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SPONTANEOUS MALE DEATH AND MONOGYNY IN THE DARK FISHING SPIDER,
DOLOMEDES TENEBROSUS HENTZ, 1843 (ARANEAE, PISAURIDAE)

by

Steven K. Schwartz

A DISSERTATION

Presented to the Faculty of
The Graduate College at the University of Nebraska
In Partial Fulfillment of Requirements
For the Degree of Doctor of Philosophy

Major: Biological Sciences
(Ecology, Evolution, and Behavior)

Under the Supervision of Professors Eileen A. Hebets and William E. Wagner Jr.

Lincoln, Nebraska

April, 2013

SPONTANEOUS MALE DEATH AND MONOGYNY IN THE DARK FISHING SPIDER,
DOLOMEDES TENEBROSUS HENTZ, 1843 (ARANEAE, PISAURIDAE)

Steven K. Schwartz, Ph.D.

University of Nebraska, 2013

Advisors: Eileen A. Hebets and William E. Wagner Jr.

Male animals typically attempt to mate with multiple females in order to increase their reproductive success. In some species, however, males instead invest in fertilizing the eggs of a single female. Monogyny (male monogamy) is found in a diverse assemblage of taxa, and recent theoretical work reveals that a male-biased sex ratio can favor the evolution of this relatively rare mating system. We integrate this theoretical framework with field observations and laboratory experiments involving the sexually size dimorphic fishing spider, *Dolomedes tenebrosus*. Results from mating trials revealed a novel form of self-sacrifice behavior where males spontaneously die when they copulate, thus all males are monogynous. Since all males experience obligate death when they mate, we set out to determine if male self-sacrifice in *D. tenebrosus* is adaptive. Self-sacrifice behavior (complicity in cannibalism or spontaneous death associated with copulation) can be adaptive if it facilitates sexual cannibalism, and if sexual cannibalism results in reproductive benefits for the self-sacrificing male. In a first series of experiments, we examine variation in female cannibalistic behavior and variation in female mating rate. We test the hypothesis that spontaneous male death facilitates postcopulatory sexual cannibalism and that sexual cannibalism reduces the likelihood of female remating. We found that spontaneous male death does indeed facilitate sexual cannibalism, as all females cannibalize males

postcopulation. However, sexual cannibalism does not reduce the likelihood of female remating, thus *D. tenebrosus* males do not appear to receive a paternity advantage from postcopulatory sexual cannibalism. In a second series of experiments, we examine how sexual cannibalism affects female fecundity. We test the hypothesis that the consumption of a male by a female postcopulation results in an increase in the quantity and/or quality of offspring. We found that sexual cannibalism causes females to produce more and higher quality offspring. Specifically, when females were allowed to consume their mating partner, it resulted in a significant increase in the number, the size, and the survivorship of offspring. Our results document a novel case of adaptive self-sacrifice in the first non-araneoid spider, *D. tenebrosus*, providing evidence that males benefit from sexual cannibalism through paternal effort. [349]

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~ Dedicated to all the males who sacrificed their lives (literally). ~

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Finally, I would like to thank my family for supporting me intellectually and financially all these years, and for literally putting a roof over my head. I would like to thank my wife for being my wife. And with my most important research tools (i.e. headlamp, chest waders, and a walking stick) I hope to continue to play around with spiders and call it research for many more years to come.

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CHAPTER 1**SPONTANEOUS MALE DEATH AND MONOGYNY IN THE DARK FISHING SPIDER**

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ABSTRACT

Monogyny (male monogamy) is found in a diverse assemblage of taxa, and recent theoretical work reveals that a male-biased sex ratio can favor the evolution of this relatively rare mating system (Fromhage et al. 2005). We integrate this theoretical framework with field observations and laboratory experiments involving the sexually size dimorphic fishing spider, *Dolomedes tenebrosus*, to test the prediction that this species exhibits monogyny. Field surveys revealed a male-biased sex ratio, likely resulting from different life history strategies (early male maturation). Results from mating trials supported our prediction of monogyny as we discovered that males mate with a single female. Unexpectedly, however, we observed that mating results in obligate male death and genital mutilation. Additional field observations of released individuals suggest that males are not limited by their ability to encounter additional females. Controlled laboratory assays demonstrated that males discriminate among virgin and non-virgin female silk cues, suggesting first-male sperm precedence. In summary, we report a novel form of male self-sacrifice in a species that exhibits female-biased sexual size dimorphism, male-biased sex ratio, genital mutilation, and a suggestion of first-male sperm precedence; all of which are consistent with theoretical predictions of the evolution of monogyny. [195]

Key words: *Dolomedes tenebrosus*, mating system, monogyny, self-sacrifice, sex ratio, sexual size dimorphism, sperm precedence

INTRODUCTION

Males of many animal taxa mate with multiple females, a phenomenon generally explained by the notion that males are only limited by the number of eggs they can access and fertilize. Males can thus increase their reproductive success by indiscriminately mating with many females (Bateman 1948). In contrast, females are thought to be limited by their ability to convert resources into eggs and, ultimately, into viable offspring. Females are thus predicted (and often observed) to mate less frequently than males and to be choosy about their mating partners (Bateman 1948; Trivers 1972; Andersson 1994). While such patterns may be common in some animal groups, such as mammals, males in other groups such as birds and fishes mate less frequently than expected; instead of mating with multiple females, these males enhance their reproductive success by caring for offspring, thereby trading offspring quantity for quality (Clutton-Brock 1991). Surprisingly, males in some groups neither mate with multiple females nor invest in paternal care, but instead invest in fertilizing the eggs of a single female (Schneider and Fromhage 2010). This phenomenon of monogyny (male monogamy) is taxonomically widespread and has been shown to have numerous independent origins (Miller 2007; Schneider and Fromhage 2010). Its existence has provided a challenge to evolutionary biologists, as it is not initially apparent what benefits males might gain by foregoing additional matings.

Recent theory provides clear and straightforward predictions of the circumstances under which monogyny can evolve – monogyny is favored over polygyny when there is a male-biased effective sex ratio (*ESR*; Fromhage et al. 2005), defined as the ratio between males and females that mate at least once. Furthermore, such male-biased sex ratios are suggested to arise in concert with female-biased sexual size dimorphism (Fromhage et al. 2005; Miller 2007; Schneider and Fromhage 2010), as males in such systems often reach sexual maturation earlier and are more likely to survive to maturity (Ghiselin 1974;

Andersson 1994; Segev et al. 2003). Such life history patterns have led scientists to predict an evolutionary correlation between female-biased sexual size dimorphisms, male-biased sex ratios, and monogyny (Schneider and Fromhage 2010). In addition, monogyny is often coincident with the evolution of extreme mating behavior (reviewed in Schneider and Fromhage 2010).

This study set out to test for monogyny in the dark fishing spider *Dolomedes tenebrosus* Hentz, 1843 (Araneae, Pisauridae), one of nine Nearctic species in the genus (Carico 1973). While females and males of most *Dolomedes* species are similarly sized, *D. tenebrosus* exhibits extreme female-biased sexual size dimorphism (Scharff and Coddington 1997; Hormiga et al. 2000): females weigh 14 times more than males and have cephalothoraxes that are 2.5 times as wide (Figure 1.1). Like most spiders, *D. tenebrosus* is relatively short-lived, but the sexes appear to have different life history strategies. Of hundreds of juveniles collected in the field and reared in the laboratory over a four-year period, males always matured the same season while females took an additional year to mature (Schwartz SK, personal observation). Additionally, within a season, males mature earlier than females (Figure 1.2a). These life history differences are predictive of a male-biased sex ratio, a hypothesized prerequisite for the evolution of monogyny. This study aimed to examine the sex ratio in the field and to conduct laboratory assays to determine the mating system of *D. tenebrosus*.

METHODS

We collected immature male and female *D. tenebrosus* over a four-year period in Lancaster County near Lincoln, Nebraska (U.S.A.) and recorded the sex ratio and individual maturation times. Over two years we ran mating trials in the laboratory to investigate mating system dynamics. Following our observation of obligate male death (see Results),

we measured the postcopulation heartbeat of males. We also dissected the pedipalps of virgin males to determine if *D. tenebrosus* males carry sperm in both their pedipalps. In the field, we monitored the mate search behavior of released males to determine the accessibility of multiple females. Finally we ran behavioral trials in the laboratory to test predictions of first-male sperm precedence by investigating whether males discriminate among virgin and non-virgin silk cues. Results are reported as means \pm SE. For the details of our methods see the supplementary material.

RESULTS and DISCUSSION

Field collections revealed that male *D. tenebrosus* outnumber females almost 3:1 at the beginning of the breeding season (Figure 1.2b), thus confirming a male-biased sex ratio. The mating behavior observed in the laboratory ($N = 24$) and once in the field was similar to that described previously (Sierwald and Coddington 1988) except that in every mating, seemingly instantaneous with sperm transfer, the male's legs curled underneath its body and it hung motionless from the female's genital opening, appearing dead (Figure 1.3, Video 1.1). All males in this position were unresponsive to touch and never recovered from this immobile state. Observations of a subset of males ($N = 15$) confirmed that heartbeats stop within a few hours (164 ± 9 minutes, Figure 1.4). Our results reveal a novel form of monogyny involving obligate male death.

Sperm transfer in spiders involves the inflation of the hematodochal bulb in the male pedipalp. Upon maturation, males ejaculate onto a sperm-web where they pick up the sperm and carry them in their pedipalps (sperm transfer organs; Foelix 1996). The hematodochal bulb expands hydraulically due to hemolymph pressure and in most spiders, after sperm transfer, the bulb subsequently contracts. In *D. tenebrosus* we found that the hematodochal bulb remains expanded (Figure 1.5) and presumably non-functional. This

type of genital disfiguration is only known to occur in one other self-sacrificing species, *Tidarren sisyphoides* (Knoflach and Benjamin 2003). Curiously, unlike *T. sisyphoides* (which removes one pedipalp), *D. tenebrosus* males retain their second, unused pedipalp. To determine if sperm is present in both, we dissected the pedipalps of virgin males. All males examined ($N = 5$) had sperm in both pedipalps, and although sperm numbers were not quantified, the amount of sperm appeared similar between the two. Thus, it appears that *D. tenebrosus* males die 'prematurely', prior to realizing their full reproductive potential (sensu Stoltz et al. 2009).

Similar to the observed single palpal discharge of *D. tenebrosus*, males of another *Dolomedes* species discharge one pedipalp and immediately dismount their mate. In the six-spotted fishing spider, *Dolomedes triton*, few females accept a second insertion from the initial suitor and over half attack the male after accepting a single insertion (Johnson 2001). Successful attacks by *D. triton* females are known to provide a reproductive advantage to both partners as females that cannibalize a male increase the likelihood that their egg sac will hatch (Johnson 2001). Sexual cannibalism associated with self-sacrifice behavior in the Australian redback spider, *Latrodectus hasselti*, has similarly been considered adaptive. Male *L. hasselti* who are successful at feeding themselves to females during mating receive two advantages over males who are not successful: (i) cannibalized males increase their paternity; and (ii) females who cannibalize are less receptive to additional matings (Andrade 1996; Snow and Andrade 2004). Currently, potential advantages to *D. tenebrosus* males have not been identified, but future studies will explore the incidence of sexual cannibalism and its potential influence on female remating rates, egg production, and offspring survival.

The pedipalps of *D. tenebrosus* are obligately disfigured during copulation, representing a form of genital mutilation. In a broad phylogenetic study of self-sacrifice

behavior and genital mutilation across araneoid (web-building) spiders, self-sacrifice was hypothesized to have evolved at least 5 or 6 times in lineages with genital mutilation (Miller 2007). Given the obligate nature of premature self-sacrifice behavior and genital mutilation in *D. tenebrosus*, our data cannot address the evolutionary timeline regarding the origin of these traits. Nonetheless, Miller (2007) suggested that when both genital mutilation and male-biased sex ratios are present, the stage is set for the evolution of adaptive male self-sacrifice behavior. Following from this, we hypothesize that early male maturation resulting in female-biased sexual size dimorphism in *D. tenebrosus* gave rise to male-biased sex ratios, which set the stage for the evolution of monogyny in this species. We further suggest that the mechanism(s) underlying the genital mutilation and self-sacrifice are connected and that they evolved in concert.

High male mortality (80-92%) during mate searching has been suggested as an important factor influencing the evolution of monogyny in *L. hasselti* (Andrade 1996; Andrade 2003). When the chance of encountering a second female is exceedingly small, males are expected to invest all their resources in the first female they encounter (see Buskirk et al. 1984). Despite its likely role in the evolution of monogyny in the redback spider, theoretical explorations into the evolution of monogyny do not find high search costs to be a necessary component (Fromhage et al. 2005). Nonetheless, in order to determine if high search costs may be a driving force in *D. tenebrosus*, we conducted field observations of released males. An astonishing fifty-percent found a female within one hour of release (50 ± 9 minutes). In one extraordinary situation, one male encountered 5 different females over a 94-minute period. We observed no instances of predation and ultimately found no evidence of high search costs in *D. tenebrosus* – supporting the theoretical findings that high search costs are not a necessary component of monogyny (Fromhage et al. 2005).

