

Spring 2014

## The Prevalence and Effect of Wolbachia Infection on the Brown Widow Spider (*Latrodectus Geometricus*)

Brittany Dane' Arrington

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THE PREVALENCE AND EFFECT OF *WOLBACHIA* INFECTION ON THE BROWN  
WIDOW SPIDER (*LATRODECTUS GEOMETRICUS*)

by

BRITTANY D. ARRINGTON

(Under the Direction of John Scott Harrison)

ABSTRACT

*Wolbachia* was recently identified in the Brown Widow Spider (*Latrodectus geometricus*). This symbiont has not previously been found in this genus, therefore the consequences on the host's population dynamics are unknown. The frequency of endosymbiont infection can provide insight into the population dynamics of the host as this bacteria is known to alter host life history. In my 1<sup>st</sup> chapter I determine the prevalence of *Wolbachia pipentis* infection in the Brown Widow Spider (*Latrodectus geometricus*) from five locations in the Southeastern United States. Infection frequency differed significantly among sampling locations, ranging from 20% to 92%. These results suggest that the strain is not an obligate symbiont and does not provide a strong fitness benefit to the spider. The observed distribution pattern also suggests recent founder events and possibly limited gene flow among sampled locations as we would expect similar infection frequencies in populations with high gene flow.

*Wolbachia* is known to alter arthropod life history patterns (e.g., male-killing, feminization, induced parthenogenesis, nutrient acquisition, etc.). As this symbiont has not previously been found in the genus *Latrodectus*, the consequences on the spider's life history are unknown. In the 2<sup>nd</sup> chapter, I examine the effects of infection on the spider's reproduction (sex ratio, clutch size, egg size, and egg mass) and juvenile development

time. No significant difference in clutch sex ratio, egg number, egg size, egg mass, or development time was found between infected and uninfected females. These results suggest that the strain is not an obligate symbiont nor does it alter the reproductive biology of the spider, as it does in most species. The lack of effect on the host suggests infection is either: i) neutral, and remains in the population by complete maternal transmission or has reached an equilibrium, or ii) affects a trait that was not analyzed in this study.

**INDEX WORDS:** Brown Widow Spider, Host-parasite Interaction, Infection Prevalence, *Latrodectus geometricus*, Life History, Population Dynamics, Reproduction Manipulation, Sex Ratio

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B.S. Biology, Georgia Southern University, 2011

A Thesis Submitted to the Graduate Faculty of Georgia Southern University in Partial

Fulfillment of the Requirements for the Degree

MASTER OF SCIENCE IN BIOLOGY

STATESBORO, GEORGIA

2014

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Electronic Version Approved:  
May 2014

## ACKNOWLEDGMENTS

I would like to thank my adviser Scott Harrison and committee member Edward Mondor for their assistance and continual support during the completion of this research project and thesis. Without such support I fear a lack of sanity would have resulted. I also wish to thank Kelly McLain for his statistical insight and editorial assistance. I would like to thank Stephanie Harper and Checo Colón-Gaud for allowing me the use of, and assistance with, the aquatic lab's imaging system microscope. I would like to also thank Janice VanDevender and David Rodriguez for their help in specimen collection.

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## INTRODUCTION

Endocellular bacteria affect their host in a multitude of ways. Some provide protection from parasites, like in Pea Aphids (*Acyrtosiphon pisum*) (Oliver, *et al.*, 2003, 2005). Others provide their host with nutrients not otherwise found in their diet (Bed Bugs, Tsetse Flies, and Pea Aphids) (Hosokawa, 2010; Nogge, 1982; Koga, 2003). One such bacteria, *Wolbachia pipentis* Hertig & Wolbach 1924, is a known manipulator of host reproduction that infects arthropods (Werren, 1997, 2008; Jeyaprakash, 2000) and filarial nematodes (Bandi *et al.*, 1998). It has been estimated to infect >65% of all insect species (Werren, 2008).

*Wolbachia* is a maternally-inherited bacterium that resides in the cytoplasm of somatic and egg cells (Shoemaker *et al.*, 2001). As males do not transmit the bacterium, and thus do not contribute to the bacterial fitness, this bacterium has developed multiple mechanisms to proliferate itself through the population by increasing the number of infected females in the population (Werren, 2008). Some of these mechanisms include: i) male killing, where male embryos fail to develop (Sakamoto, 2011); ii) induced parthenogenesis, where female haplodiploid virgins produce daughters (Stouthamer & Hamilton, 1990; Russell & Stouthamer, 2011); and iii) male feminization, where genetic males develop as functional females (Werren, 1997, 2008). These effects result in a female-biased sex ratio which increase the number of infected females in the population, thus increasing the prevalence of the bacteria (Dyer & Jenkins, 2004; Stouthamer *et al.*, 1990; Turelli, 1994; Charlat, 2004). *Wolbachia* infection has also been linked to increased fecundity (Girin & Bouleteau, 1995; Xie *et al.*, 2011), fitness (Dobson *et al.*,

2004), and life span (Dobson *et al.*, 2002). These effects certainly influence the host's ability to thrive, especially in regards to invasive species where a benefit or cost to host fitness would affect the host's ability to successfully expand its range (Reuter *et al.*, 2005; Shoemaker *et al.*, 2000; Tsutsui *et al.*, 2003).

*Wolbachia pipentis* was recently identified in the invasive Brown Widow Spider *Latrodectus geometricus* C. L. Koch 1841. Thought to have originated in South Africa, the Brown Widow is found on every continent except Antarctica (Garb *et al.*, 2004). In the U.S., its distribution was limited to South Florida for ~50 years, but has spread across the Southeast in the past 15 years (Brown *et al.*, 2008). Human transport plays a large role in this spread, but an endosymbiont infection could possibly aid its invasion should infection provide a fitness benefit. *Wolbachia* has not previously been recorded in the genus *Latrodectus*, and this study documents the first identification in this genus.

As this is the first report of infection in the Brown Widow, studies on the prevalence and effect of infection must be completed in order to fully understand this relationship. In the present study, my objectives were to determine i) the frequency of *Wolbachia* infection in Brown Widow Spiders from five locations of the Southeastern United States and ii) the effects of infection of the spider's reproduction and development. I found that i) infection was present in all sampled locations, but the proportion of infected individuals varied among populations and ii) infection did not affect reproduction or development in the Brown Widow Spider.

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## CHAPTER 1

Prevalence of *Wolbachia* Infection in the Brown Widow Spider (*Latrodectus geometricus*) from five locations of the Southeastern U.S.

### ABSTRACT

The frequency of endosymbiont infection can provide insight into the population dynamics of the host. *Wolbachia* was recently identified in the Brown Widow Spider (*Latrodectus geometricus*). This symbiont has not been previously found in this genus, therefore the consequences on the host's population dynamics are unknown. This study describes the prevalence and distribution of *Wolbachia* infections within and among Brown Widow populations in the Southeastern United States. Infection frequency differed significantly among sampling locations ranging from 20% to 92% ( $\chi^2 = 49.56$ ,  $p < 0.0001$ ). These results suggest that the strain is not an obligate symbiont and does not provide a fitness benefit. The observed distribution pattern is also consistent with limited migration and gene flow among sampled locations. These data suggest multiple founder events during the spider's invasion.

### INTRODUCTION

The population dynamics of a species can be seen as the population grows, remains stable, or goes extinct. These dynamics are affected by population size, which is influenced by the number of births, deaths, and the amount of migration in and out of the population. Factors that influence these values would also affect the stability of the population. One such factor is the infection of symbiotic endocellular bacteria (Werren,



2008). Many symbiont species are beneficial and provide their host with nutrients (e.g., Tsetse Fly and Pea Aphid) (Nogge, 1982; Koga, 2003) and increased number of offspring (e.g., Sweet Potato Fly) (Himler, 2011). These benefits would likely increase survival and/or number of young, adding to the population size. The frequency of endosymbiont infection can thus provide insight into the population dynamics of its host. One such bacteria, *Wolbachia pipentis*, has been found to infect invasive species such as the Argentine Ant, *Linepithema humile* (Reuter, 2005 & Tsutsui, 2003), the European Paper Wasp, *Polistes dominulus* (Stahlhut, 2006), and the Kudzu bug, *Megacopta cribraria* (Jenkins, 2011), and has been estimated to infect >65% of all insect species (Werren, 2008).

*Wolbachia pipentis* is an endocellular bacterial symbiont that infects filarial nematodes (Bandi *et al.*, 1998) and arthropods (Werren, 1997, 2008; Jeyaprakash, 2000). The bacterium resides in the cytoplasm of host cells and is inherited maternally through egg cytoplasm during meiosis (Shoemaker *et al.*, 2000). As sperm do not contribute cytoplasm to the zygote, infected males do not pass the bacteria on to offspring and thus do not contribute to the bacterium's fitness (Werren, 2008). *Wolbachia* has evolved multiple strategies to increase its fitness by manipulating the host. These manipulations include: i) male killing (Sakamoto, 2011), where only female embryos develop (Werren, 1997, 2008; Stouthamer *et al.*, 1999; Hurst *et al.*, 1999); ii) induction of parthenogenesis, where in haplodiploid species, *Wolbachia* causes the genome to double in unfertilized eggs leading to infected virgin females producing only infected daughters (Stouthamer & Hamilton, 1990; Russell & Stouthamer, 2011); and iii) male feminization (Narita *et al.*, 2011), whereby genetic males are hormonally induced to develop into functional and

reproductive females (Warren, 1997; Warren, 2008; Stouthamer *et al.*, 1999). These three mechanisms result in a female-biased sex ratio and have been documented in organisms such as the butterflies *Eurema hecabe* (Narita *et al.*, 2011), *Zizina emelina* (Sakamoto *et al.*, 2011), and *Talicauda nyseus* (Ankola *et al.*, 2011), Pillbugs, *Armadillidium vulgare* (Verne *et al.*, 2012), and the Two-Spot Ladybird, *Adalia bipunctata* (Majerus *et al.*, 2000). These symbiont-induced effects ensure that the majority of offspring produced are infected females, thus increasing the prevalence of the bacteria in the host population (Dyer & Jaenike, 2004; Stouthamer *et al.*, 1990; Turelli 1994; Charlat *et al.*, 2004). *Wolbachia* has also been documented to provide the host with resistance to RNA viruses (Hedges *et al.*, 2008), and an increase in fecundity (Girin & Bouletreau, 1995; Xie *et al.*, 2011), fitness (Dobson *et al.*, 2004), and life span (Dobson *et al.*, 2002). Given this, it is possible such beneficial effects can contribute to its host's successful invasion of novel habitats.

