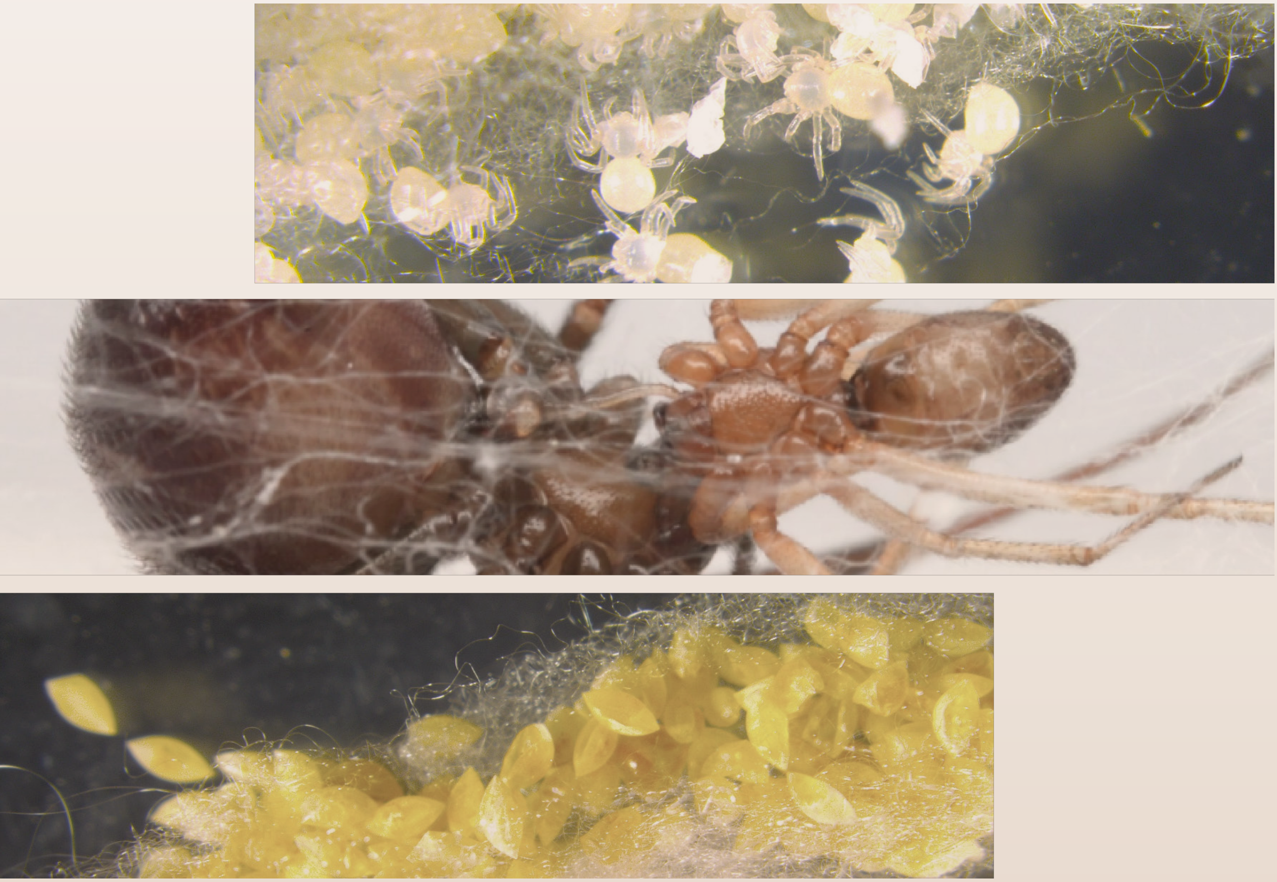
The effect of biotic and abiotic factors on reproduction in a false widow spider



Yuting Dong

Propositions

1. In female-biased sexual dimorphic animals, body mass of females is more important for fitness than of males.
   (this thesis)
2. Female spiders gain and lose half of their body mass every 2 weeks over their reproductive life.
   (this thesis)
3. Lab-based studies do represent real-world scenarios.
4. Curiosity-driven science deserves as much funding as applied science.
5. People who work with spiders are doctors for the rampant arachnophobia in society.
6. Individual cases in daily life cannot be assumed to fit the average.

Propositions belonging to the thesis, entitled

The effect of biotic and abiotic factors on reproduction in a false widow spider

Yuting Dong,

Wageningen, 22 Oct 2024

The effect of biotic and abiotic factors on reproduction in a false widow spider

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The effect of biotic and abiotic factors on reproduction in a false widow spider

Yuting Dong

Thesis

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Chapter 1

General introduction

Spiders, being key predators in many ecosystems, play an instrumental role in maintaining ecological balance, influencing prey-predator interactions, and contributing to the overall health of habitats (Rosenheim et al., 2004; Schmitz, 2003). However, the ecology of reproduction in spiders remains inadequately understood. Spider reproduction is fascinating and unique in the animal kingdom, which has already attracted scientific and societal attention. For example, males build a sperm web in advance, release the sperm onto the web and then extract them into their copulatory organs, followed by transferring it to females (Eberhard, 2004). However, biotic factors directly influencing reproduction such as body size, mating behaviour, and food availability, and the interactions among these factors, have not been thoroughly investigated. A large body size is often believed to be associated with abundant food intake, advantages in mating success and reproductive success. However, the detailed interplay and causality between these aspects require further exploration. Furthermore, although the impacts of climate change on spiders have been studied to some extent, the intricate effects of high temperatures and extreme events, such as heatwaves, on reproduction remain largely unexplored. Sudden temperature fluctuations and extreme weather events can disrupt spider physiological and behavioural reactions, potentially altering their life-history traits, including reproductive processes. By examining these factors from both biotic and abiotic perspectives, this research aims to enhance the understanding on spider reproductive ecology, and the adaptive mechanisms for navigating the challenges posed by global warming. These insights are crucial for ecological and evolutionary research on spiders, and for biodiversity conservation on a large scale.

Biotic factors influencing reproduction: food, body size, mating strategy and behaviour

Understanding the factors affecting reproductive success in animals has long fascinated evolutionary ecologists. Indeed, reproduction underpins our understanding of life-history strategies in organisms. Multiple biotic and abiotic factors can affect growth, development, reproduction and survival through effects on a wide range of parameters including body size, longevity, mating success, competitive ability and behaviour. Body size, in particular, plays a critical role as it often directly influences an individual's fitness by affecting their reproductive capacity and resource holding potentials. Biotic factors such as diet, which directly influence body size, are also important determinants of fitness. For example, numerous studies have shown that differences in the quality or quantity of plant-based diets can affect the growth, development and survival of herbivorous insects, and this in turn can affect adult fitness through size-related variation in fecundity (Awmack & Leather, 2002). Moreover, the type and amount of food contained in the diet of prey and hosts also affects fitness-related traits in natural enemies such as predatory arthropods and parasitoids respectively (Bezemer et al., 2005; Coll & Guershon, 2002). These areas have received considerable attention over many years.

Another important biotic factor that can affect reproduction involves mating strategy and behaviour. Most organisms reproduce sexually, and natural selection has generated an array of different strategies that optimize fitness through mechanisms that bias mate choice in favour of females (Andersson & Simmons, 2006; Jennions & Petrie, 1997), i.e., females tend to be more choosy than males. This is under the assumption that eggs are generally more costly to produce than sperm and female investment in reproduction is greater than male investment (Clutton-Brock, 2019; Møller & Birkhead, 1993). Numerous studies have shown that females prefer to mate with males with certain attributes (e.g., undertake intricate courtship behaviours, or else possess elaborate ornamentation, bright coloration, or larger body size) that are often correlated with male health, vigour and fitness (Andersson, 1994). Besides female choice, in many animal taxa, male-male competition often occurs and potentially influences mating success for males that have less competitive advantages (e.g., smaller body size, Qvarnström & Forsgren, 1998).

However, even when females mate with preferred males, this is no guarantee that reproduction will be optimized. For instance, other factors, such as egg- and sperm limitation can impede the ability of male and female organisms to parent maximal numbers of offspring. Although in many organisms egg limitation is generally considered to be a more limiting factor in reproduction than sperm limitation, evidence is growing suggesting that some species may also experience sperm-limitation (Boivin, 2013). Two mechanisms may underlie sperm limitation in animals. First, males may provide females with insufficient sperm during a full insemination, and thus a female may need to mate multiple times to achieve maximum fecundity. Second, when mating is disrupted prematurely, males may not be able to fully inseminate a female. This disruption may be due to factors such as wind or rain, female rejection of males, or interference by predators or rival males. In many organisms, males actively compete for access to females, leading to strong intra-sexual selection pressure among males that is often resolved through agonistic behaviours such as aggressive displays and fighting. When multiple males compete for females during mating, copulation can be temporally disrupted (Wong & Candolin, 2005), leading to the possibility of early sperm depletion in female individuals (Parker et al., 1990; Parker & Simmons, 1991). If these females are unable to find other mates later in life, this can seriously hamper their reproductive success.

Abiotic challenges influencing reproduction: High temperature from climate change

Many abiotic factors may strongly affect the expression of life-history strategies in organisms, including temperature, humidity, and light conditions. For example, artificial light plays a role in regulating circadian rhythms, reproduction, and migration (Desouhant et al., 2019; Gaston & Bennie, 2014). Temperature is one of the most important and a significant parameter in determining the niche of species (Cossins & Bowler, 1987) and its distribution (VanDerWal et al., 2013). This parameter is especially relevant under current anthropogenic climate change (ACC) with attendant climatic extremes, where individual organisms are potentially exposed to extremely high temperatures during heatwaves (Vogel et al., 2019). Climatic extremes are increasing in frequency, duration and intensity under ACC (Myhre et al., 2019; Tebaldi et al., 2006), and this together with gradual global warming is considered to be a profound threat to the biology and ecology of many species across the biosphere (Bellard et al., 2012; Easterling et al., 2000; Malcolm et al., 2006; Soroye et al., 2020).

Arthropods are ectothermic organisms of which activity and body temperature is predominantly determined by ambient temperature, rendering them especially sensitive to thermal stress (Angilletta et al., 2002; Buckley & Huey, 2016). Thus, when conditions become too cold or too hot, this may deleteriously affect important functions such as growth, development, foraging, dispersal, and reproduction (Bennett et al., 2021; Harvey et al., 2020; Harvey, et al., 2023b; Ma et al., 2021) and ultimately population dynamics. Under hot conditions, an organism may need to 'hunker down' and thus ride out the situation by seeking cooler microclimates where conditions are more optimal (Abram et al., 2017). However, this may depend on important parameters such as the motility of the organism and on its capacity to tolerate high temperatures. Furthermore, during this period of quiescence they may not feed or reproduce, which might be costly if their lifespans are short to begin with. If an organism remains active during a heatwave, exposure to high temperatures can lead to precious death if lethal upper limits are reached or exceeded (Agosta et al., 2018).

Exposure to heat can also have sublethal effects on reproduction (Dickinson, 2018; Parratt et al., 2021), for instance, by negatively influencing courtship and mating behaviour, and as result reduce reproductive success (Conradie et al., 2019; Dallas & Ross-Gillespie, 2015; Leith et al., 2021; Mak et al., 2023; Patton & Krebs, 2001; Sepulveda et al., 2023). In ectotherms, behavioral and metabolic activity are often constrained by thermal thresholds below and above which this activity, including mating, does not occur. Change in activity within this temperature window may increase gradually with temperature and drop quickly beyond the optimal temperature for this activity (Sinclair et al., 2016). These relationships with temperature may differ for various activities or metabolic functions within a species (Walsh, et al., 2019b). For example, critical thermal limits are predicted to be lower for fertility than for survival (Walsh, Parratt, Hoffmann, et al., 2019). Recent studies have suggested that male thermal fertility limits may better predict species distributions and extinctions than critical thermal limits for survival (Parratt et al., 2021; van Heerwaarden & Sgrò, 2021).

Sublethal exposure to high temperatures does not kill individuals but nevertheless can render them behaviourally or physiologically sterile when eggs or sperm inside of their bodies are negatively influenced (McCowan & Griffith, 2021; Walsh, et al., 2019b). Sperm, in particular, has been shown to be vulnerable to high temperatures (Hurley et al., 2018; Sales et al., 2018; Setchell, 2006). Moreover, in addition to sex-related differences in behaviour and (eco) physiology, sexual size dimorphism, which is displayed by many taxa, can present and become an additional factor differentiating responses to thermal stress. Evidence showed the body size of organisms is becoming smaller under global warming (Daufresne et al., 2009; Horne et al., 2015). One of the explanations behind this is that smaller organisms have a higher surface-area-to-volume ratio than larger ones and are more efficient in heat exchange, which enhances their thermal lability. Meanwhile, higher metabolic rates exhibited by smaller organisms may provide greater physiological resilience when exposed to an acute extreme temperature (Peralta-Maraver & Rezende, 2021). Therefore, the sex with a smaller body size may be more tolerant to heat stress. A recent meta-analysis showed that both sex and body size can to some extent, affect acclimation capacity, i.e., the reversible adjustment in thermal performance in response to new temperatures (Pottier et al., 2021).

Study organism

Spiders are a group of organisms that are relatively understudied, despite the fact that they are vital components of natural ecosystems for instance by consuming large amounts of prey, mainly insects (Nyffeler & Birkhofer, 2017). Many species are considered to be important in biological control programs (Cotes et al., 2018; Riechert & Lockley, 1984). Spiders are model organisms for studying a suite of different factors affecting life-history, development and reproductive strategies in ectothermic organisms. In many spider families, sexual size dimorphism is present with females being larger than males (Vollrath & Parker, 1992). However, adult body mass tends to be more variable in males than in females due to differential trade-offs between factors that affect adult fitness (Harvey, 2022).

Unlike insects and most other dioecious organisms, spiders have a unique mating strategy whereby the male transfers sperm from his gonopore into a specialized ‘sperm-web’ into which he dips structurally specialized large bulbs at the end of his palps (called emboli) that imbibe the sperm where it is stored until copulation (Foelix, 1982). During mating, one or both palp bulbs are inserted into the female epigynium, where the sperm is released and stored in the female spermatheca. Notably, sperm are encapsulated and coiled within male’s reproductive organs and females will active sperm when fertilization. Little is known how thermal stress affects reproduction in spiders.

Figure 1. A male *Steatoda grossa* (above right) copulating with a female *S. grossa* (below left). One of the pedipalps from males is attached to the female's epigynum. The pedipalp is enlarged and the tip of it is uncoiled, transferring ejaculates into the female's epigynum. Photo credit: Jasper Bergstra.