Given a male-biased sex ratio, monogyny is most likely to evolve in taxa with first-male sperm precedence (Schneider and Fromhage 2010). While sperm precedence patterns are not known for many animal taxa, particular traits and behavior are often coincident with first-male sperm precedence (e.g. early male maturation and precopulatory mate guarding; see Elias et al. 2011). Additionally, males in such systems are expected to exhibit a strong preference for virgin versus non-virgin females, as males will have an increased paternity share with the former (Elias et al. 2011). While sperm precedence patterns in *D. tenebrosus* are not known, males in the related *D. triton* guard females prior to mating and both *D. triton* and *D. tenebrosus* exhibit early male maturation (Johnson 2001; current study) – observations that are consistent with first-male sperm precedence. In *D. tenebrosus*, we tested the prediction that males discriminate among females. In support of our prediction, when exposed to virgin and non-virgin silk, males spent significantly more time exploring an arena if it had silk from a virgin female (repeated measures ANOVA: $F_{1,14} = 51.42, P < 0.001$). Generally, male spiders are attracted to virgin females and the first male to mate with a female will have significant advantages, depending on sperm precedence patterns (Gaskett 2007). However, first-male advantage is common among spiders, particularly in entelegyne spiders like *D. tenebrosus* (Austad 1984, but see Elgar 1998). So all available data are consistent with the hypothesis that *D. tenebrosus* have first-male sperm precedence – a hypothesis that remains to be tested directly.

In summary, we document a novel case of monogyny involving an unusual form of male self-sacrifice – obligate male death. While monogyny and associated self-sacrifice behavior has been reported in a few other species (Table 1.1), male *D. tenebrosus* die ‘prematurely’ (i.e. prior to realizing their full reproductive potential) with no apparent involvement from females. Additionally, while some classic unconventional mating systems are found in spiders, all prior examples come from araneoid spiders, where self-sacrifice is

hypothesized to have evolved multiple times (Miller 2007). We provide the first full description of an extraordinary form of self-sacrifice behavior in a non-araneoid spider, providing an independent evolutionary test of hypotheses relating to the evolution of monogyny. In addition, this study provides multiple avenues of data consistent with prior studies that suggest evolutionary correlations between female-biased sexual size dimorphism, male-biased sex ratio, genital mutilation, and first-male sperm precedence.

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TABLES and FIGURES

Table 1.1 The characteristics of spontaneous male death in spiders. The three published araneoid (web-building) examples of self-sacrifice behavior in the form of spontaneous death associated with copulation.

Species	Spontaneous Death Characteristics	Reference
<i>Argiope aemula</i>	Male death is associated with the insertion of their second pedipalp.	Sasaki and Iwahashi 1995
<i>Argiope aurantia</i>	Male death is triggered by the insertion and inflation of the distal bulb of their second pedipalp.	Foellmer and Fairbairn 2003
<i>Tidarren sisyphoides</i>	Male death is associated with the insertion of their first and only pedipalp (due to palpal-amputation).	Knoflach and Benjamin 2003

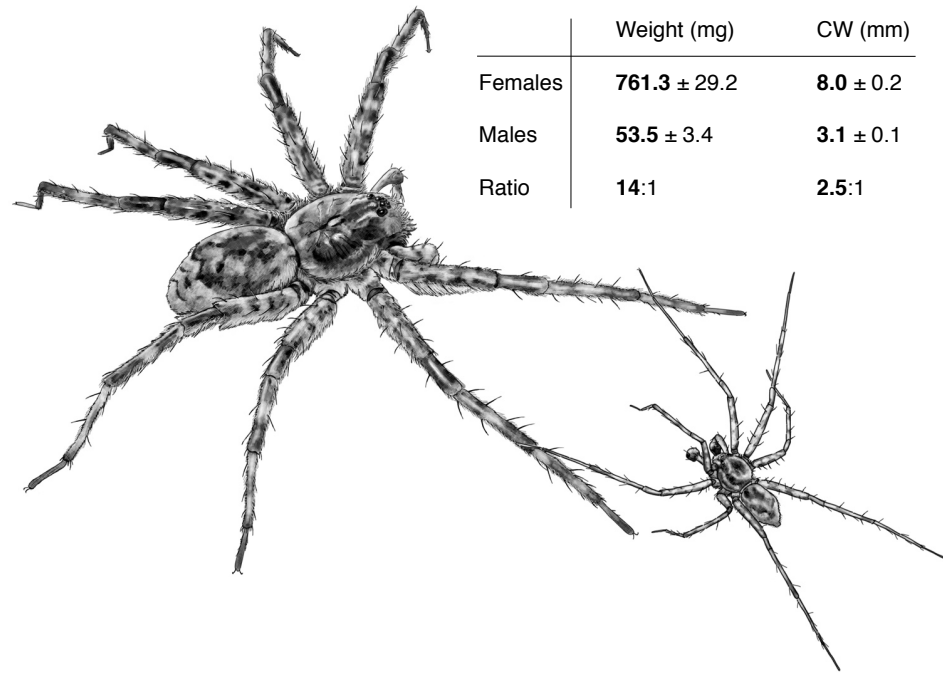


Figure 1.1 Illustration of the female-biased sexual size dimorphism in the dark fishing spider, *Dolomedes tenebrosus*. Females weigh more than males ($N = 25$) and have wider cephalothoraxes (prosoma), measured in carapace width (CW), than males ($N = 25$). Data are reported as means \pm SE. Illustration by Karina I. Helm.

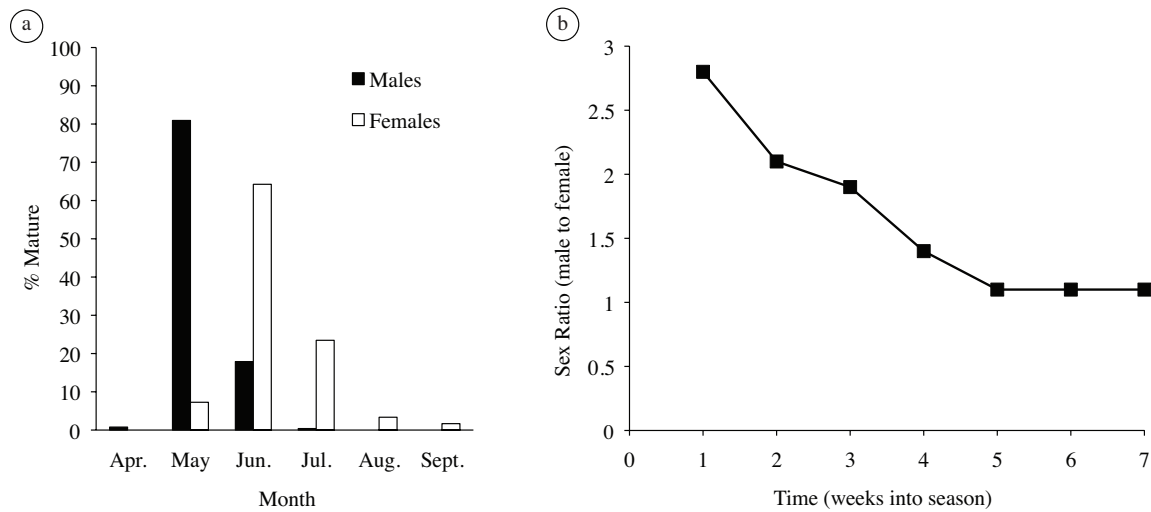


Figure 1.2 Seasonal differences in the maturation time and sex ratio in *Dolomedes tenebrosus*.

(a) Proportion of individuals collected (2006-2009) that reached maturity throughout the season

(81% males mature in May vs. 7% females).

(b) Ratio of males to females collected throughout the season.



Figure 1.3 Photograph of a *Dolomedes tenebrosus* male and female during copulation.

Postcopulation the male hangs from the female's genital opening by the single pedipalp (*circled*) that he inserted. Photograph by Steven K. Schwartz.

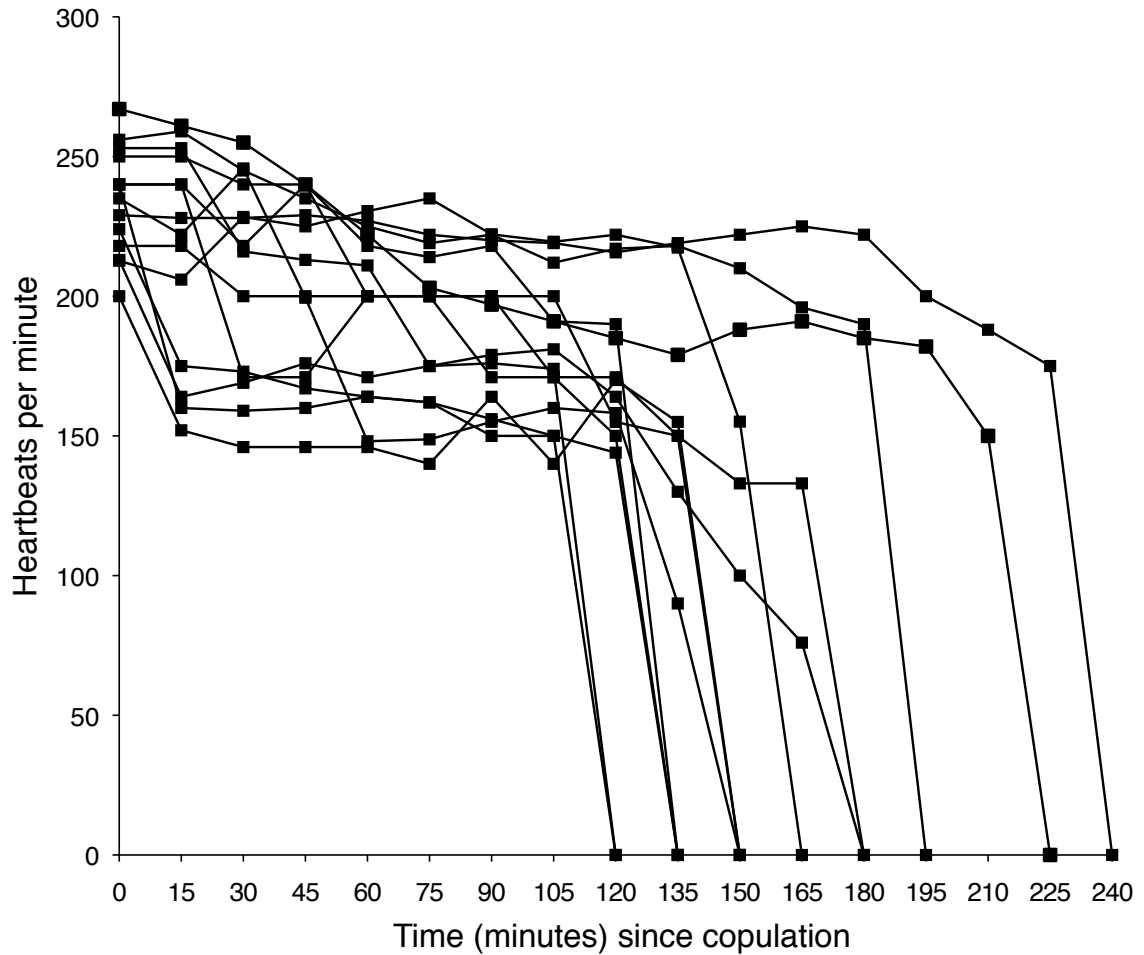


Figure 1.4 Changes in the heartbeat of *Dolomedes tenebrosus* males ($N = 15$) postcopulation. Following palpal insertion and expansion of the hematodochal bulb (i.e. copulation), the mean time to heartbeat cessation was 164 ± 9 minutes.



Figure 1.5 Photograph of a *Dolomedes tenebrosus* male after copulation. Postcopulation the hematochal bulb of the male (*circled*) remains in the expanded state and disfigured.

Photograph by Steven K. Schwartz.



Video 1.1 Screen shot from a video showing spontaneous male death in *Dolomedes tenebrosus*.

The video documents one of the first mating trials from 2006. (1) Male is introduced into the mating arena. (2) *D. tenebrosus* courtship behavior. (3) Copulation and spontaneous male death. (4) Male is detached from the female and removed from the mating arena. Video by Steven K. Schwartz (QuickTime, 9.7 MB, 480 x 360, 1:58 minutes).