Native to South Africa, the Brown Widow spider (*L. geometricus*) has been introduced to every continent except Antarctica (Garb *et al.*, 2004). In the U.S., its distribution was limited to South Florida for ~50 years, but over the last 15 years it has become well established in the Southeastern U.S. (Brown *et al.*, 2008) and Southern California (Vincent *et al.*, 2004). Its rapid spread has been attributed to human transport (Vetter, 2012), but other biological aspects such as the presence of endosymbionts could aid in its successful invasion. *Wolbachia* has not previously been found in the genus *Latrodectus*. *Latrodectus hasselti* (Redback Spider) has been screened for, but did not show any infection (Rowley, 2004). This study is the first to identify *Wolbachia* in *L. geometricus* specimens.

*Wolbachia* is classified into separate strains that are divided into supergroups (A-H) (Casiraghi *et al.*, 2005). Strains in supergroups A and B are the most commonly found and studied (Werren, 2008). Rowley (2004) demonstrated that 7 out of 10 spider families tested were infected with either an A or B strain; the remaining three were reported to belong to the G supergroup. Sequencing indicates the strain infecting *L. geometricus* is classified into supergroup F and is unique to any currently known strains, including those of its Black Widow, *L. mactans*, relative (J. S. Harrison unpublished data). Further analysis reveals this F strain to be the only strain present in this spider species (J. S. Harrison unpublished data). Little is known about the function/effect of supergroup F strains. Strains from this supergroup have been identified in Bed Bugs, *Cimex lectularius* (Hosakawa, 2012), and multiple species of Lice (Covacin, 2007), Scorpions (Baldo, 2007), and Bush Crickets (Panaram & Marshall, 2007), yet only one study has tested for effects of infection on the host. In bed bugs, *Wolbachia* endosymbionts aided in nutrient acquisition (vitamin B) that promoted successful egg development; when *Wolbachia* infection was eliminated using antibiotics, eggs were inviable, indicating an obligate relationship between host and bacterium (Hosakawa, 2012).

The aim of this study is to determine the prevalence of *Wolbachia* infection within and among populations of *L. geometricus* in the Southeast U.S. We use molecular techniques to identify the presence or absence of *Wolbachia* infection in individual spiders from five locations in the Southeastern U.S. We ask the following questions: 1) Is this a widespread infection, where all populations harbor *Wolbachia*, and 2) what proportion of the population is infected, and 3) does percent infection vary among

locations? We hypothesize that this relationship is similar to the bed bugs and predict that if infection were required for survival or bestowed a fitness benefit, infection will be nearly or completely fixed in all populations that harbored *Wolbachia*. As this spider is invasive, we also hypothesize that gene flow is unimpeded due to human transport and expect infection rates to be similar among all locations.

## MATERIAL AND METHODS

### *Sampling*

Brown Widow adults were hand collected from New Orleans, LA (n=34), Montgomery, AL (n=29), Miami-Dade County, FL (n=38), Daytona Beach, FL (n=25), and Bulloch County, GA (n=44) during the summer of 2012. Specimens were collected from different sites in each location to reduce the chance of collecting related individuals.

### *Prevalence*

Presence of *Wolbachia* was determined by Polymerase Chain Reaction (PCR) using *Wolbachia* specific primers for both the *Wolbachia* surface protein (*Wsp*) and the fructose-bisphosphate aldolase (*FbpA*) genes (Simões 2011). These primers successfully amplify these genes in all known strains of *Wolbachia*, and are commonly used in *Wolbachia* detection assays (Narita *et al.*, 2011; Sakamoto *et al.*, 2011; Vanthournout *et al.*, 2001; Simões *et al.*, 2011; Baldo *et al.*, 2006). DNA was extracted from whole leg samples (femur through tarsus) of the third leg of each specimen using the DNeasy® Blood & Tissue kit following the manufacturers protocol (Qiagen). Tissue samples that were not immediately extracted were stored in 95% ethanol, after removal, to be

extracted at a later date. Reactions were set up separately for *Wsp* and *FbpA* in 10  $\mu$ L reactions . PCR protocols for both primers followed those from Simõse et al. (2004). PCR products were detected using 1.5% agarose gel. The presence of PCR product for both genes was used to determine a positive *Wolbachia* infection status. The ratio of infected versus uninfected individuals was calculated for each sampled location. Variation within and among locations was analyzed using a G-test; a p-value of <0.05 was considered significant. Pair-wise comparisons were conducted between each location using t-tests.

## RESULTS

The frequency of infection differed among locations and no population had 100% infection. A north-south trend was observed, with New Orleans, Daytona, and Miami-Dade having the highest infection rates. The frequency of infection from each location were as follows: New Orleans, LA (LA): 50%, Montgomery, AL (AL): 28%, Miami-Dade County, FL (FL1): 92%, Daytona Beach, FL (FL2): 68%, and Bulloch County, GA (GA): 20% (Figure 1). The variation in infection rates among locations was highly significant ( $\chi^2_4=57.432$ ,  $p<0.0001$ ). Pair-wise comparisons (Table 1) were found to be significant in all cases, except between DBF and LA, and between GA and AL.

## DISCUSSION

As percent infection ranged from 20% to 90%, we determined *Wolbachia* infection in *L. geometricus* is not an obligate association. If infection was required for survival, the proportion of *L. geometricus* infected would be expected to be at fixation, as

any uninfected individuals would suffer mortality; such as observed in infected filarial nematodes (McGarry *et al.*, 2004) and parasitic wasps (Dedeine *et al.*, 2001; Stahlhut *et al.*, 2006), where infection is necessary for development and oogenesis, respectively. Additionally, sequencing indicates that the strain infecting *L. geometricus* is of the F supergroup. Bed bugs (*Cimex lectularius*) are host to a strain also classified into F supergroup, but show an obligate relationship where uninfected females produce eggs that do not develop (Hosakawa *et al.*, 2012). As infection is not fixed in any location, and varies among locations, the relationship between the spider and its *Wolbachia* symbiont are likely to be facultative or neutral.

Variation in infection among locations is also consistent with limited migration and gene flow among sampled locations. Assuming a 100% transmission rate, as shown in *Drosophila* (Charlat *et al.*, 2004), *Wolbachia* can function as a maternally-inherited genetic marker. If gene flow was common, we would expect to see little variation between populations, as infection would spread equally to all locations (Dyer & Jaenike, 2004, 2005). The observed variation, in contrast, ranged from 20% in Statesboro, GA to 90% in Miami-Dade, FL, suggesting that populations are reproductively isolated from each other. In *Drosophila innubila*, a species with a highly fragmented distribution, the prevalence of *Wolbachia* infection also varied among populations (Dyer & Jaenike, 2005). It is possible that *L. geometricus* populations have isolated distributions, given the similar infection frequency pattern.

Our data also show a trend of decreasing infection rate with increasing latitude. This variation in infection could be the result of multiple founder events, where new populations are founded by random individuals, regardless of infection status. The

resulting infection frequency would then also be random. A similar situation could occur with *L. geometricus*, where founder events by uninfected individuals could lead to the low infection frequencies seen in GA and AL.

Another hypothesis for this trend is a loss of infection as the spider enters novel environments (Reuter *et al.*, 2005; Shoemaker *et al.*, 2000; Tsutsui *et al.*, 2003). Miami-Dade's infection frequency was 90%, making the probability of new populations being founded by an infected individual also 90%. This was not observed in our data; all other populations had a significantly lower infection rate. A potential mechanism underlying this phenomenon is differing selective pressures on infection status (Reuter *et al.*, 2005; Shoemaker *et al.*, 2000; Tsutsui *et al.*, 2003). Effects of infection that would be beneficial in South Florida might become neutral or even costly in novel northern environments. For example, as this spider expands northward, seasonal climates shift towards longer and colder winters. In Pea Aphids, *A. pisum*, the frequency of infection by *S. symbiotica* reaches 80% in central California and confers a heat-tolerance advantage over their uninfected counterparts (Russell & Moran, 2006; Chen *et al.*, 1997, 2000). Infection was also found to increase fecundity when aphids were under heat-stress (Montllor *et al.*, 2002). Similarly, heat-tolerance would be beneficial in South Florida where temperatures remain mild year-round, but selection for this advantage may decrease or disappear in areas with cooler climates. Temperature can also affect maternal transmission and microbe replication rate (Douglas, 1994) and influence microbe-density, as in the wasp, *Leptopilina heterotoma* (Mouton, 2006). A change in temperature would thus change endosymbiont density and, in turn, any associated phenotype.

Communities also change, altering the species with which the spider interacts. Endosymbionts have been documented to protect against parasitism (Oliver *et al.*, 2003, 2005) and fungal infection (Ferrari *et al.*, 2001, 2004). In an environment with constant high temperatures and a wider variety of parasites, such fitness benefits to the host would gradually drive infection towards fixation, due to strong selection pressures (Charlat *et al.*, 2004; Stouthamer *et al.*, 1999). As the environment changes, however, these advantages may disappear as temperatures drop and biotic threats (e.g. parasitoids, fungi) decrease. Infection would then become either neutral or costly, gradually removing the bacterium from the population.

In this study, we have determined that the relationship between *Wolbachia* and *L. geometricus* is not obligate, and infection is variable among locations. In order to determine the true cause of this variation, we need to know what effects, if any, the bacteria has on its spider host. Dyer & Jaenike (2005) noted that in *Drosophila* infected with a male-killing strain of *Wolbachia*, both the frequency of infection and sex ratio varied among populations and mentions the possibility of differential effects by population. In *L. geometricus*, *Wolbachia* also shows differential prevalence. Should effects of infection (e.g. differential sex ratio) follow a similar pattern as *Drosophila*, this could explain limited gene flow through potential reproductive isolation. The northward trend suggests infection is random or is lost in northern populations. This could be due to founder events by random colonizers, or differential selection due to changing environments.

