent chapters of this thesis present the results of my studies measuring the effects these abiotic factors have on black soldier fly development and will aid in determining the feasibility of year-round rearing of *H. illucens* in southwestern Ontario, providing insight into the mass-rearing of black soldier flies to use in waste management industries.

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CHAPTER II

Effect of Pupation Substrate on Adult Emergence

INTRODUCTION

In many holometabolous insects, immature larvae approaching the post-feeding stage of development migrate away from the food resource in search of a suitable place to pupate. They spend little time on the pupation substrate surface before burrowing into the substrate itself (Weston and Desurmont 2008). Post-feeding larvae of many dipteran species including blow flies search for suitable substrates in which to bury themselves for protection from predation and desiccation (Lima et al. 2009). This dispersal is initiated by an innate behavioural sequence and mediated by the abiotic environment including temperature, light and soil moisture, such that the larvae tend to migrate toward cool, dark and dry substrates (Gomes, Godoy and Von Zuben 2006). Larval migration and dispersal can also be impacted by the biotic environment including larval density and the presence of buried larvae and pupae (Boldrini et al. 1997). Similar to Dipteran larval migration, Lepidopteran caterpillar dispersal within the host plant can be influenced by abiotic factors. Light and temperature can significantly affect the microclimate within the cocoon and thus affect the developing caterpillars, for example the position of spun cocoons of *Yponomeuta mahalebella* (Latr.) (Lepidoptera: Yponomeutidae) within the host plant *Prunus mahaleb* (L.) (Rosales: Rosaceae) is significantly correlated to the direction of the sun. Cocoons are spun on the south sides of trees for longer irradiance exposure, creating higher temperatures within the cocoon for optimal growth and development (Alonso 1997).

Insects colonizing patchily distributed resources often contend with a diversity of available pupation substrates. For example, ephemeral resources can be located indoors among upholstery or carpet as an available pupation substrate or outdoors where pupation substrates vary from soil, wood, detritus and grasses etc. The use of different pupation substrates may facilitate or impede adult emergence success and adult fitness. Recently, Rosati et al. (Entomological Society of America proceeding, 2008), demonstrated that the

type of pupation substrate, specifically, playing sand, wood shavings, potting soil and topsoil, impacts the length of the post-feeding stage of development within and between tribes of blow flies. Furthermore, there was evidence that pupation substrate has an effect on successful adult emergence and adult fitness. In the Viburnum Leaf Beetle, *Pyrrhalta viburni* (Paykull) (Coleoptera: Chrysomelidae), Weston and Desurmont (2008) determined that post-feeding larvae spend only a few minutes on the pupation substrate surface before burying into it for pupation. They further observed that pupation substrate did not significantly affect the depth of pupation, but that substrate type (sand, soil and sand and soil mixed) can have significant effects on pupation success with respect to interactions of temperature and moisture content (Weston and Desurmont 2008).

The black soldier fly, *Hermetia illucens* (L.) (Diptera: Stratiomyidae) is a temperate and tropical species that develops in decomposing material with three generations per year in the southeastern United States (Tomberlin et al. 2002). Adult females oviposit approximately 320-620 eggs in dry crevices near decaying organic waste with egg eclosion occurring in approximately 4 days (Tomberlin et al. 2002). Feeding on decaying organic waste, black soldier fly larvae average 22d in the feeding stages of development (Tomberlin et al. 2002), with temperature dependent pupation averaging 15-17d and temperature dependent adult longevity ranging from 12-17d (Tomberlin et al., 2009). Upon feeding completion, post-feeding larvae search for an ideal pupation site, often in a dry, shaded area away from the food resource (Lima et al. 2009).

Hermetia illucens' habitat is even more versatile and patchily distributed compared to blow flies, developing on ephemeral resources with various organic compositions including animal waste, spoiled fruits and vegetables, fish rendering waste and carrion (Diener, Zurbrugg and Tockner 2009; Liu et al. 2008, Myers et al. 2008; Pujol-Luz et al. 2008; St-Hilaire et al. 2007; Tomberlin; Sheppard and Joyce 2005; Erickson et al. 2004; Tomberlin, Sheppard and Joyce 2002; Lord et al. 1994; Sheppard et al. 1994). Their habitat may differ drastically depending on the type of resource consumed and the type of substrate serving as a habitat, which can facilitate or impede larval development (Tomberlin et al. 2005; Tomberlin et al. 2002; Lord et al. 1994). Aside from temperature, the ambient environmental effects on the post-feeding stages of development, including the post-feeding, pupal and adult stages, is not well understood

and due to the importance of abiotic factors on pupation and fitness I considered these factors in the current study by examining pupation substrates with different properties with respect to moisture content and compaction.

Of particular interest in this study was the composition of the pupation substrate and its effect on the time spent in the post-feeding and pupal stages of development. The primary objective of this study was to compare pupation substrates for use in rearing practices to optimize *H. illucens* development by increasing pupation and adult emergence success. I predicted that the presence and type of pupation substrate would have a significant impact on the time spent in the post-feeding and pupal stages of development, with pupation substrates with less compaction and higher moisture content facilitating pupation.

METHODS

Experiment: Adult Emergence

Source of Larvae

Eggs were collected from a *H. illucens* colony housed in a screen cage (1.8 x 1.8 x 1.8m with 1.5mm mesh) maintained in a greenhouse on the roof-top of the Biology Department building located at the University of Windsor, Windsor, Ontario. The greenhouse was maintained on a 14:10 [L:D] photoperiod, at ambient relative humidity (20-50%) and varied temperatures (20-40°C), depending on ambient sunlight. The colony was established in October 2008 from multiple shipments of immature *H. illucens* distributed by Phoenix Worms®; a self-sustaining colony maintained without wild population supplementation established in Tifton, Georgia by Dr. Craig Sheppard in 2002 (Sheppard, personal communication). Eggs were collected in a 1-layer, 2 x 5cm roll of corrugated cardboard, held together with 3M® masking tape with 3 x 4mm flutes (tubular holes in cardboard) used as an oviposition substrate, taped with the flutes positioned vertically, perpendicular to the substrate 5cm above the oviposition medium. Oviposition medium was composed of moist-to-liquefied 18% protein PURINATURE LAYENA® poultry laying chow (code # 6498: corn meal, soybean meal, wheat, canola meal and pork meal).

Larval Development

Eggs were collected in the corrugated cardboard for 4 hours and placed into a 946mL clear plastic Gladware® container (The Clorox Company of Canada, Brampton, Ontario) covered with a laboratory paper towel and maintained in a growth chamber at 28°C, 70-80% RH and 14:10 [L:D] photoperiod until egg eclosion. A Hobo® data logger was placed in the growth chamber to record temperature every 30 minutes throughout the duration of the experiment. Upon egg eclosion, larvae were fed 20g of dry poultry laying chow mixed with 42mL of water for 70% moisture content (as demonstrated by Nguyen et al, unpublished) *ad libitum* for four days to allow larvae to develop to a more manageable size. Four-day-old larvae were then transferred into 0.5L mason jars with a layer of black landscaping cloth as a breathable lid held in place by the hollow metal

screw cap to prevent larvae from escaping at a density of 300 larvae per jar (Tomberlin et al. 2002). Feeding continued *ad libitum* using the aforementioned ratio of dry feed to water volume until larvae reached the post-feeding stage, identified by the larvae changing colour from cream to black (Tomberlin et al. 2005).

Treatments and Sampling

Post-feeding larvae were removed from their food and divided into five pupation substrate treatments consisting of: wood shavings (NEPCO Beta chip, hardwood laboratory bedding, New York, USA), topsoil (Maidstone Garden Products, Maidstone, Ontario), potting soil (Premier Pro-Mix BX, Essex, Ontario), playing sand (Canadian Tire®, Windsor, Ontario) and the absence of a pupation substrate. Pupation substrates were chosen based on current lab practices such as the use of wood shavings (the laboratory standard) and previous research on the effect of pupation substrate on blow fly development (Rosati et al., Entomological Society of America proceedings, 2008). The wood shavings used were a virgin hardwood blend beta chip (maple, beech, birch or poplar), (NEPCO, Warrensburg, NY). The topsoil used contained nitrogen, phosphorus, potassium, calcium, magnesium and sulphur. The potting soil used contained Canadian sphagnum peat moss (80-85%/vol), Endomycorrhizae (fungi symbionts), perlite (to increase air space and water drainage), vermiculite (to hold water and fertilizer), dolomitic calcitic limestone (to regulate pH), macronutrients (a proprietary composition of nitrogen, phosphorus and potassium), micronutrients (a proprietary composition of calcium, zinc and magnesium) and a wetting agent. The sand used was pre-washed and contained < 3% crystalline silica.

Five replicates of 100 post-feeding larvae per replicate were placed into clear plastic 2.2L Ziplock® tubs, (SC Johnson Canada, Brantford, Ontario) with their corresponding lids and 15cm depth of their respective pupation substrate treatments. The centers of the lids were removed and replaced with black landscaping cloth (Home Depot, Windsor, Ontario) between tub and lid to allow air exchange and prevent wandering post-feeding larvae from escaping. Fifty millilitres of water was added to each pupation substrate, with the exception of the absence of a pupation substrate treatment, prior to the addition of larvae to maintain moisture. Five pupation substrates were tested with five replicates, for a total N = 25.

Post-feeding larvae were monitored every 12h to record time of pupation and time of adult emergence for each larva. Upon pupation, pupae were placed into 100mm x 25mm Fisherbrand® Petri dishes (Fisher Scientific Company, Ottawa, Ontario) with a layer of their respective pupation substrate on a piece of moist filter paper (Fisher Scientific Company, Ottawa, Ontario) to prevent desiccation. Petri dishes of pupae were returned to the growth chamber, with continuous 12h observations until adult emergence. The amount of time in the post-feeding and pupal stages of development as well as larval mortality was recorded.

Upon adult emergence, adults from each pupation substrate were released twice daily until all adults emerged into a 1 x 1 x 1.8m cage with 1.5mm mesh corresponding to their respective pupation substrate, for a total of five cages, one cage for each pupation substrate. The five cages were housed in the same greenhouse as the colony and arranged in a random design over a spatial area of approximately 3 x 4.5m. Tobacco plants, 15-18cm tall, with 4-5 large leaves per plant, were placed in the centre of each cage for greater surface area to facilitate male lekking behaviour (Sheppard et al. 2002, Tomberlin and Sheppard 2001). Cages were misted with water every other hour between 0800h and 2100h to ensure maximum longevity of the adult flies. A Hobo® data logger was placed away from direct sunlight, in the centre of all five cages to take temperature readings every 30 minutes.

Upon the first adults to emerge, adults in each of the five cages were monitored for ten minutes every hour between 8am and 5pm to record the elapsed time to copulation and the number of observed mating pairs (male and female *en copula*). Upon the first observation of copulation, fresh moist-to-liquefied PURINATURE LAYENA® poultry laying chow (code # 6498: corn meal, soybean meal, wheat, canola meal and pork meal) with four 2 x 5cm rolled corrugated cardboard squares as an oviposition substrate, was provided daily in each cage to collect eggs and determine female fecundity for each pupation substrate. However, due to limited available space in the greenhouse and another study taking place in close proximity during the last few days of this experiment (days with oviposition events) the females became over stimulated and began to oviposit on the crevices of the mesh cage instead of within the corrugated cardboard flutes and therefore, quantification of actual oviposition was omitted from analysis. Oviposition was

instead quantified on the basis of the number of mated females in my study and an extrapolation from the results obtained in a previous study by Tomberlin et al. (2002), where it was determined that a mated female could potentially oviposit egg clusters weighing 0.0158g±0.00012g. Furthermore, dead adults were collected from each cage daily to determine the sex ratio as well as an average daily population number.

Abiotic Factors Recorded

Post-feeding larvae and pupae remained in the growth chamber until adult emergence, with the exception of short periods of time when they were removed for progress checks (every 12 h, for not more than 1 h). A Hobo® data logger recorded the temperature within the growth chamber every 30 min and the mean temperature recorded was 26.53±0.03°C. Adults were released into their respective treatment cages upon emergence in the greenhouse. A Hobo® data logger was placed in a shaded area central to the experiment cages and recorded temperature every 30 min with a resulting mean temperature of 30.11±0.18°C.

Relative humidity was not recorded via data loggers, however output readings on the growth chamber read 70-80% throughout the course of the experiment. Daylength was maintained with lights programmed to turn on at 0700h and turn off at 2100h.

Statistics

Software:

All statistics were computed using SAS JMP® version 8.0.1 statistical software, with the exception of nonparametric two-way Kruskal-Wallis tests, which was conducted using SPSS version 14.0 statistical software and the pairwise post hoc analyses was completed using SPSS version 18.0 statistical software by Dan Edelstein, University of Windsor, Academic Data Centre.

Post-Feeding and Pupal Development:

The data failed to meet the assumptions of normality (homogeneity of variance and normality goodness-of-fit) despite data transformation attempts ($\log_{10}$ and square root transformations). Thus, a non-parametric one-way Wilcoxon/Kruskal-Wallis (Ranked Sums) was used to test both treatment effects on the mean time to pupation as well as the

mean time to adult emergence, independently. Pair-wise comparisons using Wilcoxon/Mann-Whitney U tests were conducted for both effects on post-feeding and pupal development. Alpha values were adjusted using the Dunn-Sidak procedure to correct for type I error as a result of multiple comparisons (Quinn and Keough, 2002).

Successful Adult Emergence:

A one-way analysis of variance (ANOVA) with the Tukey-Kramer HSD to compare means was used to test the statistical significance of treatment effects on percent successful adult emergence. Adult emergence was counted and analyzed as proportions of total successful pupae, performing an arcsine transformation prior to analyzing with ANOVA. The number of pupae that failed to emerge were also counted and analyzed as proportions based on total successful adult emergence and was analyzed with an ANOVA after performing a $\log_{10}$ transformation. A contingency likelihood ratio test was used to test the statistical significance of treatment effects on the sex ratio.

Adult Survivorship:

A non-parametric two-way Kruskal-Wallis (Ranked Sums) was used to test significance on a full factorial design (Factors: pupation substrate (treatment), date and the interaction of pupation substrate and date) for the proportion of living adults recorded daily as well as the proportion of adult mortalities recorded daily. Examining both the proportion of living adults separately from the proportion of adult mortalities was necessary due to a number of escaped adults that could not be accounted for, but not included in the death toll. Results from these analyses demonstrated a lack of significant interaction (Treatment*Date) for both proportion of living and dead adults. Due to the inability to perform post-hoc analyses for a two-way Kruskal-Wallis test in JMP, or SPSS, individual one-way Kruskal-Wallis tests were completed for the effect of date and treatment on both, the proportion of living and dead adults. Post-hoc pairwise comparisons were adjusted for type I error to interpret significant results as previously described.

RESULTS

Experiment: Adult Emergence

Post-feeding Development

The effect of pupation substrate on the mean time to reach the pupal stage varied between pupation substrates (Table 2.1, Figure 2.1, $\chi^2 = 253.69$, $df = 4$, $p < 0.001$), with post-feeding larvae in the no medium treatment taking longer to pupate than all other substrates. There was no difference in the length of the post-feeding stage between larvae placed in sand or topsoil. Also, post-feeding larvae in potting soil and shavings did not differ, however, post-feeding larvae in both sand and topsoil took longer to reach pupation than post-feeding larvae in potting soil and shavings.

Pupal Development

Pupae in the no medium treatment took less time to eclose than any of the other treatments (Table 2.2, Figures 2.2, $\chi^2 = 27.97$, $df = 4$, $p < 0.001$) and there were no differences between the remaining four treatments in the amount of time required to complete the pupal stage of development.

Successful Adult Emergence

The no medium treatment had fewer adults emerging than any of the other pupation substrates (Figure 2.3, $F_{4,20} = 4.62$, $p = 0.0008$). Retrospectively, no medium also had higher pupal mortalities than any other pupation substrate ($F_{4,20} = 4.25$, $p = 0.0119$). Adult mortalities were collected daily and sexed. The following sex ratios were recorded for each pupation substrate, no medium, potting soil, sand, shavings and topsoil, 208:178, 195:257, 247:201, 141:219 and 216:221 females to males, respectively. An overall sex ratio of 48.29% females to 51.71% males was not different, ($\chi^2 = 2.42$, $df = 1$, $p = 0.1196$). However, pupation substrate had an effect on sex ratio (Table 2.3, $\chi^2 = 29.975$, $df = 4$, $p < 0.001$), with a male bias in potting soil and wood shavings, and no bias in the remaining treatments.

Adult Survivorship

The proportion of living adults per day for each treatment calculated by the number of emerged adults to date, minus the number of adult mortalities per day and divided by the total number of successful emerged adults per treatment, differed by date (Appendix A: Table A.1, χ^2= 94.717, df = 27, p < 0.0001) with few adults when they initially began to emerge, a gradual increase to a peak number of adults and then a decline. Treatment influenced the proportion of living adults per day (Table 2.4, Figure 2.4, χ^2= 24.385, df = 4, p = 0.0001), however, there was no interaction between date and treatment. Wood shavings had higher proportions of living adults per day than all other treatments. The proportion of living adults from wood shavings lived longer and established a larger population over a longer period of time than the rest of the pupation substrates. The remaining pupation substrates did not vary in the proportion of living adults.

The proportion of adult mortalities per day for each treatment, calculated by the number of adult mortalities per day, divided by the total number of adult emergences per treatment, also differed by date (Appendix A: Table A.2, Figure 2.4, χ^2= 112.773, df = 27, p < 0.0001), with proportions of adult mortality higher on 30 March 2009 than the first and last three days of the experiment, however, treatment and the interaction treatment*date did not influence the proportion of adult mortalities per day. Through daily observations, it appears that adults in sand and potting soil tended to die shortly after emergence, while adults in topsoil, wood shavings and unexpectedly, no medium tended to live longer, dying several days after emergence.

Adult Mating and Oviposition Trends

Adults in the no medium treatment were first to be observed mating, followed by wood shavings, potting soil, topsoil and sand respectively (Figure 2.5). Also, there were more pairs observed mating in the no medium treatment, followed by shavings, topsoil, potting soil and sand respectively (Figure 2.6). Based on the data presented in Tomberlin et al. (2002) individual mated females oviposit between 206 and 639 eggs, using this data and the total number of mating females observed in this experiment, the total number of eggs that could potentially have been oviposited per pupation substrate, no medium,

shavings, topsoil, posting soil and sand was, 22866- 70929, 19570 - 60705, 13802 - 42813, 9270 - 28755 and 1236 - 3834, respectively.

In addition, temperature was lowest in the morning (28°C), quickly rising to 30°C by 1000h and remained between 32°C and 36°C the remainder of the day. As temperature increased above 30°C, the number of observed mating pairs also increased. A bimodal trend in mating was evident with a peak in mating at 1000h and again at 1400h, with less mating between 1000h and 1400h. The first peak of mating occurrences at 1000h corresponded to a spike in solar intensity to over 500 $\mu mol \bullet m^{-2} \bullet s^{-1}$ and the second peak of mating occurrences corresponded to solar intensities closer to 1000 $\mu mol \bullet m^{-2} \bullet s^{-1}$ (Figure 2.7).

DISCUSSION

Two factors I observed differing the most among my selected substrates were moisture content and compaction, which I suspect may have impacted post-feeding larval dispersal and pupation. Substrate compaction can have significant effects on a post-feeding larva's ability to pupate, such that higher compaction densities may be impenetrable and may lack sufficient free oxygen for post-feeding larvae to survive. Dimou et al. (2003) examined the effects soil type and compaction on pupation depths of the wild olive fruit fly, *Bactrocer*a *oleae*, (Gmel.) (Diptera: Tephritidae). They found that limestone soils, which had the lowest density when compared to higher density soils (alluvial and flysch), resulted in deeper soil penetration at pupation (Dimou et al. 2003). It was suggested that low density compaction attributed to either large porous spaces or small specific gravity of particles providing easier penetration (Dimou et al. 2003). In my experiment none of the pupation substrates used were mechanically compacted during the course of the experiment. However, each substrate had a different natural compaction density, although this was not measured. It was observed qualitatively that sand had the highest compaction density with very little free space between sand aggregates, followed by topsoil, wood shavings and potting soil. Based on my results, post-feeding larvae in lower compaction density substrates (wood shavings and potting soil) took less time to pupate than those in high compaction density substrates (sand and topsoil), suggesting that low density substrates are more penetrable, facilitating pupation, where as high density substrates are less penetrable for post-feeding larvae and therefore impede pupation.

Moisture content of the pupation substrate can also have significant impact on pupation and emergence success. Of the substrates used in this experiment, topsoil and potting soil retained water better than sand and wood shavings. This was observed at the beginning of the experiment when topsoil aggregates formed as a result of adding water. As well, the perlite and vermiculite aggregates in the potting soil enabled it to retain moisture longer than all other substrates. Studies on the olive fruit fly and the oriental fruit fly, *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae), examined the effects of moisture on pupation depths and found that wet soils supported larval pupation versus

dry soils, regardless of soil type (Chen and Shelton 2007; Ellis et al. 2004; Dimou et al. 2003; Alyokhin et al. 2001). Alyokhin et al. (2001) demonstrated using a choice design (wet versus dry soils) that larvae of the oriental fruit fly preferred moist soils to dry soils, regardless of soil type. Although the effect of moisture on pupation depth was not an objective in this study, it is evident that moisture plays a role in substrate infiltration by the post-feeding larvae and therefore may facilitate or impede pupation.

Tsitsipis and Papanikolaou (1979) (as referenced in Dimou et al., 2003) noted that substrates containing sand hindered larval penetration of the wild olive fruit fly, causing shallow pupation depths which is also consistent with my findings. The presence of moist sand in the first few days of the experiment was not an issue for post-feeding larval dispersion, however days later when the sand dried out post-feeding larvae did not penetrate the sand and were observed instead on the surface of the sand during each 12h observation check. It is important to note, that although all larvae were visible on the surface of the sand, each tub of sand containing post-feeding larvae was dumped at each check point like all other treatments for consistency and therefore, larvae were mechanically buried as a result. This was consistent across all treatments and replicates. Furthermore, despite mechanical burial, larvae in the sand treatments resurfaced and pupated on the surface of the sand.

Post-feeding larvae took the longest to pupate in the absence of a pupation substrate, as expected. A likely explanation for this is the larvae's innate behaviour strategy to bury itself for pupation as a protection mechanism from predators and desiccation. In the absence of a pupation substrate, post-feeding larvae proceeded to disperse in what is assumed a search for a substrate to bury themselves in, which appeared to delay their pupation. Furthermore, post-feeding larvae in the absence of a pupation substrate aggregated together (personal observation). This behaviour may have been an effort to increase the temperature, due to the lack of a pupation substrate to serve as an abiotic barrier blanket, or perhaps a thigmotatic response in an effort to try to bury themselves in each other. However, when post-feeding larvae pupated, pupae were dispersed throughout the tub, while post-feeding larvae still remained aggregated in corners.

Pupae in the absence of a pupation substrate completed the pupal stage and emerged faster than those in the presence of a pupation substrate regardless of the type of substrate. In addition, there was higher pupal mortality in the absence of a pupation substrate. This is not surprising since the post-feeding larvae in the absence of a pupation substrate took longer to pupate. One theory that can explain this result is energy depletion in the post-feeding stage of development due to metabolism. Insects that do not feed as adults, like the black soldier fly, have a limited amount of stored energy that is allocated to adult fitness including, mating, flight and ovarian development (Nilssen 1997).

Holometabolous insects in general have varying degrees of energy expenditures due to their very different developmental stages (Schmolz and Lamprecht 2000). Schmolz and Lamprecht (2000) outlined heat production as a result of catabolism and metabolism in holometabolous insects, including the mealworm, *Tenebrio molitor* (L.) (Coleoptera: Tenebrionidae), the greater wax moth *Galleria mellonella (*L.*)* (Lepidoptera: Galleriidae) and the honeybee *Apis mellifera* (L.) (Hymenoptera: Apidae). They noted that the most energy expensive period in development is the last larval stage, the post-feeding larval stage, in which metabolism occurs as opposed to catabolism in the feeding larval stages. When larvae moult into their last larval stage, they continue to feed and energy is stored through the process of catabolism. Under optimal conditions, energy expenditure is lowest in the post-feeding stage of development right before pupation and does not increase again until metamorphosis begins (Schmolz and Lamprecht 2000). Post-feeding larvae in the absence of a pupation substrate spend significantly more time searching for a pupation site than when under optimal conditions and as a result may have exhausted valuable energy resources needed for metamorphosis and therefore could not successfully emerge. It is worth noting however, that pupal dissections were not completed to verify this hypothesis of energy depletion. Furthermore, pupae in the no medium treatment did complete metamorphosis hours before pupae in all other treatments. Why this occurred is not well understood.

Although it was not statistically analyzed, adult longevity and fecundity trends between pupation substrates varied immensely. Adult mortality was recorded daily from each treatment cage. The March 30th date had higher adult mortalities than earlier dates and later dates because it was near the middle of the experiment, when a majority of

adults were dying after living 7-10 days which is the typical lifespan of adult *H. illucens* in colony provided water (Tomberlin et al. 2002). March 20th, 21st and 22nd were the first few days of the experiment and therefore, overall mortality was low due to adults having just emerged. April 13th, 14th and 15th were the last few days of the experiment and therefore population numbers were dwindling and mortality was also very low as a result.

Unexpectedly, I observed adults in the sand and potting soil treatments dying within a few days of emergence, with adults in the potting soil living slightly longer than those in the sand. Because adults died within a few days of emergence, the population rarely reached high enough numbers to support mating and reproduction. Female black soldier flies typically mate 2d after emergence and are ready to oviposit approximately 2d after mating (Tomberlin et al. 2002). Because of this process of adult maturation and ovarian development, mating and oviposition requires female longevity to surpass 4d. With few exceptions, females in the sand treatment died within a day or two of emergence, and therefore even those females that lived long enough to mate, died before oviposition could occur. It is also possible that because males typically emerge 1-2d before females, as the females emerge, the males have already died and therefore males were not available to the females for mating. Similarly, adults in the potting soil treatment died shortly after emergence, however, several pairs managed to survive long enough to mate successful, far more than those in the sand treatment.

It is not well understood why adults in the sand substrate died suddenly after emergence. Adults appeared dehydrated upon emergence with structures appearing dusted and brittle. As mentioned, the larvae in the sand treatment pupated on the surface of the sand once the sand dried, but were mechanically buried during each 12h check for consistency across treatments. It is likely that energy reserves allocated for metamorphosis and adult longevity were used up during the post-feeding stage of development as larvae repeatedly resurfaced after each mechanical burial. Another possible scenario is that while manoeuvring through the sand to resurface, the waxy layer of the cuticle may have been damaged by the crystalline silica in the sand and therefore, the same waxy layer on the adults may have been damaged facilitating water loss. Through rearing practices, it was observed that defects in larval segments resulted in defects in the corresponding anatomy of the adult fly (personal observation).

Nilssen (1997) studied the effects of temperature and relative humidity on weight loss, fat loss and adult longevity and fecundity on the reindeer oestrid flies *Hypoderma tarandi* (L.) (Diptera: Oestridae) and *Cephenemyia trompe* (Modeer) (Diptera: Oestridae) and noted that reduced longevity that was not a result of fat loss, could be instead due to water loss. Furthermore, studies on the swede midge, *Contarinia nasturtii* (Kieff.) (Diptera: Cecidomyiidae), found that pupation in extremely dry substrates hindered adult emergence (Chen and Shelton 2007). It has also been found that small hive beetles, *Aethina tumida* (Murray) (Coleoptera: Nitidulidae), pupating in dry soils fail to emerge as adults (Ellis et al. 2004). Black soldier fly larvae have a fairly robust cuticle that is quite leathery in appearance such that the final larval stage moults can be mistaken as larvae at first glance and therefore further studies would be necessary to verify structural damage to the cuticle resulting in short-lived adults as was observed in the sand treatment. It is not clear why many of the adults in the potting soil died shortly after emergence. However, it is possible that mortality may have resulted from unknown effects perlite or vermiculite (within the potting soil matrix) may have on the pupae's ability to retain water. Therefore, as hypothesized earlier with adults in the sand, emerged adults desiccate as a result of insufficient water retention as a pupa.

Adults in the remaining pupation substrates (topsoil, shavings and no medium) had higher success in terms of longevity than those in the sand and potting soil substrates, however some interesting trends are also apparent. Topsoil pupation substrate adults were intermediate in terms of mortality trends when compared to sand and potting soil, and wood shavings and no medium. The lower number of mating pairs in topsoil when compared to wood shavings and no medium can be attributed to the lower proportion of living adults due to broader mortality trends with some mortality occurring within two to three days of emergence to several days after adult emergence. It is unclear why many adults in the topsoil substrate died shortly after emergence and it may also have been a result of water loss and failure to retain water since the topsoil dried out significantly to a dusty powder several days into the experiment. In addition it may simply be natural mortality that exist in every natural population.

Adults reared in wood shavings and no medium had the longest longevity trends, with higher proportions of living adults per day, which allowed them to establish

populations sufficient to support mating and oviposition. Although wood shavings had significantly higher proportions of living adults per day, population and mating trends were similar between shavings and no medium, with slightly more mating pairs in the no medium treatment. Interestingly, with fewer proportions of living adults per day, adults in the no medium treatment mated more successfully than those in the wood shavings. This may be explained perhaps by the population on a spatial scale in the wood shavings treatment being too high to support optimal mating. Tingle et al. (1975) (as referenced by Sheppard et al. 2002), noted that no mating or oviposition of the black soldier fly occurred in small cages (53 x 91 x 53cm and 38 x 46 by 38cm). Furthermore, Sheppard et al. (2002) confirmed that mating and oviposition was evident in larger cages (2 by 2 by 4m), and with respect to our colony housed at the University of Windsor, successful mating and oviposition is evident in a similar sized cage (1.8 by 1.8 by 1.8-m). However, the density threshold on a spatial scale for *H. illucens* within these cages has not been determined and therefore, with wood shavings having more adults than no medium in the same spatial habitat on any given day, competition in the wood shavings treatment may have increased enough to reduce mating. That said, further studies on the effects of density on mating and oviposition are pertinent.

The trends observed in this study indicate that overall mating patterns may correspond to solar light intensity and temperature in relation to time of day. Zhang et al. (unpublished) studied artificial light sources for rearing black soldier flies and observed that most mating, both indoors (artificial lights) and outdoors (sunlight) occurred in the morning (before 1100h) with very few mating pairs in the afternoon. It was also observed that mating did not occur below light intensity levels of 70 $\mu mol \bullet m^{-2} \bullet s^{-1}$. Similarly, although light intensity levels were not observed below 76 $\mu mol \bullet m^{-2} \bullet s^{-1}$ in this study, I did observe mating below 145 $\mu mol \bullet m^{-2} \bullet s^{-1}$, regardless of time of day.

In addition, the sex ratio may affect mating and oviposition and pupation substrate may not solely be the cause of these biased sex ratios since biased sex ratios have been reported in previous *H. illucens* studies (Tomberlin et al. 2002). Post-feeding larvae were hand selected randomly and placed into their treatments and therefore it is possible, I may have inadvertently biased the sex ratio. Furthermore, not all adults could be sexed since

some escaped and others suffered from anatomical malformations and therefore sex could not be determined with certainty.

In summary, the presence of a pupation substrate has a significant impact on facilitating post-feeding larvae to pupate, regardless of the type of substrate. Taking this into consideration, the type of substrate may have positive or negative effects on adult longevity and fitness. In the absence of a pupation substrate, post-feeding larvae are slightly delayed from pupation, prolonging their post-feeding stage of development, however, adult longevity is not as negatively impacted as post-feeding larvae developing in other pupation substrates, including sand and potting soil. For the purpose of our objective, wood shavings, the current laboratory standard for blow fly rearing would be considered more feasible for mass rearing, with efficient pupation and increased successful adult emergence.