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SUPPLEMENTARY MATERIAL

METHODS

Animal Collections

Immature male and female spiders were collected during the spring (April-May) of 2006-2010 in Lancaster County near Lincoln, Nebraska (U.S.A.). Male and female *D. tenebrosus* were collected at night using a light-emitting diode (LED) headlamp. Night collection takes advantage of the eyeshine seen in *D. tenebrosus* (and other fishing spiders), which assists us to locate and capture the spiders in the field. Field caught individuals were transported back to the laboratory and housed individually in 87.3 mm x 87.3 mm x 112.7 mm plastic containers (763C, AMAC Plastics, Petaluma, CA). Containers were housed in a climate-controlled room (24-27° C) and placed on a light:dark cycle that matched the outside summer environment (April-August, 13:11-15:9 light:dark). Female spiders were fed two 2-week-old (1/4") crickets, *Acheta domesticus*, three times a week and male spiders were fed two 1-week-old (1/8") crickets, *A. domesticus*, once a week (Bassett's Cricket Ranch, Visalia, CA). In addition, all spiders were provided water *ad libitum*. All spiders were weighed (Ohaus Explorer balance 0.0001 g) twice, first when transported to the laboratory and once again prior to their assigned experimental trial.

Mating Trials

Staged mating trials were videotaped (Sony DCR-HC96 MiniDV Handycam) during the summer of 2006 ($N = 4$) and 2007 ($N = 20$). All individuals used were field caught virgins, which molted to maturity in the laboratory (spiders are not sexually mature prior to their final molt). Mature male and female spiders were used only once during the mating trials. Males were assigned to females at random and females were placed individually in a 252.4 mm x 90.5 mm (diameter x depth) plastic arena (250C, Pioneer Plastics, North Dixon,

KY) for a minimum of 24 hours prior to the introduction of the male. The arena floor was covered with a disc of filter paper (Double Rings, 102 Qualitative, 24 cm) and at the center of the arena was a 47.6 mm x 84.1 mm plastic vial (40 DRAM, Thornton Plastics, Salt Lake City, UT) covered in fiberglass mesh on which the female could climb and position herself. On average, mating trials lasted 1.5 hours from the introduction of the male to the insertion of his first pedipalp.

Heartbeat Measurements

To determine when males died following mating, we measured the time from palpal insertion to the termination of the heartbeat postcopulation during the summer of 2007 ($N = 8$), 2009 ($N = 4$), and 2010 ($N = 3$). Only male spiders that were fully intact (i.e. males that had not been punctured by the female and/or damaged when removed from the mating arena) were used for heartbeat measurements. In order to measure the heartbeat, males were removed from the arena following the insertion of their first pedipalp and their heartbeat was monitored by counting the pulse rate of the abdomen (opisthosoma) every 15 minutes via stereo microscope (Leica M216, Buffalo Grove, IL) postcopulation until it terminated.

Palpal Dissections

In the summer of 2009 we removed and dissected the pedipalps of mature virgin male spiders ($N = 5$) to determine if *D. tenebrosus* males charge both of their pedipalps with sperm. Following a simplified method similar to Bukowski and Christenson (1997), we removed the pedipalps of each male under a stereo microscope (Leica M216, Buffalo Grove, IL). The pedipalps were then crushed with metal forceps and placed on a microscope slide

with a drop of water. Finally each pedipalp was viewed using a light microscope (Leica DM4000 B, Buffalo Grove, IL) and the presence or absence of sperm cells was recorded.

Field Monitoring

In the summer of 2010 we collected males from the field and held them in the laboratory until their maturity molt, at which time they were released in the field and their mate search behavior was monitored. Following their maturity molt, male spiders were marked with a paint pen (DecoColor, Uchida of America, Torrance, CA) and returned to the field (to their original collection location) for observations. Over a series of nights, June-July 2010, male spiders ($N = 18$) were released at their initial collection points and monitored. By following individual male spiders throughout the night, we were able to quantify the probability of a *D. tenebrosus* male locating and contacting a *D. tenebrosus* female in the field.

Silk Trials

In the summer of 2009 we videotaped (Sony DCR-HC96 MiniDV Handycam) the behavior of male spiders ($N = 15$) when presented with the silk from a virgin and a non-virgin female spider. Mature virgin and non-virgin female spiders were used only once during the silk trials and mature male spiders were used twice (repeated measures design). Female spiders were placed in an individual 252.4 mm x 90.5 mm (diameter x depth) plastic arena (250C, Pioneer Plastics, North Dixon, KY) which had the floor covered with a disc of filter paper (Double Rings, 102 Qualitative, 24 cm). Female spiders (virgin and non-virgin) then spent a minimum of 24 hours in the arena laying down silk. With the female spiders removed, male spiders were individually introduced into a silken arena. The order of the presentation (virgin vs. non-virgin silk) was randomized and males were presented with

virgin or non-virgin silk a minimum of 24 hours apart. During the silk trials, we recorded male activity (i.e. time spent moving) and male courtship signals (visual and seismic) for a period of 15 minutes.

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CHAPTER 2

**MALE SELF-SACRIFICE IN THE DARK FISHING SPIDER DOES NOT AFFECT FEMALE
REMATING**

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ABSTRACT

Male dark fishing spiders (*Dolomedes tenebrosus* Araneae, Pisauridae) sacrifice themselves by spontaneously dying during their first and only copulation, thus all males are monogynous. This self-sacrifice behavior (complicity in cannibalism or spontaneous death associated with copulation) can be adaptive if self-sacrifice facilitates sexual cannibalism, and if sexual cannibalism results in reproductive benefits for the self-sacrificing male. Here we build upon the previously developed adaptive self-sacrifice framework to examine potential benefits of spontaneous male death in the dark fishing spider. In our first experiment we examine variation in female cannibalistic behavior (precopulatory and postcopulatory) and variation in female multiple mating. In our second experiment we test the hypothesis that spontaneous male death facilitates postcopulatory sexual cannibalism, and the hypothesis that sexual cannibalism reduces the likelihood of female remating. We found that spontaneous death facilitates sexual cannibalism in *D. tenebrosus*; females always cannibalize males postcopulation. We also found that females will copulate with multiple males and that the cannibalism of the male does not reduce females remating. Additionally, we confirmed that mate plugging is not common in *D. tenebrosus*, even though males may act as whole-body mating plugs immediately after they die. While studies have provided evidence that self-sacrifice behavior can provide males an advantage in sperm competition because females that consume males are less likely to remate, our results provide no support for this hypothesis. Self-sacrifice by *D. tenebrosus* males does not appear to reduce the likelihood of female remating, and thus they do not appear to receive a paternity advantage from postcopulatory sexual cannibalism. [254]

Key words: adaptive self-sacrifice, *Dolomedes tenebrosus*, mating effort, mating plug, monogyny, sexual cannibalism, sperm competition, spontaneous death

INTRODUCTION

Understanding how reproductive strategies vary within and between species (i.e. mating system variation) is essential to understanding sexual selection and its potential role in evolutionary change (Andersson 1994). While a variety of discrete mating systems have long been recognized, there is surprisingly no universal agreement on the definitions and classifications of those systems. Terms like monogamy, polygyny, and polyandry have been used in a variety of ways depending on the researcher and species studied (Andersson 1994). Within behavioral ecology, the classification of mating systems is usually described in terms of the *number of mates* per male or per female (Shuster and Wade 2003) and researchers consider the mating system at either the individual level or at the population level (Thornhill and Alcock 1983). At the population level, mating systems are commonly portrayed as mutually exclusive, but this is rarely the case. In many animals, individuals within a population may exhibit different mating systems, with some proportion of males and/or females acquiring more than one mating partner and others acquiring only one (e.g. polygynous males, polyandrous females, and monogamous males and females) (Thornhill and Alcock 1983). A handful of species show a relatively rare mating system in which all, or most, males acquire only one lifetime mating. This has been referred to as male monogamy or monogyny (Fromhage et al. 2005). In these species, males are often unable to mate with more than one partner due to the occurrence of sexual cannibalism and/or genital damage during or following mating, which prevents future mating opportunities.

Mating systems with monogynous males are relatively rare, yet taxonomically widespread and have evolved a number of times independently (Miller 2007; Schneider and Fromhage 2010). For example, in honeybees, males have evolved genitalia that explode into the female, causing males to die after a single mating (Michener 1974). In some molluscs, crustaceans, and fishes, males do not die when they mate, but remain permanently attached

to a female, rendering them unable to acquire additional matings (reviewed in Schneider and Fromhage 2010). In yet other animals, females impose monogamy on males. In the queenless ant, *Dinoponera quadriceps*, females sever the end of the copulating male's abdomen, killing the male and leaving his genitalia linked to hers (Monnin and Peeters 1998), and in burying beetles, females interfere when males attempt to attract additional females (Eggert and Sakaluk 1995). This mating system is puzzling, particularly when monogyny results from male adaptations that limit future mating opportunities. Some the most well studied examples of monogyny are in spiders, and it is from researchers working with these animals that the most recent theoretical and conceptual advances have been made. Monogyny in spiders is often associated with extreme mating behaviors such as male self-sacrifice (complicity in cannibalism or spontaneous death associated with copulation) and male self-mutilation of genitalia (reviewed in Schneider and Fromhage 2010); and it is not immediately obvious why males would (apparently) facilitate their own cannibalism or death. Theoretical and empirical explorations of the evolution of monogyny indicate that its presence is often correlated with male-biased effective sex ratios (*ESR*; Fromhage et al. 2005) and female-biased sexual size dimorphisms (Fromhage et al. 2005; Miller 2007; Schneider and Fromhage 2010). To date, most research has focused upon araneoid (web-building) spiders within the families Araneidae, Theridiidae, and Nephilidae (see Schneider and Fromhage 2010).

The first example of monogyny in a non-araneoid spider was recently discovered in the dark fishing spider, *Dolomedes tenebrosus* (Schwartz et al. in review). These males exhibit an extreme form of monogyny as the males inevitably die following the transfer of sperm. During copulation in spiders, sperm is transferred by the inflation of the hematodochal bulb in the pedipalp. This hematodochal bulb is inflated hydraulically due to an increase in hemolymph pressure and, in most species, the bulb contracts following

sperm transfer and can be reused (Foelix 1996). In *D. tenebrosus*, males inflate their first hematodochal bulb when copulating with a female, immediately curl up and become non-responsive, and their heart stops beating within a few hours (Schwartz et al. in review). All copulations of *D. tenebrosus* recorded in the laboratory and witnessed in the field have resulted in obligate male death (Schwartz et al. in review).

Consistent with patterns observed in other monogynous species, *D. tenebrosus* is unique among its Nearctic congeners as it exhibits extreme female-biased sexual size dimorphism (Scharff and Coddington 1997; Hormiga et al. 2000; Schwartz et al. in review). Furthermore, field observations reveal that *D. tenebrosus* exhibits a naturally occurring male-biased sex ratio (Schwartz et al. in review), consistent with theoretical predictions for the evolution of monogyny. This newly discovered system offers an excellent opportunity to test hypotheses regarding the potential adaptive nature of monogyny resulting from self-sacrifice.

The most well studied monogynous self-sacrificing spider species is the Australian redback, *Latrodectus hasselti*. Male redbacks actively sacrifice themselves during copulation by somersaulting their bodies onto the fangs of their mating partners in an apparent attempt to induce sexual cannibalism. Initial studies suggested that males receive two benefits from such self-sacrifice behavior: (i) increased paternity; and (ii) reduced female remating (Andrade 1996). Such research has led to the development of a framework of hypotheses regarding adaptive self-sacrifice (Andrade 1996; Andrade 1998; Andrade 2003; Snow and Andrade 2004). Self-sacrificing males who are cannibalized can increase the proportion of eggs they fertilize under sperm competition through either transferring more sperm or reducing female receptivity (Low 1978; Simmons and Parker 1989; Elgar 1992; Andrade 1996; Andrade 1998) (Table 2.1: Mating Effort). Additionally, cannibalized males can donate somatic nutrients (i.e. their bodies) to a female and ultimately to their own

offspring, thereby increasing offspring quantity or quality (Thornhill 1976; Low 1978; Parker 1979; Buskirk et al. 1984; Simmons and Parker 1989; Elgar 1992) (Table 2.1: Paternal Effort). These adaptive self-sacrifice hypotheses are not mutually exclusive. Alternatively, self-sacrificing males may also obtain an advantage in sperm competition if their bodies function as whole-body mating plugs, preventing rival males from mating (Knoflach and Van Harten 2001; Foellmer and Fairbairn 2003), however this does not require the assistance of sexual cannibalism.

In this study, we build upon the previously developed adaptive self-sacrifice framework to examine potential benefits of obligate male death in the dark fishing spider, *D. tenebrosus*. In a first experiment, we document the frequency of precopulatory and postcopulatory sexual cannibalism and determine the prevalence of female multiple mating. Multiple mating by females is a prerequisite for sperm competition. In a second experiment, we ask whether self-sacrifice behavior in *D. tenebrosus* provides males with a benefit in terms of sperm competition. Specifically, we test the hypothesis that obligate male death facilitates postcopulatory sexual cannibalism, and that postcopulatory sexual cannibalism reduces the likelihood of remating by females (Table 2.1: Mating Effort).