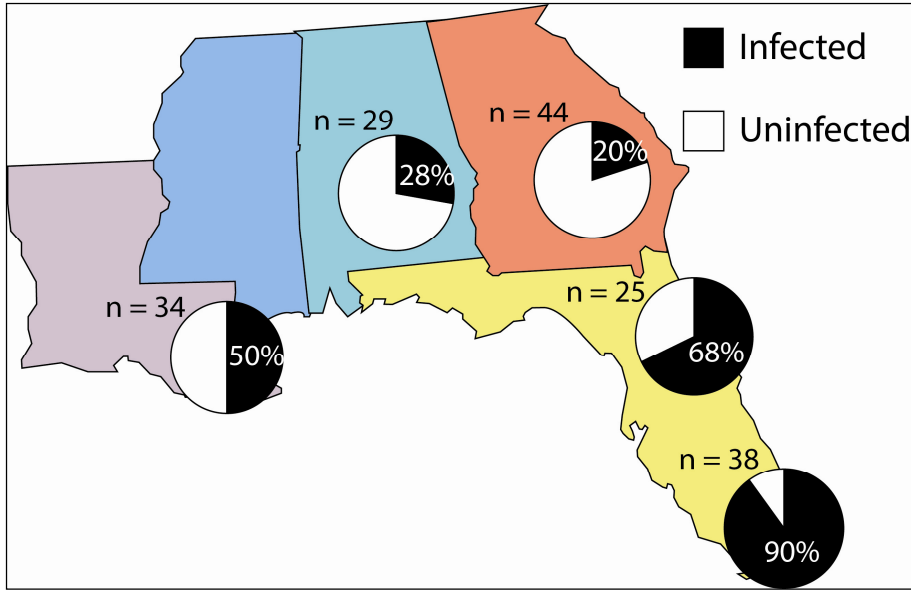


**Table 1. Pair-wise comparison of infection frequencies between sampled locations.**

	GA	AL	LA	FL1	FL2
<b>GA</b>	-	0.5894	<b>0.0107</b>	<b>&lt;0.0001*</b>	<b>0.0002*</b>
<b>AL</b>	-	-	0.0677	<b>&lt;0.0001*</b>	<b>0.0026*</b>
<b>LA</b>	-	-	-	<b>&lt;0.0001*</b>	0.1641
<b>MD</b>	-	-	-	-	<b>0.0142</b>
<b>DBF</b>	-	-	-	-	-

**Bold** = p-value significant prior to Bonferroni correction

\* = p-value significant after Bonferroni correction



**Figure 1. Prevalence of *Wolbachia* in Brown Widow Spiders (*Latrodectus geometricus*) from five Southeastern populations**

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## CHAPTER 2

### Effect of *Wolbachia* infection on Brown Widow Spider (*Latrodectus geometricus*) reproduction and juvenile development time

#### ABSTRACT

The endosymbiont bacteria, *Wolbachia pipentis*, is known to alter arthropod life history patterns (e.g., male-killing, feminization, induced parthenogenesis, nutrient acquisition, etc.). This bacteria was recently identified in the invasive Brown Widow spider (*Latrodectus geometricus*). This is the first report of a *Wolbachia* infection in the genus *Latrodectus*. Therefore, the consequences on the spider's life history patterns are unknown. This study describes the effects of *Wolbachia* on reproductive traits (clutch sex ratio, egg number, egg size, egg mass, and development time). No significant difference in clutch sex ratio, egg number, egg size, egg mass, or development time was found between uninfected and infected females. These results suggest that the *Wolbachia* strain is not an obligate symbiont nor does it alter the reproductive biology of the spider, as it does in most species. The lack of effect on the host suggests that infection is: i) neutral, and remains in the population by a) complete maternal transmission or b) has reached an equilibrium, or ii) affects a trait that was not analyzed in this study. These data indicate that while *Wolbachia* infection might not influence the spider's biology as strongly as we thought, *L. geometricus* has high reproductive output overall, which certainly aids in its successful invasion history.



## INTRODUCTION

Reproductive strategies can influence the establishment and spread of introduced species and vary widely across organisms. Life history traits that promote fast growth and rapid reproduction (e.g., age at first reproduction, number of reproductive bouts over the lifespan, number of offspring per bout) are commonly seen in successful invasive species (Stearns, 1976; Kolar *et al.*, 2001). Characterizing a species' life history gives vital information on its ecology and provides insight on how they are able to become established in their current environment and expand into new ones.

The Brown Widow Spider's (*Latrodectus geometricus*) microhabitat includes the undisturbed areas around houses and other man-made structures (Vincent, 2008). Thought to have originated in South Africa, this invasive species has now been introduced in Japan, Indonesia, Papua New Guinea, Australia (Brown, 2008), India, Brazil, Puerto Rico, Madagascar, and Jamaica (Shukla, 1972; Hernandez-Pasos, 2006; Ramialiharisoa, 1994; Baerg, 1954). In the U.S., *L. geometricus* was isolated in South Florida for ~50 years but in the last 15 years has become established in most southern states (Brown, 2008) and Southern California (Vincent, 2008).

Despite being invasive, only a handful of descriptive studies have examined the reproductive biology of *L. geometricus*. While studying sexual cannibalism, Segoli documented that the "summersault" behavior of males (where the male offers himself to the female) is more common when courting a virgin female and supports the hypothesis that males gain reproductive success by sacrificing themselves (Segoli, 2008). Bouillon (1961) analyzed the ovulation cycle, concluding that females lay an average of 22-56 egg sacs over their lifetime, but production seems to slow over time. Baerg (1954) examined

and compared the reproductive biology of *L. geometricus* with its relative *L. mactans*, demonstrating that while *L. geometricus* individuals don't live as long, the amount of development time was more rapid. It was during a population genetics study that this spider was found to harbor the bacteria, *Wolbachia* (Harrison, Pers. Comm.).

*Wolbachia* is an endosymbiotic  $\alpha$ -proteobacteria that infects filarial nematodes (Bandi *et al.*, 1998) and arthropods (Werren, 1997, 2008; Jeyaprkash, 2000). Estimated to infect > 65% of all insect species, it is among the most abundant genera of intracellular bacteria, infecting at least  $10^6$  insect species (Werren, 2008; Jeyaprkash, 2000; Dobson 2004). *Wolbachia* is inherited maternally through egg cytoplasm during meiosis (Shoemaker *et al.*, 2002). As sperm do not contribute cytoplasm to the zygote, infected males do not pass the bacteria to offspring and thus do not add to the bacterium's fitness (Werren, 2008). *Wolbachia* has evolved multiple strategies to increase its fitness by manipulating the host's life history and reproduction. The most common manipulations include: i) male killing (Sakamoto, 2011), whereby only female embryos survive (Werren, 1997, 2008; Stouthamer *et al.*, 1999; Hurst *et al.*, 1999); ii) induction of parthenogenesis, whereby, in haplodiploid species, *Wolbachia* causes the genome to double in unfertilized eggs leading to infected virgin females producing infected daughters (Stouthamer & Hamilton, 1990; Russell & Stouthamer, 2011); and iii) male feminization (Narita *et al.*, 2011), whereby genetic males are hormonally induced to develop into functional reproductive females (Werren, 1997; Werren, 2008; Stouthamer *et al.*, 1999). These three strategies result in a female-biased sex ratio and have been documented in organisms such as the butterflies *Eurema hecabe* (Narita *et al.*, 2011), *Zizina emelina* (Sakamoto *et al.*, 2011), and *Talicerca nyseus* (Ankola *et al.*, 2011),

Pillbugs, *Armadillidium vulgare* (Verne *et al.*, 2012), and the two-spot ladybird, *Adalia bipunctata* (Majerus *et al.*, 2000). These effects ensure that the majority of offspring produced are infected females, thus increasing the prevalence of the bacteria in the population (Dyer & Jaenike, 2004; Stouthamer *et al.*, 1990; Turelli 1994; Charlat *et al.*, 2004).

Endosymbiont infection has been linked to nutrient acquisition in other species (Tsetse Fly, Pea Aphid) (Nogge, 1982; Koga, 2003). In Bed Bugs (*Cimex lectularius*), *Wolbachia* provides the B vitamins necessary for larval development; females that were treated with antibiotics produced shriveled eggs that never hatch (Hosokawa, 2010). As the strain infecting *L. geometricus* is in the same supergroup (F) as that of the Bed Bug, this suggests that infection could alter development time.

Although insects comprise the majority of host species, multiple spider species have been found to harbor *Wolbachia*, (e.g., Dwarf Spider, Sheetweb Spider, Golden Orb Weaver Spider) (Vanthournout, 2001; Goodacre, 2006; Gunnarsson, 2009; Rowley, 2004; Woo Oh, 2000). Within the widow spider genus, *Latrodectus hasselti* (Redback spider) has been screened for, but did not show any infection (Rowley, 2004). To our knowledge, this study is the first account of a *Latrodectus* spider harboring a *Wolbachia* infection. As this bacteria is a known reproductive manipulator (Werren, 2008), the spider's reproductive biology must be reexamined to account for possible effects of infection.

Previous studies analyzed clutch sex ratio, as skewing of sex ratio is a consequence of feminizing, male-killing, and parthenogenesis-inducing *Wolbachia* strains (Gunnarsson, 2009; Narita *et al.*, 2011; Sakamoto *et al.*, 2011). This allows us to

identify the mechanism (e.g., male-killing, feminization) used by the symbiont to proliferate itself through the population. The dwarf spider (*Oedothorax gibbosus*) shows a female-biased sex ratio and a reduced clutch size when infected with *Wolbachia*, suggesting the strain utilizes the male-killing mechanism (Vanthournout, 2001). Previous studies have also examined the effect of infection on clutch size. Both *D. melanogaster* and *Trichogramma bourarachae* showed a significant increase in clutch size when infected with *Wolbachia*, compared to uninfected lines (Fry *et al.*, 2004; Girin *et al.*, 1995). An infected female that produces more offspring would outcompete its uninfected conspecifics and propagate the infection through the population.

In some arthropod females, quicker development time can result in increased size at sexual maturity (Higgins, 2000) and thus the numbers of offspring, as larger females tend to produce larger clutches (Marshall & Gittleman, 1994). Individuals that mature more quickly also spend less time in high-mortality juvenile stages and increase the amount of time available for reproduction (Kingsolver & Huey, 2008). Excluding genetic background, if infection decreases development time, infected females could potentially benefit from decreased juvenile mortality, larger clutches due to larger body size, and longer time spent at sexual maturity (Kingsolver & Huey, 2008).

Understanding the reproductive biology and natural history of this invasive species will not only deepen our understanding of this species, but will provide valuable insights into the characteristics allowing its rapid range expansion. *Wolbachia* infection potentially has a large impact on the reproductive ecology of *L. geometricus*, but nothing is known about this host-symbiont interaction.

