MORPHOLOGICAL AND PHYSIOLOGICAL REPRODUCTIVE ASPECTS OF BLACK SOLDIER FLY: APPLICATIONS FOR OPTIMAL INDUSTRIAL SCALE PRODUCTION

A Dissertation

by

ALINE FREITAS SPINDOLA

Submitted to the Office of Graduate and Professional Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

Chair of Committee, Jeffery K.Tomberlin
Committee Members, John D. Oswald
Hojun Song
Craig D. Coufal
Aaron M. Tarone
Head of Department, Peter D. Teel

December 2019

Major Subject: Entomology

ABSTRACT

The successful commercialization of the black soldier fly, Hermetia illucens (L.), relies on the maintenance of fecund adults. Current mass-rearing protocols has resulted in variable production of fertile eggs. The root cause for this variation relies on the lack of understanding of parameters related to their morphology, physiology, and behavior. This study was the first to describe the BSF male reproductive tract and spermatozoa, associated spermatogenesis process, the female spermatheca morphology, and the impact of age on the production of sperm, its viability and how those flies adjust thermal preferences to reach reproductive success. Using three different microscopy techniques, it was shown the gross morphology of the male reproductive tract and female spermatheca appear to be similar to those found in other brachyceran flies. Male spermatozoa are long (∼860 µm overall, ~8 µm head), apparently motile, and possess flagella with a typical 9+9+2 axoneme triplet. Germ cells go through incomplete mitotic divisions surrounded by somatic cyst cells in the testes. Spermatogenesis appears to be initiated during immature development (cryptocephalic pupa stage). From <24 h to 7 d post emergence, male aging appeared to impact sperm production. Analyzing the reproductive capacity of males through aging, age significantly impacted sperm number, which peaked (322.5(214.5-429.3)) when the adults were approximately 48-h-old and then gradually declined. Sperm viability remained consistent as the adults aged (69.7% (60.5%-76.7%). The thermal preference (Tsel) for <24-h-old male (24.3℃ (19.3℃-28.2℃)) and BSF female (20.2℃ (15.4℃-26.2℃)) were significantly different. These temperatures gradually declined over time and then increased to levels close to those initially preferred. Temperatures (20℃, 24℃, and 27℃) examined for their impact on sperm number and viability did not produce significant results. Future studies should explore a wide range of temperatures as well other physiological conditions that might impact thermal preference (i.e. starvation). Morphological data may provide clues on why females of this specie display a monogamous, rather than the polyandrous mating system, and how adults from this specie allocate resources for optimal fitness.

DEDICATION

To my parents Alexandre and Rosemi, my brother Artur, and my spouse David.

ACKNOWLEDGEMENTS

First, I want to thank myself for believing in me even when everything was falling apart. I want to thank myself to have had the endurance of leave my country and my history behind and come to the USA to pursue my dream of obtaining the highest educational degree so I could try to change the reality of Brazil and maybe have some impact in this world. I want to thank myself for doing all this hard work and for having no days off. I am really proud of women I've became.

Second, I would like to thank my committee chair, Dr. Jeffery Tomberlin, for his guidance and support throughout this painful process, for not only having waited almost 5 years until I have obtained funding for my PhD, but for also being my co-advisor during my master degree in Brazil, which allowed me to reach this point. I could write a whole acknowledgment just describing how awesome he is and how important he was for my personal and professional development and how grateful I am for his time and patience. Thank you!

I'd also like to thank my other committee members, Dr. John D Oswald, Dr. Hojun Song, Dr. Craig D Coufal, and Dr. Aaron M Tarone for their expertise, support, and suggestions for my research. Thanks to my lab mates at the F.L.I.E.S. Facility for their support and being a great group of researchers. In special, I would like to thank Dongmin for his friendship, hugs, Korean sarcasm, and tea – we did my friend! I also would like to thank Travis for all the talks, advices and constructive criticism.

Finally, I would like to thank my family, my friends in Brazil, and my spouse David. [In Portuguese]: Aos meus pais, Alexandre e Rosemi e meu irmão Artur, o suporte de vocês é e foi essencial. Não importa o quanto longe eu chegue, vocês sempre serão minha base e minha essência. Aos meus lindos e lindas, me desculpe pela ausência. Queria muito ir tomar uma com vocês, dá uma volta da rural – sinto falta disso todos os dias. Amo vocês e espero que em breve eu possa vê-los e dá um beijo em cada um.

To my spouse David, thank you for all the patience with the “crazy” and “nonsense” Aline. 2019 has been chaotic and intense for us and I am very proud of you for showing every day how strong you are. I love you!

CONTRIBUTORS AND FUNDING SOURCES

Contributors

Manuscript 1: Dr. Romano Dallai and Dr. David Mercati, both from the University of Siena, Siena, Italy in reviewing my micrographs and descriptions; Drs. John Oswald and Dr. Aaron Tarone, for the initial review of the manuscript.; Dr. Oswald for providing access to the Leica Microsystems software and binocular equipment used for gross morphology photos.

Manuscript 2: The post-doc Dr. Travis W. Rusch for his contribution to the thermal preference experiment design; The PhD candidate Elizabeth Walsh and Dr. Juliana Rangel for their collaboration in the sperm count and viability experiment; Dr. Aaron Tarone for having provided lab space and equipment.

Funding Sources

The Brazilian Ministry of Education's Federal Agency along with the The Improvement of Higher Education Personnel (CAPES) provided a scholarship to the author.

NOMENCLATURE

Biased gene conversion	BGC
Mitochondrial DNA	mtDNA
Operational Sex Ratio	OSR
Black Soldier Fly	BSF
Deoxyribonucleic Acid	DNA
Ribonucleic Acid	RNA
Heat Shock Proteins	HSP
Adenosine Triphosphate	ATP
Sterile Insect Technique	SIT
Municipal Solid Waste	MSW
Association of American Feed Control Officials	AAFCO
Forensic Laboratory for Investigative Entomological Sciences	F.L.I.E.S
Light and Fluorescence Microscopy	LFM
Scanning Electron Microscopy	SEM
Transmission Electron Microscopy	TEM
Hexamethyldisilazane	HMDS
Thermal Preference	Tsel
Thermal Fertility Limit	TFL
High Thermal Fertility Limit	TFLMax
Low Thermal Fertility Limit	TFLMin

TABLE OF CONTENTS

Page

LIST OF FIGURES

Page

LIST OF TABLES

1. INTRODUCTION

This chapter is broken into two major sections. The first section provides foundational information on the ecology and evolutionary biology of mating systems with an emphasis on sexual reproduction, sexual selection, sexual conflict, and associated temperature effects. Additional discussion is presented over the evolution of reproductive morphology, physiology (e.g., viability, adult thermal tolerance), and mating behavior as related to aggregation and lekking. The second, and more crucial, section provides an overview of the insect (black soldier fly, *Hermetia illucens* (L.) (Diptera: Stratiomyidae) used as a model to conduct the proposed research.

Black soldier fly larvae play pivotal roles in both environmental and economic aspects of waste disposal and processing (Rehman et al. 2017, Pleissner and Rumpold 2018, Mertenat et al. 2019). Sheppard (1983) reported that black soldier fly larvae can reduce dry manure biomass by 42-56% (Barragan-Fonseca et al. 2017, Rehman et al. 2017, Spranghers et al. 2017). Furthermore, nitrogen concentration and mass were reduced by 24% and 62%, respectively (Sheppard 1983, Janssen et al. 2017, Spranghers et al. 2017) while also reducing the number of environmental pollutants (Diener et al. 2015, Mertenat et al. 2019). Compost treated with BSF larvae presented enhanced available nitrogen (i.e. ammonium - NH_4^+) when compared with the untreated groups (Green and Popa 2012).

The black soldier fly is now mass produced globally to recycle organic waste and produce protein for use as food and feed. However, little is known about the adult (reviewed below). The primary impetus for conducting this research is to enhance black soldier fly mass production for the use as a waste management system to produce protein that can replace feeds for livestock, poultry, and aquaculture. Given this insect's unique mating behavior (discussed below), understanding its basic ecology could lead to more optimized systems for its mass production.

1.1. Part I. introductory information on the ecology and evolutionary biology of mating systems

1.1.1. Prevalence of sexual over asexual reproduction in eukaryotes

Presently, there still does not exist a clear explanation for why sex is pervasive. Sexual reproduction is costly for both sexes, individually, and for the species overall. Evolutionary theorists, such as John Maynard Smith and George C. Williams described through the "cost of males" and "cost of meiosis" theories, respectively, the disadvantages of sexual over asexual reproduction (Williams 1975, Smith 1971a). Assuming no investment by males in progeny (i.e., parental care, nutritional resource), asexual females would reach their maximum fitness by producing cloned daughters instead of sexual reproduction with males, where their fitness would be at most 50% (i.e., anisogamous organisms) (Gibson et al. 2017). This "two-fold cost" of sex partially describes the evolution of sexual conflict between individuals in a given population and its consequences for sexual selection (Lehtonen et al. 2012).

There are additional costs that reduce the likelihood of sexual reproduction evolving. Adults spend an incredible amount of energy in finding mates (Riebel et al.

2010), competing for access to the opposite sex (Kvarnemo and Ahnesjo 1996), and exposure to the risk of pathogens between mates as well as offspring (Tooby 1982, Sherman et al. 1988). At the molecular level, recombination is a consequence of sexual reproduction and occurs gametic cells of males as well as females. Recombination may remove positive associations between selected bundles of alleles, reducing the offspring fitness (Lewis 1987). Nonetheless, asexuality is rare in nature representing approximately 0.1% of all known species (White 1978).

1.1.1.1. Theoretical concepts for sexual reproduction prevalence

Several theories have been proposed to justify the prevalence of sexual reproduction even with its associated costs. In the early 1930s, Ronald A. Fisher (1930) argued that, in heterogenic environments, the beneficial combination between germ cells of each sex in sexually reproduced organism would confer a faster adaptation instead of relying on multiple beneficial mutations happen in the same individual under high density in order to spread them asexually throughout the population Later, Hermann J. Muller (1932, 1964) stated that, through recombination, sexual reproduction had optimized the effect and fixation of beneficial mutations toward the adaptation of individuals. Moreover, he observed that asexual populations display what he described as a “rachet mechanism” in the way of how the offspring accumulates mutations (in one possible direction). Due mutations being mainly deleterious and beneficial ones rare, under environment stimulus, asexual reproduced organisms will have accumulated large loads of deleterious mutations that could lead them to extinction. These two concepts were brought together to form the Fisher–Muller hypothesis.

Felsenstein (1974) summarized the concepts from the Hill-Robertson effect, which described a population of finite size when subjected to natural selection results in non-random association of alleles at different loci due the effects of genetic drift or mutation (Hill and Robertson 1966), and the Fisher–Muller hypothesis, using mathematical models and computer simulations inputting different selection coefficients and mutation rates, demonstrated recombination enhances the likelihood of fixation of advantageous mutations while breaking apart sub-optimal mutations. This concept is described as selection interference and it can be displayed in four different interfaces (Charlesworth 2009, Wright 2016).

Another important approach attempting to explain the maintenance of sexual reproduction can be found in the Red Queen theory, originally developed to account for Van Valen’s law of constant extinction (Van Valen 1973). This concept explains, using the co-evolutionary interplay among hosts and their parasites as a means to describe an adaptive zone for a given species must be continuously evolving to keep pace with an advancing “front” of fitness (Lively 1987). A sexually-reproducing population will potentially result in more resistant-descendants than an asexually-reproducing population (Shcherbakov 2010). This concept suggests the genetic range acquired from sexual reproduction offers the advantageous genetics necessary for a species to defend against parasites. Asexual individuals are reliant in most cases on mutation for genetic diversity, hence, under strong selection and a heterogenic environment, they will not thrive where parasites are abundant (Gandon and Michalakis 2002, Howard and Lively 2002).

Dacks and Roger (1999) after mapping the diversity of sexual cycles in a possible phylogeny of eukaryotes suggested their common ancestor was a facultative sexual unicellular species, with sex having a single evolutionary origin. Moreover, it seems the origin of obligate sex may be associated with the evolution from unicellularity to multicellularity, as these two events appears to be positively correlated (Bell 1982). An organism's complexity enhanced by the multicellularity requiring a strong plasticity under adverse environmental conditions. The "Best Man Hypothesis", proposed by Bell (1982) relied on concepts described by Emlen (1973) and Williams (1975) that assumed the occurrence of facultative sexual reproduction as a response to environmental stimuli, suggesting sexual reproduction evolved in order to provide genetic variability for progeny in a changing environment. In addition, for a population switch from facultative to obligatory sexual reproduction permanently, the environment would have to exhibit a stochastic pattern (i.e., cold/warm, food availability, high migration rate) among generations to justify the benefits of sexual reproduction over its costs (Smith 1971a, Smith 1971b).

Sexual reproduction sets a two-level recombination scenario, when the offspring are a result of recombination happening in both parents, therefore, enhancing their genetic variability and, consequently, their capability of responding rapidly to an environment stimulus. The evolution of living organisms is a consequence of two processes: The genetic variability generated by random mutations, which continuously arise within populations, and the changes in the alleles frequency within populations over time caused by natural selection, migration (gene flow), genetic drift, and biased gene conversion (BGC) (Duret 2008). However, alleles originating from mutations, recombination, or BGC do not impact positively or negatively the fitness of individuals, which is referred to as "neutral theory," which will are not targeted by natural selection and might turn out to be advantageous for the adaptation of organisms in environment with strong biotic and abiotic fluctuations (Kimura 1991, Duret 2008). An exception for this statement includes cases where the effective population size is small, which may affect the neutrality of most genetic variants (Jiménez-Mena and Bataillon 2016).

1.1.2. **Sexual selection**

Besides the costs associated with sexual reproduction, it remains being the main mechanism of reproduction in eukaryotes. However, not all individuals will reach high reproductive rate or even mate one single time during their lifetime. The reason behind the failure in reproduce displayed by some organisms within populations is related to how strong sexual selection, a type of selection based in genetic inheritance such as natural selection, will be and how those organisms, individually, will respond (Shuster 2009, Garcia-Roa et al. 2018).

Defined as an evolutionary process that has positive effects on traits related to mating opportunities promoting the expression of secondary sexual traits in the sex which contribute less to the progeny resulting in a large variance in its reproductive success. This singularity is referred to as the quantitative paradox of sexual selection (Shuster and Wade 2003) and has the function of counter balancing the initial investment made by the other sex. Such traits are indicators of sexual fitness or Darwinian fitness (Demetrius and Ziehe 2007). Due to the large investments made from each sex, sexual conflict is an inevitable consequence of sexual selection since genetically different individuals would diverge in their evolutionary direction.

1.1.2.1. Sexual selection timeline

Sexual selection differs from natural selection regarding its origin with the first as a result of differences in mating success while the second is due to variance in all other fitness components (Townsend 1989, Demetrius and Ziehe 2007). Sexual selection is often stronger than natural selection, where the trait fitness values are below the optimal for environment adaptations under the natural selection rules (Kokko and Jennions 2014). Sexual selection can be divided into two processes according to the phase of sexual interaction (Shuster 2009, Hosken and House 2011): Pre-copulatory sexual selection, which is the step related to the interaction between and among the sexes before mating and post-copulatory sexual selection, the step related to the interaction between and among the sexes after mating (Andersson and Simmons 2006, Hosken and House 2011, Clutton-Brock 2017).

1.1.2.2. Pre-copulatory mechanisms

During pre-copulatory sexual selection events, the mechanism associated with this phase is intersexual competition, with males and females performing different strategies in order to meet their optimal reproductive fitness (Andersson and Simmons 2006, Hosken and House 2011). The first strategy displayed by both sexes is related to the evaluation of the associated costs and benefits of the mate (Andersson and Simmons 2006, Kortet 2019). Mate choice involves a complexity of adaptive and non-adaptive mechanisms driven by males with promiscuity-tendency behavior and female choosiness towards reproductive compensation for itself or associated offspring (Parker 1984, Parker 2006). Secondary sexual traits are developed due to situations of contest or competition between individuals of one sex (usually males) for mates and mating preferences exerted by the opposite sex (females), where the strength of sexual selection on these traits will depends on their fitness costs and benefits (Clutton-Brock and Huchard 2013, Clutton-Brock 2017, Fitzpatrick and Servedio 2018).

Behavioral sexual traits may be assessed by females looking for signs of fidelity and domesticity prior allow copulation. The called domestic-bliss strategy, described by Dawkins (1976), states that females would delay intercourse with suitor males, obligating them to stay for a long period before allow copulation. The last male who stay will have proved his commitment toward to the offspring and, most important, his quality status. De Luca et al. (2015) had shown that intermediate mass males of the Australian redback spider, *Latrodectus hasselti*, presented higher metabolic efficiency during courtships in comparison with heavy mass males, allowing them to reach higher reproductive rate. Prolonged courtships have been described in several animal groups (Barrass 1979), such as spiders (Stoltz et al. 2008, De Luca et al. 2015), frogs (Bush and Bell 1997), ants (Mercier et al. 2007), and moths (Girling and Cardé 2006).

One of the first theories to describe sexual selection, the Fisherian runaway selection, termed by Fisher through his hypothesis called “runaway selection”, described, under theoretical quantitative genetic approach, the basic process by how females mating preference and secondary sexual traits in males coevolve (Fisher 1930).

In concordance with Darwin's assumptions about exaggerated sexually dimorphic phenotypic traits, Fisher stated the genetic basis for both preference and trait expression are related with an aesthetic sense than an indicator of genetic quality. Furthermore, mating preferences of females evolve in congruence with the exaggerated traits that are the focus of their preferences and those traits were inheritable (Ritchie 2007, Milam 2010). Therefore, under indirect selection, sons with extreme ornaments and daughters with extreme preferences for them would compose female's offspring (Andersson and Simmons 2006, Milam 2010).

Weatherhead and Robertson (1979), using the Orians-Verner-Willson model (a model that explains the evolution of polygyny based on male territorial behavior towards quality of the nest site), argued that, besides their initial reduction in their reproductive success, females who copulated with "attractive" males gained indirect fitness through the outperformance of their sons, what later they called the "sexy sons" hypothesis. Few years early, Dawkins (1976), through his "he-man strategy" concept, described females choosiness based on traits displayed by males which were attractive to females of the next generation, enhancing indirectly their reproductive rate. In a study with *Drosophila melanogaster* (M.) (Diptera: Drosophilidae), males sired by attractive males were 30% more successful on average in obtaining mates than male sired by unattractive males (Rundle et al. 2007).

Alternatively, to the runaway hypothesis, the good genes hypothesis stated there is a positive correlation between secondary sexual traits and genetic quality of males (Zahavi 1975). Quality attributed to the sexual traits displayed by males is due to the high energetic investment in developing exacerbating traits. However, this energy will not be wasted once this investment increases the chances of males being selected by females. For instance, males of gray tree frogs (*Hyla versicolor*) with longer calls resulted in offspring that performed better during larval and juvenile stages than did offspring resulting from males producing shorter calls. This study suggests that call duration may be a reliable indicator of heritable genetic quality (Welch et al. 1998).

Using similar concepts of the good genes and sexy-son, the sexy sperm hypothesis justifies the appearance and maintenance of polyandry by the means of sperm competition (Yasui 1997). In an intense male-male competition environment, males should go beyond of invest in pre-copulatory strategies to enhance their reproductive rate and invest in post-copulatory strategies, such as high sperm viability (McNamara et al. 2014). Since males would increase investment in their germ cells, polyandry would prevail over monandry (Simmons 2003).

Conversely, female choice for good genes may exist even with the lack of ornaments as cues. In pronghorns (*Antilocapra americana*), females performed an energetically expensive mate sampling process in order to identify vigorous males. As a result, a small proportion of males found to be the most sexually active sired the most of the young. In fact, resulting offspring of the attractive males were more likely to survive. However, as a counter, females, who mated with less attractive mates, compensated their low reproductive fitness by elevating rates of milk delivery to their young resulting in enhanced survivorship (Byers and Waits 2006).

Nevertheless, in a quantitative genetic study, no positive genetic correlation between paternity in sons and polyandry in daughters were observed in the field cricket, *Teleogryllus oceanicus* (Le Guillou) (Orthoptera: Gryllidae), as expected by the "sexy sperm" hypothesis (Simmons 2003). Moreover, some sperm phenotypes, such as sperm length and viability, may be expressed by mitochondrial/cytoplasmatic genes (Dowling et al. 2007) and, since mitochondrial DNA (mtDNA) is in most of the cases maternally inherited (Pizzari and Birkhead 2002), with rare exceptions (Luo et al. 2018), the male-son inheritance may not fully explain the sexually selected sperm models (Chen et al. 2016).

According to the Hardy-Weinberg law, in a randomly mating population under no selection, genotypic frequencies will meet equilibrium after one generation. However, although this is true for the individual loci, it is not true for genotypes with multi-locus. At second generation of random mating between two individuals in homozygous all genotypes will show up, but they will not occur at the frequency that was expected at equilibrium (Li 1988). The phenomenon of nonrandom association of loci is called linkage disequilibrium or gametic phase disequilibrium.

With the absence of retarding forces, loci, whether linked or not, will eventually attain linkage equilibrium, decreasing the time spent with the crossover rate. In a case where there is a small sample of individuals of a given population, the genetic correlation estimated would not be accurate in affirming if the output derives from pleiotropy (a subset of the genes that influence one trait and may also influence another trait) or from linkage disequilibrium (the genes may act independently on the two traits, but because of nonrandom mating, selection, or drift may be associated) effect (Li 1988, Guo and Thompson 1992). Overall, it seems the theoretical basis for the evolution of mate preference is the genetic correlation resulting from linkage disequilibrium (Conner and Hartl 2004).

1.1.2.3. Post-copulatory mechanisms

The second process is referred as post-copulatory sexual selection and the main mechanism observed is an intrasexual competition, however some cases of intersexual competition may happen. In mating systems where females mate with multiple males, males need to invest in strategies that will outcompete with other males after copula have took place. Geoff Parker (1970) described a type of competition that would rely on the morpho-physiological characteristics of the sperm. This competition at cellular level, initially, excludes female's decision and set males on the of the game. The pressure resulted from this intrasexual selection may impact male's reproductive anatomy, physiology, and behavior across diverse taxa, with responses varying according with the proportion of sexually mature females or/and their promiscuity level (Emlen and Oring 1977).

Early analyses made by Parker demonstrated internal fertilizations follow the "raffle principle", where the probability of fertilization of males increases with his total number of sperm produced and, since male reproductive resources are limited, the size of sperm must be small (Parker 1982, Parker 1990). Withal, the method used by males related to their contribution in a successful mating and assets available for future mates must not be the same for all of them, where adult males with extensive mating costs should designate their assets more conservatively than males with lesser mating costs (Engqvist and Sauer 2001). In populations where females are mostly monandric (i.e., female mates with just one male), a form of male cryptic choice might happen as soon as the males encounter an excessive mating effort, such as courtship rituals like nuptial gift (Smith et al. 1971a).

The different strategies displayed by males during courtship rituals might reduce simultaneously the available resources and the opportunity for future mating. Hence, it is expected males spend less reproductive resources on mates that offer low reproductive capacity, saving resources for future copulations (Engqvist and Sauer 2001). Cases can be found with crickets (Orthoptera: Gryllidae) (Gage and Morrow 2003) and beetles (Coleoptera) (García-González and Simmons 2007), where shorter sperm were more successful for fertilization. Although conflicting, positive correlations between sperm length and sperm competition were found in several groups indicating that, in special circumstances, increased sperm length can enhance the competitiveness among males, leading to select larger sperm size if larger sperm are more successful in fertilizing eggs.

Data from studies conducted in Lepidoptera (Gage and Cook 1994, Morrow and Gage 2000), some social Hymenoptera (Baer 2003), and with *Drosophila* (Luck and Joly 2005, Lüpold et al. 2016) support this assumption. Moreover, the expression of such costly feature might be a consequence of a co-evolution resulted from male-female interactions (Dybas and Dybas 1981, Minder et al. 2005). The possible coevolution of sperm size and female reproductive tract morphometry might explain the disparity in the data collected from studies focused on sperm competition implications.

Associations across different insect orders, including diving beetles, Dystiscidae (Higginson et al. 2012), featherwing beetles, Ptiliidae (Coleoptera) (Dybas and Dybas 1981), dung flies, Scathophagidae (Diptera) (Minder et al. 2005), and Drosophilidae (Pitnick et al. 1999) (Miller and Pitnick 2002) have been documented, which support the assumption that females may mediated selection on sperm morphology. Furthermore, the massive investment in sperm size made by Drosophila males may be an example of the "Fisherian runaway selection" and "sexy sons" hypothesis, where the costs involving resources allocation and maturation time for the production of such sperms promotes a honest signal about males reproductive status and, in counterpart, females provide a physiological and morphological environment that gives a fertilization advantage to those males that can produce long sperm (Pitnick 1996, Gage 2012).

In addition, females may also have some control over males that will sire their young. A case of intersexual competition during the post-copulatory phase called "cryptic female choice" (Birkhead 1998) is a concept developed by William Eberhard, which described how females prevent undesirable sperms of fertilizing their eggs throughout "hidden" morphological, behavioral, or physiological mechanisms (Eberhard 1996, Birkhead and Pizzari 2002). Firman et al. (2017) reviewed studies using several animal models that have demonstrated that mechanisms, such as time of insemination, ejaculate size, sperm ejection, neutralization, storage, dumping, activation, attraction to the eggs, and fertilization are possible mechanism behind the "cryptic female choice", with possible impacts on female fitness and sexual conflict.

1.1.3. **Sexual conflict and mating systems**

When sexual reproduction relies on the genetic variability produced by the encounter of anisogametes (i.e., unequal sizes), the “twofold cost” of sex, mentioned early in this chapter, is, at cellular level, the first explanation of why conflict arises between males and females, with direct and indirect consequences for sexual selection and appearance of unique mating systems.

1.1.3.1. Battle of sexes: The eternal argument of who invested more in the offspring

Evolutionary differentiation of males and females during the gametogenesis process lead females to yield fewer oocytes than males but invest more in each one while males produce large amounts of sperm, each one them with energy at most to reach the eggs in order to fertilize them (Krasnec et al. 2012, Lehtonen et al. 2012). However, a study conducted by Parker and Pizzari (2010) on theories of ejaculate economics, indicated ejaculates may be affected by post-copulatory sexual selection in accordance with economic principles, where the level of promiscuity of females, their quality status, and the relative costs associated with spermatogenesis would impact how males allocate their ejaculates within and among copulas.

The sex who provides the highest parental investment will tend to have the slowest potential rate of reproduction, which will result in a disproportionate availability of their sexually mature forms. Emlen and Oring (1977) proposed a model called operational sex ratio (OSR) where, differently of sex ratio, male/female distribution is based on the proportion of sexually mature males to sexually mature females at a given time. Since males and females aim to reach their own highest reproductive rate, sexual conflict is inevitable, where one or both sexes may use physical, such as persuasion, coercion, force (Emlen and Oring 1977, Eberhard 2002) and chemical, such as immune-depressed seminal peptides (Domanitskaya et al. 2007, Avila et al. 2011, Short et al. 2012), and spermicide (Bernasconi et al. 2002, Holman and Snook 2006), mechanisms in order to reach their goals.

In populations where the OSR have approximate values to Fisher's sex ratio, males tend to be less choosy toward females. Through mechanisms such as persuasion, coercion, and/or force, they will try to control mating in order to sire as many offspring as possible. Through these strategies they optimize their chances to copulate with as many mates as possible (Jennions and Fromhage 2017). On the other hand, females will counter "detrimental" male strategies effects on their reproductive fitness (i.e. male guarding, male sperm plugs, refractory substances) by becoming choosy toward males with extravagant traits in order to obtain either direct (current generation) or indirect (subsequent generation) benefits (Edward et al. 2015, Gasparini and Evans 2018).

In populations where the OSR is male-biased, females are harassed by males to achieve copulation (Rowe et al. 1994). This approach results in detrimental costs for females in terms of fecundity and survival (Iglesias-Carrasco et al. 2018). The intensity of the sexual harassment is density-dependent where in extreme cases oviposition behavior is impacted (Cordero and Andrés 2002). An alternative strategy performed by males is increasing parental investment towards the offspring, which in turn allows them to be more selective in terms of mates (Simmons 2001a, Shuster and Wade 2003, Shuster 2009, Alonzo 2012, Krasnec et al. 2012, Kokko and Jennions 2014, Lyu et al. 2017).

1.1.3.2. Mating systems and the picky sex

Mate choice strategies performed within and between sexes are positively correlated with the mating system observed in a given population (Arnqvist and Rowe 2013). Mating systems are classified based on the number of matings and frequency of copulation successes of each individual during the breeding season (Shuster and Wade 2003, Andersson and Simmons 2006, Krasnec et al. 2012). Genetically speaking, mating systems can be classified either as random or nonrandom mating, based on the genotypic frequencies for a specific locus (Castillo et al. 2016). Random mating is defined as the chance of an individual to mate with someone of equal genotype frequency (allele frequency). Nonrandom mating, as can be expected, is based on the probability of mating individuals with a different genotypic frequency. Assortative mating, a type of nonrandom mating, is related to positive (more similar) or negative (dissimilar) traits according with the parent's phenotype. Positive assertive mating involves mates that are phenotypically more similar to each other, with negative assortative mating being the opposite (Conner and Hartl 2004).

There are multiple structures to mating systems. Monogamy is where one male and one female mate exclusively with each other, displaying variable copulation frequency with a possible offspring yield every reproductive cycle. Polygamy, on the other hand, can either be an exclusive relationship of one male/female with two or more females/males. Under these circumstances, common traits include displaying a variable copula frequency with a possible offspring yield per female/reproductive cycle. Polygamy is divided in two subcategories: polyandry, which is an exclusive relationship of a female with two or more males, displaying a variable copula frequency with a possible offspring yield per male/reproductive cycle and Polygyny, which is an exclusive relationship of a male with two or more females, displaying a variable copula frequency with a possible offspring yield per female/reproductive cycle. The third category is promiscuity where members of one sex mate with any member of the opposite sex at a variable copula frequency rate with an unpredictable offspring yield per male/female/reproductive cycle (random mating) (Shuster and Wade 2003, Krasnec et al. 2012, Kvarnemo 2018).

Males from most taxa are well-known to be polygynic or promiscuous since mating with multiple females would enhance their potential rate of reproduction. Differently, females tend to be monogamous. However, there can be variation, such as serial monogamy, which is the practice of engaging in a relationship with one mate at a time over an extended time period (monandry: females; monogyny: males). Another strategy by females is extra-pair copulations, which is a promiscuous behavior in monogamous species where one of the sexes from mates outside of the pairing. Nonetheless, few studies have shown that females may enhance their fitness in polyandric mating systems, with its costs associated lower than its benefits (Kvarnemo and Simmons 2013, Parker and Birkhead 2013).

The costs involved with polygamy and promiscuity are related to several factors. Such system characteristics, such as time and resources spent searching for mates and the deleterious impact on female’s health due to the seminal peptides present in each sperm load (Domanitskaya et al. 2007) or physical injuries caused by copulation itself are known to be key factors (Morrow et al. 2003, Peinert et al. 2016). Overall, abiotic and biotic factors may have positive, neutral, or negative impacts on mating frequency, such as OSR (Emlen and Oring 1977), population density (Cordero and Andrés 2002, Cureton II et al. 2010), presence of predators, food availability (Sih et al. 1990, Travers and Sih 1991), and phenotype of previous mates (Zeh et al. 1998, Ivy et al. 2005); therefore, optimal mating is a continuum, not a fixed factor, where males and females of same species from different populations may perform different mating systems (Neff and Svensson 2013, Santos and Nakagawa 2013).

1.1.3.3. Lifespan as a limit factor

Lifespan is also an important factor to consider when studying mating system dynamics. For instance, males from monandric mating systems where females have a short lifespan and rapidly disappear from the population a few days after becoming sexually mature usually have a short lifespan as well. Some speculate male longevity is shortened in these systems due to their high initial investment to improve their reproductive success early in life (Austad and Fischer 2016). These individuals have evolved a strategy called protandry (fast immature development by males that leads them to emerge before females) (Wiklund and Fagerström 1977). This strategy enhances their potential reproductive rate by being available for females as soon as they become sexually mature (Bakker et al. 2011). For instance, males of the ant species *Cardiocondyla obscurio*r (Wheeler) (Hymenoptera: Formicidae) when exposed to a large numbers of sexual mature females had their life span significantly reduced (Metzler et al. 2016), which seems that by allocating resources in order to potentially increase reproductive output (progeny production) early in life, may cause a trade-off between early reproductive success and lifespan (Duxbury et al. 2017, Li et al. 2019).

In cases where both sexes evolve shortened lifespans, they exhibit low synchronized breeding periods. Females typically are less synchronized, and males remain sexually active during the full breeding period. In this situation, pre-copulatory sexual selection is strong. Males will invest heavily in secondary sexual traits at a cost to survivor, resulting in choosy females for particular traits and a skew OSR reaching extremes (Emlen and Oring 1977, Höglund and Alatalo 2014). As a result of the high investment made by males to be sexually mature early in the adult stage and to outcompete rivals, males do not provide any resource, besides sperm or parental care (Robert 1972, Burt et al. 2007). To optimize the encounter with sexually mature females, males may aggregate near areas where females are likely to visit or pass by (Bradbury and Gibson 1983, Lank and Smith 1992). Therefore, males may form “leks” to simply locate mates in interest of to boost their probability of copula within a short period due the short lifetime constraint (Höglund and Alatalo 2014).

1.1.4. **Lek mating system: making easy for everybody**

Lekking is described as a spatial arrangement where promiscuous males aggregate at a mating site and monogamic females visit only for copulation proposes (lek polygyny) (Bradbury and Gibson 1983, Höglund and Alatalo 2014). In a classical lekking reproductive system, the signaling between male and females is limited, as the males provide no resources to females or parental care for their offspring. This implies that females gain indirect benefits from her choice in the form of “good genes” for her offspring (Barton and Turelli 1989).

In selecting a male that excels at courtship displays, females gain genes for their offspring that might increase their survival or reproductive fitness. Moreover, male sexual features might convey beneficial to the females in cases where these traits have a handicap effect on males, therefore preventing the interference of cheaters. The basic inputs of the handicap principle are by showing how costly male ornaments are, it provides to females information about the hidden male’s inheritable fitness (Iwasa et al. 1991). The free female choice is an important aspect of lek mating patters since distinguish this form of aggregation from the typical mating swarms, where females are considered to have low or no selection power over males (Harker 1992, Shelly and Whittier 1997).

Species vary greatly regarding their level of aggregation. These variations in lekking can be classified as exploded, classic, and landmark. The first two are based on the level of dispersion found in males from an emergence site, while the third is related to species specializing in habitats that will be mating sites (Höglund and Alatalo 2014). The third type of lekking is commonly found in insects, with specific landscapes such as hilltops, sunspots or vegetation are used for male’s settlement, following a pattern or not, and females to reduce their costs in finding and choose mates (Shelly and Whittier 1997, Höglund and Alatalo 2014).

The black soldier fly (BSF), *Hermetia illucens* (L.) (Diptera: Stratiomyidae) displays a landmark lek aggregation, where males in the wild can be found resting on the surface of leaves. When other males arrive in a spot protected by a founder male, a territorial followed by aggression behavior can be observed. Transient females were grasped on flight and right after descend in copula (Tomberlin and Sheppard 2001). A Similar behavior was reported to another stratiomyid species, *Hermetia comstocki* (Williston) (Diptera: Stratiomyidae), where males were observed aggregating on agave trees and resting individually on the upper leaf surfaces. Founder males were observed being aggressive toward other approaching males, with the winner returning to the preoccupied spot and the loser leaving the area (Alcock 1990).

The intensity of female choosiness will depend on how far the lek spot is from the emergence site (Ide and Kondoh 2000). Wickman and Rutowski (1999) in an analysis of non-resource-based mating systems of different insect taxon, described the distance of encounter based on the dispersion pattern of females, whereas the closer mating occurs to the site of initial receptivity of females, the more dispersed matings will be and females being less discriminate in mate selection. Matings will become progressively concentrated based on how further females travel toward sites of highest male encounter rate before being detected. The model proposed by Parker (1978) explains how leks are expected to evolve using the logic described above.

As mentioned before, lek polygyny is performed by species where the energetic cost of searching is high (i.e., due costs associated with flight) or/and unpredictability in locating sexually mature females, and/or females display preference for clustered males

(Bradbury et al. 1986, Hibino 1986). Depending on the sex-based dominance, it can be classified in "female-preference model”, where male clustering is promoted by the dominant effect of female choice; "hotspot model," where male clustering is produced by sequential settlement of males onto sites or pathways preferentially used by females, and; “hotshot”, where combination of male-male dominance interactions added of conservative or "default" mate choice by females (Beehler and Foster 1988).

Female choice has an impact on the evolution of behaviors associated with lekking. For example, patterns of female choice for attractive males may lead other males to aggregate around these attractive males (hotshot) (Young et al. 2009). However, even within leks based around female habitat preferences (hotspot), positions within the lek may influence male mating success, with females preferring males in certain positions within the lek (Bradbury and Gibson 1983, Shelly and Whittier 1997). These position-effects may either be independent of the males present or influenced by variation in male attractiveness, behavior and density (Bateson and Healy 2005). Moreover, male success in a lek may therefore be a function of both the absolute and relative quality of a male’s courtship display as well as his geographic position in a lek.

A study by Jones et al. (1998) with sand flies, *Lutzomyia longipalpis* (Diptera: Psychodidae), tested the three main hypotheses justifying the prevalence of choosy females in lek patterns: 1) females benefit directly; 2) females gain indirect Fisherian benefits by yielding more attractive sons; or 3) females benefit indirectly due preferred males possess `good genes' that provide increased viability their offspring overall. It was found no evidence for the transmission of good-genes since offspring survival was not enhanced, but males sired by attractive males were more “attractive” when they exposed themselves in lek. The results, therefore, indicate that Fisherian benefits are at least partly responsible for *L. longipalpis* females preserve their choice pattern at leks.

1.1.4.1. Lek paradox: one single type of male?

At same time that lek patterns provide a great opportunity to study sexual selection in males and females and the sexual conflict resulted from the interaction between the sexes, it still a paradigm of how the constant strong sexual selection does not deplete the genetic variability present in this system (Höglund and Alatalo 2014). The “lek paradox”, described as the steady genetic variation in population that perform lek despite of the prevalence of choosiness females for specific traits. Sexual selection displayed by females towards males depletes genetic variation of the group by Fisherian runaway, thus excluding the possibility of gain fitness through choice (Bradbury and Gibson 1983, Rowe and Houle 1996, Bonduriansky and Day 2013).

However, the additive genetic variance is maintained and runaway does not occur. A possible explanation might be related to a mutation–selection balance mechanism, where it is assumed that mutations may generate new genetic variation as fast as it is eroded by selection (Tomkins et al. 2004). There are other two main assumptions that aim to explain the maintenance of genetic variability in lekking reproductive system. The handicap theory, developed by Zahavi (1975), assumes that the secondary sexual traits present on males that are attractive to females must be deleterious to their survivorship to prevent cheating behavior from other males during the courtship displays. Risks such as increased predator risk and high parasite infection density have a propose of allow females to assess male quality (Shuster 2009, Hosken and House 2011). Therefore, males which possess costly ornaments provide information to the females about their inheritable fitness (Zahavi 1975, Hosken and House 2011, Krasnec et al. 2012, Gibson et al. 2017).

The second, referred to as Rowe and Houle's theory of condition-dependent expression of male sexually selected traits (Rowe and Houle 1996), assumes sexually selected traits encompasses a large number of genetic loci. Therefore, due condition of dependence among locus, the genetic variation is maintained under persistent female choice, a condition later named as genic capture hypothesis (Kotiaho et al. 2001, Tomkins et al. 2004). This theory assumes a large amount of the genome will determine the expression of sexually selected traits. If males can enhance their sexually selected traits by increased investment and if this is less costly to males with more resources to invest, then covariance between male exhibition traits and condition should evolves through sexual selection (Andersson 1986, Kotiaho et al. 2001). Recently, Dugand et al. (2019) used the views described in the genic capture hypothesis (Tomkins et al. 2004) to demonstrate that the respective theory could solve the paradox of lek mating systems. Applying bidirectional changes in allele frequencies at loci under selection (i.e. 4 success-selected, 4 failure-selected, and 3 control), they found that sexual selection would erode molecular genetic variation, but the genetic variation was maintained due mutation-selection balance across the genome, supporting the concept behind of genic capture hypothesis.

Furthermore, current researchers have shown that nonadaptive processes generated by different inputs from heterogenic environments have played a central role in evolution (Bonduriansky and Day 2013, Guerrero-Bosagna 2017), demystifying the random process assumed by the neo-Darwinian evolution concept for the beginning of evolutionary novelties. The neutral theory, proposed by Kimura (1991), indicates mutations considered neutral under natural selection are the primary foundation of genetic variability observed in an organism. Recent models have indicated alleles selected under positive selection took longer to be fixed than neutral ones (Mafessoni and Lachmann 2015).

The epigenetic concepts go against those ones defended by the neo-Darwinian evolution theory and assume, considering beneficial mutations are rare and purifying selection have a small impact in reducing stochastic mutations (being these positive or negative depending of the environment context), those remaining mutations would have progressed in the genome neutrally once these genes do not impact fitness positively or negatively. In line with Kimura's neutral theory of evolution, epigenetically environment-induced mutations, such as chemical interactions of the deoxyribonucleic acid (DNA) with proteins (histones) or methyl groups (methylation) after cell divisions, can regulate gene expression independent of changes in the DNA sequence.

Accordingly, nonadaptive process caused by the inheritance of neutral genes may be related with the steady variability observed in lekking mating systems despite the intense sexual selection. Bonilla et al. (2016) described an alternative possible lek paradox resolution using environmental-induced factors, such as noncoding ribonucleic acid (RNA) expression, DNA methylation and histone modifications, to establish their assumptions using male germ line cells (sperm) (Bonduriansky and Day 2013, Bonilla et al. 2016). Due abundant replication events that occur during the spermatogenesis, sperm cells are predisposed to produce mutations called *de novo*, such as base substitutions and indels (an insertion or deletion of bases in the genome of an organism (Mullaney et al. 2010)), as a result of oxidative damage and massive chromatin rearrangements ensued in both premeiotic and post-meiotic phases, respectively (Grégoire et al. 2013). Sperm are the starting point for heritable genomic novelty occurrence in consequence of its potential for generating genetic or epigenetic variability that will be spread to unknown number of generations (Guerrero-Bosagna 2017).

Through the transmission of acquired epigenetic states via sperm epigenome, males confer a paternal environmental inheritance for the offspring. They also provide an inexhaustible source of variation in male quality. This trait resolves the lek paradox by asserting the understanding of female preference as a dichotomy between choice for direct and indirect genetic benefits (Soubry et al. 2014, Guerrero-Bosagna 2017). Costly preferences are possible if fitness is directly related to extreme mutable features that could remain in the population over multiple generations. Bonduriansky and Day (2013) in a theoretical study using a general model to predict the nongenetic inheritance displayed by males that could be subject of selection by females in lek, demonstrate that genetic variation in fitness could be maintained if genotype–environment interactions are strong, such that different alleles confer high fitness in different ambient conditions or microenvironments.

1.1.5. **Abiotic impacts on fitness**

Depending on the OSR found in a given population, sexual reproduction may impose a strong pressure for the fixation of certain genes that confers phenotypic advantages on fitness. The fixation of these genes in the males of a population is a result of biased female preferences towards certain traits that, due the disadvantages linked to them, signaling outstanding performance in comparison with males that do not display those selected traits. The negative impact on male's survival due investments that are not suitable for the environment added of initial investment made by females towards to the offspring creates a tension between the sex called sexual conflict. However, not only genotype intervenes on the expression, maintenance, and fixation of "sexual traits". Abiotic factors, such as temperature, can influence female's choice toward novel sexual traits, change male's reproductive strategy, enhancing, decreasing or extinguishing a population.