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TABLES

Table 2.1

Wilcoxon / Kruskal-Wallis Tests: Ranked Sums test results comparing mean time post-feeding larvae took to reach the pupal stage of development between the pupation substrates, no medium, potting soil, sand, wood shavings and topsoil. Dunn-Sidak adjusted $\alpha = 0.0051$

Treatments	N	Z	ChiSquare (x102)	Significance
No Medium Potting soil	486 491	13.93	1.94	p<0.0001*
No Medium Sand	486 483	-9.20	0.85	p<0.0001*
No Medium Shavings	486 475	-12.84	1.64	p<0.0001*
No Medium Topsoil	486 491	11.30	1.28	p<0.0001*
Potting soil Sand	491 483	4.17	0.17	p<0.0001*
Potting soil Shavings	491 475	-0.89	0.01	p=0.3713
Potting soil Topsoil	491 487	3.24	0.10	p=0.0012*
Sand Shavings	483 475	-4.42	0.19	p<0.0001*
Sand Topsoil	483 487	1.10	0.012	p=0.2712
Shavings Topsoil	475 487	-3.69	0.14	p=0.0002*

Table 2.2

Wilcoxon / Kruskal-Wallis Tests: Ranked Sums test results comparing the length of time spent in the pupal stage of development between pupation substrates, no medium, potting soil, sand, wood shavings and topsoil. Dunn-Sidak adjusted $\alpha = 0.0051$

Treatment Interactions	Z	ChiSquare	Significance
Potting soil*Sand	1.49	2.2339	p = 0.1350
Potting soil*Topsoil	0.83	0.6859	p = 0.4076
Potting soil*No Medium	-4.31	18.6088	p < 0.0001*
Potting soil*Shavings	-0.22	0.0473	p = 0.8278
Sand*Topsoil	-0.70	0.4870	p = 0.4853
Sand*No Medium	-3.04	9.2533	p = 0.0024*
Sand*Shavings	1.87	3.5066	p = 0.0611
Topsoil*No Medium	-3.67	13.4502	p = 0.0002*
Topsoil*Shavings	1.18	1.4011	p = 0.2365
No Medium*Shavings	-4.73	22.3514	p < 0.0001*

Table 2.3

Contingency Likelihood Ratio Tests: Results comparing pupation substrates, no medium, potting soil, sand, shavings and topsoil between sex ratios. Dunn-Sidak adjusted $\alpha = 0.0253$

Treatments	Sex Ratio (% Females)	ChiSquare	Significance
No Medium	53.64	2.04	p = 0.1529
Potting Soil	43.14	2.53	p = 0.0035 *
Sand	55.13	4.73	p = 0.0296
Shavings	39.17	17.04	p < 0.0001 *
Topsoil	49.43	0.06	p = 0.8110

Table 2.4

Wilcoxon / Kruskal-Wallis Tests: Ranked Sums test results comparing the proportion of living adults recorded daily between pupation substrates, no medium, potting soil, sand, shavings and topsoil. Dunn-Sidak adjusted $\alpha = 0.0051$

Treatment Interactions	Z	ChiSquare	Significance
Potting soil*Sand	-1.173	-12.714	p=0.241
Potting soil*Topsoil	-1.243	-13.464	p=0.214
Potting soil*No Medium	1.608	17.429	p=0.108
Potting soil*Shavings	-4.669	-50.589	p<0.001*
Sand*Topsoil	-0.069	-0.750	p=0.945
Sand*No Medium	0.435	4.714	p=0.664
Sand*Shavings	-3.495	-37.875	p<0.001*
Topsoil*No Medium	0.366	3.964	p=0.714
Topsoil*Shavings	3.426	37.125	p=0.001*
No Medium*Shavings	-3.060	-33.161	p=0.002*

FIGURES

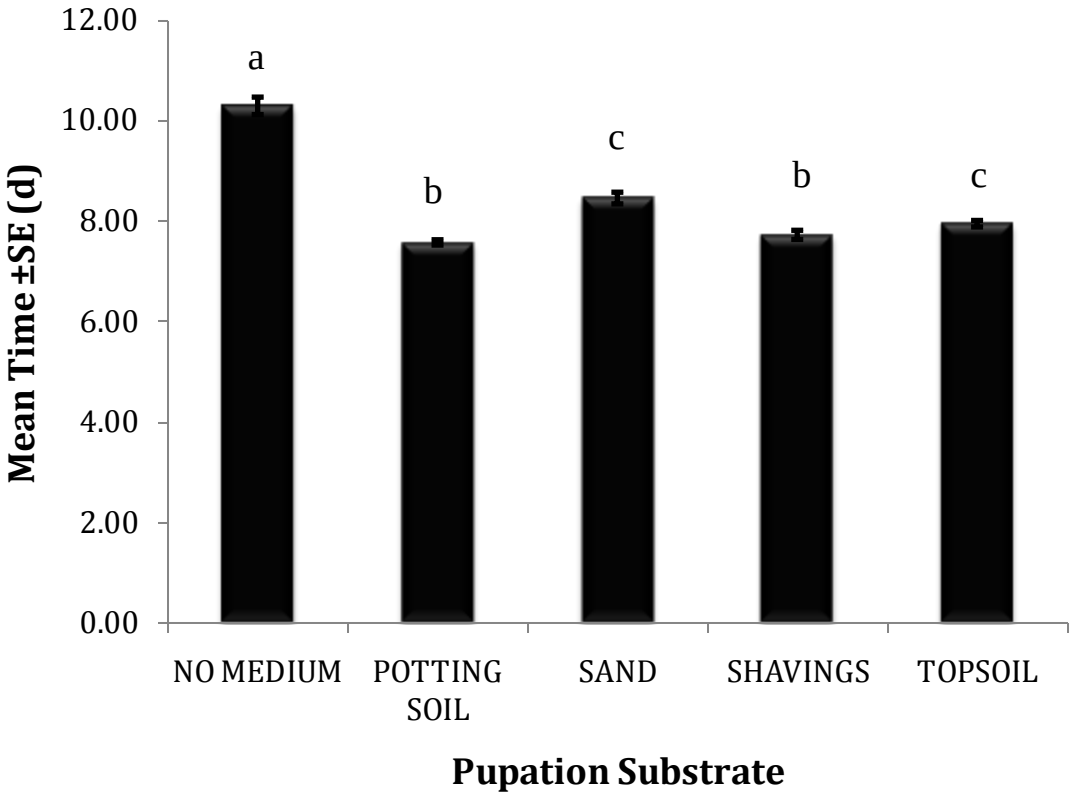

Figure 2.1

Mean (±SE) time spent in the post-feeding larval stage of development for the pupation substrates, no medium, potting soil, sand, shavings and topsoil. Means followed by the same letter are not different (P>0.05).

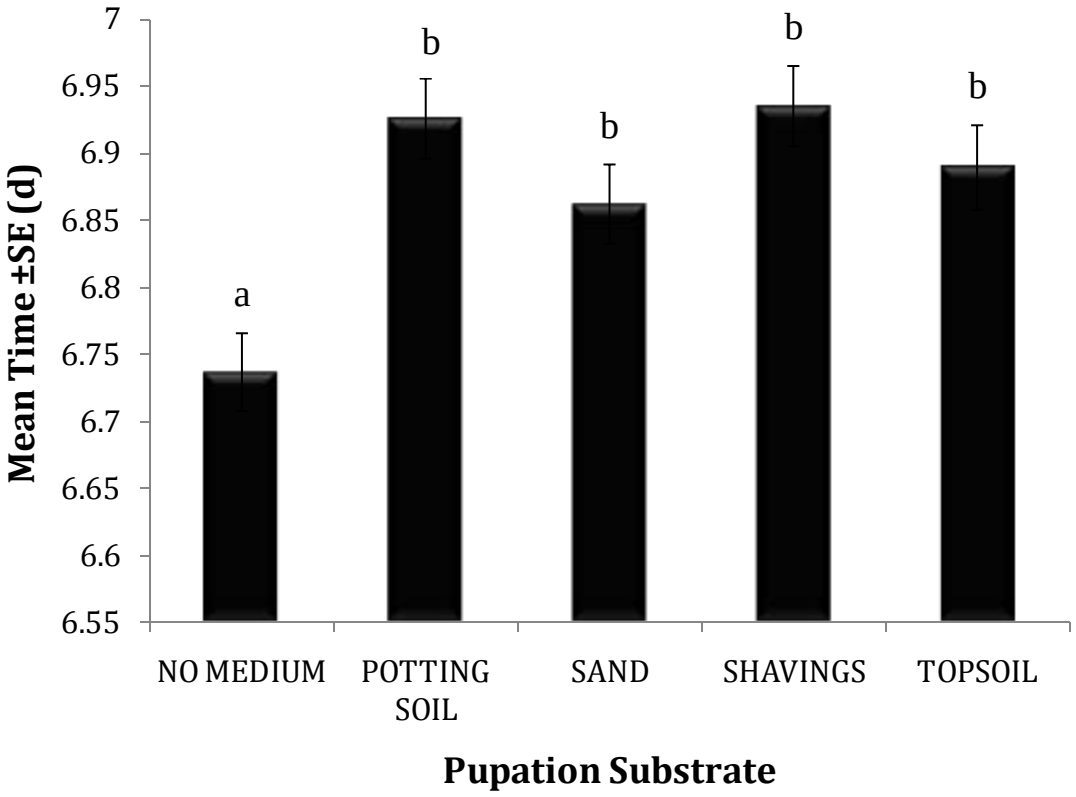

Figure 2.2

Mean (±SE) time spent in the pupal stage of development for each pupation substrate, no medium, potting soil, sand, shavings and topsoil. Means followed by the same letter are not different (P>0.05).

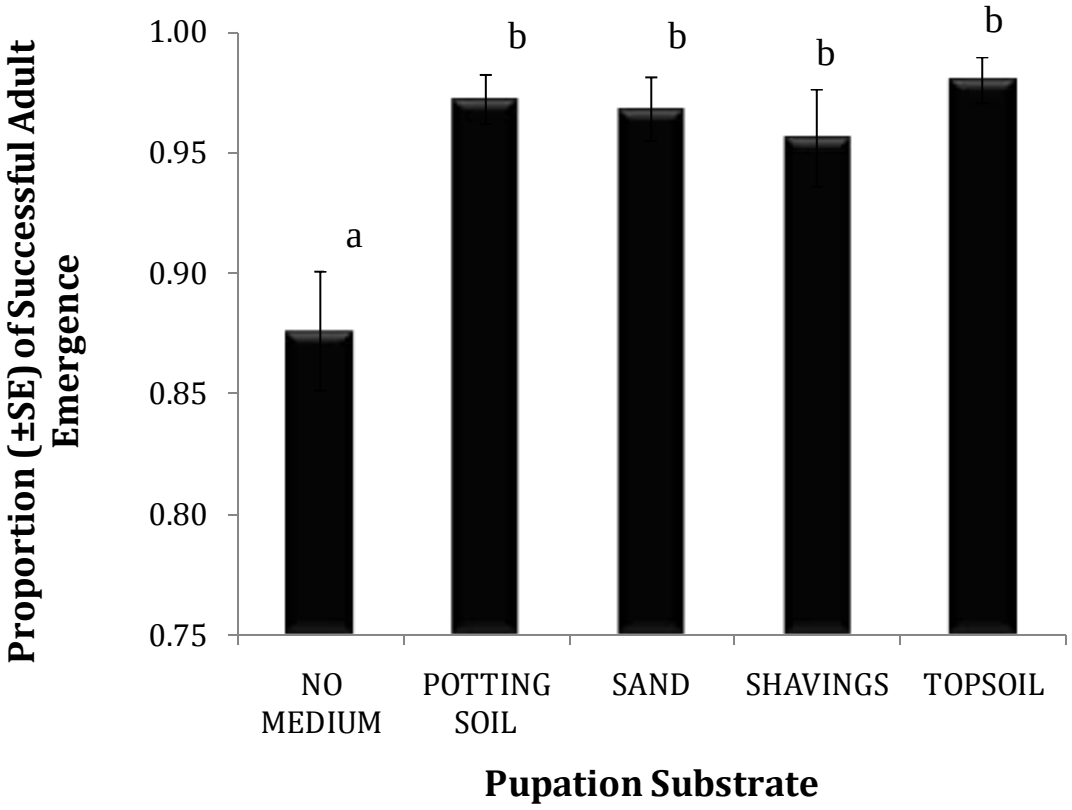

Figure 2.3

Proportion (±SE) of successful adult emergence for each pupation substrate, no medium, potting soil, sand, shavings and topsoil. Means followed by the same letter are not different *(P>0.05)*.

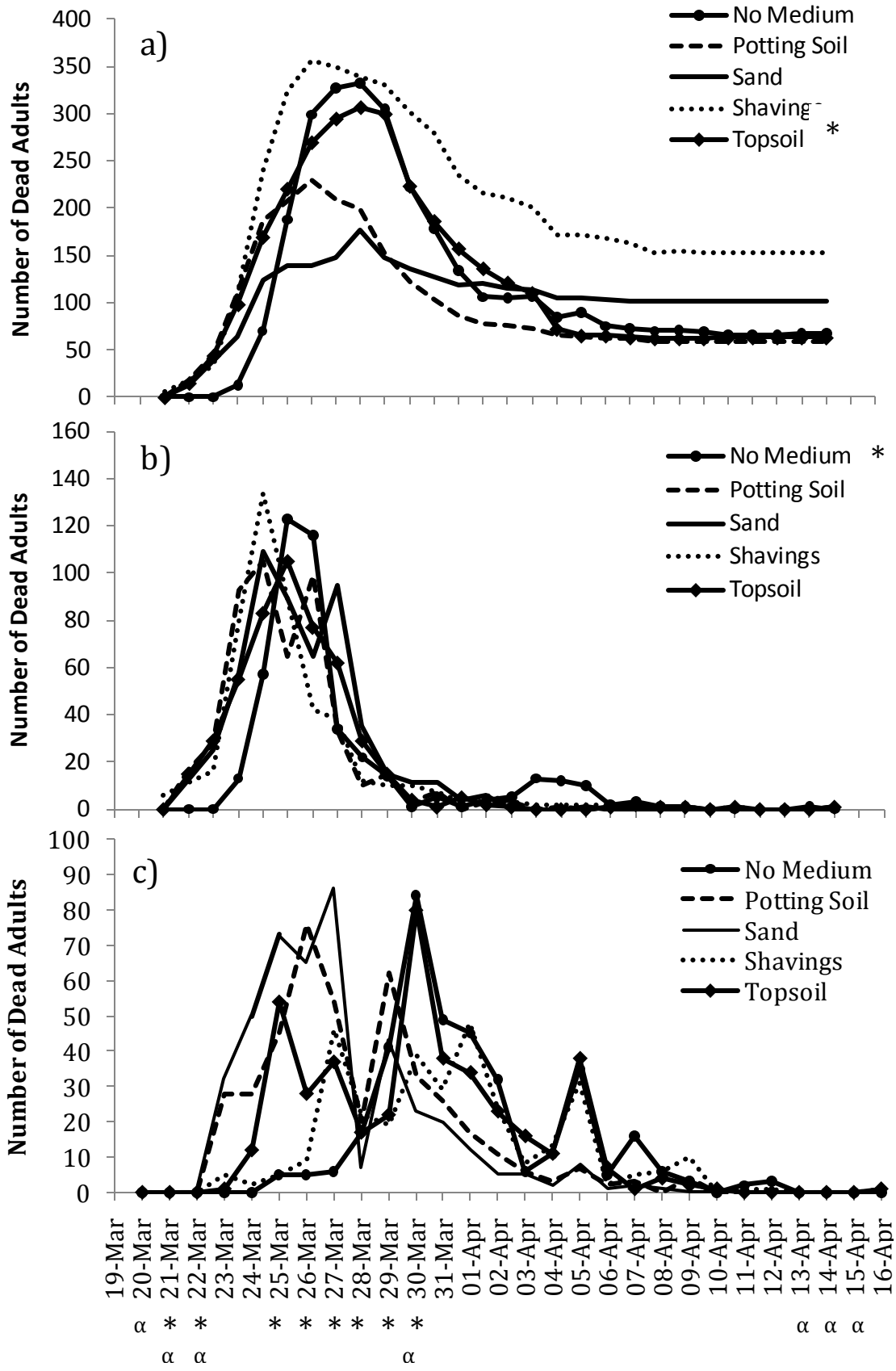
a)
No Medium
Potting Soil
Sand
Shavings
Topsoil
*
Number of Dead Adults
400
350
300
250
200
150
100
50
0
b)
No Medium
*
Potting Soil
Sand
Shavings
Topsoil
Number of Dead Adults
160
140
120
100
80
60
40
20
0
c)
No Medium
Potting Soil
Sand
Shavings
Topsoil
Number of Dead Adults
100
90
80
70
60
50
40
30
20
10
0
19-Mar
20-Mar
21-Mar
22-Mar
23-Mar
24-Mar
25-Mar
26-Mar
27-Mar
28-Mar
29-Mar
30-Mar
31-Mar
01-Apr
02-Apr
03-Apr
04-Apr
05-Apr
06-Apr
07-Apr
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Figure 2.4

Adult population trends with respect to successful adult emergence and adult mortality for each pupation substrate; no medium, potting soil, sand, wood shavings and topsoil. a) Proportion of living adults relative to the total number of emerged adults and adult mortalities for each pupation substrate. Dead adults were removed daily, and emerged adults were released into their respective treatment cage daily, therefore this graph represents the daily population count for each pupation substrate over the course of the experiment. Pupation substrates and dates marked by an asterisks (*) are different *(P>0.05)*. b) Number of successful adult emergences per pupation substrate per day. Pupation substrates marked by an asterisks (*) are different *(P>0.05)*. c) Number of dead adults collected per pupation substrate per day. Dates marked by alpha (α) are different *(P>0.05)*.

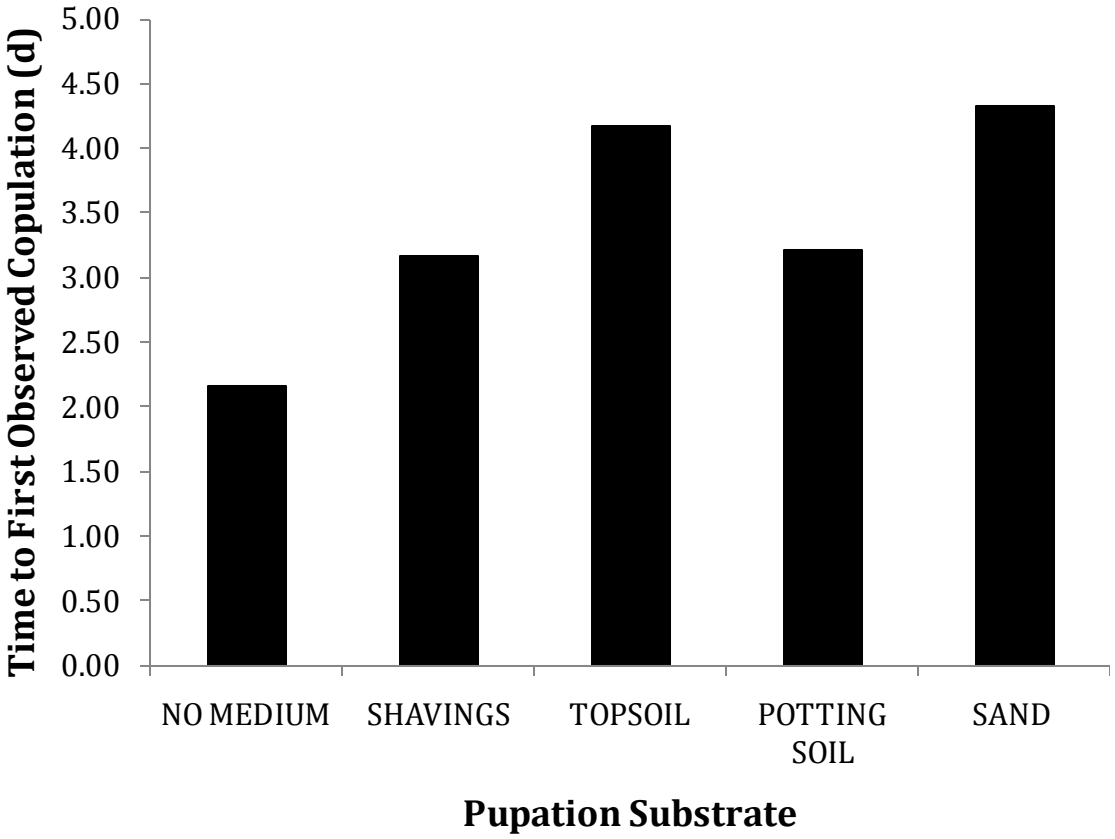

Figure 2.5

Time to first observed copulation with respect to the first adult(s) to emerge for each pupation substrate, no medium, shavings, topsoil, potting soil and sand.

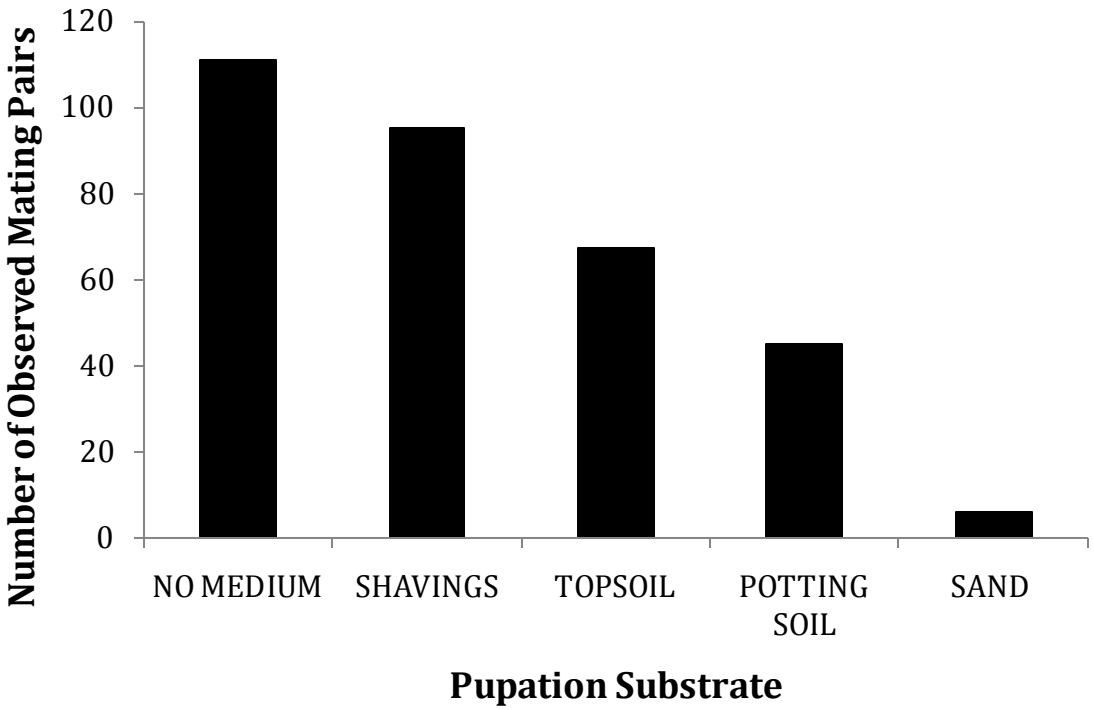

Figure 2.6

Number of observed mating pairs between pupation substrates, no medium, shavings, topsoil, potting soil and sand. Very few adults were observed mating in the sand treatment; it is speculated that this is a result of the high adult mortality and therefore low population numbers.

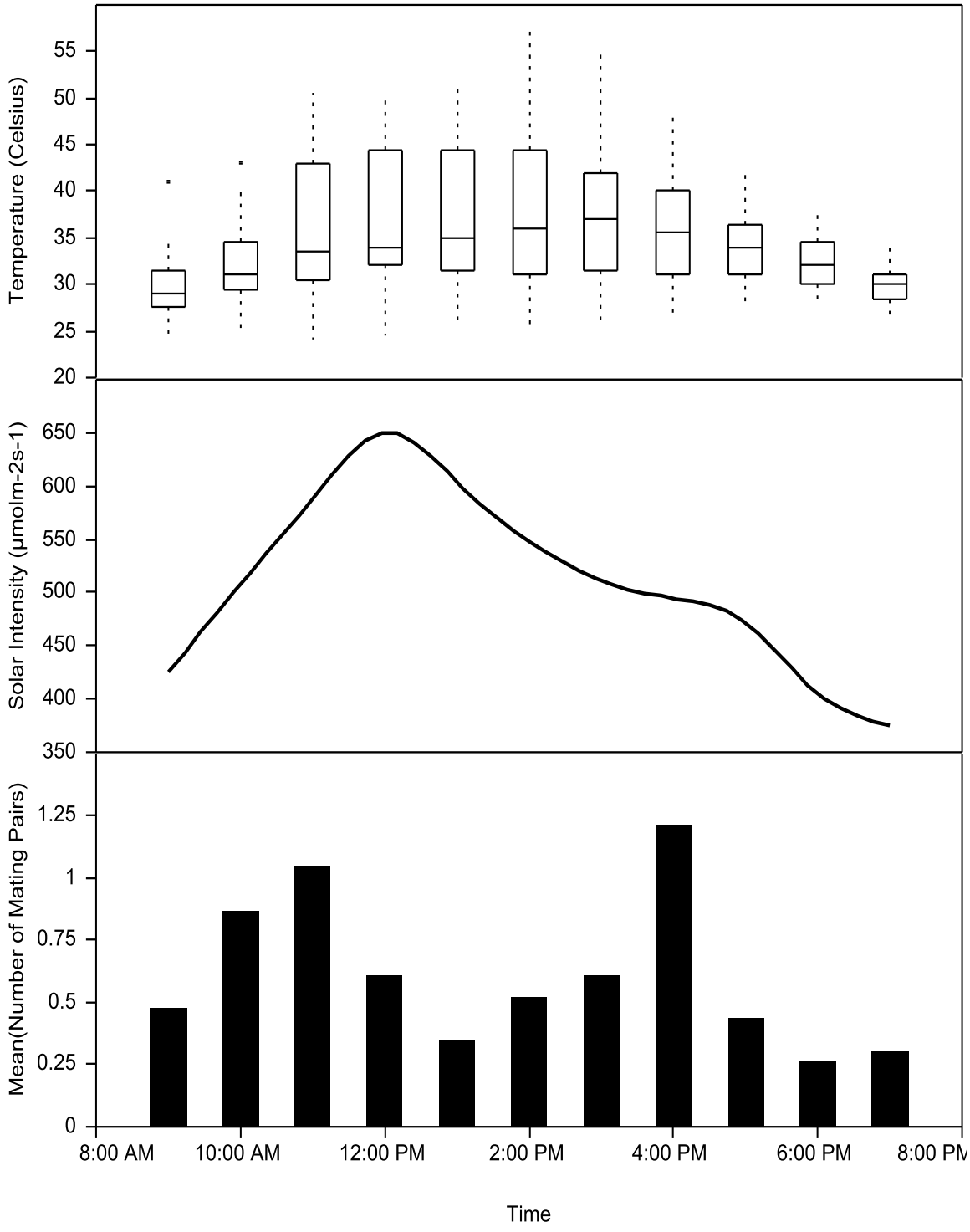

Figure 2.7

Solar light intensity ($\mu mol \bullet m^{-2} \bullet s^{-1}$) and temperature (ºC) trends and their effects on the number of mated pairs observed hourly.*

* Hourly recordings represent the mean across 36 days of observations

CHAPTER III

Effect of Relative Humidity on Egg Eclosion and Adult Emergence

INTRODUCTION

Like temperature, relative humidity is an abiotic factor affecting insect development; however, unlike temperature, the effect relative humidity has on insect development is not predominantly studied. Insect cuticles are composed of lipids and waxy molecules to withstand water loss, however water loss trade-offs are unavoidable, insects taking in oxygen through spiracles are concomitantly losing water while the wax embossed epicuticle helps retain water during respiration (Wigglesworth 1984). Unfortunately, unlike juveniles and adults who have the physiology to regulate water loss trade-offs with respiration, eggs are often immobilized and without mechanisms of controlling water loss during respiration which is a requirement of embryo development (Zrubek and Woods 2006). Egg respiration in insects should be relatively easy with their low volumes of metabolic tissues and their high surface-to-volume ratio, the concern however, is desiccation as a result of water loss (Woods, Bonnecaze and Zrubek 2005).

Woods et al. (2005) demonstrated that eggshell conductance to oxygen increased with embryo development in the hawkmoth *Manduca sexta* (L.) (Lepidoptera: Sphingidae). Thus, egg desiccation prevention strategies should be to decrease eggshell conductance, however in doing so, embryo development time would potentially be delayed, subjecting the egg to higher rates of predation for longer periods of time (Zrubek and Woods 2006). Woods et al. (2005) examined the layers composing the eggshell in the hawkmoth and concluded the location of water-loss and oxygen tradeoffs may possibly include interactions between the crystalline chorionic layer, the wax and the vitelline layer. Thus, studies examining the geographical variation in these physiological properties of eggshells would provide more insight into the evolutionary acclimation to environments of particular ambient relative humidities.

Water-loss and oxygen tradeoffs can be mediated by oviposition strategies. Laying eggs singly or in a cluster are two very different oviposition strategies and insects

typically employ one mechanism or the other (Stamp 1980). Egg clustering has been studied in many Lepidopteran species as mechanisms of aposematic colouration, (Stamp 1980), increased fecundity (Courtney 1984), larval aggregation advantages (Clark and Faeth 1997) and egg desiccation prevention (Clark and Faeth 1998). Stamp (1980) argues egg clustering is an oviposition strategy evolved secondarily to aposematism. Courtney (1984) on the other hand, argues that egg clustering is more likely an increased fecundity tactic as opposed to aposematism. Aposematism is an evolutionary pathway employed by kin selection and predator tasting and Courtney argues that neither can evolve prior to egg clustering since predator tasting would leave no viable eggs for trait selection and kin aggregated in batches is necessary for kin selection (Courtney 1998). Furthermore, females of many insect species laying their eggs in clusters have higher realized fecundities due to larval aggregation and are less susceptible to prereproductive mortality than those laying eggs singly (Courtney 1984).

Larval aggregation, a consequence of egg clustering, enables early instar larvae to overcome tough plant tissues (Stamp, 1980; Young and Moffett 1979) and to some extent, avoid predation via the concept of safety in numbers (Turner and Pitcher 1986), ultimately increasing survivorship. Additionally, Clark and Faeth (1997) studied the effects of group size in larval aggregation on larval survivorship and growth in the bordered patch butterfly, *Chlosyne lacinia* (Geyer) (Lepidoptera: Nymphalidae, Melitaenae). Results indicated that group size significantly affected survivorship and growth as larvae had higher survivorship success and faster growth rates in larger larval groups, suggesting selective pressures favour egg clustering.

Alternatively, Stamp (1980) also suggested egg clustering may be a preventative measure to egg desiccation, however, her theories on aposematism ensued. Instead, Clark and Faeth (1998) followed up on Stamp's egg desiccation hypothesis with reference to egg cluster size and composition including the number of egg layers in a cluster. They found single egg layer clusters completely desiccated at lower relative humidities (below 55% relative humidity), while multiple egg layer clusters in the same conditions had approximately 10% eclosion success. Furthermore, multiple egg layer clusters in low humidity treatments with eclosion success showed the surface eggs had desiccated,

protecting the eggs within the cluster (Clark and Faeth 1998). Therefore, it appears egg clustering may be a crucial selective force in preventing egg desiccation.