This study examines the effects of *Wolbachia* infection on clutch sex ratio, clutch size, egg size, egg mass, and hatching rate, and juvenile development time. We show infection does not influence any of the examined reproductive or developmental traits, and discuss: 1) the hypotheses underlying the infection's persistence in the population, and 2) the overall reproductive biology of *L. geometricus* in regards to its life history and *Latrodectus* relatives.

## METHODS

### *Adult Rearing*

Rearing was done in a similar manner to Vanthournout, *et al* (2001). Briefly, individual spiders were reared in separate containers. A balsa wood stick was included in each container for web construction along with a piece (~1.5cm diameter) of a cotton ball moistened with 2-3 drops of DI water for humidity. Cage bottoms were switched and cleaned, and cotton balls replaced weekly, to prevent fungal growth. Cages were kept in an incubator at 27°C with a 12h light-dark cycle at 50-60% humidity. Adult females were fed one giant mealworm once a week and adult males were given 2-3 *Drosophila melanogaster* three times a week. Once females reached maturity (~6 instars) cotton balls were removed, as preliminary trials showed increased mortality due to fungal growth.

### *Sex ratio*

A total of 23 lab-reared virgin females (10 infected, 13 uninfected) of ~3 months old were mated with lab-reared unrelated uninfected males. Their first egg sac was cut

open 2-3 days after being laid. Eggs were transferred to a Petri dish, kept in an incubator at 27°C, and allowed to hatch and molt into 2<sup>nd</sup> instars. A random subsample of 48, 2<sup>nd</sup> instar offspring from each sac were separated into individual cages. Spiderlings were fed 2-3 *Drosophila melanogaster* three times per week and raised until sex could be determined by physical examination (4<sup>th</sup> instar). At 4<sup>th</sup> instar, the width of the palpal tibia of males has been shown to be significantly wider and darker in pigment than those of females (Mahmoudi 2008). The sex ratio from each clutch was calculated by dividing the number of females by total number of offspring sampled. This proportion was compared using a t-test between uninfected versus infected clutches to determine if infected females produce a female-biased clutch compared to that of uninfected females.

### ***Reproduction***

Egg sacs from females used in the sex ratio trials were used to collect data on egg number, egg mass, and hatching rate.

#### *Egg count*

The number of eggs, from each sac, was counted for each female. Inviabile eggs (dehydrated, yellow, crystalline spheres that can occur with fertilized eggs) were included in this number as they also require energy to produce. The average number of eggs per clutch was contrasted with the female's carapace width, to determine if egg number correlated with body size; this allowed us to remove body size from the statistical analysis. The average number of eggs per clutch was then compared between uninfected versus infected females using a t-test.

### *Egg Mass and Size*

The mass of each egg was calculated by measuring the total mass of the clutch (including inviable eggs) divided by the total number of eggs. This calculation was done for each sac and compared between uninfected and infected females. To determine average egg size, the diameter of 10 eggs in every clutch in a female's lifetime was measured using a micrometer and averaged. These clutch averages were then averaged together and compared between uninfected versus infected females using a t-test.

### *Hatching Rate*

Hatching rate was calculated by dividing the number of hatchlings by the total number of eggs (including inviable eggs). This was done for each sac and compared between uninfected and infected females using a t-test. Inviabile eggs were not excluded from this calculation as doing so did not make a significant difference on the average hatching rate.

### *Reproduction across Egg Sacs*

Reproductive data was compared among the first four egg sacs of 9 females (5 infected, 4 uninfected) to determine if clutch size, egg size, egg mass, and hatching rate changed over successive egg sacs.

### ***Development Time***

During the sex ratio trials, the dates of the second and third molts were recorded for each offspring. We recorded these molts because the sex ratio trials ended when sex could be determined, which is after the 3<sup>rd</sup> molt. The number of days between: 1) hatching and 3<sup>rd</sup> instar, and 2) hatching and 4<sup>th</sup> instar, were calculated. The time between these dates was averaged for each clutch then compared between uninfected and infected. Development time was also compared between males and females regardless of infection status.

### ***Statistical Analysis***

T-tests were used to detect any difference in sex ratio, clutch size, egg mass, egg size, development time of juveniles, and development time between males and females. MANOVA was used to detect any trends in clutch size, egg mass, egg size, or hatching rate over consecutive egg sacs.

## **RESULTS**

### ***Sex Ratio***

No significant difference in sex ratio was detected between infected and uninfected clutches ( $t_{21}=0.82$ ,  $p=0.38$ ) (Figure 2). Sex ratio varied widely among females regardless of infection status. Uninfected clutches ranged from 39% to 88% female, and infected clutches ranged from 37% to 95% female. The average sex ratio (% female) for uninfected was 61%, and infected was 66%.



### *Reproduction*

No difference in clutch size ( $t_{20}=0.94$ ,  $p=0.35$ ) (Figure 3), egg mass ( $t_{20}=-0.65$ ,  $p=0.52$ ) (Figure 4), egg diameter ( $t_{20}=0.53$ ,  $p=0.6$ ) (Figure 5), or hatching rate ( $t_{20}=-0.27$ ,  $p=0.79$ ) (Figure 6). Overall, *L. geometricus* clutch size varied between 60-242 eggs ( $\bar{x}=117.5$  eggs per sac). Egg diameter varied between 0.72-0.97 mm ( $\bar{x}=0.82$  mm). Egg mass varied between 0.34-0.5mg ( $\bar{x}=0.4041$  mg) regardless of infection status. With the exception of one female, inviable eggs were present in at least one clutch per female. Egg number, egg size, egg mass, and clutch hatching rate also remained constant across clutches for each individual female.

### *Development Time*

No difference in development time was detected between uninfected and infected offspring: hatch to 3<sup>rd</sup> instar ( $t_{21}=-0.44$ ,  $p=0.66$ ), hatch to 4<sup>th</sup> instar ( $t_{21}=-0.44$ ,  $p=0.66$ ) (Figure 7). Regardless of infection status, the average number of days between hatching and 3<sup>rd</sup> instar, and hatching to 4<sup>th</sup> instar was 23 and 32, respectively. Males and females showed no difference in time between hatching and 4<sup>th</sup> instar ( $t_{854}=-1.28$ ,  $p=0.1997$ ). Development time to 4<sup>th</sup> instar for both females and males ranged from 21-51days (Figure 8).

## **DISCUSSION**

### *Host-parasite Interaction*

The relationship between *L. geometricus* and its *Wolbachia* symbiont does not appear to be one of reproductive manipulation, as there was no difference in any of the examined

reproductive traits, between infection status. While *Wolbachia* infection commonly alters reproduction, the host-symbiont relationship is not limited to these effects. Female bedbugs without *Wolbachia* produce shriveled eggs that fail to develop, and in the filarial nematode, *O. volvulus*, filariae and adults suffer mortality (Hosokawa, 2010; Hoerauf, 2008). As a result of infection, augmented development and survival increases the fitness of the bacterium through either: i) increasing host fitness (e.g., survival, reproduction) to outcompete uninfected counterparts, or ii) ensuring all females in the population are infected, by inducing a female-bias. This increase to the symbiont's fitness would drive infection in the population towards fixation. In our previous study (see chapter 1) however, we see that infection is polymorphic in every sampled population. Yet, an endosymbiont infection that has no effect on the host is not expected to persist in the population. Typically, infection is rapidly lost if: i) there is no fitness benefit for the host, ii) the infection has deleterious effects on the host, or iii) the endosymbiont lacks complete maternal transmission (Turelli, 1995). The absence of an effect on the spider suggests the presence of other mechanisms, besides reproduction manipulation, that allow the infection to remain in the population.

It is hypothesized that these types of infections can still persist if there are high levels of maternal transmission or reduced deleterious effects on the host. Studies on *Drosophila simulans* and *D. yakuba* show *Wolbachia* infection has no effect on fitness, sex ratio, nor cytoplasmic incompatibility, yet it remains in the population (Hoffmann, 1996; Turelli, 1995; Charlat, 2004). For both *Drosophila* species, maternal transmission was perfect, and infection had no deleterious effects on fitness. Preliminary data show that *Wolbachia* infection in *L. geometricus* has 100% maternal transmission (Harrison

unpublished data) and this current study reveals no difference in fitness, suggesting that the infection behaves like a neutral trait.

### *Neutrality*

Neutrality of an infection, whether viral or bacterial, can emerge through multiple mechanisms. Given perfect maternal transmission, the bacteria will propagate across generations because every egg produced contains the bacterium, resulting in 100% of the offspring being infected, without the assistance of reproductive manipulation (Hoffmann, 1996; Turelli, 1995; Charlat, 2004).

Over time, the host could also develop a “rescue mechanism”; detrimental effects of infection are reduced or eliminated by novel counter-mechanisms in the host (Bourtzis *et al.*, 1998). The Asian Butterfly, *H. bolina*, has developed resistance to its *Wolbachia* infection (Hornett *et al.*, 2006). *H. bolina* is infected with a *Wolbachia* strain that kills males in the South Pacific Islands population but not in the Southeast Asia population. This difference has been attributed to a suppressor gene present in the southeastern population and the spread of this suppressor into the South Pacific island population was marked by a shift from a 100:1 sex ratio to a near 1:1 in just 10 years (Hornett *et al.*, 2006). This suppression of male-killing has also been found in *Drosophila prosaltans* (Cavalcanti *et al.*, 1957). If the cost of suppression is “free” for the host, then infection will disappear, as the cost of losing males will be higher. If there is a cost, suppression will remain polymorphic with infection at a reduced prevalence (Engelstädter & Hurst, 2009). Our prevalence data show that infection is polymorphic in every sampled location

(see chapter 1), suggesting the possibility of an unidentified suppressor in the *L. geometricus* genome.

Virulence of *Wolbachia* can be viewed in a similar way as pathogen virulence. A pathogen that kills its host quickly benefits from high transmission rates, but at the cost of longevity in the population (i.e., spreads quickly, but quickly goes extinct in the population) (O'keef, 2002). While more virulent strains, with stronger effects on the host, will have greater immediate fitness from greater transmission (i.e., the population consists entirely of infected females), benign strains have a greater chance of persisting in a population as their effects are likely limited and non-detrimental to the host; a male-killing or feminizing strain can result in population extinction when no males are available to mate (Dyer, 2004). This selection on pathogen virulence can bring host and bacterium into an evolutionarily stable equilibrium and result in an infection that is effectively neutral (Frank, 1996; Dyer, 2004).