The majority of biochemical and physiological strategies underlying behavioral patterns, including spatial patters of body size, population density, and species biodiversity is temperature dependent (Buckley et al. 2008, Brown 2014) In fact, this abiotic factor plays a critical rule in survival and reproduction especially in ectotherms (Buckley et al. 2008, Dowd et al. 2015). Temperature variation impacts organism's life traits differently within and between species where, depending on their performance, they can be classified as thermal specialists (narrow-range tolerance over temperature fluctuations) or thermal generalists (broad-range tolerance over temperature fluctuations) (Sheth and Angert 2014, Tuff et al. 2016). Additionally, according with their capacity of regulate body temperature, thermal specialists can be called as “perfect thermoregulators” and thermal generalists as “perfect thermoconformers” (Angilletta 2009).

1.1.5.1. Temperature adaptation strategies under predictability and time length of events

Environment changes, such as temperature shift, are either cyclic (short and long-term) or acyclic (short and long-term) changes based on predictability and duration of these events (Tauber et al. 1986). Due the unpredictability of acyclic events, individual responses originate from the nervous system and random physiological and genetic mechanisms (Danks 2007). The consequences can be translated in a slow down or completely stop growth and/or reproduction, setting the individual in a dormant (i.e. diapause) state while the environment remains hostile (Tauber and Tauber 1976, Hahn and Denlinger 2011).

Insects, in general, exhibit differential thermal sensitivity depending on their developmental phase (Bowler and Terblanche 2008). *H. illucens* larvae had their metabolic and growth rate positively and development time negatively correlated to the increase in temperature (Tomberlin et al. 2009, Gligorescu et al. 2018, Shumo et al. 2019). Moreover, the longevity of the adults from this specie was reduced in both sexes (Tomberlin et al. 2009, Shumo et al. 2019) but overall fecundity (i.e. mean by the number of eggs oviposited) was improved with temperature increase (Shumo et al. 2019).

Considering such strategies are not highly evolved, they impose a high risk of failure where the negative impact of their outcomes on the individual's fitness leads to species replacement or their local extinction. Hence, insects who can possess genetic polymorphisms for some crucial morpho-physiological traits perform better than ones with less polymorphic genes (Tauber et al. 1986). These genetic variabilities presented in different loci are a product of nonadaptive processes allowing genes not filtered out by natural selection to persist (Ellegren and Galtier 2016). Consequently, the innate thermoregulation capacity, granted by the neutral genome along with life-history events, determines how well an organism adapts over a heterogenic thermal state.

Evidence of non-selected gene expression has been observed during male-female interaction of the pipefish, *Syngnathus abaster*, at breeding spots where female preference towards male sized were positively correlated with temperature (Silva et al. 2007). Moya-Laraño et al. (2007) found a correlation of body size advantage and temperature fluctuation in seed beetles males, *Stator limbatus* (Horn) (Coleoptera: Chrysomelidae) during a competition essay to assess mate preference. In cooler temperatures, small males had a significant advantage over large males (i.e., scramble competition) as a result of being faster and more intense.

1.1.5.2. Temperature acuity in insects

Insects, being ectothermic, tend to be at the mercy of temperature with direct impacts to their reproductive fitness. Therefore, thermoregulation processes have evolved to allow insects to counter temperature shifts and decrease the impact on survival and reproductive success. Abram et al. (2017) described two main processes of how insects may perceive temperature and the pathways that lead to the behavior-response: A top-down integrated effects and bottom-up kinetics effects. The top-down integrated effects translate how insects modulate behavior in response to thermal information obtained by an integrated system that includes thermo-sensory organs and the central nervous system (Abram et al. 2017). *D. melanogaster* possesses external thermo-sensing organs and internal thermo-sensory neurons that are work as an efficient network system providing accurate thermal information allowing the flies to perform precise thermoregulatory behavior (Frank et al. 2015).

Due acute variations in temperature, males of the Japanese beetle, *Popillia japonica* (Newman) (Coleoptera: Scarabaeidae), have the duration of mate guarding decreasing with temperature (Saeki et al. 2005). Similarly, in three species of *Anthocoris sp.* (Heteroptera: Anthocoridae), the mating duration is longer at lower (15 +/- 8°C) temperatures compared to higher (25 +/- 8°C) temperatures (Horton et al. 2002). These studies suggest a negative correlation between temperature and mating duration, possibly mediated by the effect of thermo-sensory behavioral adjustment (Adachi et al. 2008, Abram et al. 2017).

The second process called bottom-up kinetics effects is a result of the impact of high temperatures (high kinetic energy) on physiological systems (Abram et al. 2017). Proteins and enzymes are sensitive to high temperatures, where temperatures above 40℃ had initiated protein unfolding and inactivation of enzyme activity (Lepock 2003, Lapidus 2017). The changing in the conformation of certain proteins can have a temporary or a permanent consequence to its functionality, where depending of the protein type and density of its unfolded-protein, the individual’s fitness and survivorship may be compromised (Geiler-Samerotte et al. 2011).

1.1.5.3. Insect thermoregulation strategies

In order to cope with a heterogenic habitat composed by cyclic and acyclic abiotic changes, ectothermic insects, due its poikilothermic (i.e. body temperature is variable and depend on the temperature environment) physiology, need to develop and adjust thermoregulation strategies (Tauber and Tauber 1976). Those would be based on the thermal information acquire by the process of top-down integrated effects and bottom-up kinetics effects (discussed previously in this chapter) (Abram et al. 2017). The investment in thermoregulation is require once their physiological responses under environment stress can cause serious negative effects on fitness (discussed later in this chapter) (Angilletta et al. 2006a).

Martin and Huey (2008) proposed a model to find optimal body temperatures, based on the principle of Jensen’s inequality (Ruel and Ayres 1999), affirming that the optimal body temperature should not meet exactly the predicted body temperatures that maximize fitness. In fact, organisms would tend to maximize fitness as long as their body temperature is bellow of their optimal. Therefore, the true temperatures that maximize fitness would be a suboptimal body temperature, considering it would be bellow of the predicted temperature that maximize fitness (Martin and Huey 2008, Dowd et al. 2015).

The Jensen's inequality considers that environmental variance would increase or decrease the response of an organism based on the form of its response function, generally predicted as a nonlinear function. Due biological systems being ruled by nonlinear responses, their thermoadaptation data should always include variance (Ruel and Ayres 1999, Martin and Huey 2008). Following the coadaptation process developed by Angilletta et al. (2006a) related to thermal preferences and thermal physiology had evolved together, ectotherms would choose temperature ranges base on the trade-off between the magnitude and variability in performance and the risks imposed to them in exceeding their thermal limits (Angilletta et al. 2009).

Depending of the size of insects and stage of development, insects can display a variety of mechanisms to manage temperature inconstancy. Since immatures are not the focus of this dissertation, I will only detail strategies performed during the adult stage. In some situations, ectothermic strategies (i.e. absorb heat through radiant energy of the sun or energy from substrates) are costly and not efficient in maintaining temperatures close to the optimal for a specie. Some insects display a regional heterothermy (Loli and Bicudo 2005, Lahondère and Lazzari 2015), with heat being produced through biomechanical strategies that accumulates in certain parts of their body. Three main strategies to produce heat can be observed in these individuals: flight muscles heat production, evaporative cooling and, microhabitat selection (Casey 1988).

Winged insects produce heat using their flight muscles during pre-flight and in-flight phases (Fu et al. 2017, Wone et al. 2018). Flight is energetically demanding with massive amounts of insect’s body reserves being used to operate flight musculature (Marden 2000). During resting, the temperature of the insect flight muscles is generally below of optimal for good performance, therefore, a warm-up mechanism was evolved as a pre-flight strategy in order to establish the right temperature where the flight muscles best perform. The excess heat produced not used for wing movement is kept inside of the insect’s body if the surrounding environment is set bellow of its optimal (Dudley 2000).

During the flight, insects need to deal with the large amounts of heat produced by the constant mechanical movement of their wings and thoracic muscles. In cases where the environment is set above their optimal, during the in-flight phase, an insect in flight movement dissipates heat via hemolymph flow, from the thorax to the abdomen, using the abdomen as a heat sink and a heat radiator, which helps to maintain a stable thoracic temperature under different temperature settings (Verdú et al. 2012).

Evaporative cooling is a strategy for heat loss observed in insets, where a bubble of saliva is spewed out and heat is lost through water evaporation. Afterwards, the bubble is regurgitated to allow more heat being released to the environment. This process is repeated several times until the insect is close to its optimal. This strategy is observed in bumblebees (Nixon et al. 2016) and blow flies (Gomes et al. 2018).

Microhabitat selection is a behavioral mechanism displayed by insects where the thermoregulation is made through reallocation, within the ambient range, to a spot with temperature few degrees below or above the previous one (Kleckova and Klecka 2016).

For example, desert insects will employ a vertical migration in order to optimize their use of the surrounding environment, moving from sun exposed areas during the day and returning to those spots at night (Chappell 1983, Toolson 1987). It was observed this thermoregulation strategy in several insect groups, such as butterflies (Kleckova et al. 2014), in ladybugs (Ohashi et al. 2005), odonatans (Calvão et al. 2013), aphids (Ma and Ma 2012), among others.

Stilting behavior, a form of microhabitat selection, is performed when insect's body temperature is below the optimal (Heinrich 2013). Insects will press their thorax and abdomen against the ground in order to enhance heat uptake by conduction, lifting their body when their reach the desirable temperature (May 1979, Heinrich 2013). This pattern of thermoregulation was found in beetles (Brosius and Higley 2013), grasshoppers (Harris et al. 2015), and dragonflies (May 2017).

1.1.5.4. Molecular thermal-stress response in insects

Temperature has been shown to be the main abiotic factor to impact morphological and physiological process in insect (Bale et al. 2002, Huang et al. 2007, Walsh et al. 2019). As a thermal stress response, the performance of these individual can be affected such as by reduction or increase in the efficiency of enzymes, protein denaturation and, development rate changes (Neven 2000, Colinet et al. 2015, King and MacRae 2015, Schulte 2015, Gunderson et al. 2017). For instance, in response to thermal damage, a class of proteins called heat shock proteins (HSP) is activated and, performing a chaperone function, stabilizes new proteins to ensure correct folding or by helping to refold proteins that were damaged by the thermal stress (Haslbeck and Vierling 2015, King and MacRae 2015). Sublethal effects, such as a decrease in fertility (Arbogast 1981), particularly by sterilization of males (Saxena et al. 1992), are commonly detected in heat-stressed insects.

In order to thermoregulate themselves, and cope with short and long-terms of acyclic seasonal events, insects use a combination of top-down and bottom-up strategies to reduce the impact of detrimental temperatures and maintain a body temperature allowing optimal performance. The contact with temperatures that are high enough to provoke the heat-shock response but insufficient to create substantial damage often results in thermo-tolerance. It can lead to improvement of the lethal and sublethal effects of subsequent exposure to high temperatures. For instance, HSP usually play a major role in the local adaptation, exercising a protective outcome upon a variety of stressful stimuli, being a major determinant in the acquisition of stress resistance (Fasulo et al. 2010). However, exposure can be equally detrimental (Denlinger and Lee Jr 2010), driving a delay in mortality (Bennett and Lee Jr 1989), nervous system impairment (Yocum et al. 1994) and reduced fecundity due to increased egg mortality (Coulson and Bale 1992).

Recently, Giannetto et al. (2017) reported the relationship of the expression of two HSF subfamilies (HSP 70 and HSP90) within two key larval stages (stage II and V) of *H. illucens*, during the process of food waste bioconversion. These data suggest their involvement during their most active developmental stage, therefore, when they face more abiotic challenges related to the environment. Harnden and Tomberlin (2016) reported that black soldier fly larvae when reared at 27.6 °C and 32.2 °C had a final larval weight ≈30% greater than larvae reared at 24.9 °C and required on average ≈8.7% more degree hours to complete their development.

1.1.5.5. Temperature impact on fitness: too hot for males

Regarding sperm cells, Blanckenhorn and Hellriegel (2002) studying the yellow dung fly, *Scathophaga stercoraria* (Diptera: Scathophagidae) described that sperm size was positively correlated with an increase in temperature, with reduction in performance. These results contradict the Bergmann's rule for cell and body size where organisms from warm environments should be small or evolve to be small (Atkinson and Sibly 1997). However, it was found the Bergmann's rule may not being consist for some ectotherms and insects that display facultative endothermy (regional heterothermy), once those individuals are under selective pressure for thermoregulation or starvation resistance (Shelomi 2012, Scriven et al. 2016).

As a result of the energetic reserves of fish spermatozoa being limited, exposure to warm temperatures leads to a shorter duration of sperm motility. Similarly, Richards (1963) studying the cockroaches, *Periplaneta Americana* (L.) (Blattodea: Blattidae), found that higher temperatures negatively affect sperm velocity. Conversely, exposure to cold temperatures results in a prolonged duration of motility and reduced cell velocity (Stoss 1983).

Due the ectothermic condition, the insect’s body temperature is limited by the surrounding ambient temperature, therefore, any external temperature variation far from their optimal might impact important biological process fitness related (Saxon et al.

2018). Recently, a study conducted by Sales et al. (2018) using the red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), evaluated the impact of heat waves with different temperatures on sex-specific fitness, mating behavior, fertility and, sperm function. It was found that males were extremely sensitive to the treatment (heatwaves of 42 °C), showing a decrease in 50% of their reproductive output while females were unaffected; Increased latency during mating and a reduction of 75% in the sperm count per ejaculate.

As mentioned earlier in this review, recent studies pointed the sperm cell to be particularly susceptible in generating *de novo* mutations. Additionally, the production of germ cells by males is continuous throughout their lives (Boivin et al. 2005, Boivin and Ellers 2016), making them, in comparison with females, a best-supported mechanism through which environmentally induced condition can be transmitted to offspring. Germ cells are not only responsible for producing the genetic (Marques-Mari et al. 2009) and epigenetic (Wagner 2010) variability displayed among individuals of a population but in vertically transmitting this genetic mosaic (Marques-Mari et al. 2009).Nonetheless, the effect of temperature on reaction norms of the sperm that negatively impact performance, such as viability and motility (Appell et al. 1977), have not been well documented in insects (Hunter and Birkhead 2002, Werner and Simmons 2008).

1.1.6. **Reproductive morphological and physiological aspects of organisms and their importance for mass production under environmental controlled settings**

All of these mating systems previously described and temperature fluctuation impact the successful transfer of genetic material to the next generation. And, in many instances, previous discussions in this chapter have revolved around the animal as a whole, with some discussion of molecular mechanisms in place. Another factor that has been demonstrated to impact mating success is the reproductive morphology, with a special attention to the sperm variability, and physiology of the animal.

Morphological sperm variability across taxa has long been a scientific fascination. Depending on the taxa, phenotypic plasticity shown by sperm in response to different environments (i.e., pH, viscosity index, humidity, temperature) (Pitnick et al. 2009) and resource availability (i.e., protein, carbohydrate, and fatty acids diet content) can be quite extensive (Dávila and Aron 2017). Variability in sperm morphology (i.e., sperm length) in part corresponds with associated male reproductive tract morphology (i.e. Birkhead et al. 2005, Pitnick et al. 2009, Simmons and Fitzpatrick 2012, Vahed and Parker 2012, Manier et al. 2013, Dallai 2014, Dávila and Aron 2017, Friesen et al. 2017) and an aging effect due the trade-off between survivorship and reproductive success caused by sperm senescence (Pizzari et al. 2008, Velando et al. 2011, Johnson et al. 2018).

Moreover, specialized genitalia morphology in males may be strongly related to sex determining sperm allocation, female's sperm storage shape, and consequently, fertilization of eggs (Parker 1970, Higginson et al. 2012, Krasnec et al. 2012). Therefore, females' reproductive tract morphology may have a strong influence in the reproductive fitness of males. In a review published by Walker (1980), he pointed a positive correlation between species with spheroid spermatheca and a monogamic mating system (i.e., one insemination), contrasting species with elongate or tubular spermatheca and a polyandric mating system.

In a mating system where females mate with more than one male (polyandry), their choice plays the primary role in determining sperm allocation (Neff and Svensson 2013, Boulton et al. 2015). However, for monandrous females (i.e., female mates with just one male), male cryptic choice be selected if an “excessive” mating effort is required, such as courtship rituals like nuptial gift (Smith et al. 2017b). Moreover, when males are exposed to a high post-copulatory competition (i.e., sperm competition), resources might be allocated in accordance to their strategies for sperm production (Parker 1970, DelBarco-Trillo 2011).

Overall, males evolve different strategies resulting in enhanced fitness and overcome sperm competition (Birkhead and Møller 1998). From a morphological perspective, understanding sperm structure could lead to a greater understanding of mate selection from males and females in order to achieve an optimal fertilization rate (Neff and Svensson 2013, Friesen et al. 2017, Tinghitella et al. 2017).With regards to the evolution of some insect taxa, the shape of the flagellum has diverged remarkably from basal species or has been lost altogether resulting in aflagellate, frequently immotile sperm (Morrow 2004).

1.1.6.1. The insect sperm

The flagellate insect sperm has been recognized as a fundamental ground plan, which was first outlined for the pterygota by Phillips (1970) and Baccetti (1972), and later reviewed in detail and extended to the hexapoda entirely by Jamieson (1987) and

Jamieson et al. (1999). In regards to those studies, insect sperm is a motile filiform structure that consists of the head (acrosome and nucleus), the transitional centriole adjunct, and the flagellum. The capability to be motile resides absolutely within the flagellum containing the axoneme, mitochondrial derivatives, and accessory bodies (Jamieson 1987).

The axoneme or axial filament complex is the motile organelle within almost all spermatozoa (Jamieson et al. 1999). The insect sperm axoneme has a 9:9:2 general arrangements of the microtubules (Baccetti 1972, Pitnick et al. 2009). The central core of the axoneme is composed of two central microtubules surrounded by a circle of nine microtubule doublets that are connected to the central tubules via the radial spokes and the central sheath (Dallai and Afzelius 1990, Werner and Simmons 2008). As a feature of the insect sperm axoneme, not shared by other animal taxa, the ring of nine microtubular doublets is surrounded by nine accessory tubules. The accessory tubules, which originate as outgrowths from the B subtubule during spermatogenesis, resemble conventional microtubules (Dallai and Afzelius 1990, Dallai 2014).

The number of their protofilaments, however, varies between 13 and 20, depending on the taxa (Jamieson et al. 1999, Dallai 2014). Neighboring accessory tubules are interconnected with each other and with the adjacent doublet by an intertubular material (Meurer-Grob et al. 2001). The number of protofilaments and the cross-sectional form of the intertubular material make useful characters for taxonomic purposes (Jamieson et al. 1999). (Afzelius 1959) first suggested the bending mechanism of the axoneme is based on a sliding motion of the microtubules against each other, which confer motility to the sperm.

The theory of ‘‘sliding filaments’’ was later verified by Satir (1965) and by Summers and Gibbons (1971) who observed the reciprocal sliding of microtubule doublets in desmembranated axonemes. The sliding mechanism can be explained as the ciliary dynein motor protein that is induced by itself, attached to the A subtubule of the outer doublets in the form of two dynein arms: the inner and outer dynein arm (Satir et al. 2014). The dynein arms connect adjacent doublets in the presence of Mg2 and adenosine triphosphate (ATP). Acting as an ATPase, the dynein molecules change their structure and generate a force, which leads to the sliding motion of the doublets (Lindemann et al. 2003). Since the axonemal filaments are tied within a cell body, the sliding is interpreted into bending of the cilium (Taylor et al. 1999).

Detailed knowledge about the morphological components of sperm and differences in spermatogenesis across insect species provides some understanding as to how these cells move from the reproductive tract of a male to the female (Wu et al. 2017). The first comparative data on insect sperm motility were published by Phillips (1970) and Baccetti (1972). Both described the double helical movement pattern as the "presence of two superimposed flagellar waves with one wave being of significant amplitude and low frequency, and the opposite of small amplitude and high frequency" (Werner and Simmons 2008). In the literature, different names were given to the movement of the sperm, such as ‘‘large wave’’ and ‘‘small wave’’ or ‘‘major’’ and ‘‘minor wave’’ (Pak et al. 2012, Linck et al. 2016). Curtis and Benner (1991) call them ‘‘large bends’’ and ‘‘small bends’’, and Swan (1981) called them ‘‘small amplitude, fast wave’’ and ‘‘large amplitude, slow wave’’. Therefore, the morphology and size of the sperm play a major role in the sperm movement into the female’s reproductive tract and, subsequently, reproductive fitness of the individual (Rikmenspoel 1978).

There are several parameters applied in studying sperm biology. Examples include, but are not limited to, spermatogenesis and structure, motility parameters of the spermatozoa, motility that can be activated after storage, and fertility of stored sperm (viability) (Billard and Cosson 1992, Billard et al. 1995). Sperm viability and motility are the key pre-standards in defining the quality and fertilizing potential of semen (Stoss 1983).

Aside from sperm length, understanding sperm function is imperative to fully comprehend how sperm competition affects sperm evolution (Simmons 2001a). In polyandric females, for instance, elongated sperm potentially are beneficial due to high-density sperm storage, especially in the case where fertilization occurs at the time of oviposition (Simmons 2001b). A study of *D. melanogaster* determined the significance of sperm length in relation to sperm competition; basically, longer sperm were more successful in fertilizing eggs than shorter sperm (Miller and Pitnick 2002).

Conversely, it is possible fertilization performance selects elongate sperm for fertilization efficiency, which is independent of sperm competitiveness (García-Vázquez et al. 2016). Harris et al. (2007) reported variation in sperm length in cockroaches (Blattodea) of the same species, indicating differences in sperm length measured arose from variations in sperm produced at different times. Similar results were observed in rove beetles (Coleoptera: Staphylinidae) where young males produce smaller sperm than older males (Green 2003). Drew (1968) in a study of the morphology of reproductive tract male of *Bactrocera tryoni* (Froggatt) (Diptera: Tephritidae), found variation in the ejaculatory apodeme over time since emergence. These studies indicate sperm length and male reproductive tract variation might be repeatable within individuals but variable amongst individuals.

Moreover, individual age in some species also affects mating behavior. For instance, in the damselfly *Enallagma hageni* (Walsh) (Odonata: Coenagrionidae), mating success decreases with age (Fincke 1982). Contrastingly, Simmons and Zuk (1992) reported older crickets from the specie *Gryllus bimaculatus* (De Geer) (Orthoptera: Gryllidae) have more advantage in finding a mate than younger crickets due to their duration of mating calls being longer. Longitudinal studies that could relate the reproductive success of individuals with their physiological status (i.e. age) have a significant advantage when compare with the usual practice of associating the reproductive performance or survival of single samples of animals observed at different ages (Clutton-Brock 1988).

1.1.7. **Mass producing insects**

Such information could prove critical with systems where mass production is critical for industry success. Paoli et al. (2013) described the sperm ultrastructure of the egg parasitoid *Gryon pennsylvanicum* (Ashmead) (Hymenoptera, Platygastridae) in different ages and found that 5–20-day-old males presented skinny testes containing degenerated cysts, with some showing limited number of sperms. Furthermore, oldest males tested two months old had their testes lumen empty. Therefore, for mass production of this parasitoid, the presence of old males in the colony might drive a sexual conflict over mating, useless and which could compromise the success of rearing (Pizzari et al. 2008, Dean et al. 2010).

Moreover, the study of the sperm maturation process is primordial for the application of the sterile insect technique (SIT) in the control of insect in pest status. For instance, Paoli et al. (2014) first characterized the morphology of the male reproductive system and sperm ultrastructure of the wild-type red palm weevil *Rhynchophorus ferrugineus* (Oliv.) (Coleoptera: Dryophthoridae) and then compared with γ-irradiated sperm. They found cysts with ultrastructural aberrant spermatids in the γ-irradiated animals were observed. In the large animal breeding industry, the data about the sperm morphology and ultrastructure is already being used to enhance techniques applied for fertilization in vitro (Enciso et al. 2011, Saldívar-Hernández et al. 2015).

Another insect being mass produced is the black soldier fly (BSF), *Hermetia illucens* (L.) (Diptera: Stratiomyidae). This species is a cosmopolitan fly, with occurrence in various parts of the world (Rozkosný 1983) and, unlike other fly species (e.g., the common house fly, *Musca domestica* (Diptera: Muscidae)), the BSF is not recognized as a pest species as it is not attracted to food or houses (Furman et al. 1959). In fact, BSF larvae significantly reduce the production and establishment of this pest (e.g., house fly) near to decaying organic matter (Furman et al. 1959, Sheppard 1983). However, up to date, no information is available on its reproductive tract or sperm morphology.

1.2. Part II. Addressing behavioral ecology questions with a model system

1.2.1. General aspects of the black soldier fly

1.2.1.1. Distribution

The black soldier fly is a cosmopolitan fly, with occurrence in various parts of the world (Rozkosný 1983). The hypothesis is they are from tropical, subtropical and temperate zones of America though this is widely contested (Callan 1974, Benelli et al. 2014). It was reported in the southern portion of United States by the late 1800s. Specifically, records mention this fly in states such as Louisiana (1897), Texas (1899), South Carolina (1911), southern California (1923), Virginia (1926), Iowa (1931), Ohio (1938), northern California (1940), Maryland (1943), New York City (1945) and Warner, Merrimack County, New Hampshire (1972) (Marshall et al. 2015).

Benelli et al. (2014) indicate that international transport and commerce in the 20th century could be responsible for increasing the distribution of *H. illucens* across the world. Still, others debate that the species is probably native to Palearctic regions of the earth (Benelli et al. 2014). In Europe, black soldier fly was first reported in Malta and has since been discribed and recorded in other parts of Europe and Asia (Roháček and Hora 2013, Hauser et al. 2017).

1.2.1.2. Ecosystem services

Unlike other fly species (e.g., the common house fly, *Musca domestica* (L.) (Diptera: Muscidae)), the black soldier fly is not recognized as a pest species as it is not attracted to food or houses (Furman et al. 1959). In fact, black soldier fly larvae feeding on wastes significantly reduces the production and establishment of house flies (Furman et al. 1959, Sheppard 1983). Black soldier fly larvae have been shown to inhibit the oviposition of house fly on poultry waste, probably by overcrowding their food resource and generating short-lived interspecific chemical signals (Bradley and Sheppard 1984). Black soldier fly larvae also modify the microflora structure of organic waste, lessening the increase of unsafe bacteria, such as *Escherichia coli* (Erickson et al. 2004, Sanford et al. 2008, Kashiri et al. 2018) and *Salmonela* spp. in manure (Erickson et al. 2004, Lee et al. 2018) and faecal sludge (Lalander et al. 2013).

Moreover, food waste processed by larvae will aerate and dry more quickly, whereby lowering the production of gases such as methane, a major greenhouse gas-produced. Thus, by eliminating the production of odors that attract pests, black soldier flies can play a pivotal role in pest reduction around food waste (Čičková et al. 2015, Vanlaerhoven et al. 2015). Currently, 64.5% of total municipal solid waste (MSW) ends up in landfills in the United States (Seeberger et al. 2016). By decreasing the volume of waste by 50%, black soldier fly larvae notably drop the necessity and prices of a landfill. Particularly, natural waste composting via black soldier fly larvae permits for waste recycling and fertilizer production (Sanford et al. 2008, Diener et al. 2015). However, research into the industrialization of the black soldier fly is currently ongoing, with some research groups located in Asia, South Africa, Europe and North America.

In addition to their waste removal abilities, BSF larvae, or pre-pupae, could be used as animal feed as they are high in protein, fats, and amino acids, of which several traditional fish feeds currently lack (Makkar et al. 2014, Makkar et al. 2015, Stadtlander et al. 2017). For instance, BSF larvae reared on food waste contain approximately 42% crude protein and 35% crude fat and 4.5% of ash (Salomone et al. 2017), is comparable with the protein sources currently used in animal feeds, such as fishmeal (Nyakeri et al. 2017). A recent study with the European seabass (*Dicentrarchus labrax*) indicated that the replacement of 19% of the regular fish meal for BSF pre-pupae meal did not affect its growth performance (Magalhães et al. 2017).

Moreover, BSF meals are been developed for broiler chickens, where preliminaries results showed that it can be considered an excellent source of apparent metabolizable energy and digestible amino acids (Schiavone et al. 2017). In 2016, the Association of American Feed Control Officials (AAFCO) approved the use of BSF dried larvae as feed for industrial production of salmonid fish, being the first approval of using an insect for animal feed in the United States (AAFCO 2016).

As previously I mentioned, there is no information about their reproductive system, spermatogenesis process and how age and temperature would impact the quali-quantitative the germ cells produced. A spermatheca drawing of this species was described by Ururahy-Rodrigues and Pujol–Luz (2000) with species collected in Brazil, but no actual picture of the structure was previously displayed. Although sperm storage in females can answer various questions related to the matting system occurring and post-copulatory strategies performed by males, few studies were made to describe this structure, with none conducted with black soldier fly species.

The detailed knowledge about the morphological components of the sperm and the differences amongst the spermatogenesis of this insect through aging will help to understand how these cells move and perform from the reproductive tract male to the female (Wu et al. 2017), will lead in to a consequent maximization of the fertilization performance in indoor cages. The optimization of the techniques applied for mass production of insects will become an important industry apart in order to support the necessity of the suppliers – especially in the concern of optimize production at a low cost, which will reflect on the price of the final product. If the insect industry wants to compete with the livestock and poultry industry, it needs to find a balance of offer a high-quality product with competitive prices.

1.3. The objectives of the research presented in this dissertation were:

Objective 1 (Chapter 2): Describe the male reproductive tract and female sperm storage of *H. illucens* adults.

Ho: Reproductive morphology of male *H. illucens* is similar to Brachycera.

Ha: Reproductive morphology of male *H. illucens* is not similar to Brachycera.

Objective 2 (Chapter 2): Describe spermatogenesis of *H. illucens* adults.

Ho: There are no differences shifts in spermatogenesis of black soldier fly as adults ages.

Ha: There are shifts in spermatogenesis of black soldier fly as adults ages.

Objective 3 (Chapter 3): Determine sperm viability of *H. illucens* adult among different ages at a set temperature.

Ho: Viability of sperm in *H. illucens* does not change as the fly ages.

Ha: Viability of sperm in *H. illucens* does change as the fly ages.

Objective 4 (Chapter 3). Determine sperm viability of *H. illucens* in a single age group at different temperatures.

Ho: The viability of sperm is not impacted by temperature experienced by adults.

Ha: The viability of sperm is impacted by temperature experienced by adults.

Objective 5 (Chapter 3). Evaluate the thermoregulation of male *H. illucens* adults.

Ho: Male *H. illucens* will be distributed evenly throughout the thermogradient.

Ha: Male *H. illucens* will not be distributed evenly throughout the thermogradient.

Objective 6 (Chapter 3): Evaluate the thermoregulation of female *H. illucens* adults.

Ho: Female *H. illucens* will be distributed evenly throughout the thermogradient.

Ha: Female *H. illucens* will not be distributed evenly throughout the thermogradient.

1.4. References

AAFCO. 2016. Annual Meeting Agenda and Committee Reports; Association of American Feed Control Officials, pp. p.112, Pittsburgh, PA, US.

Abram, P. K., G. Boivin, J. Moiroux, and J. Brodeur. 2017. Behavioural effects of temperature on ectothermic animals: Unifying thermal physiology and behavioural plasticity. Biological Reviews 92: 1859-1876.

Adachi, R., T. Wakabayashi, N. Oda, and R. Shingai. 2008. Modulation of *Caenorhabditis elegans* chemotaxis by cultivation and assay temperatures. Neuroscience Research 60: 300-306.

Afzelius, B. 1959. Electron microscopy of the sperm tail: Results obtained with a new fixative. The Journal of Biophysical and Biochemical Cytology 5: 269-278.

Alcock, J. 1990. A large male competitive advantage in a lekking fly, *Hermetia comstocki* Williston (Diptera: Stratiomyidae). Psyche: A Journal of Entomology 97: 267-279.

Alonzo, S. H. 2012. Sexual selection favours male parental care, when females can choose. Proceedings. Biological Sciences 279: 1784-1790.

Andersson, M. 1986. Evolution of condition-dependent sex ornaments and mating preferences: Sexual selection based on viability differences. Evolution 40: 804-816.

Andersson, M., and L. W. Simmons. 2006. Sexual selection and mate choice. Trends in Ecology & Evolution 21: 296-302.

Angilletta, M. J. 2009. Thermal Adaptation: A Theoretical and Empirical Synthesis. University Press, Oxford, UK.

Angilletta, M. J., A. F. Bennett, H. Guderley, C. A. Navas, F. Seebacher, and R. S. Wilson. 2006a. Coadaptation: A unifying principle in evolutionary thermal biology. Physiological and Biochemical Zoology 79: 282-294.

Angilletta, M. J., R. B. Huey, and M. R. Frazier. 2009. Thermodynamic effects on organismal performance: Is hotter better? Physiological and Biochemical Zoology 83: 197-206.

Appell, R. A., Evans, P. R., and J. P. Blandy. 1977. The effect of temperature on the motility and viability of sperm. British Journal of Urology 49:751-756.

Arbogast, R. T. 1981. Mortality and reproduction of *Ephestia cautella* and *Plodia interpunctella* exposed as pupae to high temperatures. Environmental Entomology 10: 708-711.

Arnqvist, G., and L. Rowe. 2013. Sexual Conflict. Princeton University Press, NJ, US.

Atkinson, D., and R. M. Sibly. 1997. Why are organisms usually bigger in colder environments? Making sense of a life history puzzle. Trends in Ecology & Evolution 12: 235-239.

Austad, S. N., and K. E. Fischer. 2016. Sex differences in lifespan. Cell Metabolism 23: 1022-1033.

Avila, F. W., L. K. Sirot, B. A. LaFlamme, C. D. Rubinstein, and M. F. Wolfner. 2011. Insect seminal fluid proteins: Identification and function. Annual Review of Entomology 56: 21-40.

Baccetti, B. 1972. Insect sperm cells, pp. 315-397. *In* Treherne, J.E., M.J. Berridge, and V.B. Wigglesworth (eds). Advances in insect physiology, vol. 9. Academic Press, NY, US.

Baer, B. 2003. Bumblebees as model organisms to study male sexual selection in social insects. Behavioral Ecology and Sociobiology 54: 521-533.

Bakker, A., J. Campos Louçã, P. Roessingh, and S. Menken. 2011. The cost of mating: Influences of life history traits and mating strategies on lifespan in two closely related *Yponomeuta* species. International Journal of Zoology 2011: 658908 (ID).

Bale, J. S., G. J. Masters, I. D. Hodkinson, C. Awmack, T. M. Bezemer, V. K. Brown, J. Butterfield, A. Buse, J. C. Coulson, and J. Farrar. 2002. Herbivory in global climate change research: direct effects of rising temperature on insect herbivores. Global Change Biology 8: 1-16.

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

Barrass, R. 1979. The survival value of courtship in insects, pp. 403-416. *In* M. S. Blum and N.A. Blum (eds.). Sexual selection and reproductive competition. Academic Press, NY, US.

Barton, N. H., and M. Turelli. 1989. Evolutionary quantitative genetics: How little do we know? Annual Review of Genetics 23: 337-370.

Bateson, M., and S. D. Healy. 2005. Comparative evaluation and its implications for mate choice. Trends in Ecology & Evolution 20: 659-664.

Beehler, B. M., and M. S. Foster. 1988. Hotshots, hotspots, and female preference in the organization of lek mating systems. The American Naturalist 131: 203-219.

Bell, G. 1982. The Masterpiece of Nature: The Evolution and Genetics of Sexuality, Cambridge University Press, UK.

Benelli, G., A. Canale, A. Raspi, and G. Fornaciari. 2014. The death scenario of an Italian renaissance princess can shed light on a zoological dilemma: Did the black soldier fly reach Europe with Columbus? Journal of Archaeological Science 49: 203-205.

Bennett, L. E., and R. E. Lee Jr. 1989. Simulated winter to summer transition in diapausing adults of the lady beetle (*Hippodamia convergens*): Supercooling point is not indicative of cold-hardiness. Physiological Entomology 14: 361-367.

Bernasconi, G., B. Hellriegel, A. Heyland, and P. I. Ward. 2002. Sperm survival in the female reproductive tract in the fly *Scathophaga stercoraria* (L.). Journal of Insect Physiology 48: 197-203.

Billard, R., and M. P. Cosson. 1992. Some problems related to the assessment of sperm motility in freshwater fish. Journal of Experimental Zoology 261: 122-131.

Billard, R., J. Cosson, G. Perchec, and O. Linhart. 1995. Biology of sperm and artificial reproduction in carp. Aquaculture 129: 95-112.

Birkhead, T. R. 1998. Cryptic female choice: Criteria for establishing female sperm choice. International Journal of Organic Evolution 52: 1212-1218.

Birkhead, T. R., and A. P. Møller. 1998. Sperm Competition and Sexual Selection. Academic Press, New York, US.

Birkhead, T. R., and T. Pizzari. 2002. Postcopulatory sexual selection. Nature Reviews Genetics 3: 262-273.

Birkhead, T., E. Pellatt, P. Brekke, R. Yeates, and H. Castillo-Juarez. 2005. Genetic effects on sperm design in the zebra finch. Nature 434: 383-387.

Blanckenhorn, W. U., and B. Hellriegel. 2002. Against Bergmann's rule: Fly sperm size increases with temperature. Ecology Letters 5: 7-10.

Boivin, G., and J. Ellers. 2016. Replacing qualitative life-history traits by quantitative indices in parasitoid evolutionary ecology. Entomologia Experimentalis et Applicata 159: 163-171.

Boivin, G., S. Jacob, and D. Damiens. 2005. Spermatogeny as a life-history index in parasitoid wasps. Oecologia 143: 198-202.

Bonduriansky, R., and T. Day. 2013. Nongenetic inheritance and the evolution of costly female preference. Journal of Evolutionary Biology 26: 76-87.

Bonilla, M. M., J. A. Zeh, and D. W. Zeh. 2016. An epigenetic resolution of the lek paradox. BioEssays 38: 355-366.

Boulton, R. A., L. A. Collins, and D. M. Shuker. 2015. Beyond sex allocation: The role of mating systems in sexual selection in parasitoid wasps. Biological Reviews 90: 599-627.

Bowler, K., and J. S. Terblanche. 2008. Insect thermal tolerance: What is the role of ontogeny, ageing and senescence? Biological Reviews 83: 339-355.

Bradbury, J. W., and R. M. Gibson. 1983. Leks and Mate Choice, pp. 109-138. *In* P. Bateson (ed.), Mate Choice, Cambridge University Press, Cambridge, UK.

Bradbury, J., R. Gibson, and I. M. Tsai. 1986. Hotspots and the dispersion of leks. Animal Behaviour 34: 1694-1709.

Bradley, S. W., and D. C. Sheppard. 1984. House fly oviposition inhibition by larvae of *Hermetia illucens*, the black soldier fly. Journal of Chemical Ecology 10: 853-859.

Brosius, T. R., and L. G. Higley. 2013. Behavioral niche partitioning in a sympatric tiger beetle assemblage and implications for the endangered Salt creek tiger beetle. PeerJ 1: e169.

Brown, J. H. 2014. Why are there so many species in the tropics? Journal of Biogeography 41: 8-22.

Buckley, L. B., G. H. Rodda, and W. Jetz. 2008. Thermal and energetic constraints on ectotherm abundance: A global test using lizards. Ecology 89: 48-55.

Burt, D. B., P. F. Coulter, and D. Ligon. 2007. Evolution of Parental Care and Cooperative Breeding, pp. 305-336. In B.G.M. Jamieson (ed.), Reproductive Biology and Phylogeny of Birds, part B: Sexual Selection, Behavior, Conservation, Embryology and Genetics. Cambridge University Press, Cambridge, UK.

Bush, S. L., and D. J. Bell. 1997. Courtship and female competition in the Majorcan midwife toad, *Alytes muletensis*. Ethology 103: 292-303.

Byers, J. A., and L. Waits. 2006. Good genes sexual selection in nature. Proceedings of the National Academy of Sciences 103: 16343-16345.

Callan, E. 1974. *Hermetia illucens* (L.) (Diptera, Stratiomyidae), a cosmopolitan American species long established in Australia and New Zealand. Entomologist's Monthly Magazine 109: 232-234.

Calvão, L. B., M. V. C. Vital, L. Juen, G. F. d. Lima Filho, J. M. B. d. Oliveira Júnior, N. S. Pinto, and M. Júnior. 2013. Thermoregulation and microhabitat choice in *Erythrodiplax latimaculata* Ris males (Anisoptera: Libellulidae). Odonatologica 42: 97-108.

Casey, T. M. 1988. Thermoregulation and heat exchange. Advances in Insect Physiology 20: 119-146.

Castillo, D. M., A. K. Gibson, and L. C. Moyle. 2016. Assortative mating and self-fertilization differ in their contributions to reinforcement, cascade speciation, and diversification. Current Zoology 62: 169-181.

Chappell, M. A. 1983. Metabolism and thermoregulation in desert and montane grasshoppers. Oecologia 56: 126-131.

Charlesworth, B. 2009. Fundamental concepts in genetics: effective population size and patterns of molecular evolution and variation. Nature Reviews Genetics 10: 195-205.

Chen, Q., W. Yan, and E. Duan. 2016. Epigenetic inheritance of acquired traits through sperm RNAs and sperm RNA modifications. Nature Reviews Genetics 17: 733-743.

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

Clutton-Brock, T. 1988. Reproductive success: Studies of Individual Variation in Contrasting Breeding Systems. University of Chicago Press, Chicago, IL, US.

Clutton-Brock, T. 2017. Reproductive competition and sexual selection. Philosophical Transactions of the Royal Society B, Biological Sciences 372: 20160310 (ID).

Clutton-Brock, T. H., and E. Huchard. 2013. Social competition and selection in males and females. Philosophical Transactions of the Royal Society B, Biological Sciences 368: 20130074-20130074.

Colinet, H., B. J. Sinclair, P. Vernon, and D. Renault. 2015. Insects in fluctuating thermal environments. Annual Review of Entomology 60: 123-140.

Conner, J. K., and D. L. Hartl. 2004. A primer of ecological genetics. Sinauer Associates Incorporated, Sunderland, MA, US.

Cordero, A., and J. A. Andrés. 2002. Male coercion and convenience polyandry in a calopterygid damselfly. Journal of Insect Science 2:1-7.

Coulson, S., and J. Bale. 1992. Effect of rapid cold hardening on reproduction and survival of offspring in the housefly *Musca domestica*. Journal of Insect Physiology 38: 421-424.

Cureton II, J., R. Deaton, and R. Martin. 2010. Short term changes in sex ratio and density alter coercive male mating tactics. Behaviour 147: 1431-1442.

Curtis, S. K., and D. B. Benner. 1991. Movement of spermatozoa of *Megaselia Scalaris* (Diptera: Brachycera: Cyclorrhapha: Phoridae) in artificial and natural fluids. Journal of Morphology 210: 85-99.

Dacks, J., and A. J. Roger. 1999. The first sexual lineage and the relevance of facultative sex. Journal of Molecular Evolution 48: 779-783.

Dallai, R. 2014. Overview on spermatogenesis and sperm structure of Hexapoda. Arthropod Structure & Development 43: 257-290.

Dallai, R., and B. A. Afzelius. 1990. Microtubular diversity in insect spermatozoa: Results obtained with a new fixative. Journal of Structural Biology 103: 164-179.

Danks, H. 2007. The elements of seasonal adaptations in insects. The Canadian Entomologist 139: 1-44.

Dávila, F., and S. Aron. 2017. Protein restriction affects sperm number but not sperm viability in male ants. Journal of Insect Physiology 100: 71-76.

Dawkins, R. 1976. The Selfish Gene. Oxford University Press, Oxford, UK.

De Luca, P. A., J. A. Stoltz, M. C. Andrade, and A. C. Mason. 2015. Metabolic efficiency in courtship favors males with intermediate mass in the Australian redback spider, *Latrodectus hasselti*. Journal of Insect Physiology 72: 35-42.

Dean, R., C. K. Cornwallis, H. Løvlie, K. Worley, D. S. Richardson, and T. Pizzari. 2010. Male reproductive senescence causes potential for sexual conflict over mating. Current Biology 20: 1192-1196.

DelBarco-Trillo, J. 2011. Adjustment of sperm allocation under high risk of sperm competition across taxa: A meta-analysis. Journal of Evolutionary Biology 24: 1706-1714.