METHODS

Animals

Immature male and female spiders were collected during the spring (April-May) in Lancaster County near Lincoln, Nebraska (U.S.A.). Male and female *D. tenebrosus* were collected at night using a light-emitting diode (LED) headlamp. Night collection takes advantage of the eye-shine seen in *D. tenebrosus* (and other fishing spiders), which facilitates the location and capture of spiders in the field. Field caught individuals were transported back to the laboratory and housed individually in 87.3 mm x 87.3 mm x 112.7

mm plastic containers (763C, AMAC Plastics, Petaluma, CA). Containers were housed in a climate-controlled room (24-27° C) and placed on a light:dark cycle that matched the outside summer environment (April-August, 13:11-15:9 light:dark). Female spiders were fed two 2-week-old (1/4") crickets, *Acheta domesticus*, three times a week and male spiders were fed two 1-week-old (1/8") crickets, *A. domesticus*, once a week (Bassett's Cricket Ranch, Visalia, CA). In addition, all spiders were provided water *ad libitum*. Males and females matured in the laboratory, and were thus known to be virgins when used in experiments. All spiders were weighed (Ohaus Explorer balance 0.0001 g) twice, first when transported to the laboratory and once again prior to their assigned experimental trial.

Experiment 1: Sexual Cannibalism and Female Remating

Prior laboratory observations suggested that *D. tenebrosus* males are often cannibalized by females following copulation. In this experiment, we aimed to determine both the frequency of sexual cannibalism (precopulation and postcopulation) in this system and the potential for female remating. We allowed male-female pairs to interact and recorded the presence/absence of sexual cannibalism. Male *D. tenebrosus* exhibit obligate death when they mate (Schwartz et al. in review) and in this experiment, we did not remove males following mating, thus enabling females to eat their sexual partners. In order to explore the potential for female remating, all mated females were then given the opportunity to mate with two additional males 72 hours apart (i.e. 72 and 144 hours after their initial mating). Males were assigned to females at random and females were placed individually in a 252.4 mm x 90.5 mm (diameter x depth) plastic arena (250C, Pioneer Plastics, North Dixon, KY) for a minimum of 24 hours prior to the introduction of the male. The arena floor was covered with a disc of filter paper (Double Rings, 102 Qualitative, 24 cm) and at the center of the arena was a 47.6 mm x 84.1 mm plastic vial (40 DRAM,

Thornton Plastics, Salt Lake City, UT) covered in fiberglass mesh on which the female could climb and position herself. We videotaped (Sony DCR-HC96 MiniDV Handycam) staged mating trials during the summer of 2008 ($N = 114$). All individuals used were field caught virgins, which molted to maturity in the laboratory (spiders are not sexually mature prior to their final molt). Mature male spiders were used only once and female spiders were used three times during the mating trials.

Statistical Analysis

To compare the rates of cannibalism and copulation, we recorded the behavior of individual females ($N = 38$) across three mating opportunities. We used generalized linear mixed models (GLMM) that included 'female' as a random effect to account for the repeated measures and 'male' (first, second, or third) as a fixed effect allowing us to look at how female behavior (cannibalism/copulation) changed with exposure to subsequent males. In addition, we used a Fisher's exact test to determine if the proportion of females who received more or less seminal materials (i.e. copulations) differed in their likelihood to remate. All analyses were conducted using the R statistical software (R 2012) version 2.15.1. For the generalized linear mixed models we used the R statistical software package 'lme4' (Bates et al. 2012).

Experiment 2: Sexual Cannibalism and Sperm Competition

Results from Experiment 1 demonstrated the pervasiveness of sexual cannibalism as well as the potential for female remating, which sets the stage for sperm competition. Our second experiment, then, aimed to test the hypothesis that sexual cannibalism, facilitated by obligate male death, could provide males an advantage in sperm competition by reducing

the likelihood of remating in their partners. Such an advantage has been previously demonstrated in the redback spider, *L. hasselti* (Andrade 1996).

To test whether sexual cannibalism provides males an advantage in terms of sperm competition, and whether this advantage results specifically from the consumption of the male, females were randomly assigned one of three postcopulation feeding treatments: (1) male, females were allowed to cannibalize the *D. tenebrosus* male with which they were mated; (2) cricket, females were prevented from cannibalizing their mate but were provided a cricket to eat; or (3) nothing, females were prevented from cannibalizing their mate and were not provided a cricket to eat. For all females, regardless of the treatment, the curled body of the male was removed immediately following a successful mating. Following the male's removal, females were either provided with the same male's body, a cricket that matched the weight of the male removed, or nothing. Males and crickets were provided to females using large forceps (30.5 cm), and all females readily accepted their postcopulation food item when offered. Regardless of whether or not the females were still eating (treatments 1 and 2), a second male was introduced into the arena 30 minutes postcopulation and the frequency of female remating was recorded, up to 3 hours. We chose a 30 minute time window since it is known that females may encounter multiple males within a single night (Schwartz et al. in review) and, in addition, this time window enabled us to explore the potential for obligate male death to provide males a sperm competition advantage through a whole-body mating plug (see Knoflach and Van Harten 2001; Foellmer and Fairbairn 2003). We videotaped (Sony DCR-HC96 MiniDV Handycam) staged mating trials during the summer of 2009 ($N = 36$). All individuals used were field caught virgins, which molted to maturity in the laboratory.

Statistical Analysis

To investigate if the cannibalism of a male postcopulation affects the likelihood that a female will remate, we recorded the behavior of individual females ($N = 36$) under the three postcopulation feeding treatments. We used a Fisher's exact test to determine if the proportion of females who remated differed across the three treatments. All analyses were conducted using the R statistical software (R 2012) version 2.15.1 and results are reported as means \pm SE.

RESULTS

Experiment 1: Sexual Cannibalism and Female Remating

Precopulatory sexual cannibalism was a common component of *D. tenebrosus* mating behavior. Prior to mating, females cannibalized their first and second mating partners similarly (first male: 26%, second male: 29%), but there was a significant increase in precopulatory sexual cannibalism when it came to the third mating partner (third male: 68%, GLMM: $Z = 3.553$, $P < 0.001$, Figure 2.1). Postcopulatory sexual cannibalism was also a common component of *D. tenebrosus* mating behavior as all females (100%) cannibalized all of their mating partners following copulation. Mated females plucked the unresponsive male from their epigynum and proceeded to eat him (eating = 96 ± 6 minutes, $N = 61$). Even though some females were seen to bite males during copulation (22 out of 61, 36%), every instance of postcopulatory sexual cannibalism was initiated after the male had curled up following palpal insertion.

Dolomedes tenebrosus females will readily remate. When given three mating opportunities 72 hours apart, half multiply mated (i.e. mated with more than a single male during a single cycle). While 19 out of 38 (50%) copulated with only a single male, 15 out of 38 (39.5%) copulated with two males and 4 out of 38 (10.5%) copulated with three males.

Looking across these three mating opportunities, females copulated with their first and second mating partners similarly (first male: 68%, second male: 66%), however females were significantly less likely to copulate with their third mating partner (third male: 26%, GLMM: $Z = -3.553$, $P < 0.001$, Figure 2.1). These results demonstrate that *D. tenebrosus* females will mate multiply if they have the opportunity to do so, making it possible that some males may not be able to monopolize a single female and may have to compete with other males for fertilization of a single female's eggs.

Our experimental design also enabled us to ask questions about seminal materials of males and their potential role in female remating. For example, if there are substances in the seminal materials transferred during copulation that reduce female remating, then we would expect to see a difference in remating probability between females who received more or less seminal materials (i.e. copulations). If this were the case, females who copulate more should show a reduction in receptivity and be less likely to remate. In contrast to this expectation, the proportion of females that remated did not differ between the females who received more or less seminal materials (Fisher's exact test: $P = 1.0$). For females who copulated once, 4 out of 13 (31%) remated. For females who copulated twice, 5 out of 19 (26%) remated. These results suggest that doubling the amount of seminal materials received does not affect the likelihood that a female will remate.

Experiment 2: Sexual Cannibalism and Sperm Competition

If males facilitate sexual cannibalism because it reduces the likelihood that a female will remate, regardless of the underlying mechanism, we predicted that females who eat their mate would be less likely to remate than those who eat nothing. Furthermore, if the somatic material of a *D. tenebrosus* male is unique (e.g. provides specific nutrients or contains anti-aphrodisiac compounds), then we would expect to see a difference between

the remating rates of females who consumed a male versus females who consumed a cricket postcopulation. For females who consumed a *D. tenebrosus* male, 8 out of 12 (67%) remated. For females who consumed an alternative food item postcopulation (i.e. a cricket), 8 out of 12 (67%) remated. And for females who consumed nothing, 9 out of 12 (75%) remated. Overall, the proportion of females that remated did not significantly vary among the treatments (Fisher's exact test: $P = 1.0$, Figure 2.2). These results suggest that the consumption of a male postcopulation does not affect the likelihood that a female will remate.

Our experimental design also enabled us to investigate if the bodies of *D. tenebrosus* males function as whole-body mating plugs. Given that some females waited as long as 87 minutes (19 ± 3 minutes, $N = 61$, Experiment 1) until they removed the male's body from their epigynum, a dead male's body could physically prevent a rival male from copulating. Specifically, obligate male death could provide *D. tenebrosus* males an advantage in sperm competition if their bodies function as whole-body mating plugs, assuming the mating plug (i.e. their body) was in place when a rival male comes along. In our second experiment, mate plugging was not common; only 1 out of 36 males (2.8%) delayed copulation by a subsequent male. In the 'nothing' treatment, one male was successful at delaying the copulation of the second male, and in this case, the first male did not function as a whole-body mating plug. Rather, his pedipalp torn off when the female removed him and it remained securely attached to the female's epigynum postcopulation, thus obstructing the second male. These results demonstrate that spontaneous death by male *D. tenebrosus* rarely prevents other males from physically mating with a female, even within a short time frame (30 minutes).

DISCUSSION

Males of the dark fishing spider, *D. tenebrosus*, obligately die following copulation, resulting in monogyny (Schwartz et al. in review). Our first experiment established that females cannibalize their mating partners 100% of the time following spontaneous male death, and that many of these females (50%) subsequently mated with additional males. Male self-sacrifice thus appears to facilitate postcopulatory sexual cannibalism in *D. tenebrosus*. Furthermore, because females will mate with multiple males, there is the potential for sperm competition. In our second experiment, we provided mated females with a male, a cricket, or nothing following an initial mating to test whether sexual cannibalism reduces the likelihood of female remating. We found no evidence of reduced likelihood of remating following sexual cannibalism (Table 2.1: Mating Effort). Unlike males of the redback spider, *L. hasselti* (Andrade 1996), male *D. tenebrosus* do not appear to reduce the likelihood of female remating, and thus do not appear to receive a paternity advantage from postcopulatory sexual cannibalism.

Sexual cannibalism in *D. tenebrosus* differs from sexual cannibalism in the redback spider as it occurs following a male's initial hematochal bulb expansion, and thus after sperm transfer. Since these self-sacrificing males are not actively cannibalized during copulation, sexual cannibalism cannot have an effect on the transfer of their sperm, specifically the rate of sperm transfer (Table 2.1: Mating Effort). This is not to say that self-sacrifice in *D. tenebrosus* does not facilitate increased sperm transfer, but sexual cannibalism *per se* does not appear to be involved. The possibility still remains that the act of sperm transfer – for example, the hydrostatic pressure increase associated with hematochal bulb expansion – enables increased sperm transfer and thus provides an advantage in sperm competition, with a consequence of obligate male death. Such a hypothesis requires direct testing.

Our research with *D. tenebrosus* has also provided little evidence for the hypothesis that self-sacrifice is adaptive because male bodies function as whole-body mating plugs (Knoflach and Van Harten 2001; Foellmer and Fairbairn 2003; Knoflach 2004). Immediately following sperm transfer in *D. tenebrosus*, the male body curls up and hangs from the female's genital opening; this introduces the potential for male bodies to act as whole-body mating plugs. There is precedent for this among spiders (see Miller 2007). Over 5 years (2006-2010) and covering 225 copulations, only once did a *D. tenebrosus* male successfully prevent a rival male from mating via mate plugging (Experiment 2, current study). During this single case, the first male copulated and spontaneously died, at which time the female plucked him from her epigynum and proceeded to start cannibalizing him. However, when the female removed the dead male, his right pedipalp tore off and remained in the female's epigynum, effectively plugging it. The rival male was prevented from mating with the female for 166 minutes. These results confirm that mate plugging is not common in *D. tenebrosus*, and even though males may act as whole-body mating plugs until females remove them, the time frame is too short to be effective. However, we cannot rule out the possibility that *D. tenebrosus* males are using a secretion or palpal fragment mating plug (reviewed in Uhl et al. 2010).

According to our results, the body of the male does not function as a mating plug, but it is possible that something else is occurring as he hangs from the female postcopulation. Males remained attached to the female for 19 ± 3 minutes (Experiment 1) and although they are motionless and do not respond to any probing stimuli (i.e. being prodded with forceps), their heart continues to beat (Schwartz et al. chapter 1). During this period of time the male may be transferring sperm to the female and the duration of attachment may be correlated with the amount of sperm transferred. However, in a number of spider species, sperm transfer is relatively quick (i.e. a few minutes) and a lengthy

copulation does not equal the transfer of more sperm (Schneider and Andrade 2011). For example in *L. hasselti*, the majority of sperm is transferred in the first 5 minutes and it is suggested that copulation duration serves as a form of copulatory courtship, with females selectively storing/using sperm from males who copulate for longer (Snow and Andrade 2004). In the spiny orb-weaver, *Micrathena gracilis*, sperm is transferred early in copulation and is not related to copulation duration. Males vary how much sperm is transferred depending on female mating history, and transfer most of their sperm (85-90%) when mating with a virgin female, while they transfer very little to no sperm when mating with a non-virgin female (Bukowski and Christenson 1997). At this point, additional work is needed to determine if and how copulation duration (i.e. time attached after spontaneous death) is correlated with sperm transfer *D. tenebrosus*.