#### *Limited study traits*

The lack of effects due to infection could also be attributed to the limited traits examined in this study. It is possible that a trait not included in this study is strongly influenced by *Wolbachia* infection. Cytoplasmic incompatibility (CI) was not analyzed in our study, but is the most common effect seen in infected species (Werren, 2008). The presence of CI provides infected females a fitness benefit over uninfected counterparts as they are able to mate with both infected and uninfected males. Uninfected females produce viable offspring when mated with infected males and are thus limited to uninfected males (Werren, 2008).

Differential survivorship is also a possible effect as endosymbionts can alter nutrient acquisition (Nogge, 1982; Koga, 2003), protection from parasitoids (Oliver *et al*, 2003, 2005), and fungal infections (Ferrari *et al*, 2001, 2004). *Latrodectus geometricus* specimens survived a fungal outbreak that severely reduced *L. mactans* stock specimens, suggesting possible immunity (Baerg, 1954). Whether this resistance is bestowed through infection has yet to be examined, yet in nearly every case where *L. geometricus* and *L. mactans* occur simultaneously in a location, *L. geometricus* has been reported to displace the native *L. mactans* for unknown reasons (Vincent, 2008). It would then not be unreasonable to consider anti-fungal properties of infection a possible advantage.

Our study shows no benefit to reproduction or juvenile development when temperatures were 27°C, but an effect might be detected at different temperatures (i.e., average summer and winter temperatures of Central Georgia and South Florida). In aphids, the secondary endosymbionts, *S. symbiotica* and *H. defensa*, confer a heat-tolerance advantage over their uninfected counterparts (Russell & Moran, 2006; Chen *et al*, 1997, 2000). Prevalence data show a trend of reduced infection frequency in northern latitudes, where temperatures are cooler and vary seasonally more so than in South Florida. Heat-tolerance would be beneficial in South Florida where temperatures remain mild year-round, but selection for this advantage may decrease or disappear in areas with cooler climates. Temperature can also affect maternal transmission and microbe replication rate (Douglas, 1994) and influence microbe-density, as in the wasp, *Leptopilina heterotoma* (Mouton, 2006). A change in temperature would thus change endosymbiont density and in turn any associated phenotype.

## *Reproductive Biology*

### *Sex Ratio*

The average sex ratio for *L. geometricus*, regardless of infection, was 63% female, demonstrating that *L. geometricus* appears to have a slightly female-biased sex ratio when bred in lab. Males tend to be scarce in the field and this may be attributed to sexual cannibalism (Boullion, 1957). It is probable that the scarcity of males is due to both a skewed sex ratio at birth and increased male mortality in adulthood.

The mechanism behind sex determination in *L. geometricus* is a sound foundation for endosymbiont manipulation. *Latrodectus geometricus* follows an XX and XO mechanism, where females possess four copies of the sex determining chromosome and males possess only two (Zhao, 2010). This chromosome duplication is similar to the effect *Wolbachia* has on some haplodiploid species, where infection causes parthenogenesis by doubling haploid gametes to form female diploids (Charlat, 2003). It is a reasonable hypothesis that given its sex determination, feminization would be a likely effect of infection on *L. geometricus*. Sex ratio distortion due to infection has not been observed, however.

### *Egg Sac Contents*

When compared to previous studies (Baerg, 1954), our average clutch size is higher than what was previously described; 117 compared to Baerg's clutch size of 88. Clutch size, egg mass, egg diameter, and hatching success remained constant across the first four egg sacs. This agrees with findings presented by Boullion (1957) who stated that clutch size did not change across egg sacs. A similar trend was found in *L. hasselti*, suggesting that fertility and fecundity are influenced by habitat quality and food supply

(i.e., when the habitat and/or food is stable, so is reproduction) (Downes, 1985). As adult females were routinely fed giant mealworms, food supply was stable.

*Latrodectus geometricus* has the potential for high reproductive output. After a single mating, females can produce multiple clutches of ~117 eggs without any decrease over time, indicating an ability to store a large amount of sperm without it losing quality. Even when we consider that males produce a finite number of sperm per mating, females mate multiple times (Segoli, 2008). Females can refill their sperm storage and continue to produce clutches of offspring. *Latrodectus geometricus* females produce up to 29 eggs sacs in a lifetime (Bouillon, 1957). While individual sacs may have a smaller clutch size (~117) relative to other *Latrodectus* species (largest clutch size for *L. mactans* was 919; Kaston (1970), the ability to produce offspring over an extended amount of time would be advantageous over a competitor that produces fewer clutches with a marginally larger clutch size. Indeed, Bouillon (1957) reported the maximum productivity (total eggs produced) of one female was 5,761, while *L. mactans* produced a maximum of 2,132 and *L. hesperus* a maximum of 3,024 (Kaston, 1957). This reproductive ability certainly contributes to the spider's successful invasion history and is a possible reason *L. geometricus* appears to be displacing *L. hesperus* in Southern California (Vetter, 2012).

### *Development*

Juveniles develop more quickly when eggs were removed from the sacs prior to hatching versus when spiderlings were allowed to naturally emerge. Baerg (1954) reported an average of 31 days for 2<sup>nd</sup> instar juveniles to emerge from the sac, while juveniles in our study reached 4<sup>th</sup> instar in 32 days on average. This difference is most

likely due to juveniles molting and remaining inside the sac until an exit hole is made; in some cases Baerg (1954) reported no emergence, as 2<sup>nd</sup> instars were found dead inside the sac. In-sac mortality could be attributed to sibling cannibalism (Kaston, 1957; Johnson, 2010). In a separate observational study, juvenile cannibalism was witnessed when clutches were allowed to mature colonially in a Petri dish (unpublished data). Such behavior most likely improves survivorship through increased food supply (Guimaraes, 2012).

Studies have shown a trend of females having longer development time compared to males, arguing that it is adaptive for a female to delay maturation, in favor of growth, if it results in increased fecundity (Forster & Kingford, 1983; Deevy & Deevy, 1945). Our study does not show this. When comparing the development time for 856 offspring, both males and females average 32 days to reach 4<sup>th</sup> instar. From observation, the major difference between sexes was time to maturity, with males showing fully developed pedipalps at 5<sup>th</sup> instar and females developing a distinct epigynum around 7<sup>th</sup> or 8<sup>th</sup> instar.

This study provides evidence that *Wolbachia* infection has no effect on sex ratio, egg size, egg mass, or on juvenile development time. We discuss how an infection can be maintained in the population when no effect is observed, whether it be due to perfect maternal transmission, rescue mechanisms, or reduced virulence. We conclude that *L. geometricus* has high reproductive output which could contribute to its successful invasion history.



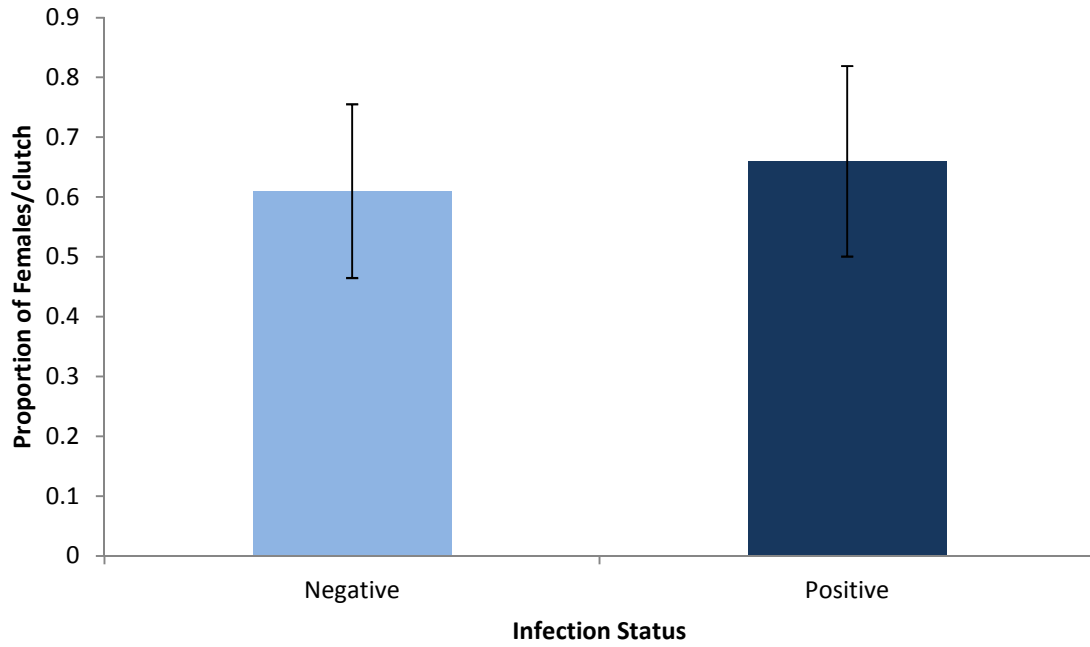


Figure 2. Average proportion of females in each clutch from uninfected and infected females. The average sex ratio of the first clutch did not differ between uninfected and infected females. ( $t_{21}=0.82$ ,  $p=0.38$ )