The black soldier fly, *Hermetia illucens*, (L.) (Diptera: Stratiomyidae) selectively oviposits in dry crevices near a moist resource (Booth and Sheppard 1984), such that hatched neonatal larvae can quickly and easily make their way to the resource before desiccating. It is been observed in colony that females mate once laying one egg cluster in their lifetime (Tomberlin et al. 2002; Sheppard et al. 1994). Adopted from Booth and Sheppard (1984), corrugated cardboard is used as substrate for *H. illucens* oviposition. Females oviposit in the cardboard flutes which are the cylindrical holes in the corrugated cardboard, often completely filling the flutes. Eggs are laid in clusters, composed of multiple egg layers and while females fill the crevice, the egg cluster takes on the form of the crevice (personal observation). Therefore, egg cluster shape, size and layer composition is manipulated by either the oviposition substrate provided or the oviposition site selected by the female (personal observation). When no oviposition substrate is provided, females will lay spherical clusters composed of multiple egg layers and are loosely packed compared to using cardboard flutes which become densely packed (personal observation).

The primary objective of this study was to determine the lower relative humidity threshold for black soldier fly development and the effects lower relative humidities have on black soldier fly development to attest if the economic costs of operating a fully functional waste management facility could be lowered by operating at lower humidities. To our knowledge, this study is the first to examine the effects of relative humidity on egg desiccation and adult emergence success in *H. illucens*. Based on the aforementioned studies on Lepidoptera, I predict black soldier fly egg clusters to succumb to desiccation at lower relative humidities ($\leq$ 30%). In addition, I expect eggs on the surface of egg clusters to desiccate before eggs within the cluster. Furthermore, black soldier fly pupae in colony have been observed flattened and dehydrated during low relative humidity conditions in Canada ($\leq$ 20% relative humidity) and therefore, I predict relative humidity may have significant effects on pupation and adult emergence success.

METHODS

Experiment: Egg Eclosion

Source of Eggs

Eggs were collected from a *H. illucens* colony housed in a cage (1.8 x 1.8 x 1.8m and 1.5mm mesh screen) maintained in a greenhouse, outside the Forensic Laboratory for Investigative Entomological Sciences (F.L.I.E.S.) Facility located at Texas A&M University in College Station, Texas. Eggs were collected in a three layer, 3 x 5cm corrugated cardboard, held together with Elmer® white glue with 3 x 4mm flutes used as an oviposition substrate, taped 5cm above the oviposition substrate with the flutes perpendicular to the substrate. Oviposition medium was composed of moist-to-liquefied Gainesville diet (5:3:2 hand mixture of wheat bran, alfalfa and corn meal, respectively), (Producers Cooperative Association, Bryan, TX), developed for rearing houseflies (Tomberlin et al. 2002; Hogsette 1992). Nutritional content for each raw material of the Gainesville diet used was 15% protein, 2.0% crude fat and 11% crude fibre, 17% protein, 1.5% crude fat and 27% crude fibre, and7% protein, 2.5% crude fat and 4% crude fibre, for wheat bran, alfalfa and corn meal, respectively).

Treatments

Each egg-containing corrugated cardboard flute was dissected to determine the number of egg clusters oviposited per flute. Egg clusters were randomly placed into 30mL clear plastic soufflé cups (Dart P100, Dart Container Corporation, Mason, MI, USA). Each soufflé cup contained one egg cluster (egg contribution from one individual female) and cups were placed into a 30-well clear plastic tray (Bio-Serv, Frenchtown, NJ) and randomly assigned a relative humidity treatment. Relative humidity treatments in this study were chosen based on Clark and Faeth's design (1998) and preliminary experiments on adjusting the relative humidity levels in the growth chambers (Percival Scientific Inc. Model I-36LLVLC8, Perry, IA). It was evident that the growth chambers could not withstand humidity levels below 30% and therefore 30% relative humidity (RH) was selected to test for the lower relative humidity threshold and 70% RH was

selected as the control. Later, a continuum of 40%, 50% and 60% RH and their effects on egg eclosion was also examined.

This experiment was composed of three independent stages. First, 10 replicates of 30% and 70% relative humidity egg clusters were compared in two separate Percival Growth Chambers (Percival Scientific Inc. Model I-36LLVLC8, Perry, IA). Second, 10 additional replicates of 30% and 70% RH treatment were compared; however, the growth chambers were switched for each RH to determine if a chamber effect existed. Finally, 20 replicates of 40%, 50% and 60% RH egg clusters were compared in three separate growth chambers (Percival Scientific Inc. Model I-36LLVLC8, Perry, IA), independent of the first two experiments. Regardless of relative humidity treatment, each growth chamber maintained a temperature of 27°C with a 12:12 [L:D] photoperiod.

A Hobo® U12-012 data logger (Onset® Computer Cooperation, Massachusetts, USA) was placed in each growth chamber on the top shelf next to the tray of egg clusters. The data logger recorded relative humidity, temperature and light intensity every 15 minutes. Eggs were monitored every 12h for successful egg eclosion. Upon first eclosion, eggs remained in their respective growth chambers under treatment conditions for an additional two days to allow eclosion in its full entirety. Two days after egg eclosion, egg clusters were stored in a -25°C chest freezer for assessment of successful egg eclosion. Time to egg eclosion and per cent successful egg eclosion was recorded for each replicate within each relative humidity treatment.

Abiotic Factors Recorded

The two data loggers in each chamber recording each abiotic factor, relative humidity, temperature and light intensity every 15 minutes were averaged. The mean relative humidity of each growth chamber was 24.4±0.01%, 35.78±0.03%, 48.96±0.08%, 58.73±0.08% and 70.43±0.08% for each treatment, 30%, 40%, 50%, 60% and 70% RH, respectively. The mean temperature of each growth chamber was 28.46±0.01°C, 27.34±0.01°C, 27.76±0.03°C, 28.77±0.02°C and 27.57±0.02°C for each treatment, 30%, 40%, 50%, 60% and 70% RH, respectively. The light intensity of each growth chamber was 5698±1.49Lux, 3907.10±4.81Lux, 4639.04±7.63Lux 6353.51±10.25Lux and 6239±1.86Lux for each treatment, 30%, 40%, 50%, 60% and 70% RH, respectively.

Furthermore, one Lux is equal to 0.0135 PPF (photosynthetic photon flux) and therefore the light intensity ranges from 52.74 $\mu mol \bullet m^{-2} \bullet s^{-1}$ to 85.76 $\mu mol \bullet m^{-2} \bullet s^{-1}$. However, this conversion changes depending on type of artificial light; equation used was based on fluorescent artificial lights (Apogee Instruments Inc., Logan, UT). Lower levels of light intensity may also be attributed to the life of the artificial light source used. The light intensity reported by the manufacturer for these growth chamber models are ~115 $\mu mol \bullet m^{-2} \bullet s^{-1}$, (Percival Scientific Inc., Perry, IA, USA).

Statistics

All statistics were computed using SAS JMP® version 8.0.1 statistical software. The data failed to meet the assumptions of normality (homogeneity of variance and normality goodness-of-fit) despite data transformation attempts (Box Cox, arcsine and $\log_{10}$ transformations). Thus, a non-parametric one-way Wilcoxon/Kruskal-Wallis (Ranked Sums) test was used for both analyses of time to egg eclosion and the percent successful egg eclosion. Pairwise comparisons using Wilcoxon/Mann-Whitney U tests were used on significant results. Alpha values were adjusted using the Dunn-Sidak procedure to correct for type I error as a result of multiple comparisons (Quinn and Keough, 2002, p 48).

Experiment: Adult Emergence

Source of Larvae

Eggs were collected from a *H. illucens* colony housed in a cage (1.8 x 1.8 x 1.8m and 1.5mm mesh screen) maintained in a greenhouse, outside the Forensic Laboratory for Investigative Entomological Sciences (F.L.I.E.S.) Facility located at Texas A&M University in College Station, Texas. Eggs were collected in a three layer, 3 x 5cm corrugated cardboard, held together with Elmer® white glue with 3 x 4mm flutes used as an oviposition substrate, taped 5cm above the oviposition substrate with the flutes perpendicular to the substrate. Oviposition medium was composed of moist-to-liquefied Gainesville diet (5:3:2 hand mixture of wheat bran, alfalfa and corn meal, respectively), (Producers Cooperative Association, Bryan, TX), developed for rearing houseflies (Hogsette 1992, Tomberlin et al. 2002). Nutritional content for each raw material of the

Gainesville diet used was 15% protein, 2.0% crude fat and 11% crude fibre, 17% protein, 1.5% crude fat and 27% crude fibre, and7% protein, 2.5% crude fat and 4% crude fibre, for wheat bran, alfalfa and corn meal, respectively).

Larval Development

Each egg-containing corrugated cardboard flute was dissected to determine the number of egg clusters oviposited per flute. Egg clusters were randomly placed into 30mL clear plastic soufflé cups (Dart P100, Dart Container Corporation, Mason, MI, USA). Each soufflé cup contained two egg clusters (egg contribution from two individual females) and cups were divided randomly among three relative humidity treatments (30%, 40% and 70%). Due to limited growth chamber availability, all replicates of eggs for all treatments were placed in a single walk-in growth chamber maintained at 70% RH, 25°C and a 14:10 [L:D] photoperiod until eclosion. Upon egg eclosion, larvae were fed 10g of dry Gainesville diet (Tomberlin et al. 2002; Hogsette 1992,) mixed with 18mL of water aliquots, *ad libitum* until larvae reached the post-feeding stage of development, indicated by their change in colour from cream to black.

Treatments

To determine the effect relative humidity has on adult emergence, all larvae from all three RH treatments (30%, 40% and 70%) were raised in the same environmental conditions until growth to the post-feeding stage of development. Since feeding neonate larvae are amongst their moist food until development to the post-feeding stage, I decided that is was not necessary to use feeding larvae to test the effects low relative humidities have on adult emergence. Instead, treatment effects started when feeding larvae left to food resource, entering their post-feeding stage of development. Upon reaching the post-feeding stage of development, 30 post-feeding larvae from each replicate per relative humidity treatment were removed from their food in the walk-in growth chamber and placed individually into 30mL clear plastic soufflé cups (Dart P100, Dart Container Corporation, Mason, MI, USA), without lids, for maximum relative humidity exposure. Cups were placed in a 30-well tray (Bio-Serv, Frenchtown, NJ) and placed into their respective growth chambers (Percival Scientific Inc. Model I-36LLVLC8, Perry, IA)

corresponding to their respective relative humidities (30%, 40% or 70%) and 25°C and 14:10 [L:D] photoperiod.

Post-feeding larvae were observed every 12h to determine time of pupation for each larva. Upon pupation, pupae remained in their soufflé cups without lids for the first five days since pupation, to maximize relative humidity exposure before adult emergence. Six-day-old pupa were capped with clear plastic lids, (100PCL25 Dart Container Corporation, Mason, MI, USA), and pierced with a 10cc (29 gauge) syringe in anticipation of adult emergence. Pupae were monitored every 12h for successful adult emergence. Time to adult emergence, percent successful adult emergence and adult longevity was recorded.

Two Hobo® U12-012 data loggers (Onset® Computer Cooperation, Massachusetts, USA) were placed in each growth chamber, one placed centered on the top shelf to record temperature, relative humidity and light intensity with an external temperature probe placed on the middle shelf and another unit placed centered on the bottom shelf to record relative humidity, temperature and light intensity every 15 minutes.

Abiotic Factors Recorded

The walk-in growth chamber, of which all treatments were subjected to for their growth to the post-feeding stage of development, was 25.14±0.03°C, 75.60±0.36% RH and 14:10 [L:D] photoperiod. When larvae reached the post-feeding stage of development and were placed into their respective growth chamber treatments; 30%, 40% or 70% RH, two data loggers were placed in each chamber to record, relative humidity, temperature and light intensity. These two data loggers were averaged for each growth chamber. The mean relative humidity of each growth chamber was 30.04±0.12%, 41.32±0.06% and 69.53±0.07%, for each treatment, 30%, 40% and 70% RH, respectively. The mean temperature of each growth chamber was 26.23±0.01°C, 26.23±0.02°C and 26.95±0.02°C for each treatment, 30%, 40% and 70% RH, respectively. The light intensity of each growth chamber was 5295.37±10.37Lux, 5963.22±13.49Lux and 6284.64±7.41Lux for each treatment, 30%, 40% and 70% RH, respectively; equating to 71.48 $\mu mol \bullet m^{-2} \bullet s^{-1}$ to 84.83 $\mu mol \bullet m^{-2} \bullet s^{-1}$. However, this

conversion changes depending on type of artificial light. Lower levels of light intensity may also be attributed to the life of the artificial light source used. The light intensity reported by the manufacturer these growth chamber models are ~115 $\mu mol \bullet m^{-2} \bullet s^{-1}$, (Percival Scientific Inc., Perry, IA, USA).

Statistics

The data failed to meet the assumptions of normality (homogeneity of variance and normality goodness-of-fit) despite data transformation attempts (Arcsine and Box Cox transformations). Therefore, a non-parametric one-way Wilcoxon/Kruskal-Wallis test with Wilcoxon/Mann-Whitney U pairwise comparisons tests were used to determine the development times in each stage of development; the post-feeding and pupal stages as well as post-feeding and pupal mortalities and the percent successful adult emergences with respect to treatment. Alpha values were adjusted using the Dunn-Sidak procedure (Quinn and Keough, 2002, p 48).

A full factorial Cox Proportional Hazard test (model effects: sex, treatment and sex*treatment) was used to compare survivorship differences with respect to adult longevity between treatments.

RESULTS

Experiment: Egg Eclosion

Lower relative humidity threshold: 30% and 70%

The number of eggs in each replicate was not different from those replicates in the control (70% RH), ($F_{1,36}$ = 0.1435, p=0.7070*).* After repeating the experiment and switching growth chambers for each treatment, there was a slight chamber effect evident with respect to time to egg eclosion for the 30% relative humidity treatment (χ^2 = *12.5057, df = 1, p=0.0004*, Dunn-Sidak adjusted α = 0.0253). Mean (±SE) time to egg eclosion for the 30% RH treatments in chamber one and two was 124.43±1.85h and 138.00±0.00h, respectively. In contrast, there was no chamber effect with respect to time to egg eclosion for the 70% relative humidity treatment *(χ^2 = 0.1330, df = 1, p = 0.7154)*. Mean (±SE) time to egg eclosion for the 70% RH treatments in chamber one and two was 87.63±2.70h and 80.78±3.75h, respectively. Regardless of the chamber effect in the 30% RH treatment, mean (±SE) time to egg eclosion varied between humidity treatments, (Figure 3.1a, χ^2= 28.7834, *df* = 1 *p* < 0.001), with eggs in the 30% RH taking longer to eclose than the control.

Contrarily, there was no chamber effect observed with respect to mean (±SE) percent successful egg eclosion for 30% and 70% RH, *(χ^2 = 3.9153, df = 1, p = 0.0478 and χ^2 = 3.9367, df = 1, p = 0.04*65, respectively, Dunn-Sidak adjusted α = 0.0253). The effect relative humidity had on successful egg eclosion varied between humidity treatments, (Figure 3.2a, χ^2= 27.8882, *df* = 1 *p*<0.001). Eggs in the 30% RH had lower eclosion success than the control.

Relative humidity continuum: 40%, 50% and 60%

The number of eggs in each replicate was not different across treatments 40%, 50% and 60% relative humidity, ($F_{2,57}$ = 2.5797, p=0.0846). The effect of relative humidity on the mean time to egg eclosion varied across treatments, (Table 3.1 and Figure 3.1b, χ^2= 43.4409, *df* = 2 *p < 0.001*). Eggs in the 40% RH took longer to eclose than those in the 50% and 60% RH treatments. Furthermore, eggs in the 50% RH took longer to eclose than those in the 60% RH treatment.

The effect of relative humidity on egg eclosion success differed across treatments, (Table 3.2 and Figure 3.2b, χ^2= 50.2911, *df* = 2 *p*<0.001). Eggs in 40% RH had lower eclosion success than those in 50% and 60% RH. Furthermore, eggs in 50% RH had lower eclosion success than those in 60% RH.

Experiment: Adult Emergence

There was no effect of sex and the interaction of treatment and sex on the baseline survival in the post-feeding stage of development (χ^2= 1.5194, *df* = 1 *p*= 0.2177 and χ^2= 1.1851, *df* = 2 *p*=0.5529, respectively). Treatment independently affected the baseline survival in the post-feeding stage of development (Figure 3.3a, χ^2= 16.4343, *df* = 2 *p*<0.001), such that a greater proportion of larvae in the 30% RH treatment spent more time in the post-feeding stage of development than larvae in the other relative humidity treatments. Similarly, there was no effect of sex and the interaction of treatment and sex on the baseline survival in the pupal stage of development (χ^2= 3.0472, *df* = 1 *p*=0.0809 and χ^2= 1.6552, *df* = 2 *p*=0.4371, respectively). Treatment independently affected the baseline survival in the pupal stage of development (Figure 3.3b, χ^2= 10.5336, *df* = 2 *p*<0.005), such that a greater proportion of larvae in the 30% RH treatment spent more time in the pupal stage of development than larvae in the any of the other relative humidity treatments.

Post-feeding larval and pupal mortality differed between relative humidity treatments, (Table 3.3 and Figure 3.4, χ^2= 37.0437, *df* = 2 *p*<0.001 and χ^2= 42.2135, *df* = 2 *p*<0.001). Post-feeding larvae and pupae in 30% and 40% RH had higher mortality than those in 70% RH.

Successful adult emergence differed between the 30%, 40% and 70% relative humidities treatments, (Table 3.4 and Figure 3.5, χ^2= 41.2807, *df* = 2 *p*<0.001). Pupae in the 70% RH treatment had higher successful adult emergences than those in the 40% and 30% RH treatments.

The sex ratio for each treatment was 76.74%, 67.60% and 71.98% male in the 30%, 40% and 70% RH treatments, respectively. The proportion of males was higher than the females within each treatment *(χ^2 = 165.9885, df = 1, p < 0.0001)*; however, treatment was not a factor on the sex ratio, (χ^2= 3.441, *df* = 2 *p = 0.1790)*.

The mean (±SE) longevity for males and females in 30%, 40% and 70% RH treatments was 5.08±0.18d and 5.3±0.21d, 6.74±0.13d and 6.51±0.16d and 8.00±0.11d and 7.77±0.14d, respectively. The effect of relative humidity on adult longevity varied between treatments, (χ^2= 104.5811, *df* = 2 *p*<0.001). Adults in the 70% RH lived longer than those in the 30% and 40% RH treatments, (χ^2= 118.5968, *df* = 1 *p*<0.001 and χ^2= 63.6374, *df* = 1 *p*<0.00, respectively). Sex did not have an effect on the date of adult emergence, (χ^2= 2.7546, *df* = 1, *p*=0.0970) and adult longevity, (χ^2= 0.5388, *df* = 1 *p*<0.4629).

There was no effect of sex and the interaction of treatment and sex on the baseline survival of adult longevity (χ^2= 1.5659, *df* = 1 *p*=0.2108 and χ^2= 0.3779, *df* = 2 *p*<0.8278, respectively). Treatment independently affected the baseline survival of adult longevity (Figure 3.6, χ^2= 103.6544, *df* = 2 *p*<0.001), such that the survival of adults in the 30% RH treatment were distributed over fewer days than those in the 40% and 70% relative humidity and the survival of adults in the 40% RH treatment were distributed over fewer days than those in the 70% relative humidity treatment.

DISCUSSION

Experiment: Egg Eclosion

Egg eclosion success at 30% RH was only 6.84% and similar to what Clark and Faeth (1998) found, eggs on the surface of egg clusters were desiccated, leaving the tightly layered eggs within the cluster protected from desiccation. Furthermore, eggs in the 30% RH took longer to eclose. Based on Woods et al (2005), eggs may be conserving water in these arid conditions, and alternatively slowing embryonic development due to the trade-off of insufficient oxygen conductance through the egg wall. Despite setting the growth chambers for 30% and 40% RH, the actual relative humidities were much lower and although I was testing 30% as the lower developmental threshold, the actual relative humidity recorded was 25% RH and since eggs in the 30% RH treatment (actually 25%RH) successfully eclosed, I cannot yet decipher the lower relative humidity threshold for black soldier fly egg eclosion and but I can conclude it is below 25% RH. To determine the lower relative humidity threshold, alternative measures, possibly using saturated salt solutions (Pappas, Broufas and Koveos 2008; Clark and Faeth 1998), would be necessary since the growth chambers used cannot maintain relative humidities below 25-30%.

Terrestrial ectotherms' tolerance to relative humidity has been demonstrated to correspond to their geographic origin (Walzer et al. 2007; Holmstrup and Loeschcke 2003; Schausberger 1998; Kramer and Hain 1989). In six species of predatory mites, it was determined that three: *Phytoseiulus longipes* (Argentinean strain) (Evans) (Acarina: Phytoseiidae), *Phytoseiulus longipes* (Chilean strain) (Evans) (Acarina: Phytoseiidae) and *Neoseiulus idaeus* (Denmark and Muma) (Acarina: Phytoseiidae), were drought tolerant; their geographic origins maintaining a moderate (50-60%) RH annually, while *Phytoseiulus macropilis* (Banks) (Acarina: Phytoseiidae), *Amblyseius swirskii* (Athias-Henriot) (Acarina: Phytoseiidae) and *Phytoseiulus Persimilis* (Athias-Henriot) (Acarina: Phytoseiidae) were drought sensitive; their geographic origins maintaining a higher (70-90%) annual relative humidity (Ferrero et al. 2010). Because black soldier flies are found more commonly in tropical and subtropical 'moist' environments (Booth and Sheppard 1984), they are more likely to have evolved a low relative humidity sensitivity than a

tolerance and my findings support this hypothesis. However, since some eclosion success was evident at lower relative humidities, and based on geographical acclimation observed in colony rearing of mites, (Ferrero et al. 2010), it would be interesting to re-evaluate our results after several generations of rearing the black soldier fly in more arid environments. Furthermore, the black soldier flies used have been in colony reared in a controlled environment for 8 years and therefore may have actually developed some tolerance to arid environments. However, the eclosion success at 30% is too low to maintain a fully functional colony for our purposes in waste conversion and therefore it is recommended to maintain humidity levels above 30%.

Time to egg eclosion appears to be inversely proportional to successful egg eclosion. Transpiration through the egg cuticle in the black soldier fly is likely higher than species acclimated to dryer environments (Wigglesworth 1944) and therefore delays in time to egg eclosion at the lower relative humidity treatments may very well be to conserve water as oxidation increases with embryo development (Woods et al. 2005). Interestingly, eggs in the 60% RH treatment eclosed before eggs in the 70% RH treatment with a comparable eclosion success, however since the temperature in the 60% RH treatment was a degree higher than the 70% treatment, it would be necessary to test these two relative humidities again in the same experiment and switch the growth chambers to test for a chamber effect.

Experiment: Adult Emergence

Our results confirm desiccation is highly evident on post-feeding larvae and pupae under low relative humidities, with only 16% adult emergence at 30% RH. Desiccation was relatively equally evident in both post-feeding larvae and pupae at 30% RH. Desiccated post-feeding larvae were identified by their brittle, truncated and flattened appearance. Desiccated pupae were identified at the end of the experiment; one month after the majority of the 70% RH adults emerged, when emergence did not occur. Desiccated pupae in the 30% and 40% RH treatments were dissected to assess evidence of dehydration. High proportions of hollowed cavities within the puparium were evident and fat bodies were completely dehydrated, however pupal mortalities in the 70% RH treatment were not dissected to confirm desiccation.

Kroeker and Walker (1991) studied the correlation of up regulated desiccation stress protein 28 (dsp28) and the up regulation of juvenile hormone in the common mealworm *Tenebrio molitor* (L.) (Coleoptera: Tenebrionidae). Juvenile hormone is up regulated during ecdysis and concomitantly, dsp28 is also up regulated during ecdysis, however, decreasing just prior to pupation and not up regulated again until adult reproduction (Kroeker and Walker 1991). Although the mealworm is in a different order, the mechanism may be similar in the black soldier fly and therefore, could potentially explain why post-feeding larvae were able to successfully pupate, however inevitably succumb to desiccation in the pupal stage of development due to a down regulation of dsp28. It is possible however, the desiccated pupae may have been misidentified as being in a state of diapause, since diapausal pupae of the black soldier fly have not been dissected for comparison, but with 16% and 59% successful adult emergences in the 30% and 40% RH, respectively, relative humidity did significantly impact *H. illucens* development.

My predictions are conclusive; relative humidity has significant effects on black soldier fly egg eclosion and adult emergence. The black soldier fly's natural tropical and subtropical habitats have likely been crucial selective pressures through evolutionary time, in their tolerance of relative humidity variation. For the purpose of my objective, although the lower relative humidity threshold could not be determined for egg eclosion or adult emergence, mortality due to desiccation provides sufficient evidence for using higher relative humidities (≥ 50% RH) in colony maintenance. Furthermore, higher successful egg eclosion was evident in the 50%, 60% and 70% RH treatments, demonstrating that egg eclosion and adult emergence success increases with relative humidity.

Finally, one drawback to this study is the lack of observations of adult fecundity. Although a proportion of adults successfully emerged in each RH treatment, relative humidity effects on successful mating and oviposition are unknown. The only indicator of adult fitness I have in this study is longevity, with adults in the control treatment (70% RH) living two to three days longer than adults subjected to lower RH environments. Despite longevity trends, I have no evidence in this study that ovarian development and successful mating followed by viable egg deposition, is correlated to adult longevity.

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TABLES

Table 3.1

Wilcoxon / Kruskal-Wallis Tests: Ranked Sums compares the mean time to egg eclosion between relative humidity treatments, 40%, 50% and 60%. Dunn-Sidak adjusted α = 0.0169.

Treatment Interactions	Z	ChiSquare	Prob>ChiSquare
40% * 50% RH	-2.851	8.207	p = 0.004*
40% * 60% RH	-5.481	30.192	p < 0.001*
50% * 60% RH	-5.449	28.845	p < 0.001*

Table 3.2

Wilcoxon / Kruskal-Wallis Tests: Ranked Sums compares percent successful egg eclosions between relative humidity treatments, 40%, 50% and 60%. Dunn-Sidak adjusted α = 0.0169.

Treatment Interactions	Z	ChiSquare	Prob>ChiSquare
40% * 50% RH	4.984	24.973	p < 0.001*
40% * 60% RH	5.402	29.323	p < 0.001*
50% * 60% RH	5.347	28.737	p < 0.001*

Table 3.3

Wilcoxon / Kruskal-Wallis Tests: Ranked Sums compares post-feeding larval and pupal mortalities between relative humidity treatments, 30%, 40% and 70%. Dunn-Sidak adjusted $\alpha = 0.0169$.

Treatment Interactions	Z	ChiSquare	Prob>ChiSquare
Post-Feeding Larval Mortality			
30% * 40% RH	-3.82	14.7220	$p < 0.001$*
30% * 70% RH	-5.15	26.7192	$p < 0.001$*
40% * 70% RH	-4.16	17.4448	$p < 0.001$*
Pupal Mortality			
30% * 40% RH	-4.08	16.8152	$p < 0.001$*
30% * 70% RH	-5.19	27.1328	$p < 0.001$*
40% * 70% RH	-4.97	24.8949	$p < 0.001$*

Table 3.4

Wilcoxon / Kruskal-Wallis Tests: Ranked Sums compares percent successful adult emergences between relative humidity treatments, 30%, 40% and 70%. Dunn-Sidak adjusted $\alpha = 0.0169$.

Treatment Interactions	Z	ChiSquare	Prob>ChiSquare
30% * 40% RH	4.28	18.4713	$p < 0.001$*
30% * 70% RH	5.13	26.4919	$p < 0.001$*
40% * 70% RH	4.61	21.3952	$p < 0.001$*

FIGURES

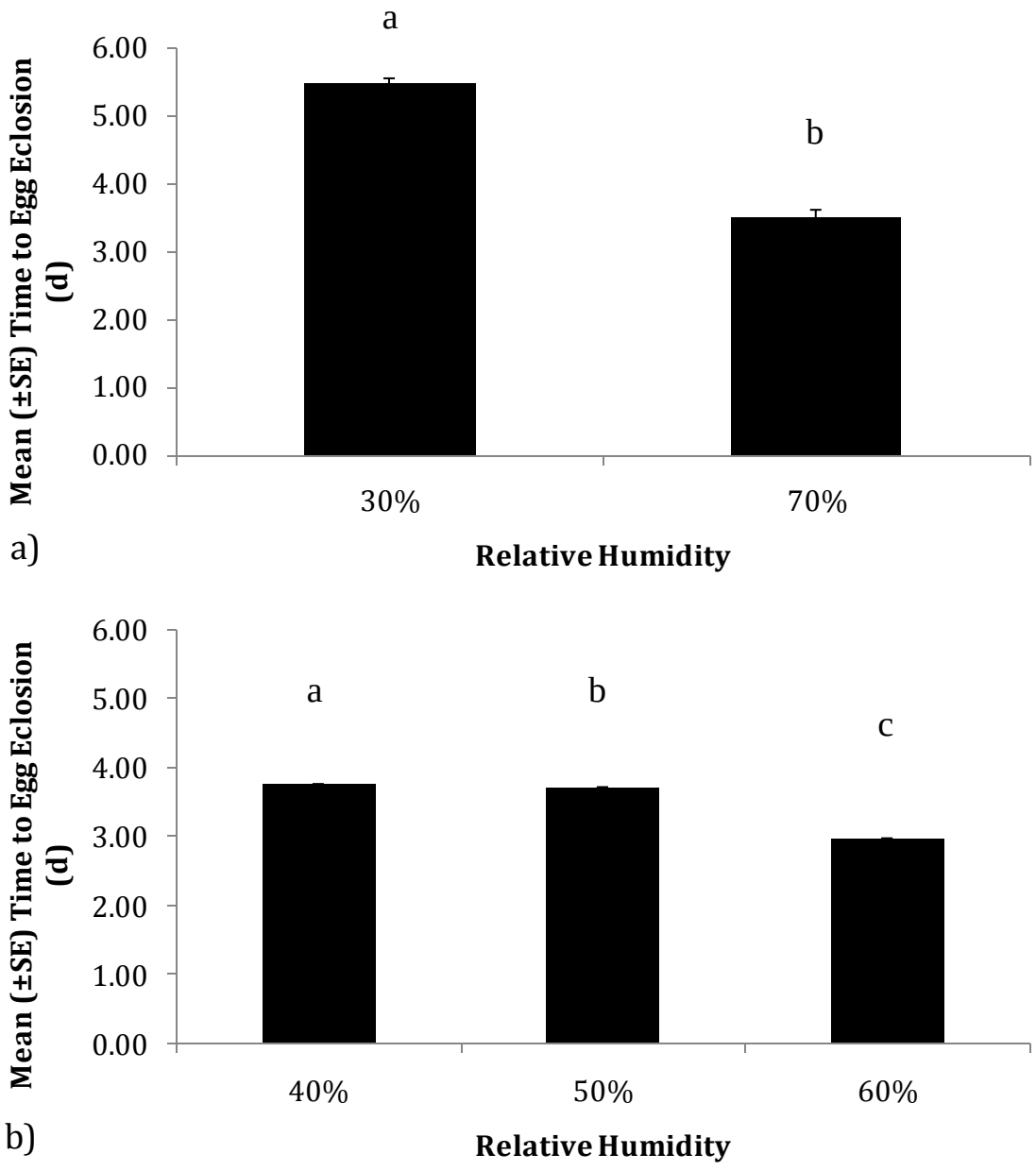

Figure 3.1

Effect of relative humidity on time to egg eclosion: a) Lower relative humidity threshold comparison, 30% and 70% RH (control). b) Relative humidity continuum comparison, 40%, 50% and 60% RH. Treatments assigned different letters are different ($P<0.05$).