Our results provide no evidence for reduced female remating following copulation and sexual cannibalism from either seminal materials transferred by males or from materials contained in the bodies of males. However, the act of sexual cannibalism itself may occupy the female, thus reducing her receptivity to subsequent courting males if she is busy feeding. We explicitly chose a half hour time frame for our remating design in order to allow us to explore the possibility that during the act of cannibalism (i.e. consuming the male), females may be less likely to remate. *Dolomedes tenebrosus* females do not immediately cannibalize the male postcopulation (mean latency to begin consumption = 19 minutes, Experiment 1), but when they do it takes some time to complete the consumption of the male (mean latency to complete consumption = 96 minutes, Experiment 1). We expected that some females would still be engaged in sexual cannibalism when the second male was introduced, and indeed, all females in Experiment 2 were busy feeding on the first male when the second male started courting. The act of sexual cannibalism does not appear to deter female remating, since 5 out of the 8 females who remated were actively consuming

the first male when they copulated with the second. Clearly, remating by female *D. tenebrosus* is not hindered by the consumption of a previous mate.

In addition to documenting the pervasiveness of postcopulatory sexual cannibalism in *D. tenebrosus* (all females), our first experiment also revealed that precopulatory sexual cannibalism was not uncommon. Precopulatory sexual cannibalism can be a risky behavior, especially for virgin females, since they may remain unmated due to their actions; assuming additional suitors do not come along (Elgar 1992; Arnqvist and Henriksson 1997).

However, precopulatory sexual cannibalism may become more likely if males are perceived to be more abundant and females may use cannibalism as a mechanism of mate choice, selectively cannibalizing unpreferred mates (Darwin 1871; Elgar and Nash 1988; Elgar 1992; Hebets 2003; Elgar and Schneider 2004; Johnson 2005; Prenter et al. 2006). Given the significant increase in the frequency of precopulatory sexual cannibalism at 144 hours with the last male (third male: 68%, Experiment 1), *D. tenebrosus* females may be using precopulatory sexual cannibalism as a means to reject additional mates at this point, whether or not the males are preferred.

In summary, in addition to establishing the prevalence of sexual cannibalism and female remating in the dark fishing spider, *D. tenebrosus*, we tested one hypothesis regarding adaptive self-sacrifice: male self-sacrifice facilitates sexual cannibalism, and sexual cannibalism reduces female remating. While self-sacrifice does seem to facilitate sexual cannibalism in *D. tenebrosus*, we found no support for the hypothesis that sexual cannibalism reduces female remating; females continued to remate at a variety of time scales following the cannibalism of a male. There are a number of additional hypotheses, however, for the benefits of male self-sacrifice. For example, sexual cannibalism may allow self-sacrificing males to redirect their reproductive effort if it leads to increased offspring quantity and/or quality (Table 2.1: Paternal Effort). That is, a male might benefit from

increasing an individual female's fecundity or offspring quality through the sacrifice of his body (i.e. from paternal investment; Simmons and Parker 1989). However, the possibility still remains that self-sacrifice in *D. tenebrosus* facilitates increased sperm transfer (Table 2.1: Mating Effort) and sexual cannibalism itself provide males no additional benefits.

Sexual cannibalism might be an incidental consequence of spontaneous male death, which is triggered by the hydraulic expansion of the hematodochal bulb during copulation.

Regardless, *D. tenebrosus* offers us an exciting new example of monogyny, and this species provides an opportunity to explore the causes and consequences of an unusual mating system where male death and postcopulatory sexual cannibalism are obligatory.

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TABLES and FIGURES

Table 2.1 The adaptive male sacrifice hypothesis*. Male self-sacrifice (complicity in cannibalism or spontaneous death associated with copulation) facilitates sexual cannibalism, resulting in reproductive benefits for the self-sacrificing male. Males can benefit from self-sacrifice via Mating Effort or Paternal Effort.

Mating Effort

The self-sacrificing male increases the proportion of eggs he fertilizes under sperm competition by:

- (1) Transferring more sperm
 - (2) Reducing female receptivity
-

Paternal Effort

The self-sacrificing male invests his somatic nutrients into his own offspring by:

- (1) Increasing offspring quantity
 - (2) Increasing offspring quality
-

**Note these hypotheses are not mutually exclusive.*

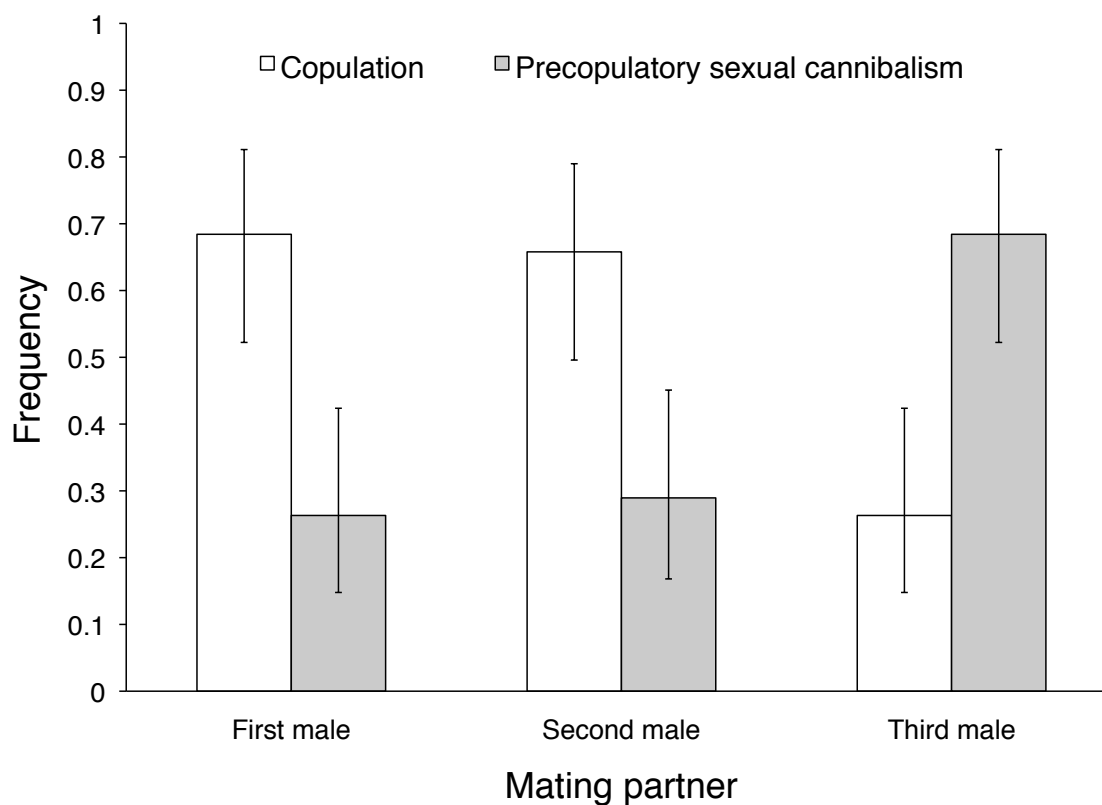


Figure 2.1 Frequency of copulation and precopulatory sexual cannibalism in *Dolomedes tenebrosus*. The mating behavior of 38 females was recorded over three mating opportunities at 0, 72, and 144 hours. Error bars represent 95% confidence limits.

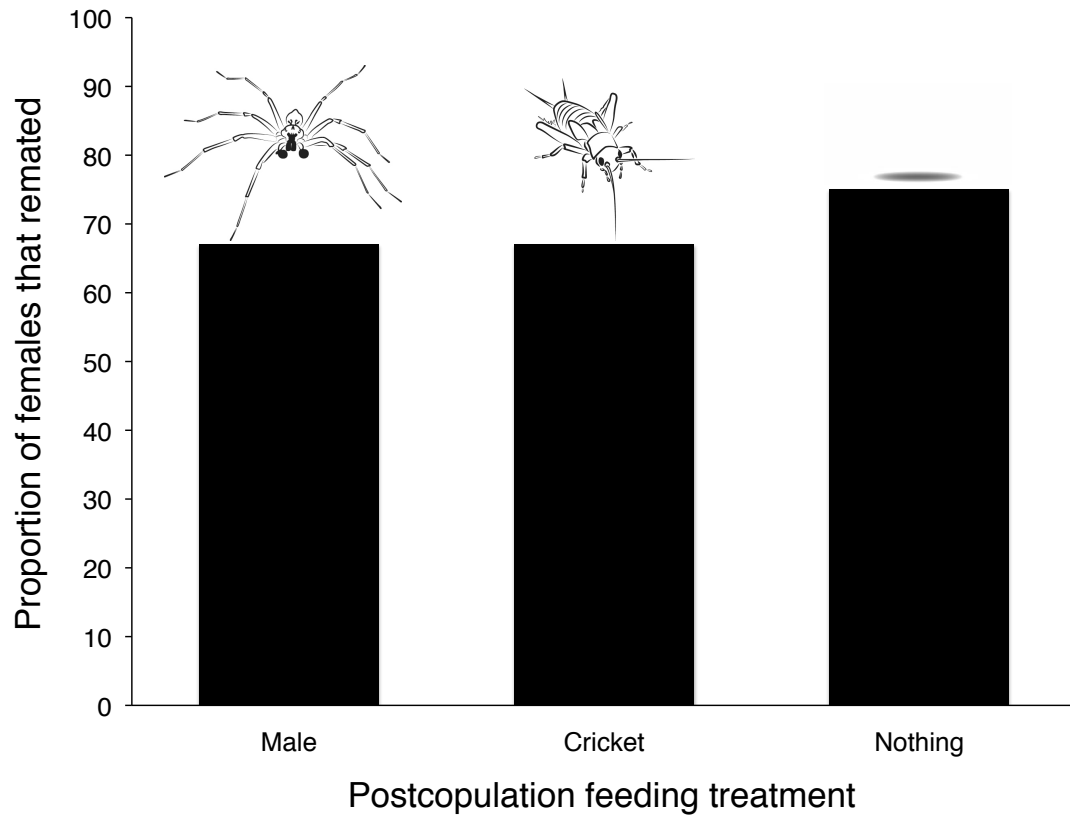


Figure 2.2 Proportion of *Dolomedes tenebrosus* females remating at 30 minutes following an initial mating. The remating rate of females was recorded under three postcopulation feeding treatments: (1) male, 8 out of 12 (67%) remated; (2) cricket, 8 out of 12 (67%) remated; and (3) nothing, 9 out of 12 (75%) remated.

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CHAPTER 3

**SEXUAL CANNIBALISM IN THE DARK FISHING SPIDER INCREASES OFFSPRING
QUANTITY AND QUALITY**

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ABSTRACT

Male self-sacrifice (complicity in cannibalism or spontaneous death associated with copulation) may be adaptive assuming sexual cannibalism occurs after sperm transfer and allows males to redirect their reproductive effort. In species that show self-sacrifice behavior, males are small relative to females and the nutritional gains from sexual cannibalism have been considered trivial, if tested at all. We studied the dark fishing spider, *Dolomedes tenebrosus*, where males are small and females are sexually cannibalistic. In this system, all males spontaneously die during copulation (self-sacrifice) and all females cannibalize males postcopulation, making this species ideal for examining the potential benefits of postcopulatory sexual cannibalism. Here, we test the hypothesis that obligate male death and subsequent sexual cannibalism is adaptive. Specifically, we test the prediction that the consumption of a male by a female postcopulation increases the number of offspring potentially sired by a male and/or increases the quality of a male's offspring. We found that the cannibalism of a male allowed females to produce more offspring and higher quality offspring. When females were allowed to consume their mating partner, it resulted in a significant increase in: (1) the number of the offspring, (2) the size of the offspring, and (3) the survivorship of the offspring compared to when females were not allowed to consume their mating partner. Our results suggest that self-sacrifice behavior (spontaneous death with copulation) is adaptive in *D. tenebrosus* and that self-sacrificing males benefit through paternal effort. To date, all examples of self-sacrifice behavior have been in araneoid (web-building) spiders and we provide the first description of adaptive self-sacrifice in a non-araneoid spider. [264]

Key words: adaptive self-sacrifice, *Dolomedes tenebrosus*, fitness benefits, monogyny, paternal effort, paternal investment, spontaneous death

INTRODUCTION

Cannibalism is defined as the killing and consumption of part or all of an individual of the same species (Elgar 1992; Edgar and Crespi 1992) and is taxonomically widespread, occurring in over 90 species of animals (Elgar 1992; Elgar and Schneider 2004; Wilder et al. 2009). A unique case of cannibalism, termed sexual cannibalism, involves the killing and consumption of an individual of one sex by the other sex (typically the killing of the male by the female) in the course of reproductive behavior; either before, during, or after sperm transfer (reviewed in Elgar and Schneider 2004; Wilder et al. 2009). The probability of sexual cannibalism during a given mating interaction can be extremely high in some species, approaching 1 (Wilder et al. 2009); sexual cannibalism can thus significantly affect population size, sex ratio, ecology, and behavior (Elgar 1992). Sexual cannibalism is taxonomically widespread but rare within most groups, but there are a few groups in which it is relatively common, such as gastropods, copepods, insects, and arachnids (see Elgar 1992; Elgar and Schneider 2004). Exploring the costs and benefits of sexual cannibalism for both males and females can lend insight into its origin and evolutionary history. Such costs and benefits will vary tremendously depending on the timing of sexual cannibalism – whether it is precopulatory or postcopulatory (Elgar and Schneider 2004).