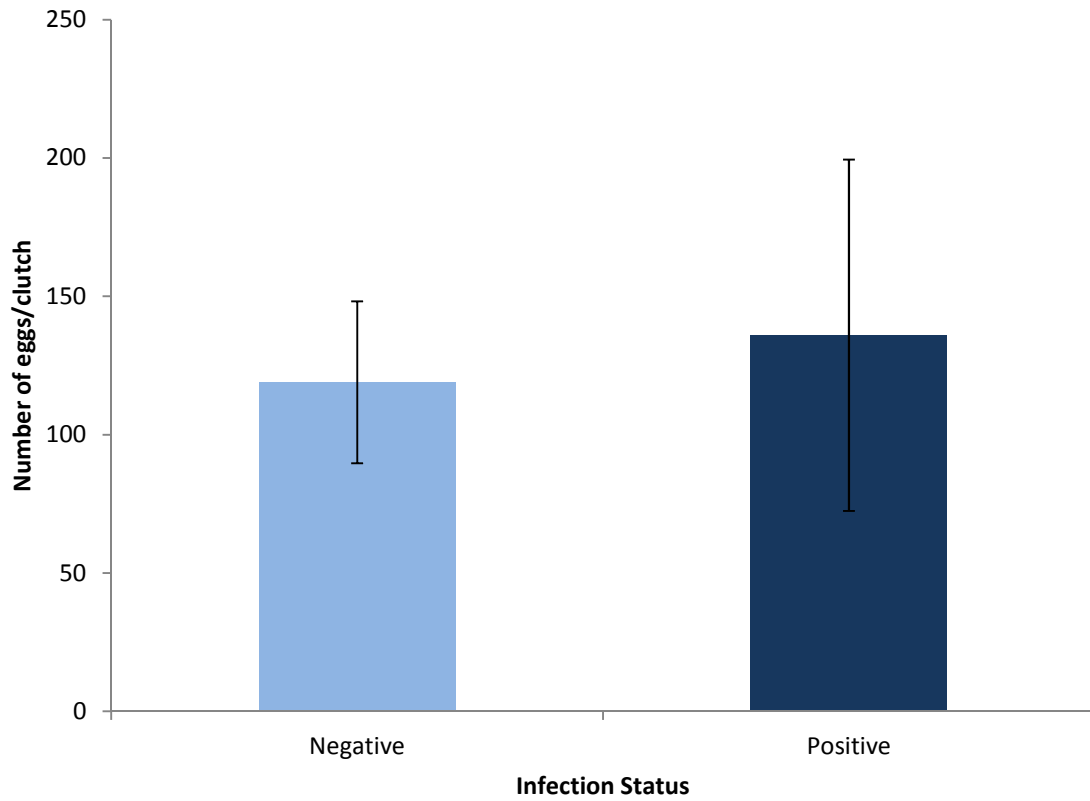


Figure 3. Average number of eggs produced in each clutch over a female's lifetime from uninfected and infected females. We detected no difference in clutch size between infected and uninfected clutches ( $t_{20}=0.94$ ,  $p=0.36$ ).

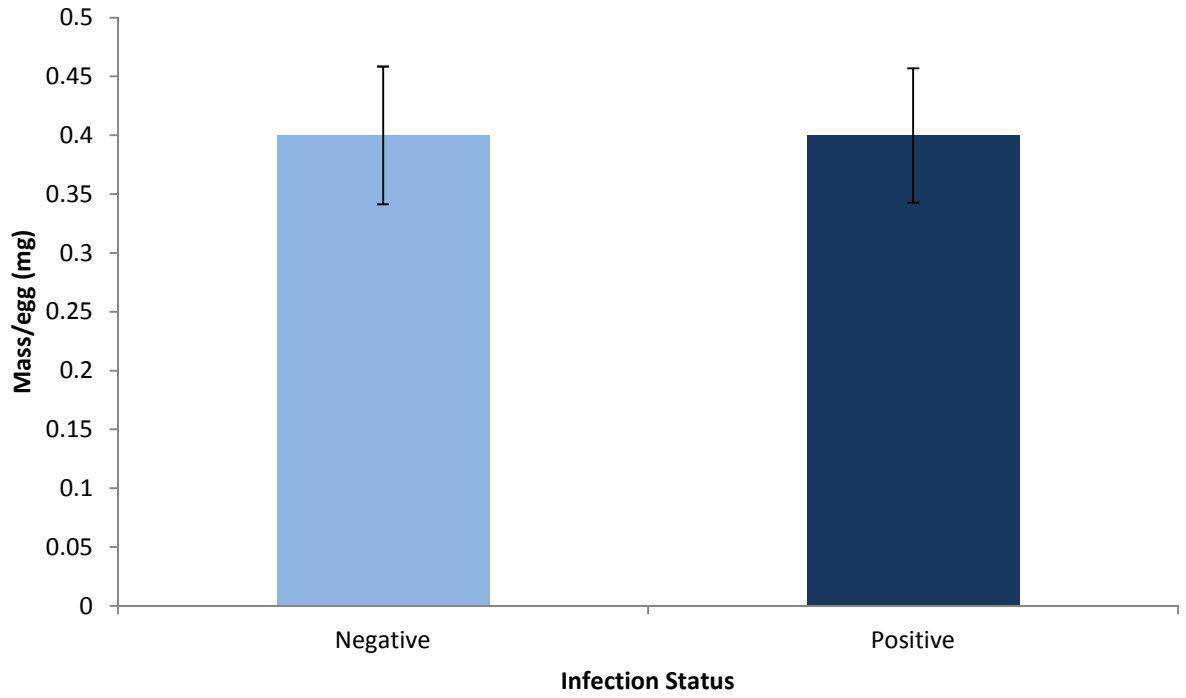


Figure 4. Average mass per egg (mg) in each clutch over a female's lifetime from uninfected and infected females. No difference in egg mass was detected ( $t_{20}=-0.65$ ,  $p=0.52$ ).

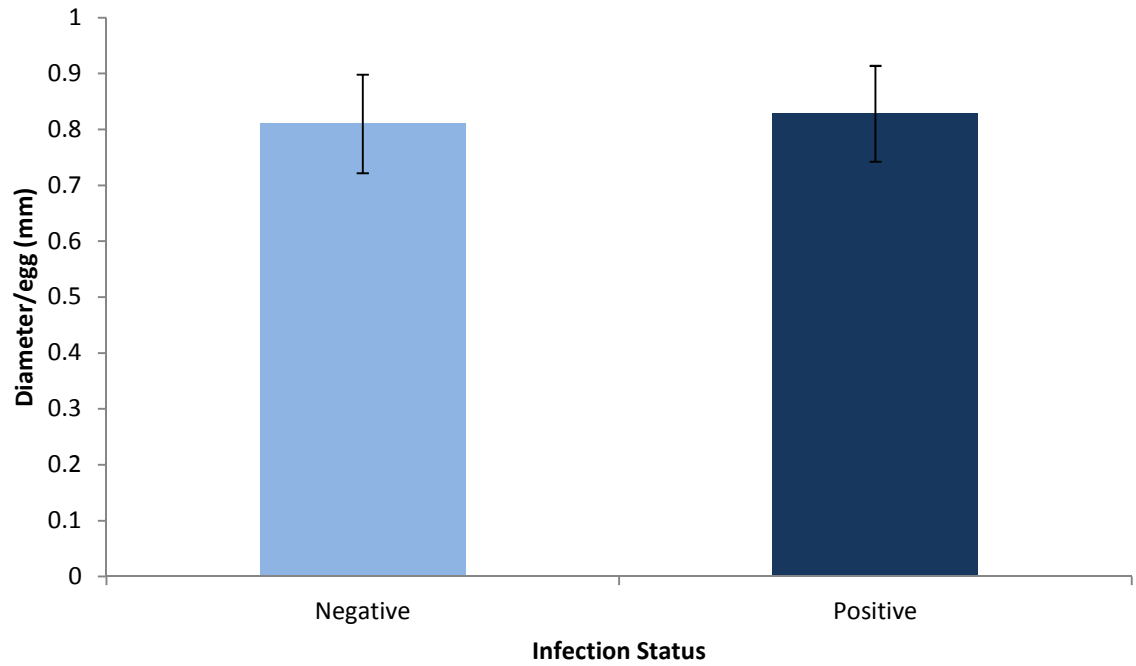


Figure 5. Average diameter of eggs in each clutch over a female's lifetime from uninfected and infected females. No difference in egg diameter was detected ( $t_{20}=0.53$ ,  $p=0.60$ ).

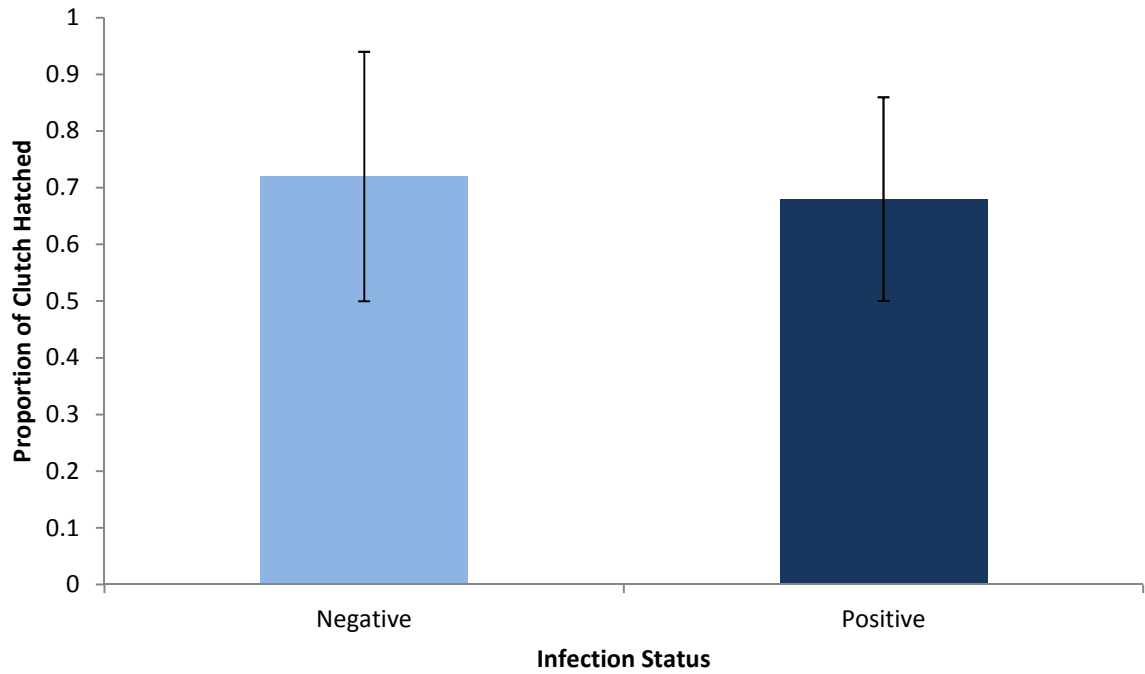


Figure 6. Average hatching success of all clutches produced over a female's lifetime from uninfected and infected females. No difference in hatching success was detected ( $t_{20} = -0.27$ ,  $p = 0.79$ ).