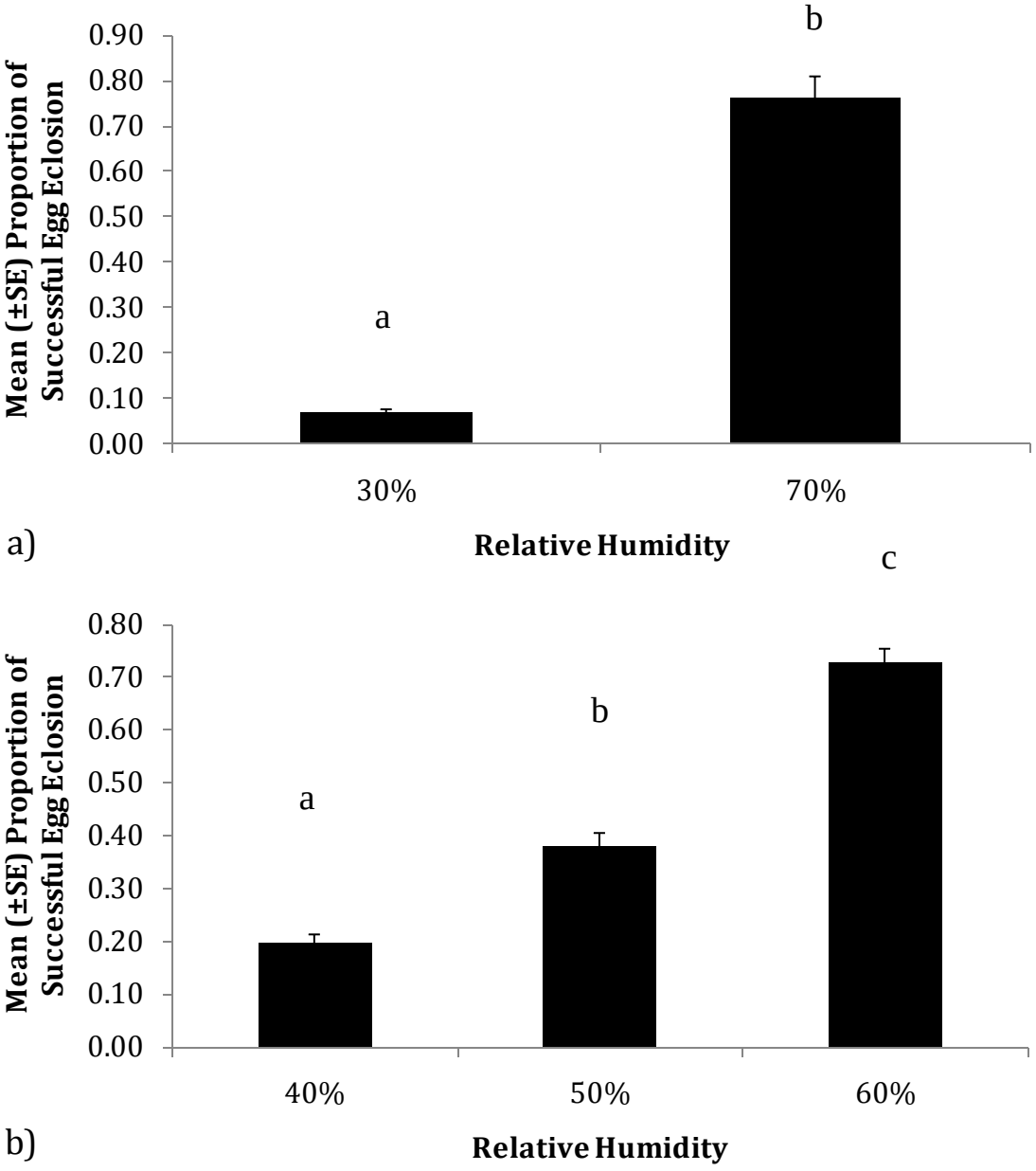

Figure 3.2

Effect of relative humidity on successful egg eclosion: a) Lower relative humidity threshold comparison, 30% and 70% RH (control). b) Relative humidity continuum comparison, 40%, 50% and 60% RH. Treatments assigned different letters are different ($P<0.05$).

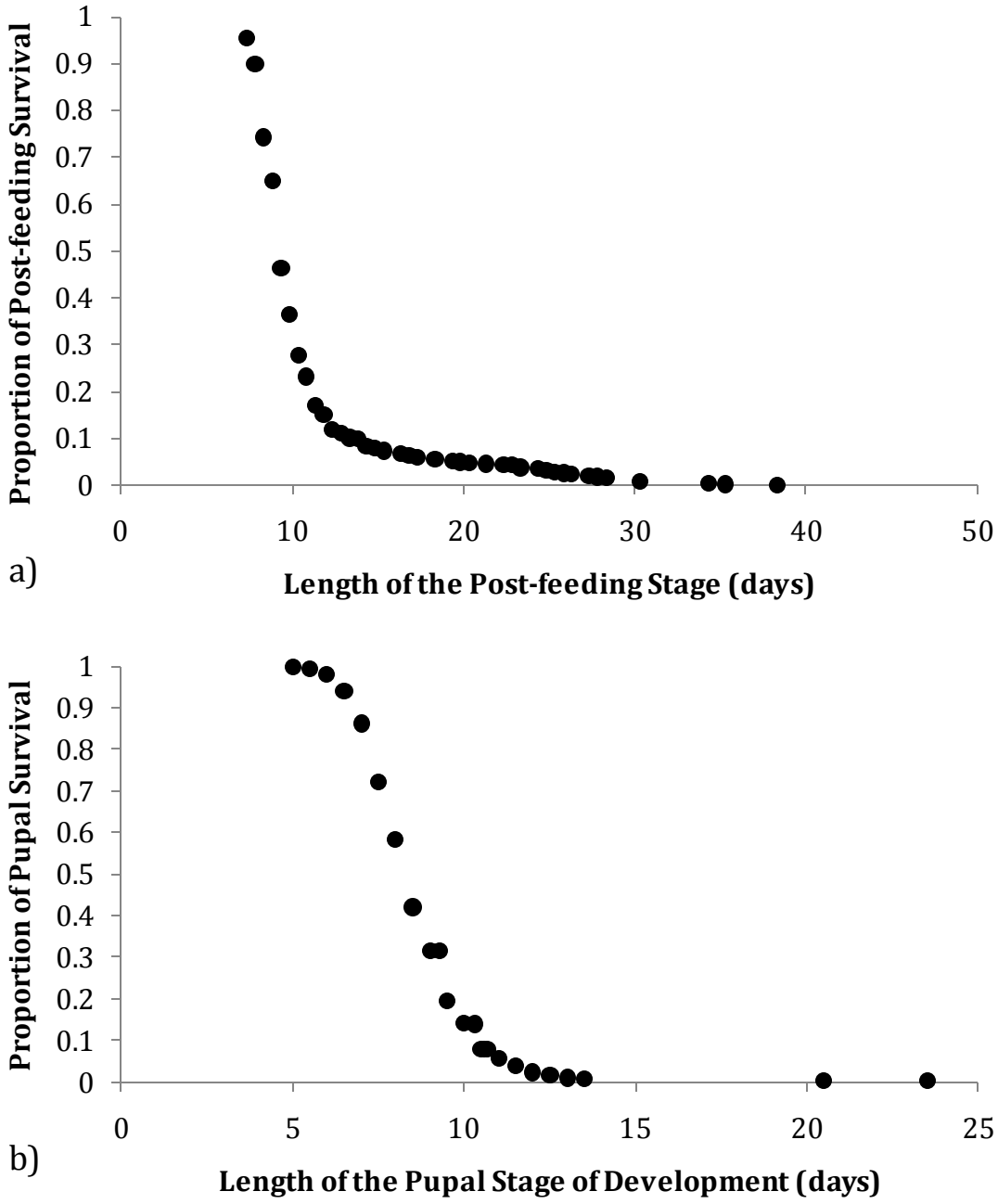

Figure 3.3

Cox Proportional Hazard fits: a) Baseline survival at the mean in the post-feeding stage of development was significantly affected by treatment, (χ^2= 16.4343, *df* = 2 p<0.001). Sex and the interaction of sex and treatment were not significant; b) Baseline survival in the pupal stage of development was significantly affected by treatment, (χ^2= 10.5336, *df* = 2 p<0.005). Sex and the interaction of sex and treatment were not significant.

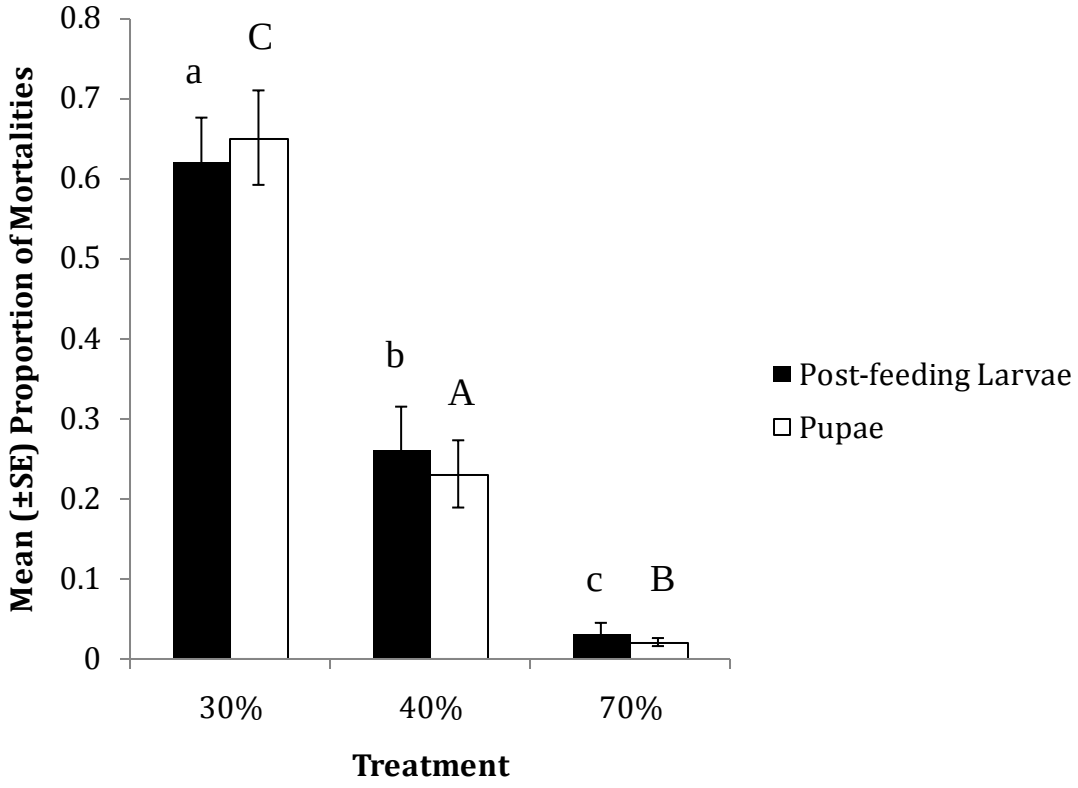

Figure 3.4

Effect of relative humidity on post-feeding larval and pupal mortalities. Proportions in the post-feeding stage of development followed by the same lower case letter are different *(P<0.05)*. Proportions in the pupal stage of development assigned different upper case letters are different (P<0.05).

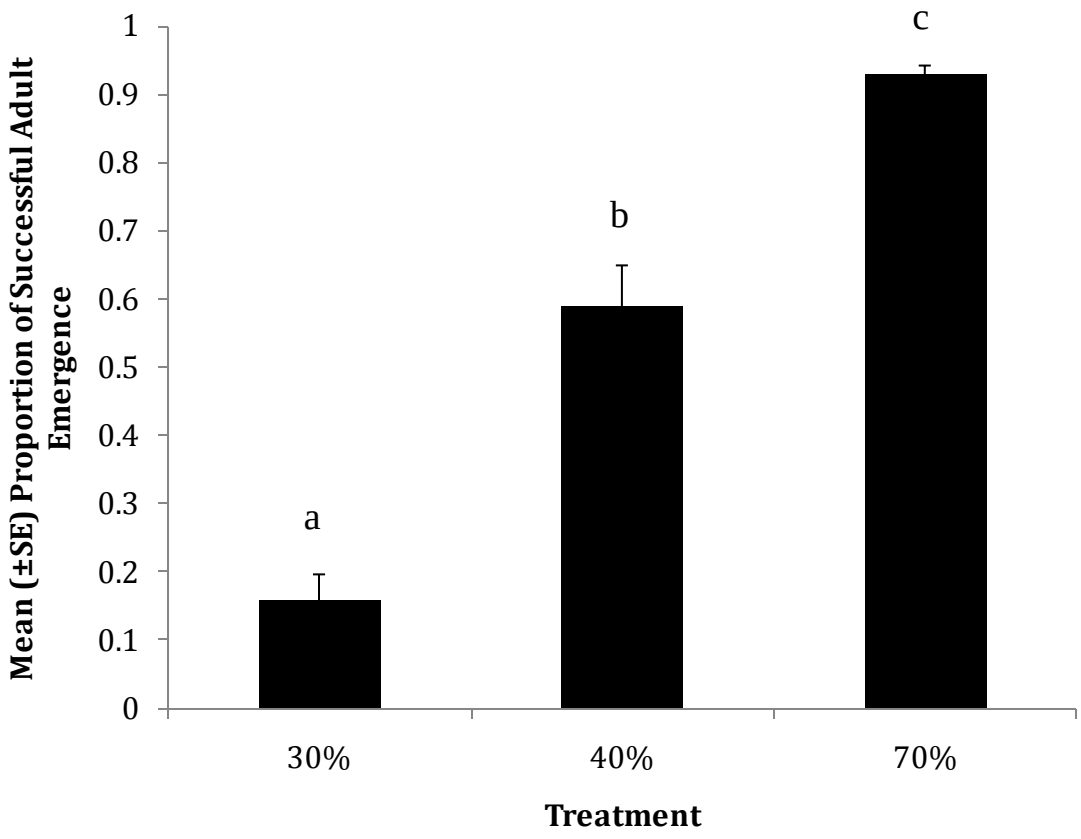

Figure 3.5

Mean (±SE) proportion of successful adult emergences by treatment. Proportions in the followed by the same letter are not different (*P>0.05*).

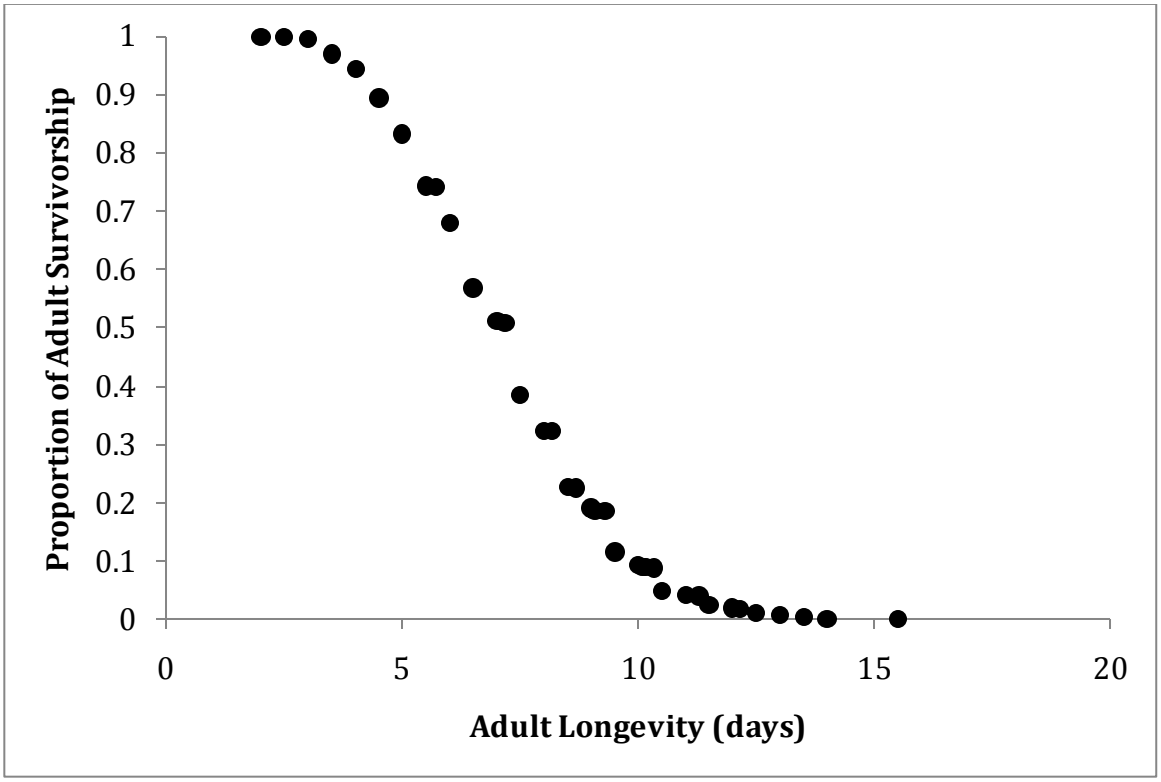

Figure 3.6

Cox Proportional Hazard fit indicates baseline survival at the mean was affected by treatment, (χ^2= 103.6544, *df* = 2 p<0.001). Sex and the interaction of sex and treatment were not different.

CHAPTER IV

Effects of Daylength on Egg Eclosion and Adult Emergence

INTRODUCTION

Insects use both internal circadian clocks, tracking their environment on a 24h scale, and photoperiodism, tracking their environment on a 12 month seasonal scale (Bradshaw and Holzapfel 2010). Internal circadian clocks enable insects to regulate rest periods with the use of light and dark periods as well as temperature fluctuations throughout the day (Saunders 2009). On longer time scales, photoperiodism enables insects to prepare for stressful environmental conditions using the shift in the length of light and dark periods throughout the year with shorter days signifying the onset of winter (Bradshaw, Quebodeaux and Holzapfel 2003). Responses to stressful environments can involve migration to more favourable conditions (Bale and Hayward 2010), entering a state of quiescence which is the direct inhibition of development due to abiotic conditions above or below developmental thresholds tolerated by cold shock or heat shock proteins (Hodek 2002) or enter a state of diapause which is a developmental suppression as a result of an abiotic signal 'token stimuli' (Hodek 2002), however, the correlation of photoperiodism and the onset of diapause varies with latitude (Danilevskii 1965).

Diapause stimuli in tropical insects tend to include changes in temperature, moisture, population density and food composition, rather than photoperiod, since photoperiod is relatively constant near the equator (Tauber, Tauber and Masaki 1986). Contrarily, in northern temperate and polar zones (above 30°N latitude), daylength, rather than temperature, is the primary trigger for diapause (Bale and Hayward 2010). Daylength, or commonly night length (Saunders 2009) varies both on temporal and latitudinal scales where higher latitudes have more temporal variation in daylength and the onset of winter arrives earlier than southern latitudes (McWatters and Saunders 1998). As a result, insects in northern latitudes often have a longer critical night length (CNL); the length of night which signals the insect to begin metabolic preparations for diapause. Furthermore, CNL were found to increase 1h for every 5° latitudinal increments to the

north in the moth, *Acronycta rumicis* (L.) (Lepidoptera: Noctuidae), (Danilevskii 1965). Regardless, shortened days indicate winter is on the horizon and many insects in these temperate zones undergo dormancy for survival (McWatters and Saunders 1998).

Photoperiod, unlike temperature, is not itself a factor of metabolism and therefore can signal weather changes well in advance without interrupting immediate metabolic functions (Saunders 2009). Saunders (2009) describes diapause as a gradual mechanism that is first initiated by an insect's reception of their CNL and from there a chain reaction of metabolic preparations ensues until the insect is ready to enter dormancy. For example, early instar larvae of the moth *Crambus tutillus* (McDunnough) (Lepidoptera: Crambidae) subjected to shortened day lengths, developed faster than those subjected to longer days (Kamm 1972). This was also the case with the Colorado potato beetle *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae), and concomitantly, larvae exposed to shorter days weighed more than those exposed to longer days (Dolezal, Habustova and Sehnal 2007). Perhaps once the CNL is reached, larvae quickly feed to reach their last larval stage and pupate, increasing their chances of survival through the winter.

Evidence of diapause in the family Stratiomyidae is very limited with only one published record found (Rozkosny and Kovac 1998). Stratiomyids typically inhabit tropical regions (Woodley 2001) and the black soldier fly, *Hermetia illucens* (L.) (Diptera: Stratiomyidae) are no exception (Booth and Sheppard 1984). Black soldier fly larvae are naturally robust consisting of 45% protein and 35% fat (Hale 1973). Adults do not feed and therefore the fat stored by the larvae support their adult life span. Aside from the effect of light on mating and oviposition (Tomberlin and Sheppard 2002; Zhang et al. unpublished), to my knowledge, the effects of light and photoperiod on black soldier fly development have not yet been studied. With respect to mating and oviposition, Tomberlin and Sheppard (2002) determined black soldier fly mating occurs 2d after adult emergence, primarily in the morning and requires a minimum light intensity of 60 $\mu mol \bullet m^{-2} \bullet s^{-1}$. Furthermore, they determined artificial light sources used did not facilitate mating whereas natural sunlight exposure did. They determined that the act of oviposition did not require light, however, the effect light has on oviposition success of subsequent generations of *H. illucens* reared under various photoperiods has yet to be determined.

The primary objective of this study was to determine the effect(s) length of light exposure has on *H. illucens* development. I also wanted to determine if light is required for larval development. In the event light is not required for larval development, larvae could be reared in an enclosed area, potentially reducing costs spent in artificial lighting. To our knowledge, this study is the first of its kind to examine the effects the presence or absence of light has on egg and larval development and adult emergence success in *H. illucens*. Furthermore, insect development studies in complete darkness are not widely studied, however, one study found the development of the Eri silk worm, *Attacus ricini* (Boisd.) (Lepidoptera: Saturiniidae) in complete darkness resulted in larger larval weights than those reared in light treatments (Gomaa and Megalla 1974). Based on the aforementioned studies demonstrating that varying lengths of daylight exposure can facilitate or impede larval development (Dolezal and Sehnal 2007; Kamm 1972), I predict different photoperiods will affect *H. illucens* development times such that if diapause is present in the black soldier fly, I would expect the rate of larval development to increase until the diapausal stage of development, in the presence of short days. If diapause is not present in the black soldier fly then I predict the rate of development to not differ greatly between short and long days.

METHODS

Experiment: Egg Eclosion

Source of Larvae

Eggs were collected from a black soldier fly colony housed in a cage (1.8 x 1.8 x 1.8m and 1.5mm mesh screen) maintained in a greenhouse, outside the United States Department of Agriculture – Agriculture Research Service building located outside Texas A&M University in College Station, Texas. Eggs were collected in a three layer, 3 x 5cm corrugated cardboard, held together with Elmer® white glue with 3 x 4mm flutes (tubular holes in cardboard). The three layer corrugated cardboard was taped 5cm above the oviposition substrate with the flutes oriented perpendicular to the substrate. Oviposition substrate was composed of moist-to-liquefied Gainesville diet (5:3:2 hand mixture of wheat bran, alfalfa and corn meal, respectively), (Producers Cooperative Association, Bryan, TX), developed for rearing houseflies (Tomberlin, Sheppard and Joyce 2002; Hogsette 1992). Nutritional content for each raw material of the Gainesville diet used was 15% protein, 2.0% crude fat and 11% crude fibre, 17% protein, 1.5% crude fat and 27% crude fibre, and7% protein, 2.5% crude fat and 4% crude fibre, for wheat bran, alfalfa and corn meal, respectively).

Treatment and Sampling

Each egg-containing corrugated cardboard flute was dissected to separate the egg clusters oviposited per flute. Egg clusters were randomly placed into 30mL clear plastic soufflé cups (Dart P100, Dart Container Corporation, Mason, MI, USA). Each soufflé cup contained one egg cluster (egg contribution from one individual females) and cups were divided evenly among three daylength treatments (0h, 8h and 14h of daylight), multiplied by 10 replicates for an N=30. Replicates for each treatment were placed in a 30-well tray (Bio-Serv, Frenchtown, NJ) and trays were placed in their individual growth chambers (Percival Scientific Inc. Model I-36LLVLC8, Perry, IA), maintained at 27°C, 70% relative humidities (RH) and their corresponding photoperiod, either 0:24, 08:16, or 14:10 [L:D]. Photoperiods of 0, 8 and 12 hours of daylight were chosen based on three factors. First, 12h day lengths in southern Texas and Georgia were long enough to sustain

successful rearing of the black soldier fly (Sheppard et al. 2002). Second, 8h of daylight represents the shorter days of the year in southern Canada (National Research Council Canada, website), which could potentially facilitate diapause, if the black soldier does in fact overwinter. Third, 0h of daylight was chosen to determine if *H. illucens* development requires the presence of light.

Night vision goggles (Eyeclops 2.0, Jakks Pacific) were used to observe progress in the 0:24 [L:D] treatment. Eggs were monitored every 12h for egg eclosion and elapsed time to egg eclosion was determined for each replicate within each daylength treatment. Two Hobo® U12-012 data loggers (Onset® Computer Cooperation, Massachusetts, USA) were placed in each growth chamber; one on the top shelf and one on the bottom shelf, to record relative humidity, temperature and light intensity variation within the growth chamber and across growth chambers. The data logger on the top shelf also had an external temperature probe which recorded temperature on the middle shelf.

0h Daylight Growth Chamber Modification

The growth chamber housing the 0h daylight treatment was externally modified with a 1.22 x 1.83 x 2.13m three sided tent to prevent light from contaminating the 0h daylight treatment when opening the growth chamber door for feeding and observation purposes. The tent frame was crafted using 1 inch PVC piping and 3-way PVC connectors (Home Depot®, College Station, TX). The tent walls were constructed using black and white poly plastic (Discount Garden Supply, Riverside, CA). The plastic walls and ceiling were taped together over the PVC frame with black Gorilla Brand® duct tape and white 3M Brand® duct tape (Home Depot®, College Station, TX). Seams were taped from both sides to reduce light penetration. The open end of the tent was taped to the top, sides and bottom of the growth chamber, ensuring no ventilation vents were covered, allowing the chamber to sit 30cm inside the tent. The tent was designed with the black side of the poly plastic on the inside of the tent, while the white side faced the outside of the tent to reflect light away from the enclosed growth chamber. The bottom of the walls were tucked under the PVC frame and taped to the cement floor with black Gorilla Brand® duct tape (Home Depot®, College Station, TX) on the inside of the tent. With respect to entry into the tent, a double door design composed of two layers of same poly plastic comprising the walls and ceiling of the tent was implemented such that the

external door can be semi-sealed upon entry before complete entry through a second door into the enclosed tent housing the growth chamber.

Statistics

Software

All statistics were computed using SAS JMP® version 8.0.1 statistical software, with the exception of nonparametric two-way Kruskal-Wallis tests, which used SPSS version 14.0 statistical software by Dr. Michelle R. Sanford, University of California-Davis and the pairwise post hoc analyses were completed using SPSS version 18.0 statistical software by Dan Edelstein, University of Windsor, Academic Data Centre.

Egg Eclosion:

The data failed to meet the assumptions of normality (homogeneity of variance and normality goodness-of-fit) despite data transformation attempts ($\log_{10}$ and Box Cox transformations). Thus, a non-parametric one-way Wilcoxon/Kruskal-Wallis (Ranked Sums) test was used to test treatment effects on the mean time to egg eclosion. Pair-wise comparisons using Wilcoxon/Mann-Whitney U tests were conducted on significant results. Alpha values were adjusted using the Dunn-Sidak procedure to correct for type I error as a result of multiple comparisons (Quinn and Keough, 2002, p 48).

Post-Feeding and Pupal:

The data failed to meet the assumptions of normality (homogeneity of variance and normality goodness-of-fit) despite a Box Cox transformation and therefore two independent full factorial non-parametric two-way Kruskal-Wallis (Ranked sums) tests were used to test treatment, sex and the interaction of treatment and sex effects on the length of post-feeding and pupal stages of development. Pairwise comparisons using Mann-Whitney U tests were adjusted for type I error using the Dunn-Sidak procedure.

Two independent full factorial Cox Proportional Hazard tests (model effects: sex, treatment and sex*treatment) were used to compare the effect of treatment on survivorship with respect to the length of the post-feeding and pupal stages of development.

Experiment: Adult Emergence

Source of Larvae

Eclosed larvae from the preceding effect of daylength on egg eclosion study were used to determine the effects on adult emergence. Replicates of each treatment were never subjected to light exposures outside their respective growth chambers. Upon egg eclosion, 10g aliquots of the same Gainesville diet used previously as an oviposition substrate (Tomberlin et al. 2002; Hogsette, 1985), mixed with 18mL of water (70% moisture, Tomberlin et al. 2002) was fed to the larvae *ad libitum* for the first 6d of development. Water was also added *ad libitum* to day-old feed to prevent moist feed from drying out.

Treatments and Sampling

A random hand-selected sub-sample of 50 6-day-old larvae was removed from the preceding replicates of hatched neonatal larvae to control the sample size for each replicate in each treatment for a total of 50 larvae per replicate, per treatment. All observations, feeding and sub-sampling in the 0h daylight treatment was done so within the confines of the poly plastic tent, preventing exposure to visible light rays. Feeding regimes of 10g of dry Gainesville diet aliquots mixed with 18mL of water continued *ad libitum* under the same treatment conditions as those in the egg eclosion experiment of 27°C, 70% RH and their respective daylength treatments 0:24, 08:24, or 14:10 [L:D] and were monitored once daily (0800h) for post-feeding larvae, pupation and adult emergence. Upon reaching post-feeding stages of development, post-feeding larvae were placed into individual 30mL clear plastic soufflé cups (Dart P100, Dart Container Corporation, Mason, MI, USA). Cups were placed into a 30-well clear plastic tray (Bio-Serv, Frenchtown, NJ) and returned to their respective growth chamber. Upon pupation, pupae in their 30mL plastic soufflé cups were capped with a clear plastic lid (100PCL25 Dart Container Corporation, Mason, MI, USA) and pierced with a 10cc (29 gauge**)** syringe. Elapsed time in the post-feeding and pupal stages of development, successful adult emergence, adult longevity and larval mortality were determined.

Statistics

Successful Adult Emergence:

Adult emergences were counted and analyzed as proportions, however, performing an arcsine transformation and a Box-Cox-Transformation did not normalize my data set and therefore a non-parametric one-way Wilcoxon/Kruskal-Wallis (Ranked sums) test was used to test treatment effects on percent successful adult emergences. Pairwise comparisons using Wilcoxon/Mann-Whitney U tests were completed and adjusted for type I error using the Dunn-Sidak procedure.

Adult Survivorship:

The data failed to meet the assumptions of normality (homogeneity of variance and normality goodness-of-fit) despite a Box Cox transformation. Thus, two independent full factorial non-parametric two-way Kruskal-Wallis (Ranked sums) tests were used to test treatment, sex and the interaction of treatment and sex effects on adult longevity. Pairwise comparisons using Wilcoxon/Mann-Whitney U tests were completed and adjusted for type I error using the Dunn-Sidak procedure.