Precopulatory sexual cannibalism is typically explored from the female's perspective, since sexual cannibalism prior to copulation and sperm transfer cannot be beneficial for males. There are four hypotheses that explain the evolution of precopulatory sexual cannibalism; two of these suggest that sexual cannibalism is non-adaptive and two suggest it is adaptive. The 'mistaken identity' hypothesis proposes that precopulatory sexual cannibalism is non-adaptive and is a result of mistaken identity since females do not identify males as non-prey (Gould 1984; Elgar 1992; Arnqvist and Henriksson 1997). Similarly, the 'aggressive-spillover' or 'genetic constraints' hypothesis proposes that

precopulatory sexual cannibalism is non-adaptive and results from selection favoring high levels of aggression during juvenile feeding that carry over into other behavioral contexts where it is not beneficial (e.g. courtship and mating), thus leaving some females unmated due to their aggressive behavior (Arnqvist and Henriksson 1997; Elgar and Schneider 2004; Johnson and Sih 2005; Wilder et al. 2009). In contrast to these two non-adaptive hypotheses of precopulatory sexual cannibalism, the 'mate rejection' or 'female choice' hypothesis proposes that females use precopulatory sexual cannibalism as a mechanism of mate choice and selectively cannibalize unpreferred mates (Darwin 1871; Elgar and Nash 1988; Elgar 1992; Hebets 2003; Elgar and Schneider 2004; Prenter et al. 2006). Finally, the 'feeding opportunism' or 'adaptive foraging' hypothesis proposes that precopulatory sexual cannibalism is an adaptive female foraging strategy in which females cannibalize males when the male encounter rate is high and prey availability is low (Buskirk et al. 1984; Newman and Elgar 1991; Elgar 1992; Andrade 1998; Johnson 2001; Elgar and Schneider 2004). There is varying support for each of these hypotheses.

In contrast to precopulatory sexual cannibalism, postcopulatory cannibalism occurs during and/or following copulation and sperm transfer. Given that males are capable of transferring sperm prior to their consumption in these situations, there is the potential for males, as well as females, to benefit from sexual cannibalism (Buskirk et al. 1984; Elgar 1992; Elgar and Schneider 2004), and thus far most hypotheses proposed for postcopulatory sexual cannibalism are adaptive hypotheses (but see Gould 1984; Jamieson 1986). While putative fitness benefits of postcopulatory sexual cannibalism are still often approached from the female's perspective (e.g. by assessing the number/size of eggs, hatching success, offspring survival, female body condition, etc.), they are often interpreted in terms of male advantages as well. Multiple adaptive hypotheses exist for how postcopulatory sexual cannibalism might benefit male paternity and/or female fecundity.

For example, sexual cannibalism could result in increased sperm transfer or a decreased likelihood of female remating (mating effort hypothesis: Low 1978; Simmons and Parker 1989; Elgar 1992; Andrade 1996; Andrade 1998) (Table 3.1), though the mechanisms underlying these benefits may not be initially obvious. In the Australian redback, *Latrodectus hasselti*, for example, cannibalized males experience an advantage in sperm competition, increasing their paternity share as (i) they are able to transfer more sperm than uncannibalized males and (ii) their mates show a reduced likelihood of remating (Andrade 1996; Snow and Andrade 2004). In addition to increasing sperm transfer or decreasing the likelihood of remating, the nutrients provided to females by male bodies could also lead to increased offspring numbers or quality (paternal effort hypothesis: Thornhill 1976; Low 1978; Parker 1979; Buskirk et al. 1984; Simmons and Parker 1989; Elgar 1992), resulting in selection for increased paternal effort (Table 3.1). While there is some support for the mating effort hypotheses, there is relatively little support for the paternal effort hypotheses. One major argument against the paternal effort hypotheses has been the extreme sexual size dimorphism often observed in sexually cannibalistic taxa – males are argued to be too small to be a significant source of nutrients to the female (Elgar 1992; Elgar 1998; Fromhage et al. 2003). Using the example above, redback males are only about 2% the female's body mass (Andrade 1996), which is similar to other self-sacrificing species (e.g. in *Argiope aemula*, male mass is less than 10% of female mass; Sasaki and Iwahashi 1995). In redbacks, there is no evidence that sexual cannibalism results in female fecundity benefits in terms of increased offspring quantity (egg number) or quality (egg mass) (Andrade 1996); there is thus no evidence for selection for paternal effort.

Despite the general lack of general support for the hypothesis that females receive a fecundity benefit from consuming males (either precopulatory or postcopulatory), especially the cannibalism of a single male (Elgar and Nash 1988; Andrade 1996; Arnqvist

and Henriksson 1997; Fahey and Elgar 1997; Elgar 1998; Fromhage et al. 2003), a few examples do exist where females appear to receive fecundity benefits from sexual cannibalism (see Table 1 in Elgar and Schneider 2004; Barry et al. 2008). These species all tend to have proportionally larger males. For example, in mantids, sexual cannibalism has been shown to increase female fecundity in *Hierodula membranacea*, where males are 61% the body mass of females (Birkhead et al. 1988), and in *Pseudomantis albofimbriata*, where males are ~40% the body mass of females (Barry et al. 2008). In spiders, sexual cannibalism has been shown to increase female body weight in *Araneus diadematus* (males are 25% the mass of females; Elgar and Nash 1988), egg sac weight in *Dolomedes triton* (males are ~27% the mass of females; Johnson 2005), and hatching success in *D. triton* (Johnson 2001). Confusingly, however, results are often conflicting, even within a single species. For example, Fromhage et al. (2003) found that the consumption of one or two males did not result in any detectable fitness benefits for females of the sexually dimorphic spider, *Argiope bruennichi*, where males are ~11% the mass of females (Fromhage and Schneider 2005; Schneider et al. 2006; Welke et al. 2012). However, Welke et al. (2012) found that *A. bruennichi* females who cannibalized (independent of the number of males consumed) produced larger clutches with heavier eggs and produced offspring that survived longer. What we can conclude from these prior studies is that even in species with sexual size dimorphism (i.e. in species in which male size is a small percentage of female size; see Horminga et al. 2000), males as meals should not be overlooked when considering the benefits of sexual cannibalism.

Here, we test the hypothesis that obligate male death and subsequent sexual cannibalism in the dark fishing spider, *Dolomedes tenebrosus*, is adaptive for males. Specifically, we test the prediction that the consumption of a male by a female following mating results in increased offspring quantity and/or quality. *Dolomedes tenebrosus* males

appear to passively facilitate their own cannibalism; they spontaneously die upon sperm transfer and are obligately cannibalized by their female partner (Schwartz et al. in review; Schwartz et al. chapter 2). Unlike the redback, *L. hasselti*, where males benefit from sexual cannibalism, in part, through a reduced likelihood of female remating, prior research on *D. tenebrosus* has suggests that the consumption of a male does not reduce the probability that a female will remate (Schwartz et al. chapter 2). Irrespective of the time frame during which females were provided with an additional mating opportunity following copulation and sexual cannibalism, female *D. tenebrosus* readily accepted a second mate (67% remated 30 minutes after copulating with and consuming a male, while 50% remated 72 hours after copulating with and consuming a male; Schwartz et al. chapter 2). Furthermore, self-sacrifice (spontaneous death) in this species does not appear to physically prevent females from remating as only one out of 225 copulations (0.4%) resulted in successful mate plugging (Schwartz et al. chapter 2).

Unlike the redback system in which males are only about 2% the female's body mass, *D. tenebrosus* males range from 7-13% of the female's mass (Schwartz et al. in review; current study). Given that (i) all *D. tenebrosus* males sacrifice themselves by exhibiting obligate death during copulation, (ii) all females cannibalize their mating partners postcopulation, and (iii) when females cannibalize, they appear to consume a large portion of the self-sacrificing male (Schwartz SK, personal observation), we asked the following question: Do self-sacrificing males gain benefits from being cannibalized in terms of increased female fecundity or increased offspring quality? We tested this paternal effort hypothesis by comparing the number, size, and survivorship of offspring that resulted from matings in which females were either allowed to consume their mate or prevented from consuming their mate. We also quantified the proportion of the male consumed during sexual cannibalism. Finally, in order to examine potential benefits of sexual cannibalism

from the female's perspective, we examined the relationship between number of mates, number of mates eaten, and offspring number using data from this study and unpublished data from a previous study (Schwartz et al. chapter 2).

METHODS

Animals

Immature male and female spiders were collected during the spring (April-May) in Lancaster County near Lincoln, Nebraska (U.S.A.). Male and female *D. tenebrosus* were collected at night using a light-emitting diode (LED) headlamp. Collecting at night allowed us to exploit the eye-shine seen in *D. tenebrosus* (and other fishing spiders) to help locate the spiders in the field, thus expediting their collection. Field caught individuals were transported back to the laboratory and housed individually in 87.3 mm x 87.3 mm x 112.7 mm plastic containers (763C, AMAC Plastics, Petaluma, CA). Containers were housed in a climate-controlled room (24-27° C) and placed on a light:dark cycle that matched the outside summer environment (April-August, 13:11-15:9 light:dark). Female spiders were fed two 2-week-old (1/4") crickets, *Acheta domesticus*, three times a week and male spiders were fed two 1-week-old (1/8") crickets, *A. domesticus*, once a week (Bassett's Cricket Ranch, Visalia, CA). In addition, all spiders were provided water *ad libitum*. All spiders were weighed (Ohaus Explorer balance 0.0001 g) twice, first when transported to the laboratory and once again prior to their assigned experimental trial.

Mating Trials

In the summer of 2010, we videotaped (Sony DCR-HC96 MiniDV Handycam) staged mating trials between *D. tenebrosus* males and females. All individuals used were field caught virgins, which molted to maturity in the laboratory (spiders are not sexually mature

prior to their final molt). Mature male and female spiders were used only once during the mating trials. Males were assigned to females at random and females were placed individually in a 252.4 mm x 90.5 mm (diameter x depth) plastic arena (250C, Pioneer Plastics, North Dixon, KY) for a minimum of 24 hours prior to the introduction of the male. The arena floor was covered with a disc of filter paper (Double Rings, 102 Qualitative, 24 cm) and at the center of the arena was a 47.6 mm x 84.1 mm plastic vial (40 DRAM, Thornton Plastics, Salt Lake City, UT) covered in fiberglass mesh on which the female could climb and position herself.

To test whether males might benefit from sexual cannibalism in terms of offspring quality or quantity, females were randomly assigned one of two treatments postcopulation: (1) cannibalism, in which females were allowed to cannibalize their mating partner and (2) no cannibalism, in which females were not allowed to cannibalize their mating partner. For all females, regardless of the treatment, the curled body of the male was removed immediately following a successful copulation. Following the male's removal, females were provided with either the same male's body (cannibalism) or nothing (no cannibalism). For the remainder of the experiment (i.e. until an egg sac was produced), all females continued on a diet of two 2-week-old crickets three times a week with water *ad libitum* (as above). This feeding regime ensured that all females, regardless of their treatment, had sufficient access to food resources, thus any differences seen would be due to the treatments (cannibalism, no cannibalism) and not due to food limitations. In addition, this feeding regime resulted in females being satiated in the laboratory; females often killed but did not consume all of the crickets they were allotted each week, and there was no obvious difference between females in the cannibalism and no cannibalism treatments in the number of crickets consumed (Schwartz SK, personal observation).

Offspring Measurements

Following a single mating, females were maintained in their individual containers and monitored daily for egg sac production. When egg sacs hatched, all spiderlings were counted and two subsets of 10 spiderlings were selected at random for additional measurements. The first subset of 10 spiderlings from each egg sac were used to measure spiderling mass (Ohaus Explorer balance 0.0001 g). Since spiderlings were too small to use for individual measurements, the first subset of 10 spiderlings were measured three separate times and the average was divided by 10 to obtain the mean spiderling mass. The second subset of 10 spiderlings from each egg sac were used in a survival experiment, where spiderlings were placed on the day of hatching in individual 60 mm x 15 mm plastic Petri dishes (Fisherbrand, Thermo Fisher Scientific Inc.) with no food or water and monitored daily until death.