Demetrius, L., and M. Ziehe. 2007. Darwinian fitness. Theoretical Population Biology 72: 323-345.

Denlinger, D. L., and R. E. Lee Jr. 2010. Low Temperature Biology of Insects. Cambridge University Press, Cambridge, UK.

Diener, S., C. Zurbrügg, and K. Tockner. 2015. Bioaccumulation of heavy metals in the black soldier fly, *Hermetia illucens* and effects on its life cycle. Journal of Insects as Food and Feed 1: 261-270.

Domanitskaya, E. V., H. Liu, S. Chen, and E. Kubli. 2007. The hydroxyproline motif of male sex peptide elicits the innate immune response in *Drosophila* females. The FEBS Journal 274: 5659-5668.

Dowd, W. W., F. A. King, and M. W. Denny. 2015. Thermal variation, thermal extremes and the physiological performance of individuals. Journal of Experimental Biology 218: 1956-1967.

Dowling, D. K., A. L. Nowostawski, and G. Arnqvist. 2007. Effects of cytoplasmic genes on sperm viability and sperm morphology in a seed beetle: Implications for sperm competition theory? Journal of Evolutionary Biology 20: 358-368.

Dudley, R. 2000. The Biomechanics of Insect Flight: Form, Function, Evolution. Princeton University Press, NJ.

Dugand, R. J., J. L. Tomkins, and W. J. Kennington. 2019. Molecular evidence supports a genic capture resolution of the lek paradox. Nature Communications 10: 1359.

Duret, L. 2008. Neutral theory: The null hypothesis of molecular evolution. Nature Education 1: 803-806.

Duxbury, E. M. L., W. G. Rostant, and T. Chapman. 2017. Manipulation of feeding regime alters sexual dimorphism for lifespan and reduces sexual conflict in *Drosophila melanogaster*. Proceedings Biological Sciences 284: 20170391.

Dybas, L. K., and H. S. Dybas. 1981. Coadaptation and taxonomic differentiation of sperm and spermathecae in featherwing beetles. International Journal of Organic Evolution 35: 168-174.

Eberhard, W. G. 1996. Female control: Sexual Selection by Cryptic Female Choice, Princeton University Press, Princeton, NJ, US.

Eberhard, W. G. 2002. The function of female resistance behavior: intromission by male coercion vs. female cooperation in sepsid flies (Diptera: Sepsidae). Revista de Biologia Tropical 50: 485-505.

Edward, D. A., P. Stockley, and D. J. Hosken. 2015. Sexual conflict and sperm competition. Cold Spring Harbor Perspectives in Biology 7: a017707.

Ellegren, H., and N. Galtier. 2016. Determinants of genetic diversity. Nature Reviews Genetics 17: 422-433.

Emlen, J. M. 1973. Ecology: An Evolutionary Approach. Addison-Wesley, Boston, MA, US.

Emlen, S. T., and L. W. Oring. 1977. Ecology, sexual selection, and the evolution of mating systems. Science 197: 215-223.

Enciso, M., M. Iglesias, I. Galán, J. Sarasa, A. Gosálvez, and J. Gosálvez. 2011. The ability of sperm selection techniques to remove single-or double-strand DNA damage. Asian Journal of Andrology 13: 764-768.

Engqvist, L., and K. P. Sauer. 2001. Strategic male mating effort and cryptic male choice in a scorpionfly. Proceedings. Biological Sciences 268: 729-735.

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

Fasulo, S., A. Mauceri, M. Maisano, A. Giannetto, V. Parrino, F. Gennuso, and A. D'Agata. 2010. Immunohistochemical and molecular biomarkers in *Coris julis* exposed to environmental contaminants. Ecotoxicology and Environmental Safety 73: 873-882.

Felsenstein, J. 1974. The evolutionary advantage of recombination. Genetics 78: 737-756.

Fincke, O. M. 1982. Lifetime mating success in a natural population of the damselfly, *Enallagma hageni* (Walsh) (Odonata: Coenagrionidae). Behavioral Ecology and Sociobiology 10: 293-302.

Firman, R. C., C. Gasparini, M. K. Manier, and T. Pizzari. 2017. Postmating Female Control: 20 Years of Cryptic Female Choice. Trends in Ecology & Evolution 32: 368-382.

Fisher, R. 1930. The Genetical Theory of Natural Selection. Clarendon Press, Oxford, UK.

Fitzpatrick, C. L., and M. R. Servedio. 2018. The evolution of male mate choice and female ornamentation: A review of mathematical models. Current Zoology 64: 323-333.

Frank, D. D., G. C. Jouandet, P. J. Kearney, L. J. Macpherson, and M. Gallio. 2015. Temperature representation in the *Drosophila* brain. Nature 519: 358-361.

Friesen, C. R., E. J. Uhrig, E. J. Bentz, L. A. Blakemore, and R. T. Mason. 2017. Correlated evolution of sexually selected traits: Interspecific variation in ejaculates, sperm morphology, copulatory mate guarding, and body size in two sympatric species of garter snakes. Behavioral Ecology and Sociobiology 71: 180.

Fu, X.-W., H. Chang, L.-M. He, S.-Y. Zhao, and K.-M. Wu. 2017. Flight performance of *Macdunnoughia crassisigna* (Lepidoptera: Noctuidae). Bulletin of Entomological Research 107: 715-723.

Furman, D. P., P. E. Catts, and R. D. Young. 1959b. *Hermetia illucens* (Linnaeus) as a Factor in the Natural Control of *Musca domestica* Linnaeus. Journal of Economic Entomology 52: 917-921.

Gage, M. J. G. 2012. Complex sperm evolution. Proceedings of the National Academy of Sciences 109: 4341-4342.

Gage, M. J. G., and P. A. Cook. 1994. Sperm size or numbers? Effects of nutritional stress upon eupyrene and apyrene sperm production strategies in the moth *Plodia interpunctella* (Lepidoptera: Pyralidea). Functional Ecology 8: 594-599.

Gage, M. J., and E. H. Morrow. 2003. Experimental evidence for the evolution of numerous, tiny sperm via sperm competition. Current Biology 13: 754-757.

Gandon, S., and Y. Michalakis. 2002. Local adaptation, evolutionary potential and host-parasite coevolution: interactions between migration, mutation, population size and generation time. Journal of Evolutionary Biology 15: 451-462.

García-González, F., and L. W. Simmons. 2007. Paternal indirect genetic effects on offspring viability and the benefits of polyandry. Current Biology 17: 32-36.

Garcia-Roa, R., M. Serra, and P. Carazo. 2018. Ageing via perception costs of reproduction magnifies sexual selection. Proceedings Biological Sciences 285: 1892

García-Vázquez, F. A., J. Gadea, C. Matás, and W. V. Holt. 2016. Importance of sperm morphology during sperm transport and fertilization in mammals. Asian Journal of Andrology 18: 844-850.

Gasparini, C., and J. P. Evans. 2018. Female control over multiple matings increases the opportunity for postcopulatory sexual selection. Proceedings Biological Sciences 285(1888): 20181505 (ID).

Geiler-Samerotte, K. A., M. F. Dion, B. A. Budnik, S. M. Wang, D. L. Hartl, and D. A. Drummond. 2011. Misfolded proteins impose a dosage-dependent fitness cost and trigger a cytosolic unfolded protein response in yeast. Proceedings of the National Academy of Sciences of the United States of America 108: 680-685.

Giannetto, A., S. Oliva, L. Mazza, G. Mondello, D. Savastano, A. Mauceri, and S. Fasulo. 2017. Molecular characterization and expression analysis of heat shock protein 70 and 90 from *Hermetia illucens* reared in a food waste bioconversion pilot plant. Gene 627: 15-25.

Gibson, A. K., L. F. Delph, and C. M. Lively. 2017. The two-fold cost of sex: Experimental evidence from a natural system. Evolution Letters 1: 6-15.

Girling, R. D., and R. T. Cardé. 2006. Analysis of the courtship behavior of the navel orangeworm, *Amyelois transitella* (Walker) (Lepidoptera: Pyralidae), with a commentary on methods for the analysis of sequences of behavioral transitions. Journal of Insect Behavior 19: 497-520.

Gligorescu, A., S. Toft, H. Hauggaard-Nielsen, J. A. Axelsen, and S. A. Nielsen. 2018. Development, metabolism and nutrient composition of black soldier fly larvae (*Hermetia illucens*; Diptera: Stratiomyidae) in relation to temperature and diet. Journal of Insects as Food and Feed 4: 123-133.

Gomes, G., R. Köberle, C. J. Von Zuben, and D. V. Andrade. 2018. Droplet bubbling evaporatively cools a blowfly. Scientific Reports 8: 5464.

Green, K. 2003. Age-related variation in mean sperm length, in the rove beetle *Aleochara bilineata*. Journal of Insect Physiology 49: 993-998.

Green, T. R., and R. Popa. 2012. Enhanced ammonia content in compost leachate processed by black soldier fly larvae. Applied Biochemistry and Biotechnology 166: 1381-1387.

Grégoire, M.-C., J. Massonneau, O. Simard, A. Gouraud, M.-A. Brazeau, M. Arguin, F. Leduc, and G. Boissonneault. 2013. Male-driven de novo mutations in haploid germ cells. MHR: Basic Science of Reproductive Medicinevol 19: 495–499.

Guerrero-Bosagna, C. 2017. Evolution with no reason: A neutral view on epigenetic changes, genomic variability, and evolutionary novelty. BioScience 67: 469-476.

Gunderson, A. R., E. E. King, K. Boyer, B. Tsukimura, and J. H. Stillman. 2017. Species as stressors: Heterospecific interactions and the cellular stress response under global change. Integrative and Comparative Biology 57: 90-102.

Guo, S. W., and E. A. Thompson. 1992. Performing the exact test of Hardy-Weinberg proportion for multiple alleles. Biometrics 48: 361-372.

Hahn, D. A., and D. L. Denlinger. 2011. Energetics of insect diapause. Annual Review of Entomology 56: 103-121.

Harker, J. E. 1992. Swarm behaviour and mate competition in mayflies (Ephemeroptera). Journal of Zoology 228: 571-587.

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

Harris, R. M., P. McQuillan, and L. Hughes. 2015. The effectiveness of common thermo-regulatory behaviours in a cool temperate grasshopper. Journal of Thermal Biology 52: 75-83.

Harris, W. E., A. J. Moore, and P. J. Moore. 2007. Variation in sperm size within and between ejaculates in a cockroach. Functional Ecology 21: 598-602.

Haslbeck, M., and E. Vierling. 2015. A first line of stress defense: small heat shock proteins and their function in protein homeostasis. Journal of Molecular Biology 427: 1537-1548.

Hauser, M., N. Woodley, and D. Fachin. 2017. Stratiomyidae (Soldier Flies), pp. 919–979. *In* Manual of Afrotropical Diptera, Vol. 2: Nematocerous Diptera and lower Brachycera. SANBI Graphics & Editing, Pretoria, South Africa.

Heinrich, B. 2013. The Hot-Blooded Insects: Strategies and Mechanisms of Thermoregulation. Springer Science & Business Media, Berlin, Germany.

Hibino, Y. 1986. Female choice for male gregariousness in a stink bug, *Megacopta punctissimum* (Montandon) (Heteroptera, Plataspidae). Journal of Ethology 4: 91-95.

Higginson, D. M., K. B. Miller, K. A. Segraves, and S. Pitnick. 2012. Female reproductive tract form drives the evolution of complex sperm morphology. Proceedings of the National Academy of Sciences 109: 4538-4543.

Hill, W. G., and A. Robertson. 1966. The effect of linkage on limits to artificial selection. Genetical Research 8: 269-294.

Höglund, J., and R. V. Alatalo. 2014. Leks. Princeton University Press, Princeton, NJ.

Holman, L., and R. R. Snook. 2006. Spermicide, cryptic female choice and the evolution of sperm form and function. Journal of Evolutionary Biology 19: 1660-1670.

Horton, D. R., T. M. Lewis, and T. Hinojosa. 2002. Copulation duration in three species of *Anthocoris* (Heteroptera: Anthocoridae) at different temperatures and effects on insemination and ovarian development. Pan Pacific Entomologist 78: 43-55.

Hosken, D. J., and C. M. House. 2011. Sexual selection. Current Biology 21: 62-65.

Howard, R. S., and C. M. Lively. 2002. The ratchet and the red queen: The maintenance of sex in parasites. Journal of Evolutionary Biology 15: 648-656.

Huang, L.-H., B. Chen, and L. Kang. 2007. Impact of mild temperature hardening on thermotolerance, fecundity, and Hsp gene expression in *Liriomyza huidobrensis*. Journal of Insect Physiology 53: 1199-1205.

Hunter, F. M. and T. R. Birkhead. 2002. Sperm viability and sperm competition in insects. Current Biology 12: 121-123.

Ide, J., and M. Kondoh. 2000. Male–female evolutionary game on mate-locating behaviour and evolution of mating systems in insects. Ecology Letters 3: 433-440.

Iglesias-Carrasco, M., G. Bilgin, M. D. Jennions, and M. L. Head. 2018. The fitness cost to females of exposure to males does not depend on water availability in seed beetles. Animal Behaviour 142: 77-84.

Ivy, T. M., C. B. Weddle, and S. K. Sakaluk. 2005. Females use self-referent cues to avoid mating with previous mates. Proceedings of the Royal Society B, Biological Sciences 272: 2475-2478.

Iwasa, Y., A. Pomiankowski, and S. Nee. 1991. The evolution of costly mate preferences II. The "handicap" principle. Evolution 45: 1431-1442.

Jamieson, B. G. M. 1987. The Ultrastructure and Phylogeny of Insect Spermatozoa. Cambridge University Press, Cambridge, UK.

Jamieson, B. G. M., R. Dallai, and B. A. Afzelius. 1999. Insects: Their spermatozoa and phylogeny. Science Publishers Inc., New Hampshire, US.

Janssen, R. H., J.-P. Vincken, L. A. M. van den Broek, V. Fogliano, and C. M. M. Lakemond. 2017. Nitrogen-to-protein conversion factors for three edible insects: *Tenebrio molitor*, *Alphitobius diaperinus*, and *Hermetia illucens*. Journal of Agricultural and Food Chemistry 65: 2275-2278.

Jennions, M. D., and L. Fromhage. 2017. Not all sex ratios are equal: The Fisher condition, parental care and sexual selection. Philosophical Transactions of the Royal Society B, Biological Sciences 372: 20160312(ID).

Jiménez-Mena, B., and T. Bataillon. 2016. Heterogeneity in effective population size and its implications in conservation genetics and animal breeding. Conservation Genetics Resources 8: 35-41.

Johnson, S. L., S. Zellhuber-McMillan, J. Gillum, J. Dunleavy, J. P. Evans, S. Nakagawa, and N. J. Gemmell. 2018. Evidence that fertility trades off with early offspring fitness as males age. Proceedings of the Royal Society B, Biological Sciences 285: 20172174.

Jones, T., R. Quinnell, and A. Balmford. 1998. Fisherian flies: Benefits of female choice in a lekking sandfly. Proceedings of the Royal Society of London B, Biological Sciences 265: 1651-1657.

Kashiri, M., C. Marin, R. Garzón, C. M. Rosell, D. Rodrigo, and A. Martínez. 2018. Use of high hydrostatic pressure to inactivate natural contaminating microorganisms and inoculated *E. coli* O157:H7 on *Hermetia illucens* larvae. PLOS One 13: e0194477.

Kimura, M. 1991. The neutral theory of molecular evolution: A review of recent evidence. The Japanese Journal of Genetics 66: 367-386.

King, A. M., and T. H. MacRae. 2015. Insect heat shock proteins during stress and diapause. Annual Review of Entomology 60: 59-75.

Kleckova, I., and J. Klecka. 2016. Facing the heat: Thermoregulation and behaviour of lowland species of a cold-dwelling butterfly genus, Erebia. PloS One 11: e0150393.

Kleckova, I., M. Konvicka, and J. Klecka. 2014. Thermoregulation and microhabitat use in mountain butterflies of the genus *Erebia*: Importance of fine-scale habitat heterogeneity. Journal of Thermal Biology 41: 50-58.

Kokko, H., and M. D. Jennions. 2014. The relationship between sexual selection and sexual conflict. Cold Spring Harbor Perspectives in Biology 6: a017517.

Kortet, R., Niemela, P. T., Vainikka, A., and J. Laakso. 2012. Females prefer bold males: An analysis of boldness, mate choice, and bacterial resistance in the field cricket *Gryllus integer*. Ecological Parasitology and Immunology 1: 235580(ID).

Kotiaho, J. S., L. W. Simmons, and J. L. Tomkins. 2001. Towards a resolution of the lek paradox. Nature 410: 684-686.

Krasnec, M., C. Cook, and M. Breed. 2012. Mating systems in sexual animals. Nature Education Knowledge 3: 72.

Kvarnemo, C. 2018. Why do some animals mate with one partner rather than many? A review of causes and consequences of monogamy. Biological Reviews 93: 1795-1812.

Kvarnemo, C., and I. Ahnesjo. 1996. The dynamics of operational sex ratios and competition for mates. Trends in Ecology & Evolution 11: 404-408.

Kvarnemo, C., and L. W. Simmons. 2013. Polyandry as a mediator of sexual selection before and after mating. Philosophical Transactions of the Royal Society B, Biological Sciences 368: 20120042.

Lahondère, C., and C. R. Lazzari. 2015. Thermal effect of blood feeding in the telmophagous fly *Glossina morsitans* morsitans. Journal of Thermal Biology 48: 45-50.

Lalander, C., S. Diener, M. E. Magri, C. Zurbrügg, A. Lindström, and B. Vinnerås. 2013. Faecal sludge management with the larvae of the black soldier fly (*Hermetia illucens*) - From a hygiene aspect. Science of the Total Environment 458: 312-318.

Lank, D. B., and C. M. Smith. 1992. Females prefer larger leks: Field experiments with ruffs (*Philomachus pugnax*). Behavioral Ecology and Sociobiology 30: 323-329.

Lapidus, L. J. 2017. Protein unfolding mechanisms and their effects on folding experiments. Faculty 1000 Research 6: 1723.

Lee, J.-A., Y.-M. Kim, Y.-K. Park, Y.-C. Yang, B.-G. Jung, and B.-J. Lee. 2018. Black soldier fly (*Hermetia illucens*) larvae enhances immune activities and increases survivability of broiler chicks against experimental infection of *Salmonella gallinarum*. Journal of Veterinary Medical Science 80: 736-740.

Lehtonen, J., M. D. Jennions, and H. Kokko. 2012. The many costs of sex. Trends in Ecology & Evolution 27: 172-178.

Lepock, J. R. 2003. Cellular effects of hyperthermia: Relevance to the minimum dose for thermal damage. International Journal of Hyperthermia 19: 252-266.

Lewis W.M. 1987. The Cost of Sex, pp 33-57. In S. C. Stearns (ed), The Evolution of Sex and Its Consequences, vol 55. Experientia Supplementum, Birkhäuser, Basel.

Li, C. 1988. Pseudo-random mating populations. In celebration of the 80th anniversary of the Hardy-Weinberg law. Genetics 119: 731-737.

Li, F., H. Tong, M. A. A. Omar, W. Ye, Y. Ao, Y. Wang, Z. Li, and M. Jiang. 2019. Male mating and female postmating performances in cotton mealybug (Hemiptera: Pseudococcidae): Effects of female density. Journal of Economic Entomology, toz030.

Linck, R. W., H. Chemes, and D. F. Albertini. 2016. The axoneme: The propulsive engine of spermatozoa and cilia and associated ciliopathies leading to infertility. Journal of Assisted Reproduction and Genetics 33: 141-156.

Lindemann, C., A. Hunt, C. Lindemann, and A. Hunt. 2003. Does axonemal dynein push, pull or oscillate? Cell Motility and the Cytoskeleton 56: 237-244.

Lively, C. M. 1987. Evidence from a New Zealand snail for the maintenance of sex by parasitism. Nature 328: 519-521.

Loli, D., and J. E. P. Bicudo. 2005. Control and regulatory mechanisms associated with thermogenesis in flying insects and birds. Bioscience Reports 25: 149-180.

Luck, N., and D. Joly. 2005. Sexual selection and mating advantages in the giant sperm species, *Drosophila bifurca*. Journal of Insect Science 5: 10.

Luo, S., C. A. Valencia, J. Zhang, N.-C. Lee, J. Slone, B. Gui, X. Wang, Z. Li, S. Dell, J. Brown, S. M. Chen, Y.-H. Chien, W.-L. Hwu, P.-C. Fan, L.-J. Wong, P. S. Atwal, and T. Huang. 2018. Biparental inheritance of mitochondrial DNA in humans. Proceedings of the National Academy of Sciences 115: 13039-13044.

Lüpold, S., M. K. Manier, N. Puniamoorthy, C. Schoff, W. T. Starmer, S. H. B. Luepold, J. M. Belote, and S. Pitnick. 2016. How sexual selection can drive the evolution of costly sperm ornamentation. Nature 533: 535–538.

Lyu, N., M. R. Servedio, H. Lloyd, and Y.-H. Sun. 2017. The evolution of postpairing male mate choice. International Journal of Organic Evolution 71: 1465-1477.

Ma, G., and C.-S. Ma. 2012. Effect of acclimation on heat-escape temperatures of two aphid species: implications for estimating behavioral response of insects to climate warming. Journal of Insect Physiology 58: 303-309.

Mafessoni, F., and M. Lachmann. 2015. Selective strolls: Fixation and extinction in diploids are slower for weakly selected mutations than for neutral ones. Genetics 201: 1581-1589.

Magalhães, R., A. Sánchez-López, R. S. Leal, S. Martínez-Llorens, A. Oliva-Teles, and H. Peres. 2017. Black soldier fly (*Hermetia illucens*) pre-pupae meal as a fish meal replacement in diets for European seabass (*Dicentrarchus labrax*). Aquaculture 476: 79-85.

Makkar, H. P. S., G. Tran, V. Heuzé, and P. Ankers. 2014. State-of-the-art on use of insects as animal feed. Animal Feed Science and Technology 197: 1-33.

Makkar, H. P. S., V. Heuzé, and G. Tran. 2015. Insects in fish diets. Animal Frontiers 5: 37-44.

Manier, M. K., J. M. Belote, K. S. Berben, S. Lüpold, O. Ala-Honkola, W. F. Collins, and S. Pitnick. 2013. Rapid diversification of sperm precedence traits and processes among three sibling *Drosophila* species. Evolution 67: 2348-2362.

Marden, J. H. 2000. Variability in the size, composition, and function of insect flight muscles. Annual Review of Physiology 62: 157-178.

Marques-Mari, A.I., Lacham-Kaplan, O., Medrano, J.V., Pellicer, A., and C. Simón. 2009. Differentiation of germ cells and gametes from stem cells. Human Reproduction Update 15: 379–390.

Marshall, S., N. Woodley, and M. Hauser. 2015. The historical spread of the black soldier fly, *Hermetia illucens* (L.) (Diptera, Stratiomyidae, Hermetiinae), and its establishment in Canada. The Journal of the Entomological Society of Ontario 146.

Martin, T. L., and R. B. Huey. 2008. Why "suboptimal" is optimal: Jensen's inequality and ectotherm thermal preferences. The American Naturalist 171: 102-118.

May, M. L. 1979. Insect thermoregulation. Annual Review of Entomology 24: 313-349.

May, M. L. 2017. Body temperature regulation in the dragonfly, *Arigomphus villosipes* (Odonata: Anisoptera: Gomphidae). International Journal of Odonatology 20: 151-163.

McNamara, K. B., E. van Lieshout, and L. W. Simmons. 2014. A test of the sexy-sperm and good-sperm hypotheses for the evolution of polyandry. Behavioral Ecology 25: 989-995.

Mercier, J.-L., J.-C. Lenoir, A. Eberhardt, S. Frohschammer, C. Williams, and J. Heinze. 2007. Hammering, mauling, and kissing: Stereotyped courtship behavior in Cardiocondyla ants. Insectes Sociaux 54: 403-411.

Mertenat, A., S. Diener, and C. Zurbrügg. 2019. Black Soldier Fly biowaste treatment-Assessment of global warming potential. Waste Management 84: 173-181.

Metzler, S., J. Heinze, and A. Schrempf. 2016. Mating and longevity in ant males. Ecology and Evolution 6: 8903-8906.

Meurer-Grob, P., J. Kasparian, and R. H. Wade. 2001. Microtubule structure at improved resolution. Biochemistry 40: 8000-8008.

Milam, E. L. 2010. Looking for a few good males: Female choice in evolutionary biology. Johns Hopkins University Press, Baltimore, MD, US.

Miller, G. T., and S. Pitnick. 2002. Sperm-female coevolution in *Drosophila*. Science 298: 1230-1233.

Minder, A. M., D. J. Hosken, and P. I. Ward. 2005. Co-evolution of male and female reproductive characters across the Scathophagidae (Diptera). Journal of Evolutionary Biology 18: 60-69.

Morrow, E. H. 2004. How the sperm lost its tail: the evolution of aflagellate sperm. Philosophical Transactions of the Royal Society B, Biological Sciences 79: 795-814.

Morrow, E. H., and M. J. Gage. 2000. The evolution of sperm length in moths. Proceedings. Biological Sciences 267: 307-313.

Morrow, E. H., G. Arnqvist, and S. Pitnick. 2003. Adaptation versus pleiotropy: Why do males harm their mates? Behavioral Ecology 14: 802-806.

Moya-Laraño, J., M. E. T. El-Sayyid, and C. W. Fox. 2007. Smaller beetles are better scramble competitors at cooler temperatures. Biology Letters 3: 475-478.

Mullaney, J. M., R. E. Mills, W. S. Pittard, and S. E. Devine. 2010. Small insertions and deletions (INDELs) in human genomes. Human Molecular Genetics 19: 131-136.

Muller, H. J. 1932. Some genetic aspects of sex. The American Naturalist 66: 118-138.

Muller, H. J. 1964. The relation of recombination to mutational advance. Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis 1: 2-9.

Neff, B. D., and E. I. Svensson. 2013. Polyandry and alternative mating tactics. Philosophical Transactions of the Royal Society B, Biological Sciences 368: 20120045 (ID).

Neven, L. G. 2000. Physiological responses of insects to heat. Postharvest Biology and Technology 21: 103-111.

Nixon, A. E., M. Scholar, and H. Hines. 2016. Bumblebee thermoregulation: Understanding the thermal properties of physical features of bumblebees. The Penn State McNair Journal 21: 99-115.

Nyakeri, E., H. Ogola, M. Ayieko, and F. Amimo. 2017. An open system for farming black soldier fly larvae as a source of proteins for smallscale poultry and fish production. Journal of Insects as Food and Feed 3: 51-56.

Ohashi, K., Y. Sakuratani, N. Osawa, S. Yano, and A. Takafuji. 2005. Thermal microhabitat use by the ladybird beetle, *Coccinella septempunctata* (Coleoptera: Coccinellidae), and its life cycle consequences. Environmental Entomology 34: 432-439.

Pak, O. S., S. E. Spagnolie, and E. Lauga. 2012. Hydrodynamics of the double-wave structure of insect spermatozoa flagella. Journal of The Royal Society Interface 9: 1908-1924.

Paoli, F., M. Gottardo, R. Dallai, and P. F. Roversi. 2013. Morphology of the male reproductive system and sperm ultrastructure of the egg parasitoid *Gryon pennsylvanicum* (Ashmead) (Hymenoptera, Platygastridae). Arthropod Structure & Development 42: 297-308.

Paoli, F., R. Dallai, M. Cristofaro, S. Arnone, V. Francardi, and P. F. Roversi. 2014. Morphology of the male reproductive system, sperm ultrastructure and γ-irradiation of the red palm weevil *Rhynchophorus ferrugineus* Oliv. (Coleoptera: Dryophthoridae). Tissue and Cell 46: 274-285.

Parker, G. 1978. Evolution of competitive mate searching. Annual Review of Entomology 23: 173-196.

Parker, G. 1984. Sperm competition and the evolution of animal mating strategies, pp. 1-60. *In* R. Smith, Sperm Competition and the Evolution of Animal Mating Systems. Academic Press, New York, US.

Parker, G. A. 1970. Sperm competition and its evolutionary consequences in the insects. Biological Reviews 45: 525-567.

Parker, G. A. 1982. Why are there so many tiny sperm? Sperm competition and the maintenance of two sexes. Journal of Theoretical Biology 96: 281-294.

Parker, G. A. 1990. Sperm competition games: Raffles and roles. Proceedings of the Royal Society of London B, Biological Sciences 242: 120-126.

Parker, G. A. 2006. Sexual conflict over mating and fertilization: an overview. Philosophical Transactions of the Royal Society B, Biological Sciences 361: 235-259.

Parker, G. A., and T. Pizzari. 2010. Sperm competition and ejaculate economics. Biological Reviews 85: 897-934.

Parker, G. A., and T. R. Birkhead. 2013. Polyandry: The history of a revolution. Philosophical Transactions of the Royal Society B, Biological Sciences 368: 20120335.

Peinert, M., B. Wipfler, G. Jetschke, T. Kleinteich, S. N. Gorb, R. G. Beutel, and H. Pohl. 2016. Traumatic insemination and female counter-adaptation in Strepsiptera (Insecta). Scientific Reports 6: 25052.

Phillips, D. M. 1970. Insect sperm: Their structure and morphogenesis. The Journal of Cell Biology 44: 243.

Pitnick, S. 1996. Investment in testes and the cost of making long sperm in *Drosophila*. The American Naturalist 148: 57-80.

Pitnick, S., D. J. Hosken, and T. R. Birkhead. 2009b. Sperm morphological diversity, pp. 69-149. In T. R. Birkhead, D. J. Hosken and S. Pitnick (eds.), Sperm Biology. Academic Press, London, UK.

Pitnick, S., T. Markow, and G. S. Spicer. 1999. Evolution of multiple kinds of female sperm-storage organs in *Drosophila*. International Journal of Organic Evolution 53: 1804-1822.

Pizzari, T., and T. R. Birkhead. 2002. The sexually-selected sperm hypothesis: Sex-biased inheritance and sexual antagonism. Biological Reviews 77: 183-209.

Pizzari, T., R. Dean, A. Pacey, H. Moore, and M. B. Bonsall. 2008. The evolutionary ecology of pre-and post-meiotic sperm senescence. Trends in Ecology & Evolution 23: 131-140.

Pleissner, D., and B. A. Rumpold. 2018. Utilization of organic residues using heterotrophic microalgae and insects. Waste Management 72: 227-239.

Rehman, K. u., A. Rehman, M. Cai, L. Zheng, X. Xiao, A. A. Somroo, H. Wang, W. Li, Z. Yu, and J. Zhang. 2017. Conversion of mixtures of dairy manure and soybean curd residue by black soldier fly larvae (*Hermetia illucens* L.). Journal of Cleaner Production 154: 366-373.

Richards, A. G. 1963. The rate of sperm locomotion in the cockroach as a function of temperature. Journal of Insect Physiology 9: 545-549.

Riebel, K., M.-J. Holveck, S. Verhulst, and T. Fawcett. 2010. Are high-quality mates always attractive? State-dependent mate preferences in birds and humans: State-dependent mate preferences in birds and humans. Communicative & Integrative Biology 3: 271-273.

Rikmenspoel, R. 1978. The equation of motion for sperm flagella. Biophysical Journal 23: 177-206.

Ritchie, M. G. 2007. Sexual selection and speciation. Annual Review of Ecology, Evolution, and Systematics 38: 79-102.

Robert, T. 1972. Parental Investment and Sexual Selection, pp.136-179. In B. Campbell (ed.), Sexual Selection and the Descent of Man, Aldine de Gruyter, New York, US.

Roháček, J., and M. Hora. 2013. A northernmost European record of the alien black soldier fly *Hermetia illucens* (Linnaeus, 1758) (Diptera: Stratiomyidae). Casopis Slezskeho Zemskeho Muzea 62: 101-106.

Rowe, L., and D. Houle. 1996. The lek paradox and the capture of genetic variance by condition dependent traits. Proceedings of the Royal Society of London B, Biological Sciences 263: 1415-1421.

Rowe, L., G. Arnqvist, A. Sih, and J. J.Krupa. 1994. Sexual conflict and the evolutionary ecology of mating patterns: water striders as a model system. Trends in Ecology & Evolution 9: 289-293.

Rozkosný, R. 1983. A biosystematic study of the european Stratiomyidae (Diptera): Clitellariinae, Hermediinae, Pachygasterinae and Bibliography - Series entomologica, vol. 2. Springer Science & Business Media, Berlin, Germany.

Ruel, J. J., and M. P. Ayres. 1999. Jensen's inequality predicts effects of environmental variation. Trends in Ecology & Evolution 14: 361-366.

Rundle, H. D., A. Ödeen, and A. Ø. Mooers. 2007. An experimental test for indirect benefits in *Drosophila melanogaster*. BMC Evolutionary Biology 7: 36.

Saeki, Y., K. C. Kruse, and P. V. Switzer. 2005. The social environment affects mate guarding behavior in Japanese beetles, *Popillia japonica*. Journal of Insect Science 5: 18.

Saldívar-Hernández, A., M. E. González-González, A. Sánchez-Tusié, I. Maldonado-Rosas, P. López, C. L. Treviño, F. Larrea, and M. Chirinos. 2015. Human sperm degradation of zona pellucida proteins contributes to fertilization. Reproductive Biology and Endocrinology 13: 99.

Sales, K., R. Vasudeva, M. E. Dickinson, J. L. Godwin, A. J. Lumley, Ł. Michalczyk, L. Hebberecht, P. Thomas, A. Franco, and M. J. Gage. 2018. Experimental heatwaves compromise sperm function and cause transgenerational damage in a model insect. Nature Communications 9: 4771.

Salomone, R., G. Saija, G. Mondello, A. Giannetto, S. Fasulo, and D. Savastano. 2017. Environmental impact of food waste bioconversion by insects: Application of life cycle assessment to process using *Hermetia illucens*. Journal of Cleaner Production 140: 890-905.

Sanford, M. R., Z. Yu, Q. Liu, J. K. Tomberlin, and J. A. Brady. 2008. Black soldier fly (Diptera: Stratiomyidae) larvae reduce *Escherichia coli* in dairy manure. Environmental Entomology 37: 1525-1530.

Santos, E. S. A., and S. Nakagawa. 2013. Breeding biology and variable mating system of a population of introduced dunnocks (*Prunella modularis*) in New Zealand. PLOS One 8: e69329.

Satir, P. 1965. Studies on cilia: II examination of the distal region of the ciliary shaft and the role of the filaments in motility. Journal of Cell Biology 26: 805-834.

Satir, P., T. Heuser, and W. S. Sale. 2014. A structural basis for how motile cilia beat. Bioscience 64: 1073-1083.

Saxena, B., P. Sharma, R. Thappa, and K. Tikku. 1992. Temperature induced sterilization for control of three stored grain beetles. Journal of Stored Products Research 28: 67-70.

Saxon, A., E. O'brien, and J. Bridle. 2018. Temperature fluctuations during development reduce male fitness and may limit adaptive potential in tropical rainforest *Drosophila*. Journal of Evolutionary Biology 31: 405-415.

Schiavone, A., M. De Marco, S. Martínez, S. Dabbou, M. Renna, J. Madrid, F. Hernandez, L. Rotolo, P. Costa, and F. Gai. 2017. Nutritional value of a partially defatted and a highly defatted black soldier fly larvae (*Hermetia illucens* L.) meal for broiler chickens: Apparent nutrient digestibility, apparent metabolizable energy and apparent ileal amino acid digestibility. Journal of Animal Science and Biotechnology 8: 51.

Schulte, P. M. 2015. The effects of temperature on aerobic metabolism: towards a mechanistic understanding of the responses of ectotherms to a changing environment. Journal of Experimental Biology 218: 1856-1866.

Scriven, J. J., P. R. Whitehorn, D. Goulson, and M. C. Tinsley. 2016. Bergmann's body size rule operates in facultatively endothermic insects: Evidence from a complex of cryptic bumblebee species. PloS One 11: e0163307.

Seeberger, J., R. Grandhi, S. S. Kim, W. A. Mase, T. Reponen, H. Shuk-mei, and C. Aimin. 2016. E-waste management in the United States and public health implications. Journal of Environmental Health 79:8-16.

Shcherbakov, V. P. 2010. Biological species is the only possible form of existence for higher organisms: the evolutionary meaning of sexual reproduction. Biology Direct 5: 14-14.

Shelly, T. E., and T. S. Whittier. 1997. Lek Behavior of Insects. *In* J.C. Choe and B.J. Crespi (eds), The Evolution of Mating Systems in Insects and Arachnids, Cambridge University Press, Cambridge, UK.

Shelomi, M. 2012. Where are we now? Bergmann’s rule sensu lato in insects. The American Naturalist 180: 511-519.

Sheppard, C. 1983. House fly and lesser fly control utilizing the black soldier fly in manure management systems for caged laying hens. Environmental Entomology 12: 1439-1442.

Sherman, P. W., T. D. Seeley, and H. K. Reeve. 1988. Parasites, pathogens, and polyandry in social Hymenoptera. The American Naturalist 131: 602-610.

Sheth, S. N., and A. L. Angert. 2014. The evolution of environmental tolerance and range size: a comparison of geographically restricted and widespread Mimulus. International Journal of Organic Evolution 68: 2917-2931.

Short, S. M., M. F. Wolfner, and B. P. Lazzaro. 2012. Female D*rosophila melanogaster* suffer reduced defense against infection due to seminal fluid components. Journal of Insect Physiology 58: 1192-1201.

Shumo, M., F. M. Khamis, C. M. Tanga, K. K. Fiaboe, S. Subramanian, S. Ekesi, A. van Huis, and C. Borgemeister. 2019. Influence of temperature on selected life-history traits of black soldier fly (*Hermetia illucens*) reared on two common urban organic waste streams in Kenya. Animals 9: 79.

Shuster, S. M. 2009. Sexual selection and mating systems. Proceedings of the National Academy of Sciences 106: 10009-10016.

Shuster, S., and M. Wade. 2003. Mating systems and strategies. Princeton University Press, Princeton, NJ, USA.

Sih, A., J. Krupa, and S. Travers. 1990. An experimental study on the effects of predation risk and feeding regime on the mating behavior of the water strider. The American Naturalist 135: 284-290.

Silva, K., M. N. Vieira, V. C. Almada, and N. M. Monteiro. 2007. The effect of temperature on mate preferences and female–female interactions in *Syngnathus abaster*. Animal Behaviour 74: 1525-1533.

Simmons, L. 2001a. Sperm competition and its evolutionary consequences in the insects. Princeton University Press, Princeton, NJ, US.

Simmons, L. 2003. The evolution of polyandry: patterns of genotypic variation in female mating frequency, male fertilization success and a test of the sexy-sperm hypothesis. Journal of Evolutionary Biology 16: 624-634.

Simmons, L. W. 2001b. The evolution of polyandry: An examination of the genetic incompatibility and good-sperm hypotheses. Journal of Evolutionary Biology 14: 585-594.

Simmons, L. W., and J. L. Fitzpatrick. 2012. Sperm wars and the evolution of male fertility. Reproduction 144: 519-534.

Simmons, L. W., and M. Zuk. 1992. Variability in call structure and pairing success of male field crickets, *Gryllus bimaculatus*: the effects of age, size and parasite load. Animal Behaviour 44: 1145-1152.

Smith, C. C., R. B. Srygley, E. I. Dietrich, and U. G. Mueller. 2017a. Partitioning the effects of mating and nuptial feeding on the microbiome in gift-giving insects. Environmental Microbiology Reports 9: 104-112.

Smith, C. C., R. B. Srygley, E. I. Dietrich, and U. G. Mueller. 2017b. Partitioning the effects of mating and nuptial feeding on the microbiome in gift-giving insects. Environmental Microbiology Reports 9: 104-112.

Smith, J. M. 1971a. What use is sex? Journal of Theoretical Biology 30: 319-335.

Smith, J. M. 1971b. The Origin and Maintenance of Sex, pp. 163–175. In G. C. Williams (ed.), Group Selection. Aldine-Atherton, Chicago, IL, US.

Soubry, A., C. Hoyo, R. L. Jirtle, and S. K. Murphy. 2014. A paternal environmental legacy: Evidence for epigenetic inheritance through the male germ line. Bioessays 36: 359-371.

Spranghers, T., M. Ottoboni, C. Klootwijk, A. Ovyn, S. Deboosere, B. De Meulenaer, J. Michiels, M. Eeckhout, P. De Clercq, and S. De Smet. 2017. Nutritional composition of black soldier fly (*Hermetia illucens*) prepupae reared on different organic waste substrates. Journal of the Science of Food and Agriculture 97: 2594-2600.

Stadtlander, T., A. Stamer, A. Buser, J. Wohlfahrt, F. Leiber, and C. Sandrock. 2017. *Hermetia illucen*s meal as fish meal replacement for rainbow trout on farm. Journal of Insects as Food and Feed 3: 165-175.

Stoltz, J., D. Elias, and M. Andrade. 2008. Females reward courtship by competing males in a cannibalistic spider. Behavioral Ecology and Sociobiology 62: 689-697.

Stoss, J. 1983. Fish Gamete Preservation and Spermatozoan Physiology, pp. 305-350. In W. S. Hoar, D. J. Randall, and E. M. Donaldson (eds.), Fish Physiology, vol. 9. Academic Press, Cambridge, MA.

Summers, K. E., and I. R. Gibbons. 1971. Adenosine Triphosphate-induced sliding of tubules in trypsin-treated flagella of sea-urchin sperm. Proceedings of the National Academy of Sciences 68: 3092-3096.

Swan, M. A. 1981. The generation and propagation of double waves in mosquito (*Aedes notoscriptus*) sperm-tails. Gamete Research 4: 241-250.

Tauber, M. J., and C. A. Tauber. 1976. Insect seasonality: Diapause maintenance, termination, and postdiapause development. Annual Review of Entomology 21: 81-107.

Tauber, M. J., C. A. Tauber, and S. Masaki. 1986. Seasonal adaptations of insects. University Press on Demand, Oxford, UK.

Taylor, H. C., P. Satir, and M. E. Holwill. 1999. Assessment of inner dynein arm structure and possible function in ciliary and flagellar axonemes. Cell Motility and the Cytoskeleton 43: 167-177.

Tinghitella, R. M., A. C. Lackey, M. Martin, P. D. Dijkstra, J. P. Drury, R. Heathcote, J. Keagy, E. S. Scordato, A. M. Tyers, and L. Simmons. 2017. On the role of male competition in speciation: A review and research agenda. Behavioral Ecology 29: 783-797.

Tomberlin, J. K., and D. C. Sheppard. 2001. Lekking behavior of the black soldier fly (Diptera: Stratiomyidae). Florida Entomologist 84: 729.

Tomberlin, J. K., P. H. Adler, and H. M. Myers. 2009. Development of the black soldier fly (Diptera: Stratiomyidae) in relation to temperature. Environmental Entomology 38: 930-934.

Tomkins, J. L., J. Radwan, J. S. Kotiaho, and T. Tregenza. 2004. Genic capture and resolving the lek paradox. Trends in Ecology & Evolution 19: 323-328.

Tooby, J. 1982. Pathogens, polymorphism, and the evolution of sex. Journal of Theoretical Biology 97: 557-576.

Toolson, E. C. 1987. Water profligacy as an adaptation to hot deserts: Water loss rates and evaporative cooling in the sonoran desert cicada, *Diceroprocta apache* (Homoptera: Cicadidae). Physiological Zoology 60: 379-385.

Townsend, D. S. 1989. Sexual selection, natural selection, and a fitness trade-off in a tropical frog with male parental care. The American Naturalist 133: 266-272.

Travers, S. E., and A. Sih. 1991. The influence of starvation and predators on the mating behavior of a semiaquatic insect. Ecology 72: 2123-2136.

Tuff, K., T. Tuff, and K. Davies. 2016. A framework for integrating thermal biology into fragmentation research. Ecology Letters 19: 361-374.

Ururahy-Rodrigues, A., and J. R. Pujol–Luz. 2000. Notas sobre a espermateca de *Hermetia illucens* (L., 1758) (Diptera, Stratiomyidae). Ururahy-Rodrigues, A., and J. R. Pujol–Luz. 2000. Contribuições avulsas sobre a história natural do brasil. Série Zoologia 17: 1-5.