The research challenge here is to understand immature development, mating behaviour and reproduction in the false widow or cupboard spider, *Steatoda grossa* (Araneae: Theridiidae). This synanthropic species has a cosmopolitan distribution. It is one of the most invasive spiders in the world, and its distribution ranges from temperate to tropical regions. This suggests that the species is thermally labile. It is probably native to Eurasia (Levi, 1967), although its origins remain somewhat controversial (Nentwig et al., 2022; World Spider Catalog, 2023). It is closely related to other well-studied spiders due to similarities in morphology and venom composition, including the American house spider, *Parasteatoda tepidoriarum*, and species of true widow spiders in the genus *Latrodectus*. True widow spiders produce neurotoxins that are of medical significance to humans (Grishin, 1998), whereas bites of *P. tepidoriarum* and *Steatoda* are less toxic because the venom proteins are less concentrated than in *Latrodectus spp* (Dunbar et al., 2021). Like other theridiids, *S. grossa* is a 'comb-footed spider', with distinctive bristles on the tarsi of its 4th pair of legs through which a single silk thread extends from the spinnerets that is thrown over prey during capture. Sexual-size dimorphism is pronounced among species in the Theridiidae, although it varies considerably from one species to another. Adult virgin females of *S. grossa* weigh some 5 to 10 times more than males. While females of this species are sedentary and spent most of their adult life in their cobwebs, males move around and actively search for female mates. Mating takes place in the female's web. Females typically produce their first egg sac 2-3 weeks after mating containing 50-200 eggs, and, if well-fed, continue to produce additional egg sacs every 2-3 weeks. Males have a shorter lifespan and only few survive beyond 1 year (Bradley, 2012; Harvey, 2022). Mature females can live more than 2 years in the laboratory (Levy & Amitai, 1982) and, if mated, females can produce 10 or more egg sacs from a single copulation event (Khandelwal & Sharma, 2014)

Thesis aims and outline

The aim of this thesis is to comprehensively understand the reproduction of *S. grossa* under the influence of biotic and abiotic factors. This thesis dives into the ecology of reproduction in *S. grossa*, which is a widespread synanthropic species, providing insights for population dynamics in spiders and contributing to biodiversity conservation knowledge. Initially, I explore body size and mating behaviour in this species. Following these foundational understanding, I introduced one biotic factor, food availability and one abiotic factor, thermal stress, to examine their effects on reproduction. **Chapter 2 to 4** primarily focus on biotic factors, and I investigate the effects of an abiotic factor, thermal stress, in **Chapter 5**. As little is known about courtship behaviour in this species, I investigated this first (**Chapter 2)**. As already mentioned, sexual dimorphism is pronounced in *S. grossa.* Notably, the variation in male body size is larger than in females (Harvey, 2022). Male-male competition over access to females appears to be an important driver to select for large body size in many spiders (Elgar 1998). Furthermore, the duration of copulation can be influenced by body size when smaller males need more time to transfer sufficient amounts of sperm (Elgar 1995). **In Chapter 2,** I studied the role of male body size in determining mating success and copulation duration both in the absence and presence of male rivals.

In chapter 2, I showed that *S. grossa* displays a ritualized and hierarchal courtship behaviour. Competition for mates, may interfere with the sequence leading up to successful insemination of the female or limit the amount of sperm that is transferred. Natural copulation usually lasts 40-60 minutes, typically including 1-4 distinct copulatory bouts, which I defined as the period between successful insertions of pedipalps into one of the female's epigynes and its subsequent removal. In **Chapter 3**, I investigated mating success and offspring production when copulation is disrupted prematurely. I simulated mating disruption by varying the duration of insemination and determined how this affected offspring production in the female.

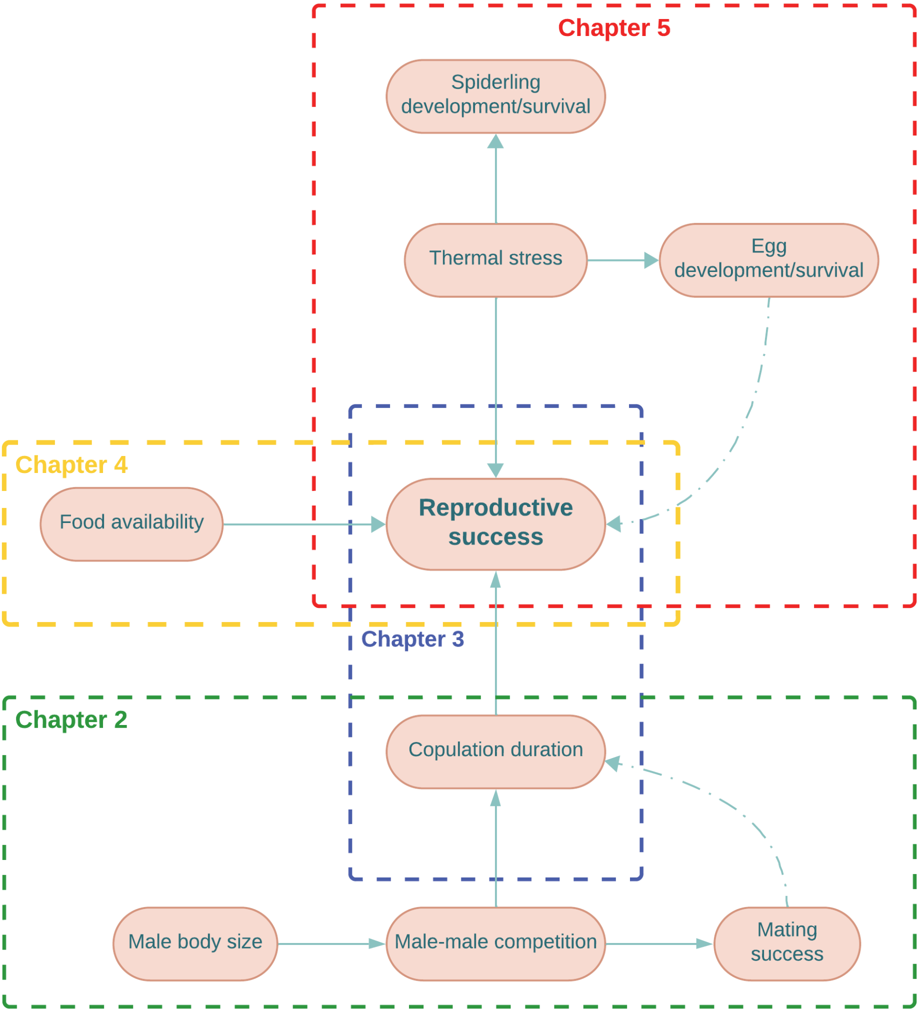

Figure 2. Conceptual scheme of the thesis on the reproduction of *S. grossa*. This scheme illustrates the overarching aim of the thesis to understand the effects of biotic and abiotic factors on reproduction in *S. grossa*. Chapter 2 focuses on male body size and mating behaviours, serving as a foundation for Chapter 3, which explores the relationship between copulation duration and reproductive success. Chapter 4 investigates the influence of the food availability on reproduction. Finally, in Chapter 5, thermal stress was examined for its impact on reproductive success, as well as the development and survival of eggs and spiderlings.

Food resources are fundamental for survival and reproduction. As mentioned above, *S. grossa* exhibits a female-biased sexual size dimorphism. Males mature faster and with less food, trading off developmental time with a smaller body size. In contrast, females develop more slowly and require substantial food intake to achieve a large body size, essential for reproduction. Females remain their growth strategy even when food is limited (Harvey, 2022). Under regular food regimes in lab conditions, females invest heavily in reproduction, losing almost half of their body mass to produce a healthy egg sac. Will females still invest enormously in reproduction when food is limited, especially for a starvation-resistant animal? **In Chapter 4**, I studied the effects of food availability on reproductive success by monitoring female body mass changes before and after reproduction. Different quantities of prey were provided to females and the reproductive outcome was determined.

Global warming is extensively reported and experienced across the globe. In the Netherlands, for example, extreme events, such as heatwaves, are getting more common and severe (KMNI, 2023). How *S. grossa* and spiders in general respond to heat exposure is little studied. **In Chapter 5,** I used *S. grossa* originating from the Netherlands to test thermal lability to summer and heatwave conditions representative for the Netherlands. Various life stages of arthropods (e.g., eggs, immatures and adults) differ in their vulnerability to heat stress (Barnes et al., 2019; Jia et al., 2020; Klockmann et al., 2017). I explored the effects of thermal stress on egg survival and spiderling development. In addition, I exposed adults (males, females or both) to a simulated heatwave before or after mating and recorded reproductive success in the females.

Chapter 2

The role of male body size in mating success and male-male competition in a false widow spider (*Steatoda grossa*)

Yuting Dong, Jeffrey A. Harvey, Robin Steegh, Rieta Gols, Melissah Rowe

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Abstract

In many animals, body size is correlated with reproductive success. Selection sometimes generates striking differences in body size between males and females (i.e., sexual size dimorphism SSD). SSD is common in spiders (Araneae), and is typically explained by selection for larger, more fecund females and rapidly maturing and smaller males. Within a species males and females also often vary in body size. In a false widow spider, *Steatoda grossa*, females are larger than males and males trade body size for rapid development and early maturation. Moreover, males vary considerably in body size, suggesting that under certain conditions there may be advantages to large size. Here, we tested the role of male body size on mating success under non-competitive and competitive mating conditions (i.e., male-male competition) in *S. grossa*. We found that body size did not influence mating success or copulation duration under noncompetitive conditions. Larger males obtained most mating success under competitive mating conditions. Additionally, we found that total copulation duration was significantly lower when a rival male was present. Our results show a large male advantage under male-male competition, which may contribute to the high variation in male body size observed in *S. grossa*. We further suggest that the reduced copulation duration observed under competitive conditions may have potential ramifications for male and female reproductive success and we discuss how patterns of selection acting on male body size might limit the extent of SSD in this species.

Keywords

copulation duration, reproduction, sexual selection, sexual size dimorphism, *Steatoda grossa*, Theridiidae

Introduction

Body size is a key phenotypic trait influencing mating and reproductive success in many animal species (Andersson, 1994; Andersson & Iwasa, 1996). For females, larger body size is often positively correlated with the number of eggs a female is able to produce or the size of the eggs she produces, and thus larger females enjoy a fecundity advantage (Head, 1995; Honěk, 1993; Roff, 1993; Shine, 1988). In contrast, larger male body size is frequently attributed to sexual selection (i.e., selection arising from differences in reproductive success resulting from the competition for access to gametes for fertilization; Andersson, 1994; Shuker & Kvarnemo, 2021). For example, larger males may gain greater mating success because they outcompete smaller males during direct male-male contests for access to females (Arnott & Elwood, 2009). Larger males may also have higher fitness because they are more successful at defending females or resources important for reproduction (Moore et al., 2009; Shuster & Wade, 2019), because females prefer larger males (Charlton et al., 2007; Lank & Smith, 1992) or because large male body size is positively correlated with endurance and thus the amount of time invested in gaining matings (Ospina-L. et al., 2017). Alternatively, selection may favour a smaller body size if small males have greater success in scramble competition for mates because they are more mobile, more agile or can maintain longer search times due to higher energy efficiency (Blanckenhorn et al., 1995; Crompton et al., 2003; Kelly, 2020).

At the same time, male body size may be constrained by trade-offs with other traits that contribute to fitness. One such trade-off is that between size at maturity and the time taken to reach sexual maturity (i.e., development time): larger body sizes can be obtained by extending the period of growth (Blueweiss et al., 1978). However, longer development times can also incur costs, such as an increased risk of predation or parasitism (Benrey & Denno, 1997; Blanckenhorn, 2000; Fordyce & Shapiro, 2003; Stamps, 2007; Uesugi, 2015). Male body size may also be linked to survival in some animal taxa (Alcock, 1996). For example, larger individuals may be more susceptible to predation if they are less agile or manoeuvrable, they are more visible to predators (le Roux et al., 2019) or they face increased mortality risk if they require more food and resources are limited (Blanckenhorn, 2000). Thus, body size can impact fitness in a variety of ways.

Understanding how selection acts on body size has been of particular interest in animals exhibiting sexual size dimorphism (SSD). SSD is a common phenomenon in the animal kingdom, but in some taxa the size difference between males and females can be extreme. Such extreme SSD (ESSD) is prevalent in many spider families, where females are much larger than males (Head, 1995; Vollrath & Parker, 1992). ESSD may be the result of selection for larger and more fecund females, selection for reduced male size or a combination of the two (Coddington et al., 1997; Head, 1995; Prenter et al., 1999; Uhl et al., 2004; Vollrath & Parker, 1992). At the same time, male-male competition over access to females appears to be an important driver of large body size in many spiders (reviewed in Elgar, 1998). Several studies have shown that large males usually outcompete smaller males during direct contests or that larger males can occupy a favourable position on the female's web and prevent smaller rivals from accessing the female (Dodson & Schwaab, 2001; Foellmer & Fairbairn, 2005; Golobinek et al., 2021; Hoefler, 2007; Maklakov et al., 2004; Rittschof, 2010; Schmitt et al., 1992). Importantly, however, the advantages of large body size for male mating success may be influenced by variation in the local operational sex ratio (OSR) (Dodson et al., 2015; Vollrath & Parker, 1992). For example, at increasingly male-biased OSR, the form of male-male competition can switch from interference competition to scramble competition (Weir et al., 2011). Under scramble competition, small male size may be advantageous. For example, small males may be better able to locate receptive females because they mature sooner (i.e., selection for protandry, Danielson-François et al., 2012), or because they are better able to climb (i.e., gravity hypothesis, Moya-Laraño, 2002) or disperse (i.e., bridging gravity hypothesis, Corcobado et al., 2010). Additionally, it has been posited that small body size may be favoured because smaller males are better able to avoid sexual cannibalism or because they have lower metabolic requirements enabling them to spend more time and energy searching for females (Foellmer & Moya-Laraño, 2007). Thus, selective pressures acting on male body size in spiders may be complex. Yet, compared to many other taxonomic groups (e.g., insects, fish, anurans, mammals), the role of male body size in mating success remains relatively unexplored in spiders (see Andersson 1994).