A full factorial Cox Proportional Hazard test (model effects: sex, treatment and sex*treatment) was used to compare the effect of treatment on survivorship with respect to adult longevity.

Abiotic Factors Recorded

Two Hobo® U12-006 data loggers (Onset® Data Loggers) recorded temperature, relative humidity and light intensity on the top and bottom shelves within each growth chamber. The resulting output read a mean (±SE) temperature and relative humidity of 26.44±0.01ºC, 72.43±0.02%RH, 27.08±0.01ºC, 74.75±0.06%RH, and 28.02±0.02ºC, 74.75±0.08%RH on the top shelf and 26.53±0.01ºC, 71.49±0.02%RH, 27.41±0.02ºC, 74.95±0.07%RH, 28.73±0.02ºC, 72.07±0.08%RH, on the bottom shelf for each treatment, 0h, 8h and 12h of daylight, respectively.

The mean (±SE) light intensity for the 0h treatment was 2.82±0.05Lux and 1.94±0.24Lux on the top and bottom shelves, respectively. The mean (±SE) light intensity for the 8h and 12h daylight treatments during daylight hours was 2536.08±16.87Lux and 4924.88±18.96Lux for the top shelf, and 6207.38±19.05Lux and

6698.48±24.07Lux for the bottom shelf, respectively. The mean (±SE) light intensity for the 8h and 12h daylight treatments during non daylight hours was 12.10±0.03Lux and 15.49±0.10Lux for the top shelf, and 11.80±0.00Lux and 11.70±0.02Lux for the bottom shelf, respectively.

Temperature and relative humidity did vary across treatments *(χ^2 = 4710.5961, df 2, p < 0.0001* and *χ^2 = 1643.0382, df = 2, p < 0.0001,* respectively*).* The 0h of daylight treatment had lower temperatures and relative humidities than the 8h and 12h treatments. The 12h of daylight treatment had higher temperatures than the 8h treatment, but did not differ in relative humidities (Table 4.1).

RESULTS

Experiment: Egg Eclosion and Adult Emergence

Egg Eclosion

Mean (±SE) time to egg eclosion differed between treatments, 0h, 8h and 12h, (Table 4.2, Figure 4.1, $\chi^2 = 20.57$, $df = 2$ $p < 0.001$). Eggs in the 12h treatment took less time to eclose than eggs in the 8h and 0h treatments, but there was no difference in egg eclosion times between the 0h and 8h treatments, ($\chi^2 = 1.6836$, $df = 1$, $p=0.1945$).

Post-feeding and Pupal Development

Total post-feeding larval and pupal mortality for the 0h, 8h and 12h was 4.60±2.23%, 0.20±0.20%, 0.0±0.0% and 4.97±%1.23, 4.00±0.79% 3.20±0.61%, respectively. Mortalities in the post-feeding stage of development were higher in the 0h daylight treatment ($\chi^2 = 8.9870$, $df = 2$ $p = 0.0112$), however mortalities in the pupal stage of development did not differ among treatments ($\chi^2 = 1.8558$, $df = 2$, $p = 0.3954$).

Although there was no treatment*sex interaction effect on the time it took post feeding larvae to pupate ($\chi^2 = 2.0590$, $df = 2$ $p = 0.3572$), both main effects of treatment (Table 4.3, $\chi^2 = 660.1800$, $df = 2$, $p < 0.0001$) and sex ($\chi^2 = 10.2780$, $df = 1$ $p = 0.0012$) independently influenced the length of the post feeding stage. Post-feeding larvae in the 0h treatment took longer to pupate than those in either of the 8h and 12h treatments, regardless of sex (Figure 4.2, $\chi^2 = 454.3929$, $df = 1$ $p < 0.0001$ and $\chi^2 = 509.8959$, $df = 1$ $p < 0.0001$, respectively). Post-feeding larvae in the 8h treatment took longer to pupate than those in the 12h treatment, regardless of sex ($\chi^2 = 136.1754$, $df = 1$ $p < 0.0001$). With respect to sex, males took longer to pupate than females, regardless of treatment (Figure 4.3, $\chi^2 = 42.0310$, $df = 1$ $p < 0.0001$).

There was an interaction between treatment and sex on the baseline survival in the post-feeding stage of development (Figure 4.4, $\chi^2 = 11.1714$, $df = 2$ $p=0.0038$) such that males spent more time in the post-feeding stage of development than females, larvae in the 0h treatment took longer to pupate than the 8h and 12h treatments and post-feeding larvae in the 8h treatment took longer than the 12h treatment, with the exception of

females in the 0h and 8h treatments, which did not differ from males in the 12h treatment.

The interaction between treatment and sex on the baseline survival had no effect on the pupal stage of development *(χ^2 = 3.6840, df = 2 p = 0.1585)*, however both treatment (Table 4.4, χ^2 = 203.9980, *df* = 2 *p* < *0.0001*) and sex (χ^2 = 10.0330, *df* = 1 *p* = 0.0015) independently influenced the amount of time spent in the pupal stage. Pupae in the 0h treatment took longer to emerge than both the 8h and 12h treatments, regardless of sex, (Figure 4.2, χ^2 = 139.7973, *df* = 1 *p* < 0.0001 and χ^2 = 175.3156, *df* = 1 *p* < 0.0001, respectively). There was no difference in pupation times between 8h and 12h treatments, regardless of sex, (χ^2 = 0.5520, *df* = 1 *p* = 0.4575). With respect to sex, females took longer to emerge than males, regardless of treatment, (Figure 4.3, χ^2 = 10.2124, *df* = 1 *p* = *0.0061*).

There was no interaction between treatment and sex on the baseline survival in the pupal stage of development (χ^2 = 3.6293, *df* = 2 *p*=0.1629), however both treatment and sex independently influenced survival (Figure 4.5, χ^2 = 159.5779, *df* = 2 *p*<0.001 and χ^2 = 4.5424, *df* = 1 *p* = 0.0331, respectively) , such that a greater proportion of adults in the 0h treatment survived over a longer period of time, regardless of sex, while a greater proportion of females survived over a longer period of time, regardless of treatment.

Adult Emergence

Mean successful adult emergence for 0h, 8h and 12h treatments was 73.00±3.00%, 95.80±0.76% and 96.80±0.61%, respectively. Successful adult emergence differed between treatments, (*χ^2 = 20.4526, df = 1 p < 0.001*). The 0h treatment had lower successful adult emergence than both 8h and 12h, (*χ^2 = 14.5594, df = 1 p < 0.001* and *χ^2 = 14.4487, df = 1 p < 0.001*), respectively). There was no difference in adult emergence success between 8h and 12h, (*χ^2 = 1.4905, df = 1 p < 0.2221*).

The sex ratio for each treatment was, 48.86% to 51.14%, 49.05% to 50.95% and 48.14% to 51.86%, males to females in the 0h, 8h and 12h treatments, respectively. Sex ratio was not different between treatments, *(χ^2 = 0.3780, df = 2, p = 0.8276)*.

Treatment and sex interacted to influence adult longevity *(*Table 4.5, Figure 4.6, *χ^2 = 10.0440, df = 1, p < 0.0066)* such that males in all treatments lived longer than females in all treatments, with the exception of females in the 0h and 8h daylight

treatments, which did not differ from the males in the 12h daylight treatment and females in the 0h treatment did not differ from females in the 8h treatment.

Baseline survival in adult longevity was affected by the interaction of treatment and sex (Figure 4.7, $\chi^2 = 20.5596$, $df = 2$ $p < 0.0001$) such that a greater proportion of males lived longer than females, with a higher proportion living longer in the 0h treatment than in the 8h and 12h of daylight and a higher proportion living longer in the 8h treatment than in the 12h treatment, with the exception of females in the 0h and 8h treatments which did not differ from each other or from males in the 12h treatment.

The percent of virgin females to lay non-viable eggs in the 0h, 8h and 12h of daylight treatments (N = 178, 239 and 251, respectively), was 25.28%, 19.67% and 11.16%, respectively. The number of virgin females to lay eggs was different across photoperiod treatments ($\chi^2 = 13.294$, $df = 2$, $p = 0.0013$). Virgin females reared in complete darkness laid more eggs than those reared in 12h of daylight ($\chi^2 = 14.5150$, $df = 1$ $p < 0.0001$). In addition, virgin females reared in 8h of daylight laid more eggs than those reared in 12h of daylight ($\chi^2 = 6.8900$, $df = 1$ $p = 0.0087$), however, the number of virgin females to oviposit did not differ between the 0h and 8h of daylight treatments ($\chi^2 = 1.8590$, $df = 1$ $p = 0.1728$).

DISCUSSION

Experiment: Egg Eclosion and Adult Emergence

Dolezal et al. (2007) found the Colorado potato beetle developed faster when reared at short day photoperiods (12h of daylight) than when reared under long day photoperiods (18h of daylight). However, they noted that development times in the first larval instar did not differ between short or long day photoperiods. In addition, as mentioned, females typically oviposit in dry crevices near the food resource and these crevices may naturally be shielded from light to prevent desiccation. Therefore, although my results indicate longer periods of daylight (12h) may shorten the time to egg eclosion, since the temperature in this treatment was 1-2ºC warmer than the other two treatments, this increase in temperature may explain the increased development time in the 12h daylight treatment and may not necessarily be attributed to the daylength exposure.

Post-Feeding and Pupal Development

It is not clear why the absence of light caused the post-feeding larval stage of development to vary so much in the 0h of daylight treatment. If diapause had been invoked in the post-feeding larval stage, it is not clear what signalled the larvae to pupate or emerge, since abiotic conditions did not change over the course of the experiment. It may be possible that *H. illucens* utilizes a biological clock based on some genetically controlled time mechanism, similar to that suggested by Rozkosny and Kovac (1998) in other Stratiomyids.

More commonly, studies examining the effects of daylength on insect development tend to observe one of two things: 1) insects develop faster at decreasing photoperiods resulting in diapausal adults as occurs in the eight-toothed spruce bark beetle, *Ips typographus* (L.) (Coleoptera: Scolytidae), (Dolezal and Sehnal 2007); or 2) insect development does not change when reared under decreased photoperiods and diapause is not induced such as in *Orius niger* (Wolff), (Hemiptera: Anthocoridae) (Bahsi and Tunc 2008). However, these findings can vary drastically depending on the experimental design. Experimental designs looking strictly at decreasing daylight exposure at constant photoperiods are less likely to induce diapause whereas insects reared at a steady decrease in daylength photoperiod (i.e. x number of minutes of daylight

reduced per day) can stimulate diapause (Kamm 1972). Therefore, as defined by photoperiodism, exposing insects to a steady decrease in daylength will likely provide more indicative results on the parameters that induce diapauses in insects.

In addition, a higher proportion of mortality occurred in the post-feeding stage of development in the 0h treatment than all other treatments. I have deduced four plausible explanations for this: 1) post-feeding larvae wandered excessively long periods before pupating in complete darkness and therefore some of these larvae may have exhausted their fat resources and inevitably died; 2) it is possible that the post-feeding larvae wandering for excessive amounts of time suffered increased water-loss due to increased respiration in the post-feeding stage of development; 3) Arachnid and Blattidae populations inside the 0h of daylight growth chamber were substantially higher than in the 8h and 12h growth chambers. I observed in one cup (each cup contains a single larva) in the 0h treatment, the presence of a cockroach egg capsule *Periplaneta americana* (L.) (Blattodea: Blattidae), instead of the post-feeding larva originally placed in the cup. The missing post-feeding larva may or may not have been predated upon by the American cockroach, however this larva was not included in my analysis. I also suspect the possibility of other post-feeding larval fatalities to be an act of Arachnid predation due to the over abundance of arachnid activity in the 0h growth chamber and the dead post-feeding larvae collected were flattened and hollow-like; evident of fat body removal. However, this is merely my observations, and since mortalities in the daylight treatments were not dissected for comparison, I cannot confirm the aforementioned mortalities in the 0h treatment were a result of arachnid predation; 4) it may also be possible that post-feeding larvae in the 0h treatment did not reach their critical weight required to pupate before being pulled from their food resource. Beetle larvae of, *Anoplophora glabripennis* (Motschulsky) (Coleoptera: Cerambycidae) cannot pupate successfully until the larvae feed to a critical weight (Keena 2005). Post-feeding larvae in this experiment that may not have achieved their critical weight could possibly be a matter of human error. Using the night vision goggles did take some getting used to, deciphering the position of the goggles to reduce shadows in the Infrared light rays (not visible to the human eye), casted by the observer. Therefore, some of the post-feeding larvae appeared dark (post-feeding colouration) when in actuality, they still had not moulted their last moult, however this

confusion was completely rectified by the second day of the first sign of post-feeding larvae, affecting only 10, or 2% of the post-feeding larvae.

The presence of light significantly affected the pupal stage of development, specifically, those pupae reared in the presence of light emerged 1-1.5 days before pupae reared in complete darkness. It is not clear why pupae reared in complete darkness only took a day to day and a half longer to emerge than those reared in the presence of light but perhaps the adults are able to detect infrared light. Shintani et al. (2009) found the carabid beetle, *Leptocarabus kumagaii* (Kimura & Komiya) (Coleoptera: Carabidae), when exposed to white light after surgical removal of the compound eye, is unable to respond to photoperiodism. They also determined the stemmata in the immatures enable the larvae to respond to photoperiodism, but the stemmata derived organs in the adult brain are not necessary for photoperiodism (Shintani, Shiga and Numata 2009). Perhaps, in *H. illucens,* the stemmata in the larvae cannot detect certain wavelengths of light. In the same study, Shintani et al. (2009) found that some larvae did not respond to photoperiods of blue, green or yellow light. Therefore the photoreception of the stemmata may differ from the photoreception of the compound eye. *Hermetia illucens* differ from other Dipteran species reared in colony in that mating is not stimulated by some artificial lights (Tomberlin and Sheppard 2002; Zhang et al.unpublished) and therefore the compound eye of the black soldier fly may be unique in its photoreception. As pupae complete metamorphosis and the photoreceptors in the compound eye develop, infrared light rays emitted from the night vision goggles may be detectable by the non-emerged adult and serve as an emergence cue.

Sex was determined when adults emerged, however since each post-feeding larva was placed individually in a cup, sex effects on the post-feeding and pupal stages of development can be determined. Tomberlin et al. (2009) found females took longer to emerge than males, suggesting females spend more time feeding, resulting in higher weights and increased fecundity. However, random, hand-selected females in this experiment did not feed longer than males, but did emerge after males. This could possibly be a pre-emptive measure of increased fecundity by being sought out quickly by males that have already emerged and therefore time and resources are not wasted waiting for males to emerge.

Adult Longevity

Similar to effects on pupation, the presence of light affected adult longevity. Pupae reared in the presence of light had higher rates of emergence success than pupae reared in complete darkness. However, the unsuccessful adult emergence in the 0h treatment are primarily due to post-feeding larval mortality and failure to pupate as discussed earlier; energy exhaustion, water-loss, predation and not attaining critical weights required for pupation caused potentially by human error. In addition, adult longevity increased with decreasing exposure to light with adults living the longest in complete darkness. Adults are stimulated by light and in addition, sunlight is required for successful mating (Tomberlin and Sheppard 2002). Thus adults reared in the presence of light are more stimulated, and were observed flying around their tiny cups within minutes of the lights turning on inside the growth chamber. On the other hand, adults reared in complete darkness were very docile throughout the duration of their adult life such that even with the disruption of tapping the cups to assess for mortality, the adults did not appear alarmed and instead of flying around the cup, would simply walk around the cup. Therefore, it is possible adults stimulated by light, observed by flying around the cups, exhausted more energy resources than those reared in complete darkness and as a result did not live as long.

Additionally, more females exposed to decreased hours of daylight (8h and 0h) oviposited more non-viable eggs than those females reared in 12h of daylight. As a comparison, less than 1% of virgin females in Tomberlin et al. (2002) laid non-viable eggs, whereas 18% of virgin females laid non-viable eggs in my study. If successful oviposition by virgin females is a sign of increased fecundity, then these results suggest females reared in 12h of daylight have decreased fecundity when compared to females reared in 8h or 0h of daylight. However, because I did not determine mating and oviposition success on adults reared at decreased hours of daylight (0h and 8h), I cannot attest to higher fecundity in virgin female oviposition reared in 0h and 8h of daylight because of the possibility of inducing reproductive diapause in decreased hours of daylight (0h and 8h).

Males lived longer than females, regardless of treatment. In comparison, males and females (not provided water) reared in 12h of daylight in this study lived longer at

9.71d and 8.61d, respectively, than those males and females (not provided water) in Tomberlin et al. (2002) at 6.0d and 6.1d, also reared with the same diet, at 27°C, 60-70% RH, but reared in a photoperiod of 14:10 [L:D]. However, as previously mentioned, Tomberlin et al. (2002) used wild caught black soldier flies where as my flies have been in colony for 8 years at 27°C and a 12:12 [L:D] photoperiod and therefore my flies in colony may have acclimated to these conditions with improved development.

In summary, photoperiod significantly affected *H. illucens* development. Although black soldier flies reared in complete darkness took several weeks longer than those reared in the presence of light to complete development, 73% did successfully complete development. In addition, because the development time in the 0h treatment varied from 30d and 40d (similar to the 8h and 12h treatments) to over a 100d, it is difficult to draw any conclusions on whether diapause was induced or not. Based on previous discussions, it would be of interest to determine if *H. illucens* photoreceptors can detect infrared light rays. Such a study would provide insight into our results and perhaps pupae may have entered diapause had infrared light rays not been introduced via the night vision goggles. In addition, black soldier flies reared in 8h of daylight did develop slightly slower (4d slower) than those reared in 12h of daylight, however pupation, adult longevity, and inferred fecundity based on virgin female oviposition was comparable between the two treatments. Despite my findings, it would be necessary to allow adults subjected to these experimental conditions to mate before inferring the presence or absence of reproductive diapause. As this study did not look at incrementally decreasing or increasing daylength, it would be difficult to assess diapause induction in immatures without subjecting them to progressively decreasing photoperiods. Regardless, it is not recommended that *H. illucens* be reared in complete darkness in anticipation of a mass rearing facility.

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TABLES

Table 4.1

Wilcoxon / Kruskal-Wallis Tests: Ranked sums comparing the temperature, relative humidity and light intensity between photoperiod treatments 0h, 8h and 12h. Dunn-Sidak adjusted $\alpha = 0.0169$.

Treatment Interactions	Z	ChiSquare ($x10^2$)	Prob>ChiSquare
Temperature			
0h * 8h	40.17	16.13	p < 0.0001*
0h * 12h	62.19	38.67	p < 0.0001*
*8h * 12h*	*38.97*	*15.18*	*p* < 0.0001*
Relative Humidity			
0h * 8h	40.34	16.27	p < 0.0001*
0h * 12h	25.30	6.40	p < 0.0001*
*8h * 12h*	*-1.62*	*0.0026*	*p = 0.1052*
Light Intensity			
0h * 8h	77.56	60.16	p < 0.0001*
0h * 12h	-3.79	14.6832	p = 0.0001*
*8h * 12h*	*3.76*	*14.4707*	*p = 0.0001**

Table 4.2

Wilcoxon / Kruskal-Wallis Tests: Ranked sums comparing the time to egg eclosion between photoperiod treatments 0h, 8h and 12h. Dunn-Sidak adjusted $\alpha = 0.0169$.

Treatment Interactions	Z	ChiSquare	Prob>ChiSquare
0h * 8h	-1.26	1.6836	p = 0.1945
0h * 12h	-3.79	14.6832	p = 0.0001*
8h * 12h	3.76	14.4707	p = 0.0001*

Table 4.3

Wilcoxon / Kruskal-Wallis Tests: Ranked sums comparing the mean (±SE) time in the post-feeding stage of development between photoperiod treatments 0h, 8h and 12h with respect to treatment. Dunn-Sidak adjusted $\alpha = 0.0169$.

Treatment Interactions	Z	ChiSquare	Prob>ChiSquare
0h * 8h	21.31	454.3929	p < 0.0001*
0h * 12h	22.58	509.8959	p < 0.0001*
8h * 12h	11.66	136.1754	p < 0.0001*

Table 4.4

Wilcoxon / Kruskal-Wallis Tests: Ranked sums comparing the mean (±SE) in the pupal stage of development between photoperiod treatments 0h, 8h and 12h with respect to treatment. Dunn-Sidak adjusted $\alpha = 0.0169$.

Treatment Interactions	Z	ChiSquare	Prob>ChiSquare
0h * 8h	11.82	139.7973	p < 0.0001*
0h * 12h	13.24	175.3156	p < 0.0001*
8h * 12h	0.74	0.5520	p = 0.4575

Table 4.5

Wilcoxon / Kruskal-Wallis Tests: Ranked sums comparing the mean (±SE) adult longevity between photoperiod treatments 0h, 8h and 12h, with respect to treatment and the interaction of treatment and sex. Dunn-Sidak adjusted α for the treatment and treatment and sex interaction pairwise comparisons are 0.0169 and 0.0034, respectively.

Treatment Interactions	Z	ChiSquare	Prob>ChiSquare
0h * 8h	2.73	7.4643	p = 0.0063*
0h * 12h	10.01	100.24	p < 0.0001*
8h * 12h	9.43	88.8814	p < 0.0001*
M @ 0h * M @ 8h	4.64	21.5387	*p < 0.0001**
M @ 8h * M @ 12h	-6.71	44.9963	*p < 0.0001**
M @ 12h * M @ 0h	9.09	82.6303	*p < 0.0001**
M @ 0h * F @ 0h	7.89	62.2705	*p < 0.0001**
M @ 0h * F @ 8h	8.79	77.3226	*p < 0.0001**
M @ 0h * F @ 12h	11.80	139.3421	*p < 0.0001**
M @ 8h * F @ 0h	-5.83	34.0298	*p < 0.0001**
M @ 8h * F @ 8h	-6.97	48.6501	*p < 0.0001**
M @ 8h * F @ 12h	11.44	131.1063	*p < 0.0001**
M @ 12h * F @ 0h	1.54	2.3845	*p = 0.1225*
M @ 12h * F @ 8h	-1.47	2.1520	*p = 0.1424*
M @ 12h * F @ 12h	4.19	17.5322	*p < 0.0001**
F @ 0h * F @ 8h	0.41	0.1706	*p = 0.6796*
F @ 8h * F @ 12h	6.21	38.6018	*p < 0.0001**
F @ 12h * F @ 0h	5.83	33.9762	*p < 0.0001**

FIGURES

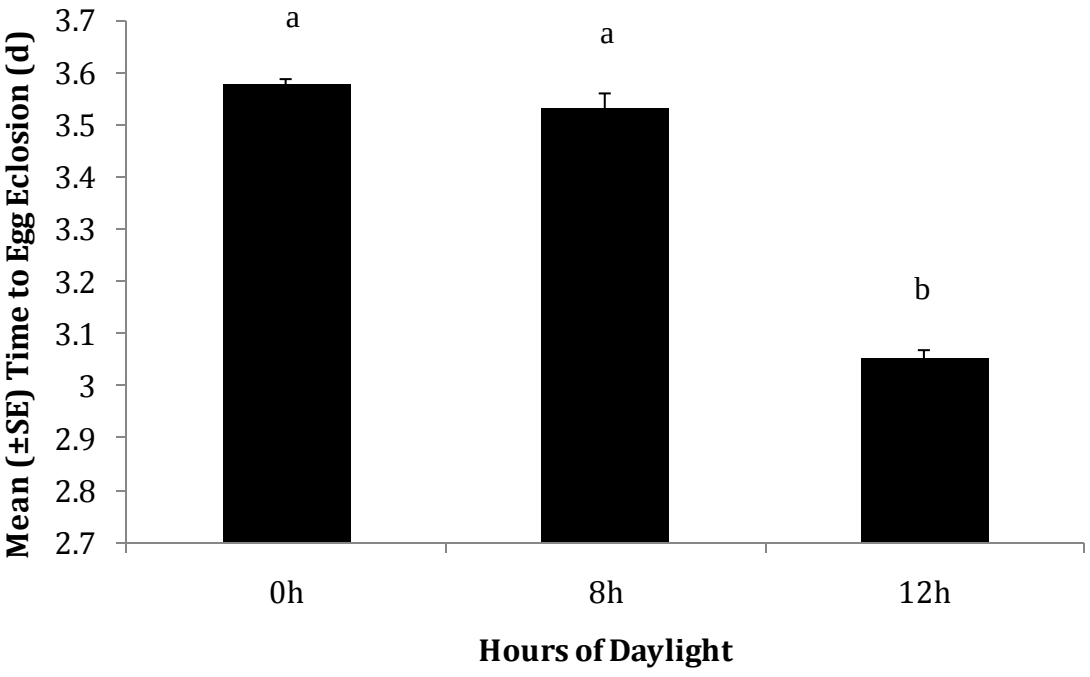


Figure 4.1

Mean (±SE) time to egg eclosion for the 0h, 8h and 12h treatments. Means followed by the same letter are not different (P>0.05).

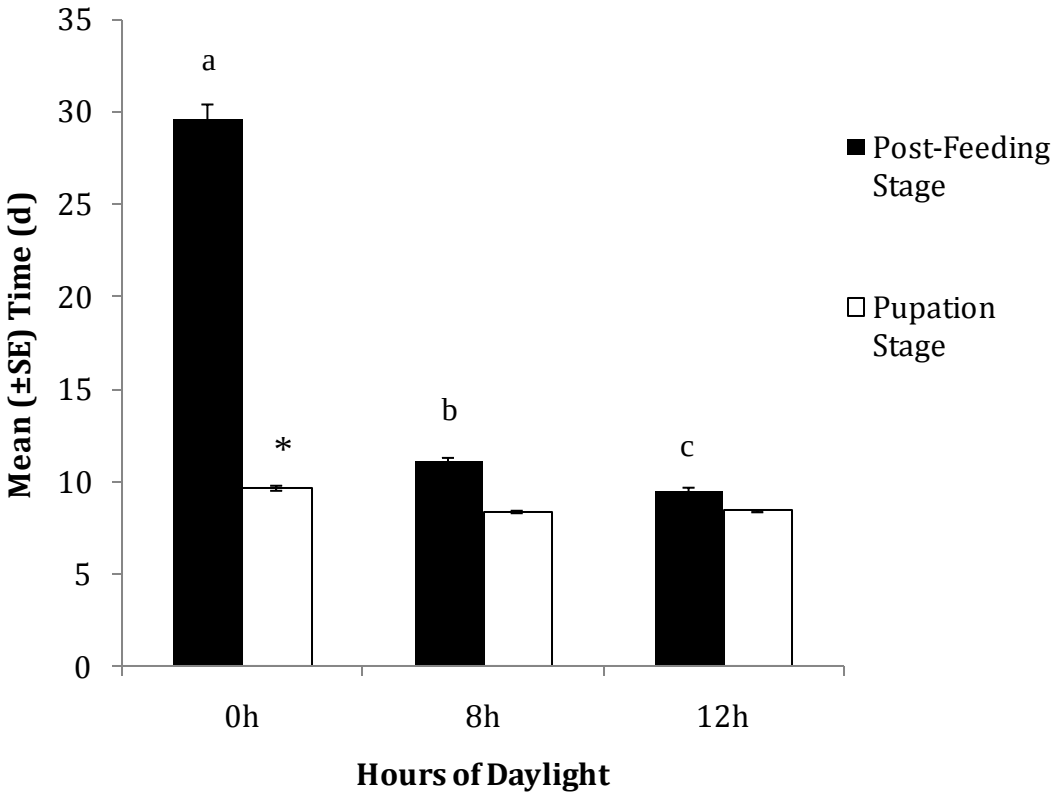


Figure 4.2

Mean (±SE) time in each stage of immature development. Mean times in the post-feeding stage of development followed by the same letter are not different *(P>0.05)*. Mean times in the pupal stage of development followed by an asterisks are different (P<0.05).

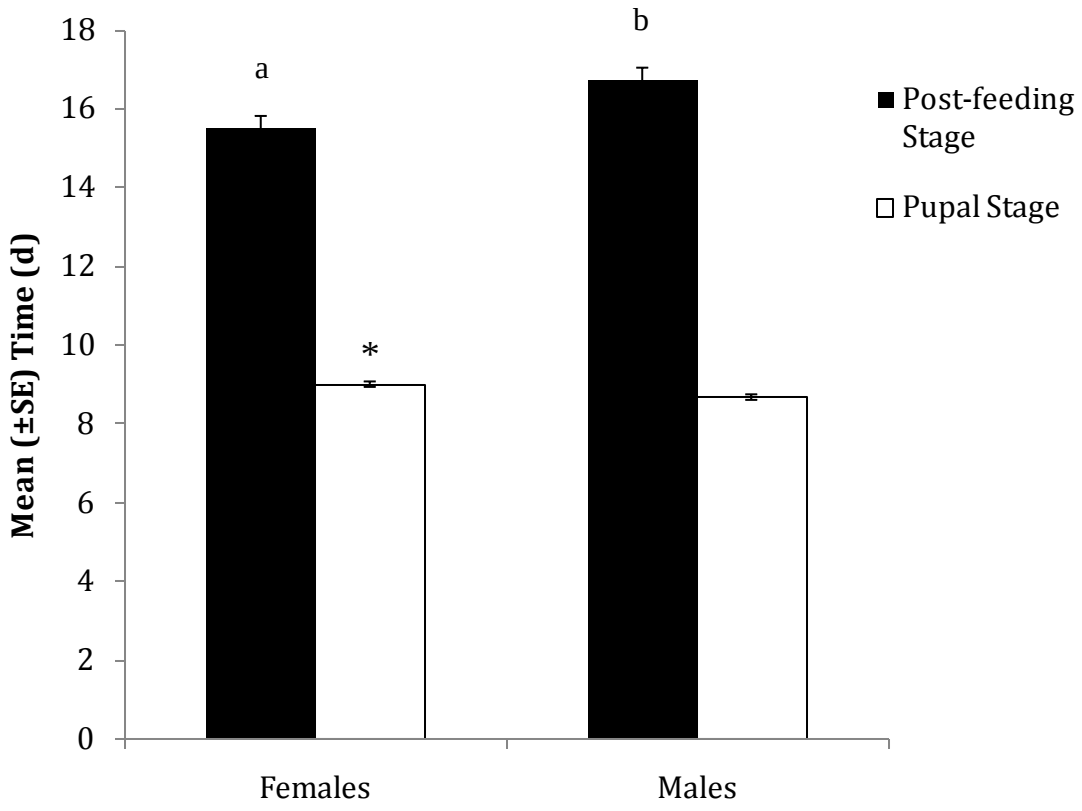
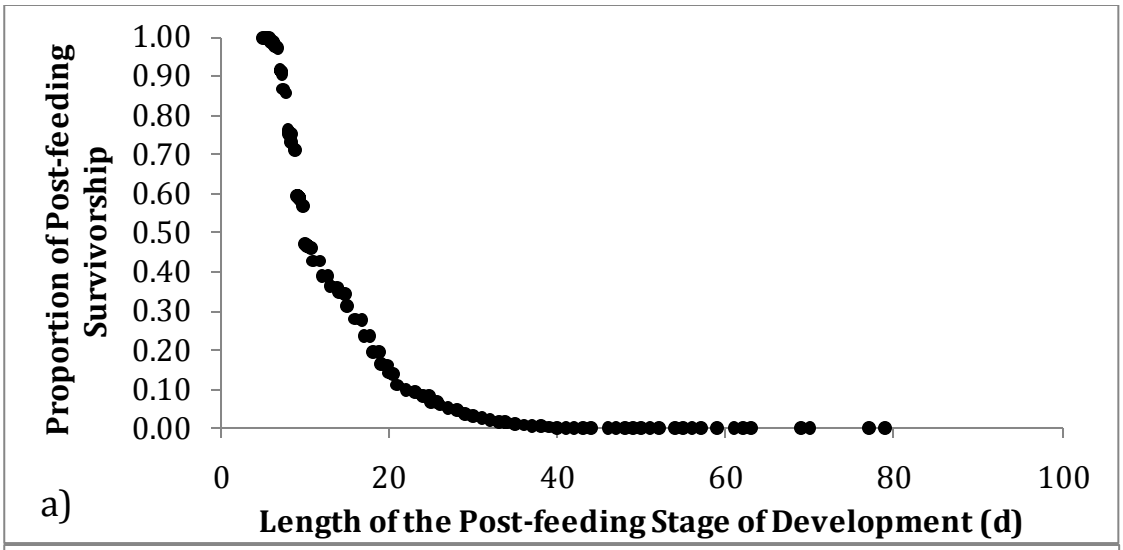
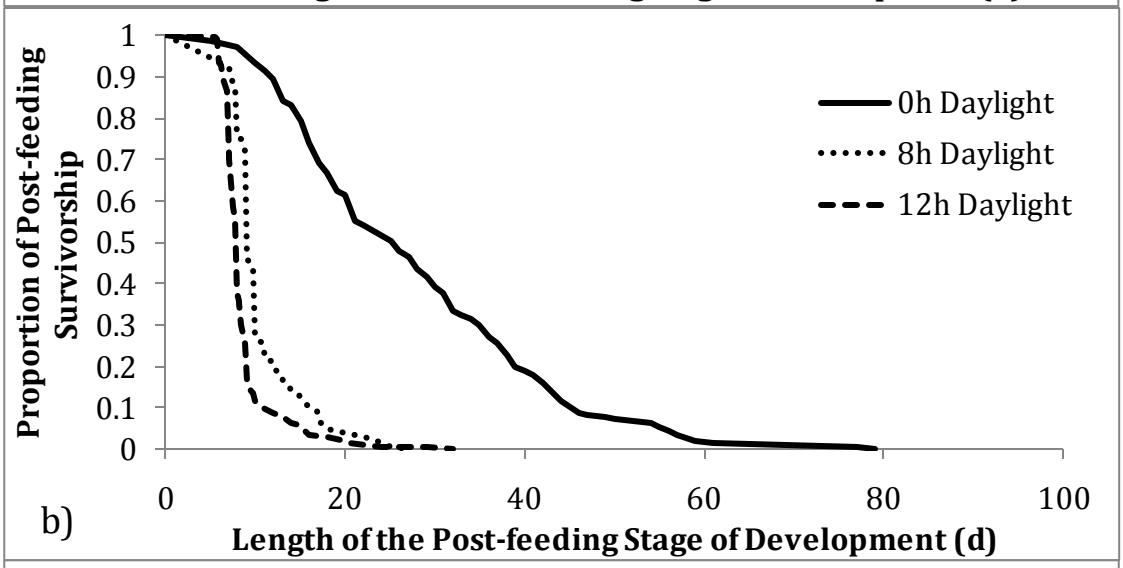


Figure 4.3

Mean (±SE) time in the post-feeding and pupal stages of development with respect to sex. Mean times in the post-feeding stage of development followed by the same letter are not different *(P>0.05)*. Mean times in the pupal stage of development followed by an asterisks are different (P<0.05*)*.