Vahed, K., and D. J. Parker. 2012. The evolution of large testes: Sperm competition or male mating rate? Ethology 118: 107-117.

Van Valen, L. 1973. A new evolutionary law. Evolutionary Theory 1: 1-30.

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

Velando, A., J. Noguera, H. Drummond, and R. Torres. 2011. Senescent males carry premutagenic lesions in sperm. Journal of Evolutionary Biology 24: 693-697.

Verdú, J. R., J. Alba-Tercedor, and M. Jiménez-Manrique. 2012. Evidence of different thermoregulatory mechanisms between two sympatric *Scarabaeus* species using infrared thermography and micro-computer tomography. PloS One 7: e33914.

Wagner, C. R. 2010. Germ cells and epigenetics. Nature Education 3:64

Walker, W. F. 1980. Sperm utilization strategies in nonsocial insects. The American Naturalist 115: 780-799.

Walsh, B. S., S. R. Parratt, A. A. Hoffmann, D. Atkinson, R. R. Snook, A. Bretman, and T. A. Price. 2019. The impact of climate change on fertility. Trends in Ecology & Evolution 34: 249-259.

Weatherhead, P. J., and R. J. Robertson. 1979. Offspring quality and the polygyny threshold: The sexy son hypothesis. The American Naturalist 113: 201-208.

Welch, A. M., R. D. Semlitsch, and H. C. Gerhardt. 1998. Call duration as an indicator of genetic quality in male gray tree frogs. Science 280: 1928-1930.

Werner, M., and L. W. Simmons. 2008. Insect sperm motility. Biological Reviews 83: 191-208.

White, M. J. 1978. Modes of speciation. Paleobiology 4: 373-379.

Wickman, P.-O., and R. L. Rutowski. 1999. The evolution of mating dispersion in insects. Oikos 84: 463-472.

Wiklund, C., and T. Fagerström. 1977. Why do males emerge before females? A hypothesis to explain the incidence of protandry in butterflies. Oecologia 31: 153-158.

Williams, G. C. 1975. Sex and evolution, Princeton University Press, Princeton, NJ, US.

Wone, B. W., J. Pathak, and G. Davidowitz. 2018. Flight duration and flight muscle ultrastructure of unfed hawk moths. Arthropod Structure & Development 47: 457-464.

Wright, S. I. 2016. Charlesworth *et al.* on background selection and neutral diversity. Genetics 204: 829-832.

Wu, Y.-F., L.-S. Wei, M. Anthony Torres, X. Zhang, S.-P. Wu, and H. Chen. 2017. Morphology of the male reproductive system and spermiogenesis of *Dendroctonus armandi* Tsai and Li (Coleoptera: Curculionidae: Scolytinae). Journal of Insect Science 17.

Yasui, Y. 1997. A" good-sperm" model can explain the evolution of costly multiple mating by females. The American Naturalist 149: 573-584.

Yocum, G. D., J. Žďárek, K. H. Joplin, R. E. Lee Jr, D. C. Smith, K. D. Manter, and D. L. Denlinger. 1994. Alteration of the eclosion rhythm and eclosion behavior in the flesh fly, *Sarcophaga crassipalpis*, by low and high temperature stress. Journal of Insect Physiology 40: 13-21.

Young, K. A., M. J. Genner, D. A. Joyce, and M. P. Haesler. 2009. Hotshots, hot spots, and female preference: Exploring lek formation models with a bower-building cichlid fish. Behavioral Ecology 20: 609-615.

Zahavi, A. 1975. Mate selection - A selection for a handicap. Journal of Theoretical Biology 53: 205-214.

Zeh, J. A., S. D. Newcomer, and D. W. Zeh. 1998. Polyandrous females discriminate against previous mates. Proceedings of the National Academy of Sciences 95: 13732-13736.

2. ADULT REPRODUCTIVE TRACT MORPHOLOGY AND SPERMATOGENESIS IN THE BLACK SOLDIER FLY, *HERMETIA ILLUCENS* (L.) (DIPTERA: STRATIOMYIDAE)*

Successful commercialization of BSF relies on optimizing the production of fecund adults. However, current mass-rearing protocols result in variable production of fertile eggs. To help lay a foundation for a better understanding of factors that may play a role in this variability, the morphology of the BSF male reproductive tract and spermatozoa, associated spermatogenesis process, impact of age on the process, and the female spermatheca morphology were examined with various microscopic techniques (e.g., scanning electron microscope, transition electron microscope, and dissecting scope). The gross morphology of the male reproductive tract and female spermatheca appear to be similar to those found in other brachyceran flies. Male spermatozoa are long (~860 µm overall, ~8 µm head), apparently motile, and possess flagella with a typical 9+9+2 axoneme triplets. Germ cells go through incomplete mitotic divisions surrounded by somatic cyst cells in the testes. Spermatogenesis appears to be initiated during immature development (cryptocephalic pupa stage). From <24 h to 7 d post emergence, male aging appeared to impact sperm production.

*Reprinted with permission from "Adult Reproductive Tract Morphology and Spermatogenesis in the Black Soldier Fly, Hermetia illucens (L.) (Diptera: Stratiomyidae)" by Aline S. Malawey, David Mercati, Charles C. Love, and Jeffery K. Tomberlin, 2019. Annals of the Entomological Society of America [in press] by Oxford University Press.

2.1. Introduction

A challenge for mass production of insects is the regulation of their reproductive behavior in order to optimally produce offspring. Aspects of the reproductive biology of an organism, such as the anatomy of the reproductive tract and the gametogenesis process, could provide information about mating frequency (i.e., polygamy vs. monogamy) (Ridley 1988) behavioral preference (i.e., gregarious vs. non-gregarious) (Matthews and Matthews 2009), frequency of oviposition, and timeframe from sexual maturation to senescence (Sen et al. 2016). For example, male reproductive tract and associated sperm morphology (i.e., sperm length) are known to be under selective pressure to optimize sperm allocation (Krasnec et al. 2012, Dallai 2014, Dávila and Aron 2017). Similarly, females can possess specialized anatomical structures for optimizing sperm storage (Walker 1980) and egg fertilization (Parker 1970, Higginson et al. 2012, Krasnec et al. 2012).

Senescence is known to play an important role in resource allocation by insects, as do trade-offs between survivorship and germ cell production (Pizzari et al. 2008, Velando et al. 2011, Johnson et al. 2018). Adult male insects generally emerge with mature sperm in place and continue producing sperm throughout adult life. Sperm production rate is impacted by factors such as adult age and size as well as the availability of mature females (Boivin and Ellers 2016).

Some morphological features of the female reproductive tract may also impact the reproductive fitness of males. For instance, in mating systems where females mate with multiple males, female choice can play a primary role in determining subsequent utilization of sperm acquired (Neff and Svensson 2013, Boulton et al. 2015) resulting in the evolution of male strategies to overcome sperm competition (Birkhead and Møller 1998). Hence, understanding sperm morphology, behavior, quality, and quantity produced over time could lead to insights into how to optimize fertilize egg production under mass rearing conditions (Neff and Svensson 2013, Friesen et al. 2017, Tinghitella et al. 2017).

In the livestock industry, data on sperm morphology are already being used to enhance *in vitro* fertilization (Enciso et al. 2011, Saldívar-Hernández et al. 2015) by ensuring paired adults are more likely to be reproductively successful. Insect males may use sperm production strategies characterized by different spermatogenic indices (e.g., continuous or one-time sperm production) depending on the abiotic and biotic stimuli (including individual physiological status) in a given environment (Boivin et al. 2005). In mass-rearing facilities, sperm-depleted males could continue to mate (Boivin 2013) and compete with non-depleted males for access to females, but only transfer seminal fluids to females leading to suboptimal offspring production.

The black soldier fly (BSF), *Hermetia illucens* (L.) (Diptera: Stratiomyidae), is currently being mass produced and has been proposed as a sustainable method for recycling animal and food wastes on an industrial scale (Lalander et al. 2019). BSF larvae can reduce dry manure biomass by 42-56% (Sheppard 1983, Myers et al. 2014); however, more recent research indicates a reduction of poultry manure by 85% (Lalander et al. 2019). Reductions of organic nitrogen concentration up to 62% also have been reported (Sheppard et al. 1998).

Resulting larvae can be used as animal feed (Sheppard et al. 1998, Wang and Shelomi 2017, Schiavone et al. 2017). In some cases, BSF larvae are high in protein and fat– nutrients that are poorly represented in several traditional fish feeds (Makkar et al. 2014, Henry et al. 2015). In fact, the Association of American Feed Control Officials approved the use of BSF dried larvae as feed for industrial production of salmonid fish in 2016 (AAFCO 2016) and poultry in 2019 (AAFCO 2019).

Digestion of organic wastes with BSF larvae offers a number of benefits besides the production of feed. Compost resulting from BSF digestion of waste still contains large amounts of available nitrogen (i.e., ammonium - NH_4^+) (Green and Popa 2012), which can be used as a fertilizer. Moreover, BSF recycling of wastes can reduce environmental toxins (i.e., heavy metals such as cadmium, lead, and zinc) (Diener et al. 2015) as well as pathogens (i.e., *Salmonella* spp. and *Escherichia coli*; Lalander et al. 2015, Erickson et al. 2004, and Liu et al. 2008) and noxious odors (Beskin et al. 2018). Cai et al. (2018), recently reported that BSF larvae suppress the persistence of antibiotic resistance genes in pathogenic bacteria associated with such wastes.

To date, there are no known published data on the reproductive tract, spermatogenesis, or spermatozoa morphology of this species. Therefore, this study aimed to describe the: 1) morphology of male reproductive tract, 2) morphology of spermatheca, 2) sperm morphology and size, 4) ultrastructural morphology of mature spermatozoa, 5) spermatogenesis process, and 6) impact of male aging on sperm production. Detailed knowledge about the morphological components of the sperm and shifts in spermatogenesis throughout the life cycle could lead to an understanding as to how these cells move and perform in the male, as well as female, reproductive tract (Wu et al. 2017). This understanding could be used to optimize the production of fertile eggs by BSF reared indoors at an industrial scale.

2.2. Materials and Methods

Black Soldier Fly Colony. Adults used in this study were from a colony established in January 2014 from eggs received from Phoenix Worm, Inc., Tifton, GA, USA, which was produced from a laboratory colony located at the Coastal Plains Experiment Station, University of Georgia, Tifton, GA, USA. This colony has been maintained at the Forensic Laboratory for Investigative Entomological Sciences (F.L.I.E.S.) Facility at Texas A&M University in College Station, TX, USA with wild-caught flies periodically added to maintain genetic variability. Methods applied for the collection of eggs and maintenance of immature and adult BSF were previously described by Cammack and Tomberlin (2017). Resulting neonates (~ 10,000) were placed in a 500 ml plastic container with 200 g Gainesville diet at 70% of moisture and stored in a walk-in environmental chamber (30°C, 60% RH, and 16:8 L:D) for 7 d. The 7-d-old larvae were counted and placed in 500 ml plastic containers, with a total of 500 larvae per container, with 60 g Gainesville diet at 70% moisture provided daily until 40% of the larvae in each container have reached the prepupae stage (i.e., larvae turn black). Feeding was terminated at this point (Tomberlin et al. 2002). Resulting prepupae were sifted and partitioned in equal number in individual 500 ml plastic cups stored inside 27 x 27 x 27 cm plastic cages (Bioquip®, Rancho Dominguez, CA, USA). These cages were placed in Percival incubators set to 27°C, 70% RH, and 12:12 (L:D) (Tomberlin et al. 2009, Holmes et al. 2017). Pupae were monitored every 12 h for adult emergence. Adults emerging between 24-36 h after initiation of emergence were sexed (Oliveira et al. 2016) and segregated by sex in glass mason jars (1 L) (n = 10/jar) covered with organza fabric, which was held in place with a rubber band. A wet cotton ball was placed in each jar to provide water ad libitum. Jars were placed in the Percival incubator previously described. Jars were rotated among the shelves to avoid spatial bias.

Experimental design. Three different microscopy-imaging techniques were used: 1) light and fluorescence microscopy (LFM), 2) scanning electron microscopy (SEM), and 3) transmission electron microscopy (TEM). Images were obtained using equipment located in the College of Veterinary Medicine & Biomedical Sciences (1) and Microscopy and Imaging Group (2), both located at Texas A&M University, College Station, TX, USA, and Department of Life Sciences, University of Siena, Siena, Italy (3). Five males and five females were examined per procedure.

Light and fluorescence microscopy (LFM). The gross morphology of the reproductive tract of 2-d-old males was assessed under light microscopy. Preliminary studies using TEM indicated 2-d-old males had sperm present in all stages of development. A binocular microscope was used to remove the reproductive tract, which was then placed in a petri dish containing 3 ml of Dulbecco’s Phosphate Buffered Saline solution (Sigma-Aldrich™, St. Louis, MO, USA) where it was photographed using Leica Microsystems software combined with a binocular microscope attached to a digital camera (Leica DFC480, Leica Microsystems™, Wetzlar, Germany). To assess spermatogenesis as well as the impact of senescence on this species, the right testis from cryptocephalic pupae identified in phase II up to pharate adult in phase VII (Barros-Cordeiro et al. 2014, Li et al. 2016) and adults in seven age groups (< 24 h, 2, 3, 4, 5, 6, and 7-d-old) were removed and fixed with Davidson's fixative (Bell and Lightner 1988). Samples were infiltrated with paraffin embedded and sectioned at a thickness of 4–5 µm. Slices were stained with hematoxylin and eosin. Each section was examined with an interference-contrast microscopy Zeiss Axioplan 2 (Type 00-24-473-0000, Carl Zeiss™) set at three different magnifications: 40x, 200x, and 1000x. Photographs were taken with a Zeiss AxioCam MRc5 digital camera.

Scanning Electron Microscopy (SEM). Using a light microscope, testes were removed from 2-d-old males (n = 6) and placed in labeled (i.e., male – sample1) Eppendorf tubes containing 30 µL Dulbecco’s Phosphate Buffered Saline solution. In lower Diptera, the seminal vesicles are typically not evident (Matsuda 1976, Hunter and Birkhead 2002); therefore, testes, and the vasa deferentia were isolated for examination. Dissecting needles were used to cut the tissue and release the sperm. Approximately, 3 µL of the free sperm bundles were collected from the Eppendorf and spread onto coverslips previously treated with Poly-L-lysine solution 0.1% (w/v) (p8920, Sigma-Aldrich™). For females, spermathecae were removed from 3-d-old females (n = 6) and placed, separately, in labeled (i.e., female – sample1) Eppendorf tubes containing 30 µL of Dulbecco’s Phosphate Buffered Saline solution. The spermathecae samples were removed from each Eppendorf tube, placed on round coverslips (10mm), and immediately immersed in 2.5% glutaraldehyde in phosphate buffer at pH 7.4 and 4°C to be fixed overnight. Samples were then rinsed with phosphaste buffer at pH = 7.4 and processed through a dehydration series (30%, 50%, 75%, 90%, and 100% acetone) (adapted from (McDowell and Trump 1976)). Following dehydration, both coverslips and spermatheca were immersed in two concentrations of hexamethyldisilazane (HMDS) solution (100% HMDS + 100% ethanol; 1:2, 2:1) for 10 min each, followed by one application of absolute HMDS and then air-dried at room temperature under a ventilation hood for 1h (Adapted from Shively and Miller, 2009). The coverslips were gently attached to aluminum stubs using double-stick tape and coated with 15 nm of Iridium using a Cressington 208HR sputter coater (Systems Inc., Las Vegas, NV, USA) connected to an Argon gas cylinder supply. The sample was then before viewed with a FEI Quanta 600 FE-SEM (JEOL, MA, USA) scan electron microscope.

Transmission electron microscopy (TEM). Using the dissection methods previously described, the right testis from 2-d-old males (n = 5) was removed and placed in labeled (i.e., male – sample1) Eppendorf tubes containing 30 μL Dulbecco’s Phosphate Buffered Saline solution. The testis samples were removed from each Eppendorf tube, placed in labeled petri dishes, and fixed overnight at 4°C in 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH = 7.2). Tissues then were washed in 0.1M phosphate buffer, post fixed in 1% osmium tetroxide in 0.1 M phosphate buffer for 90 min at room temperature, dehydrated in five ethanol concentrations (10%, 30%, 50%, 70%, 90%, 100%) (Dallai and Afzelius 1990) and embedded in Epon 812 resin. Dallai and Afzelius (1990) reported this fixation and post-fixation method efficiently preserved proteinaceous structures. Ultrathin (silver–gold) sections were cut using a diatome diamond knife at a

Reichert Ultracut II E ultramicrotome (Leica Microsystems™, Wetzlar, Germany) and collect on 300 mesh copper grids before staining with uranyl acetate and lead citrate (Adapted from Dykstra (1993)). Sections were examined and photographed with a Philips CM10 (Philips Electron Optics™, Eindhoven, The Netherlands) electron microscope operating at 80 kV.

2.3. Results

Gross morphology of the male reproductive tract and female spermatheca. The male reproductive system consists of a pair of tubular-shape testes (size (right one): 3.9–7.6 mm). They are linked to a pair of vasa deferentia, which is then connected to a well-developed tube-like accessory gland (Fig.1A). The accessory gland enters at the anterior apex of the ejaculatory duct, which extends to the sperm pump (Fig.1B). The seminal vesicle was not present. The sperm pump consists of a rigid disc with small muscles associated and an ejaculatory apodeme (Fig.1B and C). The ejaculatory duct enters in the diverticulum at the junction of the apodeme and continuing through the hypandrium and epandrium where meet a narrow phallotrema from where the sperm are expelled inside of the female reproductive tract during copula (Fig.1C). The hypandrium and epandrium are separate (Fig.1C). The reproductive system occupies mostly the IV-V abdominal segments and is surrounded by a well-developed body fat. Sperm storage by BSF females (spermatheca) consists of three individual capsules with size ~ 200µm (195-234 µm), each one of them connected to a capsular duct. At the end of each capsular duct, valves attach to an ejector ducts that lead to a common larger duct inserted between the furca arms, which opens to the bursa copulatrix to receive the sperm during copula (Fig.2A). Ejection ducts are divided in two sections: proximal and distal. The ejection ducts are bulbous at their base and covered with glandular tissue. They gradually widened, reaching their maximum calibers in the median region (Fig.2A). At the distal portions, the tubes are covered with glandular cells, joining the proximal portions of the valves. The valves are bent in the form of an elbow, with basal sclerotization and glandular filaments emerging from the distal portions, connecting to the capsular ductal bases. The capsular ducts are thin, long, and slightly wider at the distal with heavily sclerotized walls (Fig.2A). The capsules are semi-spherical in shape, with the surface slightly grooved (Fig.2B).

Spermatozoon and its ultrastructure. The spermatozoon tail (flagellum) of BSF is long, motile, slender and filiform, measuring ~860 µm in total length (Fig. 3A). The mature sperm head has an elliptical shape, measuring ~8 µm (Fig. 3B). From the SEM micrographs, it seems the sperm display a bending wave propagation pattern, movement associated with motile sperm (Fig. 3C). The TEM micrographs cross sections revealed an ultrastructure configuration with an elliptical nucleus (0.4 x 0.35 µm) displaying two lateral infoldings of the membrane envelope (Fig. 4A). The basal body is hosted in a nuclear cavity and is characterized by an axoneme with 9 triplets, with each triplet composed of a microtubule doublet connected to an incomplete microtubule (C-tubule) which will become the accessory tubule (Fig. 4B). Moreover, this basal body is embedded, at this level, in a scanty centriole adjunct material (Fig. 4B). Further behind in the centriolar region, the nucleus progressively reduces its size (Fig. 4C and D) and, the 9 + 9 + 2 axoneme is flanked by the centriole adjunct material that shows areas of different densities (Fig. 4C, D, F), two of which merging together will give rise to a u-shaped structure (Fig. 4F). Immediately below, in a very short area of the flagellum, a dense structure of microtubules is observed and small crystallized mitochondrial derivatives appear (Fig. 4E and G). Lateral two thick dense laminae layers (~1.0 μm thick) progressively extend all around the periphery of the sperm flagellum giving rise to two peculiar wing-like structures filled with dense material, with the two mitochondrial derivatives displaying an increase in their size (Fig. 4E, H, I). All these materials could be part of an expanded centriole adjunct. This particular wing-like pattern of the centriole adjunct must be of short length as it is not easy to find among other sections. Black soldier fly sperm display two elliptical mitochondrial derivatives that become smaller at sperm tail end (Fig. 4J).

Spermatogenesis and the impact of aging. Overall, the spermatogenesis process takes place inside of the testis, from the apical part (germ line - hub), where it is possible to distinguish spherical cells (spermatogonia), to the basal part, in which spermatids may be observed in their last stage of maturation (Fig. 5A-H). Each germ line cell goes through incomplete mitotic divisions to form the spermatogonia cells, which progress through four mitotic divisions surrounded by somatic cyst cells, yielding 16 early spermatocytes (Fig. 5A-C). The spermatocyte cells undergo two more mitotic divisions, resulting in 64 early spermatids. All mitotic divisions occur in a synchronized pattern, where each cyst contains undeveloped sperm cells at the same stage of development (Fig. 5C and D). They progressively moved away from the tip, where cysts with primary spermatocytes move down and entering in meiosis, and at the end of the two incomplete meiotic divisions, the maturation and consequently differentiation phase started (Fig. 5D and H). During the differentiation phase, the sperm cells were found in bundles inside of the cyst cells (Fig. 5E, F and H). Moreover, spermatogenesis in this species potentially is initiated during the early stages of larval development. In the current study, the testicular tubules of cryptocephalic and phanerocephalic (phase II) pupae were full of spermatocyte cyst cells in mitosis; however, no mature cells (spermatids) were observed (Fig. 5A and B). While not quantified in the current study, age appears to impact sperm production with younger flies producing more cells than older flies (Fig. 5A-H).

2.4. Discussion

Gross morphology of the male reproductive tract and female spermatheca. We provide a morphological description BSF reproductive tract, size and ultrastructure of their spermatozoon and spermatogenesis of aging male, as well as the female sperm storage morphology. Overall, the reproductive system of brachyceran males described thus far generally consists of one pair of oval or tubular-shaped testes, with each one containing a series of testicular tubes or follicles in which spermatozoa are produced, a paired vasa differentia, also called vas deferens, the lack of an evident seminal vesicle structure (Matsuda 1976, Hunter and Birkhead 2002), a tube-like and long paired accessory glands, a long and slender ejaculatory duct starting from the junction of the accessory glands, and vasa deferentia that extends to the sperm pump, a structure with muscular tissue found inside a parameral sheath and lying free in base of phallus exposed on a concave aedeagal sclerite. The BSF males presented all of these components in their reproductive tract commonly found in other Brachycera and displayed similar morphological description with a testicular shape and sperm pump configuration commonly found in the Stratiomyomorpha (Matsuda 1976, Hunter and Birkhead 2002).

The BSF females possessed three semi spheroid-shaped spermatheca capsules, corroborating with the previous description made by Ururahy-Rodrigues and Pujol–Luz (2000). Moreover, the arrangement of capsules, capsular ducts, valve, ejector ducts, and furca arms are common for several species of the Hermetiinae (Godoi 2006). Interestingly, the size of the spermatheca is four times smaller comparing with the flagellum (~200 µm and ∼860 µm, respectively). A similar pattern is observed in the ground louse Zorotypus impolitus (Insecta: Zoraptera) regarding to the size of the spermatheca and spermatozoon (i.e., 1mm and 3 mm, respectively) (Dallai et al. 2014). It is assumed that sperm with large size should more efficiently block the female spermatheca entrance, thus preventing the storage of new ejaculates (Simmons and Fitzpatrick 2012).

Additionally, Walker (1980), have described a positive correlation between species with spheroid spermatheca and being monogamic (i.e., one single male/insemination), contrasting with species presenting elongate or tubular spermatheca and being polyandric (Ridley 1989, Pitnick et al. 2009). Recently, Giunti et al. (2018) described BSF females as monogamist since multiple mating attempts were not observed by mated females in the presence of virgin males. Tomberlin and Sheppard (2001) observed a wild population where females usually left the lek arena once mating was terminated. No data were reported for wild or laboratorial populations indicating if females would copulate more than once with the same male.

Spermatozoon and spermatogenesis. With respect to male germ cells, the individuals BSF apparently invest considerably in spermatozoa as the gametes have relatively long flagella compared to other Diptera (Dallai 2014). Some examples of male investment in sperm production can be found in Polypedilum nubeculosum (M.) (Diptera: Chironomidae) that have flagella measuring 130 μm (Dallai et al. 2007) and the muscoid dung fly species, Scathophaga stercoraria (L.) (Diptera: Scathophagidae) with flagella measuring 201μm (Sharma et al. 2013). Parker (1990), using the "raffle principle", have demonstrated that for internal fertilizations, the probability of a male to sire an offspring increases with its total number and associated cost of sperm produced; therefore, sperm size must be small (Parker 1982, Parker 1990, Parker and Pizzari 2010). However, positive correlations between spermatozoon length and sperm competition have been documented for various taxa (LaMunyon and Samuel 1999, Lattao et al. 2012) indicating, under certain circumstances, exaggerated sperm length is a competitive advantage (Snook 2005, Pitnick et al. 2009, Immler et al. 2011, Dallai et al. 2014). Lüpold et al. (2016) revolutionized the undestanding of post-copulatory sexual selection, showing that male Drosophila melanogaster (M.) (Diptera: Drosophiladae) tend to invest in producing long sperm cells since those enhace their capability of siring a majority of progeny produced by a female.

Regarding the ultrastructure of BSF sperm identified in the current study, most of the structures described for other Brachycera have been conserved (Dallai et al. 2016a, Dallai 2018). The acrosome was not found in the cross sections. Therefore, it is suspected to be very short and may consist of a simple apical acrosome vesicle. The 9+2+2 axoneme arrangement is similar to the ones described for D. melanogaster and Bactrocera oleae (= Dacus oleae) (Diptera: Tephritidae) (Dallai and Afzelius 1991). However, while BSF presented a 9 triplet formation, members of Drosophila display a 9 microtubule doublet connected to a single microtubule ring (Greenan et al. 2018). While 13 protofilaments are conserved in all Brachycera groups described (Dallai et al. 1993, Dallai 2018), the axoneme triplet pattern seem to be symplesiomorphic (i.e., ancestor) features since they are also observed in Tipulidae germ cells (Jamieson 1987, Dallai et al. 2008, Dallai et al. 2016a).

The outer microtubule (C-tubule) presented in BSF basal body is incomplete and will further become the accessory tubule in the flagellum. The mitochondria derivatives of this species displayed a small crystallization, feature also presented in Tipulidae (Jamieson 1987). Moreover, the large mitochondria derivatives found in this species, being observed even at the sperm tail end, were also described for D. melanogaster, where those may perform a secondary function related to the sperm elongation process by providing a structural platform for microtubule reorganization that would supports the strong elongation taking place at the tip of the long sperm tail (Noguchi et al. 2011).

Furthermore, BSF sperm ultrastructure displays some peculiar features. In the anterior cross-sections, a peculiar U-shaped structure, formed by the centriole adjunct material is the most peculiar feature of the sperm. Although not commonly found in other dipterans, this same structure was described in cross-sections of Megaselia scalaris (Loew) (Diptera: Phoridae) sperm flagellum (Curtis et al. 1989). In posterior cross-sections, this region beneath the nucleus presented a denser centriole adjunct surrounding the axoneme, forming a wing-like structure lateral to it. Dallai et al. (2008) described a similar structure for a Chionea sp. (Diptera: Limoniidae). The centriole adjunct found surrounding the basal body and the two mitochondrial derivatives may reinforce the junction between the sperm head and the flagellum, including the two mitochondrial derivatives, allowing a better integration of the sperm components, making the sperm movement more uniform and stable (Dallai et al. 2016b, Avidor-Reiss 2018).

The spermatogenesis process presented incomplete cytokinesis phenomenon, which is common in insects (Dumser 1980). It is characterized by the development of germ cells in a synchronized pattern, where inside of each cyst the undeveloped spermatozoa are found at the same stage of development. The hub area consisted of stem cells, similar to D. melanogaster (Demarco et al. 2014), and are present from early pupal phase (cryptocephalic pupae) until the oldest age assessed in this study (7-d-old males), with primary spermatogonium and secondary spermatogonia cell presenting two and 16 (8-16 cells) cells per cyst cell, respectively. Based on these data, BSF virgin males are capable of produce viable sperm cells (Malawey, A.S. unpublish data) late in life, however in reduced amounts. However, the mechanism involved in the maintenance of spermatogenesis in virgin BSF males is unknown. In D. melanogaster, the elevated expression of a microRNA is correlated to senescence and it down regulates molecules linked with the production of stem cells (Epstein et al. 2017). In addition, whether mated males still produce sperm remains in question.

Due to the importance of the BSF for recycling organic wastes to mass produce protein for use as animal feed, developing reliable mass-production techniques is required. Current colony methods have not evolved much from those initially described (Sheppard et al. 2002). Adults emerge and are placed in cages. Overtime, different age classes of adults are present in the cage. However, the impact of such an approach on the production of fertile eggs is not fully understood.

Data presented in this manuscript provide greater insight as to the potential mechanisms partially responsible for variability in fertile egg production encountered when rearing BSF in colony. More specifically, males appear to possess functional sperm at the time of emergence; however, the ability to produce viable sperm decrease over the lifespan of the fly. Consequently, older adult males persisting in a colony potentially are not capable of providing sufficient quantities of sperm to fertilize all eggs in a female; however, additional research is needed to verify if true before recommending shifts in the current methods (e.g., Sheppard et al. 2002) employed.

2.5. References

AAFCO. 2016. Annual meeting agenda and committee reports; Association of american feed control officials, p.112. Pittsburgh, PA, USA.

AAFCO. 2019. Annual meeting agenda and committee reports; Association of american feed control officials, p.42. Savannah, GA, USA.

Avidor-Reiss, T. 2018. Rapid evolution of sperm produces diverse centriole structures that reveal the most rudimentary structure needed for function. Cells 7: 67.

Barros-Cordeiro, K. B., S. N. Báo, and J. R. Pujol-Luz. 2014. Intra-puparial development of the black soldier-fly, *Hermetia illucens*. J. Insect Sci. 14: 1-10.

Bell, T. A., and D. V. Lightner. 1988. A Handbook of Normal Penaeid Shrimp Histology. World Aquaculture Society, Baton Rouge, CA.

Beskin, K. V., C. D. Holcomb, J. A. Cammack, T. L. Crippen, A. H. Knap, S. T. Sweet, and J. K. Tomberlin. 2018. Larval digestion of different manure types by the black soldier fly (Diptera: Stratiomyidae) impacts associated volatile emissions. Waste Management 74: 213-220.

Birkhead, T. R., and A. Møller. 1998. Sperm Competition. Academic Press, New York, US.

Boivin, G. 2013. Sperm as a limiting factor in mating success in Hymenoptera parasitoids. Entomol. Exp. Appl. 146: 149-155.

Boivin, G., and J. Ellers. 2016. Replacing qualitative life-history traits by quantitative indices in parasitoid evolutionary ecology. Entomol. Exp. Appl. 159: 163-171.

Boivin, G., S. Jacob, and D. Damiens. 2005. Spermatogeny as a life-history index in parasitoid wasps. Oecologia 143: 198-202.

Boulton, R. A., L. A. Collins, and D. M. Shuker. 2015. Beyond sex allocation: The role of mating systems in sexual selection in parasitoid wasps. Biol. Rev. 90: 599-627.

Cai, M., S. Ma, R. Hu, J. K. Tomberlin, C. Yu, Y. Huang, S. Zhan, W. Li, L. Zheng, and Z. Yu. 2018. Systematic characterization and proposed pathway of tetracycline degradation in solid waste treatment by *Hermetia illucens* with intestinal microbiota. Environ. Pollut. 242: 634-642.

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

Curtis, S. K., D. B. Benner, and G. Musil. 1989. Ultrastructure of the spermatozoon of *Megaselia scalaris* Loew (Diptera: Phoridae). J. Morphol. 200: 47-61.

Dallai, R. 2014. Overview on spermatogenesis and sperm structure of Hexapoda. Arthropod. Struct. Dev. 43: 257-290.

Dallai, R. 2018. The Insect Spermatozoon, pp. 321-329. *In* M.K. Skinner (ed.), Encyclopedia of Reproduction, 2nd ed. Academic Press, Oxford, UK.

Dallai, R., and B. A. Afzelius. 1990. Microtubular diversity in insect spermatozoa: results obtained with a new fixative. J. Struct. Biol. 103: 164-179.

Dallai, R., and B. A. Afzelius. 1991. Sperm flagellum of *Dacus oleae* (Gmelin) (Tephritidae) and *Drosophila melanogaster* Meigen (Drosophilidae) (Diptera). Int. J. Insect Morphol. Embryol. 20: 215-222.

Dallai, R., B. M. Lombardo, and P. Lupetti. 2007. Sperm ultrastructure in Chironomoidea (Insecta, Diptera). Tissue and Cell 39: 179-194.

Dallai, R., B. M. Lombardo, D. Mercati, S. Vanin, and P. Lupetti. 2008. Sperm structure of Limoniidae and their phylogenetic relationship with Tipulidae (Diptera, Nematocera). Arthropod. Struct. Dev. 37: 81-92.

Dallai, R., M. Gottardo, and R. G. Beutel. 2016a. Structure and evolution of insect sperm: New interpretations in the age of phylogenomics. Annu. Rev. Entomol. 61: 1-23.

Dallai, R., F. Paoli, D. Mercati, and P. Lupetti. 2016b. The centriole adjunct of insects: Need to update the definition. Tissue & cell 48: 104-113.

Dallai, R., M. Gottardo, D. Mercati, R. Machida, Y. Mashimo, Y. Matsumura, and R. G. Beutel. 2014. Giant spermatozoa and a huge spermatheca: A case of coevolution of male and female reproductive organs in the ground louse *Zorotypus impolitus* (Insecta, Zoraptera). Arthropod. Struct. Dev. 43: 135-151.

Dallai, R., P. L. Bellon, S. L. Vecchia, and B. A. Afzelius. 1993. The dipteran sperm tail: Ultrastructural characteristics and phylogenetic considerations. Zoologica Scripta 22: 193-202.

Dávila, F., and S. Aron. 2017. Protein restriction affects sperm number but not sperm viability in male ants. J. Insect Physiol. 100: 71-76.

Demarco, R. S., Å. H. Eikenes, K. Haglund, and D. L. Jones. 2014. Investigating spermatogenesis in *Drosophila melanogaster*. Methods (San Diego, Calif.) 68: 218-227.

Diener, S., C. Zurbrügg, and K. Tockner. 2015. Bioaccumulation of heavy metals in the black soldier fly, *Hermetia illucens* and effects on its life cycle. J. Insects Food Feed 1: 261-270.

Dumser, J. B. 1980. The regulation of spermatogenesis in insects. Annu. Rev. Entomol. 25: 341-369.

Dykstra, M. J. 1993. A Manual of Applied Techniques for Biological Electron Microscopy. Springer Science & Business Media, Plenum Press, New York.

Enciso, M., M. Iglesias, I. Galán, J. Sarasa, A. Gosálvez, and J. Gosálvez. 2011. The ability of sperm selection techniques to remove single-or double-strand DNA damage. Asian J. Androl. 13: 764.

Epstein, Y., N. Perry, M. Volin, M. Zohar-Fux, R. Braun, L. Porat-Kuperstein, and H. Toledano. 2017. MiR-9a modulates maintenance and ageing of *Drosophila* germline stem cells by limiting N-cadherin expression. Nature Communications 8: 600.

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

Friesen, C. R., E. J. Uhrig, E. J. Bentz, L. A. Blakemore, and R. T. Mason. 2017. Correlated evolution of sexually selected traits: Interspecific variation in ejaculates, sperm morphology, copulatory mate guarding, and body size in two sympatric species of garter snakes. Behav. Ecol. Sociobiol. 71: 180.

Giunti, G., O. Campolo, F. Laudani, and V. Palmeri. 2018. Male courtship behaviour and potential for female mate choice in the black soldier fly *Hermetia illucens* L. (Diptera: Stratiomyidae). Entomol. Gen. 38: 29-46.

Godoi, F. S. P. d. 2006. Morfologia da Espermateca e Forquilha Genital de Algumas Espécies das Subfamílias Neotropicais de Stratiomyidae (Insecta, Diptera). M.S. thesis, Federal University of Amazonas, Manaus.

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

Greenan, G., B. Keszthelyi, R. D. Vale, and D. A. Agard. 2018. Insights into centriole biogenesis and evolution revealed by cryoTomography of doublet and triplet centrioles. bioRxiv: 300525.

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

Higginson, D. M., K. B. Miller, K. A. Segraves, and S. Pitnick. 2012. Female reproductive tract form drives the evolution of complex sperm morphology. Proc. Natl. Acad. Sci. 109: 4538-4543.

Höglund, J., and R. V. Alatalo. 2014. Leks. Princeton University Press, New Jersey, US.

Holmes, L. A., S. L. VanLaerhoven, and J. K. Tomberlin. 2017. Photophase duration affects immature black soldier fly (Diptera: Stratiomyidae) development. Environ. Entomol. 46: 1439-1447.

Hunter, F. M., and T. R. Birkhead. 2002. Sperm viability and sperm competition in insects. Curr. Biol. 12: 121-123.

Ide, J., and M. Kondoh. 2000. Male–female evolutionary game on mate-locating behaviour and evolution of mating systems in insects. Ecol. Lett. 3: 433-440.

Immler, S., S. Pitnick, G. A. Parker, K. L. Durrant, S. Lüpold, S. Calhim, and T. R. Birkhead. 2011. Resolving variation in the reproductive tradeoff between sperm size and number. Proc. Natl. Acad. Sci. U.S.A. 108: 5325-5330.

Jamieson, B. G. M. 1987. The Ultrastructure and Phylogeny of Insect Spermatozoa. Cambridge University Press, UK.

Johnson, S. L., S. Zellhuber-McMillan, J. Gillum, J. Dunleavy, J. P. Evans, S. Nakagawa, and N. J. Gemmell. Year. 2018. Evidence that fertility trades off with early offspring fitness as males age. Proc. R. Soc. Lond. B. Biol. Sci. 20172174 (id).

Krasnec, M., C. Cook, and M. Breed. 2012. Mating systems in sexual animals. Nature Education Knowledge 3: 72.

Lalander, C. H., J. Fidjeland, S. Diener, S. Eriksson, and B. Vinnerås. 2015. High waste-to-biomass conversion and efficient *Salmonella* spp. reduction using black soldier fly for waste recycling. Agron. Sustain. Dev. 35: 261-271.

Lalander, C., S. Diener, C. Zurbrügg, and B. Vinnerås. 2019. Effects of feedstock on larval development and process efficiency in waste treatment with black soldier fly (*Hermetia illucens*). J. Clean Prod. 208: 211-219.

LaMunyon, C. W., and W. Samuel. 1999. Evolution of sperm size in nematodes: Sperm competition favours larger sperm. Proc. R. Soc. Lond. B. Biol. Sci. 266: 263-267.

Lattao, R., S. Bonaccorsi, and M. Gatti. 2012. Giant meiotic spindles in males from Drosophila species with giant sperm tails. J. Cell Sci. 125: 584-588.

Li, L., Y. Wang, and J. Wang. 2016. Intra-puparial development and age estimation of forensically important *Hermetia illucens* (L.). J. Asia. Pac. Entomol. 19: 233-237.

Liu, Q., J. K. Tomberlin, J. A. Brady, M. R. Sanford, and Z. Yu. 2008. Black soldier fly (Diptera: Stratiomyidae) larvae reduce *Escherichia coli* in dairy manure. Environ. Entomol. 37: 1525-1530.

Lüpold, S., M. K. Manier, N. Puniamoorthy, C. Schoff, W. T. Starmer, S. H. B. Luepold, J. M. Belote, and S. Pitnick. 2016. How sexual selection can drive the evolution of costly sperm ornamentation. Nature 533: 535.

Makkar, H. P. S., G. Tran, V. Heuzé, and P. Ankers. 2014. State-of-the-art on use of insects as animal feed. Anim. Feed. Sci. Tech. 197: 1-33.

Matsuda, R. 1976. Morphology and Evolution of the Insect Abdomen, with Special Reference to Developmental Patterns and Their Bearings upon Systematics. Pergamon Press Ltd., Oxford, UK.

Matthews, R. W., and J. R. Matthews. 2009. Insect Behavior. Springer Science & Business Media, Berlin, Germany.

McDowell, E. M., and B. F. Trump. 1976. Histologic fixatives suitable for diagnostic light and electron microscopy. Arch. Pathol. Lab. Med. 100: 405-414.

Myers, H. M., J. K. Tomberlin, B. D. Lambert, and D. Kattes. 2014. Development of black soldier fly (Diptera: Stratiomyidae) larvae fed dairy manure. Environ. Entomol. 37: 11-15.

Neff, B. D., and E. I. Svensson. 2013. Polyandry and alternative mating tactics. Proc. R. Soc. Lond. B. Biol. Sci. 368: 20120045-20120045.

Noguchi, T., M. Koizumi, and S. Hayashi. 2011. Sustained elongation of sperm tail promoted by local remodeling of giant mitochondria in *Drosophila*. Curr. Biol. 21: 805-814.

Oliveira, F., K. Doelle, and R. Smith. 2016. External morphology of *Hermetia illucens* Stratiomyidae: Diptera (L. 1758) based on electron microscopy. Annu. Res. Rev. Biol. 9: 10.

Parker, G. A. 1970. Sperm competition and its evolutionary consequences in the insects. Biol. Rev. 45: 525-567.

Parker, G. A. 1982. Why are there so many tiny sperm? Sperm competition and the maintenance of two sexes. J. Theor. Biol. 96: 281-294.

Parker, G. A. 1990. Sperm competition games: raffles and roles. Proc. R. Soc. Lond. B. Biol. Sci. 242: 120-126.

Parker, G. A., and T. Pizzari. 2010. Sperm competition and ejaculate economics. Biol. Rev. 85: 897-934.

Pitnick, S., M. F. Wolfner, and S. S. Suarez. 2009. 7 - Ejaculate–Female and Sperm–Female Interactions, pp. 247-304. *In* T. R. Birkhead, D. J. Hosken and S. Pitnick (eds.), Sperm Biology. Academic Press, London, UK.

Pizzari, T., R. Dean, A. Pacey, H. Moore, and M. B. Bonsall. 2008. The evolutionary ecology of pre-and post-meiotic sperm senescence. Trends Ecol. Evol. 23: 131-140.

Ridley, M. 1988. Mating frequency and fecundity in insects. Biol. Rev. 63: 509-549.

Ridley, M. 1989. The incidence of sperm displacement in insects: four conjectures, one corroboration. Biol. J. Linn. Soc. Lond. 38: 349-367.

Saldívar-Hernández, A., M. E. González-González, A. Sánchez-Tusié, I. Maldonado-Rosas, P. López, C. L. Treviño, F. Larrea, and M. Chirinos. 2015. Human sperm degradation of zona pellucida proteins contributes to fertilization. Reprod. Biol. Endocrinol. 13: 99.

Schiavone, A., M. De Marco, S. Martínez, S. Dabbou, M. Renna, J. Madrid, F. Hernandez, L. Rotolo, P. Costa, and F. Gai. 2017. Nutritional value of a partially defatted and a highly defatted black soldier fly larvae (*Hermetia illucens*

L.) meal for broiler chickens: apparent nutrient digestibility, apparent metabolizable energy and apparent ileal amino acid digestibility. J. Anim. Sci. 8: 51.

Sen, P., P. P. Shah, R. Nativio, and S. L. Berger. 2016. Epigenetic mechanisms of longevity and aging. Cell 166: 822-839.

Sharma, M., A. Minder, and D. Hosken. 2013. No association between sperm competition and sperm length variation across dung flies (Scathophagidae). J. Evol. Biol. 26: 2341-2349.

Sheppard, C. 1983. House fly and lesser fly control utilizing the black soldier fly in manure management systems for caged laying hens. Environ. Entomol. 12: 1439-1442.