In this study we investigated the potential role of male body size in determining mating success and copulation duration in a false widow spider, *Steatoda grossa* (Araneae: Theridiidae). This is a synanthropic, cosmopolitan species that is frequently found living in buildings and in close association with humans, and it reproduces year round (Nentwig et al., 2022). Female *S. grossa* appear to be sedentary, living and reproducing in their cobwebs, as is common in many comb-footed spiders (family Theridiidae; Knoflach, 2004). Webs of adult virgin females disseminate a sex pheromone that attracts adult males (Scott et al., 2018), and females show a preference for building webs in sites that already contain cobwebs of conspecifics, perhaps as this indicates microhabitat suitability (Fischer et al., 2019). In contrast to females, male *S. grossa* appear to be relatively more mobile and spend their time actively searching for females. Importantly, *S. grossa* exhibits pronounced SSD, with adult female body mass being 5-10 times greater than adult male body mass (Harvey, 2022; Wilczek et al., 2017). As in other spider taxa, the pronounced SSD observed in *S. grossa* indicates a fitness advantage for large female size and small male size (Foellmer & Moya-Larano 2007). Indeed, SSD in *S. grossa* may be, at least in part, due to fecundity selection in females and selection for early maturation in males (i.e., protandry) resulting in reduced male body size, as is postulated for other spider taxa (Prenter et al., 1999; Vollrath & Parker, 1992). Males of *S. grossa* indeed mature earlier than females, and rapid development time trades off against size at maturity, resulting in small male body sizes of typically less than 15 mg (Harvey, 2022). In that study, small male size was argued to be favoured due to reduced predation risk and earlier access to females, and it was suggested that rapid development is more important than larger male body size in this species (Harvey 2022). Yet, despite the apparent importance of rapid development and early maturation for male *S. grossa*, male body size is highly variable in this species (Fischer, Goh, et al., 2020; Harvey, 2022). For example, male body mass varies with food availability: mature virgin males weigh 7-35 mg (Harvey, 2022). Moreover, male body mass varies considerably even when males are raised under identical conditions in the laboratory (see Fig S1, S2).

Given selection for rapid maturation, the observed variation in male body size is surprising and suggests that additional selection pressures may be acting on male size, or alternatively that selection on body size is lacking, in this species. We therefore examined the potential role of body size in male mating success and copulation duration. Specifically, we tested for an association between male body size and both mating success and copulation duration under noncompetitive and competitive mating conditions. Given the lack of information on the mating system of this species, we have refrained from making specific predictions concerning the potential role of male body size in mating success when matings occur under noncompetitive conditions. In contrast, given the prevalence of large male advantage under male-male competition (Hoefler, 2007; Rittschof, 2010), we predicted that male body size may influence mating success under competitive mating conditions. Specifically, we predicted that large males would outcompete small males and thus gain the greater share of mating opportunities.

Methods

Spider Collection and Maintenance

Several hundred juvenile and adult male and female spiders were collected from the buildings and grounds of Wageningen University & Research (51.9863°N, 5.6680°E) and the Netherlands Institute of Ecology (51.9876°N, 5.6706°E) in Wageningen, The Netherlands, during 2019 and 2020. All field-collected juvenile spiders were reared in the laboratory in Petri dishes (8 cm diameter) until their final moult. Upon reaching adulthood, males were transferred to individual Petri dishes, while females, which are several times larger, were transferred to individual plastic boxes (11 x 11 cm and 6 cm high). These adults were then mated to produce an F1 laboratory cohort. Matings were established by placing a male in a female's home container, and pairs were created using individuals from different locations to minimize the chance of inbreeding. Mated females typically produced an egg sac approximately 2-3 weeks after mating. In addition, some field-collected female spiders produced egg sacs. Egg sacs from both the field-collected females and the laboratory crosses were transferred into individual Petri dishes (8 cm diameter) until hatching (ca. 30-40 days later). After hatching, spiderlings were separated into individual Petri dishes (8 cm diameter) with a damp cotton ball (ca. 1 cm diameter). The spiderlings were provided with two to three freshly killed (by freezing) fruit flies (*Drosophila melanogaster*) once every 1-2 weeks, then after two to three moults, they were given five to six freshly killed *Drosophila hydei* fruit flies (which are larger than *D. melanogaster*) every 1-2 weeks. Once spiderlings had moulted to L3, they received one small house cricket (*Acheta domestica*), nymph every 1-2 weeks. Fruit flies and crickets were obtained from commercial retailers. Finally, when females reached adulthood, they were transferred to individual plastic boxes, where they constructed a web, and were provided with one late-instar *A. domestica* nymph weekly. In contrast, males remained in their individual Petri dishes and, because adult males eat little food, were only provided with live *D. hydei* flies once every 2 weeks. Adult males and females were subsequently used for the mating experiments (described below). All spiders were maintained at 22 ± 2 °C, 16:8 h light:dark cycle with 50% relative humidity.

Mating Experiments

We performed mating trials under two conditions: (1) a non-competitive mating scenario (i.e., one male/one female, N = 47) and (2) a competitive mating scenario (i.e., two males/one female, N = 34). All mating trials were conducted with virgin males and females that had moulted to adulthood 2-4 weeks prior to the experiment. Mating trials were performed in the female's housing container after ensuring that the female had successfully constructed a web. Prior to the mating trials, adult males were narcotized for ca. 10 min using CO2 and were weighed to the nearest 0.001 mg using a Mettler Toledo Microbalance MT5 (Columbus, OH, U.S.A.). Once the males became active again, they were introduced into the plastic container with the adult female. Mating trials were conducted in the laboratory during the day at room temperature; unlike most species of true widow spiders in the genus *Latrodectus*, which are nocturnal, light does not affect courtship and copulation behaviour in *S. grossa* (Scott et al. 2018). Successful copulations typically included one to four distinct copulatory bouts, which we defined as the period between the successful insertion of the male's pedipalp into one of the female's epigynes and its subsequent removal. Following a copulatory bout, males frequently repeat their courtship behaviour, which, when the female is receptive, can lead to an additional copulatory bout. Typically, males appear to alternate the use of their left and right pedipalps between successive copulatory bouts. Upon successful palp insertion, the male habitually 'lifts' the body of the female and both sexes remain quiescent until mating is terminated. Spiders were observed for 90 min or until the palp was removed. Thereafter, males were removed from the female's box and returned to their original Petri dishes. In some instances, observations exceeded 90 min. Specifically, when males and females were in copula at the end of the 90 min period, observations were continued until the mating terminated naturally.

In the noncompetitive mating experiment, males were randomly assigned to a female trial. In each trial, we observed male courtship behaviour and recorded the duration of all copulatory bouts (i.e., the period between pedipalp insertion and removal). We then summed the duration of copulation across all copulatory bouts to obtain total copulation duration for each mating. Males were selected randomly in this experiment. In these trials, average male body mass was 20.5 ± 9.2 mg (range 7.2-53.9 mg).

For the competitive mating experiment, two size-mismatched males (one ‘small’ and one ‘large') were simultaneously introduced, at the same location, into the female’s home container. To select male pairs for the competitive mating experiment, we randomly selected males from the population that were visually size-mismatched (see Fig. S2). We then weighed males to ensure the two males indeed differed in size. Importantly, the two male rivals always differed considerably in size (mean ± SD large:small male body mass ratio= 2.5 ± 0.7, range 1.7-5.0). Males classified as ‘small’ averaged 11.2 ± 3.1 mg (range 5.3-16.1 mg), while males classified as ‘large’ averaged 27.3 ± 7.6 mg (range 13.9-47.9 mg). Each pair was then assigned randomly to a female for a competitive mating trial. In these trials, we recorded (1) the duration of each copulatory bout, (2) the identity of the successful male (i.e., ‘large’ or ‘small’) in each copulatory bout, and (3) the total number of fights between males observed across the entire mating trial. We defined a fight as any direct interaction between the males. These interactions were characterized either by the males facing one another and extending their first two pairs of legs to ‘push’ against the other male, or by the unmated male making physical contact with the mating male’s body. Such interactions often resulted in the two males ‘tussling’ or holding each other tightly with their legs contracted. We then summed the duration of copulation across all copulatory bouts (regardless of male identity) to obtain the total time a female spent in copula.

Statistical Analysis

For noncompetitive mating trials, we assessed the relationship between copulation duration (s) and male body mass using a linear model, using data from successful trials (i.e., trials in which there was a copulation, N = 45 of 47 trials) only. Under competitive mating conditions, six trials did not result in a copulation. We therefore tested whether the body mass ratio of competing males (large:small male size) or the number of fights between males differed between unsuccessful and successful trials using permutation tests. Next, considering only successful competitive mating trials, and given that we predicted that larger males would win more matings, we used a binomial test (one tailed) to determine whether mating success of large males was greater than expected by random chance. We then tested for an effect of male body mass ratio on the number of fights occurring during a trial using a linear model. Additionally, we examined the relationship between total copulation duration and the number of fights using a linear model, with copulation duration as the dependent variable and both the number of fights, male body mass ratio and their two-way interaction as predictor variables (using data from successful trials only). The non-significant interaction term ($F_{1,30} = 0.14$, $P = 0.73$) was removed from the model.

Next, we combined data from the two mating scenarios (non-competitive and competitive matings) and compared female mating success (i.e., whether or not a female successfully mated) between the two scenarios using a binomial proportion test. Finally, we compared total female copulation duration (using data from successful trials only) between the two mating scenarios using a Mann-Whitney U test. All values given are mean ± SD. All statistical analysis was performed using R

4.1.3 (R Core Team, 2023) and RStudio (RStudio Team, 2020), and model assumptions were examined using the package DHARMa (Hartig & Lohse, 2022).

Ethical Note

All spiders were maintained under standard rearing conditions, using offspring from field-collected individuals and egg sacs. Although we did not require any institutional or governmental agency review for this species, we made every effort to conduct all our work in accordance with the ASAB/ABS standards for the use of invertebrates in research. All field collections and laboratory experiments were performed with the aim of minimizing any negative effect on the wellbeing and behaviour of the animals and the source population. We gently moved males between their home Petri dish and the female's container using brushes, and back again by gently grasping them with forceps. Competition among males for mating access to females is a common occurrence in many spiders, and following mating experiments all spiders were placed back into the laboratory population and reared under standard housing conditions with access to sufficient food.

Results

Across all mating experiments, average male body mass was 19.8 ± 9.6 mg (range 7.2-53.9 mg). We observed a ritualized and hierarchal courtship sequence typically consisting of five sequential behaviours. (1) Approach and stroking: the male approached the female and used his forelegs to 'stroke' the female. (2) Web reduction: the male moved around and used his chelicerae to cut the female's silk threads. (3) Web building: the male added fresh silk near the female. (4) Vibration: the male was positioned with second and third leg pairs folded underneath his body and vibrated rapidly. At this point, the female, if receptive, moved towards the male and oriented her body vertically in the web with the ventral surface of the abdomen facing the male. (5) The male approached the female, similar to step (1). Finally, copulation occurred when the male successfully inserted one of his pedipalps into the female's epigynum. At this point the male clasped the female with his pedipalp and physically raised her body. Typical matings included three to four pedipalp insertions/copulatory bouts, after which mating was terminated naturally and males no longer showed interest in the females. All males in our experiments showed ritualized courtship behaviours except for one pair of males in the competitive mating condition because they were continuously engaged in conflicts.

Under noncompetitive mating conditions, 96% of males (45 of 47 trials) were successful in obtaining copulations with the female. In the two trials in which males failed to mate, body mass did not appear to play a role in the male's failure (male body mass: 31.0 and 11.8 mg; failed trials 1 and 2, respectively). In addition, male courtship behaviour did not appear to influence mating success in these two cases, as both males exhibited typical courtship behaviour (J. A. Harvey, *personal observation*). Across all successful mating trials, average total copulation duration was 45.5 ± 17.4 min, and the average number of copulatory bouts per mating trial was 2.3 ± 1.0. Finally, total copulation duration was not influenced by male body mass under noncompetitive mating conditions ($F_{1,43} < 0.001$, $P = 0.98$).

Under competitive mating conditions, 28 of 34 trials resulted in successful copulation, with an average total copulation duration of 25.17 ± 16.5 min. Successful and unsuccessful trials did not differ in either the body mass ratio of the competing males ($z = 0.88$, $P = 0.38$) or the number of fights between the competing males ($z = 0.47$, $P = 0.67$). Considering only trials that resulted in a successful mating, large males 'won' significantly more in terms of gaining access to females than expected by chance (one-tailed binomial test: $P < 0.001$). In 25 of 28 successful trials, the larger male was the only male to successfully copulate. In one of the trials, the smaller male was the only male that achieved a copulation, whereas in the other two trials, the smaller male achieved a single copulation, while the larger male always achieved a greater number of copulatory bouts during a trial (number of copulatory bouts for large versus small males: seven versus one and four versus one). Fighting was commonly observed between males and across all trials males engaged in an average of 8.8 ± 6.1 fights. The number of fights, however, was not influenced by male body mass ratio ($F_{1,32} = 0.96$, $P = 0.34$). Similarly, female copulation duration was not influenced by male body mass ratio ($F_{1,25} = 0.003$, $P = 0.96$), nor was it influenced by the number of fights occurring between the competing males ($F_{1,25} = 1.22$, $P = 0.29$).

Comparing mating success across the two mating scenarios, we found that the presence of a male competitor did not significantly influence mating success (i.e., successful copulation; 96% versus 82%, noncompetitive and competitive mating conditions, respectively; proportion test: $X_1^2 = 2.61$, $P = 0.11$). However, total female copulation duration was significantly reduced under competitive mating conditions compared to noncompetitive mating conditions (Mann-Whitney test: $W = 249$, $P < 0.0001$; Fig 1).

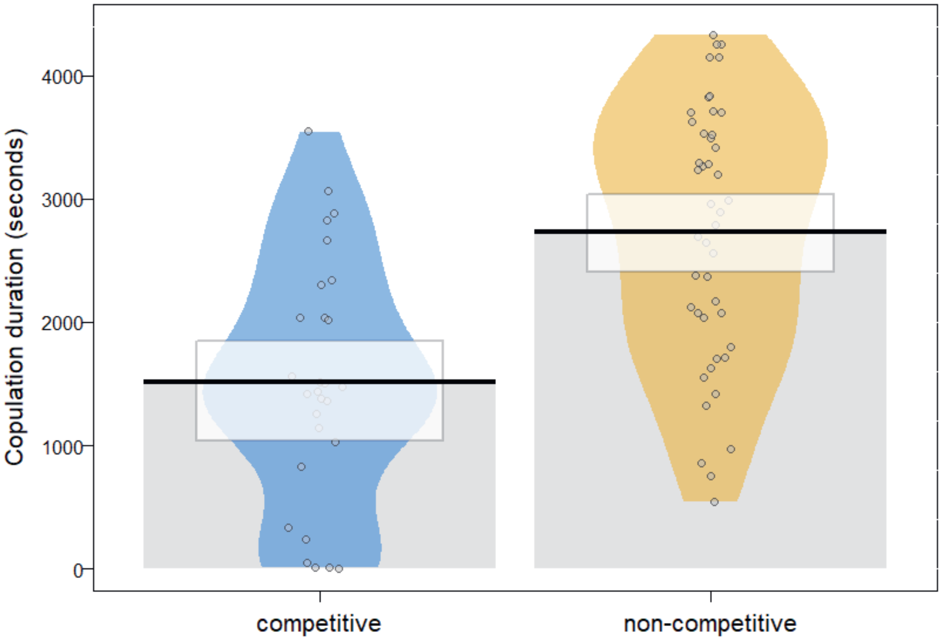

Figure 1. The difference in total copulation duration of females under competitive and noncompetitive conditions presented in violin plots. Circles show the data. The solid line is the median. The bean shape represents density. The box indicates a 95% confidence interval.