Proportion of Post-feeding Survivorship
1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
0
20
40
60
80
100
a)
Length of the Post-feeding Stage of Development (d)


Proportion of Post-feeding Survivorship
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0h Daylight
8h Daylight
12h Daylight
0
20
40
60
80
100
b)
Length of the Post-feeding Stage of Development (d)

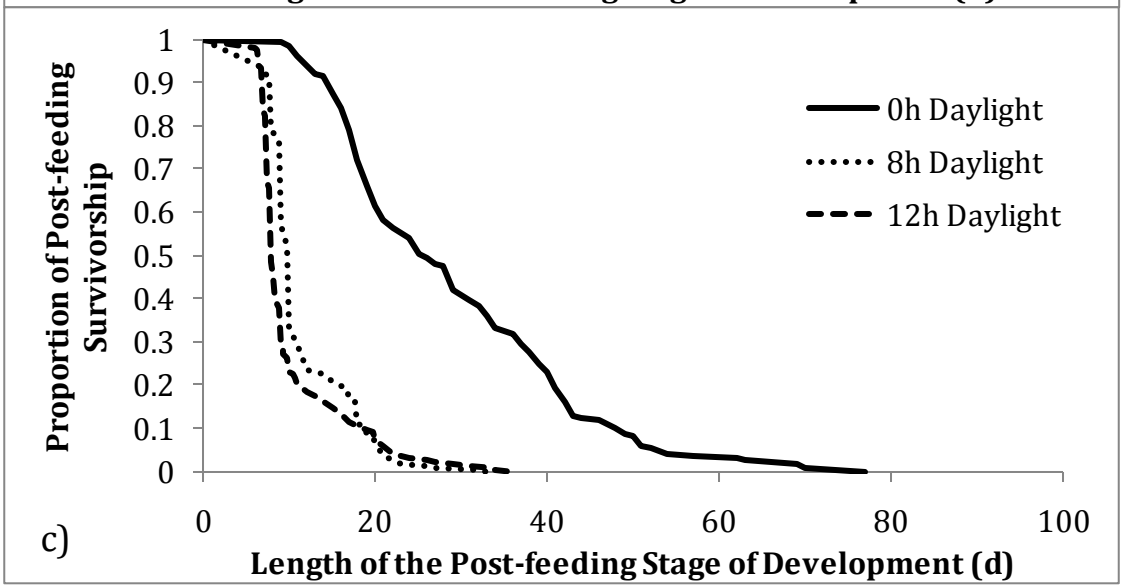
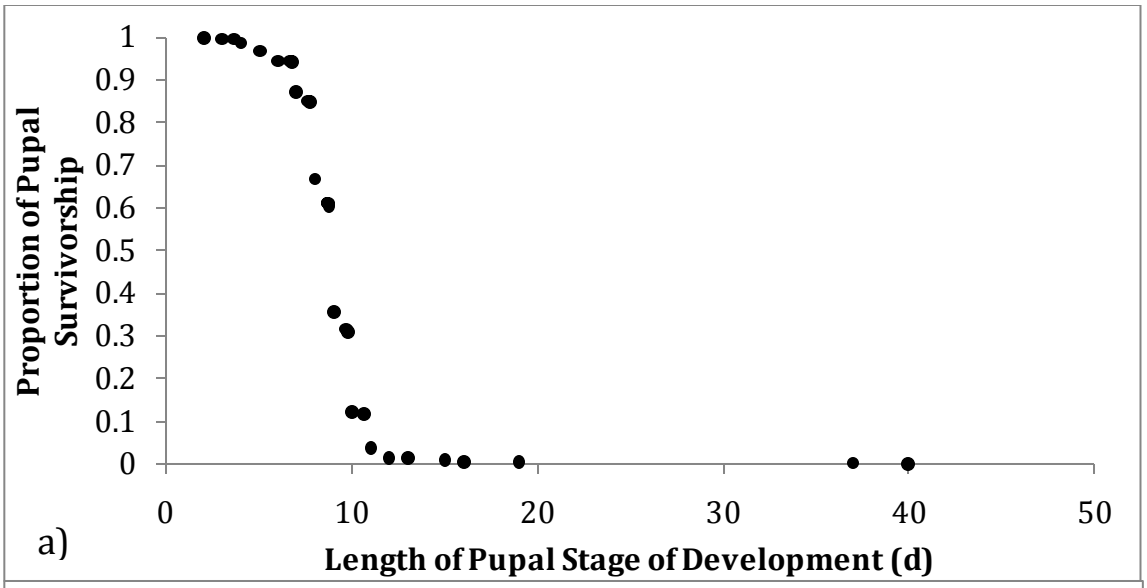
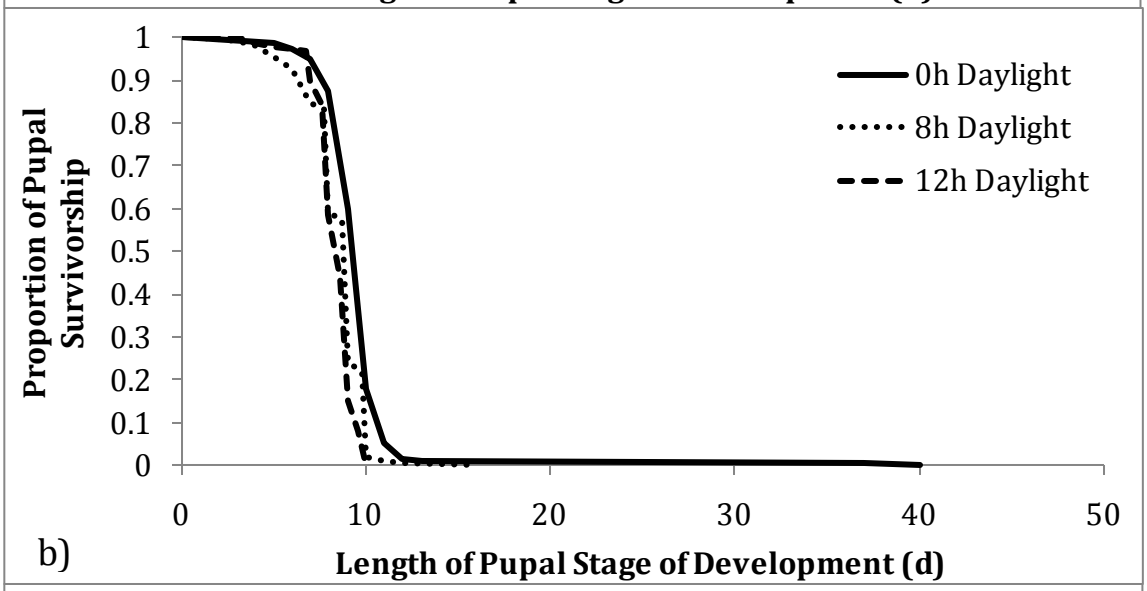

Proportion of Post-feeding Survivorship
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0h Daylight
8h Daylight
12h Daylight
0
20
40
60
80
100
c)
Length of the Post-feeding Stage of Development (d)

Figure 4.4

Cox Proportional Hazard fits indicating baseline survivorship throughout the post-feeding stage of development. The y-axis represents the proportion of post-feeding larvae surviving x number of days (x-axis). a) Combined survival curve at the mean: treatments (0h, 8h and 12h) and sex (males and females) in the post-feeding stage of development. b) Female survival curve for treatments 0h, 8h and 12h for larvae in the post-feeding stage of development. c) Male survival curve for treatments 0h, 8h and 12h for larvae in the post-feeding stage of development.


Proportion of Pupal Survivorship
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
10
20
30
40
50
a)
Length of Pupal Stage of Development (d)


Proportion of Pupal Survivorship
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
10
20
30
40
50
0h Daylight
8h Daylight
12h Daylight
b)
Length of Pupal Stage of Development (d)

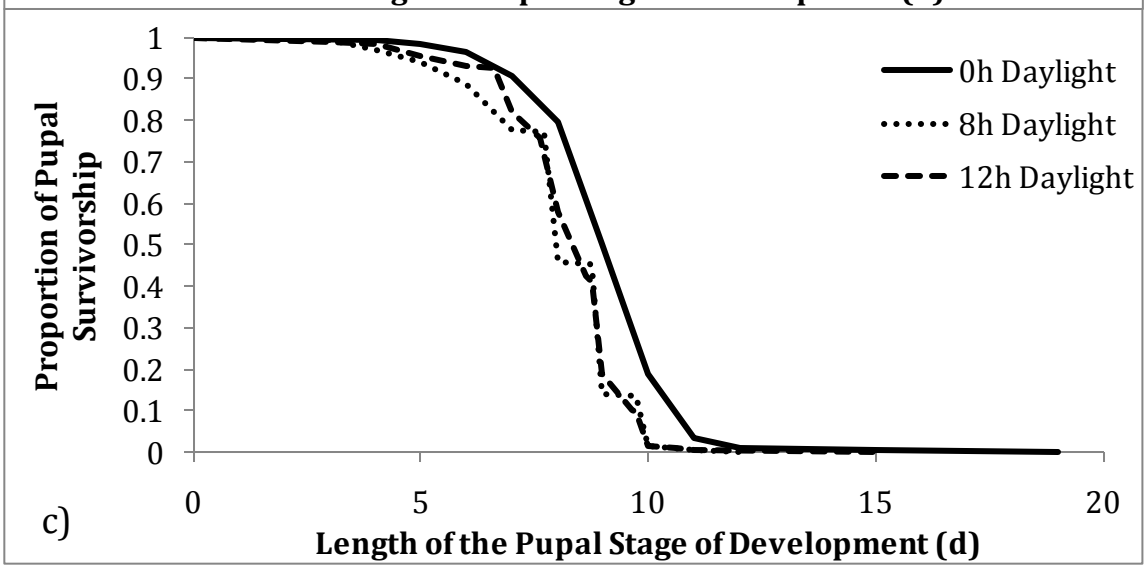

Proportion of Pupal Survivorship
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
5
10
15
20
0h Daylight
8h Daylight
12h Daylight
c)
Length of the Pupal Stage of Development (d)

Figure 4.5

Cox Proportional Hazard fits indicating baseline survivorship throughout the pupal stage of development. The y-axis represents the proportion of pupae surviving x number of days (x-axis). a) Combined baseline survival at the mean: treatments (0h, 8h and 12h) and sex (males and females) in the pupal stage of development. b) Female survival curve for treatments 0h, 8h and 12h for pupae in the pupal stage of development. c) Male survival curve for treatments 0h, 8h and 12h for pupae in the pupal stage of development.

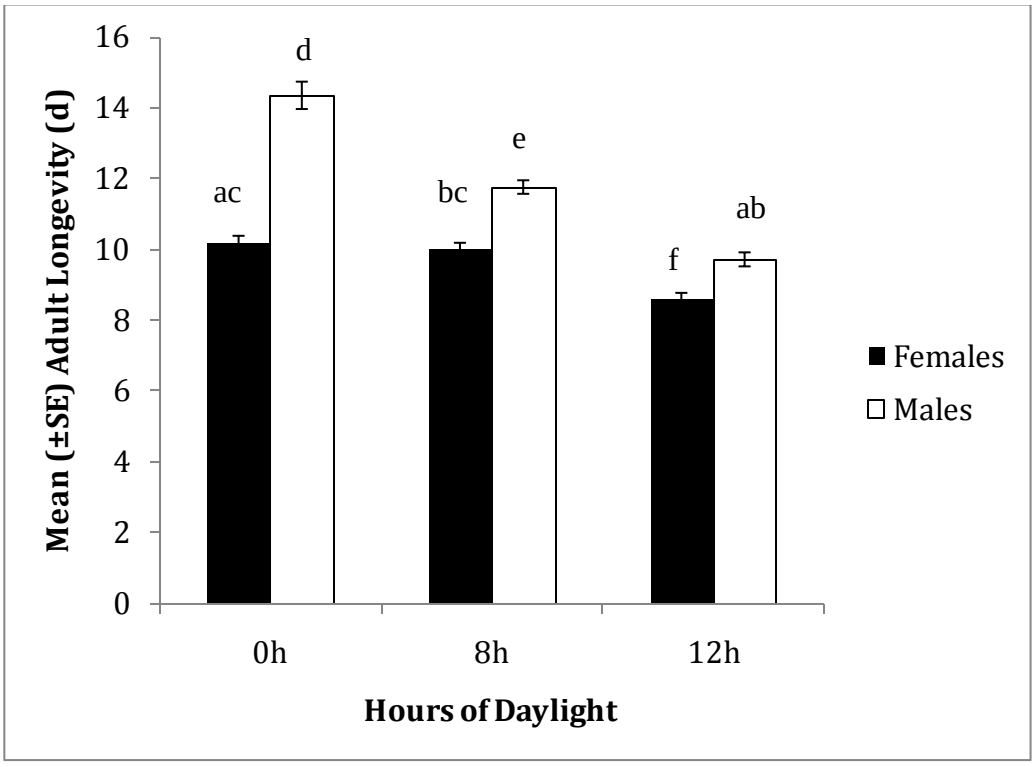


Figure 4.6

Mean (±SE) adult longevity in days across daylight treatments, 0h, 8h and 12h. Mean adult longevities followed by the same letter are not different *(P>0.05)*. Males lived significantly longer than females, regardless of treatment, (χ^2 = 110.5830, *df* = 1 *p*<0.001).

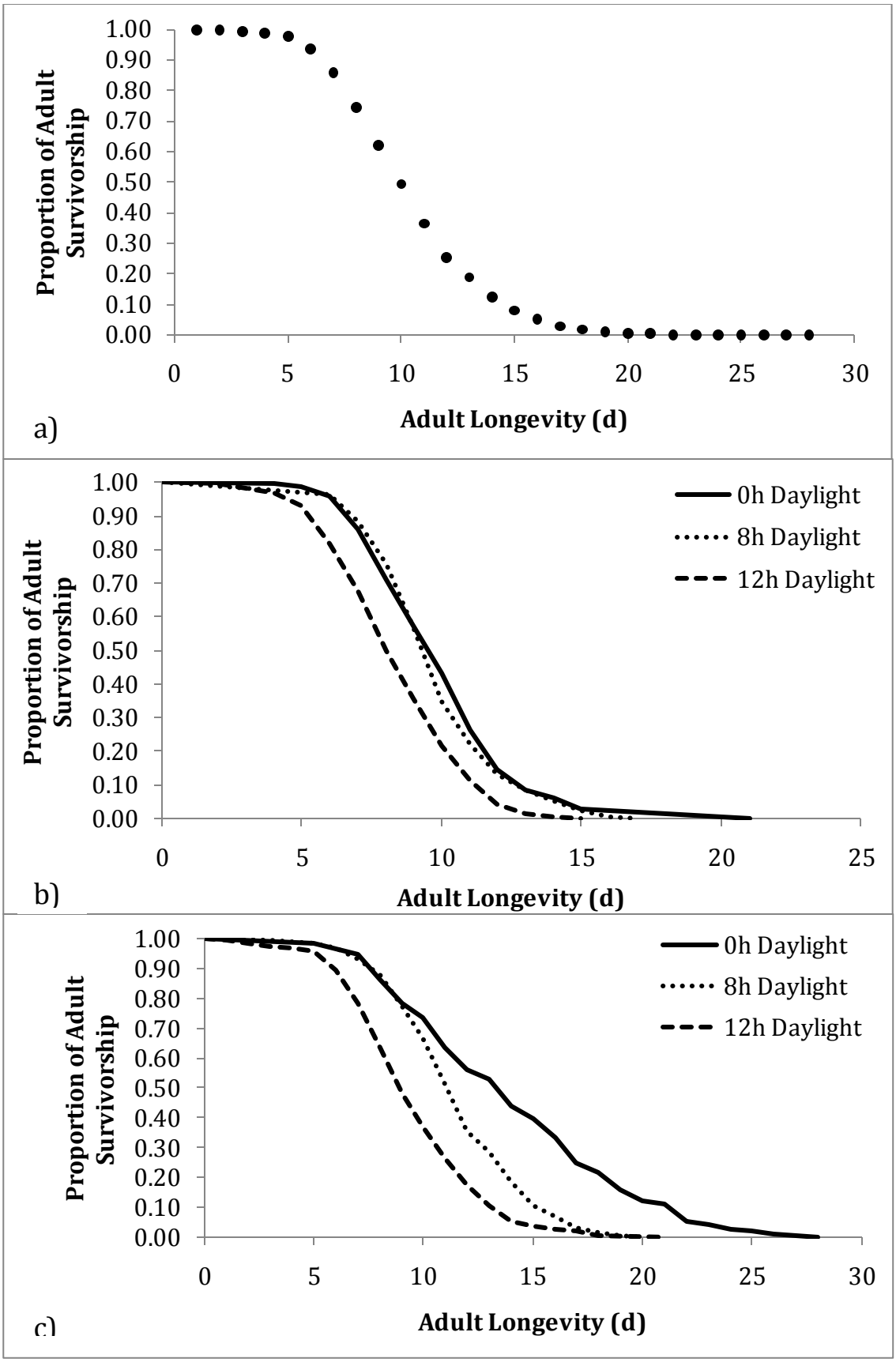

Proportion of Adult Survivorship
1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
0
5
10
15
20
25
30
Adult Longevity (d)
a)
0h Daylight
8h Daylight
12h Daylight
b)
c)

Figure 4.7

Cox Proportional Hazard fits indicating baseline survivorship at the mean for adult longevity. The y-axis represents the proportion of adults surviving x number of days (x-axis). a) Combined adult longevity survivorship curve: treatments (0h, 8h and 12h) and sex (males and females). b) Female adult longevity survivorship curve for treatments 0h, 8h and 12h. c) Male adult longevity survivorship curve for treatments 0h, 8h and 12h.

CHAPTER V

Effect of Temperature on Egg Eclosion and Adult Emergence

INTRODUCTION

Ectotherms rely on heat energy from their environment for growth and development. Ambient temperature largely regulates their metabolism and rate of development (Jarosik et al. 2004). Insect habitats can vary drastically, thriving in extreme environments due to an adaptive suite of behavioural and physiological mechanisms. However, mechanisms of tolerance to the most extreme habitats are not well understood (Dixon et al. 2009). Even across very large geographic distances, insects of the same species can have different physiological tolerances to the same unfavourable temperatures, with populations in the northern latitudes having better tolerances to low temperatures than populations in southern latitudes (Dixon et al. 2009). Higher latitude inhabiting insects are more temporally versatile than those in lower latitudes as a result of greater temperature variation in higher latitudes than regions at lower latitudes (Addo-Bediako, Chown and Gaston 2000). It has been proposed using forecast modelling that as climate change progresses and the Earth's temperature increases, those terrestrial ectotherms in the southern latitudes surrounding the equator where little temperature variation occurs will suffer drastic decreases in diversity as those insects intolerable to temperature increases will not survive (Deutsch et al. 2008). Insects in the northern latitudes will also be affected, however, their variable tolerances to changing temperature will better equip them to acclimate as climate warming continues.

In climates that undergo periods of below freezing temperatures insects will employ cold hardiness strategies either by freeze avoidance or freeze tolerance (Bale and Hayward 2010). For example, the temperate hoverfly, *Syrphus ribesii, (L.)* (Diptera: Syrphidae) (Hart and Bale 1997) can survive stressful temperatures as low as -25°C. Insects can also employ mechanisms of diapause, which as defined in a review by Bale and Hayward (2010), is a genetically programmed pre-emptive response to seasonal change in which insects make preparation for a prolonged period of low resources and

reduced metabolism to survive winter temperatures. This is often indicated to the insects via changes in daylength as opposed to temperature because this is a cue that precedes the actual time of reduced temperature (Bale and Hayward 2010). However, McWatters and Saunders (1998) found that temperature can modulate diapausal cues in insects inhabiting 51°N compared to 65°N in times when low temperatures are evident during long days. Furthermore, *Calliphora vicina* (Robineau-Desvoidy) (Diptera: Calliphoridae) inhabiting a latitude of 51°N have a diapause cue at 14.5h of daylight, whereas inhabitants at a latitude of 65°N have a diapause cue at 16.5h of daylight (McWatters and Saunders 1998). This change in latitude and the differential response in diapausal cues are indicative of longer winters occurring at higher latitudes. On the other hand, temperature, rather than photoperiod, is one of the main driving forces invoking diapause in tropical insects because daylength does not vary much throughout the year at these latitudes (Bale and Hayward 2010; Tauber, Tauber and Masaki 1986).

The black soldier fly, *Hermetia illucens (L.)* (Diptera: Stratiomyidae) has a native range that includes the southeastern United States (Sheppard et al. 2002; Tomberlin, Sheppard and Joyce 2002), Europe, Asia as far north as 45°N latitude, the northern United States including, New York, Pennsylvania, New Jersey and Maryland (Leclercq 1997) and Canada, particularly in southern Ontario (Morgan Jackson and Steven Paiero, University of Guelph, personal communication). Black soldier flies are well adapted to tropical and subtropical environments and therefore it is not known if *H. illucens* inhabiting the temporal regions during the summer months are capable of diapause, migrating south for the winter, or perhaps have the ability to expand and contract their tolerance to temporal range on an annual basis through acclimation. Furthermore, if diapause is invoked, the abiotic factors serving as cues to initiate this process are not known.

Despite *H. illucens'* greater prevalence in tropical and subtropical habitats, waste management industries throughout the temporal regions of the United States have shown much interest in *H. illucens* and their ability to consume organic waste, and demonstrated promise in bio-conversion industries (Diener, Zurbrugg and Tockner 2009; Myers et al. 2008; Sheppard and Newton 2000; Sheppard et al. 1994; Booram et al. 1977). As a result, black soldier fly colonies have been successfully maintained in different parts of

temperate North America on a year round basis. Dr. Craig Sheppard has successfully reared a continuous black soldier fly colony (>3 years) in Tifton, Georgia year round in an outdoor greenhouse facility, using the natural ambient photoperiod of the sun (Sheppard et al. 2002). In Stephenville, Texas, Dr. Jeffery Tomberlin has successfully maintained a colony using a similar facility (Tomberlin, Adler and Myers 2009). More recently, operations have harvested black soldier flies for the purpose of animal feedstuff in Idaho (St-Hilaire et al. 2007) and a colony has been maintained in Windsor, Ontario, Canada. Additionally with the use of indoor facilities and artificial lighting, efforts are ongoing in Yellow Springs, Ohio to maintain a colony for waste management and food supplementation (Glen Courtright, personal communication).

Utilizing the black soldier fly in temperate regions of Canada for the purpose of year round waste management may be a promising proposition since the success in operations at bovine, poultry and swine facilities in the southern United States (Diener et al. 2009, Myers et al. 2008, Sheppard and Newton 2000; Sheppard et al. 1994; Booram et al. 1977). However, determining the abiotic parameters limiting black soldier fly development is crucial for determining the potential for mass rearing and the economic costs associated with the energy input required for maintaining an active population of this insect through the Canadian winter when it would not normally be active.

The primary objective of this study was to determine the lower temperature developmental threshold for black soldier fly development and the effect of cool temperatures on black soldier fly development to determine the lowest operating temperatures for a fully functional facility through the winter.

The life history of the black soldier fly has been studied with respect to diet (Tomberlin et al. 2002) and temperature (Tomberlin et al. 2009) within the range of 27°C to 36°C. It was determined that 27°C was optimal for growth and development, and that temperature is directly proportional to their rate of development (Tomberlin et al. 2009). Therefore, I predicted that development would cease close to 12°C and the rate of development would drastically decrease at 15°C and 18°C when compared to development at 27°C. With that in mind I sought to determine the lower developmental threshold of the black soldier fly to determine the heat input required to maintain a year-round actively feeding population in Canada.

METHODS

Experiment: Egg Eclosion

Source of Eggs

Eggs were collected from a black soldier fly colony housed in a screen mesh cage (1.8 x 1.8 x 1.8m with 1.5mm mesh) maintained outdoors, outside the Forensic Laboratory for Investigative Entomological Sciences (F.L.I.E.S.) Facility located at Texas A&M University in College Station, Texas. Eggs were collected in a 3-layer, 3 x 5cm corrugated cardboard rectangle, held together with Elmer® white glue such that the 3 x 4mm flutes (tubular holes in cardboard) can be used as an oviposition substrate. These were taped with the flutes perpendicular to the oviposition substrate, 5cm above the oviposition medium. Oviposition medium was composed of moist-to-liquefied Gainesville diet (5:3:2 hand mixture of wheat bran, alfalfa and corn meal, respectively), (Producers Cooperative Association, Bryan, TX), developed for rearing houseflies (Hogsette 1992, Tomberlin et al. 2002). Nutritional content for each raw material of the Gainesville diet used was 15% protein, 2.0% crude fat and 11% crude fibre, 17% protein, 1.5% crude fat and 27% crude fibre, and7% protein, 2.5% crude fat and 4% crude fibre, for wheat bran, alfalfa and corn meal, respectively).

Treatments

Each egg-containing corrugated cardboard flute was dissected to determine the number of egg clusters oviposited per flute. Egg clusters were randomly placed into 30mL clear plastic soufflé cups (Dart P100,Dart Container Corporation, Mason, MI, USA) placed into 680mL clear plastic sandwich containers (Rubbermaid® TakeAlongs, USA). Each soufflé cup contained two egg clusters (egg contribution from two individual females) and cups were divided evenly among three separate I-36LLVLC8 Percival Scientific Growth Chambers®. All three growth chambers were maintained at 70% relative humidity and a 14:10 [L:D] dial cycle, but with unique temperature settings representative of each treatment (12°C, 15°C and 18°C respectively). Each temperature treatment was composed of 24 replicates, for an N = 72. Temperatures in this study were chosen based on preliminary experiments on the black soldier fly using the egg stage of

development only. It was evident that black soldier fly eggs could eclose at 18°C and with winter temperatures in southern Ontario often below 18ºC; 12ºC and 15ºC were chosen for comparisons.

Hobo® U12-006 data loggers (Onset® Computer Cooperation, Massachusetts, USA) with 3 temperature probes were placed in each growth chamber (one probe per shelf) to record potential temperature variation every 15 minutes within the growth chamber and across growth chambers.

Eggs were checked for eclosion twice daily. Upon first eclosion, eggs remained in their respective growth chambers under treatment conditions for an additional two days to allow the entire egg clutch to fully eclose. Two days after the first egg eclosion, empty and or non-viable egg clusters were stored in a -25°C chest freezer for assessment of successful egg eclosion. Time to egg eclosion and percent successful egg eclosion was recorded for each replicate within each temperature treatment.

Statistics

All statistics were computed using SAS JMP® version 8.0.1 statistical software. The data failed to meet the assumptions of normality (homogeneity of variance and normality goodness-of-fit) despite box-cox transformation, thus a non-parametric one-way Wilcoxon/Kruskal-Wallis test (Ranked Sums) was used to test both treatment effects on the mean time to egg eclosion (including only those replicates to eclose in the analysis) as well as the mean percent successful egg eclosion, independently.

Experiment: Adult Emergence

Source of Larvae

Eclosed larvae from the preceding effect of temperature on egg eclosion study were used to determine temperature effects on adult emergence. Upon egg eclosion, 10g aliquots of Gainesville Diet (Tomberlin et al. 2002; Hogsette 1992) mixed with 18mL of water (70% moisture), (Tomberlin et al. 2002) was fed to the larvae *ad libitum* until development to the post-feeding stage. Water was also added *ad libitum* to day-old feed to prevent drying and desiccation.

Treatments and Sampling

Larvae continued development under the same treatment conditions as those in the egg eclosion experiment (70% RH, 14:10 [L:D] photoperiod and their respective temperature; 12°C, 15°C and 18°C respectively) and monitored daily for pupation and adult emergence. Upon pupation, pupae were placed into 30mL plastic soufflé cups (Dart P100, Dart Container Corporation, Mason, MI, USA) and capped with a clear plastic lid (Dart 100PCL25, Dart Container Corporation, Mason, MI, USA), pierced with a 10cc (29 gauge) syringe. Upon emergence, adults were provided water in 0.125mL aliquots *ad libitum* at daily observations using a 10cc (29 gauge) syringe to maximize adult longevity (Tomberlin et al. 2002). Time to adult emergence, percent successful adult emergence and larval mortality was determined for each replicate per treatment. Adult longevity was also determined for the first and last 10% of emerged adults per replicate per treatment.