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

Sheppard, D., G. Newton, J. Davis, G. Gascho, S. Thompson, S. Savage, and K. Bramwell. 1998. Using soldier flies as a manure management tool for volume reduction, house fly control and feedstuff production (AS 93-9 and LS 93-5). Southern Regional SARE Program, Georgia, US.

Simmons, L. W., and J. L. Fitzpatrick. 2012. Sperm wars and the evolution of male fertility. Reproduction 144: 519-534.

Snook, R. R. 2005. Sperm in competition: Not playing by the numbers. Trends. Ecol. Evol. 20: 46-53.

Tinghitella, R. M., A. C. Lackey, M. Martin, P. D. Dijkstra, J. P. Drury, R. Heathcote, J. Keagy, E. S. Scordato, A. M. Tyers, and L. Simmons. 2017. On the role of male competition in speciation: a review and research agenda. Behav. Ecol. 29: 783-797.

Tomberlin, J. K., and D. C. Sheppard. 2001. Lekking behavior of the black soldier fly (Diptera: Stratiomyidae). Fla. Entomol. 84: 729.

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

Tomberlin, J. K., P. H. Adler, and H. M. Myers. 2009. Development of the black soldier fly (Diptera: Stratiomyidae) in relation to temperature. Environ. Entomol. 38: 930-934.

Ururahy-Rodrigues, A., and J. R. Pujol–Luz. 2000. Notas sobre a espermateca de *Hermetia illucens* (l., 1758) (Diptera, Stratiomyidae). Ururahy-Rodrigues, A., and J. R. Pujol–Luz. 2000. Contribuições avulsas sobre a história natural do brasil, Série Zoologia. Seropédica, BR 17: 1-5.

Velando, A., J. Noguera, H. Drummond, and R. Torres. 2011. Senescent males carry premutagenic lesions in sperm. J. Evol. Biol. 24: 693-697.

Walker, W. F. 1980. Sperm utilization strategies in nonsocial insects. Am. Nat. 115: 780-799.

Wang, Y.-S., and M. Shelomi. 2017. Review of black soldier fly (*Hermetia illucens*) as animal feed and human food. Foods 6: 91.

Wu, Y.-F., L.-S. Wei, M. Anthony Torres, X. Zhang, S.-P. Wu, and H. Chen. 2017. Morphology of the male reproductive system and spermiogenesis of *Dendroctonus armandi* Tsai and Li (Coleoptera: Curculionidae: Scolytinae). J. Insect. Sci. 17: 20.

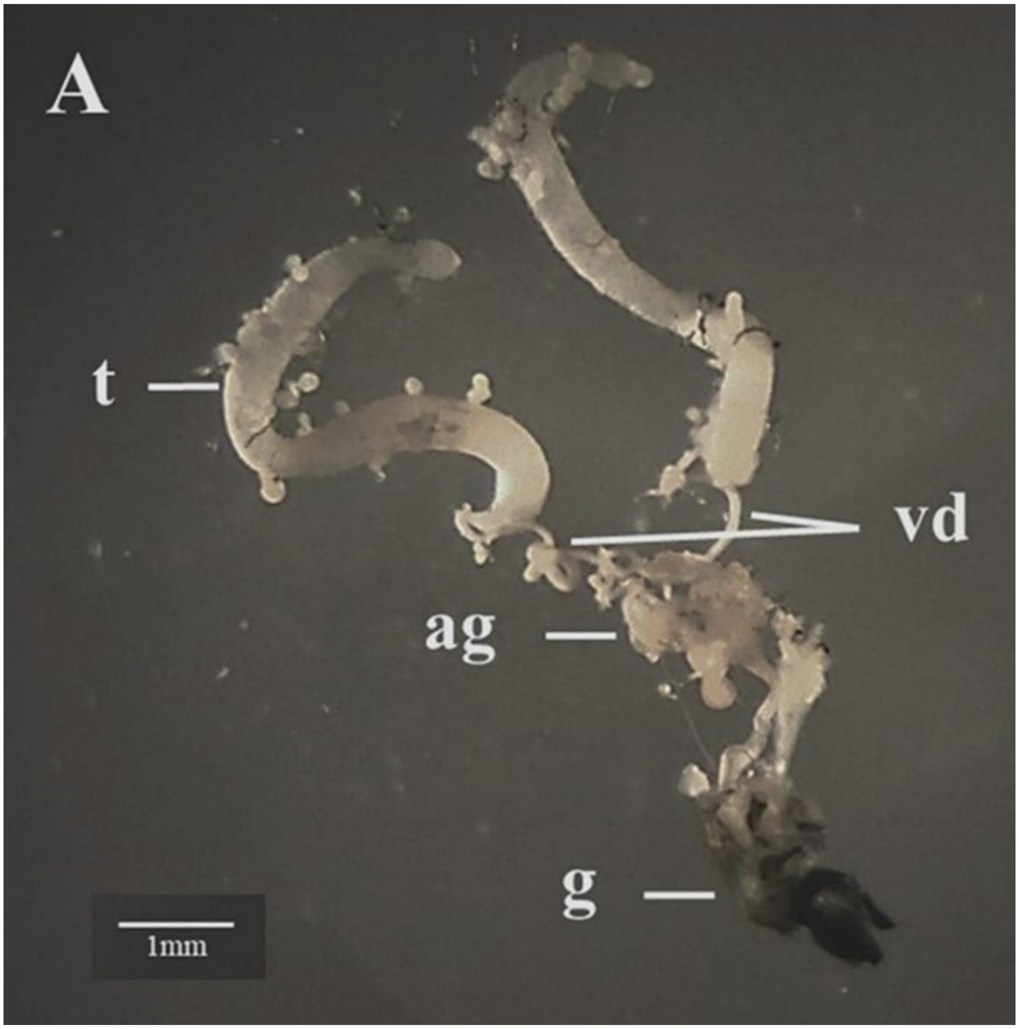

Figure 2-1 A. Light microscopy of the male reproductive tract of *H. illucens*. General composition of the male reproductive organs: Two tubular-shape testis and vasa deferentia, a tube-like accessory gland and a common ejaculatory duct connecting to the male genitalia (aedeagal complex). Abbreviations: t, testes; vd, vasa deferentia; ag, accessory gland; g, genitalia; sp, sperm pump; epm, epandrium; hyp, hypandrium; gsty, gonostylus; ac, aedeagal complex.

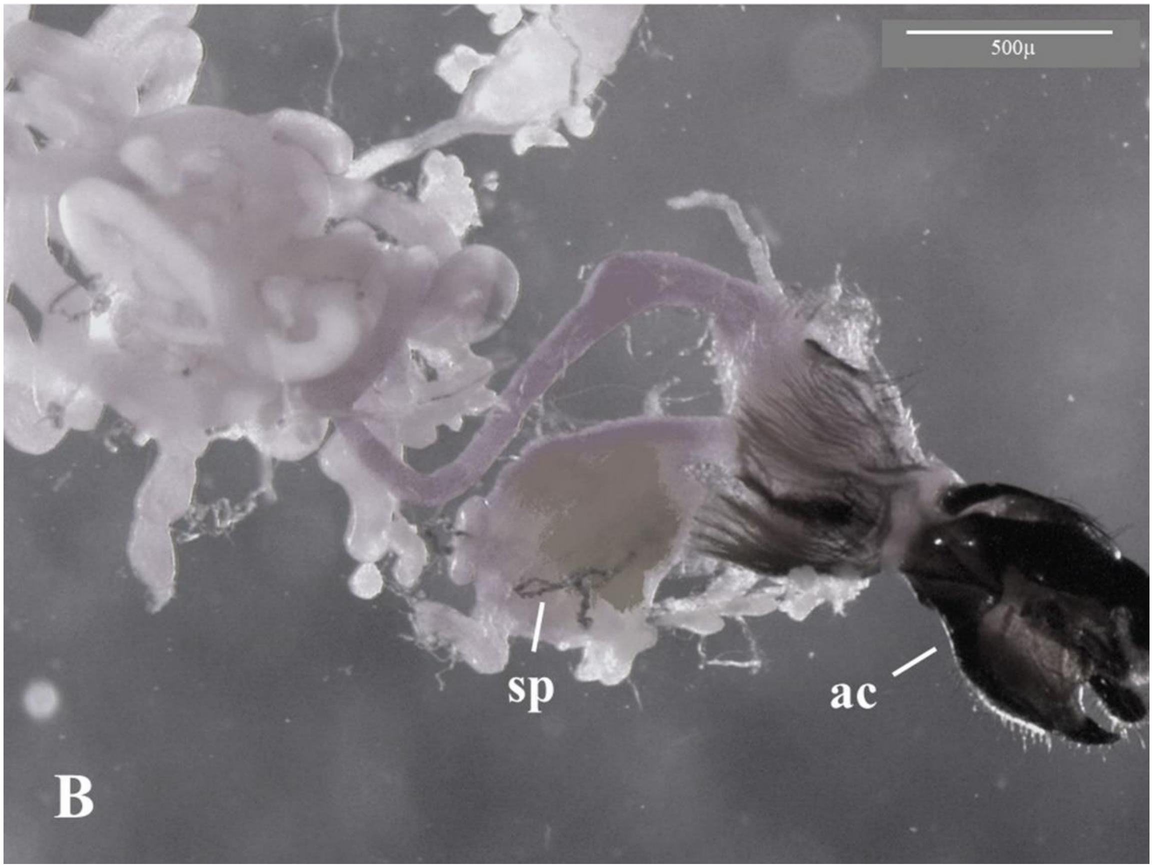

Figure 2-1 B. Light microscopy of the male reproductive tract of *H. illucens*. The sperm pump and aedeagal complex. Abbreviations: t, testes; vd, vasa deferentia; ag, accessory gland; g, genitalia; sp, sperm pump; epm, epandrium; hyp, hypandrium; gsty, gonostylus; ac, aedeagal complex.

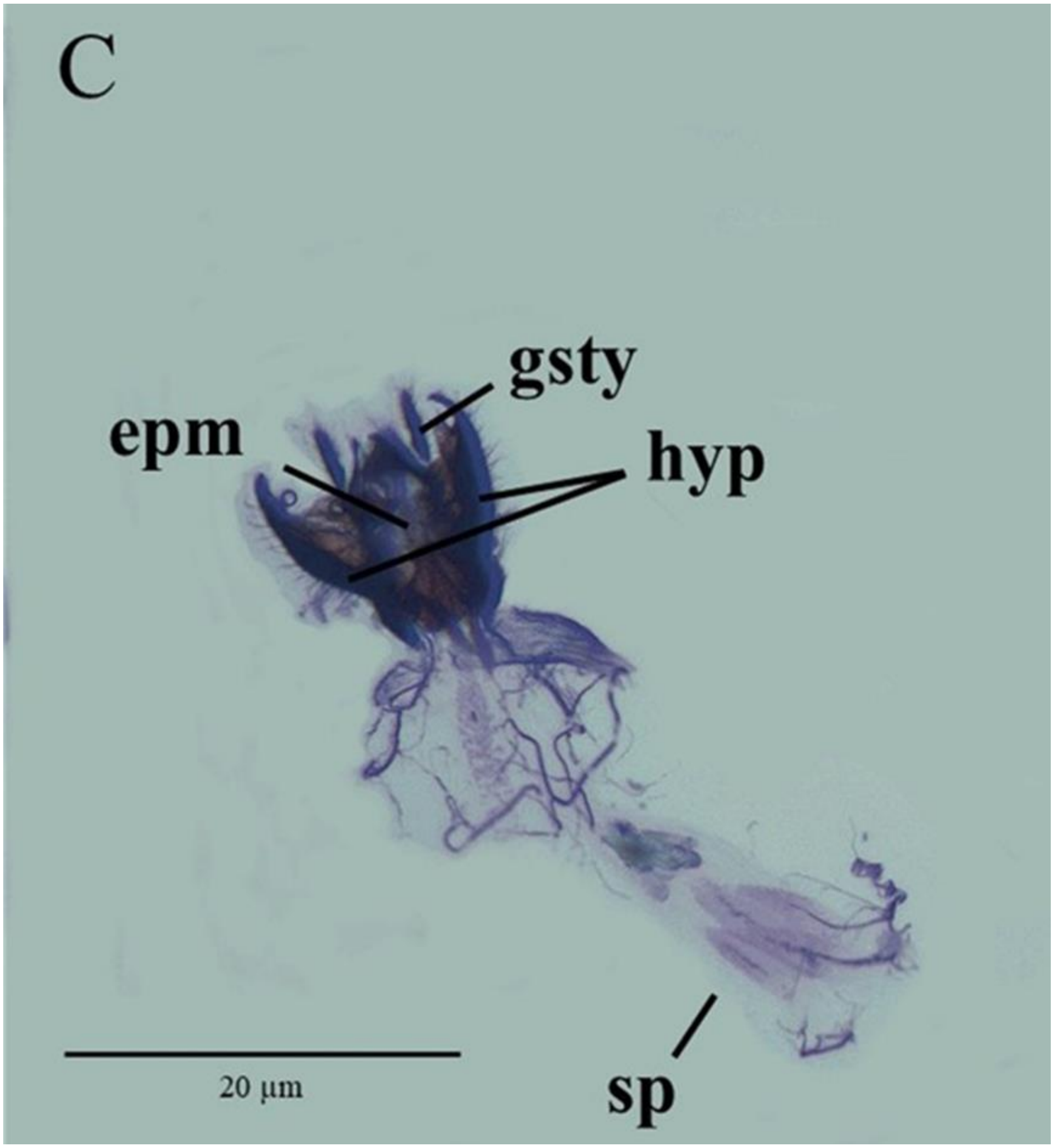

Figure 2-1 C. Light microscopy of the male reproductive tract of *H. illucens*.The aedeagal complex (hypandrium + epandrium) and the sperm pump showing small muscles associated with it. Note the evident separation of the epandrium and hypandrium structures. Abbreviations: t, testes; vd, vasa deferentia; ag, accessory gland; g, genitalia; sp, sperm pump; epm, epandrium; hyp, hypandrium; gsty, gonostylus; ac, aedeagal complex.

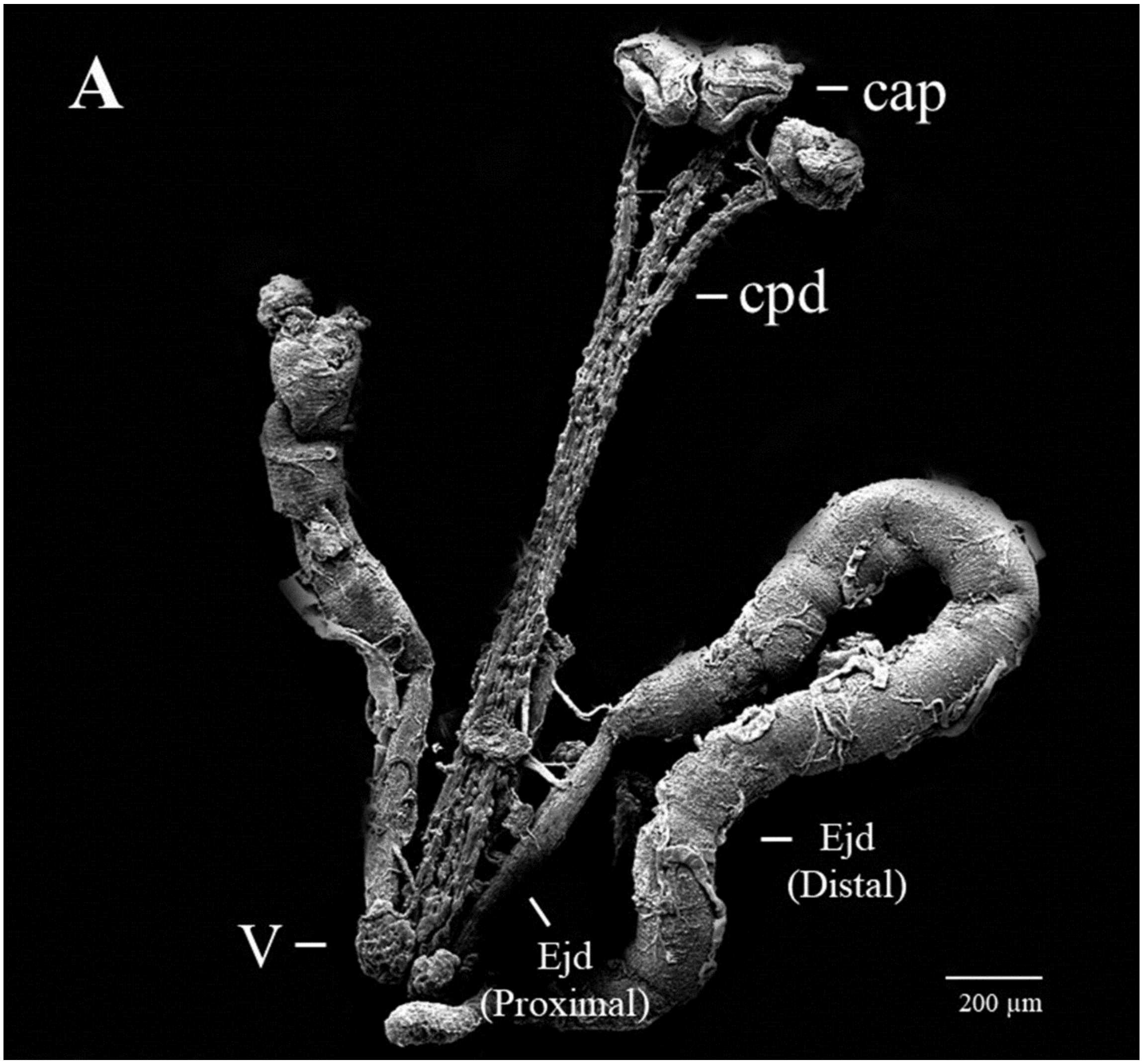

Figure 2-2 A. SEM side image of the female sperm storage (spermatheca) of *H. illucens*. The three individual capsules, each one of them connected to a capsular duct. Note the valve at the base of each capsular duct attaching to an ejector ducts that lead to a common larger duct inserted between the furca arms (not included in the image). Abbreviations: cap, capsule; cpd, capsular duct; Ejd, ejector duct; V, valve.

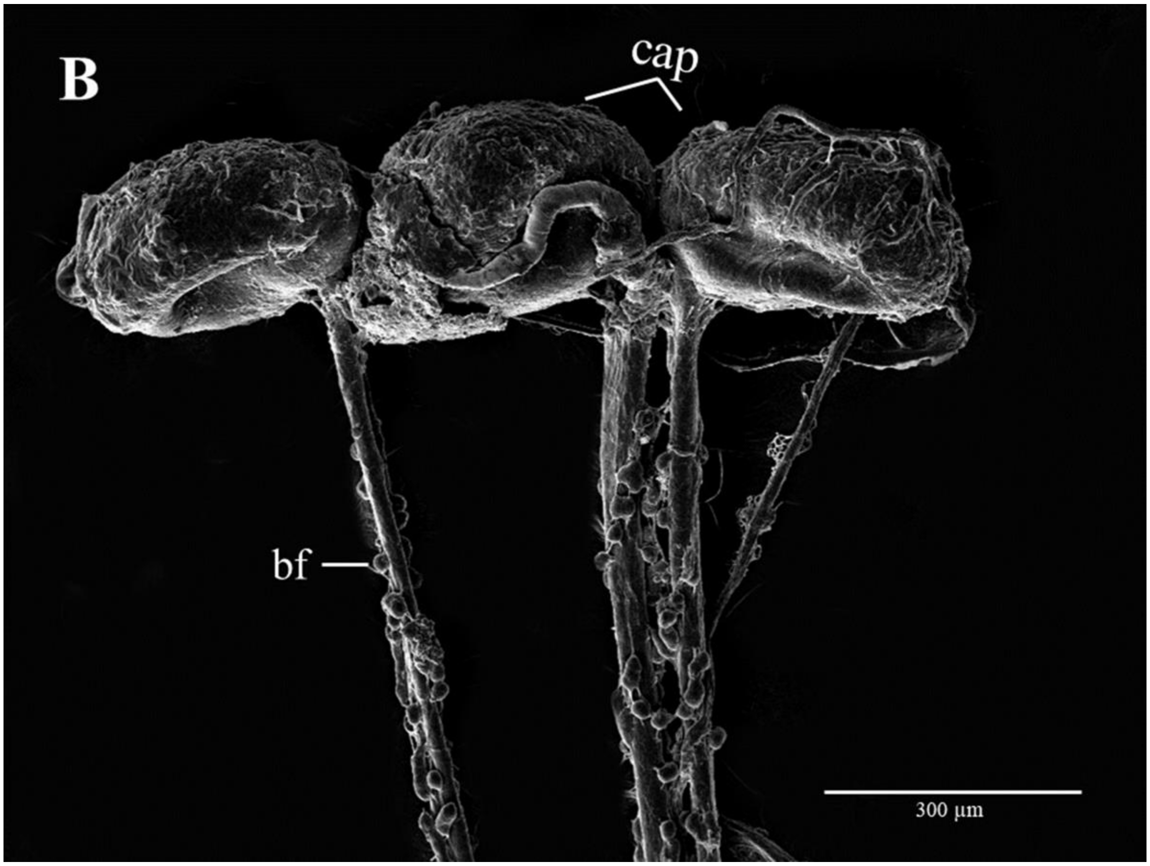

Figure 2-2 B. SEM side image of the female sperm storage (spermatheca) of *H. illucens*. The three individual capsules, each one of them connected to a capsular duct. Note the valve at the base of each capsular duct attaching to an ejector ducts that lead to a common larger duct inserted between the furca arms (not included in the image). Abbreviations: cap, capsule; cpd, capsular duct; Ejd, ejector duct; V, valve.

Figure 2-2 C SEM side image of the female sperm storage (spermatheca) of *H. illucens*. Detailed image of the semi-spherical spermatheca capsules with the surface slightly grooved. Abbreviations: cap, capsule; cpd, capsular duct; Ejd, ejector duct; V, valve.

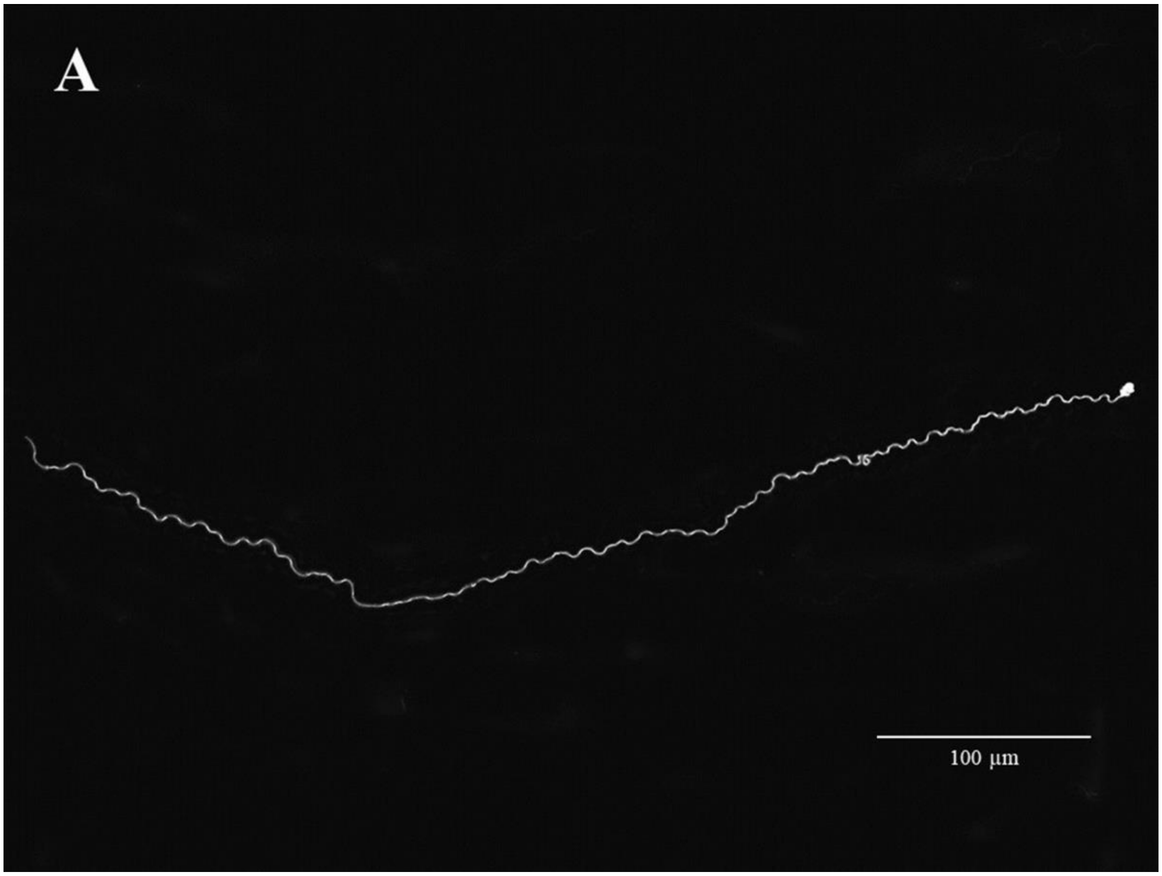

Figure 2-3 A. SEM image of the spermatozoon of *H. illucens*. The total length of the spermatozoon.

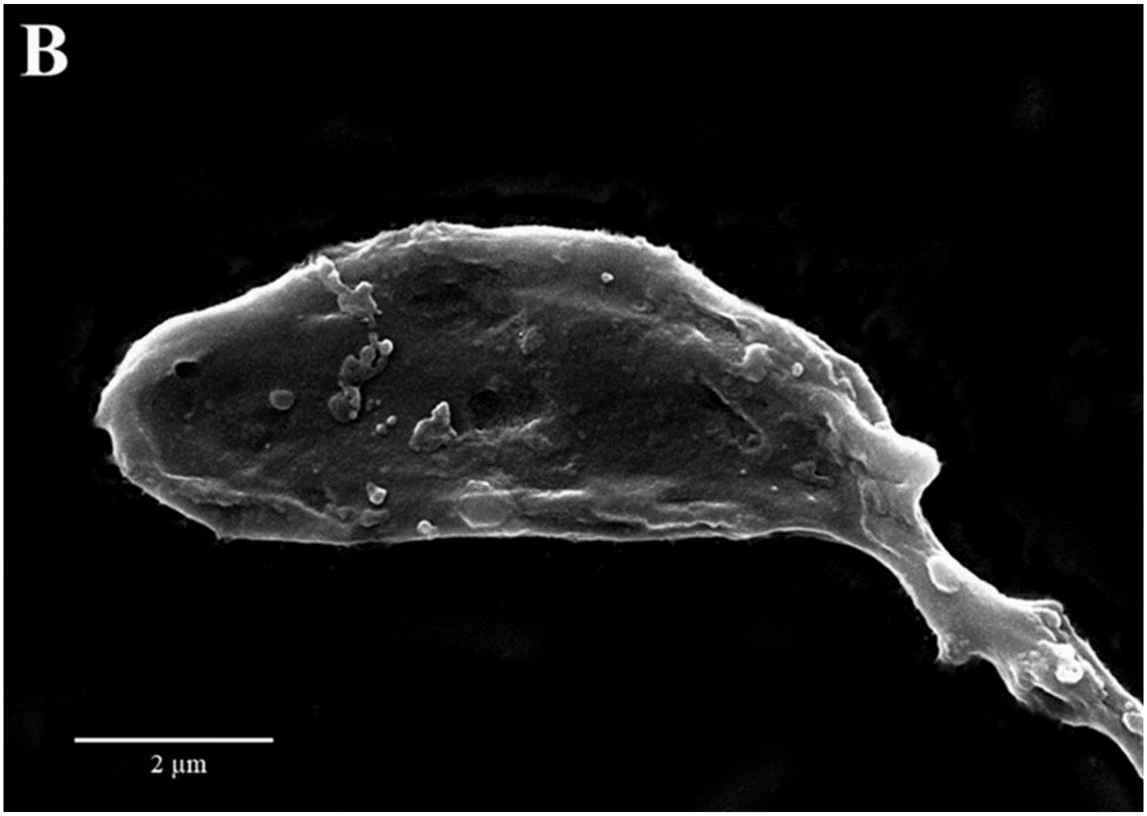

Figure 2-3 B. SEM image of the spermatozoon of *H. illucens*. The side view of the spermatozoon head and acrosome.

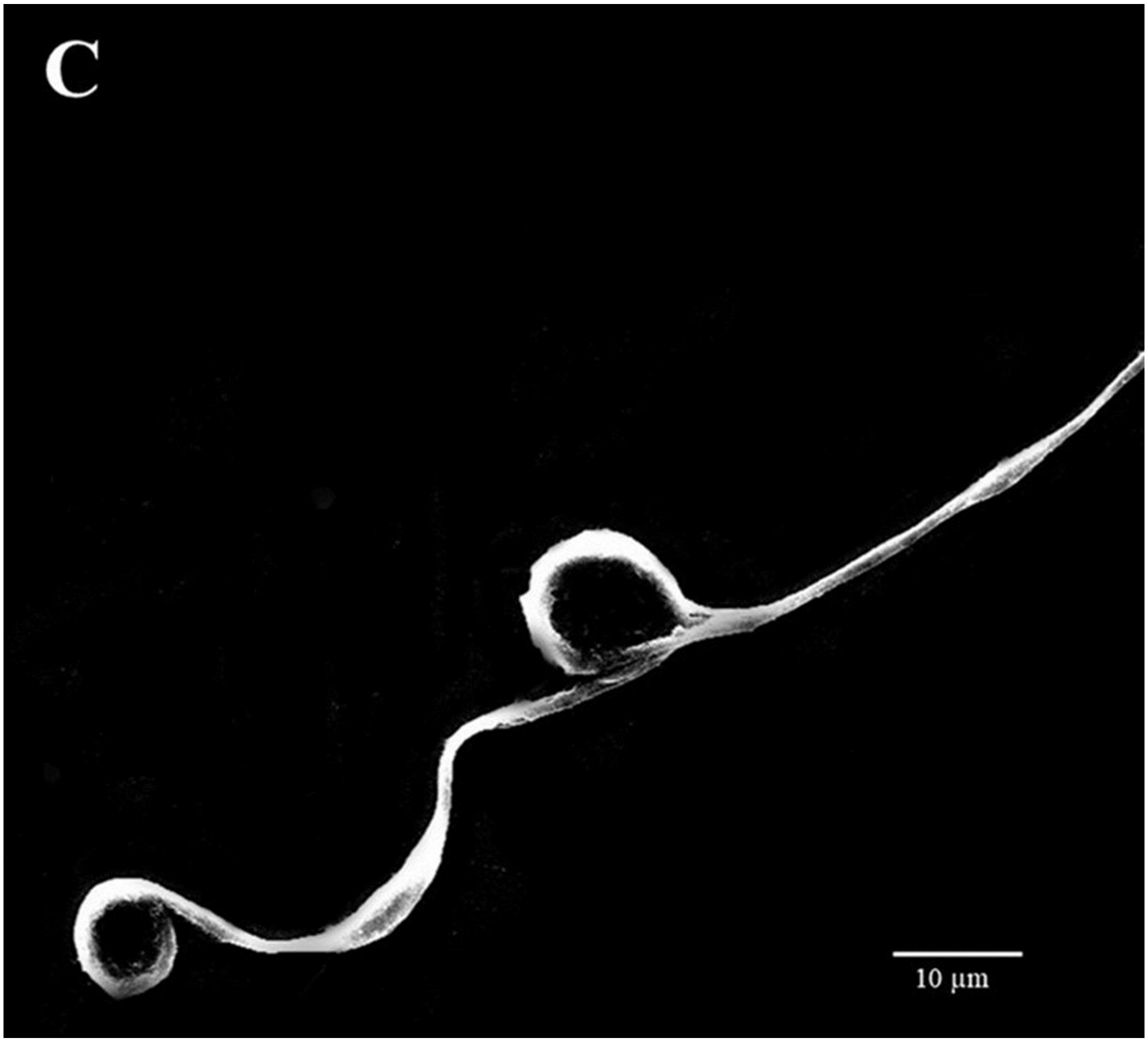

Figure 2-3 C. SEM image of the spermatozoon of *H. illucens*. The sperm displaying a bending wave propagation.

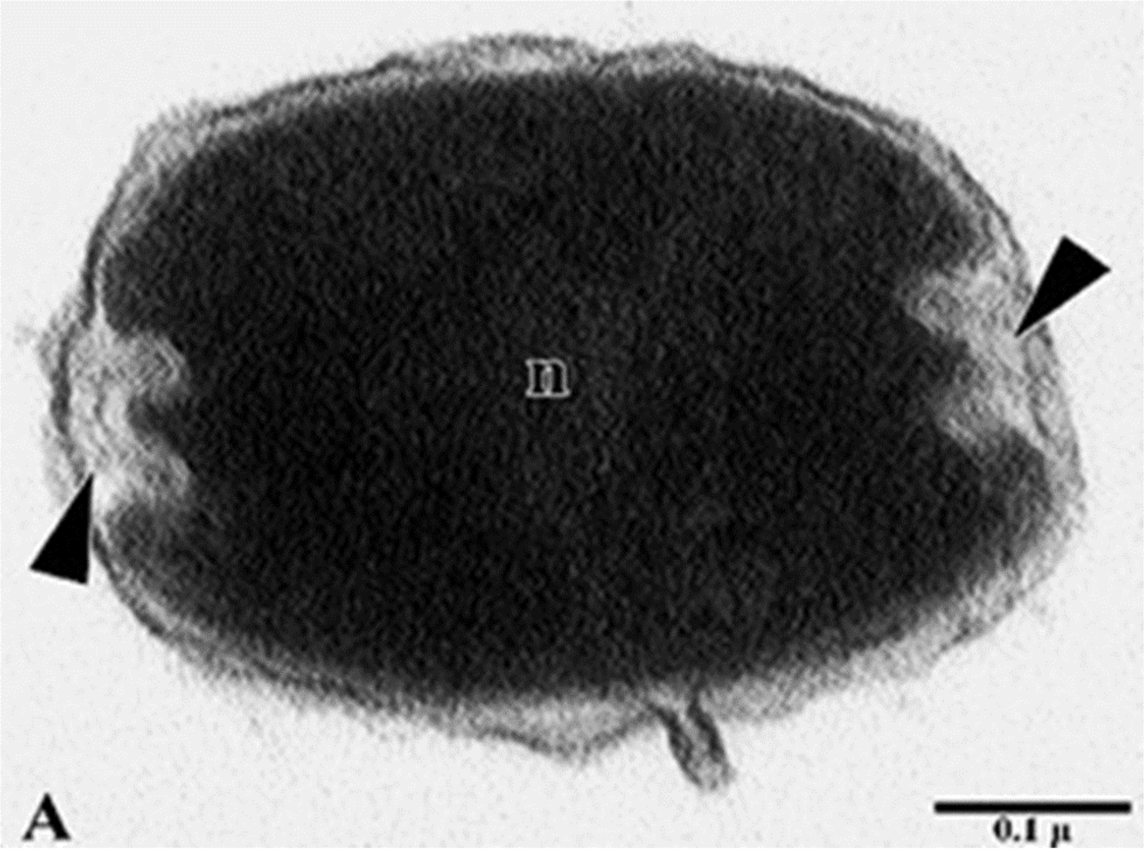

Figure 2-4 A. TEM image of *H. illucens* sperm. Cross section of an elliptical nucleus (n) with two lateral membrane infoldings (arrowheads).

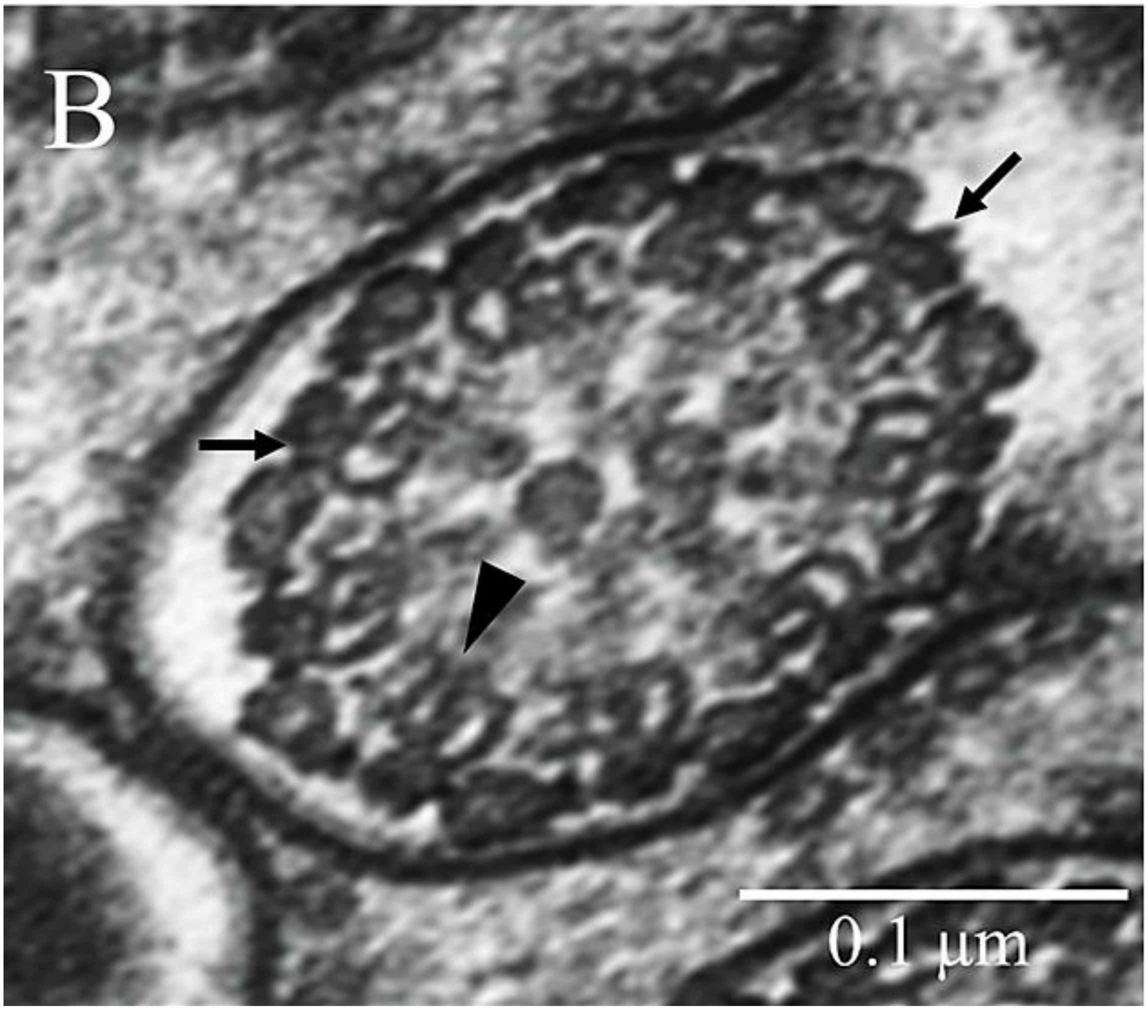

Figure 2-4 B. TEM image of *H. illucens* sperm. The axoneme showing 9 triplets (arrowheads) with a 9 + 9 + 2 microtubules arrangement pattern, with each triplet composed of a microtubule doublet connected to an incomplete microtubule (C-tubule) (arrow).

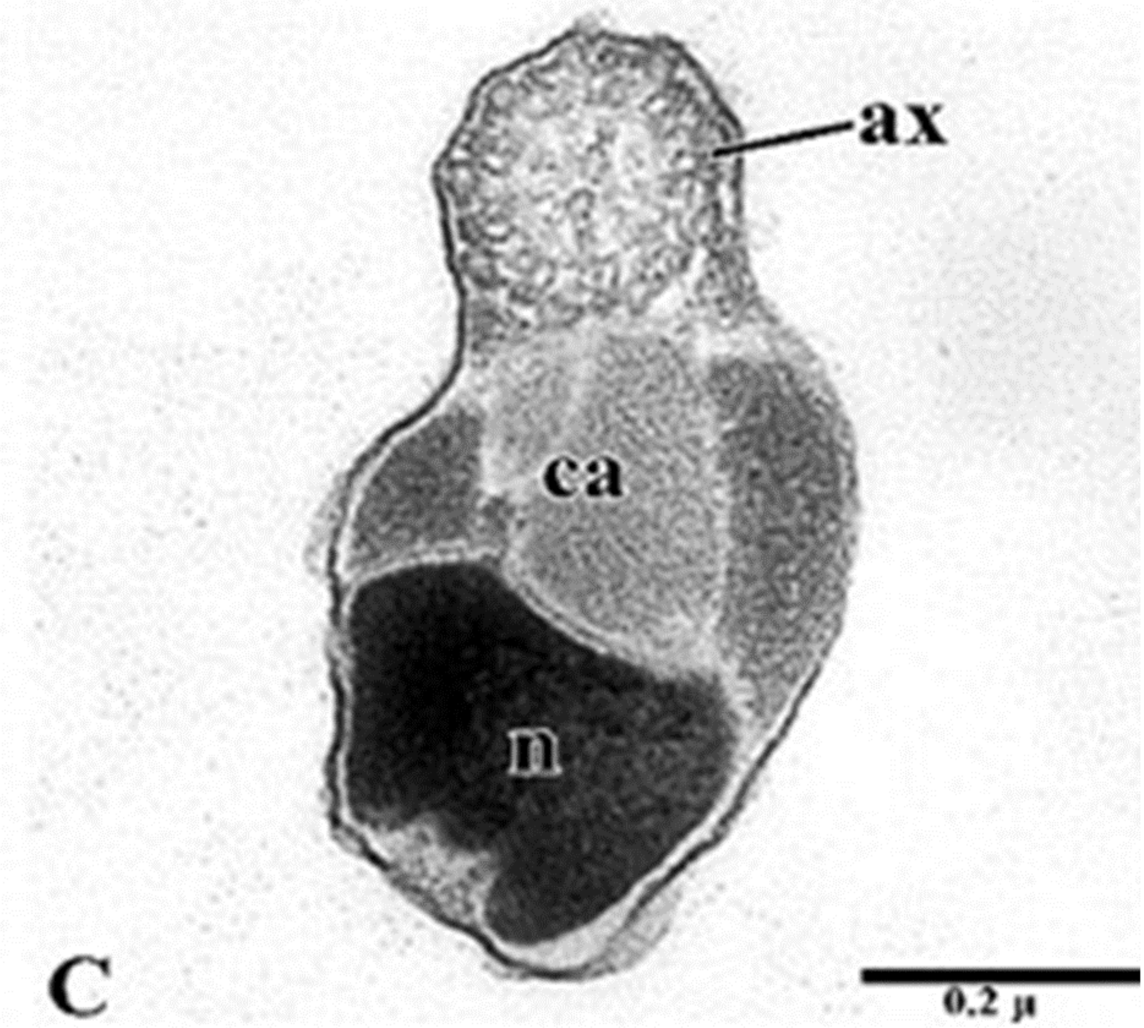

Figure 2-4 C. TEM image of *H. illucens* sperm. Cross section through the centriole adjunct material (ca). In this zone of the flagellum the nucleus (n) starts to became smaller. Centriole adjunct material (ca) with its different densities is visible between the axoneme (ax) and the nucleus (n).