Discussion

In this study, we showed that male body size in *S. grossa* does not impact male mating success or copulation duration under noncompetitive mating conditions. However, under competitive mating conditions, larger males won contests significantly more often. Interestingly, when males competed for mating opportunities, small males did not appear to avoid conflict, but instead male-male interactions typically escalated into physical fights regardless of how much larger ‘large' males were. These fights were characterized by males facing each other and spreading their first appendages as widely as possible to exaggerate their size to their opponent, possibly acting as a form of intimidation, and frequently escalated into physical tussles between rivals. We also found that copulation duration was reduced when matings took place in the presence of a rival male. Finally, we observed a highly ritualized courtship behaviour in *S. grossa*, consistent with previous descriptions in this species (Knoflach 2004; Scott et al. 2018) and with courtship behaviours observed in a range of spider taxa (Fisher & Price, 2019; Schmitt et al., 1992; Wignall & Herberstein, 2013).

Under noncompetitive conditions, male body size did not appear to influence mating success or copulation duration in *S. grossa*, which is consistent with findings in several spider taxa (Kotiaho et al., 1996; Schäfer et al., 2008; Schneider, 1997). Although we did not explicitly test female choice here, the lack of a relationship between male body size and mating success suggests that precopulatory female choice (at least for body size) is perhaps absent or minimal in this system. Our findings do not exclude the possibility that other male traits may play a role in female choice in this system (e.g., acoustic, vibratory, tactile or chemical signals; Huber 2005), as they do in other spider species (e.g., Wignall & Herberstein 2013). However, we found that almost all virgin females accepted the first male they encountered. Under conditions in which male encounter rate is low (Andrade, 2003) and given the potential costs of choosiness (e.g., the risk of remaining unmated; Kokko & Mappes, 2005), females may be under strong selection to accept the first male they encounter to ensure their reproductive success.

Under competitive conditions, we found that larger males gained a competitive advantage in direct contests for access to mates, which suggests that sexual selection via male-male competition may be important in *S. grossa*. Large male advantage under male-male competition has also been observed in the closely related Mediterranean black widow spider, *Latrodectus tredecimguttatus* (Golobinek et al., 2021) and indeed a number of spider species (reviewed in Elgar, 1998; Dodson &

Schwaab, 2001; Foellmer & Fairbairn, 2005; Hoefler, 2007; Maklakov et al., 2004; Rittschof, 2010; Schmitt et al., 1992; but see Neumann & Schneider, 2015). Thus, our study contributes to a body of literature showing large male advantage in a range of different spider families. Intriguingly, a previous study of *S. grossa* suggested that selection for rapid development was more important than increasing male body size (Harvey, 2022). Despite this, some males in this species are relatively large (> 50 mg), and our findings suggest that this may be due to sexual selection for larger size under competitive scenarios.

We found that the presence of a rival male reduced total copulation duration. Although we did not examine reproductive success in terms of offspring production in the current study, our findings may have implications for male or female fitness. Specifically, reproductive success may be correlated with copulation duration if males that copulate longer transfer more sperm (Snow & Andrade, 2004) or if copulation duration positively affects the number of sperm stored by a female (Bukowski & Christenson, 1997). This pattern has been confirmed in several species of arachnids (Andrade, 1996; Austad, 1982; Cohn, 1990; Kiss et al., 2019; Schneider et al., 2006; Schneider & Lesmono, 2009), although in some species the relationship between sperm transfer and copulation duration is nonlinear (Snow & Andrade, 2004; Szirányi et al., 2005). Alternatively, if sperm transfer takes place early during copulation, extended copulation duration may reflect a form of physiological mate guarding, which may be important for the transfer of substances that ensure fertilization or render females less receptive to other males (e.g., seminal fluid proteins, insertion of mating plugs). Scott et al. (2018) showed that males never obtain copulations with previously mated females, suggesting that they may be monandrous or at least unreceptive to additional matings in the short term, although to what extent this is caused by male-derived substances transferred during copulation is unknown. Regardless, under both scenarios, a shorter copulation duration may result in the production of fewer offspring.

The factors driving intra- and intersexual differences in body size in animals include sexual conflict and a range of ecological factors such as predator-induced early mortality, variable exposure to abiotic stresses such as heat and ease of finding mates (Blanckenhorn, 2000). In spider taxa, female-biased SSD is commonly observed in many species and reaches its zenith in families such as the Araneidae and Theridiidae. For instance, the body mass ratio of females to males in the genera Trichonephila and Latrodectus may be 50 to 1 or even greater (Cheng & Kuntner, 2014; Kleinteich & Schneider, 2010; Kuntner & Coddington, 2020). In Australian redback spiders, *Latrodectus hasselti*, large males were more successful than smaller males in competition for females, but small males still had 10 times higher fitness than larger males because of their significantly faster development time to adulthood (Kasumovic & Andrade, 2009), supporting the ‘first come, first served’ hypothesis. This competitive advantage is apparently a major factor driving ESSD in *L. hasselti* and perhaps other widow spiders.

It is also important to recognize that SSD appears to be most exaggerated in web-building spiders, where the ecology of adult males and females generally differs significantly. By contrast, SSD appears to be only marginal in many, although not all, non web-building spider species in the Lycosidae, Pisauridae, Salticidae, Zoropsidae and other cursorial ambush-feeding spiders where both sexes have significantly overlapping niches. Variation in SSD is therefore generated by multiple biotic and abiotic selective forces acting on each sex, although broadly speaking SSD in spiders appears driven by fecundity selection for large female size and selection for small male size (Foellmer & Moya-Larano 2007). Compared to other closely related spiders in the *Latrodectinae* (i.e., widow spiders) that display ESSD (Kuntner & Coddington 2020), SSD in *S. grossa* is less pronounced. This may be due to lack of selection for morphological or behavioural traits that influence mate location (e.g., climbing ability and dispersal, Corcobado et al., 2010; Grossi & Canals, 2015; Moya-Laraño, 2002), low levels of sexual cannibalism (Elgar & Fahey, 1996) and lower mortality of immature spiders in urban habitats with a low predation risk. In females, selection for large body size is generally correlated with increased fecundity (Head, 1995), whereas for males, we suggest that large male size is advantageous in male-male competition, which may occur more frequently in urban habitats where these spiders are most abundant and immature survival is higher.

Different forms of male courtship behaviour have been demonstrated across many phylogenetically unrelated groups of vertebrates and invertebrates (Reynolds, 1996). This reveals evolutionary convergence among many animals in the differing roles played by males and females in reproduction. Females usually invest much more in reproduction than males, and hence selection has been geared towards female choosiness for fitter males. In spiders, courtship behaviour can be influenced by the presentation of nuptial gifts, such as prey, from the male to the female (Stålhandske, 2001), colourful displays in species with good vision (Girard et al., 2011) or, as in *S. grossa* and widow spiders, a combination of multiple physical and tactile cues (Knoflach 2004; Scott et al. 2018; Fischer et al. 2020; Sivalinghem & Mason, 2021). Complex courtship behaviours in spiders have been little studied in most taxa, and should be the focus of future research, along with how balancing selection on reproduction and survival generates variation in body size of males in different spider families.

Supplementary figures

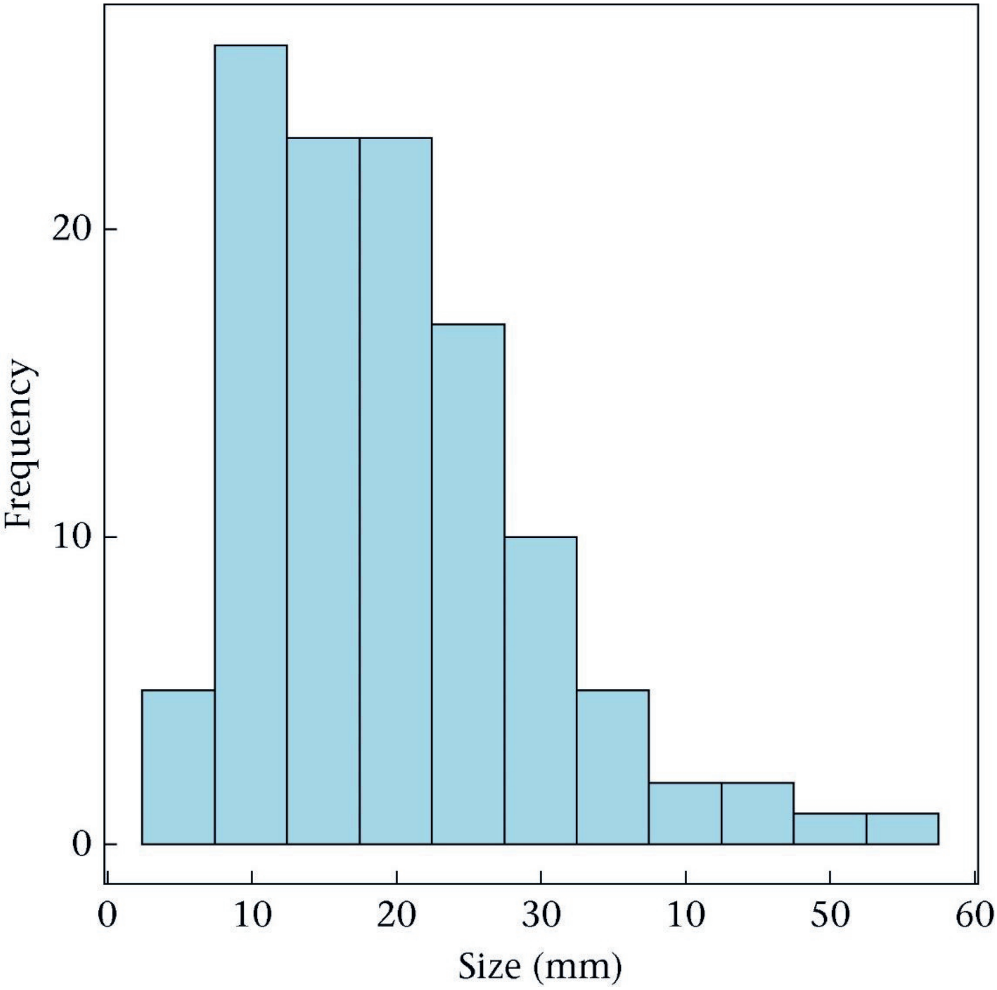

Figure S1. The body mass distribution of 115 males reared under identical conditions in the laboratory (mean ± SD: 19.82 ± 9.61 mg; range 5.3–53.9 mg).

Figure S2. Variation in male body size in *S. grossa*. These two males were randomly selected from the spider rearing; both males are mature and of similar age (9–10 months) and were raised and maintained under identical laboratory conditions and feeding regimes (large one = 27.5 mg, small one = 5.2 mg). Photo: Mark Hillaert.

Author Contributions

J.A.H., and R.G. conceived of the study; J.A.H., Y.D. and R.S. conducted the experiment and collected data with suggestions from R.G.; Y.D. and M.R. analysed the data; Y.D. wrote the initial manuscript draft with support from M.R.; M.R reviewed and edited the manuscript with support from J.A.H. and R.G. All authors approved the final version of the manuscript.

Data Availability

Data are deposited in Dryad: https://doi.org/10.5061/dryad.ksn02v79j

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Chapter 3

Mating disruption decreases female reproductive success in a false widow spider

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This chapter has been submitted to a journal

Abstract

Copulation is essential for transferring sperm from males to females in most terrestrial animals. During copulation, males and females are often static and thus prone to predation or other threats. Its duration should therefore be brief to minimize costs but sufficient to ensure the fertilization of eggs. Here, we investigated reproductive behaviour and success in the false widow spider, *Steatoda grossa* (Araneae: Theridiidae), under a sequence of disrupted copulation duration (1 min, 3 min, 5 min, 10 min, 20 min) with a control treatment (i.e., uninterrupted mating). In *S. grossa,* undisrupted copulations typically last for 40-60 minutes. We found that within the first 5 minutes, copulation disruption negatively affected reproductive success (i.e., egg sac number and mass, spiderling number and aggregate body mass), and delayed production of the first egg sac (in some extreme instances by over 200 days). However, when copulation duration was 10 minutes or longer, reproductive outcome was unaffected by copulation duration. Copulation duration also did not influence the mean weight of spiderlings, showing that it does not alter the ejaculates content which influences development. In the 1-minute treatment, offspring number and mass varied greatly among individuals which indicates considerable variation among males with respect to the speed and efficiency of sperm transfer. Extended copulation duration did not influence female receptivity to fresh males 24 h later, but might influence oviposition and fertilization processes. We discuss the costs and benefits of extended copulation duration on female reproduction and on male and female fitness.

Keywords

Copulation duration, egg sac, offspring, *Steatoda grossa*, *Theridiidae*, mating disruption

Introduction

One of the most significant transitions in evolution was the shift from asexual to sexual reproduction (Crow, 1994; Smith & Szathmary, 1997). This transition was enormously successful, as most eukaryotic organisms in nature now reproduce sexually (Lively & Morran, 2014). Sexual reproduction in animals involves a remarkable spectrum of behaviors that profoundly impact reproductive success, including copulation (Anholt et al., 2020). The primary function of copulation is sperm transfer from the male to the female and its duration varies greatly among sexually reproducing animals with internal fertilization. In many animals, copulation is relatively short in duration. For example, many bird and rodent species copulate for only a few seconds or less (Birkhead et al., 1993; Dewsbury, 1972). At the other end of the extreme are animals that copulate for several hours, such as in some dasyurid marsupials in the genus *Antechinus* (up to 18 hours; Taggart et al., 1998). In arthropods, such as insects, copulation is extremely variable both intra- and inter-specifically (Sears et al., 2020). For example, it can be as short as a few seconds (e.g., 13 seconds in the yellow fever mosquito, *Aedes aegypti*; Spielman, 1964), up to 5 hours in the damselfly *Ischnura graellsii*; Cordero, 1990), or even longer (could be 12 h-7 days in fire bug *Pyrrhochis apterus;* Schöfl & Taborsky, 2002). This variation in copulation duration suggests that there are costs and benefits and that is strongly influenced by natural or sexual selection (Daly, 1978).