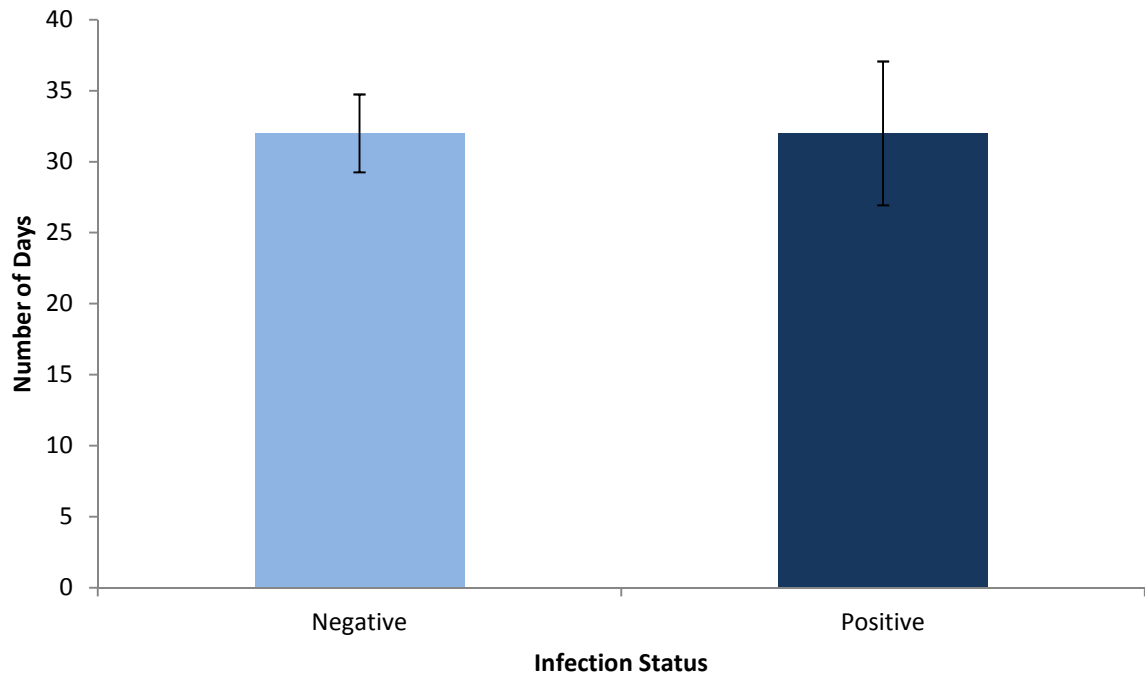


Figure 7. Average number of days between hatching and 4<sup>th</sup> instar for the first egg sac of infected and uninfected females. No difference in development time was detected ( $t_{20}=-0.44$ ,  $p=0.66$ ).

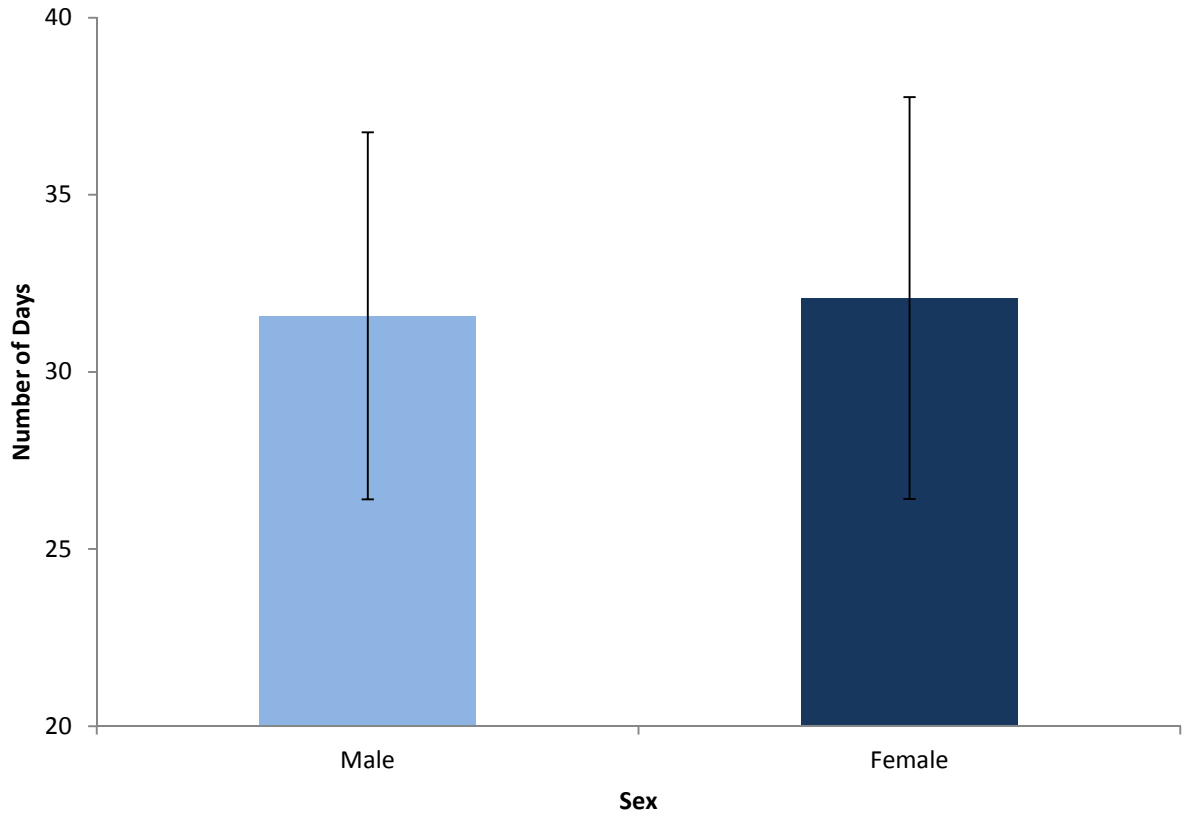


Figure 8. Number of days between hatching and 4<sup>th</sup> instar for males and females regardless of infection status. No difference in development time was detected between males and females ( $t_{854}=-1.28$ ,  $p=0.20$ ). Development time for both females and males ranged from 21-51days.

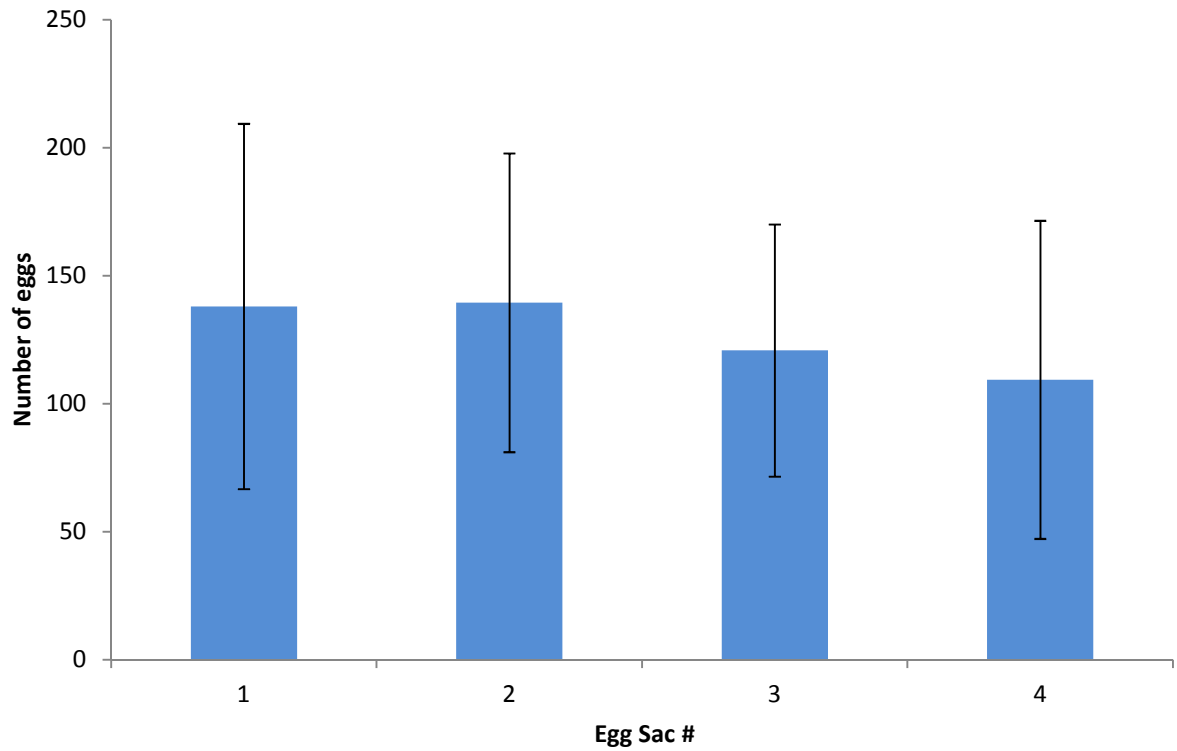


Figure 9. Number of eggs per clutch for the first four sacs. Only data from females that produced 4+ sacs were used. Egg number did not differ among the first four clutches ( $F_{3,32}=0.45$ ,  $p=0.72$ ). No interaction between infection status and egg sac number was detected ( $F_{3,5}=2.87$ ,  $p=0.14$ ).