Male Consumption

To look at the amount of the male's body that a female consumes during sexual cannibalism, we used unpublished data from a previous study where we measured male mass (mg) before and after mating trials in which females were allowed to mate and cannibalize freely (see Schwartz et al. chapter 2). By comparing the mass of the male before and after the trial (i.e. after the female was finished feeding), we were able to determine the proportion of a male that a female consumes when she cannibalizes.

Statistical Analysis

We conducted all statistical tests using SPSS version 11.0 (SPSS 2005) and the R statistical software version 2.15.1 (R 2012) with consultation from Zar (1999), Field (2009), and Field et al. (2012). All variables were tested for homogeneity of variance and normality.

Four out of seven variables were found to violate the assumptions of parametric data. Specifically, the number of spiderlings and spiderling mass had variances that were significantly different between treatments ($P < 0.05$, Levene's test), so we chose to analyse these variables using unequal variance t -tests (see Ruxton 2006). In addition, male mass and time to egg sac deviated significantly from normality ($P < 0.05$, Shapiro-Wilk test), so we chose to analyse these variables using Mann-Whitney U tests. Given that sample sizes were small and unequal and that some variables violated parametric assumptions, we chose to analyze most variables using nonparametric methods. However, for those variables that were normally distributed and had equal variances (female size, female mass, time to hatching), the results from the nonparametric test (Mann-Whitney U) did not differ qualitatively from their parametric equivalent (t -test). The use of Kendall's tau rank correlation is indicated by the symbol τ for the correlation coefficient. All data are reported as means \pm SE.

To compare the survival of offspring from cannibalistic and non-cannibalistic matings, we used a survival regression model with a Weibull distribution and included female identity ('female') as a cluster term in the model to account for the fact that the spiderlings from the same sibship were sub-samples and not independent. We conducted the survival analyses using the R statistical software package 'survival' (Therneau 2012).

RESULTS

Benefits of Cannibalizing a Single Male

There was not a significant difference in female size, male size, time to egg sac production, or time to spiderling hatching between the cannibalism ($N = 10$) and no cannibalism ($N = 6$) treatments (Table 3.2). However, females that cannibalized a male produced significantly more spiderlings, and those spiderlings were significantly larger

compared to the spiderlings from females who were precluded from cannibalizing their mating partner (Table 3.2). In addition, this difference in spiderling mass at hatching appears to have translated into a survival advantage for the spiderlings of cannibalized males. Specifically, the spiderlings of cannibalized males survived significantly longer when starved (50% mortality at 13.6 days) than the spiderlings of males who were not cannibalized (50% mortality at 8.4 days, $Z = -2.73$, $P = 0.00627$, Figure 3.1). These data provide support for the hypothesis that males benefit from being cannibalized and that the sexual cannibalism of a single male can significantly affect offspring quantity (producing more spiderlings) and offspring quality (greater mass and increased survival).

We did not find any correlation of the number of spiderlings or spiderling mass with either female mass or male mass within the two treatments. In the cannibalism treatment, female mass was not correlated with the number of spiderlings, $\tau = -0.07$, $P = 0.79$ or with spiderling mass, $\tau = -0.16$, $P = 0.53$. Similarly, male mass was not correlated with the number of spiderlings, $\tau = -0.07$, $P = 0.79$ or with spiderling mass, $\tau = -0.16$, $P = 0.53$. In the no cannibalism treatment, female mass was not correlated with the number of spiderlings, $\tau = -0.33$, $P = 0.35$ or with spiderling mass, $\tau = 0.47$, $P = 0.19$. Similarly, male mass was not correlated with the number of spiderlings, $\tau = 0.47$, $P = 0.19$ or with spiderling mass, $\tau = -0.33$, $P = 0.35$.

Male Consumption

The bodies of males that were cannibalized by females decreased in mass by 87% after female consumption (before trial: 92.5 ± 3.4 mg, after trial: 12.5 ± 1.1 mg, $N = 105$), indicating that *D. tenebrosus* females consume most of their mates. The proportion of the male's body consumed did not differ whether the male was cannibalized prior to or following copulation (Mann-Whitney U test: $U = 1326.00$, $Z = -0.239$, $P = 0.811$, $r = -0.023$).

Benefits to Females of Cannibalizing Multiple Males

All prior results are indicative of fitness benefits that both males and females can achieve from sexual cannibalism. Here, we focus solely on *D. tenebrosus* females and explore the benefits to females of sexual cannibalism beyond the consumption of a single male. To do this, we took advantage of data from a prior study (i.e. spiderling counts from a subset of females) where females mated with and cannibalize multiple males (Schwartz et al. chapter 2). We were curious to see if additional benefits existed for females and their mating partners from the consumption of more than one male. By comparing singly mated females who were allowed to either cannibalize ($N = 10$) or not cannibalize ($N = 6$) a *D. tenebrosus* male postcopulation (current study) with females who mated with and cannibalized multiple *D. tenebrosus* males precopulation/postcopulation ($N = 18$), we were able to test whether the number of mates and/or the number of males consumed affects offspring production. The number of spiderlings produced by females was not affected by the number of mates (Kruskal-Wallis test: $H(2) = 5.05$, $P = 0.080$), however, the number of spiderlings produced was significantly affected by the number of males consumed (Kruskal-Wallis test: $H(2) = 15.45$, $P < 0.001$). To follow up this finding we used a Jonckheere-Terpstra test that revealed a significant trend in the data: as more males are consumed, the number of spiderlings produced increases ($J = 295$, $P < 0.0001$, Figure 3.2). When *D. tenebrosus* females cannibalize a single male it improves their fecundity (number of offspring) and some components of offspring fitness (size and survivorship), and we see an additional improvement in fecundity (a 77% increase in the number of offspring) when females cannibalize multiple males (Figure 3.2).

DISCUSSION

Dolomedes tenebrosus males experience obligate death upon mating and are subsequently consumed by their female partners (Schwartz et al. in review; Schwartz et al. chapter 2). Prior work found no evidence of a benefit for such self-sacrifice behavior in terms of male mating effort – females that did and did not consume a male were equally likely to remate (Schwartz et al. chapter 2). In this paper we established that *D. tenebrosus* males do benefit from self-sacrifice through paternal effort (Table 3.1). Specifically, when females cannibalize males postcopulation, it resulted in a significant increase in: (1) the number of the offspring (a 94% increase), (2) the size of the offspring (a 25% increase), and (3) the survivorship of the offspring when starved (a 62% increase) compared to when females were prevented from cannibalizing males postcopulation. This is the first empirical study to support the idea that self-sacrifice (spontaneous death with copulation) is adaptive via paternal effort (Thornhill 1976; Low 1978; Parker 1979; Buskirk et al. 1984; Simmons and Parker 1989; Elgar 1992). Even with the extreme sexual size dimorphism in *D. tenebrosus* (males are only 7-13% the mass of females), the cannibalism of a single male can result in significant benefits for females and their mating partners.

Although similar effects of postcopulatory sexual cannibalism have recently been found in the orb-web spider, *Argiope bruennichi*, it is not clear that *A. bruennichi* males exhibit self-sacrifice behavior (complicity in cannibalism or spontaneous death associated with copulation) (see Fromhage et al. 2003; Schneider et al. 2006; Welke et al. 2012). Regardless, in this species males are small (males are 8-14% the mass of females; Fromhage and Schneider 2005; Schneider et al. 2006; Welke et al. 2012), and their consumption results in larger clutches, heavier eggs, and increased offspring survival (Welke et al. 2012). Interestingly, earlier work on this same species (*A. bruennichi*) found no detectable fitness benefits for females that consumed one or two males (Fromhage et al. 2003). Similarly,

unlike our results and those from Welke et al. (2012), numerous other studies encompassing diverse taxonomic groups have specifically tested for potential fitness benefits of sexual cannibalism in terms of offspring quantity and/or offspring quality. Such studies have explored both precopulatory and postcopulatory sexual cannibalism, and found no support (see Table 1 in Barry et al. 2008). However, substantial evidence exists (in spiders) for fitness benefits of sexual cannibalism in terms of male paternity (Schneider and Fromhage 2010). As suggested by prior authors, it is unlikely that any single explanation is sufficient to completely explain sexual cannibalism given that it is hypothesized to have a number of evolutionary origins in highly divergent taxa (Elgar and Schneider 2004).

During sexual cannibalism, *D. tenebrosus* females consume a significant portion of their partner's body (87% of the male), which may contribute to our observed fitness benefits. This amount of consumption is in contrast to studies on other spider species where females consume relatively less of their mate than a similarly sized prey item (51% of a male vs. 72% of a cricket; Wilder and Rypstra 2010). Prior work on spiders has also suggested that the ratio of lipid to protein in crickets is more consistent with the requirements of females for egg production than the ratio of lipid to protein in males (Wilder and Rypstra 2010). Our study did not quantify lipid to protein ratios in male *D. tenebrosus*, but future work exploring the mechanism underlying the fitness benefits of male cannibalism are now needed, as our data clearly indicate that the consumption of a single male increases female fitness as measured through our proxies (number, size, and survivorship of offspring). Further, it is the consumption of the male and not anything transferred during copulation that best explains our results. This can be concluded from our additional analysis of female benefits from multiple mates/male meals. We found no relationship between the number of mates and the number of offspring produced, but we

did see a significant relationship between the number of mates cannibalized and the number of offspring a female produced (Figure 3.2).

Our results suggest that there is something special about *D. tenebrosus* males; that they are somehow different than other prey items (crickets), and that this difference (e.g. lipid to protein ratio, trace elements, specific nutrients, etc.) leads to the benefits that we observed. In *A. keyserlingi*, the consumption of a male postcopulation results in an increase in egg energy density (kJ/g), which can translate into enhanced offspring development and survivorship (Blamires 2011). In *A. bruennichi*, the consumption of a male postcopulation results in an increase in egg size, clutch size, and offspring survival (Welke et al. 2012). At this time it is not clear how the consumption of a male increases female fecundity in these species. However, Blamires (2011) suggests it is the act of feeding on high-protein prey (i.e. a male) postcopulation that initiates an undescribed physiological process, which induces females to invest more in reproduction. Given this, there may be a similar process at work in *D. tenebrosus*, where the consumption of a male postcopulation induces females to invest more in reproduction, resulting in the fecundity benefits that we observed. Clearly, additional work is needed to determine the specifics of the relationship between sexual cannibalism and female fecundity in *D. tenebrosus*.

An alternative explanation for our results is that females in the ‘cannibalism’ treatment simply received more food than females in the ‘no cannibalism’ treatment, and that their additional meal is responsible for the benefits we observed in terms of offspring quantity and offspring quality. We argue that this alternative explanation is extremely unlikely. A single *D. tenebrosus* male (85 ± 10 mg, $N = 16$; current study) is roughly equivalent in mass to a single laboratory meal (two 2-week-old crickets = 84-105 mg). Thus, a single male meal represents only 1/3 of the food resources a female received per week in the laboratory (see Methods). Females took, on average 49 ± 9 days to produce their first

egg sac, thus females in the ‘cannibalism’ treatment received 22 meals, while females in the ‘no cannibalism’ treatment received 21 meals during the time period between copulation and egg sac production. Given the negligible proportion of food that a single male represents, the addition of a single meal (cricket or *D. tenebrosus* male) seems unlikely to cause the major differences that we observed between our two treatments. Additionally, we found not relationship between female mass and any of our proxies of fitness in either treatment. However, we cannot rule out the possibility that it is the timing of the meal rather than the composition of the meal postcopulation that is important, suggesting that we may see a similar increase in offspring quantity and/or quality if females were allowed to consume an alternative food item postcopulation (i.e. a cricket). Research is now needed to explore the composition of male *D. tenebrosus* in an attempt to gain insight into the relationship between the consumption of males and offspring number and quality.

Our data demonstrate that *D. tenebrosus* males can benefit from sacrificing themselves to their mating partner and that females can receive additional fitness benefits by consuming additional males. Exploring the benefits of sexual cannibalism from the female’s perspective, as well as the male’s perspective, enabled us to uncover this unexpected pattern of increased fitness benefits to females with increased numbers of males consumed. This finding opens up the possibility that even males who are second to copulate with a female may receive significant fitness payoffs from self-sacrifice (up to a 77% increase in offspring number when females consume multiple mates). For females, this means that precopulatory, as well as postcopulatory sexual cannibalism can be extremely beneficial once sperm is acquired. For males, this means that facilitating your own cannibalism, after sperm transfer, to a previously mated female can be potentially more beneficial. Female multiple mating is common in *D. tenebrosus* (see Figure 2.1; Schwartz et al. chapter 2) and it is intriguing to imagine that males who copulate with a non-virgin

female may benefit more than those who copulate with a virgin female. However, these ideas rest upon knowledge of the sperm precedence patterns in the species and while prior authors have suggested first-male sperm precedence in *D. tenebrosus* (Schwartz et al. in review), this remains to be tested directly.