Copulation can be energetically and ecologically costly, thus favouring a reduction in copulation duration. Time spent *in copula* cannot be used for other activities such as foraging or searching for other potential mates, and it can increase the incidence of disease transmission and enhance the risk of predation (Daly, 1978; Sheldon, 1997). Extended copulation, on the other hand, can increase reproductive success of males, when prolonged copulation enhances fertilization success (Andrés & Cordero Rivera, 2000; Parker, 1970; Wang et al., 2008). When more sperm are transferred during extended copulation, this can influence the outcome of sperm competition when males mate with polyandrous females (Parker & Pizzari, 2010; Wedell et al., 2002). Prolonged copulation can also prevent females from mating with other males (i.e., mate guarding) (Kelly & Jennions, 2011; Pérez-Staples & Abraham, 2023; Prenter et al., 2006) or allow a male to remove the sperm of rivals (i.e., sperm displacement) (Michiels, 1992; Siva-Jothy, 1987). In addition to sperm, other substances can be transferred during copulation that can also reduce female receptiveness to other mating partners (i.e., physiological mate guarding) and increase male fertilization success (Avila et al., 2011; Poiani, 2006; Vahed, 1998). These 'nuptial gifts' may benefit females, either directly, e.g., by providing nutrients or carbohydrates that enhance fecundity, or indirectly, e.g., through chemicals that protect eggs against predation (South & Lewis, 2011). In the European nursery web spider *Pisaura mirabilis,* for example, males facultatively provide females with a nuptial gift (i.e., prey wrapped in silk) during mating. This gift-giving extends copulation duration in this species and females that had mated with gift-giving males stored more sperm and experienced greater hatching success of their offspring compared to females that mated without a nuptial gift (Albo et al., 2013). Similar results were found for gift-giving male scorpionflies (*Panorpa cognata*; Engqvist & Sauer, 2001). Thus, copulation duration can influence reproductive success of both males and females.

The risk of early termination of copulation by external factors is higher in animals with relatively long copulation durations. A number of factors can affect copulation duration (Birkhead et al., 1987; Corbet, 1980). Environmental conditions, such as physical disturbance (i.e., resulting from wind and rain), the presence of predators or rival males, and variation in mating behaviours, such as sexual conflicts, can reduce copulation duration (Pellegrino et al., 2013; Schaefer & Uhl, 2003; Schneider et al., 2006**.** In *Drosophila melanogaster*, copulation duration was reduced at higher temperatures (Stazione et al., 2023), whereas in a predatory mite, a short period of heat stress extended the copulation period (Zhang et al., 2016). The consequences of disrupted copulation on reproduction can be significant, in particular, when sperm transfer continues throughout copulation and thus early termination of copulation results in fewer sperm being inseminated. This phenomenon has been described in a range of insect taxa such as flies and beetles (Lorch et al., 1993; Pérez-Staples & Abraham, 2023; Qazi et al., 1996; Takami, 2002). In the bruchid beetle *Callosobruchus maculatus,* copulation duration had a significant positive effect on lifetime offspring production (Edvardsson & Canal, 2006). However, in many species, copulation often lasts longer than is necessary for the transfer of sufficient amounts of sperm to fertilize all eggs (Linn et al., 2007; Sears et al., 2020; Snow & Andrade, 2004). This suggests that prolonged copulation occurs for reasons other than direct sperm transfer, and is most likely related to sperm competition and enhancement of fertilization success of the male (Herberstein et al., 2011; Schneider et al., 2006).

Spiders have variable copulation duration that varies both within and across species in different families (from less than 1 minute in many web-building spiders to 15 hours in salticid *Pseudicios sp.*; Elgar, 1995), and many spiders copulate longer than required for the successful insemination of females (Linn et al., 2007; Stratton et al., 1996). The terminal ends of the male palps possess copulatory bulbs with internal spiral spermaphors. Sperm that was ejaculated from the epigastric furrow onto an intricately constructed sperm web is then taken up into the spermaphor when the pedipalp bulbs are dipped into it. Courtship consists of species-specific ritualized behavioural sequences which is an important part of mating assessment and ensure that the male is recognized as a conspecific mating partner and not as prey (Robinson, 1982). This is followed by alternate insertions of the tips of the pedipalps into the female copulatory opening(s) (Elgar, 1998; Foelix, 1982). Studies that examined copulation duration in spiders have focused on the effect of copulation duration on sperm transfer and/or intra-sexual competition. For example, in the orb-weaver spider *Trichonephila clavipes* (Linn et al., 2007) copulation duration did not influence sperm number transferred to females. In the wolf spider *Pardosa agrestis*, copulation duration affected male paternity when two males were present at the same time, i.e., siring success from both males (Kiss et al., 2019). For some spider species, it has been shown that most sperm are transferred early during copulation (Snow & Andrade, 2004; Szirányi et al., 2005). How copulation duration affects life-time reproductive outcome, however, has been little studied. For *P. agrestis,* which usually produces a single egg sac after copulation, it was found that copulation duration did not affect the number and size of offspring (Szirányi et al., 2005). However, the number of egg sacs that were abandoned prematurely, supposedly due to sterility, was significantly higher in females with shorter copulation duration. Thus, when mating disturbance reduces sperm transfer, this can have more profound consequences on reproductive outcome in spider species that produce multiple eggs sacs following mating.

In this study, we investigated the effect of mating disruption on reproductive success in the false widow spider, *Steatoda grossa* Koch (Araneae; Theridiidae), a synanthropic species that likely originated in Eurasia but which is now widely introduced across the world. Adult male *S. grossa* spiders are significantly smaller than females (Harvey, 2022) and undisturbed copulation duration usually lasts about 40-60 minutes (Dong et al., 2023). A recent study with an introduced Canadian strain of *S. grossa* showed that females were less receptive to remating after an initial copulation (Scott et al., 2018). Females after maturity can live for over 2 years or more in the laboratory (Levy & Amitai, 1982) and if mated, females produce 10 or more egg sacs from a single copulation event (Khandelwal & Sharma, 2014). Males, on the other hand, are relatively short-lived and few survive beyond a single year, irrespective of mating status (Bradley, 2012). Other biotic and abiotic factors may also negatively affect mating behaviour and duration. For example, a previous study showed that male-male competition leads to significantly reduced copulation duration in *S. grossa* (Dong et al., 2023).

We hypothesize that copulation duration in *S. grossa* is positively correlated with offspring production because it might influence the amount of sperm transfer. In another *Latrodectinae* species, the Australian redback spider (*Latrodectus hasselti*), it was shown that most sperm were transferred within the first 5 minutes of copulation (Snow & Andrade, 2004). To investigate this, we paired freshly-matured virgin females with virgin males and either artificially ended copulation prematurely after 1, 3, 5, 10, or 20 minutes by tapping the mating chamber or allowed mating to terminate naturally (= control). Males were thereafter removed, and egg sac production was recorded until the female died or stopped producing egg sacs over the course of 6 months. We also remated several females from different treatments when they stopped producing egg sacs for over 6 months. The receptivity of previously-mated females was also determined. The main aim of the study is to determine the reproductive costs of disrupted mating in *S. grossa*.

Materials and methods

Animal collection and maintenance

A laboratory population of *S. grossa* was established from individuals and egg sacs collected from several sites in Gelderland, the Netherlands between 2019 and 2021. All of the sites are physically separated by are located within 10-20 km of each other. Egg sacs were placed in 8 cm dia. Petri dishes until the spiderlings hatched. Spiderlings were reared individually in Petri dishes in a climate room at 22 ± 2 °C, 16:8 h light:dark cycle, and 50% relative humidity. They were fed on a standardized diet of fruit flies (*Drosophila spp*.) and house crickets (*Acheta domesticus*) obtained from commercial providers. Males mature after 5-7 molts while females have 1-2 additional molts. At adulthood, females were transferred and placed individually in plastic boxes (11 x 11 x 6 cm) with two crossed wooden sticks to allow web building and the release of sex pheromones on webs, which has been demonstrated in this species (Fischer et al., 2022). Males remained in Petri dishes until mating. Females remained in plastic boxes with perforated lids for at least 2 weeks, during which time they had produced a prey-capture web and had thus released sex pheromones that stimulate mate-finding behaviour in males (Scott et al., 2018). Males and females used in experiments had molted into adults over the previous 2-4 weeks. For the mating experiment, virgin adult females and males produced by ca. 15 different unrelated mothers were selected.

Experimental design

We assigned randomly selected females to one of the following five mating disruption treatments (mating disruption after 1, 3, 5, 10, or 20 min) and a control treatment. One male was thereafter introduced into the box with a female. The pair of spiders was observed until copulation was initiated, i.e., when the male successfully inserted the embolus from one of the pedipalps into the female epigynum (Note that copulations under 10 min are copulations that only has one palp insertion). Mating was allowed to continue under one of the assigned treatments. Mating was terminated by tapping the box manually or by gently touching the web around the in-copula pair using forceps. A control consisted of allowing mating to proceed until it was terminated by the spiders. In total we established 94 spider couples (n = 15-16 per treatment). After mating, males were removed from the box, while females were left undisturbed and subsequently provided with one house cricket nymph (*A. domesticus*, 6th instar) as food per week until the end of the experiment.

Boxes with mated females were monitored daily for egg sac production until the female died or no more egg sacs were produced over at least 6 months. Egg sacs were removed from the boxes by gently cutting the silk threads with micro-scissors, weighed on a Mettler-Toledo Microbalance MT5 (accuracy 0.001mg, Columbus, Ohio, USA), and placed individually in Petri dishes (8 cm diam.). The dishes were maintained in a climate chamber set at 22 ± 2°C during daytime and 12 ± 2°C during nighttime, a relative humidity of 60%, and a 16:8 light-dark photoperiod. The number of spiderlings that hatched from each egg sac was counted and spiderling count was summed across all egg sacs to give total offspring production per female. Mean spiderling mass was determined by the total mass of all spiderlings in egg sac divided by spiderling number. In addition, the time interval between mating and production of the first egg sac was recorded.

To test whether females that did not or stopped to produce egg sacs was due to sperm limitation, females in the 1-min copulation treatment were allowed to remate with a virgin mature male after at least 6 months of reproductive inactivity and monitored for egg production for an additional 3 months. The same procedure was repeated with females that mated normally (control) and that after producing multiple egg sacs did not produce a new egg sac for at least 6 months.

We tested if mated females are willing to mate again when their copulation are disrupted or not. Virgin females mated with a virgin male naturally (n = 6) or for a disrupted copulation (disrupted before 10 min, n = 5), and males were removed after copulation ended. After 24 hours, we introduced another virgin male into the box of the female. Mating status was recorded (Yes / No for another copulation).

Statistical Analysis

All analyses were carried out using R 4.1.3 (R Core Team, 2023). We assessed the relationship between copulation duration (in min) and several response variables associated with reproductive success of a female. These variables included the time interval between mating and production of the first egg sac, total number of egg sacs, cumulative mass of all egg sacs, total number of spiderlings, and mean weight of spiderlings. Firstly, we visualized data pints with copulation duration as a continuous variable to explore the general pattern between each variable and copulation duration. For the time interval between mating and production of the first egg sac, total number of egg sacs, and total mass of all egg sacs and total number of spiderlings, these relationships appeared to be asymptotic. The relationship between these variables and copulation duration was then fitted using asymptotic regression (R: non-linear least squares (nls) function). We defined the asymptotic regression model as:

$$Y = a - b^{-cX}$$

where a is the asymptote, $b = a - R_0$ (R_0 is the Y value at $X = 0$), c = relative rate of Y increase/decreases while X (= copulation duration) increases. We fitted data for these four variables using this asymptotic regression model because the data followed an asymptotic pattern, and validated the models by visual inspection of the residuals. We compared the asymptotic model with a linear model and tested which model fitted the data better using a likelihood ratio test (R package: *lrtest*). Since the mean weight of spiderlings didn't show an asymptotic trend, we fitted it with a linear model. All calculated values are presented by mean ± SE.

Results

The copulation duration in *S. grossa* pairs in the control trials was 54.1 ± 4.8 min (range from 31-73 min, n = 16). The male usually inserts one palp into the epigyne of the female for 15-30 minutes, afterward removes it, and then repeats this with the other palp before mating is discontinued. We first analyzed the effect of copulation duration on the time interval until first egg sac production. An asymptotic model fitted the data significantly better than a linear model (likelihood ratio test; $X^2 = 55.5$, $p < 0.001$). The time interval between mating and production of the first egg sac decreased exponentially and leveled off at approximately 15 days, when copulation duration was 10 min or longer (Fig 1). The longest time lapse until first egg sac production was 276 days for a female that was *in copula* for only 1 min, with a mean for 138 days. Most females that mated for 1 minute produced no or only a few egg sacs (it is 2 egg sacs on average, Fig 2).

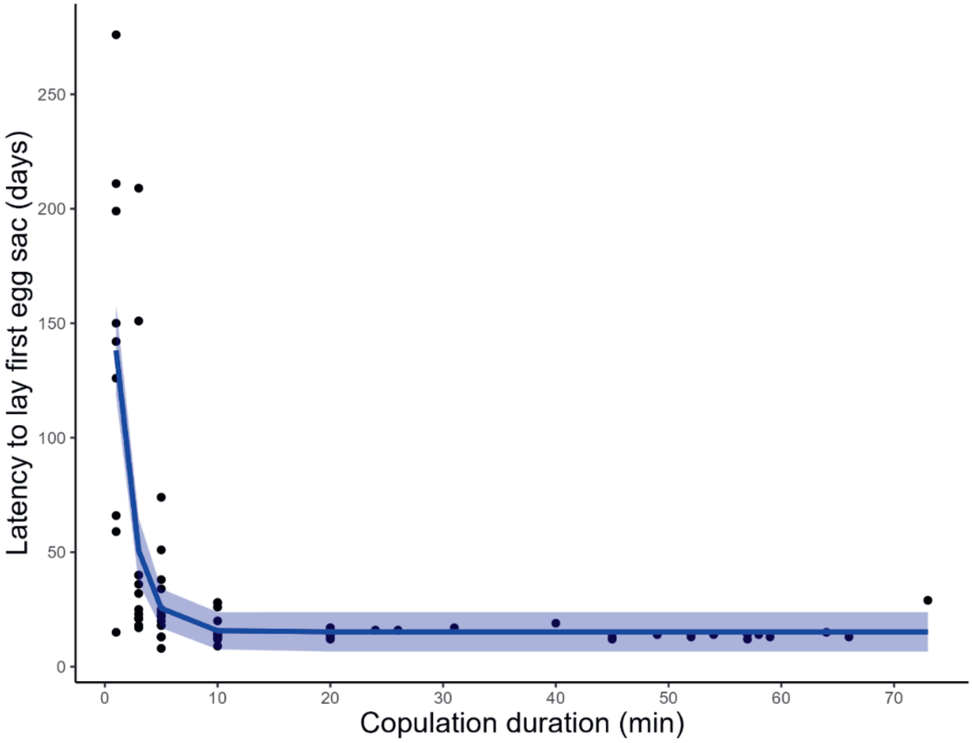

Figure 1. Asymptotic relationship between the time latency for first egg sac production following mating and copulation duration. The blue line is the predicted relationship based on the estimated model parameters [Interval (days) $= 15.24 + 228.88^{-0.62 \times Copulation\ duration(mins)}$, $R^2 = 0.54$] with a 95% confidence interval in light blue.