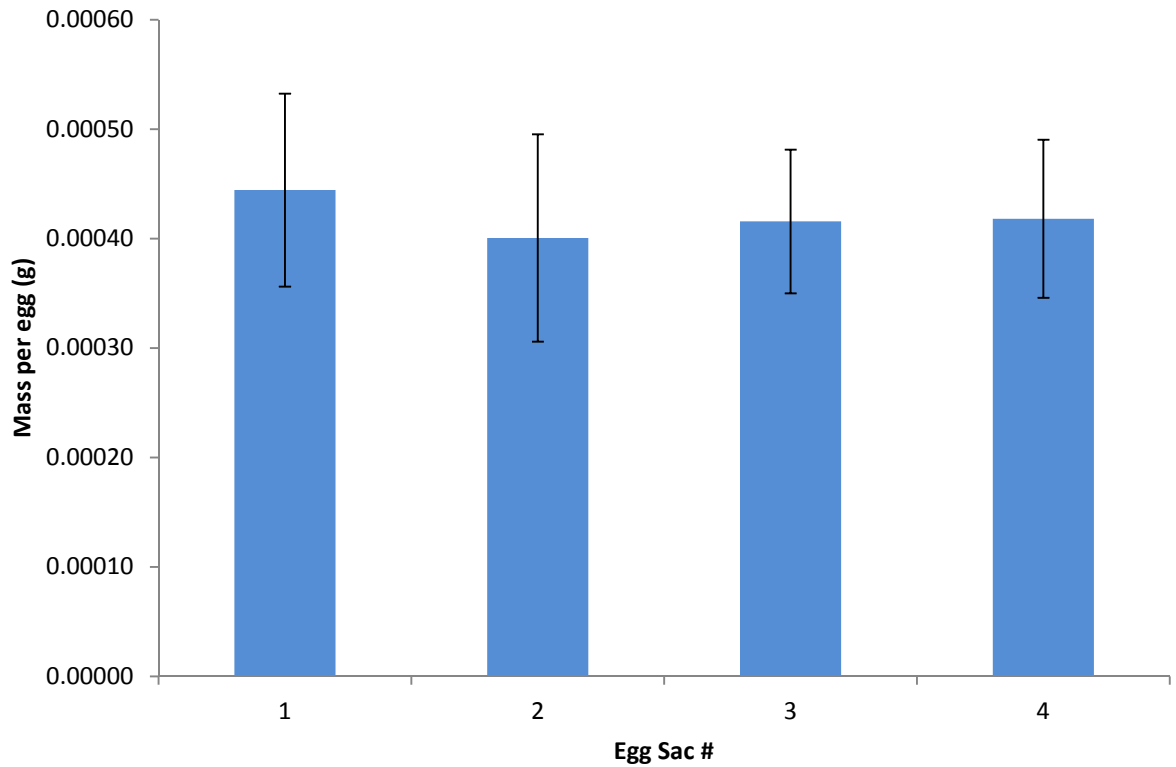


Figure 10. Average egg mass per clutch compared across the first four sacs. Only data from females that produced  $\geq 4$  sacs were used. No difference in egg mass between clutch number was detected ( $F_{3,32}=0.39$ ,  $p=0.76$ ). No interaction between infection status and egg sac number was detected ( $F_{3,5}=0.42$ ,  $p=0.74$ ).

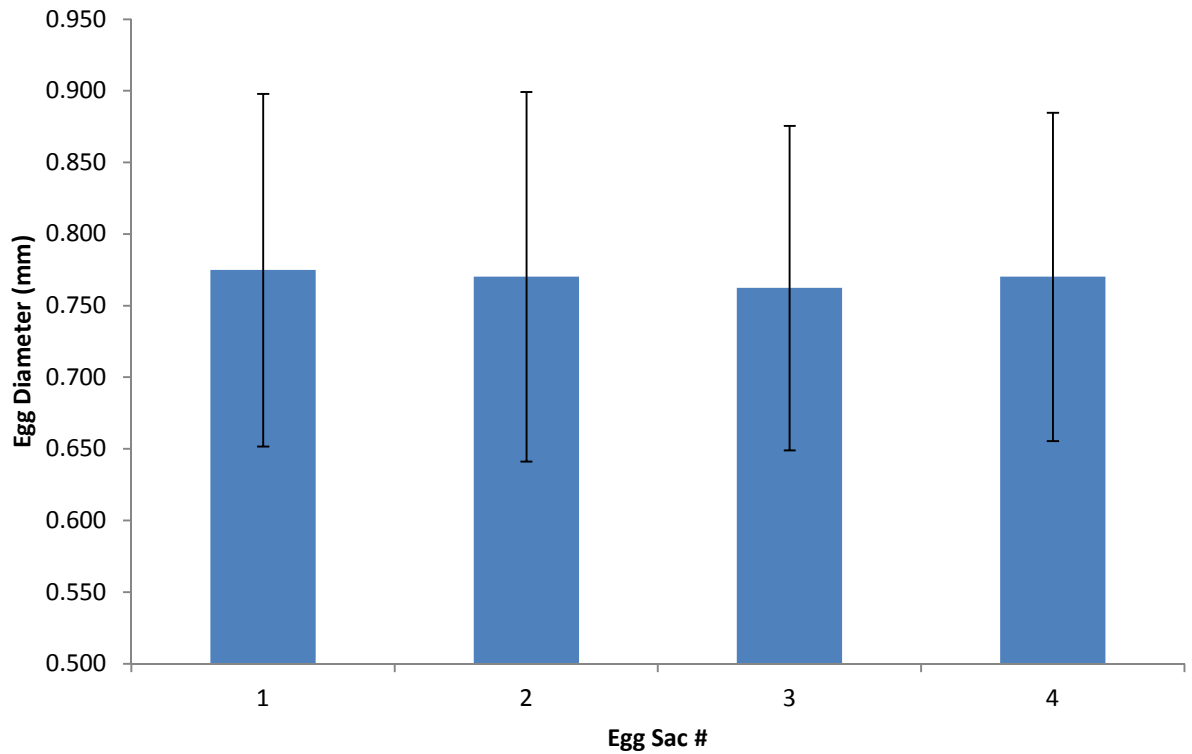


Figure 11. Average egg diameter per clutch for the first four sacs. Only data from females that produced  $\geq 4$  sacs were used. No difference in egg diameter was detected among egg sacs ( $F_{3,32}=0.01$ ,  $p=1.00$ ). No interaction between infection status and egg sac number was detected ( $F_{3,5}=1.05$ ,  $p=0.45$ ).

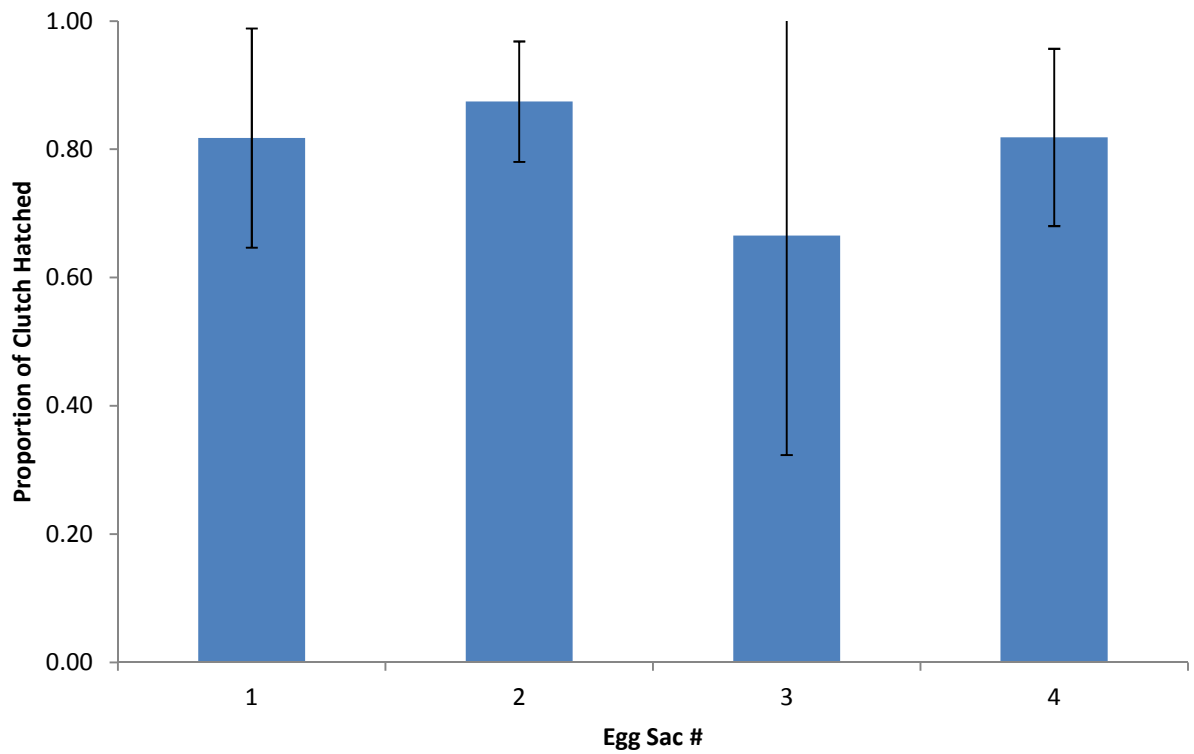


Figure 12. Proportion of clutch hatched for the first four sacs. Only data from females that produced  $\geq 4$  sacs were used. Hatching success did not change across egg sacs ( $F_{3,32}=1.46$ ,  $p=0.24$ ). No interaction between infection status and egg sac number was detected ( $F_{3,5}=0.43$ ,  $p=0.74$ ).

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## CONCLUSION

Determining the prevalence of *Wolbachia* infection in Southeastern populations of Brown Widow spider (Chapter 1) gives us insight into the interactions between host and symbiont and reveals information on the movement of this non-native spider as it expands its range. Additionally, examining the effects of infection on reproduction and development of the spider (Chapter 2) provides insight into the history of this relationship and into the life history and ecology of the spider itself.

In Chapter 1, I found that *Wolbachia* infection occurred at varying frequencies in all of the sampled locations, demonstrating that the relationship between the Brown Widow Spider (*Latrodectus geometricus*) and its endosymbiont is not obligate. This finding was supported as both uninfected and infected offspring of wild-caught females showed no significant difference in regards to reproduction or development time. There also appears to be a trend of lower infection frequency in northern populations. Possible explanations include: i) multiple founder events; or ii) loss of infection in northern populations due to differential selective pressures, where infection is more beneficial in southern populations (e.g. heat tolerance).

In Chapter 2, I found that *Wolbachia* infection did not affect spider reproduction or development, suggesting it is maintained in the population through other mechanisms besides reproductive manipulation. It is possible that this infection has reached a neutral equilibrium, being proliferated solely due to efficient meiosis; preliminary data show that infection in this spider is 100% inheritable. Neutral equilibrium could also be reached through rescue mechanisms (e.g., suppressor genes) and decreased virulence due to a long infection.

These experiments demonstrate that, regardless of infection, the Brown Widow Spider has high reproductive output. We found the average clutch size to be around 117 eggs per sac, and that reproduction does not change over the first four egg sacs. Though its clutch size is smaller in comparison to the Southern Black Widow (*Latrodectus mactans*), the Brown Widow has more clutches over its lifetime. If the Brown Widow's reproduction follows the observed trend over all egg sacs, one female could produce nearly 6,000 eggs in her lifetime. This reproductive ability certainly contributes to the spider's successful invasion history. It also provides a possible reason why Brown Widows appear to be displacing native Western Black Widows (*Latrodectus hesperus*) in Southern California.