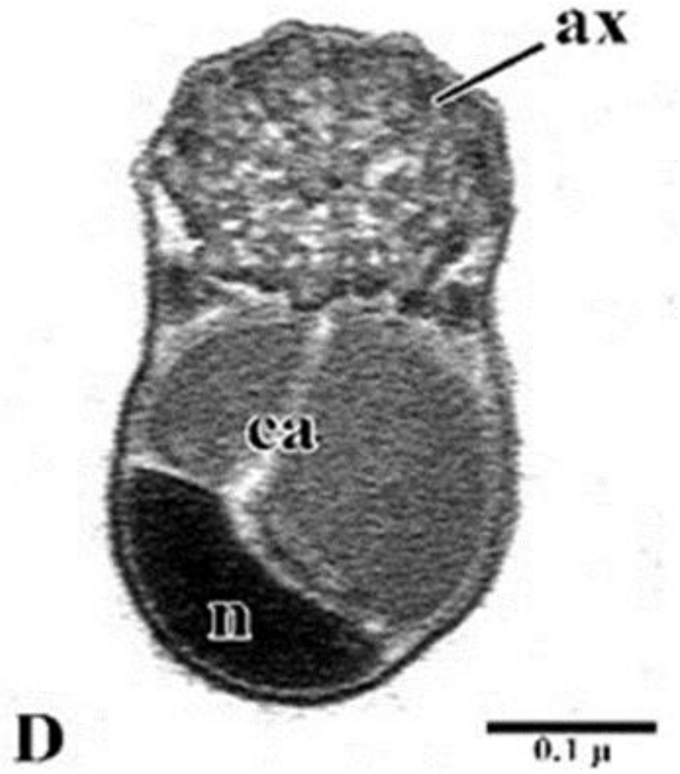

Figure 2-4 D. TEM image of *H. illucens* sperm. Cross section through the centriole adjunct material (ca). In this zone of the flagellum the nucleus (n) starts to became smaller. Centriole adjunct material (ca) with its different densities is visible between the axoneme (ax) and the nucleus (n).

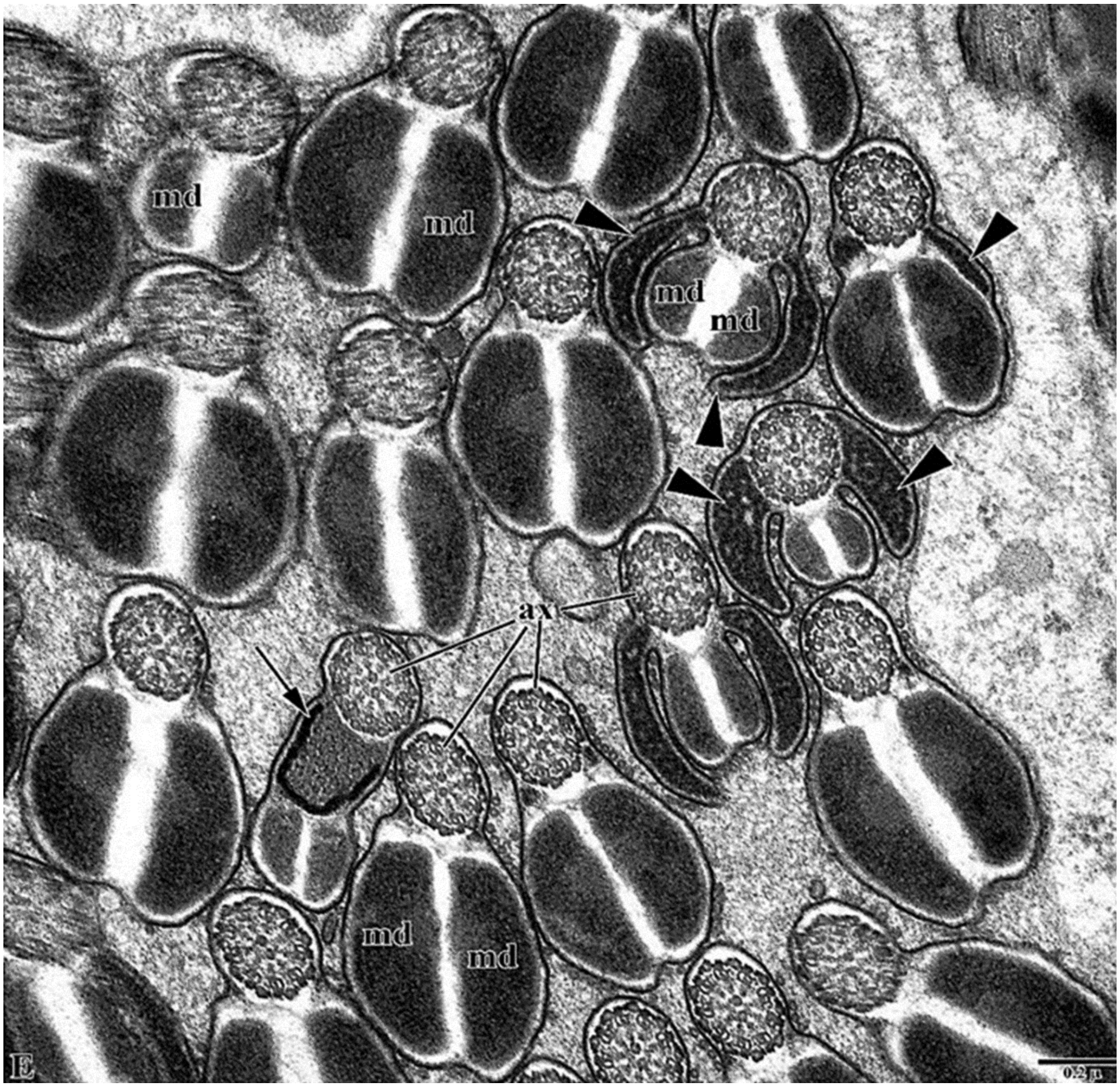

Figure 2-4 E. TEM image of *H. illucens* sperm. E) Cross section showing the two-lateral expansion (arrowheads) at different stages of formation, the short structure filled with microtubule (arrows) and the increasing size of mitochondrial derivatives (md). ax, axoneme.

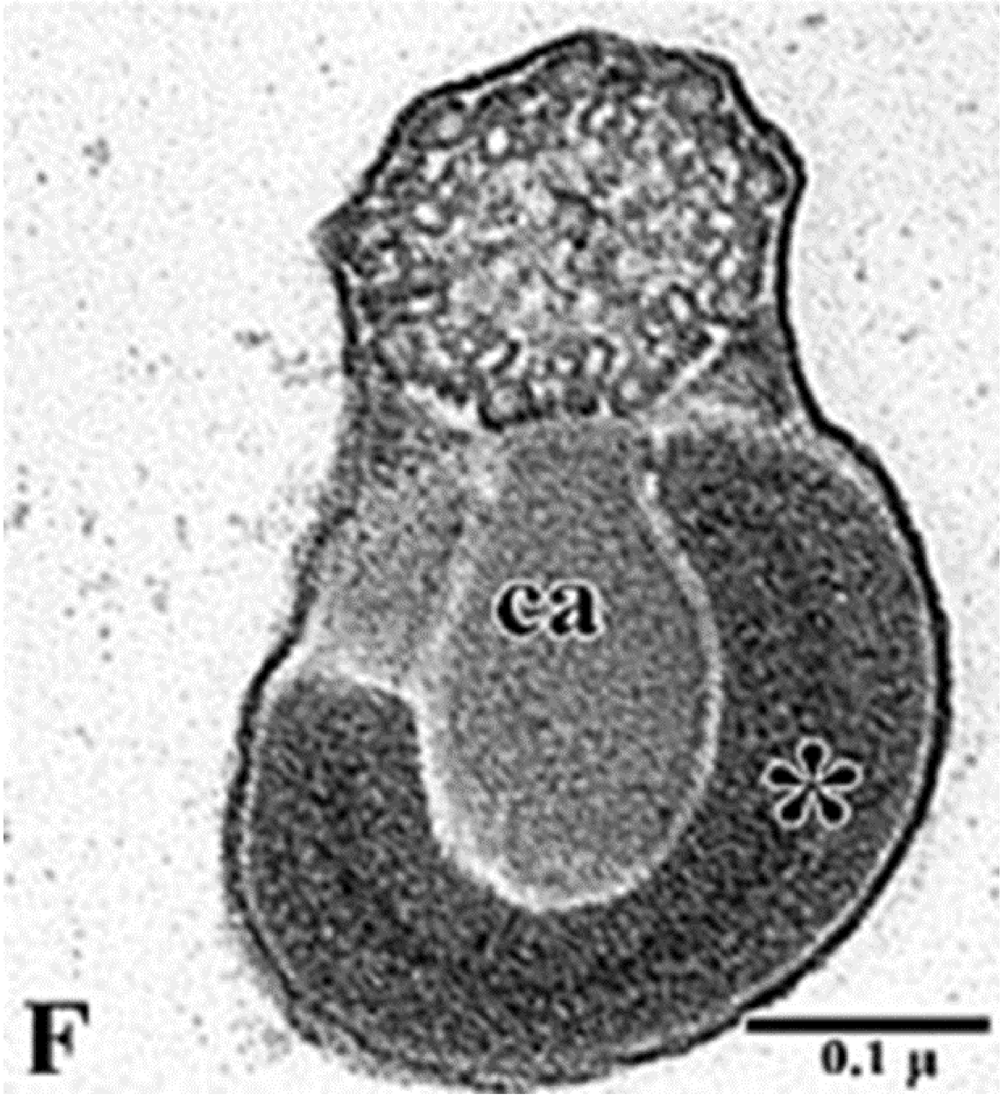

Figure 2-4 F. TEM image of *H. illucens* sperm. Cross section of the centriole adjunct (ca) zone. Note the U-shape structure (asterisk).

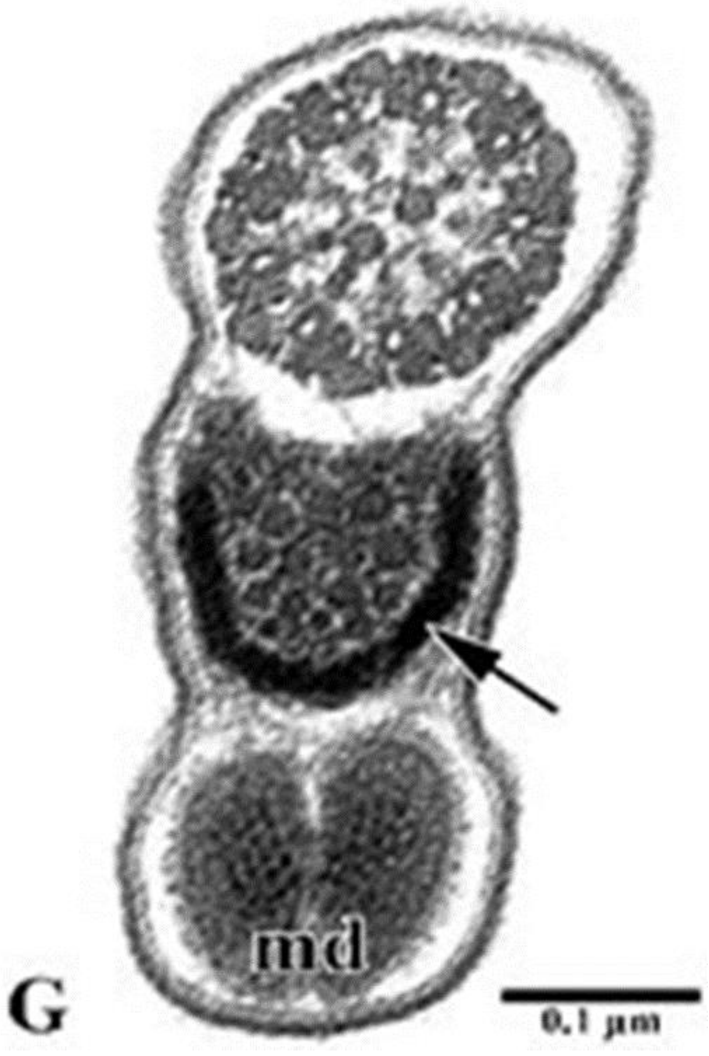

Figure 2-4 G. TEM image of *H. illucens* sperm. Cross section through the short zone presenting the structure fill of tubules flanked by a thin electron-dense U-shaped lamina (arrows). At this level the two mitochondria derivatives (md) appear and their matrix is crystallized.

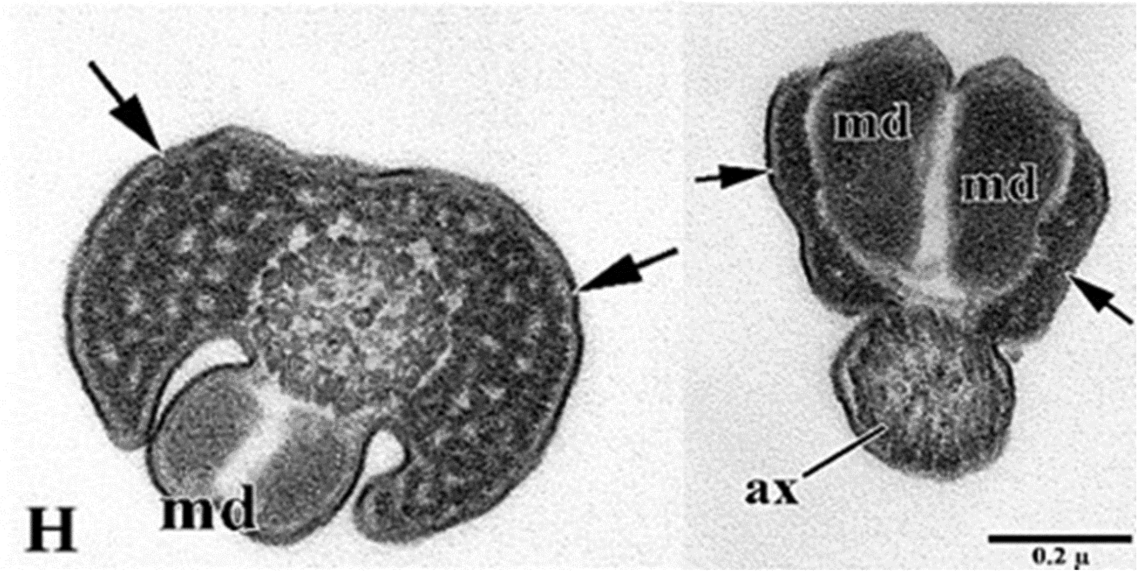

Figure 2-4 H. TEM image of *H. illucens* sperm. Cross section through the winged structure (arrows). These latter present a different pattern inside and became thinner. At this level the mitochondrial derivatives (md) increase their size. ax, axoneme.

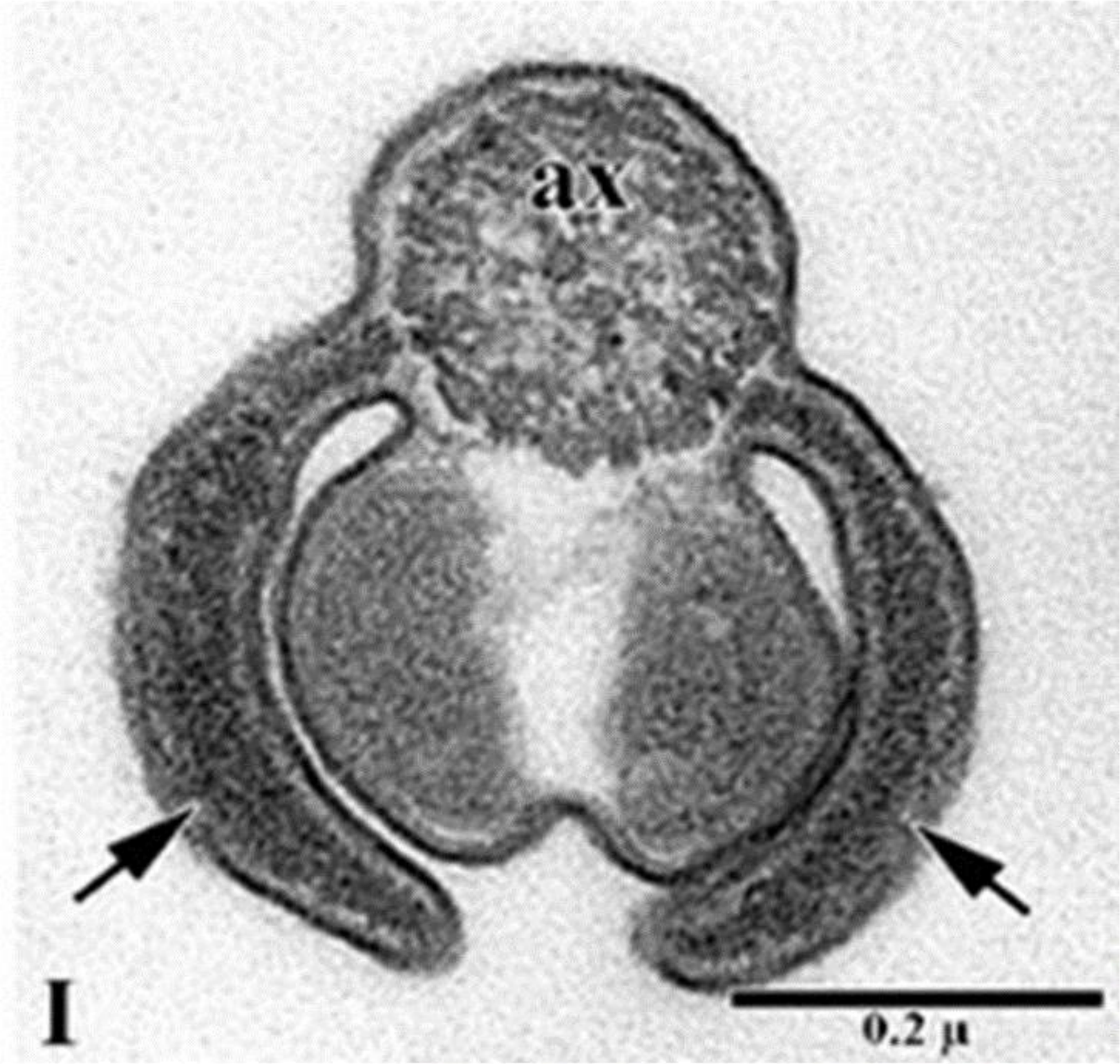

Figure 2-4 I. TEM image of *H. illucens* sperm. Cross section through the winged structure (arrows). These latter present a different pattern inside and became thinner. At this level the mitochondrial derivatives (md) increase their size. ax, axoneme.

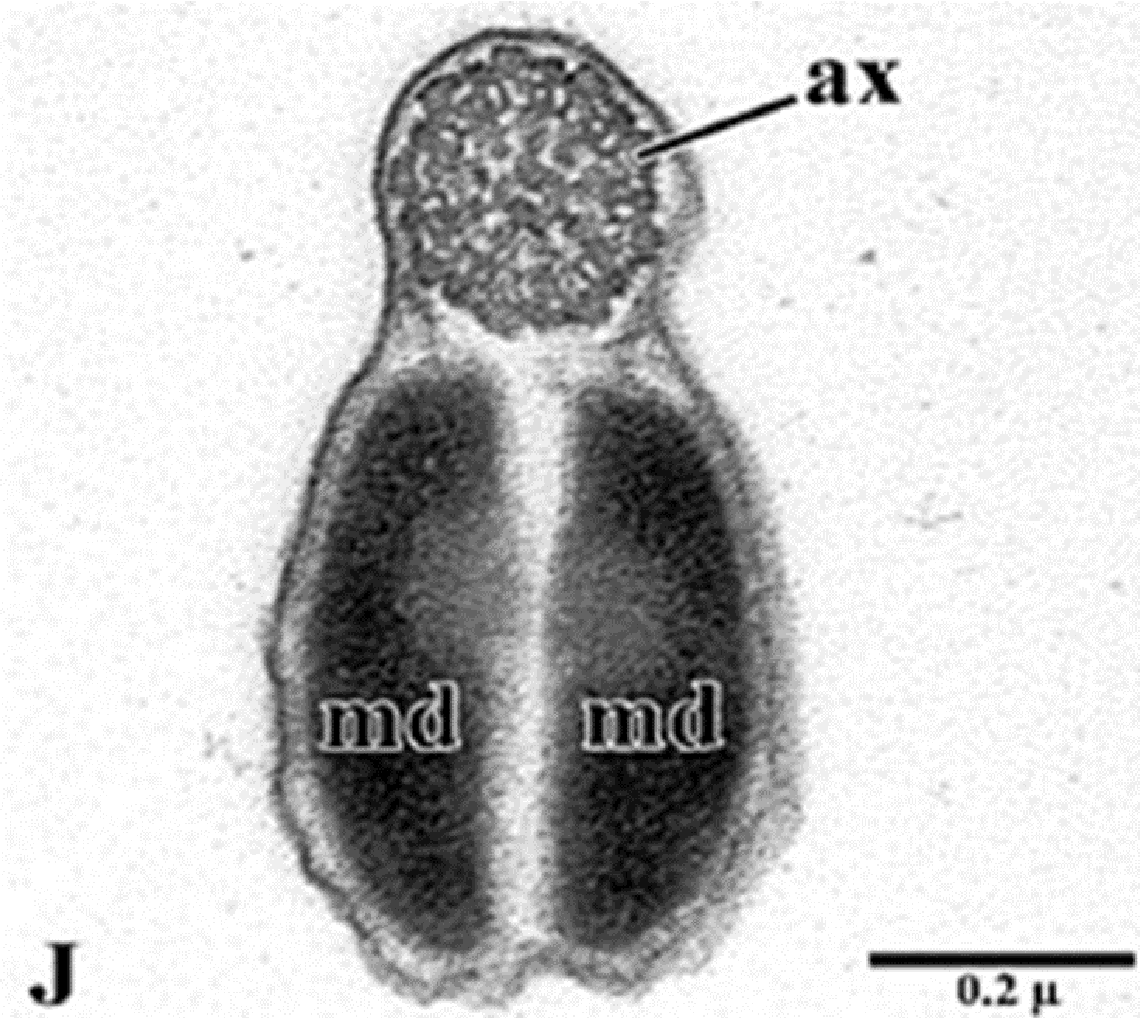

Figure 2-4 J. TEM image of *H. illucens* sperm. J) Cross section through the posterior end of the flagellum showing the ovoid mitochondrial derivatives (md) with the axoneme (ax).

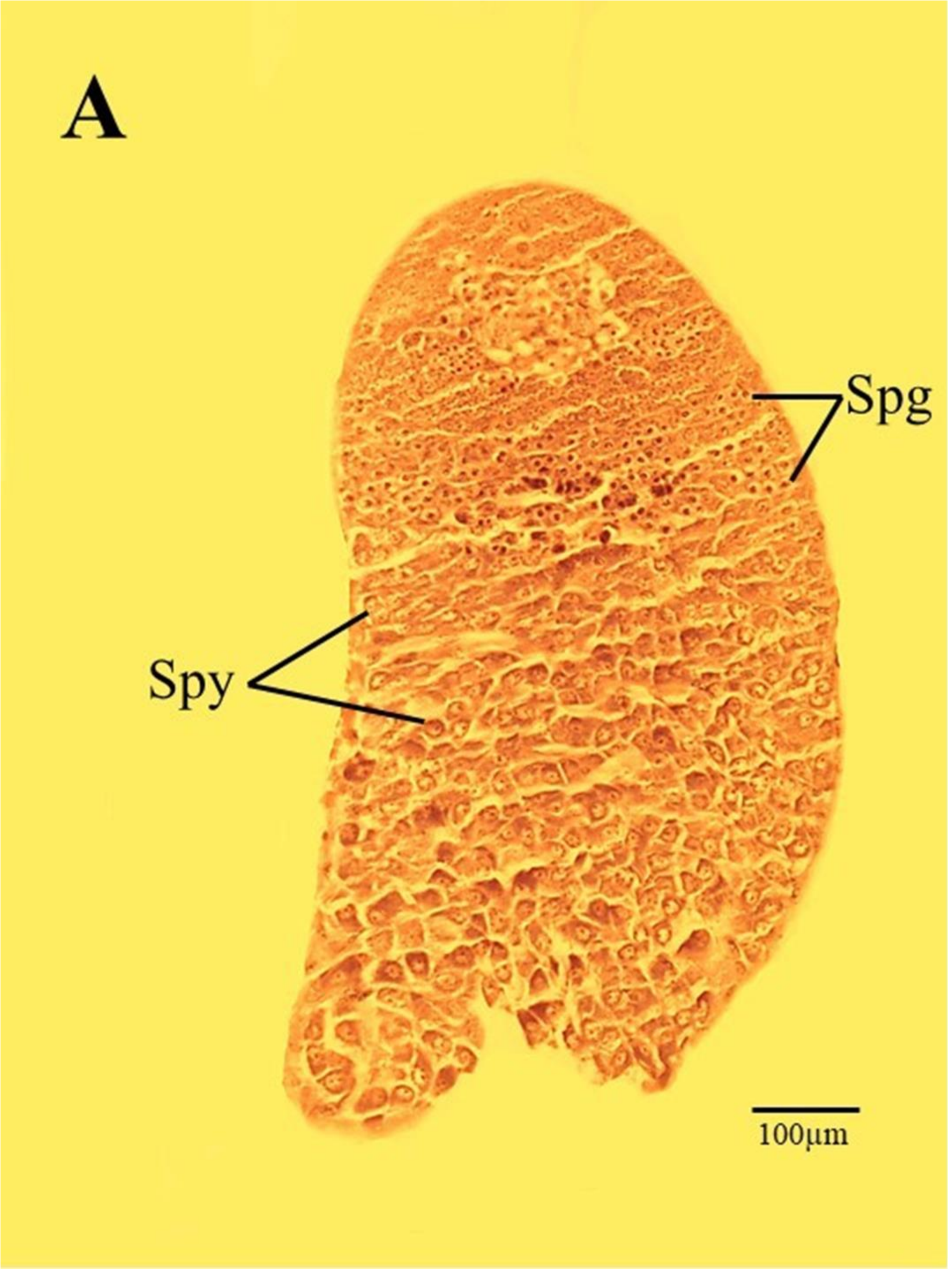

Figure 2-5 A. Longitudinal histological sections of the testis (right) of *H. illucens* staining with H/E. Cryptocephalic pupae showing the germ line (hub) (asterisk), where only pre-meiotic and meiotic stages are found. Spermatogonia cells (Spg) can be found right next to the hub, with the remaining testis filled with spermatocyte cell cysts (Spy). Abbreviations: Spg, spermatogonia; Spy, Spermatocytes; SpdI, Spermatids phase I (Maturation); SpdII, Spermatids phase II (Differentiation); sp, Sperm bundles; (asterisk), germ line (hub/ opened circle).

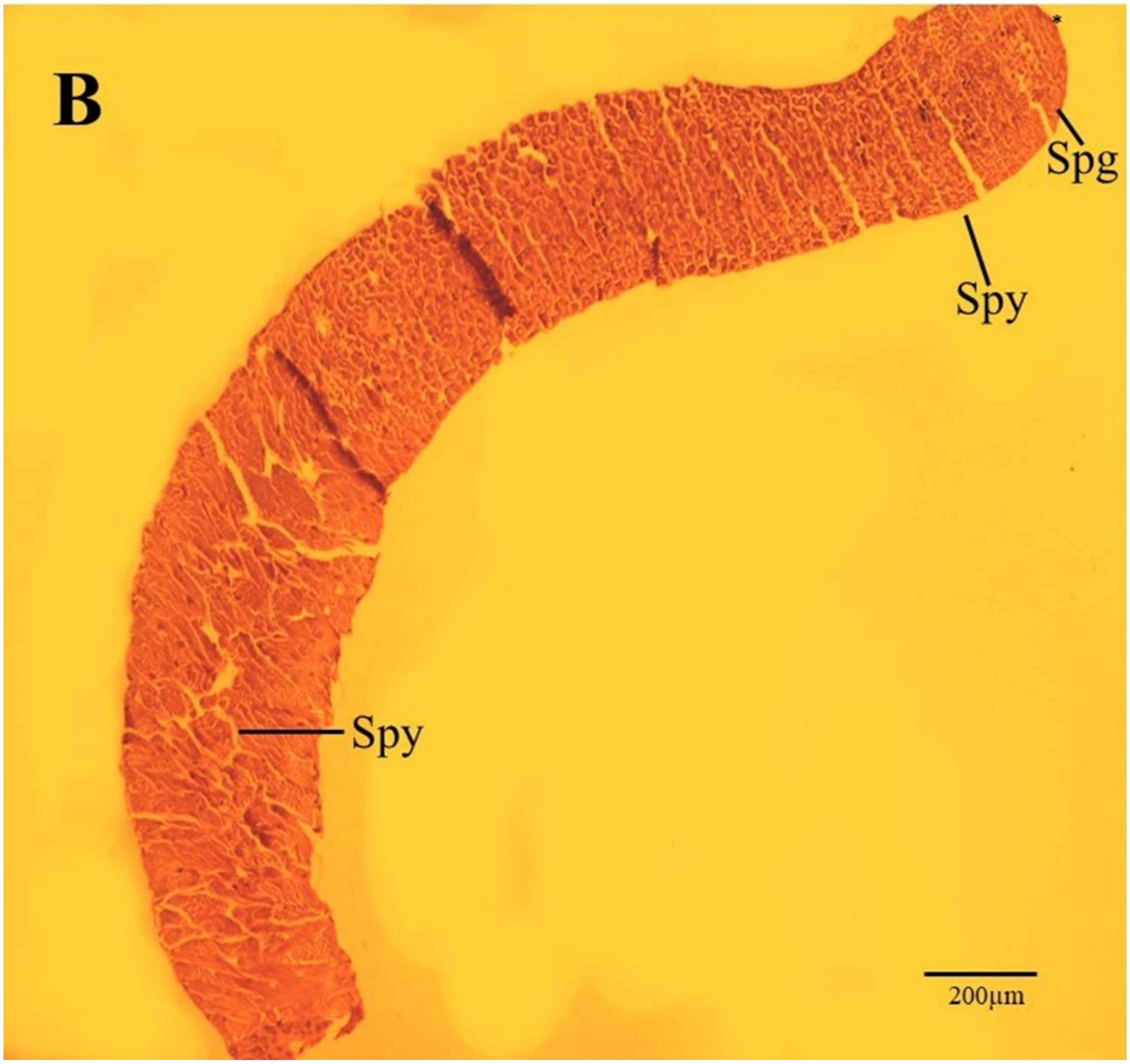

Figure 2-5 B. Longitudinal histological sections of the testis (right) of *H. illucens* staining with H/E. Phanerocephalic (phase II) pupae showing the germ line (hub) (asterisk), where only pre-meiotic and meiotic stages are found. Spermatogonia cells (Spg) can be found right next to the hub, with the remaining testis filled with spermatocyte cell cysts (Spy). Abbreviations: Spg, spermatogonia; Spy, Spermatocytes; SpdI, Spermatids phase I (Maturation); SpdII, Spermatids phase II (Differentiation); sp, Sperm bundles; (asterisk), germ line (hub/ opened circle).

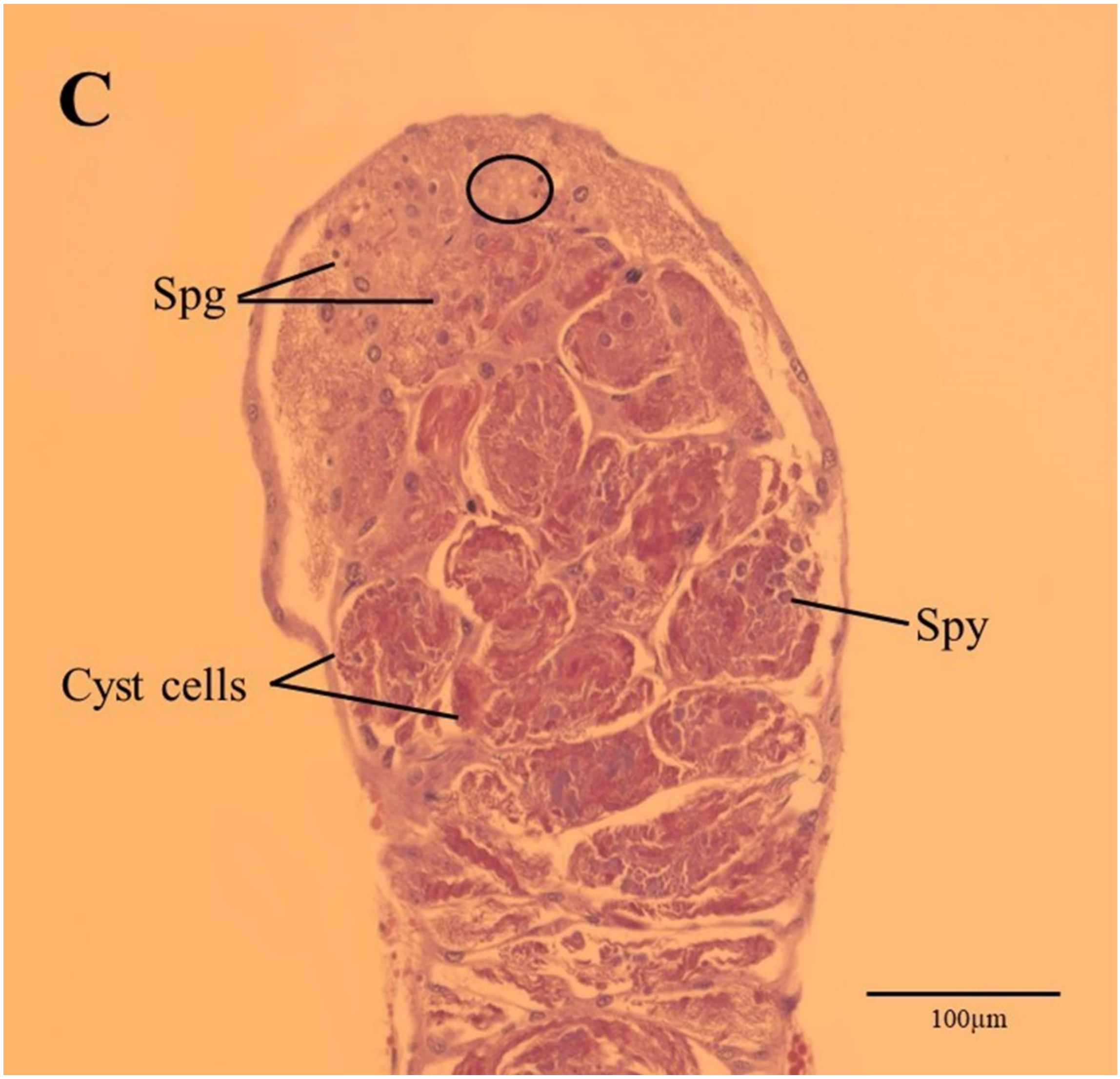

Figure 2-5 C. Longitudinal histological sections of the testis (right) of *H. illucens* staining with H/E. < 24h males. Note the germ line (opened circle), where right bellow spermatogonial (Spg) and primary spermatocyte (Spy) cells can be seen inside of cysts cells. Abbreviations: Spg, spermatogonia; Spy, Spermatocytes; SpdI, Spermatids phase I (Maturation); SpdII, Spermatids phase II (Differentiation); sp, Sperm bundles; (asterisk), germ line (hub/ opened circle).

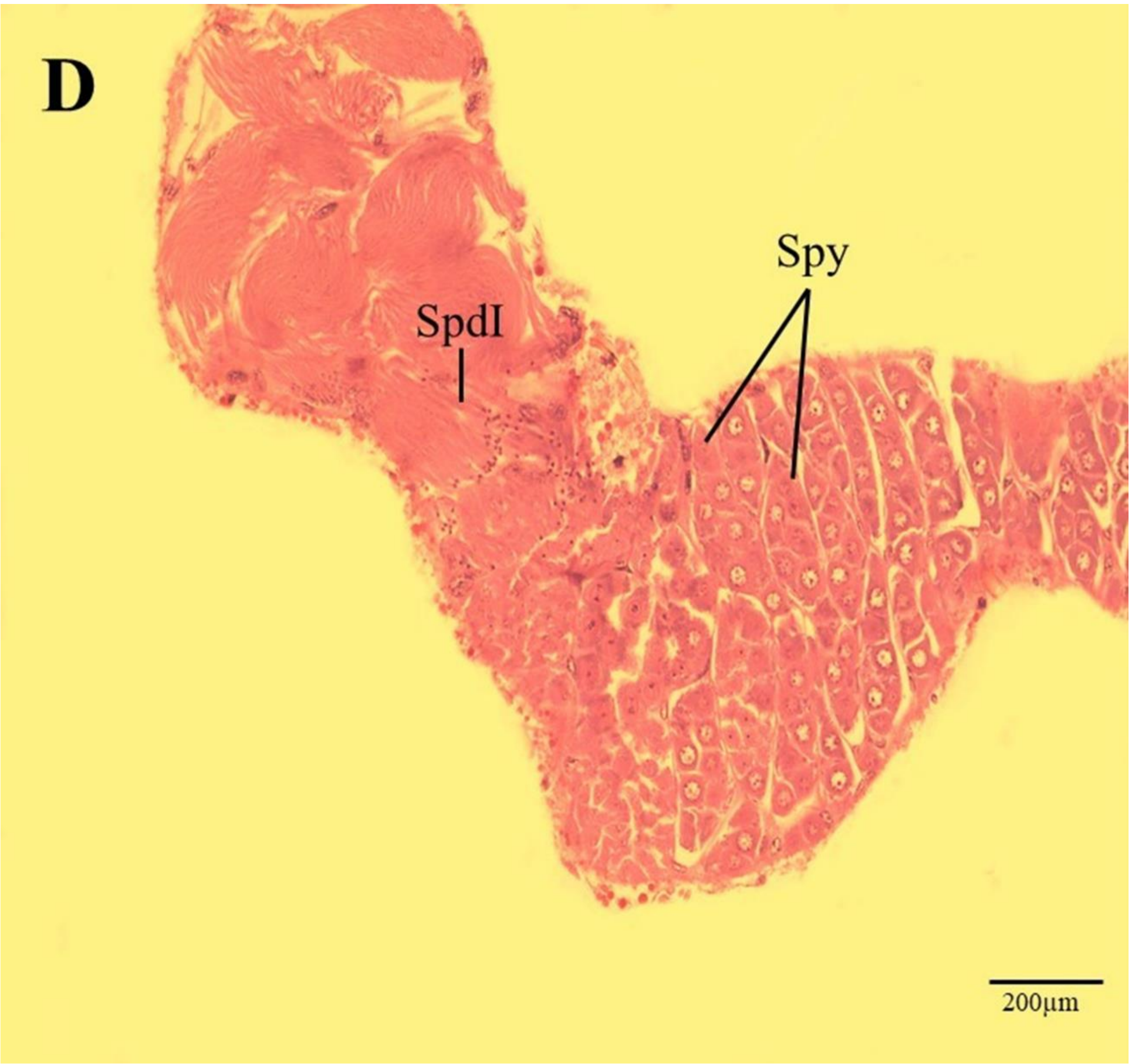

Figure 2-5 D. Longitudinal histological sections of the testis (right) of *H. illucens* staining with H/E. < 24h males. Early spermatids are visible in bundles in the posterior section of the testes. Abbreviations: Spg, spermatogonia; Spy, Spermatocytes; SpdI, Spermatids phase I (Maturation); SpdII, Spermatids phase II (Differentiation); sp, Sperm bundles; (asterisk), germ line (hub/ opened circle).

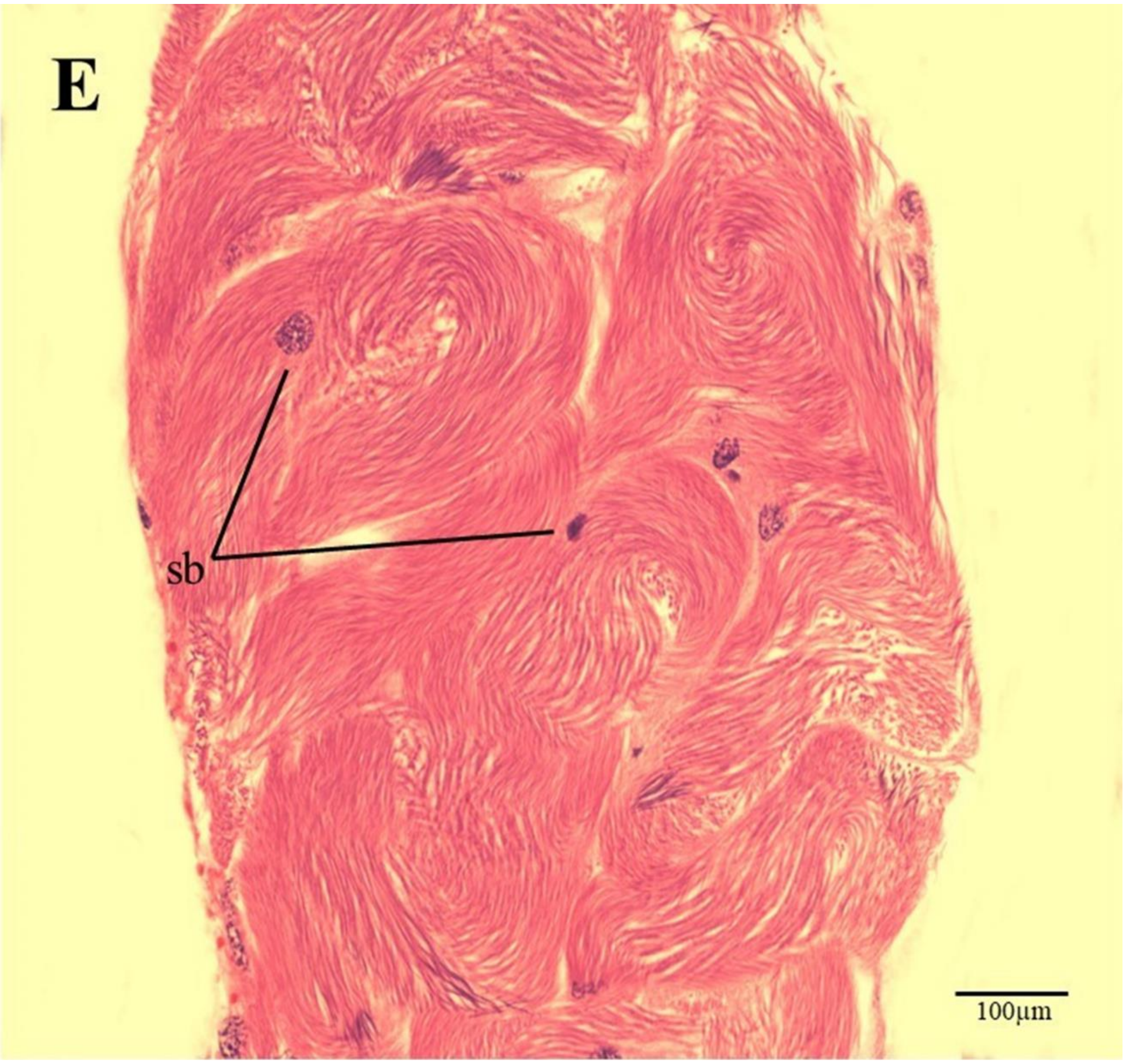

Figure 2-5 E. Longitudinal histological sections of the testis (right) of *H. illucens* staining with H/E. 72h males showing spermatids (SpdI) during the differentiation phase. Note the sperm bundles (sb), where the sperm heads aggregate within the cysts. Abbreviations: Spg, spermatogonia; Spy, Spermatocytes; SpdI, Spermatids phase I (Maturation); SpdII, Spermatids phase II (Differentiation); sp, Sperm bundles; (asterisk), germ line (hub/ opened circle).