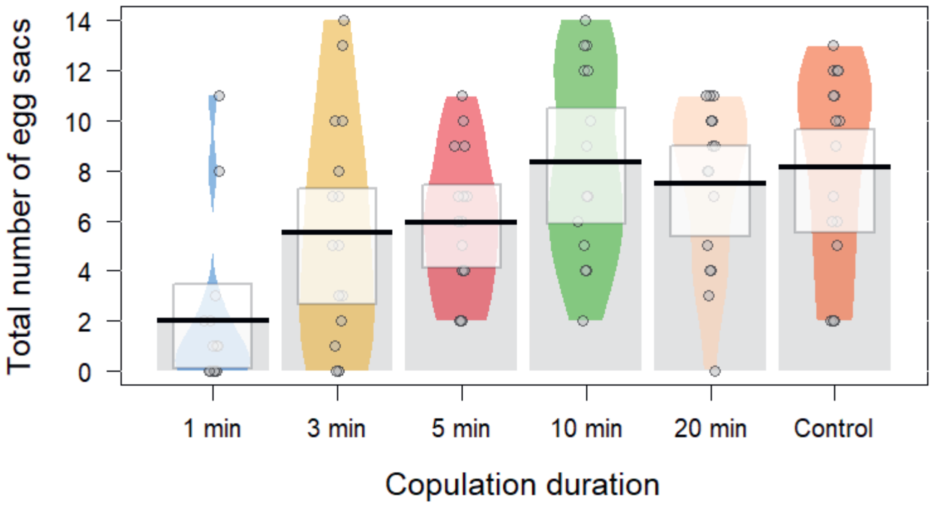

Figure 2. The relationship between the total number of egg sacs produced per female and copulation duration. The round dots show the raw data. The band shows the confidence interval, and the solid line is the median. The bean shape represents density.

Furthermore, the relationships between copulation duration and egg sac number, total egg sac mass, and number of spiderlings, respectively, were fitted best using asymptotic regression compared to linear models (egg sac number; $X^2 = 19.1$, $p < 0.001$; egg sac mass: $X^2 = 17.3$, $p < 0.001$; spiderling number: $X^2 = 24.9$, $p < 0.001$; Fig 3). The total number of egg sacs produced per female increased exponentially with copulation duration and leveled off to approximately 8 egg sacs in females with longer copulation (i.e., > 10 min; Fig 3a). The total number of egg sacs produced per females was highly variable (25% of the variance was explained by copulation duration). The total mass of the egg sacs (Fig 3b) and the cumulative number of spiderlings produced by a female (Fig 3c) showed the same pattern as was found for the total number of egg sacs produced per female (Fig 3a). Total egg sac mass and the number of spiderlings increased rapidly with copulation duration until 10 min and then maximized (at 500 mg for eggs sac mass and 500 for the number of spiderlings) when copulation lasted longer. For all variables shown in Fig 3, the amount of variation was much greater than for the results on time interval until first egg sac production shown in Fig 1. The explained variance for the variables in Fig 3 ranged between 22 and 26% and was 54% for the time interval results. Finally, mean mass of individual spiderling was not affected by copulation duration (0.37 ± 0.01 mg, linear model: $F_{1,79} = 1.95$, $p = 0.167$).

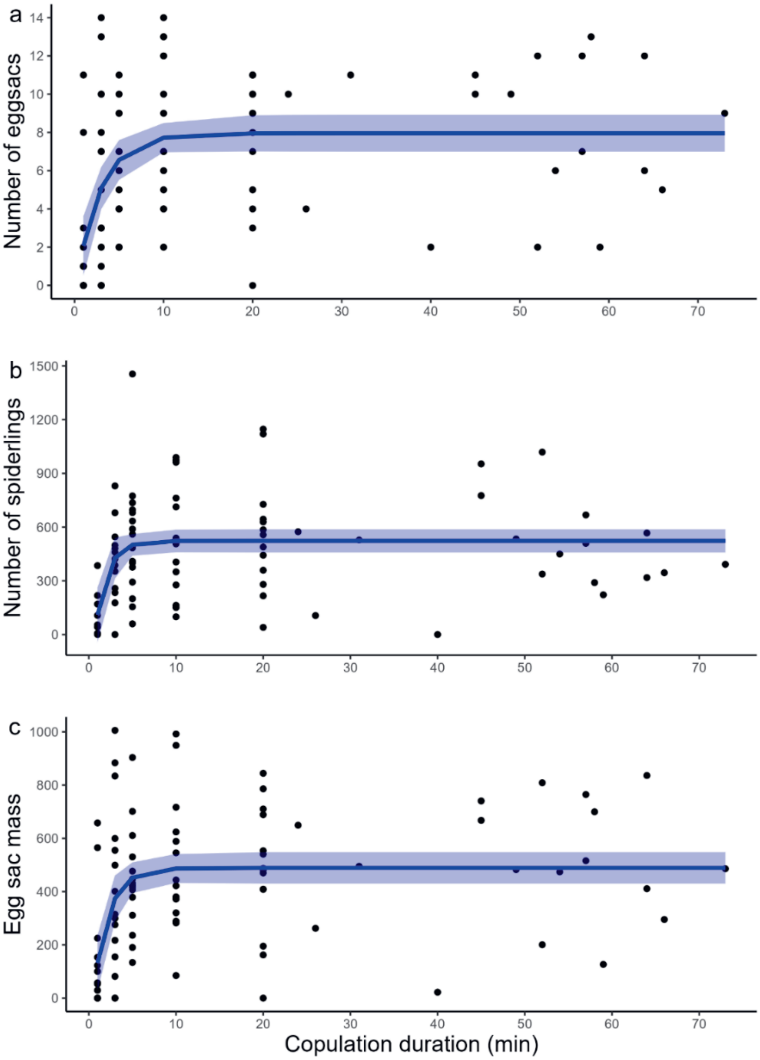

Figure 3. Asymptotic relationship between copulation duration and total number of egg sacs (a), copulation duration and total egg sac mass (b) and copulation duration and the number of spiderlings (c) produced per female. The blue line depicts the predicted relationship based on the estimated model parameters with a 95% confidence interval in light blue [a, total number of egg sacs = $7.97 - 8.35^{-0.36 \times copulation\ duration(mins)}$, $R^2 = 0.25$; b, total number of spiderlings = $512.57 - 805.27^{-0.58 \times copulation\ duration(mins)}$, $R^2 = 0.26$; c, egg sac mass (mg) = $488.13 - 629.35^{-0.57 \times copulation\ duration(mins)}$, $R^2 = 0.22$].

In the remating trials, the females from 1 min copulation treatment copulated for 45 ± 4.4 (n = 8) min while control females copulated on average 63 ± 6.0 (n = 5) min when mated for a second time. 88% of the females (7/8) in the 1 min treatment produced egg sacs while 40% of control females (2/5) did. These five control females had already produced 9.4 ± 1.3 egg sacs and mated 55.4 ± 3.1 min copulation when mated for the first time. These two control females both produced 3 egg sacs from which most egg sacs were not viable (for both female eggs from 2 out of 3 egg sacs were not viable), while the five females from the 1-min copulation treatment, the 5 females produced 28 egg sacs of which 25 developed into spiderlings.

All mated females (5/5 females in <10 min copulation, 6/6 females in natural copulation) copulated again after 24 hours from the previous copulation. Both naturally mating females and the disrupted females that were disrupted before 10 mins were interested in another virgin male's copulatory behaviors and conducted another copulation.

Discussion

Our results show that undisturbed mating pairs of *S. grossa* generally copulated for around 40 to 60 minutes. Moreover, we found that when copulation was disrupted within the first 5 minutes of initiation in which only one palp was used, female reproductive performance was negatively affected, with females producing fewer egg sacs and fewer offspring over their lifetimes. Copulation that lasted 10 minutes or longer did not significantly affect reproductive success. This suggests that sperm transfer from the male to the female increases with copulation duration over the first 10 minutes, and that is copulation duration of 10 minutes and longer is sufficient for females to fertilize most or all of their eggs. Furthermore, females that copulated for 10 minutes or longer typically produced their first egg sac around 2 weeks after mating. In contrast, early disruption of copulation significantly delayed production of the first egg sac, and in some instances, this was extreme, extending to over 200 days (or almost 7 months). On the other hand, copulation duration did not influence the mean weight of spiderlings when they hatched from the egg sac. This reveals that copulation duration does not alter the contents of ejaculates that can influence spiderling body mass.

Studies with other insects have reported positive, but not always linear, relationships between copulation duration and sperm transfer (Bukowski & Christenson, 1997; Danielson-François, 2006; Hinn & Niedzlek-Feaver, 2001; Linn et al., 2007; Shandilya et al., 2021; Tong et al., 2021). For instance, the mean number of sperm transferred increases with copulation duration in the leafhopper *Balclutha incisa* (Bailey & Nuhardiyati, 2005). Similar results were found for the black field cricket (*Teleogryllus commodus*), but in that species the relationship is asymptotic (Bussiégre et al., 2006). In spiders, a positive linear relationship was found in *Pisaura mirabilis* (Albo et al., 2013) and the golden orb-weaver *Trichonephila edulis* (Ceballos et al., 2015). However, in Australian red back spiders, *L. hasselti*, sufficient sperm for fertilization is transferred within the first 5 minutes of copulation (Snow & Andrade, 2004). This is similar to the asymptotic trend we report here for *S. grossa*, and suggests that sufficient sperm is transferred within the first 10 minutes of copulation in *S. grossa* regardless of the number of pedipalp insertions. Some *S. grossa* females that had copulated for 1 minute either did not produce offspring at all, or took an extended time to produce their first viable egg sac, and readily remated with a new male. These females always produced fresh egg sacs with viable offspring. This reveals that 1-minute copulation does not provide sufficient sperm for these females to fulfill reproductive potential. In contrast, females from the unlimited copulation cohort also readily remated, but produced no egg sacs or a few of poor quality, i.e., they were ‘dummy’ egg sacs did not develop into spiderlings.

To what extent products transferred during mating (i.e., seminal fluids) other than sperm contribute to egg production has been little studied in spiders (Michalik & Ramírez, 2014). For insects, it has been shown that substances from male ejaculate can influence female reproductive processes such as remating propensity, sperm storage, oögenesis, ovulation, and oviposition (Avila et al., 2011; Chapman, 2001; Parker & Pizzari, 2010; Shuker & Simmons, 2014; Vahed, 1998). These processes might also occur in spiders. A study with wolf spiders showed that substances in male ejaculate reduced female receptivity to other males (Estramil & Costa, 2007). Thus, when mating is disrupted early, two scenarios emerge: first, females appear to not have received sufficient amounts of sperm and or other essential substances to initiate early (or any) egg laying; second, females delay producing offspring from this male and wait for other, potentially fitter males to court and mate with them. Once a critical temporal threshold is passed and no subsequent male mates with a female, then some of these females 'hedge their bets' and produce their first egg sac. Our data show that the mean weight of spiderlings when they emerged from egg sacs was not affected by copulation duration, showing that products in the ejaculate other than sperm did not play an important role in offspring body mass.

Copulation disruption delayed production of the first egg sac, in particular when copulation lasted less than 3 minutes. Sperm transferred during copulation are immobile, encapsulated and stored in the spermatheca of the female (Tuni et al., 2020). In spiders, sperm is activated and eggs are fertilized when the female initiates egg-laying, which can take place for up to several months after copulation (Foelix, 1982; Useta et al., 2007; Vöcking et al., 2013). Sperm activation requires a sequence of endocrinological and or neurological signals (Herberstein et al., 2011). Thus, the initiation of egg fertilization appears to be actively controlled by the females. Females can wait for another male when insufficient amounts of sperm or other products are transferred during the previous copulation. Courtship behaviors, such as nuptial gift-giving and web vibration may also be an indicator of male quality (Andrés & Cordero Rivera, 2000; Eberhard et al., 2020; Pilastro et al., 2007). Females may wait for a high-quality male to sire their offspring in order to maximize their fitness. However, delay of reproduction can have severe fitness costs if the female dies prematurely.

Therefore, some females under very short copulation duration eventually produced egg sacs to ensure at least some reproductive success. Little is known how often *S. grossa* females encounter males in nature. However, given that the synanthropic lifestyle and its presence are often restricted to anthropogenic habitats, and their mobility and dispersal will be limited if the habitat is enclosed, we expect that multiple males may compete for access to females, leading to intra-sexual conflicts that disrupt mating.

Since *S. grossa* can achieve maximum reproductive success when mating duration is as low as 10 minutes, an important question is why copulation extends well beyond this time. Long copulation decreases time spent in other activities such as foraging or feeding, and also increases the risk of precocious mortality from natural enemies via predation or parasitism or from other factors such as extreme weather events (Daly, 1978). In many organisms, copulation behavior is relevant to mate guarding and paternity (such as insects, Alcock, 1994; birds, Møller & Birkhead, 1991). In spiders, copulation duration has mostly been studied in relation to sexual cannibalism. For example, females often initiate cannibalism of males which reduces copulation duration and thus influences paternity (Elgar et al., 2000; Herberstein et al., 2011). In some cases, however, sexual cannibalism prolongs copulation duration (Andrade, 1996; Schneider et al., 2006). Another exceptional behavior from *Tidarren argo* sp. nov. is that the males' palp is excised once copulation starts but the palp still functions independently for several hours while females feed on the palpless male in the meantime (Knoflach & Harten, 2001). However, in spiders where sexual cannibalism is rare, the function of extended copulation duration is poorly understood. A study by Scott et al. (2018) found that *S. grossa* females were less receptive for another mate after extended copulation. However, we found that disrupted copulation did not have the same effect on receptivity because females that copulated naturally were still highly receptive to new virgin males presented to them 24 h later, and readily mated with them. Extended copulation, however, may be beneficial for males in other ways. For example, it might affect the outcome of sperm competition because of increased insemination by the first male that numerically dilutes sperm inseminated by subsequent males (Arnqvist & Nilsson, 2000; Bukowski & Christenson, 1997; Snow & Andrade, 2004).