Statistics

Statistical comparisons for adult emergence were not conducted due to the 18°C treatment being the only treatment to complete development to the adult stage. Instead, time to adult emergence and successful adult emergence are reported for the 18°C treatment. Adult longevity was determined for the first and last 10% of the total number of adults to emerge using a non-parametric one-way Wilcoxon/Kruskal-Wallis (Ranked Sums) test. Sex ratio was also compared using a non-parametric one-way Wilcoxon/Kruskal-Wallis (Ranked Sums) test.

Abiotic Factors Recorded

A Hobo® U12-006 data logger (Onset® Data Loggers) recorded the temperature within each growth chamber, a separate probe on each shelf for a total of 3 temperature recordings per treatment, every hour. The resulting output read a mean temperature of 11.71±0.02°C, 16.03±0.03°C and 19.02±0.02°C, for each treatment, 12°C, 15°C and 18°C, respectively. A nonparametric one-way Kruskal-Wallis was used to verify temperature treatments were significantly different, ($\chi^2 = 2031.4358$, $df = 2$ $p<0.001$, Table 5.1).

Relative humidity was not recorded via data loggers, however output readings on the growth chamber read ~70% (± humidity loss when doors were open) throughout the

course of the experiment. Daylength was not recorded via data loggers either, however lights were programmed to turn on at 0700h and turn off at 2100h. Furthermore, light intensity for these growth chamber models were ~115 $\mu mol \bullet m^{-2} \bullet s^{-1}$, as per the manufacturer's specifications for these specific models.

Accumulated Degree Hours Calculation

Development data from chapters II and III were used to calculate the number of accumulated degree hours for each stage of development in *H. illucens*. Accumulated degree hours were calculated using the following equation:

$$\sum (T_h - T_b)$$

Where T_h is the mean hourly temperature and T_b is the base temperature; in this case, two calculations were completed using 10ºC and 15ºC as base temperatures; 10ºC being the standard base temperature used and 15ºC being my determined base temperature (beyond egg eclosion). When the mean hourly temperature was below the base temperature, zero accumulated degree hours were accumulated. Accumulated degree hours were calculated for each stage of development, using the minimum, mean and maximum times of development (Table 5.2).

RESULTS

Experiment: Egg Eclosion

The number of eggs in each replicate was evaluated to ensure consistency across treatments and was not different across treatments ($F_{2,42} = 2.3154$, $p = 0.1112$). The percentage of replicates to successfully eclose in the 18°C, 15°C and 12°C treatments was 100%, 73% and 0% respectively. After 30d, eggs in the 12°C treatment were observed under a dissection microscope to determine viability, however, the eggs were completely collapsed and deemed not viable.

Mean (±SE) time to egg eclosion for those replicates reared at 15°C was 15.26±0.11d, taking longer to eclose than eggs reared at 18°C, which eclosed in 7.75±0.02d ($\chi^2 = 18.5764$, $df = 1$ $p < 0.001$). Mean (±SE) successful egg eclosion for all replicates in the 12°C, 15°C and 18°C treatments was, 0±0.00%, 12.85±3.32% and 75.40±2.88%, respectively. The percent successful egg eclosion for eggs reared at 18°C was higher than for eggs reared at 15°C and 12°C, (Table 5.3, $\chi^2 = 37.4321$, $df = 2$ $p<0.001$). Unexpectedly, successful egg eclosion at 18°C was comparable to egg eclosion success at 25°C, which had a mean percentage of 76±2.28 (chapter II). Furthermore, hatched neonate larvae in the 15°C treatment suffered 100% mortality within 3d of eclosion.

Experiment: Adult Emergence

Mean (±SE) time to adult emergence was 72.07±0.08d for the 18°C treatment. With a mean (±SE) larval mortality and pupal mortality of 63.53±7.16% and 16.47±2.76%, respectively, the overall mean (±SE) percent successful adult emergence was 31.90±6.56%.

Male and female emergence distributions were not different, however, the total emerged adult population (N=2426) was male biased with 62.98% males, (Figure 5.0, $\chi^2 = 27.1639$, $df = 1$, $p < 0.0001$). The mean (±SE) adult longevity calculated for the first and last 10% of emerged females and males (N=233) was 25.37±0.58d and 25.47±0.53d, respectively. Male and female longevity did not differ ($\chi^2 = 0.0012$, $df = 1$, $p = 0.9727$),

however, a male biased sex ratio of 61.37% was also evident within this sub population ($\chi^2 = 9.6009$, $df = 1$, $p = 0.0019$).

Although not statistically tested, using development data from the preceding studies in this thesis (*See chapters III and IV*), the trends across temperatures with reference to accumulated degree hours (ADH) are compared. Accumulated degree days were calculated based on the assumed lower development threshold for most insects (10°C), as well as the determined lower development threshold (15°C), (Table5.1).

DISCUSSION

My results indicate two distinct lower developmental thresholds; one for successful egg eclosion and one for successful adult emergence. Eggs successfully eclosed at 15°C and 18°C, however hatched neonate larvae reared at 15°C did not develop beyond 3d. The degree of development was not confirmed for these neonate larvae, but larvae were noticeably larger upon death than when first eclosed (personal observation). A fraction of the hatched neonate larvae reared at 18°C did successfully pupate and emerge.

Adult longevity was dramatically longer in 18°C versus rearing at higher temperatures; for example in colony rearing and the preceding experiments in this thesis (*see chapters II and III, as well as* Tomberlin et al. (2009)). Because black soldier flies do not feed as adults, their energy is acquired entirely via feeding during the larval stages. It is possible that 18°C is too cold to catabolise these energy reserves for flight, mating or in the case for females, for egg production, enabling them to live longer. Furthermore, Tomberlin et al. (2009) noted that length of larval development is an excellent predictor of adult longevity for the black soldier fly such that the longer the larval development, the longer the longevity of the emerged adult.

It is hypothesized by some that temperature and the rate of development is a linear relationship, and that the proportion of time spent in each stage of development is independent of temperature, thus in a state of isomorphy (Jarosik, Honek and Dixon 2002). If temperature and rate of development are truly isomorphic, then minimum and maximum thresholds can theoretically be calculated using linear regression models because isomorphic insects have common lower developmental threshold for all stages of development (Jarosik et al. 2002). Accumulated degree hours vary drastically depending on the lower development threshold used and the lower developmental threshold, or the temperature below which insect development stops, is species specific (Amendt et al 2007). My results demonstrate the need for determining insects' lower development threshold, with accumulated degree hours more than doubled when using 10ºC as opposed to 15ºC as a base temperature, it is important to have accurate lower

development thresholds when predicting insect development based on temperature and accumulated degree hours. In addition, using an accumulated degree hour model to predict black soldier fly development, one could extrapolate this information to anticipate development and waste conversion in a waste management system. Knowing the accumulated degree hours required to reach certain life stages over a range of constant temperatures, climate control in a waste management system can be further manipulated to optimize waste consumption.

The theoretical lower development threshold and thermal constant K, have been calculated for each stage of development (egg, larval and pupal) in several insects using temperature and rate of development in conjunction with linear and non-linear regression models. These insects include an aphidophagous ladybeetle, *Propylea dissecta* (Mulsant) (Coleoptera: Coccinellidae), the codling moth, *Cydia pomonella* (L.) (Lepidoptera: Tortricidae), four Hawaiian fruit flies, *Bactrocera cucurbitae* (Coquillett) (Diptera: Tephritidae), *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae), *Bactrocera latifrons* (Hendel) (Diptera: Tephritidae) and *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) and blow flies, *Calliphora* vicina (Robineau-Desvoidy) (Diptera: Calliphoridae) and Protophormia *terraenovae* (Robineau-Desvoidy) (Diptera: Calliphoridae), (Aghdam et al. 2009; Donovan et al. 2006; Omkar and Pervez 2004; Grassberger and Reiter 2002; Vargas et al. 1996). However, experimental confirmation of these theoretical lower development thresholds is not evident. Furthermore, the calculated lower development threshold is lower in the larval stage than in the egg and pupal stages in the aforementioned examples; the codling moth and Hawaiian fruit flies, as well as in the African fig fly, *Zaprionus indianus* (Gupta) (Diptera: Drosophilidae), (Nava et al. 2007). In contrast, the calculated lower development threshold is lowest in the egg and pupal stages of development in the tephritid fruit fly in Africa, *Bactrocera invadens* (Drew, Tsuruta & White) (Diptera: Tephritidae), (Rwomushana et al. 2008) and the Rattlebox moth, *Utetheisa ornatrix* (L.) (Lepidoptera: Arctiidae), (Signoretti et al. 2008) using experimental methods. It is important to assess the accuracy of these calculated lower developmental thresholds first hand by subjecting the insect to the calculated lower developmental threshold for verification.

In my study, the lower developmental threshold for *H. illucens* is likely between 12°C and 15°C for eggs and between 15°C and 18°C for the larvae. Lower developmental thresholds may depend on the insect's temporal variation, their number of generations per year and their cold hardiness (Signoretti et al. 2008). For example, the codling moth overwinters in its last larval stage (Higbee, Calkins and Temple 2001), therefore it would be beneficial for the larvae to have a lower development threshold for the larval stage of its development, such that early instar larvae may still have time develop to the last larval stage prior to overwintering.

It is not known if *H. illucens* overwinters, however during the first attempt of rearing a colony in the winter in Windsor, Ontario, Canada, *H. illucens* development was only successful to the pupal stage. When adult emergence still had not occurred weeks after pupation, it was hypothesized that the pupae had desiccated, since an artificial photoperiod theoretically conducive to *H. illucens* development was in place. However, months later, the following spring, the same pupae believed to have dissecated began to emerge, suggesting an induction of diapause several months earlier (personal observation). Furthermore, the oriental soldier fly, *Ptecticus flavifemoratus* (Rozkosny & Kovac) (Diptera: Stratiomyidae: Sarginae) in Malaysia was discovered to diapause during the 'young' bamboo shoot offseason, in October to July (Rozkosny and Kovac 1998). It was found that only half of the pupae collected emerged during the shoot season (*P. flavifemoratus* colonizes young bamboo shoots), while the other half emerged the following shoot season, a year later (Rozkosny and Kovac 1998). Therefore, if the black soldier fly does overwinter, it would likely be in the pupal stage of development, like that of the oriental soldier fly and previous observations in colony rearing. Furthermore, it is not known if the mechanism of diapause induction for the oriental soldier fly is temperature or photoperiod (Rozkosny and Kovac 1998).

In summary, it is not surprising that temperature has a significant impact on black soldier fly development. Tomberlin et al. (2009) determined that the maximum developmental threshold for black soldier flies is between 30°C and 36°C, with optimal rearing at 27°C. Our study has determined that the minimum developmental threshold for black soldier flies is between 15°C and 18°C. Although adults did emerge at 18°C, it was not unexpected upon emergence that adults lived longer. Insects undergoing slow

development typically accumulate more weight and with greater fat reserves allocated to adult fitness, longer adult longevities result (Andrewartha 1952). On the contrary, it is not known if mating and oviposition is successful at 18°C. For the purpose of determining the feasibility of long term year-round production of black soldier flies, it would be necessary to subject adult black soldier flies reared at 18°C and the optimal 27°C to determine if other life history traits are affected by low temperatures, such as mating and oviposition. Furthermore, results from such an experiment may provide insight into potential diapause cues and mechanisms.

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TABLES

Table 5.1

Wilcoxon / Kruskal-Wallis Tests: Ranked Sums compares the temperature readings between each treatment recorded via Hobo® data loggers placed in each growth chamber, 12°C, 15°C and 18°C. Dunn-Sidak adjusted $\alpha = 0.0169$

Treatments	Z	ChiSquare	Significance
12°C * 15°C	33.81	1143.0837	$p < 0.001$*
12°C * 18°C	33.80	1143.0654	$p < 0.001$*
15°C * 18°C	33.77	1140.9305	$p < 0.001$*

Table 5.2

Accumulated degree hour (ADH) calculations for total development time egg to adult emergence using both 10°C and 15°C as the lower development threshold.

* Mean temperatures recorded using a Hobo® data logger (Onset® Data Loggers).

** Each temperature represents an individual experiment; no comparisons were made statistically between temperatures

***19.02±0.02°C**		**Minimum ADH**	**Median ADH**	**Maximum ADH**
Lower Development Threshold (10°C)		*16731.31*	*18010.40*	*20829.06*
	Total Development			
Lower Development Threshold (15°C)		*7757.00*	*8316.79*	*9340.15*
	Total Development			
***26.30±0.05°C**		**Minimum ADH**	**Median ADH**	**Maximum ADH**
Lower Development Threshold (10°C)				
	Egg	-	1481.03	-
	Larval	7807.49	8552.06	9287.13
	Post-feeding	11223.42	11638.98	25591.39
	Pupal	13810.56	15356.46	26721.96
Lower Development Threshold (15°C)				
	Egg	-	981.03	-
	Larval	5242.49	5747.06	6247.13
	Post-feeding	7588.42	7883.98	17751.39
	Pupal	9455.56	10581.46	18526.96
***27.97±0.03°C**		**Minimum ADH**	**Median ADH**	**Maximum ADH**
Lower Development Threshold (10°C)				
	Egg	1317.86	1356.59	1394.83
	Larval	7049.58	8369.50	21235.55
	Post-feeding	10443.89	11668.77	23496.59
	Pupal	13751.21	14987.89	26205.75
Lower Development Threshold (15°C)				
	Egg	957.86	986.59	1014.83
	Larval	5159.58	6119.50	15265.55
	Post-feeding	7593.89	8458.77	16926.59
	Pupal	9941.21	10817.89	18915.75

Table 5.3

Wilcoxon / Kruskal-Wallis Tests: Ranked Sums compares the mean (±SE) successful egg eclosion between temperature treatments, 12°C, 15°C and 18°C. Dunn-Sidak adjusted $\alpha = 0.0169$

Treatments	Z	ChiSquare	Significance
12°C * 15°C	3.93	15.7073	$p < 0.001$*
12°C * 18°C	4.96	24.8793	$p < 0.001$*
15°C * 18°C	4.65	21.8471	$p < 0.001$*

FIGURES

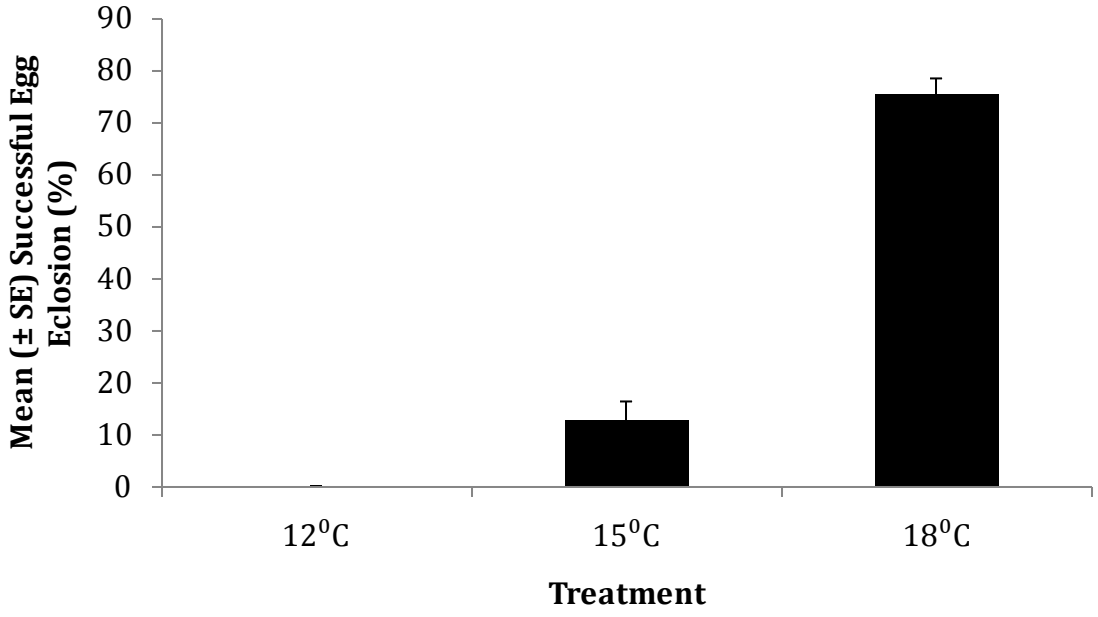

Figure 5.1

Mean (±SE) percent successful egg eclosion in the 12°C, 15°C and 18°C treatments, respective of all replicates. Eggs in the 18°C treatment had significantly higher egg eclosion success than those in the 12°C and the 15°C treatments, (χ^2= 37.4321, *df* = 2 p<0.001).

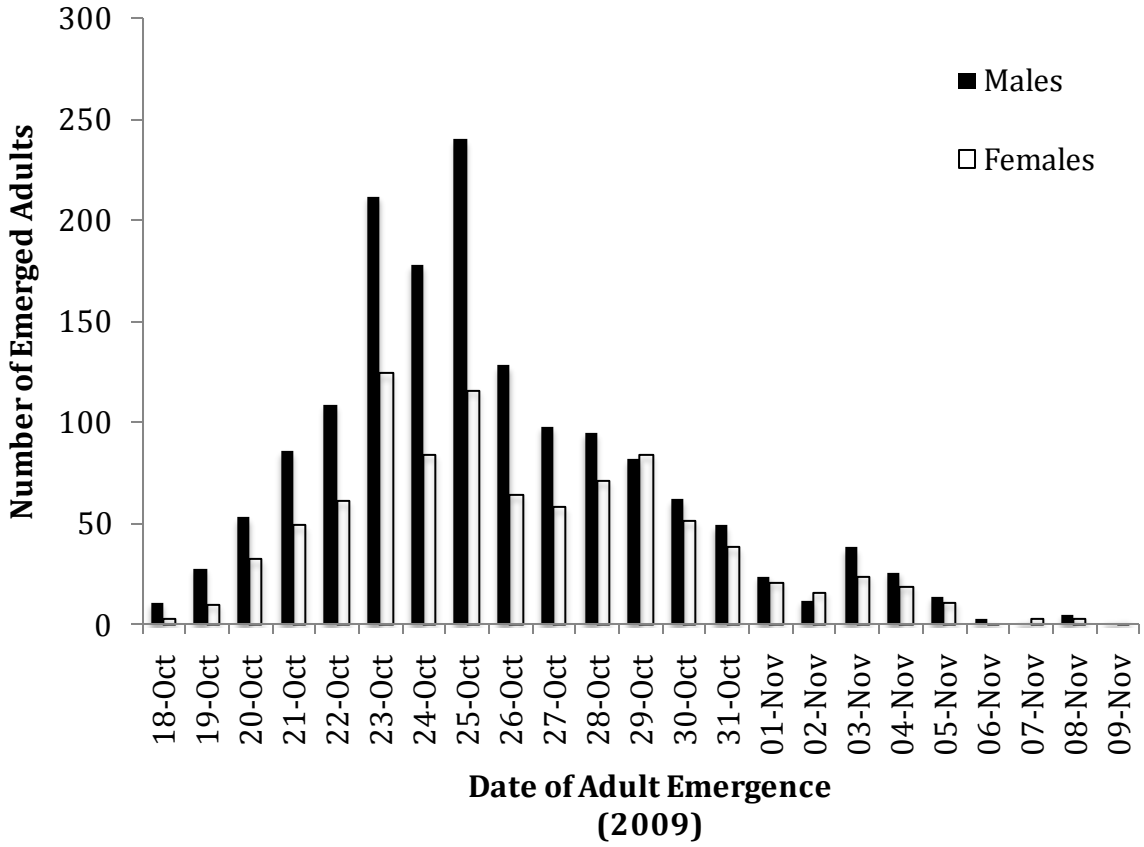

Figure 5.2

Male and female emergence trends with respect to date of emergence for the entire population of emerged adults. Sex ratio was slight male biased, 62.98%.

CHAPTER VI
CONCLUSION

Black soldier fly growth and development depends on their ambient environment. Abiotic factors including, temperature, relative humidity, daylength and pupation substrate and their effects on *Hermetia illucens* development were examined in the studies presented here. I found the best pupation substrate to use in rearing *H. illucens* was our laboratory standard, wood shavings. I determined less than ideal relative humidity levels for both egg eclosion and adult emergence is 60% and 40%, respectively, yielding at least 50% survival, with optimal growth and development at 60-70% RH, yielding 70% survival or higher. I found egg eclosion and adult emergence are successful in complete darkness, however, the time it took to develop is not conducive to mass rearing and therefore using photoperiods of at least 8h of daylight are recommended. Lastly, I discovered two lower temperature thresholds conducive to development; the first at 15°C, supporting successful egg eclosion, while the second at 18°C, supporting successful adult eclosion.

I discussed several factors that may have impacted my results. One of the most surprising aspects of this study was the complete development, including oviposition by virgin females in the complete darkness treatment. I did not anticipate it taking so long for the post-feeding larvae to pupate and eclose in complete darkness, but what was more surprising was the oviposition by virgin females in this experiment. This was the only experiment in this entire study, by which virgin females oviposited inside their capped cups. I considered this a sign of fecundity, which led me to believe that larval density impacts adult fitness and fecundity, since even virgin females in the relative humidity experiment at 25°C, 70% RH and 14:10 [L:D] photoperiod fed *ad libitum* did not oviposit, but larvae in this experiment were reared at a much higher density. In the same spatial environment as all other experiments, with only fifty larvae per replicate in the photoperiod experiment, as opposed to hundreds per replicate (one egg cluster) in the temperature and relative humidity experiments, oviposition was observed and therefore I am convinced population density may have an impact on adult fitness and fecundity.

As discussed previously, adult fitness was drastically affected by pupation substrate. I proposed substrate compaction can affect a substrate's ability to retain water, as well as a post-feeding larva's ability to penetrate, both of which could impede adult fitness. Pupae attempting to penetrate densely compacted substrates, like sand, may inevitably damage their cuticle, resulting in water loss and susceptibility to desiccation. They may also find respiration difficult and burrow out of the substrate, expending more energy uncovering itself and possibly resulting in less energy allocated to adult fitness and longevity.

Despite the small percentage of successful egg eclosions at low relative humidities, interestingly, I found not all eggs and post-feeding larvae desiccated at low relative humidities. This supports the egg clustering hypothesis and theories of geographic acclimation. When counting egg eclosions, I noted eggs on the surface of egg clusters were desiccated, while eggs in the centre of the cluster successfully eclosed. In addition, my colony was reared from Dr. Sheppard's colony in Tifton, Georgia, which has not been supplemented with wild caught *H. illucens* since 2002, and therefore my colony may be more tolerant to cooler temperatures and lower relative humidities than those closer to the equator.

The results of my study are not without limitations and therefore I offer suggestions for future experiments. Firstly, the adult mating and oviposition aspect of the pupation substrate experiment was not replicated and therefore more replications would provide more insight into the observed detrimental effects sand had on adult longevity. In addition, although treatment cages were set up in the same room of the greenhouse, because of lack of replication, the effect of sun directionality could not be accounted for and therefore more replications would eliminate position effects with respect to directionality of the sun.

Secondly, the lower development thresholds for temperature were determined, however, the prospect of diapause was not. When eggs in the 12°C treatment did not eclose within 30d, I placed them in a walk-in growth chamber with a temperature of 25°C and approximately 60-70% RH, to determine if the eggs had entered diapause or some phase of dormancy. However, not allowing the eggs to slowly acclimate to 25°C, I fear if the eggs were viable and just in diapause then the eggs may have experienced heat shock

and died. As a result, I propose a study to determine diapause induction in the black soldier fly by progressively increasing and decreasing temperatures at various life stages of development. Similarly, insects using photoperiodism as a cue for diapause are subjected to progressively increasing and decreasing lengths of daylight and therefore to determine the overwintering capabilities of *H. illucens*; if they are capable, it would be necessary to mimic the ambient environment that signals diapause by subjecting them to progressively decreasing lengths of daylight instead of just constant short daylengths.

In summary, with environmental manipulation, the black soldier fly can be reared successfully in temperate zones and with further experimentation, landfill wastes could potentially serve as a suitable diet, potentially reducing greenhouse gas emissions. That said, heating appliances and humidifiers would be necessary in the cool, dry seasons impeding *H.illucens* development. The issue of sunlight inducing mating is still an obstacle. Excessive cloud cover in the fall and winter in southern Ontario reduces mating, however, colleagues have had some mating success on cloud cover days and therefore, the black soldier has many secrets to unveil still (VanLaerhoven, 2010 personal communication). That said, egg production would evidently decrease in the fall and winter and waste reduction would in turn decrease and therefore further research on the effect of sunlight and the interaction of biotic effects would be worth investigating. Although, reducing the ambient temperature could potentially slow development, but continue reducing waste during the lulls of egg production. Further experimentation with these abiotic factors would be necessary in an applied setting, with limiting levels of temperature, relative humidity and photoperiod simultaneously regulating development. Therefore, although this contribution to the black soldier fly's life history is valuable, offering detailed observations on abiotic factors affecting growth and development, there is still much to learn with regard to these abiotic factors and furthermore, the endless biotic factors governing their development.

APPENDIX A

Table A.1

Wilcoxon / Kruskal-Wallis Tests: Ranked Sums test results comparing the proportion of living adults recorded daily between dates; 20 March 2009 and 16 April 2009.

Date Interactions	Test Statistic	Significance p-value	Adjusted Significance p-value
March 20*March 21	-4.700	0.855	1.000
March 20*March 22	-8.100	0.752	1.000
March 20*March 23	-46.400	0.070	1.000
March 20*April 11	-47.100	0.066	1.000
March 20*April 09	-47.400	0.065	1.000
March 20*April 12	-47.400	0.065	1.000
March 20*April 13	-47.400	0.065	1.000
March 20*April 14	-47.400	0.065	1.000
March 20*April 15	-47.900	0.062	1.000
March 20*April 16	-47.900	0.062	1.000
March 20*April 10	-48.900	0.057	1.000
March 20*April 08	-52.400	0.041	1.000
March 20*April 07	-55.200	0.031	1.000
March 20*April 06	-60.500	0.018	1.000
March 20*April 05	-60.600	0.018	1.000
March 20*April 04	-73.600	0.004	1.000
March 20*April 03	-77.200	0.003	0.985
March 20*April 02	-80.400	0.002	0.648
March 20*April 01	-85.600	0.001	0.319
March 20*March 24	-90.100	0.000	0.167

March 20*March 31	-96.400	0.000	0.064
March 20*March 30	-105.800	0.000	0.014
March 20*March 25	-109.900	0.000	0.007
March 20*March 29	-112.300	0.000	0.004
March 20*March 26	-117.500	0.000	0.002
March 20*March 27	-117.900	0.000	0.002
March 20*March 28	-120.400	0.000	0.001
March 21*March 22	-3.400	0.895	1.000
March 21*March 23	-41.700	0.104	1.000
March 21*April 11	-42.400	0.098	1.000
March 21*April 09	-42.400	0.096	1.000
March 21*April 12	-42.700	0.096	1.000
March 21*April 13	-42.700	0.096	1.000
March 21*April 14	-42.700	0.096	1.000
March 21*April 15	-43.200	0.092	1.000
March 21*April 16	-43.200	0.092	1.000
March 21*April 10	-44.200	0.085	1.000
March 21*April 08	-47.700	0.063	1.000
March 21*April 07	-50.500	0.049	1.000
March 21*April 06	-55.600	0.030	1.000
March 21*April 05	-55.900	0.029	1.000
March 21*April 04	-68.900	0.007	1.000
March 21*April 03	-72.500	0.005	1.000
March 21*April 02	-75.700	0.003	1.000
March 21*April 01	-80.900	0.002	0.607

March 21*March 24	-85.400	0.001	0.323
March 21*March 31	-91.700	0.000	0.132
March 21*March 30	-101.100	0.000	0.030*
March 21*March 25	-105.200	0.000	0.015*
March 21*March 29	-107.600	0.000	0.010*
March 21*March 26	-112.800	0.000	0.004*
March 21*March 27	-113.200	0.000	0.004*
March 21*March 28	-115.700	0.000	0.002*
March 22*March 23	-38.300	0.135	1.000
March 22*April 11	-39.000	0.128	1.000
March 22*April 09	-39.300	0.125	1.000
March 22*April 12	-39.300	0.125	1.000
March 22*April 13	-39.300	0.125	1.000
March 22*April 14	-39.300	0.125	1.000
March 22*April 15	-39.800	0.121	1.000
March 22*April 16	-39.800	0.121	1.000
March 22*April 10	-40.800	0.112	1.000
March 22*April 08	-44.300	0.084	1.000
March 22*April 07	-47.100	0.066	1.000
March 22*April 06	-52.400	0.041	1.000
March 22*April 05	-52.500	0.041	1.000
March 22*April 04	-65.500	0.011	1.000
March 22*April 03	-69.100	0.007	1.000
March 22*April 02	-72.300	0.005	1.000
March 22*April 01	-77.500	0.003	0.948

March 22*March 24	-82.000	0.001	0.523
March 22*March 31	-88.300	0.001	0.217
March 22*March 30	-97.700	0.000	0.052
March 22*March 25	-101.800	0.000	0.027*
March 22*March 29	-104.200	0.000	0.018*
March 22*March 26	-109.400	0.000	0.008*
March 22*March 27	-109.800	0.000	0.007*
March 22*March 28	-112.300	0.000	0.004*
March 23*April 11	-0.700	0.978	1.000
March 23*April 09	-1.000	0.969	1.000
March 23*April 12	-1.000	0.969	1.000
March 23*April 13	--1.000	0.969	1.000
March 23*April 14	-1.000	0.969	1.000
March 23*April 15	-1.500	0.953	1.000
March 23*April 16	-1.500	0.953	1.000
March 23*April 10	-2.500	0.922	1.000
March 23*April 08	-6.000	0.815	1.000
March 23*April 07	-8.800	0.731	1.000
March 23*April 06	-14.100	0.582	1.000
March 23*April 05	-14.200	0.580	1.000
March 23*April 04	-27.200	0.289	1.000
March 23*April 03	-30.800	0.230	1.000
March 23*April 02	-34.000	0.185	1.000
March 23*April 01	-39.200	0.126	1.000
March 23*March 24	-43.700	0.088	1.000

March 23*March 31	-50.000	0.051	1.000
March 23*March 30	-59.400	0.021	1.000
March 23*March 25	-63.500	0.013	1.000
March 23*March 29	-65.900	0.010	1.000
March 23*March 26	-71.100	0.006	1.000
March 23*March 27	-71.500	0.005	1.000
March 23*March 28	-74.000	0.004	1.000
April 11*April 09	-0.300	0.991	1.000
April 11*April 12	-0.300	0.991	1.000
April 11*April 13	-0.300	0.991	1.000
April 11*April 14	-0.300	0.991	1.000
April 11*April 15	-0.800	0.975	1.000
April 11*April 16	-0.800	0.975	1.000
April 11*April 10	1.800	0.944	1.000
April 11*April 08	5.300	0.836	1.000
April 11*April 07	8.100	0.752	1.000
April 11*April 06	13.400	0.601	1.000
April 11*April 05	13.500	0.599	1.000
April 11*April 04	26.500	0.301	1.000
April 11*April 03	30.100	0.240	1.000
April 11*April 02	33.300	0.194	1.000
April 11*April 01	38.500	0.133	1.000
April 11*March 24	43.000	0.094	1.000
April 11*March 31	49.300	0.055	1.000
April 11*March 30	58.700	0.022	1.000