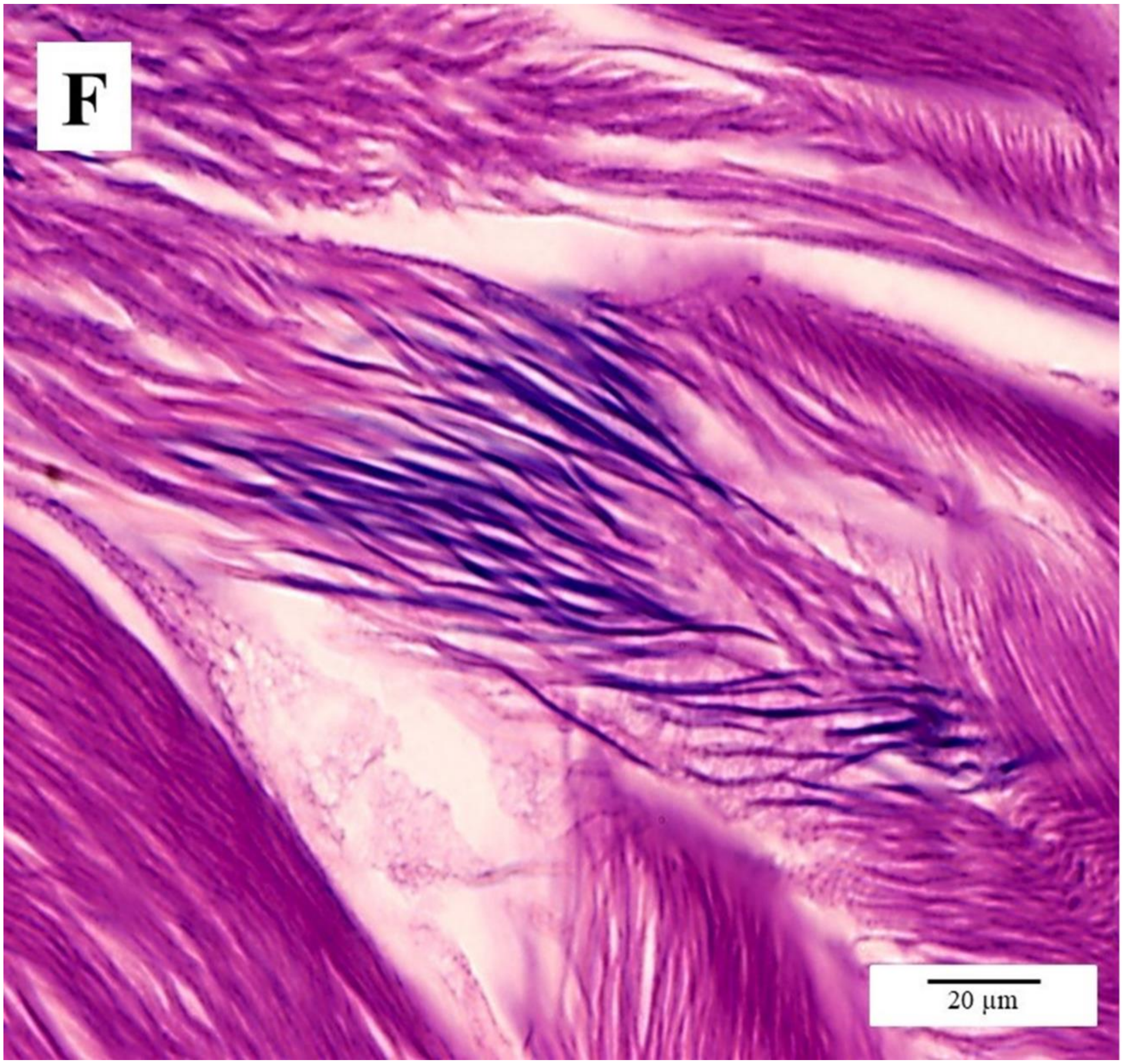

Figure 2-5 F. Longitudinal histological sections of the testis (right) of *H. illucens* staining with H/E. 72h males showing spermatids (SpdI) during the differentiation phase. Note the sperm bundles (sb), where the sperm heads aggregate within the cysts. Abbreviations: Spg, spermatogonia; Spy, Spermatocytes; SpdI, Spermatids phase I (Maturation); SpdII, Spermatids phase II (Differentiation); sp, Sperm bundles; (asterisk), germ line (hub/ opened circle).

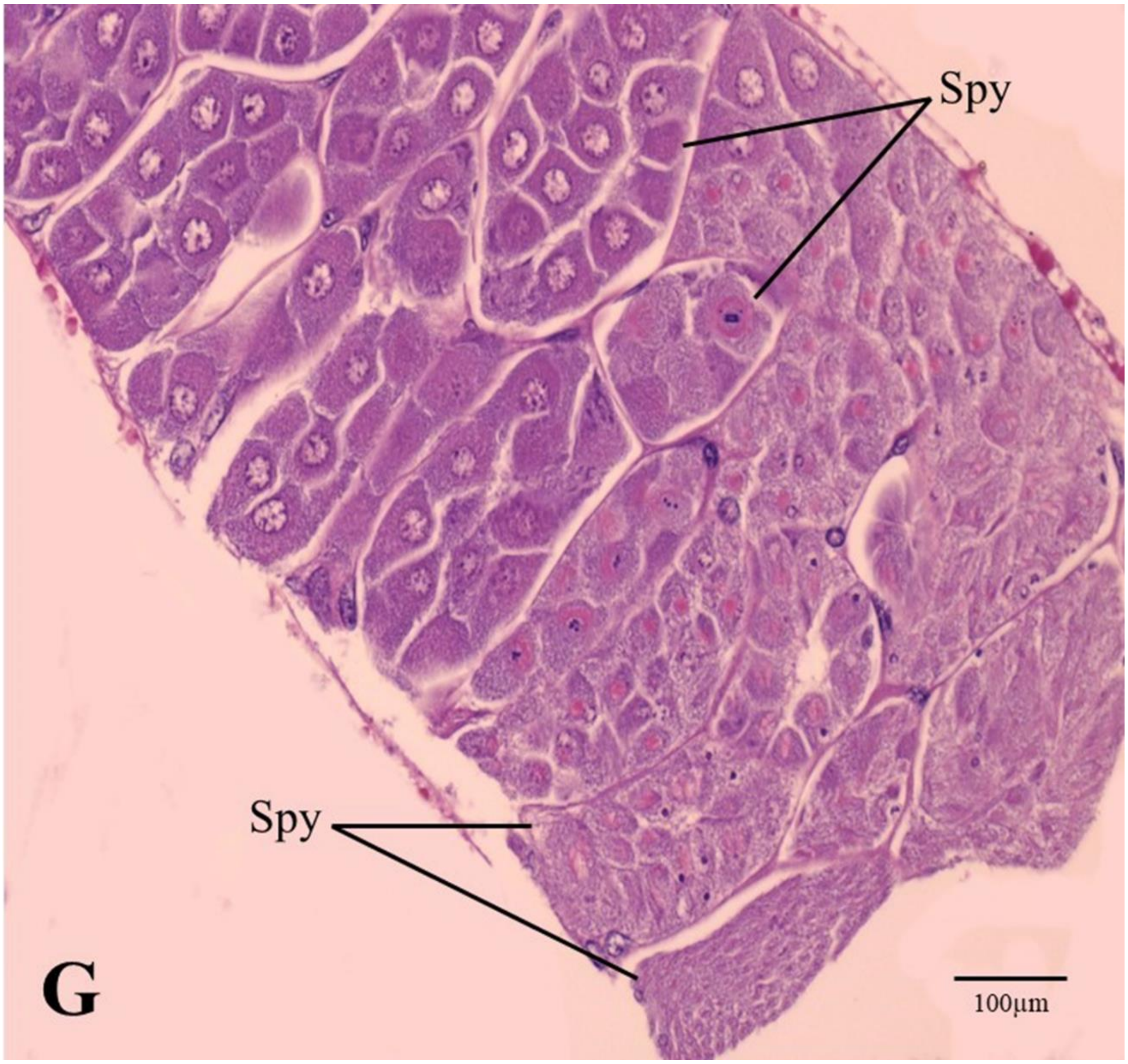

Figure 2-5 G. Longitudinal histological sections of the testis (right) of *H. illucens* staining with H/E. 168h males with visible spermatocytes (Spy). Abbreviations: Spg, spermatogonia; Spy, Spermatocytes; SpdI, Spermatids phase I (Maturation); SpdII, Spermatids phase II (Differentiation); sp, Sperm bundles; (asterisk), germ line (hub/ opened circle).

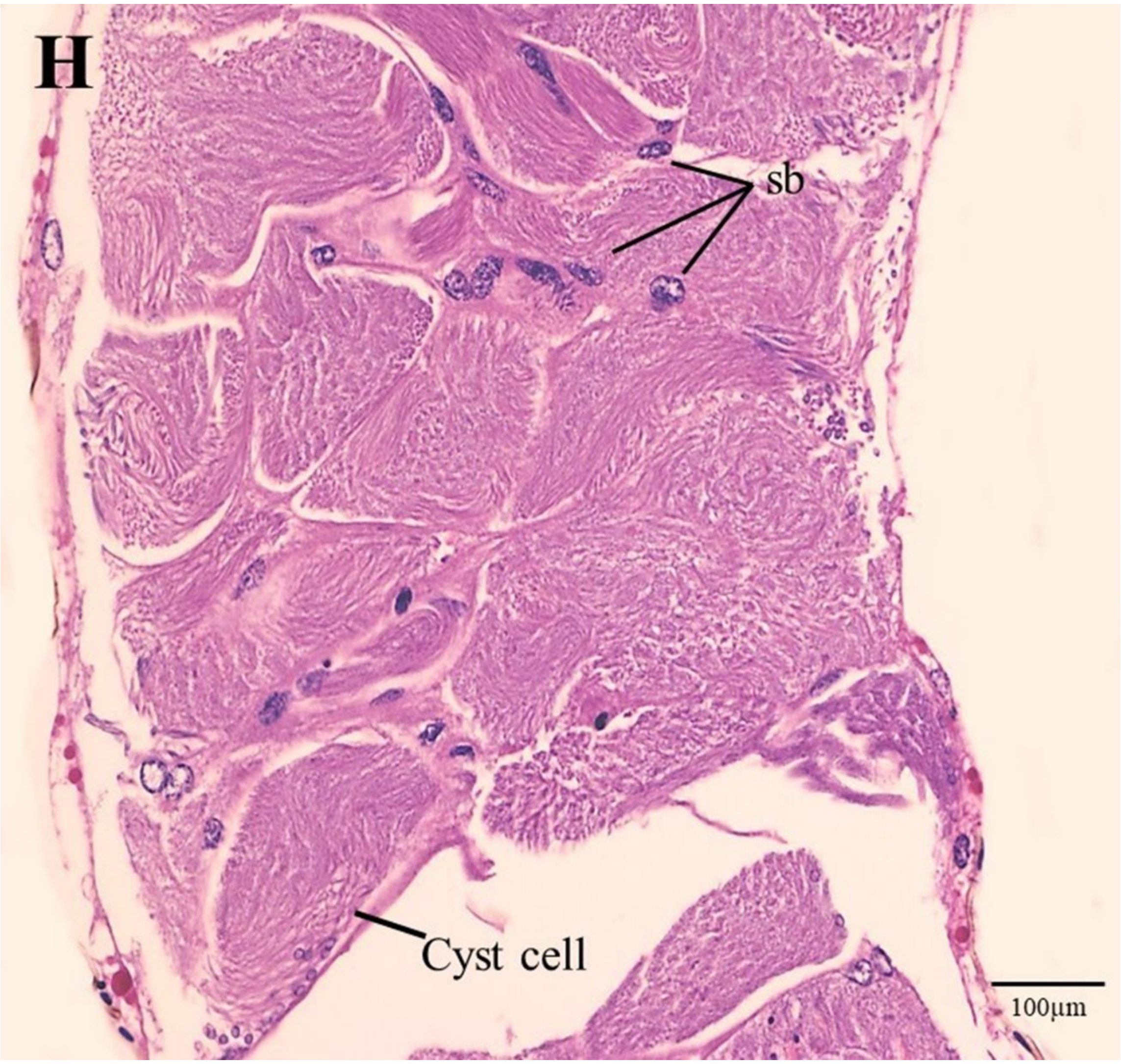

Figure 2-5 H. Longitudinal histological sections of the testis (right) of *H. illucens* staining with H/E. Abbreviations: Spg, spermatogonia; Spy, Spermatocytes; SpdI, Spermatids phase I (Maturation); SpdII, Spermatids phase II (Differentiation); sp, Sperm bundles; (asterisk), germ line (hub/ opened circle).

3. PHYSIOLOGICAL AND BEHAVIORAL RESPONSE OF BLACK SOLDIER FLIES: EFFECT OF AGE ON THERMAL PREFERENCE AND SPERM COUNT AND SPERM VIABILITY UNDER VARYING TEMPERATURES

3.1. Introduction

Ectothermic animals, particularity insects, are vulnerable to shifts in temperature (Thomas and Blanford 2003, Burdine and McCluney 2019). Many insects live in thermally variable environments; therefore, they have evolved a suite of thermoregulatory adaptations including, but not limited to, morphological, physiological, and behavioral mechanisms, to avoid or cope with low and high temperature or select for the optimal temperature (Fuller et al. 2010, Heinrich 2013). In particular, insects can thermoregulate using evaporative cooling, movement between microhabitats, and the contraction of flight muscles (Casey 1988, Heinrich 2013). If not regulated, variation in body temperature can impact many physiological (i.e., digestion, locomotion) and developmental processes (Huey and Stevenson 1979, Angilletta Jr et al. 2002), including reproduction (Zeng et al. 2018). Consequently, insects will move within a range of temperatures to achieve their thermal preference (Tsel) (Hertz et al. 1993, Kirchhof et al. 2017).

As previously mentioned, exposure to sub-optimal temperatures may cause irrevesible negative effects. One key biological function impacted by temperature is fertility. This topic has been well described with the thermal fertility limit (TFL), both high (TFmax) and low (TFmin), being described as the temperature extremes resulting in thermal stress and potential sterility (Walsh et al. 2019). Interestingly, males, rather than females, appear to be more vulnerable to temperature-driven fertility loss (Zizzari and Ellers 2011, Sales et al. 2018). Sales et al. (2018) evaluated the impact of heatwaves on sex-specific fitness, mating behavior, fertility, and sperm function of the red flour beetle, *Tribolium castaneum* (H.) (Coleoptera: Tenebrionidae). They determined males exposed to 42℃ suffered a 60% decrease in reproductive output (i.e., by means of hatched eggs) due to a 75% reduction in sperm count per ejaculate. Furthermore, they determined competitiveness of sperm from thermally stressed individuals decreased from 80% to 30%. However, reproductive success of females exposed to the same treatments was unaffected. Similar results were recorded for male *Drosophila buzzatii* (Patterson & Wheeler) (Diptera: Drosophilidae) where 80% of individuals exposed to 38.5℃ for six hours (i.e., TFmax) suffered permanent sterility (Vollmer et al. 2004).

Exposure to temperatures below the lower temperature threshold (i.e., TFmin) also impact insect fecundity and fitness. Rinehart (2000) reported an 80% reduction in male fertility of *Sarcophaga crassipalpis* (M.) (Diptera: Sarcophagidae) exposed to 10℃ for one hour. And again, with Drosophilia, but a different species, Boulétreau-Merle et al. (2003) demonstrated male *Drosophila melanogaster* (M.) (Diptera: Drosophilidae) exposed to 11 °C for 20 days became sterile. In both cases, experiencing extreme temperatures (i.e., TFmax and TFmin) can have long-lasting impacts on associated insect populations. However, temperature is only one factor impacting ectotherm fecundity and fertility.

Aging can have adverse effects on physiological processes in ectotherms, especially insects, as well (McHugh and Gil 2018). In fact, the detrimental impact of thermal stress on an insect as it ages can intensify (Rikke and Johnson 2004, Conti 2008). For example, older (16 days old) honey bee, *Apis mellifera* (L) (Hymenoptera: Apidae), drones had 50% less viable sperm than younger ones (10 days old). Furthermore, thermal stress negatively impacted sperm viability among individuals (Stürup et al. 2013). And, most importantly as related to the study presented here, thermal stress and age significantly. As drones aged, the negative impact of thermal stress was greater. Consequently, managing these factors is crucial in the honey industry. The same can be said for other industries, such as production of biocontrol agents (Parra 2009) or with the sterile insect release programs (Rubink 1987), or the black soldier fly (BSF), *Hermetia. illucens* (L.) (Diptera: Stratiomyidae).

The BSF is currently being mass produced for waste management (Miranda et al. 2019, Shumo et al. 2019) and protein production (Barragan-Fonseca et al. 2017). The BSF can digest several organic wastes (ur Rehman et al. 2017, Pleissner and Rumpold 2018, Mertenat et al. 2019). And, through this process, it reduces associated pollutants, such as heavy metals (i.e., cadmium, lead, and zinc) (Diener et al. 2015), noxious odors (Beskin et al. 2018), and greenhouse gas (GHG) emissions (Mertenat et al. 2019). This species is a cosmopolitan fly (Rozkosný 1983) and is not recognized as a pest species as it is not synanthropic (Furman et al. 1959). In addition to its ability to recycle wastes, BSF larvae, or prepupae, can be used as animal feed as they are high in protein, fats, and amino acids, of which several traditional fish feeds currently lack (Makkar et al. 2014, Tran et al. 2015, Stadtlander et al. 2017). However, little is known about the adult biology (e.g., one publication on beahvior of adults in wild population, Tomberlin and Sheppard 2001).

To date, no studies have determined the thermal preference of adult BSF or the relationship of temperature experienced with sperm viability as adults age. Consequently, the BSF industry lacks important information about the adult physiology that could impact reproduction and resulting production. The objectives of this study were to determine, 1) evaluate thermal preference of male and female *H. illucens* adults, and 2) sperm count and viability of H. illucens as they age under different temperature regimes.

3.2. Materials and Methods

Black soldier fly colony. *Hermetia illucens* adults used in the following experiments came from a colony maintained at the Forensic Laboratory for Investigative Entomological Sciences (F.L.I.E.S.) Facility at Texas A&M University in College Station, TX, USA. Wild-caught flies periodically were added to maintain genetic diversity. This colony was established in January 2014 from eggs received from Phoenix Worm, Inc., Tifton, GA, USA, which originated in 1998 from a laboratory colony maintained at the Coastal Plains Experiment Station, University of Georgia, Tifton, GA, USA. The colony procedures applied in these experiments follow those outlined by Sheppard et al. (2002) and Cammack and Tomberlin (2017). Resulting neonates (~ 10,000) <24-h-old were placed in a 500 ml plastic container with 200 g Gainesville diet

(Hogsette 1992) at 70% moisture and stored in a walk-in environmental chamber (30.0°C±1.7, 80% RH, and 16:8 L:D) (Cammack and Tomberlin 2017). After seven days, 500 larvae allotments were removed from this container and placed in 1-L plastic containers (n = 10 containers). Approximately, 60 g Gainesville diet at 70% moisture were provided daily until 40% of the larvae reached the prepupae stage. At this point, feeding was terminated (Tomberlin et al. 2002). Resulting prepupae were sifted and consolidated into a 35 (L) x 21 (W) x 12 (H) cm plastic pans. Each plastic pan was placed inside a 34 x 34 x 61 cm plastic cage (Bioquip®, Rancho Dominguez, CA). Plastic cages containing pans with prepupae were then placed in a Percival incubator set at 27°C, 70% RH, and 12:12 (L:D) (Tomberlin et al. 2009, Holmes et al. 2017). Prepupae were monitored every 12 h for adult emergence. Adults to emerge with the initial 24 h of emergence were excluded from the experiment due to insufficient numbers (Lin 2016). Adults emerging during the subsequent 12 h were collected, sexed using external morphological characteristics (Oliveira et al. 2016), and segregated by sex in glass Mason jars (1 L) (n = 10 flies per jar) covered with organza fabric. A wet cotton ball was placed in each jar to provide water ad libitum. A total of 10 jars (i.e., technical replicates) were used per sex/treatment (see below). Jars containing flies were maintained in the Percival incubator previously described (Tomberlin et al. 2009, Holmes et al. 2017). Jars were randomly rotated inside of the incubators daily to avoid spatial biases.

Thermal gradient device. The thermal gradient used in this assay was adapted from the design described by Anderson et al. (2013). It consisted of an aluminum sheet (85 x 20 cm) with one end placed into an ice bucket, and the other in a water bath (WB02A11B Digital General-Purpose Water Bath, 2 L Capacity, 120V/60 Hz, PolyScience®, Illinois, USA). This setup provided a 10-50°C (±2°C, SD) range. Temperatures along the gradient were recorded using four fine-wire fast-response digital thermocouples (Model A0188598; Gain Express Inc., Kowloon, Hongkong) affixed to the aluminum plate at 2, 17, 37, and 51.5 cm along its length. To keep flies in contact with the aluminum plate, a closed arena (53 x 18 x 4 cm) constructed of Plexiglass was placed on top of the aluminum plate. Vents were cut to the sides of the arena to allow airflow but not allow flies to escape (Figure 1A). Preliminary studies were conducted to ensure stability of temperature regime employed (Figure 1B) (Schilman and Lazzari 2004).

Thermal preference. Males and females from seven age groups (i.e., <24 h, 48 h, 72 h, 96 h, 120 h, 144 h, and 168 h) were sampled from the jars and tested. These ages were selected as they represent active adults up and through the primary mating period (Tomberlin and Sheppard 2002). For each sex/age group, a jar was randomly selected from the incubator. Ten individuals were removed from the jar and placed in the middle of the thermal gradient to prevent them being cold-trapped in the low temperature range (Giraldo et al. 2019) located in a room set at 27°C, 50% RH, and 12:12 L:D. This density was selected as it represents BSF aggregation under natural conditions (i.e. lekking behavior) (Tomberlin and Sheppard 2001). A digital video camera (Canon® VIXIA HF R700, Tokyo, Japan) was placed 1.5 m directly above the thermal gradient to record the spatial positions of flies (i.e., the value on the x axis within the range 0 to 51.5 cm of the thermal gradient). To estimate the temperature at any position of the thermal gradient, a third-degree polynomial equation was established for each replicate/trial/sex within the age categories using the temperatures recorded from each fine-wire fast-response digital thermocouples. Videos were analyzed using Ethovision software (Noldus©, Wageningen, Netherlands) where the datapoint position of each fly on the surface of the thermal gradient arena was recorded every 5 s. A Cartesian coordinate system was established in Ethovision, with 0 cm starting from the extreme cold side and 51.5 cm reaching the extreme hot side, to track the position of flies on the surface of the thermal gradient. Before initiating an experiment (i.e., replicate), flies were allowed to explore the thermal arena for 10 min in order to diminish possible influence of stress due to handling and placement in a new environment. A total of four replicates per age group/per sex across two total trials were performed, with males and females tested independently.

Sperm count and Viability. The design previously described for rearing, selecting, and housing adult BSF was used for this experiment as well. However, jars containing adults (n = 10/jar) were placed in Percival incubators set at either 20ºC, 24 ºC, or 27 ºC (± 0.5°C, SD). Temperatures were selected based on the data available regarding of life table and physiology process of this species under variable temperatures (Booth and Sheppard 1984, Sheppard et al. 2002, Tomberlin and Sheppard 2002, Tomberlin et al. 2009, Harnden and Tomberlin 2016, Holmes et al. 2016, Gligorescu et al. 2018, Shumo et al. 2019). A total of 70 jars were placed in each incubator. The humidity and photoperiod were kept at optimal conditions described for this species (27°C; 12:12 L:D; 70% RH) (Tomberlin et al. 2009, Holmes et al. 2017). Flies were exposed, at least, for

12 h to the treatments prior to initiating sperm viability measures to allow temperature to have a potential effect on the subjects and to prevent possible influence of stress due manipulation and changing of the surrounding physical conditions on the final results (personal observation). After this period, jars from each temperature treatment were randomly selected at the same ages used in the thermal preference assay. To diminish the possible impact of the laboratorial room temperature on the results, the flies per jar were processed within a range of 10 -15 min, with each jar exposed for ~15 min at most. Over the course of the experiment, jars were randomly rotated daily within incubators to avoid spatial biases.

Semen Collection and Sample Preparation. Males from the same seven age groups (<24 h, 48 h, 72 h, 96 h, 120 h, 144 h, and 168 h) were examined for sperm number and viability when exposed to 12 h in incubators set at 20ºC, 24ºC, or 27ºC, with all at 70% RH, and 12:12 (L:D) A single jar (jar = 10 adult males) from each temperature was removed daily from each temperature. All the flies from each jar were dissected following methodology of Eckel et al. (2017). Testis and seminal vesicles were removed from individuals using dissecting tools under a binocular stereoscope (SW-2B13-V331, AmScope™, Stephens city, VA, USA) and samples from each age category/temperature were placed into labeled Eppendorf tubes (1ml) containing 30 µL of saline solution (0.24 g HEPES, 0.88 g NaCl and 1 g BSA diluted in 100 mL of diH2O) to extend spermatozoa longevity for dual florescent staining and counting with a Cellometer Vision® (Nexcelom, Massachusetts, USA) (Rzymski et al. 2012, Tofilski et al. 2012). Dissecting needles (Insect pins morpho black enameled No. 2, catalog number: 1208B2, BioQuip©, Rancho Dominguez, CA, USA) were used to cut the submerged tissues (testis and seminal vesicles) to release sperm. After allowing the sperm to flow into the saline solution for 10 s, 6 µL of the sperm + saline solution were collected and then combined with 3 µL of sybr-14 (1 mM in DMSO) (Life Technologies™, Carlsbad, CA, USA) and 3 µL of propidium iodide (PI, 2.4mM in water) (Life Technologies™, Carlsbad, CA, USA), which differentially stained viable (green) and non-viable spermatozoa (red), respectively (Collins and Donoghue 1999). The prepared tubes were then gently inverted to homogenize the samples and kept in darkness for approximately 10 min to allow interaction between spermatozoa, sybr-14 and PI (Eckel et al. 2017). The semen from three males per age category/temperature were pooled for the sperm count and viability analysis following procedures also adapted from Eckel et al. (2017). The viability of the spermatozoa and sperm counting of each treatment were analyzed using the Nexcelom Cellometer® Vision CBA Image Cytometer (Nexcelom Biosciences LLC, Lawrence, MA, USA).

Statistical Analysis. Nonparametric tests were used for all assays since the groups tested did violate the assumption of normal distribution and homogeneity of variance. For the thermal preference, as well as sperm count and viability assay, the Kruskal-Wallis test (H) was used to determine significant differences among ages; when significant differences were determined, a Wilcoxon method for comparisons was used to determine differences between two independent treatments. Bonferroni corrections were applied to all p-values to compensate for multiple comparisons (Newson 2002). Significance prior to Bonferroni corrections was set at an alpha = 0.05. The thermal preference (Tsel) was established based on the central 50% (median) of the thermal gradient data of all individual per age/sex based on distribution. The 25% and 75% quartiles were set as the lower and upper limits of the Tsel range (Hertz et al. 1993, Kirchhof et al. 2017) and were presented as interquartile range (IQR). With regards to sperm count and viability, the correlation between the responses of the two dependent variables (sperm count and viability) was tested using Spearman's correlation (r) analysis. A Nominal Logistic Regression was performed to assess interaction effects between temperature and age on sperm count and viability. As with the previous statistical analysis, significance prior to Bonferroni corrections was set at an alpha = 0.05. All analyses were conducted in JMP pro 14.3 (SAS©).

3.3. Results

Thermal preference (Tsel) of BSF adults. Overall, males and females presented a similar average Tsel of 22.3℃ (16.6℃-28.6℃); median (Q1-Q3) and 21.5℃ (16.4℃-25.4℃); median (Q1-Q3), respectively across ages (Table 1). Aging significantly impacted the Tsel displayed by males ($H = 15841.5$, $df = 6$, $p < 0.0001$) and females ($H = 5089.8$, $df = 6$, $p < 0.0001$). Males <24-h-old preferred 24.3℃ (19.3℃-28.2℃); median (Q1-Q3) (Table 2; Fig. 1) while females at the same age displayed a Tsel of 20.2℃ (15.4℃-26.2℃); median (Q1-Q3) (Table 3; Fig. 2). Moreover, males <24-h-old displayed the highest Tsel, with it being reduced until age 72-h-old by 3.4℃ ($p= 0.0023$), their lowest Tsel; 20.9℃ (14.3℃-24.2℃); median (Q1-Q3). Males with 96-h-old increased by 1.8℃ their Tsel to 22.7℃ (19.7℃-24.5℃); median (Q1-Q3), reaching a Tsel of 23.6°C (20.6°C-26.3°C); median (Q1-Q3) at age 168-h-old ($p < 0.0001$). Females <24-h-old displayed their lowest Tsel, with it being increased by 3.8°C from age <24-h-old to 48-h-old ($p < 0.0001$), their highest Tsel; 24.0°C (18.2°C-26.7°C); median (Q1-Q3). From age 48-h-old to 72-h-old females reduced by 3.3°C their Tsel ($p < 0.0001$), set at 20.7°C (15.2°C-23.4°C); median (Q1-Q3).

Sperm count and Viability. Age impacted sperm count and viability in BSF males ($H = 39.88$, $df = 6$, $p < 0.0001$ and $H = 17.35$, $df = 6$, $p = 0.0081$, respectively) (Tables 4 and 5). Adults 48-h-old presented the highest sperm count with 322.5 (214.5-429.3); median (Q1-Q3) cells, while males 168-h-old had the lowest sperm count with 167.0 (96.0-275.3); median (Q1-Q3) cells ($p = 0.0023$) (Table 4; Fig. 3). Regarding sperm viability, 48-h-old males presented the greatest sperm viability with 78.3% (70.5%-81.2%); median (Q1-Q3) while 144-h-old males had the lowest sperm viability with 59.5% (55.9-63.1) ($p < 0.0001$); median (Q1-Q3) (Table 5; Fig. 4). Sperm count and sperm viability were statistically significant positive correlated ($r = 0.3961$, $p < 0.0001$), presenting a monotonic relationship (Fig. 5). Across ages, only males with 48-h-old and 96-h-old presented a statistically significant positive correlation between sperm count and sperm viability ($r = 0.593$, $p = 0.0309$ and $r = 0.5996$, $p = 0.0085$, respectively), with both presenting a monotonic relationship. There was no statistically significant effect of temperature on sperm count and viability ($H = 1.1102$, $df = 2$, $p = 0.5740$ and $H = 0.4525$, $df = 2$, $p = 0.7975$, respectively). Moreover, there was not a statistically significant interaction between age and temperature on sperm count and viability ($F12,126 = 03668$, $p = 0.9723$ and $F12,126 = 1.2979$, $p = 0.2308$, respectively).

3.4. Discussion

Thermal preference (Tsel) of BSF adults. BSF were demonstrated to have a thermal preference, and this preference varied based on sex and age. On average, males and females presented a similar Tsel value of 22.3℃ (16.6℃-28.6℃) and 21.5℃ (16.4℃-25.4℃), respectively, regardless of age; median (Q1-Q3) (Table 1). These finding are similar to those determined for other insect species. For example, male and female kissing bugs, *Rhodnius prolixus* Stål (Hemiptera: Reduviidae), which has a distribution the Western hemisphere similar to BSF, displayed an average preference of 25.0°C, and 25.4°C, respectively (Schilman and Lazzari 2004). The same was determined for male and female *Drosophila tripunctata* Loew (Diptera: Drosophilidae) (Dillon et al. 2009).

Age and sex of the BSF impacted Tsel. While adult (<24-h) males preferred a warmer Tsel of 24.3℃ (Table 2; Fig. 1), females preferred a cooler Tsel of 20.2℃ (Table 3; Fig. 2); however, while females displayed an increase in their Tsel until they reach 48-h-old, males progressively selected cooler temperatures. A similar pattern was observed for male and female *D. virilis*, where males selected significantly lower temperatures on a thermal gradient than did females (Yamamoto 1994).

Female preference for a higher Tsel than males during the initial 48 h could be due to differences in physiological mandates. Females, being synovigenic (Jervis et al. 2001), must mature eggs during the first 48 h prior to mating (Tomberlin and Sheppard 2002), while males emerge with mature sperm (Malawey et al. in press). The wasp *Ooencyrtus nezarae Ishii* (Hymenoptera: Encyrtidae), which is also synovigenic, also responds similarly. Females are more fecund at 25℃ than when forced to develop eggs at 22℃ (Aung et al. 2011). The study conducted with BSF females by Chia et al. (2018) supports the results found here, where adults maintained at 30°C had more viable eggs (80%) than when kept at 15℃ (below 11%).

Male preference for lower temperatures as they age could enhance sperm viability over time. Males used in the present study were virgin and are known to emerge with a full batch of functional sperm (Malawey et al. in press) and they are not known to be able to replenish sperm after mating. Consequently, maintain a full complement of sperm for the greatest duration could result in greater fitness. These results do not come as a surprise as temperature is a well-known factor impacting sperm viability in insects (David 2008, Sales et al. 2018).

Conversely, adult male BSF 96-h-old or older demonstrated a preference for warmers Tsel (e.g., 23.6℃ at age 168-h-old). This pattern could reflect a last attempt by males to locate females aggregated in warm environments maturing their eggs. This behavioral strategy is thought to exist as warmer temperatures enhance reproductive success of some species (Kindle et al. 2006). Larsson and Kustvall (1990) reported male mating success of the longhorn beetle, *Stenurella melanura* L. (Coleoptera: Cerambycidae), was positively correlated with temperature. Moreover, males of the red mason bee, *Osmia bicornis* L. (Hymenoptera: Megachilidae) at high temperatures (22℃-26℃) showed an increase in their modulation range (Hz) in comparison with males that were at low temperatures (17℃-21℃), which had conferred to them better communication with females with consequently reproductive performance.

Additionally, studies conducted with BSF have demonstrated that those individuals present a preference for warmer environments within the range of 27℃-30℃, where most of their physiological and behavior processes occurs at their highest rates (Harnden and Tomberlin 2016, Nakamura et al. 2016, Giunti et al. 2018, Gligorescu et al. 2018, Hoc et al. 2019, Miranda et al. 2019, Shumo et al. 2019). We hypothesize that the lower Tsel displayed by males and females across ages may be lower than optimum temperatures because, since performance curves are asymmetric, it is expected that performance would decrease rapidly above the optimum temperature, thus, the thermal preference should be for lower temperatures to minimize the risk of have their performance reduced once thermoregulation is imperfect in these individuals (Martin and Huey 2008). Moreover, optimum temperatures may diverge among physiological states and behavior processes, therefore the concept of a single optimal thermal preference is not sustained (Huey and Stevenson 1979). Thermal preference may depend on the physiological status (i.e., age) or major constraints for fitness, such as the absence of mates.

Sperm count and Viability. Sperm number (Table 4) (Fig. 3) decreased as BSF adults aged (<24-h-old male with 297 sperm/individual to 168-h-old male with 167 sperm/individual), while sperm viability remained relatively consistent at approximately 70% (Table 5) (Fig. 4). The positive correlation between sperm count and sperm viability (Fig. 5) across ages demonstrates the capability of males in produce new sperm cells without affect viability despite their limited resources. This became clear when we found a positive correlation between these two variable responses in 48-h-old and 96-h-old males. These two ages were the ones to present the two consecutive peaks in sperm count while displaying a sperm viability above 70%. Nevertheless, these flies were maintained in a small space (mason jar) and were sexually constrained, therefore, males might have allocated extra resources for sperm nourishment that otherwise would have been used for flight and copula attempts.

Sperm count was greatest for BSF 48-h-old and gradually decreased as they aged. However, percent viability remained relatively consistent, with few one-off falls. Malawey et al. (in press) determined spermatogenesis in BSF is initiated during pupation, and males emerge with full complement of mature sperm, with the number of mature cells increasing during the first 48h. They also determined males continue producing sperm as they age but at low levels. But, based on results from the current study, sperm production in older flies must not be at a level capable of compensating for natural morality since spermatogenic function may ceased after emergence, especially in short-lived species, by the degeneration of the stem cells (Dumser 1980). In D. melanogaster males, their sperm production declines as they aged, however, in sexually-constrained males, their seminal fluid proteins (Sfps) are accumulated over time (Sepil et al. 2019). Therefore, sperm deprived males may impact overall fecundity by mating with high fecund females without fertilize them (Paoli et al. 2013).

The temperatures selected to determine their impact on sperm viability were selected a priori based on published literature and the range used in behavior (Booth and Sheppard 1984, Tomberlin and Sheppard 2001, Sheppard et al. 2002, Tomberlin and Sheppard 2002, Giunti et al. 2018) and development (Sheppard et al. 2002, Harnden and

Tomberlin 2016, Chia et al. 2018, Gligorescu et al. 2018, Shumo et al. 2019) studies for this specie. The justification being these temperatures would most likely be used in mass production facilities. Therefore, determining if these temperatures impact sperm viability would be valuable information for mass production facilities aiming to optimize fertile egg production. Unfortunately, the temperatures examined in the current study were not outside the thermal preference and did not significantly impact sperm viability. It should be noted the thermal tolerance data presented here were determined after the sperm viability study was completed.

Future studies should establish the thermal preference range first and then explore the impact of temperatures outside this range on sperm viability. Doing so, should allow for the detection of such effects as they have been determined for other insects (Dickinson 2018, Houston et al. 2018, McAfee et al. 2019). In order to fully investigate the relationship between temperature and sperm viability, future studies should also incorporate exposure time as part of the study. In the current study, exposure duration was dictated by age (e.g., 168-h-old were exposed for that amount of time to an assigned temperature). It would be interesting to determine how variable temperature exposure impacts sperm viability (Zizzari and Ellers 2011, Dickinson 2018, Houston et al. 2018).

Implications of temperature on mass production of black soldier fly. Selecting an appropriate temperature range for BSF is critical for optimal mass production. As demonstrated in the current study, adults have preferred temperatures that shift as they age. Furthermore, the number of viable sperms decreases as adult males age. Thus, developing methods allowing adult males and females to aggregate, or lek in the case of the BSF, at the appropriate time is critical to maximize colony fitness.

Unfortunately, published methods continuously mass release adults into a cage over time resulting in various age cohorts commingled (Sheppard et al. 2002, Tomberlin and Sheppard 2002, Hoc et al. 2019, Jucker et al. 2019, Miranda et al. 2019). This strategy potentially is sub-optimal with regards to producing viable eggs. Given females are not ready to mate until two days post emergence (Tomberlin and Sheppard 2001) and males emerge with sperm (Malawey et al. in press), such a setup could result in disruptive mating occurring with males attempting to mate with females not ready or that have already mated. By having two distinct temperature environments in the cage, segregation could occur allowing for females to mature without male harassment, and males to not wasting energy attempting to mate with unreceptive females. Such an approach could enhance mating success and thus the collection of fertilized eggs. Another potential solution could be to have a gradient of temperatures or shifting temperatures in the cage over time to allow cohorts to remain segregated in the cage.

Furthermore, future studies should evaluate the impact of different temperature regimes along with different larval densities on the fitness of these flies, since larval density may increase the temperature experience by these individuals (Paz et al. 2015). Larval density by itself have a negative impact on final larval weight and crude protein of this specie (Barragan-Fonseca et al. 2018) and it may, consequently, impact sperm quality and ovarium size (Cresoni-Pereira and Zucoloto 2012). Temperature has also been reported of having impacted adult's body size (Harnden and Tomberlin 2016, Chia et al. 2018).

Adults emerged from larvae reared under low or high larval densities may display variations in their morpho-physiological traits, such as antennae, eye, testis, and ovarium size (Birrell 2018) that would impact fitness. Therefore, if all these traits, apparently sexually selected, are correlated (positively or negatively) with adult body size (Birrell 2018), temperature may have a role on their establishment as well. Regardless, results presented here indicate more research is needed to understand temperature fluctuations in cages as related to thermal preference, sperm viability, and optimal adult mating success.

3.5. References

Angilletta Jr, M. J., P. H. Niewiarowski, and C. A. Navas. 2002. The evolution of thermal physiology in ectotherms. Journal of Thermal Biology 27: 249-268.

Aung, K. S. D., T. Ueno, and M. Takagi. 2011. Effect of temperature on lifetime reproduction of the egg parasitoid *Ooencyrtus nezarae* (Ishii)(Hymenoptera: Encyrtidae). Journal Faculty of Agriculture-Kyushu University 56: 67-70.

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

Barragan-Fonseca, K. B., M. Dicke, and J. J. van Loon. 2018. Influence of larval density and dietary nutrient concentration on performance, body protein, and fat contents of black soldier fly larvae (*Hermetia illucens*). Entomologia Experimentalis et Applicata 166: 761-770.

Beskin, K. V., C. D. Holcomb, J. A. Cammack, T. L. Crippen, A. H. Knap, S. T. Sweet, and J. K. Tomberlin. 2018. Larval digestion of different manure types by the black soldier fly (Diptera: Stratiomyidae) impacts associated volatile emissions. Waste Management 74: 213-220.

Birrell, N. 2018. Sensory Morphology, Investment and Plasticity in the Black Soldier Fly (*Hermetia illucens*). ResearchSpace, Auckland.

Booth, D. C., and C. Sheppard. 1984. Oviposition of the black soldier fly, *Hermetia illucens* (Diptera: Stratiomyidae): Eggs, masses, timing, and site characteristics. Environmental Entomology 13: 421-423.

Boulétreau-Merle, J., P. Fouillet, and J. Varaldi. 2003. Divergent strategies in low temperature environment for the sibling species *Drosophila melanogaster* and *D. simulans*: Overwintering in extension border areas of France and comparison with African populations. Evolutionary Ecology 17: 523-548.

Burdine, J. D., and K. E. McCluney. 2019. Differential sensitivity of bees to urbanization-driven changes in body temperature and water content. Scientific Reports 9: 1643.

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

Casey, T. M. 1988. Thermoregulation and heat exchange. Advances in Insect Physiology 20: 119-146.

Chia, S. Y., C. M. Tanga, F. M. Khamis, S. A. Mohamed, D. Salifu, S. Sevgan, K. K. Fiaboe, S. Niassy, J. J. van Loon, and M. Dicke. 2018. Threshold temperatures and thermal requirements of black soldier fly Hermetia illucens: Implications for mass production. PloS one 13: e0206097.

Collins, A., and A. Donoghue. 1999. Viability assessment of honey bee, Apis mellifera, sperm using dual fluorescent staining. Theriogenology 51: 1513-1523.

Conti, B. 2008. Considerations on temperature, longevity and aging. Cellular and Molecular Life Sciences 65: 1626-1630.

Cresoni-Pereira, C., and F. S. Zucoloto. 2012. Fruit flies (Diptera). Insect bioecology and nutrition for integrated pest management. CRC Press, Boca Raton: 451-472.

David, J. 2008. Male sterility at high and low temperatures in Drosophila. Journal de la Societe de Biologie 202: 113-117.

Dickinson, M. 2018. The impacts of heat-wave conditions on reproduction in a model insect, *Tribolium castaneum*. University of East Anglia.

Diener, S., C. Zurbrügg, and K. Tockner. 2015. Bioaccumulation of heavy metals in the black soldier fly, *Hermetia illucens* and effects on its life cycle. Journal of Insects as Food and Feed 1: 261-270.

Dillon, M. E., G. Wang, P. A. Garrity, and R. B. Huey. 2009. Thermal preference in Drosophila. Journal of Thermal Biology 34: 109-119.

Dumser, J. B. 1980. The regulation of spermatogenesis in insects. Annual Review of Entomology 25: 341-369.

Eckel, B. A., R. Guo, and K. Reinhardt. 2017. More pitfalls with sperm viability staining and a viability-based stress test to characterize sperm quality. Frontiers in Ecology and Evolution 5: 165.

Fuller, A., T. Dawson, B. Helmuth, R. S. Hetem, D. Mitchell, and S. K. Maloney. 2010. Physiological mechanisms in coping with climate change. Physiological and Biochemical Zoology 83: 713-720.

Furman, D. P., R. D. Young, and P. E. Catts. 1959. *Hermetia illucens* (Linnaeus) as a factor in the natural control of *Musca domestica* Linnaeus. Journal of Economic Entomology 52: 917-921.

Giraldo, D., A. Adden, I. Kuhlemann, H. Gras, and B. R. Geurten. 2019. Correcting locomotion dependent observation biases in thermal preference of Drosophila. Scientific Reports 9: 3974.

Giunti, G., O. Campolo, F. Laudani, and V. Palmeri. 2018. Male courtship behaviour and potential for female mate choice in the black soldier fly *Hermetia illucens* L.(Diptera: Stratiomyidae). Entomologia Generalis 38: 29-46.

Gligorescu, A., S. Toft, H. Hauggaard-Nielsen, J. A. Axelsen, and S. A. Nielsen. 2018. Development, metabolism and nutrient composition of black soldier fly larvae (*Hermetia illucens*; Diptera: Stratiomyidae) in relation to temperature and diet. Journal of Insects as Food and Feed 4: 123-133.

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

Heinrich, B. 2013. The hot-blooded insects: strategies and mechanisms of thermoregulation, Springer Science & Business Media.

Hertz, P. E., R. B. Huey, and R. Stevenson. 1993. Evaluating temperature regulation by field-active ectotherms: the fallacy of the inappropriate question. The American Naturalist 142: 796-818.