This study has reported how copulation duration influences reproductive outcome in the false widow spider, *S. grossa*, under laboratory conditions. These experiments reflect natural constraints on copulation duration that may occur through natural factors such as male-male competition, the presence of a predator, physical disturbance by another organism or by weather-related factors such as wind and rain. For example, a previous study showed that copulation duration is significantly shorter when two males compete for access to an individual *S. grossa* female than when a male was alone (Dong et al., 2023). The 'winner' was frequently attacked by the 'loser', leading to early termination in mating. Here, a minor stimulus – tapping – was also sufficient for male and female spiders to terminate copulation early. Physical disruptions happening during the early stages of copulation leads to decreased reproductive outcome and thus generates fitness costs for females and males. Given the prevalence of potentially disruptive factors, we anticipate that selection favours rapid and efficient transfer of sperm during copulation, maximizing reproductive output even under very short copulation duration. However, this was only partially true in *S. grossa* since in this species copulation is up to several times longer than necessary in terms of maximizing reproductive output. Extended copulation duration also did not decrease the female receptivity for other males. This suggests that it has other functions which might influence cryptic female choice and fertilization processes, thus influencing the reproductive success of males. Even though this study did not explore mechanical parameters in detail, it is important to understand not only the relationship between copulation duration and female reproductive processes (i.e., sperm activation, oviposition, fertilization, pheromone changes), but also the costs and benefits of extended copulation in terms of male and female fitness across a broad range of organisms.

Author Contributions

All authors contributed to the idea for the study. Y.D., M.H. and A. Z. performed the experiments and collected data with suggestions from J.A.H. Y.D. and R.G. analyzed data. Y.D wrote the manuscript with suggestions from M.R. and R.G. and J.A.H.. J.A.H., M.R., and R.G. reviewed and edited the manuscript, and all authors approved the final version of the manuscript.

Data Availability

Data is deposit in Dryad website, and now is temporarily available for reviewers. Please see https://datadryad.org/stash/share/wpcJrHkXZGEyFd46Sk4iUcYradZB51futC_zlssK9QM

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Chapter 4

The effects of food availability on female reproductive success

Yuting Dong, Rieta Gols, Melissah Rowe and Jeffrey A. Harvey

This chapter has been submitted to a journal

Abstract

All animals require food in order to survive, grow, reproduce and thus optimize fitness in nature. Food availability and quality can profoundly affect demographic parameters such as longevity and fecundity. Here, we compared reproductive parameters in the false widow spider, *Steatoda grossa* (Araneae, Theridiidae) when availability and size of prey (the house cricket, *Acheta domestica*) was manipulated. Adult mated female spiders that were fed weekly (constant prey treatment) produced more progeny during their lifetime than females fed every three weeks (intermittent prey treatment). Furthermore, the monitoring of fecundity schedules showed that the mean number of neonate spiderlings per egg sac was around 40% higher in constantly fed spiders over the first 10 egg sacs. Time intervals between egg sac production were generally higher when prey availability was lower. Some females lost more than 50% of their body mass after production of the first egg sac, although reproductive investment tended to decrease thereafter. The size of prey offered to female spiders significantly affected mass gain between reproductive events, but fewer progeny was produced by females per egg sac as well as cumulatively over the first three egg sacs when they were only fed small prey. Starved females that had not produced egg sacs in several months exhibited total reproductive recovery when fed with large crickets. Our results demonstrate the importance of prey attributes on *S. grossa* reproduction. Furthermore, although this species is iteroparous, females invest remarkably large amounts of resources during each reproductive cycle and over the course of a lifetime.

Key words

Egg sac, fecundity schedule, hunger, starvation, reproduction

Introduction

All organisms require sufficient food in order to perform vital physiological and metabolic functions in nature. Food availability and quantity affect growth, survival, immunity, longevity and reproduction in animals, thus ultimately affecting fitness (Briga et al., 2017; Dmitriew, 2011; Houston et al., 2007; White, 2008) and these effects are well-documented in vertebrates including amphibians (Courtney Jones et al., 2015), reptiles (Mugabo et al., 2011), birds (Grames et al., 2023), and mammals (Speakman, 2007). Similarly, food availability and quality have been reported to have significant effects on growth and reproduction in insects (Bauerfeind & Fischer, 2005; Clissold & Simpson, 2015; Gols et al., 2022) and other arthropods, such as scorpions (Bradley, 1984; Seiter et al., 2020). In nature, food resources may vary spatially and temporally, and this unpredictability may be influenced by such factors as competition among species in the same functional guild, climate, and phenology between the resource and consumer (Closs & Lake, 1994; Dmitriew, 2011; White, 2008). Periodic shortages of food generate potential trade-offs among different and potentially competing fitness functions such between growth rate and survival or between body size and reproduction (Adler et al., 2013; Kaitala, 1991; Moore & Attisano, 2011; Stearns, 1989; Zera & Harshman, 2001).

The number of times an organism reproduces during its lifetime – described as its mode of 'parity' – is a fundamental trait that reflects a range of intrinsic and extrinsic selection pressures on the life-history evolution of a species or clade (Hughes, 2017). Semelparous species are those where the females have only a single reproductive event, whereas females of iteroparous species exhibit two or more repeated bouts of reproduction throughout life, which is often extended (Bell, 1980; Cole, 1954; Hughes, 2017; Ranta et al., 2002; Trumbo, 2013). Unsurprisingly, many semelparous species appear to invest disproportionate amounts of metabolic resources into a single reproductive event, thereby depleting internal metabolic resources beyond a critical threshold for extended survival, or else invest in offspring provisioning without feeding. Iteroparous species, by contrast, retain enough resources to produce offspring at discrete intervals, and are thus able to fully or partially recover after each reproductive event. The two modes of parity, however, are not necessarily binary but may constitute part of a reproductive continuum (Hughes, 2017).

Among arthropods, such as insects, semel- and iteroparous reproductive strategies are well-described (Fritz et al., 1982). The expression of each strategy may be linked with environmental parameters, such as the reliability of food resources and appropriate habitat. For instance, univoltine semelparous insects may have evolved this strategy as a means of exploiting conditions that are only favorable during a short temporal window over the course of a season (Corkum et al., 1997). Under these conditions, insects may have to complete their life-cycle very rapidly and timing is of crucial importance in mating and reproduction, so everything is invested into one reproductive bout (Fritz et al., 1982). Extended maternal care may also favor semelparity or low iteroparity in insects with comparatively short lifespans (Ratz et al., 2016; Stegmann & Eduard Linsenmair, 2002). Despite this, most current evidence suggests that a majority of insects are iteroparous (Fritz et al., 1982).

Spiders (Araneae) are one of the most diverse and abundant groups of generalist predators in most terrestrial ecosystems (Wilder, 2011). Many spiders make webs that are especially constructed in order to trap prey (Han et al., 2019; Rypstra, 1982; Uetz et al., 1978), whereas other species are active, motile predators and search for prey on the ground or in vegetation (Beydizada et al., 2022; Eggs et al., 2015; Nentwig, 1987). As with other organisms, the availability of prey for spiders can be highly variable (Greenstone, 1984; Halaj et al., 1998), and this can affect spider survival, growth, and reproduction (Toft, 2013; Wilder, 2011; Wise, 2006). Spiders, depending on taxa, also exhibit both semel- and iteroparous lifestyles (Futami & Akimoto, 2005; Marchetti & Persons, 2020; Rádai et al., 2018; Schneider & Lubin, 1997; Stoltz et al., 2010).

Reproduction in spiders differs in one major way from most predatory insects. Whereas many female insect predators, such as ladybirds, hoverflies and ground beetles attach their eggs directly onto a substrate, such as on the soil or to a plant surface (Almohamad et al., 2009; Barbosa-Andrade et al., 2019; Chapman, 1998; Seagraves, 2009), female spiders possess specialized silk glands and lay egg clusters into intricately woven silken sacs that are either attached to the web or else placed into a cryptic site (Sethy & Ahi, 2022). Spider silk contains proteins composed of amino acids that are costly to synthesize, and place a greater strain on energy resources than other proteins which consist of ingested amino acids or that are metabolically less costly to produce (Craig et al., 1999; Vollrath, 1992). Although some insects also produce silk, it is rarely used for reproduction and plays a far less important role in insect than spider lifestyle.

In this study we examine egg sac production, fecundity schedules and lifetime reproductive success in the iteroparous spider, *Steatoda grossa* (Araneae: Theridiidae) under different prey availability and size treatments. *S. grossa* is a cosmopolitan and synanthropic spider (World Spider Catalog, 2023) that exhibits strong sexual size dimorphism (Harvey, 2022) and mated adult females lay multiple egg sacs within a tangled web over the course of their lifetime (Fischer, Hung, et al., 2020). Since the lifestyle of *S. grossa* is closely associated with human-created environments, such as cupboards, sheds, and cellars, periodic prey scarcity in these habitats may affect reproductive output in this species. By manipulating temporal access and size of prey to mated adult female spiders, we predict that there will be trade-offs between their own metabolism maintenance and egg production over the course of reproductive life. The main aim of this study is to understand how food availability influences reproductive investment and success in *S. grossa*.

Methods

Spider collection and rearing

Individuals and egg sacs from *S. grossa* were collected from multiple sites around Wageningen, the Netherlands in 2019 and 2020. All animals were reared at NIOO-KNAW (Netherlands Institute of Ecology). Egg sacs collected from the field or produced by individuals in a climate room (22 ± 2 °C, 50% relative humidity, 16:8 h light:dark cycle) were placed in Petri dishes (8 cm diam.). After the spiderlings hatched from egg sacs, they were placed individually in Petri dishes. Spiderlings and adult spiders were fed on a diet of fruit flies (*Drosophila spp.*) and house crickets (*Acheta domestica*) that were obtained from commercial providers (cricket body sizes were considered ranking by numbers, and indicated by the categories "size 2" (1^{st} instar nymph), through "size 6" (fully grown nymph)). A standardized amount of prey was provided based on the life stage and body size of the spiders. Males undergo 5-7 molts until maturation while females have 1-3 additional molts. Adult males remained in Petri dishes until mating and were provided with 3-4 fruit flies every 2-3 weeks. To obtain fresh body mass, spiders and crickets were anaesthetized for 10-15 minutes in a chamber with CO2 and then weighed on a Mettler-Toledo Microbalance (accuracy ± 1 ug). When females weighed approximately 100 mg, they were individually transferred to plastic boxes (11 x 11 x 6 cm) with 2 wooden sticks as a frame on which to facilitate web-building. Adult females were fed one or two late-instar cricket nymphs every week until death. Random mating was applied to reduce inbreeding. For mating, one virgin adult male was introduced into a female's box. After mating the male was removed from the box. In general, females produce their first egg sac ~two weeks after mating and they can produce 10 egg sacs or occasionally more during their lifetime from a single copulation. Adults used in the experiments were generated from the second or third generation of spiders reared in the lab.

Experimental design

This study investigates the effects of food availability on reproductive success. The study was divided into three experiments: 1) lifetime reproduction investigating the long-term effect of food availability on the number of emerging spiderlings, and reproductive intervals between the production of subsequent egg sacs until death, 2) dynamic changes in the body mass of female spiders before and after laying egg sacs, and egg sac mass as a measure of reproductive investment over time, and 3) the effect of providing different amount of prey (house crickets) on spiderling production for 4 months and body mass changes before reproduction. A final part of this experiment determined if female spiders under food limitation that stopped producing egg sac can produce egg sacs after being fed with a late instar cricket nymph. All male and female spiders used in the experiments had been reared under the same conditions from neonate to adult, and were fed *ad libitum*.

Experiment 1

In experiment 1, adult females were fed one late instar cricket nymph (‘size 6’) per week before they were mated according to the protocol described in the *Animal collection and rearing* section. Following mating they were randomly divided into two feeding groups: 1) Intermittent feeding (n = 36) where spiders received one cricket every 3 weeks, and 2) Constant feeding (n = 57) where spiders received one cricket weekly. Late-instar cricket nymphs (‘size 6’) weigh 150-200 mg. Egg sacs were carefully removed from the boxes using dissection scissors and were placed into labelled Petri dishes (8 cm dia.) with female number, egg sac number and treatment written onto the lid. The dishes were placed on plastic trays and kept in the climate room under culture conditions (22 ± 2 °C, 50% relative humidity, 16:8 h light:dark cycle) until spiderling hatching. Neonate spiderlings were anesthetized using CO2 and the number of spiderlings that hatched from each egg sac was counted and recorded. The duration (in days) of intervals between egg sac production was also recorded. These variables were monitored until females died in the two feeding treatments, except when unhealthy females died before producing 2 egg sacs (n=5 from constant feeding treatment, and n=2 from intermittent feeding treatment, excluded in the dataset).

Experiment 2

In the second experiment, we closely monitored the temporal variation in mass gain and loss of female spiders during the early reproductive cycle (over the first 5 egg sacs). Ten virgin females and males were randomly selected from rearing and were mated in plastic boxes. The females had been reared as in experiment 1. Late nymph house crickets (weighing 150-200 mg) were offered once weekly to females up until production of the first egg sac. Newly mated females were weighed on the microbalance. When they produced their first egg sac, they were immediately re-weighed on the microbalance, and their egg sacs were also carefully removed and weighed. The females were returned to their boxes and immediately presented with a late nymphal instar cricket, and this was repeated in subsequent weeks. After 12 days (just prior to producing the second egg sac) females were re-weighed and returned to their boxes. Immediately after producing their second egg sac, the females were again re-weighed along with the excised egg sac. This procedure was repeated until all females had produced 5 egg sacs.

Experiment 3

In this experiment, the quantity of food offered to females was varied. Adult females were placed separately into five groups (with 8 to 11 individuals per group), each receiving varying sizes of crickets. The mass of crickets was weighed before they were given to the spiders and cricket nymphs were divided into three categories based on the size categories of the supplier, as ‘small’ (size 2), ‘medium’ (size 4), and ‘large’ (size 6) cricket nymphs. Mean (± SE) fresh body mass were 8.0 ± 0.45; 63 ±

3.48; 113 ± 4.93, for small, medium and large cricket nymphs, respectively; Supplemental Excel file1, cricket size). As it was easy to visually categorize the crickets into the three size categories, crickets for use in the experiment were selected visually. The food treatments were as follows: 1) two large crickets per week (~240 mg per week); 2) one large cricket per week (~120 mg per week); 3) one medium cricket per week (~60 mg per week); 4) one medium cricket every 2 weeks (~30 mg per week), and 5) one small cricket every 2 weeks (~5 mg per week). The amount of prey (crickets in mg) fed per week were used as treatment categories.