April 11*March 25	62.800	0.014	1.000
April 11*March 29	65.200	0.011	1.000
April 11*March 26	70.400	0.006	1.000
April 11*March 27	70.800	0.006	1.000
April 11*March 28	73.300	0.004	1.000
April 09*April 12	0.000	1.000	1.000
April 09*April 13	0.000	1.000	1.000
April 09*April 14	0.000	1.000	1.000
April 09*April 15	-0.500	0.984	1.000
April 09*April 16	-0.500	0.984	1.000
April 09*April 10	-1.500	0.953	1.000
April 09*April 08	5.000	0.845	1.000
April 09*April 07	7.800	0.761	1.000
April 09*April 06	13.100	0.609	1.000
April 09*April 05	13.200	0.607	1.000
April 09*April 04	26.200	0.307	1.000
April 09*April 03	29.800	0.245	1.000
April 09*April 02	33.000	0.198	1.000
April 09*April 01	38.200	0.136	1.000
April 09*March 24	42.700	0.096	1.000
April 09*March 31	49.000	0.056	1.000
April 09*March 30	58.400	0.023	1.000
April 09*March 25	62.500	0.015	1.000
April 09*March 29	64.900	0.011	1.000
April 09*March 26	70.100	0.006	1.000

April 09*March 27	70.500	0.006	1.000
April 09*March 28	73.000	0.004	1.000
April 12*April 13	0.000	1.000	1.000
April 12*April 14	0.000	1.000	1.000
April 12*April 15	-0.500	0.984	1.000
April 12*April 16	-0.500	0.984	1.000
April 12*April 10	1.500	0.953	1.000
April 12*April 08	5.000	0.845	1.000
April 12*April 07	7.800	0.761	1.000
April 12*April 06	13.100	0.609	1.000
April 12*April 05	13.200	0.607	1.000
April 12*April 04	26.200	0.307	1.000
April 12*April 03	29.800	0.245	1.000
April 12*April 02	33.000	0.198	1.000
April 12*April 01	38.200	0.136	1.000
April 12*March 24	42.700	0.096	1.000
April 12*March 31	49.000	0.056	1.000
April 12*March 30	58.400	0.023	1.000
April 12*March 25	62.500	0.015	1.000
April 12*March 29	64.900	0.011	1.000
April 12*March 26	70.100	0.006	1.000
April 12*March 27	70.500	0.006	1.000
April 12*March 28	73.000	0.004	1.000
April 13*April 14	0.000	1.000	1.000
April 13*April 15	-0.500	0.984	1.000

April 13*April 16	-0.500	0.984	1.000
April 13*April 10	1.500	0.953	1.000
April 13*April 08	5.000	0.845	1.000
April 13*April 07	7.800	0.761	1.000
April 13*April 06	13.100	0.609	1.000
April 13*April 05	13.200	0.607	1.000
April 13*April 04	26.200	0.307	1.000
April 13*April 03	29.800	0.245	1.000
April 13*April 02	33.000	0.198	1.000
April 13*April 01	38.200	0.136	1.000
April 13*March 24	42.700	0.096	1.000
April 13*March 31	49.000	0.056	1.000
April 13*March 30	58.400	0.023	1.000
April 13*March 25	62.500	0.015	1.000
April 13*March 29	64.900	0.011	1.000
April 13*March 26	70.100	0.006	1.000
April 13*March 27	70.500	0.006	1.000
April 13*March 28	73.000	0.004	1.000
April 14*April 15	-0.500	0.984	1.000
April 14*April 16	-0.500	0.984	1.000
April 14*April 10	1.500	0.953	1.000
April 14*April 08	5.000	0.845	1.000
April 14*April 07	7.800	0.761	1.000
April 14*April 06	13.100	0.609	1.000
April 14*April 05	13.200	0.607	1.000

April 14*April 04	26.200	0.307	1.000
April 14*April 03	29.800	0.245	1.000
April 14*April 02	33.000	0.198	1.000
April 14*April 01	38.200	0.136	1.000
April 14*March 24	42.700	0.096	1.000
April 14*March 31	49.000	0.056	1.000
April 14*March 30	58.400	0.023	1.000
April 14*March 25	62.500	0.015	1.000
April 14*March 29	64.900	0.011	1.000
April 14*March 26	70.100	0.006	1.000
April 14*March 27	70.500	0.006	1.000
April 14*March 28	73.000	0.004	1.000
April 15*April 16	0.000	1.000	1.000
April 15*April 10	1.000	0.969	1.000
April 15*April 08	4.500	0.861	1.000
April 15*April 07	7.300	0.776	1.000
April 15*April 06	12.600	0.623	1.000
April 15*April 05	12.700	0.620	1.000
April 15*April 04	25.700	0.316	1.000
April 15*April 03	29.300	0.253	1.000
April 15*April 02	32.500	0.205	1.000
April 15*April 01	37.700	0.141	1.000
April 15*March 24	42.200	0.100	1.000
April 15*March 31	48.500	0.059	1.000
April 15*March 30	57.900	0.024	1.000

April 15*March 25	62.000	0.016	1.000
April 15*March 29	64.400	0.012	1.000
April 15*March 26	69.600	0.007	1.000
April 15*March 27	70.000	0.006	1.000
April 15*March 28	72.500	0.005	1.000
April 16*April 10	1.000	0.969	1.000
April 16*April 08	4.500	0.861	1.000
April 16*April 07	7.300	0.776	1.000
April 16*April 06	12.600	0.623	1.000
April 16*April 05	12.700	0.620	1.000
April 16*April 04	25.700	0.316	1.000
April 16*April 03	29.300	0.253	1.000
April 16*April 02	32.500	0.205	1.000
April 16*April 01	37.700	0.141	1.000
April 16*March 24	42.200	0.100	1.000
April 16*March 31	48.500	0.059	1.000
April 16*March 30	57.900	0.024	1.000
April 16*March 25	62.000	0.016	1.000
April 16*March 29	64.400	0.012	1.000
April 16*March 26	69.600	0.007	1.000
April 16*March 27	70.000	0.006	1.000
April 16*March 28	72.500	0.005	1.000
April 10*April 08	3.500	0.891	1.000
April 10*April 07	6.300	0.806	1.000
April 10*April 06	11.600	0.651	1.000

April 10*April 05	11.700	0.648	1.000
April 10*April 04	24.700	0.335	1.000
April 10*April 03	28.300	0.270	1.000
April 10*April 02	31.500	0.219	1.000
April 10*April 01	36.700	0.152	1.000
April 10*March 24	41.200	0.108	1.000
April 10*March 31	47.500	0.064	1.000
April 10*March 30	56.900	0.026	1.000
April 10*March 25	61.000	0.017	1.000
April 10*March 29	63.400	0.013	1.000
April 10*March 26	68.600	0.007	1.000
April 10*March 27	69.000	0.007	1.000
April 10*March 28	71.500	0.005	1.000
April 08*April 07	2.800	0.913	1.000
April 08*April 06	8.100	0.752	1.000
April 08*April 05	8.200	0.749	1.000
April 08*April 04	21.200	0.408	1.000
April 08*April 03	24.800	0.333	1.000
April 08*April 02	28.000	0.275	1.000
April 08*April 01	33.200	0.195	1.000
April 08*March 24	37.700	0.141	1.000
April 08*March 31	44.000	0.086	1.000
April 08*March 30	53.400	0.037	1.000
April 08*March 25	57.500	0.025	1.000
April 08*March 29	59.900	0.019	1.000

April 08*March 26	65.100	0.011	1.000
April 08*March 27	65.500	0.011	1.000
April 08*March 28	68.000	0.008	1.000
April 07*April 06	5.300	0.752	1.000
April 07*April 05	5.400	0.836	1.000
April 07*April 04	18.400	0.833	1.000
April 07*April 03	22.000	0.473	1.000
April 07*April 02	25.200	0.391	1.000
April 07*April 01	30.400	0.236	1.000
April 07*March 24	34.900	0.173	1.000
April 07*March 31	41.200	0.108	1.000
April 07*March 30	50.600	0.048	1.000
April 07*March 25	54.700	0.033	1.000
April 07*March 29	57.100	0.026	1.000
April 07*March 26	62.300	0.015	1.000
April 07*March 27	62.700	0.014	1.000
April 07*March 28	65.200	0.011	1.000
April 06*April 05	0.100	0.997	1.000
April 06*April 04	13.100	0.609	1.000
April 06*April 03	16.700	0.515	1.000
April 06*April 02	19.900	0.436	1.000
April 06*April 01	25.100	0.328	1.000
April 06*March 24	29.600	0.248	1.000
April 06*March 31	35.900	0.161	1.000
April 06*March 30	45.300	0.077	1.000

April 06*March 25	49.400	0.054	1.000
April 06*March 29	51.800	0.043	1.000
April 06*March 26	57.000	0.026	1.000
April 06*March 27	57.400	0.025	1.000
April 06*March 28	59.900	0.019	1.000
April 05*April 04	13.000	0.612	1.000
April 05*April 03	16.600	0.517	1.000
April 05*April 02	19.800	0.440	1.000
April 05*April 01	25.000	0.330	1.000
April 05*March 24	29.500	0.250	1.000
April 05*March 31	35.800	0.163	1.000
April 05*March 30	45.200	0.078	1.000
April 05*March 25	49.300	0.055	1.000
April 05*March 29	51.700	0.044	1.000
April 05*March 26	56.900	0.026	1.000
April 05*March 27	57.300	0.025	1.000
April 05*March 28	59.800	0.020	1.000
April 04*April 03	3.600	0.888	1.000
April 04*April 02	6.800	0.791	1.000
April 04*April 01	12.000	0.640	1.000
April 04*March 24	16.500	0.520	1.000
April 04*March 31	22.800	0.374	1.000
April 04*March 30	32.200	0.209	1.000
April 04*March 25	36.300	0.157	1.000
April 04*March 29	38.700	0.131	1.000

April 04*March 26	43.900	0.087	1.000
April 04*March 27	44.300	0.084	1.000
April 04*March 28	46.800	0.068	1.000
April 03*April 02	3.200	0.901	1.000
April 03*April 01	8.400	0.743	1.000
April 03*March 24	12.900	0.615	1.000
April 03*March 31	19.200	0.454	1.000
April 03*March 30	26.600	0.265	1.000
April 03*March 25	32.700	0.202	1.000
April 03*March 29	35.100	0.171	1.000
April 03*March 26	40.300	0.116	1.000
April 03*March 27	40.700	0.112	1.000
April 03*March 28	43.200	0.092	1.000
April 02*April 01	5.200	0.839	1.000
April 02*March 24	9.700	0.705	1.000
April 02*March 31	16.000	0.533	1.000
April 02*March 30	25.400	0.322	1.000
April 02*March 25	29.500	0.250	1.000
April 02*March 29	31.900	0.213	1.000
April 02*March 26	37.100	0.148	1.000
April 02*March 27	37.500	0.144	1.000
April 02*March 28	40.000	0.119	1.000
April 01*March 24	4.500	0.861	1.000
April 01*March 31	10.800	0.674	1.000
April 01*March 30	20.200	0.431	1.000

April 01*March 25	24.300	0.343	1.000
April 01*March 29	26.700	0.298	1.000
April 01*March 26	31.900	0.213	1.000
April 01*March 27	32.300	0.208	1.000
April 01*March 28	34.800	0.175	1.000
March 24*March 31	-6.300	0.806	1.000
March 24*March 30	-15.700	0.540	1.000
March 24*March 25	-19.800	0.440	1.000
March 24*March 29	-22.200	0.387	1.000
March 24*March 26	-27.400	0.285	1.000
March 24*March 27	-27.800	0.278	1.000
March 24*March 28	-30.300	0.237	1.000
March 31*March 30	9.400	0.714	1.000
March 31*March 25	13.500	0.599	1.000
March 31*March 29	15.900	0.535	1.000
March 31*March 26	21.100	0.411	1.000
March 31*March 27	21.500	0.402	1.000
March 31*March 28	24.000	0.349	1.000
March 30*March 25	4.100	0.873	1.000
March 30*March 29	6.500	0.800	1.000
March 30*March 26	11.700	0.648	1.000
March 30*March 27	12.100	0.637	1.000
March 30*March 28	14.600	0.569	1.000
March 25*March 29	-2.400	0.925	1.000
March 25*March 26	-7.600	0.767	1.000

March 25*March 27	-8.000	0.755	1.000
March 25*March 28	-10.500	0.682	1.000
March 29*March 26	5.200	0.839	1.000
March 29*March 27	5.600	0.827	1.000
March 29*March 28	8.100	0.752	1.000
March 26*March 27	-0.400	0.988	1.000
March 26*March 28	-2.900	0.910	1.000
March 27*March 28	-2.500	0.922	1.000

Table A.2

Wilcoxon / Kruskal-Wallis Tests: Ranked Sums test results comparing the proportion of adult mortalities recorded daily between dates; 20 March 2009 and 16 April 2009.

Date Interactions	Test Statistic	Significance p-value	Adjusted Significance p-value
March 20*March 21	0.000	1.000	1.000
March 20*March 22	0.000	1.000	1.000
March 20*April 13	0.000	1.000	1.000
March 20*April 14	0.000	1.000	1.000
March 20*April 15	0.000	1.000	1.000
March 20*April 10	-5.500	0.827	1.000
March 20*April 16	-11.500	0.648	1.000
March 20*April 11	-14.100	0.575	1.000
March 20*April 12	-14.800	0.556	1.000
March 20*April 08	-36.300	0.149	1.000
March 20*April 09	-37.500	0.136	1.000
March 20*April 06	-42.000	0.095	1.000
March 20*April 07	-45.100	0.073	1.000
March 20*March 23	-50.600	0.044	1.000
March 20*April 04	-56.400	0.025	1.000
March 20*April 03	-58.700	0.020	1.000
March 20*March 24	-59.200	0.019	1.000
March 20*March 28	-75.100	0.004	1.000
March 20*April 02	-75.100	0.003	1.000
March 20*April 05	-81.700	0.001	0.441
March 20*March 25	-84.200	0.001	0.309

March 20*March 26	-85.000	0.001	0.276
March 20*April 01	-88.800	0.000	0.158
March 20*March 31	-91.300	0.000	0.108
March 20*March 29	-94.400	0.000	0.066
March 20*March 27	-95.800	0.000	0.053
March 20*March 30	-100.600	0.000	0.024*
March 21*March 22	0.000	1.000	1.000
March 21*April 13	0.000	1.000	1.000
March 21*April 14	0.000	1.000	1.000
March 21*April 15	0.000	1.000	1.000
March 21*April 10	-5.500	0.827	1.000
March 21*April 16	-11.500	0.648	1.000
March 21*April 11	-14.100	0.575	1.000
March 21*April 12	-14.800	0.556	1.000
March 21*April 08	-36.300	0.149	1.000
March 21*April 09	-37.500	0.136	1.000
March 21*April 06	-42.000	0.095	1.000
March 21*April 07	-45.100	0.073	1.000
March 21*March 23	-50.600	0.044	1.000
March 21*April 04	-56.400	0.025	1.000
March 21*April 03	-58.700	0.020	1.000
March 21*March 24	-59.200	0.019	1.000
March 21*March 28	-75.100	0.004	1.000
March 21*April 02	-75.100	0.003	1.000
March 21*April 05	-81.700	0.001	0.441

March 21*March 25	-84.200	0.001	0.309
March 21*March 26	-85.000	0.001	0.276
March 21*April 01	-88.800	0.000	0.158
March 21*March 31	-91.300	0.000	0.108
March 21*March 29	-94.400	0.000	0.066
March 21*March 27	-95.800	0.000	0.053
March 21*March 30	-100.600	0.000	0.024*
March 22*April 13	0.000	1.000	1.000
March 22*April 14	0.000	1.000	1.000
March 22*April 15	0.000	1.000	1.000
March 22*April 10	-5.500	0.827	1.000
March 22*April 16	-11.500	0.648	1.000
March 22*April 11	-14.100	0.575	1.000
March 22*April 12	-14.800	0.556	1.000
March 22*April 08	-36.300	0.149	1.000
March 22*April 09	-37.500	0.136	1.000
March 22*April 06	-42.000	0.095	1.000
March 22*April 07	-45.100	0.073	1.000
March 22*March 23	-50.600	0.044	1.000
March 22*April 04	-56.400	0.025	1.000
March 22*April 03	-58.700	0.020	1.000
March 22*March 24	-59.200	0.019	1.000
March 22*March 28	-75.100	0.004	1.000
March 22*April 02	-75.100	0.003	1.000
March 22*April 05	-81.700	0.001	0.441

March 22*March 25	-84.200	0.001	0.309
March 22*March 26	-85.000	0.001	0.276
March 22*April 01	-88.800	0.000	0.158
March 22*March 31	-91.300	0.000	0.108
March 22*March 29	-94.400	0.000	0.066
March 22*March 27	-95.800	0.000	0.053
March 22*March 30	-100.600	0.000	0.024*
April 13*April 14	0.000	1.000	1.000
April 13*April 15	0.000	1.000	1.000
April 13*April 10	5.500	0.827	1.000
April 13*April 16	-11.500	0.648	1.000
April 13*April 11	14.100	0.575	1.000
April 13*April 12	14.800	0.556	1.000
April 13*April 08	36.300	0.149	1.000
April 13*April 09	37.500	0.136	1.000
April 13*April 06	42.000	0.095	1.000
April 13*April 07	45.100	0.073	1.000
April 13*March 23	50.600	0.044	1.000
April 13*April 04	56.400	0.025	1.000
April 13*April 03	58.700	0.020	1.000
April 13*March 24	59.200	0.019	1.000
April 13*March 28	75.100	0.004	1.000
April 13*April 02	75.100	0.003	1.000
April 13*April 05	81.700	0.001	0.441
April 13*March 25	84.200	0.001	0.309

April 13*March 26	85.000	0.001	0.276
April 13*April 01	88.800	0.000	0.158
April 13*March 31	91.300	0.000	0.108
April 13*March 29	94.400	0.000	0.066
April 13*March 27	95.800	0.000	0.053
April 13*March 30	100.600	0.000	0.024*
April 14*April 15	0.000	1.000	1.000
April 14*April 10	5.500	0.827	1.000
April 14*April 16	-11.500	0.648	1.000
April 14*April 11	14.100	0.575	1.000
April 14*April 12	14.800	0.556	1.000
April 14*April 08	36.300	0.149	1.000
April 14*April 09	37.500	0.136	1.000
April 14*April 06	42.000	0.095	1.000
April 14*April 07	45.100	0.073	1.000
April 14*March 23	50.600	0.044	1.000
April 14*April 04	56.400	0.025	1.000
April 14*April 03	58.700	0.020	1.000
April 14*March 24	59.200	0.019	1.000
April 14*March 28	75.100	0.004	1.000
April 14*April 02	75.100	0.003	1.000
April 14*April 05	81.700	0.001	0.441
April 14*March 25	84.200	0.001	0.309
April 14*March 26	85.000	0.001	0.276
April 14*April 01	88.800	0.000	0.158

April 14*March 31	91.300	0.000	0.108
April 14*March 29	94.400	0.000	0.066
April 14*March 27	95.800	0.000	0.053
April 14*March 30	100.600	0.000	0.024*
April 15*April 10	5.500	0.827	1.000
April 15*April 16	-11.500	0.648	1.000
April 15*April 11	14.100	0.575	1.000
April 15*April 12	14.800	0.556	1.000
April 15*April 08	36.300	0.149	1.000
April 15*April 09	37.500	0.136	1.000
April 15*April 06	42.000	0.095	1.000
April 15*April 07	45.100	0.073	1.000
April 15*March 23	50.600	0.044	1.000
April 15*April 04	56.400	0.025	1.000
April 15*April 03	58.700	0.020	1.000
April 15*March 24	59.200	0.019	1.000
April 15*March 28	75.100	0.004	1.000
April 15*April 02	75.100	0.003	1.000
April 15*April 05	81.700	0.001	0.441
April 15*March 25	84.200	0.001	0.309
April 15*March 26	85.000	0.001	0.276
April 15*April 01	88.800	0.000	0.158
April 15*March 31	91.300	0.000	0.108
April 15*March 29	94.400	0.000	0.066
April 15*March 27	95.800	0.000	0.053

April 15*March 30	100.600	0.000	0.024*
April 10*April 16	-6.000	0.812	1.000
April 10*April 11	-8.600	0.733	1.000
April 10*April 12	-9.300	0.712	1.000
April 10*April 08	30.800	0.221	1.000
April 10*April 09	32.000	0.203	1.000
April 10*April 06	36.000	0.147	1.000
April 10*April 07	39.600	0.116	1.000
April 10*March 23	45.100	0.073	1.000
April 10*April 04	50.900	0.043	1.000
April 10*April 03	53.200	0.034	1.000
April 10*March 24	53.700	0.033	1.000
April 10*March 28	67.900	0.007	1.000
April 10*April 02	69,600	0.006	1.000
April 10*April 05	76.200	0.002	1.000
April 10*March 25	78.700	0.002	0.666
April 10*March 26	79.500	0.002	0.597
April 10*April 01	83.300	0.001	0.352
April 10*March 31	85.800	0.001	0.246
April 10*March 29	88.900	0.000	0.155
April 10*March 27	90.300	0.000	0.126
April 10*March 30	95.100	0.000	0.059
April 16*April 11	2.600	0.918	1.000
April 16*April 12	3.300	0.896	1.000
April 16*April 08	24.800	0.324	1.000

April 16*April 09	26.000	0.301	1.000
April 16*April 06	30.500	0.225	1.000
April 16*April 07	33.600	0.182	1.000
April 16*March 23	39.100	0.120	1.000
April 16*April 04	44.900	0.074	1.000
April 16*April 03	47.200	0.061	1.000
April 16*March 24	47.700	0.058	1.000
April 16*March 28	61.900	0.014	1.000
April 16*April 02	63,600	0.011	1.000
April 16*April 05	70.200	0.005	1.000
April 16*March 25	72.700	0.004	1.000
April 16*March 26	73.500	0.003	1.000
April 16*April 01	77.300	0.002	0.803
April 16*March 31	79.800	0.002	0.573
April 16*March 29	82.900	0.001	0.372
April 16*March 27	84.300	0.001	0.305
April 16*March 30	89.100	0.000	0.151
April 11*April 12	-0.700	0.978	1.000
April 11*April 08	22.200	0.378	1.000
April 11*April 09	23.400	0.352	1.000
April 11*April 06	27.900	0.267	1.000
April 11*April 07	31.000	0.218	1.000
April 11*March 23	36.500	0.147	1.000
April 11*April 04	42.300	0.093	1.000
April 11*April 03	44.600	0.076	1.000

April 11*March 24	45.100	0.073	1.000
April 11*March 28	59.300	0.018	1.000
April 11*April 02	61.000	0.015	1.000
April 11*April 05	67.600	0.007	1.000
April 11*March 25	70.100	0.005	1.000
April 11*March 26	70.900	0.005	1.000
April 11*April 01	74.700	0.003	1.000
April 11*March 31	77.200	0.002	0.814
April 11*March 29	80.300	0.001	0.535
April 11*March 27	81.700	0.001	0.441
April 11*March 30	86.500	0.001	0.222
April 12*April 08	21.500	0.393	1.000
April 12*April 09	22.700	0.367	1.000
April 12*April 06	27.200	0.280	1.000
April 12*April 07	30.300	0.228	1.000
April 12*March 23	35.800	0.155	1.000
April 12*April 04	41.400	0.098	1.000
April 12*April 03	43.900	0.081	1.000
April 12*March 24	44.400	0.078	1.000
April 12*March 28	58.600	0.020	1.000
April 12*April 02	60.300	0.017	1.000
April 12*April 05	66.900	0.008	1.000
April 12*March 25	69.400	0.006	1.000
April 12*March 26	70.200	0.005	1.000
April 12*April 01	74.700	0.003	1.000

April 12*March 31	76.500	0.002	0.893
April 12*March 29	79.600	0.002	0.589
April 12*March 27	81.000	0.001	0.486
April 12*March 30	85.800	0.001	0.246
April 08*April 09	-1.200	0.962	1.000
April 08*April 06	5.700	0.821	1.000
April 08*April 07	8.800	0.727	1.000
April 08*March 23	14.300	0.570	1.000
April 08*April 04	20.100	0.424	1.000
April 08*April 03	22.400	0.373	1.000
April 08*March 24	22.900	0.363	1.000
April 08*March 28	37.100	0.140	1.000
April 08*April 02	38.800	0.123	1.000
April 08*April 05	45.400	0.071	1.000
April 08*March 25	47.900	0.057	1.000
April 08*March 26	48.700	0.053	1.000
April 08*April 01	52.500	0.037	1.000
April 08*March 31	55.000	0.029	1.000
April 08*March 29	58.100	0.021	1.000
April 08*March 27	59.500	0.018	1.000
April 08*March 30	64.300	0.011	1.000
April 09*April 06	4.500	0.858	1.000
April 09*April 07	7.600	0.763	1.000
April 09*March 23	13.100	0.603	1.000
April 09*April 04	18.900	0.453	1.000

April 09*April 03	21.200	0.399	1.000
April 09*March 24	21.700	0.388	1.000
April 09*March 28	35.900	0.154	1.000
April 09*April 02	37.600	0.135	1.000
April 09*April 05	44.200	0.079	1.000
April 09*March 25	46.700	0.063	1.000
April 09*March 26	47.500	0.059	1.000
April 09*April 01	51.300	0.041	1.000
April 09*March 31	53.800	0.032	1.000
April 09*March 29	56.900	0.024	1.000
April 09*March 27	58.300	0.020	1.000
April 09*March 30	63.100	0.012	1.000
April 06*April 07	-3.100	0.902	1.000
April 06*March 23	8.600	0.733	1.000
April 06*April 04	14.400	0.567	1.000
April 06*April 03	16.700	0.507	1.000
April 06*March 24	17.200	0.494	1.000
April 06*March 28	31.400	0.212	1.000
April 06*April 02	33.100	0.188	1.000
April 06*April 05	39.700	0.115	1.000
April 06*March 25	42.200	0.094	1.000
April 06*March 26	43.000	0.087	1.000
April 06*April 01	46.800	0.063	1.000
April 06*March 31	49.300	0.050	1.000
April 06*March 29	52.400	0.037	1.000

April 06*March 27	53.800	0.032	1.000
April 06*March 30	58.600	0.020	1.000
April 07*March 23	5.500	0.827	1.000
April 07*April 04	11.300	0.653	1.000
April 07*April 03	13.600	0.589	1.000
April 07*March 24	14.100	0.575	1.000
April 07*March 28	28.300	0.261	1.000
April 07*April 02	30.000	0.233	1.000
April 07*April 05	36.600	0.146	1.000
April 07*March 25	39.100	0.120	1.000
April 07*March 26	39.900	0.113	1.000
April 07*April 01	43.700	0.082	1.000
April 07*March 31	46.200	0.066	1.000
April 07*March 29	49.200	0.050	1.000
April 07*March 27	50.700	0.044	1.000
April 07*March 30	55.500	0.027	1.000
March 23*April 04	-5.800	0.818	1.000
March 23*April 03	-8.100	0.748	1.000
March 23*March 24	-8.600	0.733	1.000
March 23*March 28	-22.800	0.365	1.000
March 23*April 02	-24.500	0.330	1.000
March 23*April 05	-31.100	0.216	1.000
March 23*March 25	-33.600	0.182	1.000
March 23*March 26	-34.400	0.172	1.000
March 23*April 01	-38.200	0.129	1.000

March 23*March 31	-40.700	0.106	1.000
March 23*March 29	-43.800	0.082	1.000
March 23*March 27	-45.200	0.072	1.000
March 23*March 30	-50.000	0.047	1.000
April 04*April 03	2.300	0.927	1.000
April 04*March 24	3.800	0.911	1.000
April 04*March 28	17.000	0.499	1.000
April 04*April 02	18.800	0.457	1.000
April 04*April 05	-25.300	0.315	1.000
April 04*March 25	27.800	0.269	1.000
April 04*March 26	28.600	0.256	1.000
April 04*April 01	32.400	0.198	1.000
April 04*March 31	34.900	0.165	1.000
April 04*March 29	38.000	0.131	1.000
April 04*March 27	39.400	0.117	1.000
April 04*March 30	44.200	0.079	1.000
April 03*March 24	0.500	0.984	1.000
April 03*March 28	14.700	0.559	1.000
April 03*April 02	16.400	0.515	1.000
April 03*April 05	-23.000	0.361	1.000
April 03*March 25	25.500	0.311	1.000
April 03*March 26	26.300	0.296	1.000
April 03*April 01	30.100	0.232	1.000
April 03*March 31	32.600	0.195	1.000
April 03*March 29	36.700	0.156	1.000

April 03*March 27	37.100	0.140	1.000
April 03*March 30	41.900	0.096	1.000
March 24*March 28	-14.200	0.573	1.000
March 24*April 02	-15.900	0.527	1.000
March 24*April 05	-22.500	0.371	1.000
March 24*March 25	-25.000	0.320	1.000
March 24*March 26	-25.800	0.305	1.000
March 24*April 01	-29.600	0.239	1.000
March 24*March 31	-32.100	0.202	1.000
March 24*March 29	-35.200	0.162	1.000
March 24*March 27	-36.600	0.146	1.000
March 24*March 30	-41.400	0.100	1.000
March 28*April 02	-1.700	0.946	1.000
March 28*April 05	-8.300	0.741	1.000
March 28*March 25	10.800	0.668	1.000
March 28*March 26	11.600	0.645	1.000
March 28*April 01	-15.400	0.540	1.000
March 28*March 31	-17.900	0.477	1.000
March 28*March 29	-21.000	0.793	1.000
March 28*March 27	22.400	0.718	1.000
March 28*March 30	-27.200	0.694	1.000
April 02*April 05	-6.600	0.586	1.000
April 02*March 25	9.100	0.520	1.000
April 02*March 26	9.900	0.443	1.000
April 02*April 01	13.700	0.411	1.000

April 02*March 31	16.200	0.311	1.000
April 02*March 29	19.300	0.921	1.000
April 02*March 27	20.700	0.896	1.000
April 02*March 30	25.500	0.778	1.000
April 05*March 25	2.500	0.921	1.000
April 05*March 26	3.300	0.896	1.000
April 05*April 01	7.100	0.778	1.000
April 05*March 31	9.600	0.703	1.000
April 05*March 29	12.700	0.614	1.000
April 05*March 27	14.100	0.575	1.000
April 05*March 30	18.900	0.453	1.000
March 25*March 26	-0.800	0.975	1.000
March 25*April 01	-4.600	0.855	1.000
March 25*March 31	-7.100	0.778	1.000
March 25*March 29	-10.200	0.685	1.000
March 25*March 27	-11.600	0.645	1.000
March 25*March 30	-16.400	0.515	1.000
March 26*April 01	-3.800	0.880	1.000
March 26*March 31	-6.300	0.802	1.000
March 26*March 29	-9.400	0.709	1.000
March 26*March 27	-10.800	0.668	1.000
March 26*March 30	-15.600	0.535	1.000
April 01*March 31	2.500	0.921	1.000
April 01*March 29	5.600	0.824	1.000
April 01*March 27	7.000	0.781	1.000

April 01*March 30	11.800	0.639	1.000
March 31*March 29	3.100	0.902	1.000
March 31*March 27	4.500	0.858	1.000
March 31*March 30	9.300	0.712	1.000
March 29*March 27	1.400	0.956	1.000
March 29*March 30	-6.200	0.805	1.000
March 27*March 30	-4.800	0.849	1.000

VITA AUCTORIS

NAME:	Leslie Ann Holmes
PLACE OF BIRTH:	Belleville, Ontario
YEAR OF BIRTH:	1984
EDUCATION:	East Northumberland Secondary School, Brighton, Ontario 1998-2003 University of Waterloo, Waterloo, Ontario 2003-2005 University of Windsor, Windsor, Ontario 2005-2008 B.F.Sc. University of Windsor, Windsor, Ontario 2008-2010 M. Sc.