While prior work has found no support for a decreased likelihood of female remating following sexual cannibalism in *D. tenebrosus* (Schwartz et al. chapter 2), the possibility remains that males experience an increase in sperm transfer correlated with their death. Self-sacrifice itself may still be the result of selection for mating effort if, for example, the hydraulic pressure involved in sperm transfer is somehow causally linked to male death. Even if future work finds support for this hypothesis, however, it is unlikely that this increased sperm transfer is linked to sexual cannibalism *per se*, since females consume the males following sperm transfer. All work to date on *D. tenebrosus* suggest that mating effort (Table 3.1) cannot explain sexual cannibalism, in contrast to support found for these hypotheses in another self-sacrificing spider species (e.g. redbacks; Andrade 1996; Snow and Andrade 2004).

Dolomedes tenebrosus from the focal geographic area (Nebraska U.S.A.) represents a monogynous mating system in which males experience obligate death followed by sexual cannibalism. While our study documents potential fitness benefits for males from obligate death and sexual cannibalism, selection for sexual cannibalism itself seems unlikely to be the main factor influencing the evolution of monogyny in this system. This supposition is due solely to the sequence of events – males immediately curl up upon sperm transfer and females can take up to 87 minutes (19 ± 3 ; Schwartz et al. chapter 2) to begin consuming the male. As such, sexual cannibalism appears more a byproduct of the mating system than the driving selective force. In other sexually cannibalistic spiders, selection appears to have favored male complicity in cannibalism (i.e. self-sacrifice behavior), as all males actively

move their bodies near the female's mouthparts in an apparent attempt to aid the female in their consumption (e.g. redbacks; Andrade 1996). In still others, male complicity appears more passive as males experience obligatory death during copulation, thus providing their mate with an easy meal (Sasaki and Iwahashi 1995; Knoflach and Van Harten 2000; Knoflach and Van Harten 2001; Knoflach 2002; Knoflach 2004). We question whether such passive complicity is truly the result of selection for sexual cannibalism or whether sexual cannibalism is a byproduct of selection for mating effort. Future studies examining the details of sperm transfer, in addition to comparative studies examining the mating system of geographically distinct populations and closely related species (see Carico 1973), may provide more insights into the evolutionary patterns of self-sacrifice and sexual cannibalism.

In summary, we provide evidence that self-sacrifice is adaptive in *D. tenebrosus*; obligate male death facilitates sexual cannibalism (Schwartz et al., chapter 2), and sexual cannibalism results in an increase in both offspring quantity and offspring quality (this study). This is the first study to demonstrate that males of a self-sacrificing species benefit via paternal effort. In addition, our results imply that we should not assume the benefits or lack thereof in sexually cannibalistic species that show extreme sexual size dimorphism.

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TABLES and FIGURES

Table 3.1 The adaptive male sacrifice hypothesis*. Male self-sacrifice (complicity in cannibalism or spontaneous death associated with copulation) facilitates sexual cannibalism, resulting in reproductive benefits for the self-sacrificing male. Males can benefit from self-sacrifice via Mating Effort or Paternal Effort.

Mating Effort

The self-sacrificing male increases the proportion of eggs he fertilizes under sperm competition by:

- (1) Transferring more sperm
- (2) Reducing female receptivity

Paternal Effort

The self-sacrificing male invests his somatic nutrients into his own offspring by:

- (1) Increasing offspring quantity
 - (2) Increasing offspring quality
-

**Note these hypotheses are not mutually exclusive.*

Table 3.2 Comparison between treatments in which *Dolomedes tenebrosus* females were and were not allowed to cannibalize their mating partner postcopulation. Values are means \pm SE. *P*-values are from Mann-Whitney *U* tests or unequal variance *t*-tests.

Variable	Treatment		Test Statistic			Effect Size
	cannibalism (<i>N</i> = 10)	no cannibalism (<i>N</i> = 6)	<i>U</i>	<i>z</i>	<i>P</i>	<i>r</i>
Female CW* (mm)	7.7 \pm 0.2	7.3 \pm 0.3	20.00	-1.085	0.313	-0.27
Female mass (mg)	758.3 \pm 47.1	680.1 \pm 61.1	21.00	-0.976	0.368	-0.24
Male mass (mg)	91.6 \pm 10.3	74.1 \pm 21.3	15.00	-1.627	0.118	-0.41
Time to egg sac (days)	42 \pm 13	57 \pm 15	15.00	-0.862	0.445	-0.22
Time to hatching (days)	27 \pm 1	28 \pm 1	18.00	-0.436	0.731	-0.11
			<i>t</i>	<i>df</i>	<i>P</i>	<i>r</i>
Number of spiderlings	135.4 \pm 17.4	69.7 \pm 6.8	-3.53	11.49	0.004	0.72
Spiderling mass (mg)	0.69 \pm 0.038	0.55 \pm 0.014	-3.31	11.38	0.007	0.70

*Carapace Width (CW), measured at the widest part of the cephalothorax (prosoma).

*Significant differences (*P* < 0.05) are given in bold.

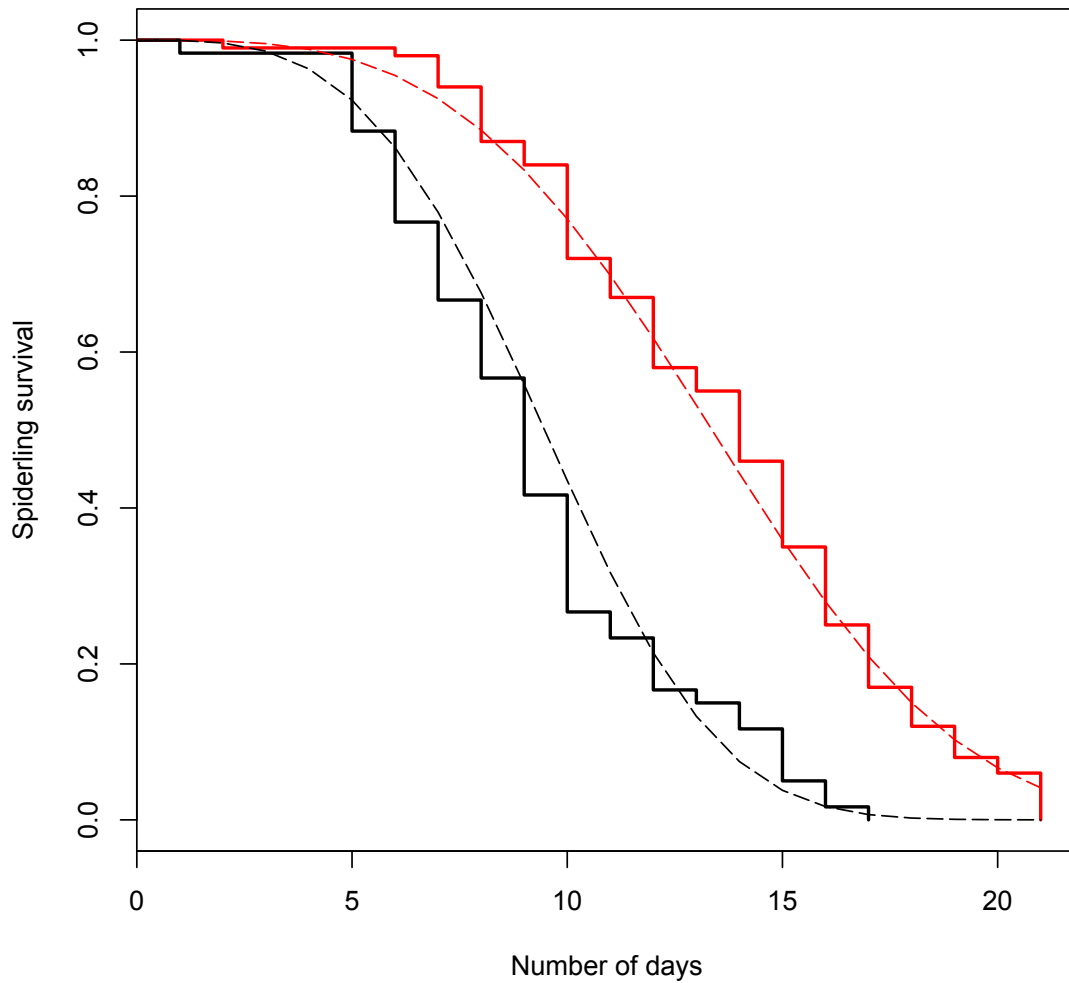


Figure 3.1 Kaplan-Meier survival function for the proportion of *Dolomedes tenebrosus* spiderlings surviving after hatching. Spiderlings resulting from matings where females were allowed to consume (cannibalism: $N = 100$, 10 per female, solid red line) and not consume (no cannibalism: $N = 60$, 10 per female, solid black line) their mating partner. A Weibull distribution (dashed lines) was fitted using our model and plotted on top of the Kaplan-Meier estimates above.

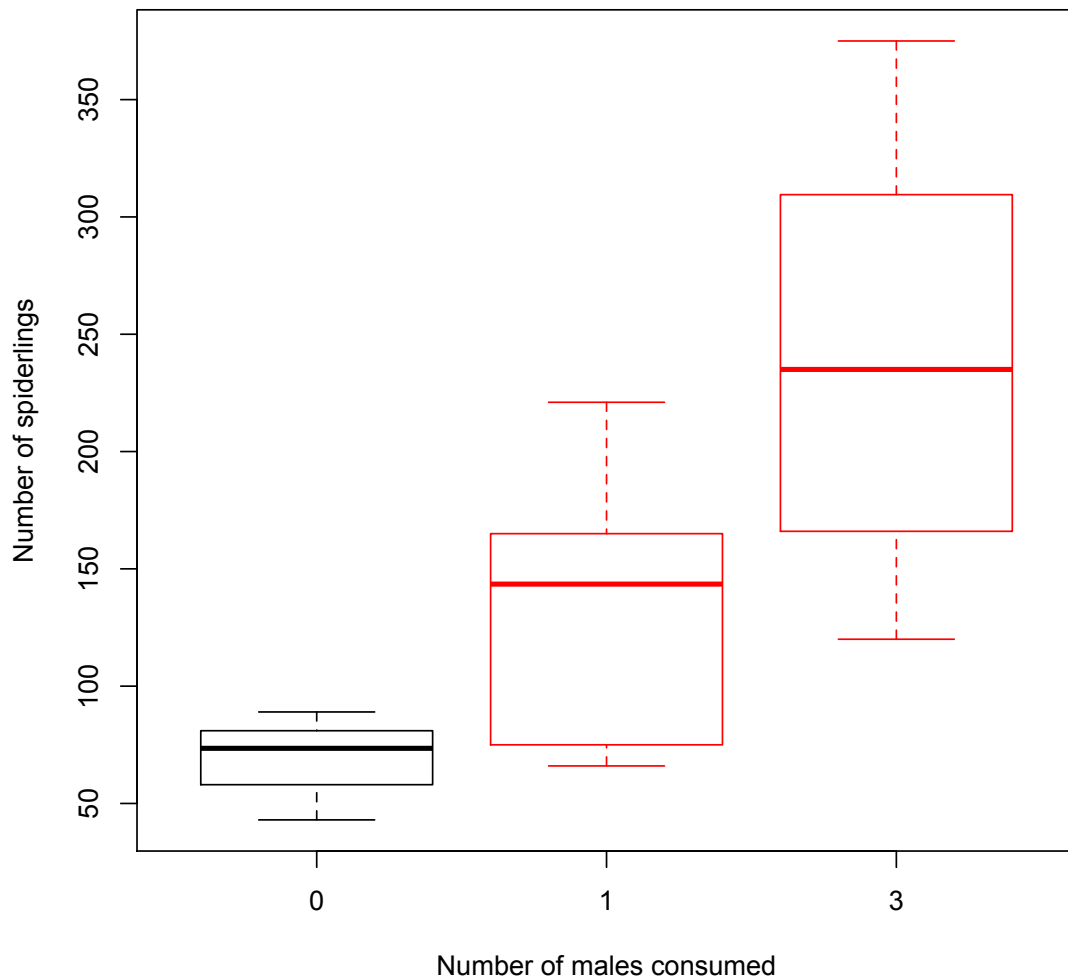


Figure 3.2 The number of spiderlings produced by females depending on the number of *Dolomedes tenebrosus* males they consumed. Data: (0) females who did not consume a male postcopulation ($N = 6$; current study), (1) females who consumed a single male postcopulation ($N = 10$; current study), and (3) females who consumed three males over three mating opportunities ($N = 18$; Schwartz et al. chapter 2). Treatments with cannibalism (red) and treatments without cannibalism (black). Box plots show median, interquartiles, and range.

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APPENDIX



Figure A.1 Photograph of the very first *Dolomedes tenebrosus* spider (circled) I found during my first field season. This was the first spider of hundreds that I collected for my Ph.D. research. This particular spider was a female and I found her in Pioneers Park (Lincoln, Nebraska) on May 26th, 2006. Photograph by Steven K. Schwartz.