Hoc, B., G. Noël, J. Carpentier, F. Francis, and R. C. Megido. 2019. Optimization of black soldier fly (*Hermetia illucens*) artificial reproduction. PloS one 14: e0216160.

Hogsette, J. A. 1992. New diets for production of house flies and stable flies (Diptera: Muscidae) in the laboratory. Journal of Economic Entomology 85: 2291-2294.

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

Holmes, L. A., S. L. VanLaerhoven, and J. K. Tomberlin. 2017. Photophase Duration Affects Immature Black Soldier Fly (Diptera: Stratiomyidae) Development. Environmental Entomology 46: 1439-1447.

Houston, B. J., B. Nixon, J. H. Martin, G. N. De Iuliis, N. A. Trigg, E. G. Bromfield, K. E. McEwan, and R. J. Aitken. 2018. Heat exposure induces oxidative stress and DNA damage in the male germ line. Biology of Reproduction 98: 593-606.

Huey, R. B., and R. Stevenson. 1979. Integrating thermal physiology and ecology of ectotherms: A discussion of approaches. American Zoologist 19: 357-366.

Jervis, M. A., G. E. Heimpel, P. N. Ferns, J. A. Harvey, and N. A. Kidd. 2001. Life-history strategies in parasitoid wasps: A comparative analysis of 'ovigeny'. Journal of Animal Ecology 70: 442-458.

Jucker, C., D. Lupi, S. Savoldelli, and M. G. Leonardi. 2019. Feeding in the adult of *Hermetia illucens* (Diptera Stratiomyidae): Reality or fiction? Journal of Entomological and Acarological Research 51:1.

Kindle, T. K., K. M. Johnson, T. M. Ivy, C. B. Weddle, and S. K. Sakaluk. 2006. Female mating frequency increases with temperature in two cricket species, *Gryllodes sigillatus* and *Acheta domesticus* (Orthoptera: Gryllidae). Canadian Journal of Zoology 84: 1345-1350.

Kirchhof, S., R. S. Hetem, H. M. Lease, D. B. Miles, D. Mitchell, J. Müller, M. O. Rödel, B. Sinervo, T. Wassenaar, and I. W. Murray. 2017. Thermoregulatory behavior and high thermal preference buffer impact of climate change in a Namib Desert lizard. Ecosphere 8(12): e02033.

Larsson, F., and V. Kustvall. 1990. Temperature reverses size-dependent male mating success of a cerambycid beetle. Functional Ecology 4: 85-90.

Lin, Y. 2016. Rearing Black Soldier Fly to Supplement Natural Populations in Waste Composting Systems. Clemson University.

Makkar, H. P., G. Tran, V. Heuzé, and P. Ankers. 2014. State-of-the-art on use of insects as animal feed. Animal Feed Science and Technology 197: 1-33.

Malawey, A. S., D. Mercati, C. C. Love, and J. K. Tomberlin. 2019. Adult Reproductive Tract Morphology and Spermatogenesis in the Black Soldier Fly, *Hermetia illucens* (L.) (Diptera: Stratiomyidae). Annals of the Entomological Society of America. (in press).

Martin, T. L., and R. B. Huey. 2008. Why "suboptimal" is optimal: Jensen's inequality and ectotherm thermal preferences. The American Naturalist 171: E102-E118.

McAfee, A., H. Higo, R. Underwood, J. Milone, L. J. Foster, M. M. Guarna, J. S. Pettis, and D. R. Tarpy. 2019. Queen honey bees combat heat stress-induced loss of stored sperm viability with ATP-independent heat shock proteins. bioRxiv: 627729.

McHugh, D., and J. Gil. 2018. Senescence and aging: Causes, consequences, and therapeutic avenues. Journal of Cell Biology 217: 65-77.

Mertenat, A., S. Diener, and C. Zurbrügg. 2019. Black Soldier Fly biowaste treatment–Assessment of global warming potential. Waste Management 84: 173-181.

Miranda, C. D., J. A. Cammack, and J. K. Tomberlin. 2019. Life-History Traits of the Black Soldier Fly, *Hermetia illucens* (L.)(Diptera: Stratiomyidae), Reared on Three Manure Types. Animals 9: 281.

Nakamura, S., R. T. Ichiki, M. Shimoda, and S. Morioka. 2016. Small-scale rearing of the black soldier fly, *Hermetia illucens* (Diptera: Stratiomyidae), in the laboratory: Low-cost and year-round rearing. Applied Entomology and Zoology 51: 161-166.

Newson, R. 2002. Parameters behind "nonparametric" statistics: Kendall's tau, Somers' D and median differences. The Stata Journal 2: 45-64.

Oliveira, F., K. Doelle, and R. Smith. 2016. External morphology of *Hermetia illucens* Stratiomyidae: Diptera (L. 1758) based on electron microscopy. Annual Research & Review in Biology 9(5): 1-10.

Paoli, F., M. Gottardo, R. Dallai, and P. F. Roversi. 2013. Morphology of the male reproductive system and sperm ultrastructure of the egg parasitoid *Gryon pennsylvanicum* (Ashmead)(Hymenoptera, Platygastridae). Arthropod Structure & Development 42: 297-308.

Parra, J. R. P. 2010. Mass rearing of egg parasitoid for biological control programs, pp. 267–292. In F L Consoli, J R P Parra, R A Zucchi (eds.), Egg parasitoid in agroecosystems with emphasis on Trichogramma. Progress in biological control, Springer, Dordrecht, Netherlands.

Paz, A. S. P., N. S. Carrejo, and C. H. G. Rodríguez. 2015. Effects of larval density and feeding rates on the bioconversion of vegetable waste using black soldier fly larvae *Hermetia illucens* (L.),(Diptera: Stratiomyidae). Waste and Biomass Valorization 6: 1059-1065.

Pleissner, D., and B. A. Rumpold. 2018. Utilization of organic residues using heterotrophic microalgae and insects. Waste Management 72: 227-239.

Rikke, B. A., and T. E. Johnson. 2004. Lower body temperature as a potential mechanism of life extension in homeotherms. Experimental Gerontology 39: 927-930.

Rinehart, J. P. 2000. Molecular and organismal aspects of insect diapause and acquired resistance to temperature stress. The Ohio State University.

Rozkošný, R. 1982. A biosystematic study of the European Stratiomyidae (Diptera). Volume 1. Introduction, Beridinae, Sarginae, Stratiomyinae. The Hague, Netherlands.

Rubink, W. L. 1987. Thermal ecology of the screwworm larva, Cochliomyia hominivorax (Coquerel)(Diptera: Calliphoridae). Environmental Entomology 16: 599-604.

Sales, K., R. Vasudeva, M. E. Dickinson, J. L. Godwin, A. J. Lumley, Ł. Michalczyk, L. Hebberecht, P. Thomas, A. Franco, and M. J. Gage. 2018. Experimental heatwaves compromise sperm function and cause transgenerational damage in a model insect. Nature Communications 9: 4771.

Schilman, P., and C. Lazzari. 2004. Temperature preference in *Rhodnius prolixus*, effects and possible consequences. Acta Tropica 90: 115-122.

Sepil, I., B. R. Hopkins, R. Dean, E. Bath, S. Friedman, B. Swanson, H. Ostridge, N. Buehner, M. Wolfner, and R. Konietzny. 2019. An exposition of ejaculate senescence and its inhibition in Drosophila. BioRxiv: 624734.

Sheppard, D. C., J. K. Tomberlin, J. A. Joyce, B. C. Kiser, and S. M. Sumner. 2002. Rearing methods for the black soldier fly (Diptera: Stratiomyidae). Journal of Medical Entomology 39: 695-698.

Shumo, M., F. M. Khamis, C. M. Tanga, K. K. Fiaboe, S. Subramanian, S. Ekesi, A. van Huis, and C. Borgemeister. 2019. Influence of temperature on selected life-history traits of black soldier fly (*Hermetia illucens*) reared on two common urban organic waste streams in kenya. Animals 9: 79.

Stadtlander, T., A. Stamer, A. Buser, J. Wohlfahrt, F. Leiber, and C. Sandrock. 2017. *Hermetia illucens* meal as fish meal replacement for rainbow trout on farm. Journal of Insects as Food and Feed 3: 165-175.

Stürup, M., B. Baer-Imhoof, D. R. Nash, J. J. Boomsma, and B. Baer. 2013. When every sperm counts: Factors affecting male fertility in the honeybee *Apis mellifera*. Behavioral Ecology 24: 1192-1198.

Thomas, M. B., and S. Blanford. 2003. Thermal biology in insect-parasite interactions. Trends in Ecology & Evolution 18: 344-350.

Tomberlin, J. K., and D. C. Sheppard. 2001. Lekking behavior of the black soldier fly (Diptera: Stratiomyidae). Florida Entomologist 84: 729.

Tomberlin, J. K., and D. C. Sheppard. 2002. Factors influencing mating and oviposition of black soldier flies (Diptera: Stratiomyidae) in a colony. Journal of Entomological Science 37: 345-352.

Tomberlin, J. K., D. C. Sheppard, and J. A. Joyce. 2002. Selected life-history traits of black soldier flies (Diptera: Stratiomyidae) reared on three artificial diets. Annals of the Entomological Society of America 95: 379-386.

Tomberlin, J. K., P. H. Adler, and H. M. Myers. 2009. Development of the black soldier fly (Diptera: Stratiomyidae) in relation to temperature. Environmental Entomology 38: 930-934.

Tran, G., V. Heuzé, and H. Makkar. 2015. Insects in fish diets. Animal Frontiers 5: 37-44.

ur Rehman, K., A. Rehman, M. Cai, L. Zheng, X. Xiao, A. A. Somroo, H. Wang, W. Li, Z. Yu, and J. Zhang. 2017. Conversion of mixtures of dairy manure and soybean curd residue by black soldier fly larvae (*Hermetia illucens* L.). Journal of Cleaner Production 154: 366-373.

Vollmer, J., P. Sarup, C. Kaersgaard, J. Dahlgaard, and V. Loeschcke. 2004. Heat and cold-induced male sterility in *Drosophila buzzatii*: Genetic variation among populations for the duration of sterility. Heredity 92(3): 257-262.

Walsh, B. S., S. R. Parratt, A. A. Hoffmann, D. Atkinson, R. R. Snook, A. Bretman, and T. A. Price. 2019. The impact of climate change on fertility. Trends in ecology & evolution.

Yamamoto, A. H. 1994. Temperature preference of *Drosophila immigrans* and *D. virilis*: Intra-and inter-population genetic variation. The Japanese Journal of Genetics 69: 67-76.

Zeng, B., W. Zhu, Y. Fu, and S. Zhou. 2018. Influence of high-temperature exposure on the mating, oviposition and thermotaxis of *Bactrocera cucurbitae* (Coquillet)(Diptera: Tephritidae). PloS one 13: e0204065.

Zizzari, Z. V., and J. Ellers. 2011. Effects of exposure to short-term heat stress on male reproductive fitness in a soil arthropod. Journal of Insect Physiology 57: 421-426

Table 1. Quartile (Q1, Q2, and Q3)* and the interquartile range (IQR) of the temperature selection by *H. illucens* (N= 280 (♂), 208(♀) per trial (n= 2)) adults across ages examined (24-168-h-old) with a thermogradient assay.

Sex	Q1 (℃)	Q2 (℃)	Q2 (℃)	IQR (Lower) Q1-Q2	IQR (Upper) Q2-Q3
Males	16.6	22.3	28.6	5.7	6.3
Females	16.4	21.5	25.4	5.1	3.9

*Q1 = Lower 25% T_{set}; Q2 = median T_{set} (T_{sel}); Q3 = 75% Upper T_{set}.

Table 2. Quartile (Q1, Q2, and Q3)* and interquartile range (IQR) of the temperature preferences by adult male *H. illucens* (N= 10 per age/replicate (n= 4)/trial (n= 2)) at different ages (h) as determined with a thermogradient assay.

Age (h)	*Q1 (℃)	Q2 (℃)	Q3 (℃)	IQR (Lower) Q1-Q2	IQR (Upper) Q2-Q3
<24	19.3	24.3	28.2	5.0	3.9
48	16.2	21.6	25.0	5.4	3.4
72	14.3	20.9	24.2	6.6	3.3
96	19.7	22.7	24.5	3.0	1.8
120	16.6	22.5	24.7	5.9	2.2
144	16.4	21.9	24.6	5.5	2.7
168	20.6	23.6	26.3	3.1	2.6

*Q1 = Lower 25% T_{set}; Q2 = median T_{set} (T_{sel}); Q3 = 75% Upper T_{set}.

Table 3. Quartile (Q1, Q2, and Q3)* and interquartile range (IQR) temperature preferences by adult female *H. illucens* (N= 10 per age/replicate (n= 4)/trial (n= 2)) at different ages (h) as determined with a thermogradient assay.

Age (h)	Q1 (℃)	Q2 (℃)	Q3 (℃)	IQR (Lower) Q1-Q2	IQR (Upper) Q2-Q3
24	15.4	20.2	26.2	4.8	6.0
48	18.2	24.0	26.7	5.8	2.6
72	15.2	20.7	23.4	5.5	2.7
96	16.7	21.4	24.3	4.7	2.9
120	17.0	21.3	24.0	4.3	2.7
144	14.5	22.1	27.2	7.6	5.2
168	17.5	21.7	27.0	4.2	5.3

*Q1 = Lower 25% T_{set}; Q2 = median T_{set} (T_{sel}); Q3 = 75% Upper T_{set}.

Table 4. Quartile (Q1, Q2, and Q3)* and interquartile range (IQR) for sperm counts (N) of adult male *H. illucens* (N= 11 per age/trial (n= 2)) at different ages (h) held at 27.0°C ± 1.7, 70% RH, and 12:12 L:D. IQR: median-Q1 (lower T_{set}); IQR: Q3-median (Upper T_{set}).**

Age (h)	Q1	Q2	Q3	IQR (Lower) Q1-Q2	IQR (Upper) Q3-Q2
24	95.5	292.5	743.0	197.0	450.5
48	214.5	322.5	429.3	108.0	106.8
72	103.3	186.0	204.5	82.8	18.5
96	125.0	283.5	445.3	158.5	161.8
120	166.8	223.5	475.5	56.8	252.0
144	120.3	174.5	240.3	54.3	65.8
168	96.0	167.0	275.3	71.0	108.3

*Q1 = Lower 25% T_{set}; Q2 = median T_{set} (T_{sel}); Q3 = 75% Upper T_{set}.

** The semen from three males per age category/temperature were pooled following procedures adapted from (Eckel et al., 2017).

Table 5. Quartile (Q1, Q2, and Q3)* and the interquartile range (IQR) for sperm viability (%) displayed by adult male *H. illucens* (N= 11 per age/trial (n= 2)) at different ages (h) held at 27.0°C ± 1.7, 70% RH, and 12:12 L:D. IQR: median-Q1 (lower T_{set}); IQR: Q3-median (Upper T_{set}.).**

Age (h)	Q1	Q2	Q3	IQR (Lower) Q1-Q2	IQR (Upper) Q3-Q2
24	50.5	71.6	77.8	21.1	6.2
48	70.5	78.3	81.2	7.7	3.0
72	50.4	60.9	65.8	10.5	4.9
96	62.2	70.4	85.0	8.1	14.7
120	69.1	73.2	82.9	4.1	9.7
144	55.9	59.5	63.1	3.7	3.6
168	64.7	73.9	81.2	9.2	7.4

*Q1 = Lower 25% T_{set}; Q2 = median T_{set} (T_{sel}); Q3 = 75% Upper T_{set}.

** The semen from three males per age category/temperature were pooled following procedures adapted from (Eckel et al., 2017).

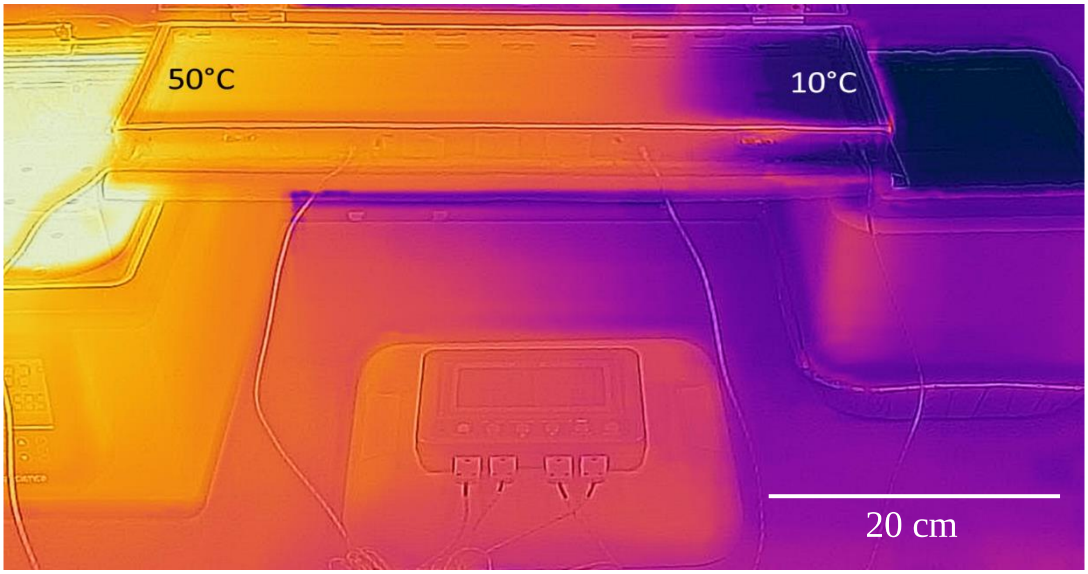

Figure 3- 1. Thermal gradient design used to assess thermal preference of *H. illucens* adult males and females. Note the thermal gradient represented by the arrow pentagon created by a water bath and an ice bucket placed in each extremity of the metal plate, providing a temperature range of 10-50 °C (± 2°C). The temperatures of the gradient were recorded using four equidistant fine-wire fast-response digital thermocouples affixed to the aluminum plate. (Photography: Travis W. Rusch)

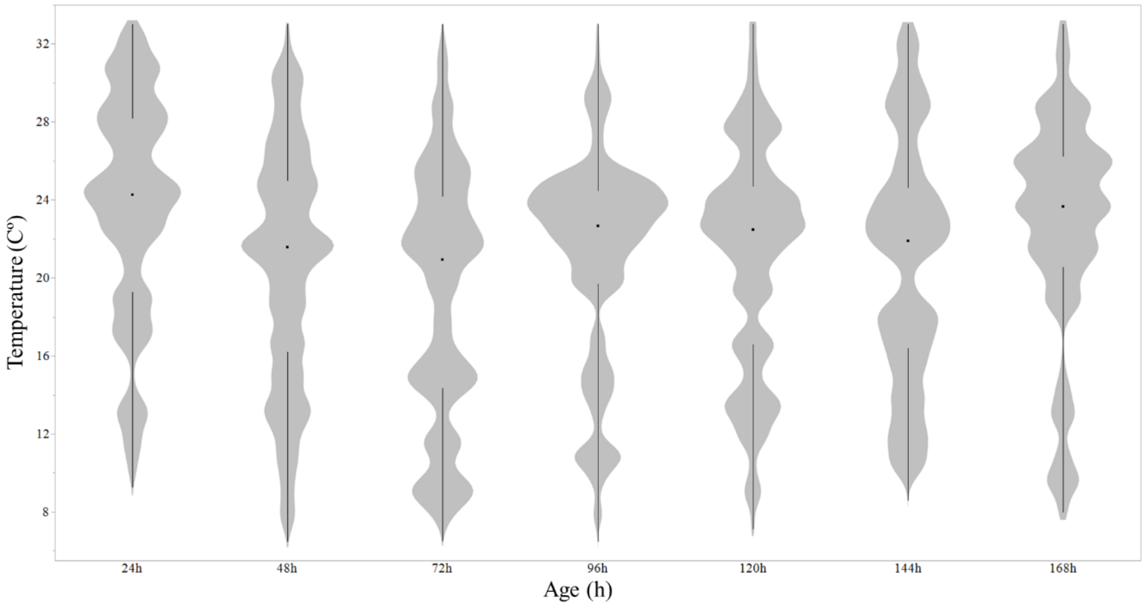

Figure 3- 2. Violin plots of the thermal preference (*T_{sel}*) represented by the median for aging (h) adult male of *H. illucens* at 27.0°C ± 1.7, 70% RH, and 12:12 L:D. The variance is given in interquartile range (IQR); IQR: median-Q1 (lower values); IQR: Q3-median (Upper values).

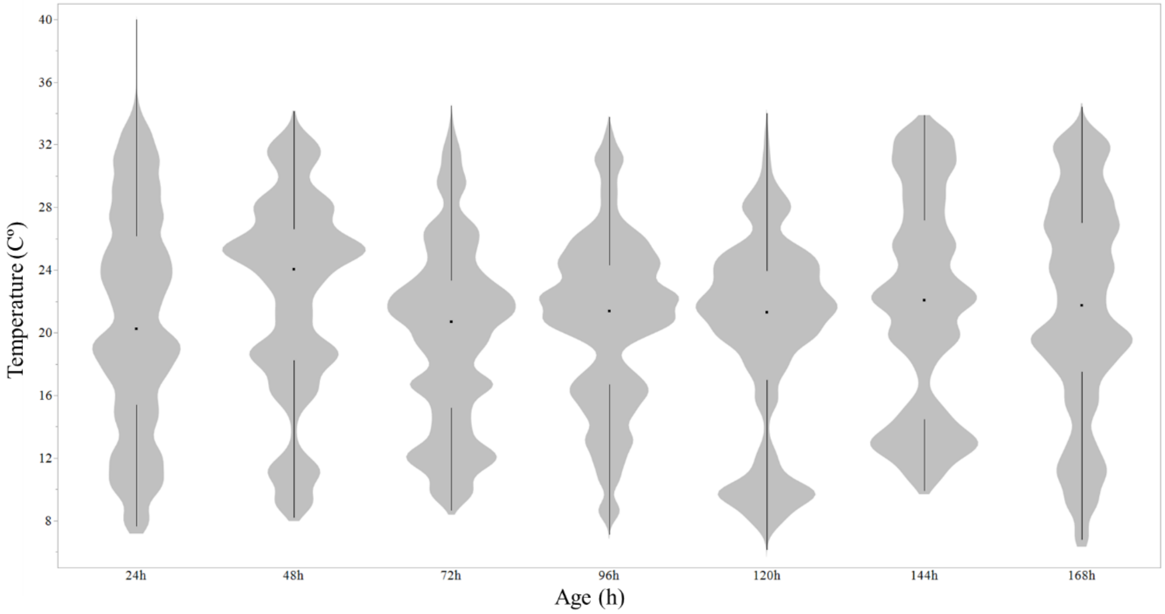

Figure 3- 3. Violin plots of thermal preference (*T_{sel}*) represented by the median for aging (h) adult female of *H. illucens* at 27.0°C ± 1.7, 70% RH, and 12:12 L:D. The variance is given in interquartile range (IQR); IQR: median-Q1 (lower values); IQR: Q3-median (Upper values).

Figure 3- 4. Violin plots of sperm count represented by the median for aging (h) adult male of *H. illucens* at 27.0°C ± 1.7, 70% RH, and 12:12 L:D. The variance is given in interquartile range (IQR); IQR: median-Q1 (lower values); IQR: Q3-median (Upper values).

Figure 3- 5. Violin plots of sperm viability represented by the median for aging (h) adult male of *H. illucens* at 27.0°C ± 1.7, 70% RH, and 12:12 L:D. The variance is given in interquartile range (IQR); IQR: median-Q1 (lower values); IQR: Q3-median (Upper values).

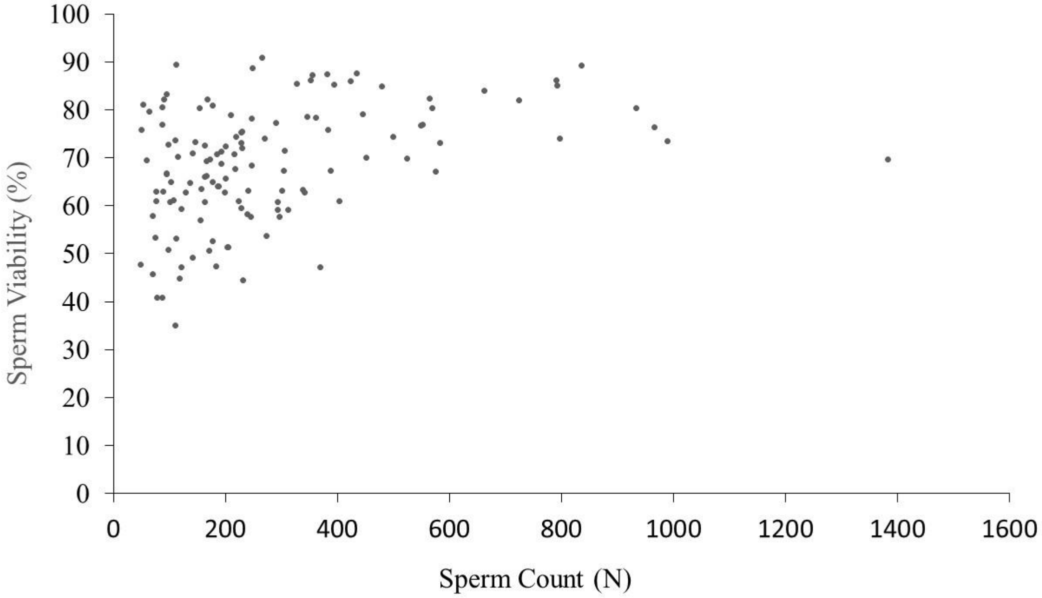

Figure 3- 6. Correlation between sperm count and sperm viability for aging (h) adult male of *H. illucens* at 27.0°C ±1.7, 70% RH, and 12:12 L:D. Spearman's correlation (*r*)= 0*.3961*, p < *0.0001*.

4. CONCLUSIONS

4.1. Summary and Future Studies

The initial two studies completed as part of my dissertation provided descriptions of various morphological elements of the adult male and female black soldier fly (BSF), *Hermetia illucens* (L.) (Diptera: Stratiomyidae). I provided the first morphological description of the adult male BSF reproductive tract (Chapter 2), spermatogenesis process, as well as the size and ultrastructure of the spermatozoon (Chapter 2). I determined the ultrastructure arrangement of the axoneme (9+2+2), shape (elliptical) and size of the sperm head (~8µ), flagellum length (~860 µm), and shape of the spermatheca capsules (semi-spherical) (Chapter 2). Furthermore, I expanded my work by providing images of the BSF spermatheca, which confirmed a previous description by Ururahy-Rodrigues et al. (2000).

The third and fourth studies completed as part of my dissertation determined adult male and female temperature preferences as they aged. The central 90% thermal preference (Tsel) for males (28.6℃ ± 1.8) was lower than that recorded for females (30.1℃ ± 1.9). Furthermore, male and female preferences shifted as they aged to cooler temperatures and then over time returned to temperatures preferred initially (Males: 24-h (30.8℃), 48-h (28.6℃), 72-h (26.5℃), 96-h (25.6℃), 120-h (27.9℃), 144-h (29.3℃) and 168-h-old (28.9℃); Females: 24-h (30.6℃), 48-h (30.1℃), 72-h (29.9℃), 96-h (26.5℃), 120-h (27.0℃), 144-h (31.1℃) and 168-h-old (30.5℃)) (Chapter 3).

Based on data generated through my research, I hypothesize the shift in Tsel to lower temperatures (from 30.8°C to 25.6 °C) as males initially age could enhance sperm longevity (McAfee et al. 2019). Such a strategy could also conserve fat (i.e., energy) (Reinhold 1999) by reducing metabolism (Kührt et al. 2006), which could allow males to be more competitive for mates over a greater period of time. Interestingly, virgin females selecting warmer Tsel during the initial 48-h than males, which could enhance egg production. In order to elucidate the relevance of these data as related to mass production of BSF, future investigations should assess metabolic responses (i.e., CO2 emission) (Reinhold 1999, Käfer et al. 2015) in combination with sperm viability and production (McAfee et al. 2019) as well as egg production (Rosenheim and Rosen 1991, Berger et al. 2008) when experiencing different temperature regimes other than those used in the current work.

The fifth study completed as part of my dissertation examined the impact of age on sperm count and percent viability. Of the age groups tested, males 48-h-old had the highest sperm count and viability (Chapter 3), which is when males have the highest frequency of mating after emergence (Tomberlin and Sheppard 2002). The sixth study examined the relationship between age and temperature (i.e., thermal stress) experienced as related to sperm count and percent viability. Unfortunately, the temperatures explored did not have a significantly impact on either variable (Chapter 3).

Data generated from my research adds substantially to our understanding of adult BSF biology. Surprisingly, few studies to date have been conducted on adult BSF. Scant data are available on the external morphology of males and females (Oliveira et al. 2016, Birrell 2018), reproductive physiology (Oonincx et al. 2016, Heussler et al. 2018, Bertinetti et al. 2019), adult behavior (Tomberlin and Sheppard 2001, 2002, Giunti et al. 2018, Hoc et al. 2019), and digestion capability (Bertinetti et al. 2019, Bruno et al. 2019). Regarding the BSF reproductive morphology, Tomberlin and Sheppard (2002) represents the only study, to my knowledge, describing the female reproductive tract and germ cell maturation process.

Data provided in Chapters 2 and 3 could potentially be used to optimize BSF production in colony. Current methods result in adult males and females of different ages being mixed within a cage set at a single temperature. Given that male BSF have a spermatogenic index close to 1, which indicates males emerge with most of their sperm being mature, this species can be classified as synspermatogenic (Boivin and Ellers 2016). This information indicates the introduction of females not receptive to mating (i.e., 24-48-h-old) potentially results in low mating success, wasted energy, and consequently, low production of fertilized eggs (Giebultowicz et al. 1990, Ringo 1996, Cangussu and Zucoloto 1997). Furthermore, data presented in Chapter 3 demonstrate young males have a higher sperm count and viability in comparison with older BSF males. One thing to note is the sperm count and viability assessment were made with virgin males removed from the presence of females, which is a limitation. Hence, future work should explore the impact of detecting female presence on sperm production and maintenance as the threat of sperm competition could induce significant shifts (Parker et al. 1997).

Based on current mass production practices, and as previously indicated in the previous paragraph, young and old males are forced together within a cage. In instances where older males do not possess a full complement of sperm, their value is limited due to low number of eggs being fertilized after mating (Paoli et al. 2013). This physiological aspect of the BSF resonates even more when one considers females typically mate once (Tomberlin and Sheppard 2002, Giunti et al. 2018). The updated description of the spermatheca morphology of BSF presented in Chapter 2 supports the previous reports about the monogamic nature of females since spheroid spermatheca and being monogamic were described to be positively correlated (Walker 1980). Further studies with wild population may provide clues on why females display a monogamic, rather than polyandric, mating system, which would enhance their fitness by increase the genetic variability of their offspring (Arnqvist and Nilsson 2000).

Based on previous work by Tomberlin and Sheppard (2001), females depart lekking sites once mated, suggesting current methods of forces occupation is not conducive for optimal egg production. Thus, age variation and mating history of adults in cages could impact the success of the facility as far as producing fertile eggs. Future studies might attempt to integrate methods for segregating age cohorts as a means of paring males and females when both are optimal for mating. A stabilized colony is imperative for the development and management of an industrial-scale larval production. Predictably producing a constant number of fertile eggs on a regular basis is the cornerstone of any BSF factory. Data provided in Chapters 2 and 3 regarding male production of sperm and the thermal preference of males and females through senescence could aid in enhancing egg production. Moreover, current techniques used to mass produced BSF (i.e., small cages, artificial diets, and reduced genetic variability; (Tomberlin et al. 2002, Azrag et al. 2016, Nakamura et al. 2016), respectively) may hamper production due to less than suitable conditions for their reproductive physiology and behavior (i.e., males lekking, monogamic females). For instance, small size cages do not allow recently emerged females to mature in isolation from reproductive males; such engagements could select for a simple swarming aggregation pattern, as observed in mosquitos (Diptera: Culicidae) (Helinski and Harrington 2012) and mayflies (Ephemeroptera) (Vilela and Sanmartín-Villar 2019), where high levels of male harassment reduce female choice. If segregation based on age is not possible, keeping a greater proportion of high-quality males in the colony under an operational sex ratio male-biased could prevent abrupt drop-offs in fertile egg production (Nakamura et al. 2016, Hoc et al. 2019). Understand the rules of the system (i.e., sperm morphology, physiology) and how it operates under certain conditions (i.e., environment stress, nutrient restriction) are the pillars to successfully up scale the production of BSF in an industrial setting.

All literature available, to my knowledge, on the BSF outside of Tomberlin and Sheppard (2001) and Birrell (2018) is based on laboratory colonies. As stated previously, additional research is needed on wild BSF populations to determine the accuracy of the published literature. For example, some speculate adults do not need to feed and rely on reserves accumulated during the larval stage (Newton et al. 2005). Non-feeding adults presumably evolved from free-living ancestral species that were capable of growth and reproduction on stored, desiccated and often nutritionally deficient foodstuffs (Levinson and Levinson 1995). Rozkošný (1982) described Stratiomyidae flies as nectar-feeders, presenting sponge-like mouthparts, a structure later confirmed by Oliveira et al. (2016), where both sexes obtain carbohydrates for short-term energy needs, especially during periods of peak activity such as swarming, mating and oviposition, dispersal, and migration (Woodcock et al. 2014).

Besides sugars, nectar contain various amino acids, proteins, lipids, and vitamins (Baker and Baker 1983). It should be noted Bruno et al. (2019) described a prestomal tooth-like structures on the mouthparts of BSF adults that could be used to scrape semi-solid feeding substrates. In fact, histological analysis of the BSF digestive tract, they determined BSF can digest solid food substrates. Point being, additional research is needed to determine if adults actually do feed and if such nutrition bolsters sperm or egg production.

Accordingly, additional studies should determine if providing sex-specific nutrients to adults enhances fertile egg production. The formulation of such diets would need to consider differences in the sexual maturation process. For instance, while males produce sperm during the first phases of pupation (Chapter 2) (Malawey et al. in press), females only mature their germ cells after emergence (Tomberlin et al. 2002); therefore, females may benefit from being provided protein and carbohydrates resources at emergence (Bertinetti et al. 2019) in combination with optimal temperatures (Chapter 3) as it could accelerate egg production. Moreover, the inclusion of amino acids such as methionine into the larval diet might increase fecundity (Chang et al. 2001, Hoedjes et al. 2017). Males might enhance their fitness by increasing sperm viability with inclusion of micronutrients such as amino acids and vitamins at larval stage (Cabrita et al. 2011, Phasomkusolsil et al. 2017).

In summary, the research presented here provides a greater understanding of adult BSF physiology as related to sperm production and viability as well as thermal preference as adults age. And, such data could potentially enhance industrialized production of the BSF. However, this research is only the beginning, and as outlined in this chapter, a tremendous amount of research is still needed on these topics to stabilize the industry as related to egg production.

4.2. References

Arnqvist, G., and T. Nilsson. 2000. The evolution of polyandry: multiple mating and female fitness in insects. Animal Behaviour 60: 145-164.

Azrag, R. S., K. Ibrahim, C. Malcolm, E. El Rayah, and B. El-Sayed. 2016. Laboratory rearing of *Anopheles arabiensis*: impact on genetic variability and implications for Sterile Insect Technique (SIT) based mosquito control in northern Sudan. Malaria Journal 15: 432.

Baker, H. G., and I. Baker. 1983. A brief historical review of the chemistry of floral nectar, pp. 126-152. In B Bentley, T Elias and T S Elias (eds), The biology of nectaries, Columbia University Press, US.

Berger, D., R. Walters, and K. Gotthard. 2008. What limits insect fecundity? Body size-and temperature-dependent egg maturation and oviposition in a butterfly. Functional Ecology 22: 523-529.

Bertinetti, C., A. C. Samayoa, and S.-Y. Hwang. 2019. Effects of Feeding Adults of *Hermetia illucens* (Diptera: Stratiomyidae) on Longevity, Oviposition, and Egg Hatchability: Insights Into Optimizing Egg Production. Journal of Insect Science 19(1): 19.

Birrell, N. 2018. Sensory Morphology, Investment and Plasticity in the Black Soldier Fly (*Hermetia illucens*). ResearchSpace, Auckland.

Boivin, G., and J. Ellers. 2016. Replacing qualitative life-history traits by quantitative indices in parasitoid evolutionary ecology. Entomologia Experimentalis et Applicata 159: 163-171.

Bruno, D., M. Bonelli, A. G. Cadamuro, M. Reguzzoni, A. Grimaldi, M. Casartelli, and G. Tettamanti. 2019. The digestive system of the adult *Hermetia illucens* (Diptera: Stratiomyidae): Morphological features and functional properties. Cell and Tissue Research: 1-18.

Cabrita, E., S. Ma, P. Diogo, S. Martínez-Páramo, C. Sarasquete, and M. T. Dinis. 2011. The influence of certain aminoacids and vitamins on post-thaw fish sperm motility, viability and DNA fragmentation. Animal Reproduction Science 125: 189-195.

Cangussu, J., and F. Zucoloto. 1997. Effect of protein sources on fecundity, food acceptance, and sexual choice by *Ceratitis capitata* (Diptera, Tephritidae). Revista Brasileira de Biologia 57: 611-618.

Chang, C. L., C. Albrecht, S. S. El-Shall, and R. Kurashima. 2001. Adult reproductive capacity of *Ceratitis capitata* (Diptera: Tephritidae) on a chemically defined diet. Annals of the Entomological Society of America 94: 702-706.

Giebultowicz, J. M., A. K. Raina, and E. C. Uebel. 1990. Mated-like behaviour in senescent virgin females of gypsy moth, *Lymantria dispar*. Journal of Insect Physiology 36: 495-498.

Giunti, G., O. Campolo, F. Laudani, and V. Palmeri. 2018. Male courtship behaviour and potential for female mate choice in the black soldier fly *Hermetia illucens* L.(Diptera: Stratiomyidae). Entomologia Generalis 38: 29-46.

Helinski, M. E., and L. C. Harrington. 2012. The role of male harassment on female fitness for the dengue vector mosquito *Aedes aegypti*. Behavioral Ecology and Sociobiology 66: 1131-1140.

Heussler, C. D., A. Walter, H. Oberkofler, H. Insam, W. Arthofer, B. C. Schlick-Steiner, and F. M. Steiner. 2018. Influence of three artificial light sources on oviposition and half-life of the Black Soldier Fly, *Hermetia illucens* (Diptera: Stratiomyidae): Improving small-scale indoor rearing. PloS one 13: e0197896.

Hoc, B., G. Noël, J. Carpentier, F. Francis, and R. C. Megido. 2019. Optimization of black soldier fly (*Hermetia illucens*) artificial reproduction. PloS one 14: e0216160.

Hoedjes, K. M., M. A. Rodrigues, and T. Flatt. 2017. Amino acid modulation of lifespan and reproduction in Drosophila. Current Opinion in Insect Science 23: 118-122.

Käfer, H., H. Kovac, B. Oswald, and A. Stabentheiner. 2015. Respiration and metabolism of the resting European paper wasp (*Polistes dominulus*). Journal of Comparative Physiology B 185: 647-658.

Kührt, U., J. Samietz, and S. Dorn. 2006. Thermal response in adult codling moth. Physiological Entomology 31: 80-88.

Levinson, A., and H. Levinson. 1995. Reflections on structure and function of pheromone glands in storage insect species. Anzeiger für Schädlingskunde, Pflanzenschutz, Umweltschutz 68: 99-118.

Malawey, A. S., D. Mercati, C. C. Love, and J. K. Tomberlin. in press. Adult Reproductive Tract Morphology and Spermatogenesis in the Black Soldier Fly, *Hermetia illucens* (L.) (Diptera: Stratiomyidae). Annals of the Entomological Society of America.

McAfee, A., H. Higo, R. Underwood, J. Milone, L. J. Foster, M. M. Guarna, J. S. Pettis, and D. R. Tarpy. 2019. Queen honey bees combat heat stress-induced loss of stored sperm viability with ATP-independent heat shock proteins. BioRxiv: 627729.

Nakamura, S., R. T. Ichiki, M. Shimoda, and S. Morioka. 2016. Small-scale rearing of the black soldier fly, *Hermetia illucens* (Diptera: Stratiomyidae), in the laboratory: Low-cost and year-round rearing. Applied Entomology and Zoology 51: 161-166.

Newton, L., C. Sheppard, D. W. Watson, G. Burtle, and R. Dove. 2005. Using the black soldier fly, *Hermetia illucens*, as a value-added tool for the management of swine manure. Animal and Poultry Waste Management Center, North Carolina State University, Raleigh, NC 17.

Oliveira, F., K. Doelle, and R. Smith. 2016. External morphology of *Hermetia illucens* Stratiomyidae: Diptera (L. 1758) based on electron microscopy. Annual Research & Review in Biology 9: 1-10.

Oonincx, D., N. Volk, J. Diehl, J. Van Loon, and G. Belušič. 2016. Photoreceptor spectral sensitivity of the compound eyes of black soldier fly (*Hermetia illucens*) informing the design of LED-based illumination to enhance indoor reproduction. Journal of Insect Physiology 95: 133-139.

Paoli, F., M. Gottardo, R. Dallai, and P. F. Roversi. 2013. Morphology of the male reproductive system and sperm ultrastructure of the egg parasitoid *Gryon pennsylvanicum* (Ashmead) (Hymenoptera, Platygastridae). Arthropod Structure & Development 42: 297-308.

Parker, G., M. Ball, P. Stockley, and M. Gage. 1997. Sperm competition games: a prospective analysis of risk assessment. Proceedings of the Royal Society of London. Series B: Biological Sciences 264: 1793-1802.

Phasomkusolsil, S., K. Pantuwatana, J. Tawong, W. Khongtak, Y. Kertmanee, N. Monkanna, S. Khaosanorh, E. W. Wanja, and S. A. Davidson. 2017. Sugar and multivitamin diet effects on the longevity and mating capacity of laboratory-reared male anopheline mosquitoes. Journal of the American Mosquito Control Association 33: 175-184.

Reinhold, K. 1999. Energetically costly behaviour and the evolution of resting metabolic rate in insects. Functional Ecology 13: 217-224.

Ringo, J. 1996. Sexual receptivity in insects. Annual Review of Entomology 41: 473-494.

Rosenheim, J. A., and D. Rosen. 1991. Foraging and oviposition decisions in the parasitoid *Aphytis lingnanensis*: Distinguishing the influences of egg load and experience. The Journal of Animal Ecology 60: 873-893.

Rozkošný, R. 1982. A biosystematic study of the European Stratiomyidae (Diptera). Volume 1. Introduction, Beridinae, Sarginae, Stratiomyinae. The Hague, Netherlands.

Tomberlin, J. K., and D. C. Sheppard. 2001. Lekking behavior of the black soldier fly (Diptera: Stratiomyidae). Florida Entomologist 84: 729.

Tomberlin, J. K., and D. C. Sheppard. 2002. Factors influencing mating and oviposition of black soldier flies (Diptera: Stratiomyidae) in a colony. Journal of Entomological Science 37: 345-352.

Tomberlin, J. K., D. C. Sheppard, and J. A. Joyce. 2002. Selected life-history traits of black soldier flies (Diptera: Stratiomyidae) reared on three artificial diets. Annals of the Entomological Society of America 95: 379-386.

Ururahy-Rodrigues, A., J. Pujol-Luz, and P. Iide. 2000. Notas sobre a espermateca de *Hermetia illucens* (L., 1758)(Diptera, Stratiomyidae). Contribuições avulsas sobre a História Natural do Brasil, Série Zoologia, Seropédica 17: 1-5.

Vilela, D. S., and I. Sanmartín-Villar. 2019. Reproductive Behavior and Sexual Selection, pp. 263-293, Aquatic Insects. Springer.

Walker, W. F. 1980. Sperm utilization strategies in nonsocial insects. The American Naturalist 115: 780-799.

Woodcock, T. S., B. M. Larson, P. G. Kevan, D. W. Inouye, and K. Lunau. 2014. Flies and flowers II: floral attractants and rewards. Journal of Pollination Ecology 12: 63-94.