Female spiders (around 20 months old) were exposed to these feeding regimes 2 months before mating, following mating and until they had produced 3 egg sacs or stopped producing egg sacs for 2 months. The first 3 egg sacs showed significantly positive relations to lifetime reproduction (egg sac mass: $F_{1,30} = 31.77$, $p < 0.001$; number of spiderlings: $F_{1,30} = 11.01$, $p = 0.002$; data from Supplemental Excel file2, the first three egg sacs and lifetime reproductive success). Body mass of the females was measured on the microbalance at the beginning of the experiment (day0), 50 days after initiation of the feeding regime (day50), and 7 days after mating (day86). Freshly produced egg sacs were carefully removed from boxes, weighed on the microbalance, and the date and female treatment and number were recorded. Egg sacs were maintained individually in Petri dishes in the rearing climate room until spiderling hatching. The hatching status of egg sacs was checked daily. The number of spiderlings and the mean weight of spiderlings per egg sac was also recorded. If females did not produce egg sacs 2 months after mating (this only occurred in the 5 mg cricket treatment), they were offered large crickets weekly and monitored for 1 month or until production of the first egg sac. Females that died prior to completion of the experiment were excluded from the analyses (1 female from 5 mg treatment, 30 mg treatment, 240 mg treatment and 2 females from 120 mg treatment).

Statistical analyses

For experiment 1, we calculated, per female the total number of emerging neonate spiderlings, total number of egg sacs, and the mean number of spiderlings per egg sac, and the mean duration of intervals between egg sac production. These measurements served as response variables in the linear model analyses for testing the effects of feeding regimes (constant and intermittent).

For experiment 2, we observed female body mass before and after producing egg sacs for 5 times of reproductive events (i.e., in each event females produced one egg sac) and calculated the body mass loss after producing an egg sac. We applied a one sample t-test to test whether the body mass loss deviates from 0 (Shapiro-Wilk normality test was applied in advance). Two linear mixed models were fitted for testing the changes in 1) the body mass loss for producing an egg sac and 2) the egg sac mass over the course of reproduction. In these models, body mass loss and the egg sac mass were included as a response variable, and reproductive events were included as the explanatory variable with female ID as random factor.

In experiment 3, we compared female body mass in all treatments at the initiation of the experiment using an ANOVA (Shapiro-Wilk normality test was applied in advance). To assess whether the changes in body mass between day0 and day86 were influenced by different food regimes, we applied a linear model, in which body mass changes is included as the response variable and the food regime is included as the explanatory variable. We used a Kruskal-Wallis rank test for the differences of time interval between mating and the first egg sac production among food treatment. We determined the effect of food availability on offspring production (i.e., total mass of egg sacs, total number of spiderlings, mean number of spiderlings per egg sac, mean weight of spiderlings, and these were included in the following models as response variables). Linear models were applied for all variables to assess the impact of feeding regime (5 levels) on each of the response variables. In all models, post hoc contrasts among feeding regimes were conducted if the effects of feeding regime were significant using ‘emmeans’ with Tukey HSD correction (Lenth et al., 2024). If females in 5 mg food treatment had not produced an egg sac, but produced an egg sac after food availability improved, their first reproduction after increased food was compared with the first reproduction from treatment 120 mg using linear models. In these models, food treatment (5 mg and 120 mg) was included as explanatory variable. Egg sac mass, number of neonate spiderlings and mean weight of spiderlings were included as response variables. All analyses were conducted in R 4.3.1 (R Core Team, 2023). All model assumptions were checked by ‘DHARMa’ package (Hartig & Lohse, 2022). Calculated numbers shown as a form of Mean ± SE.

Results

Experiment 1

Feeding regime had a significant effect on all measured variables (mean number of spiderlings per egg sac, $F_{1,75} = 74.2$, $p < 0.001$; total number of spiderlings produced over the course of a lifetime, $F_{1,75} = 6.06$, $p = 0.016$, and latency between egg sac production, $F_{1,75} = 6.10$, $p = 0.02$). Females fed weekly produced on average 7.5 (± 0.5) egg sacs and 131(± 4.4) live spiderlings per egg sac over the course of their lives, whereas females fed intermittently produced 8.9 (± 0.7) egg sacs with 80 (± 3.7) live spiderlings. Three out of 57 females (5%) produced at least 12 or more egg sacs in the constant feeding treatment, whereas 7 out of 36 intermittently-fed females (19.4%) produced more than 12 egg sacs. This shows that intermittently-fed females produced more egg sacs than constantly-fed females. Intervals between egg sac production were longer for intermittently fed females (24 ± 1.8 days) than for weekly fed females (20 ± 1.3 days). Mean total number of spiderlings was 1010.1 ± 78.7 for constantly fed females and 738.8 ± 72 for intermittently fed females. The fecundity schedules (mean number of spiderlings per egg sac) of females exposed to the two treatments is shown in Fig 1.

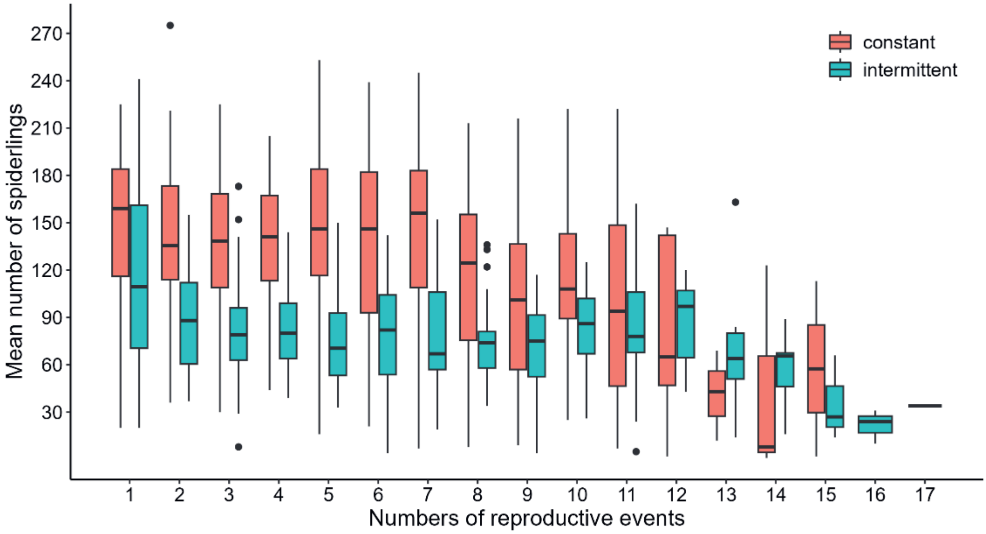

Figure 1. Fecundity schedules (mean number of spiderlings per female) of female *S. grossa* spiders fed constantly (red boxes) or intermittently (blue boxes). Boxes represent the interquartile range (IQR) of the data with the top and bottom edges of the box indicating the first and third quartiles. Black lines inside boxes represent the median. The whiskers extend from the box to the minimum and maximum values within a certain range, and data points beyond whiskers are considered outliers.

Experiment 2

Females weighed significantly less after producing an egg sac ($t = 37.63$, $df = 49$, $p < 0.001$). Females lost almost half of their body mass after laying their first egg sac (before, 194.9 ± 3.8 mg; after, 98.7 ± 2.4 mg), and egg sac mass was similar to the remaining body mass of females (Egg mass, 73.9 ± 2.4; Fig S1). The mass loss of females decreased with consecutive reproductive events ($X^2 = 5.55$, df=1, P = 0.02). Furthermore, egg sac mass decreased with consecutive reproductive events ($X^2 = 36.78$, $df = 1$, $p < 0.001$).

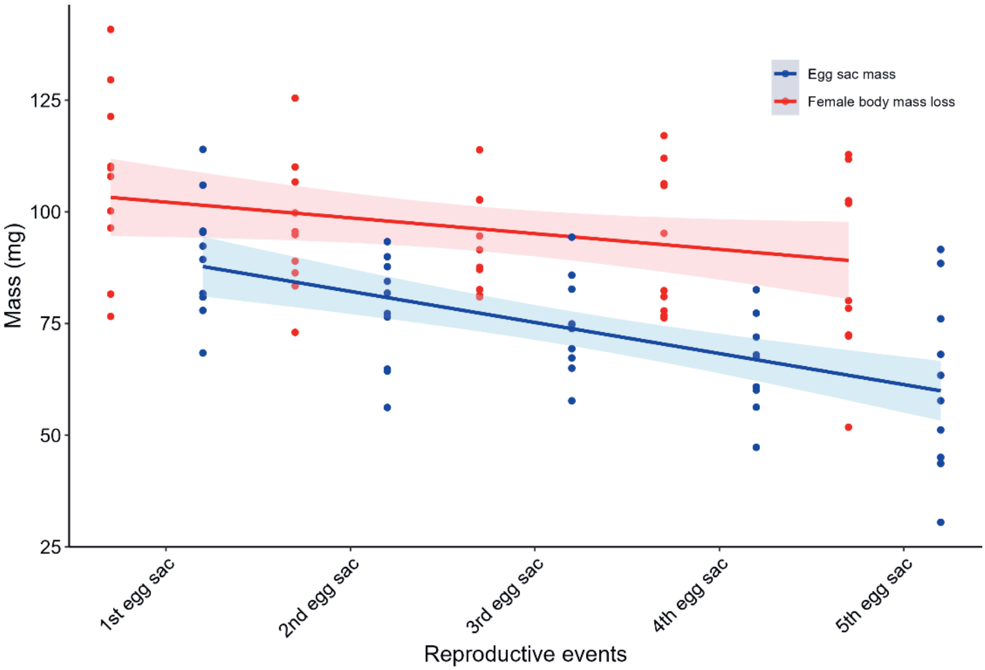

Figure 2. Female body mass loss after each reproductive event and the egg sac mass in each reproductive event. All the dots are original data points, and the two lines were fitted with linear model methods. The light blue and light red areas around the lines represent the 95% confidence intervals.

Experiment 3

Mean female body mass was similar at the start of the experiment ($F_{1,42}$ = 0.76, p = 0.39) but decreased with decreasing food availability when measured between day0 and day86 ($F_{1,42} = 25.59$, $p < 0.001$; Fig 3).

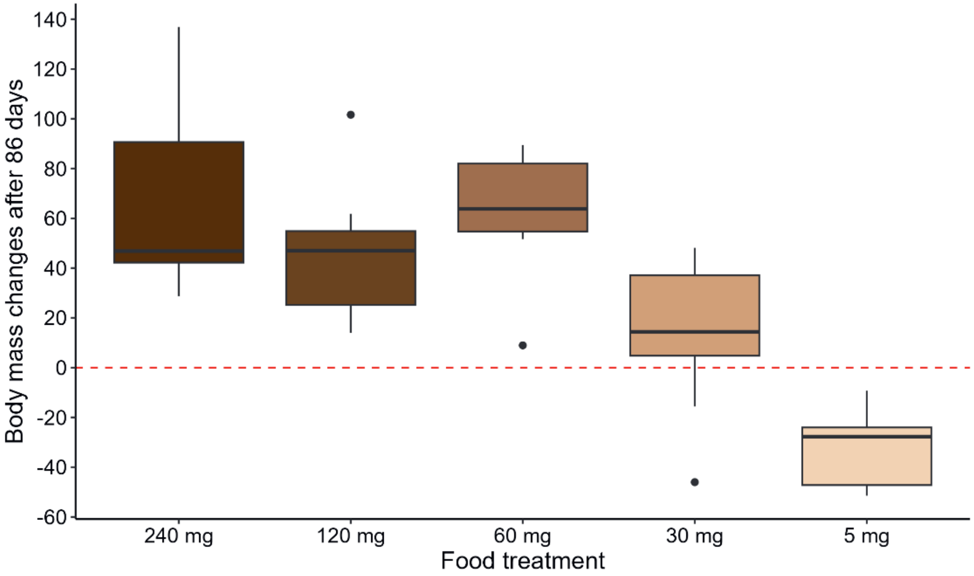

Figure 3. Female body mass changes of *S.grossa* between day 0 (the initiation of different feeding regimes) and day 86 across different feeding treatments (approx. 240 mg, 120 mg 60 mg, 30 mg, 5 mg food provided weekly to female spiders). Black lines inside boxes represent the median, and the boxes show the interquartile range (IQR). The whiskers extend from the minimum and maximum values, and data points beyond the whiskers are outliners.

The time interval between mating and the first egg sac production was produced showed no difference among feeding regimes ($X^2 = 1.28$, $df = 4$, $p = 0.86$; overall mean ± SE, 16.6 ± 0.8 days). Feeding regime significantly affected the total number of spiderlings ($F_{4,34} = 12.34$, $p < 0.001$, Fig 4A). Females in 240 mg prey treatment produced a higher total number of spiderlings than those in 30 mg and 5 mg prey treatments (240 mg vs. 30 mg, $t_{34} = 3.07$, $p = 0.03$; 240 mg vs. 5 mg, $t_{34} = 5.99$, $p < 0.001$). Females in the 120 and 60 mg prey treatment also produced significantly more spiderlings than females in the 5 mg treatment (120 mg vs. 5 mg, $t_{34} = 5.43$, $p < 0.001$; 60 mg vs. 5 mg, $t_{34} = 4.96$, $p < 0.001$). Other treatments were not different in total number of spiderlings with each other (Table S1). Females in 60, 120, 240 mg treatments produced larger egg sacs with a higher mean number of spiderlings than females in the 5 mg food treatment ($F_{4,34} = 5.85$, $p = 0.001$, Fig 4B). The differences between 5 mg and 240 mg, and between 60 mg and 120 mg were significant (Table S2). Furthermore, the mean weight of spiderlings decreased with an increase in the severity of prey deprivation ($F_{4,33} = 2.81$, $p = 0.04$, Fig 4C). However, there were no significant results from the post hoc test, but spiderlings hatching from the 5 mg prey treatment tended to be smaller than spiderlings from 120 and 240 mg treatment ($t_{33} = 2.75$, $p = 0.07$, and $t_{33} = 2.78$, $p = 0.06$ respectively; Table S3). Females from the 60, 120 and 240 mg treatments showed no difference in these three variables (Fig 4).

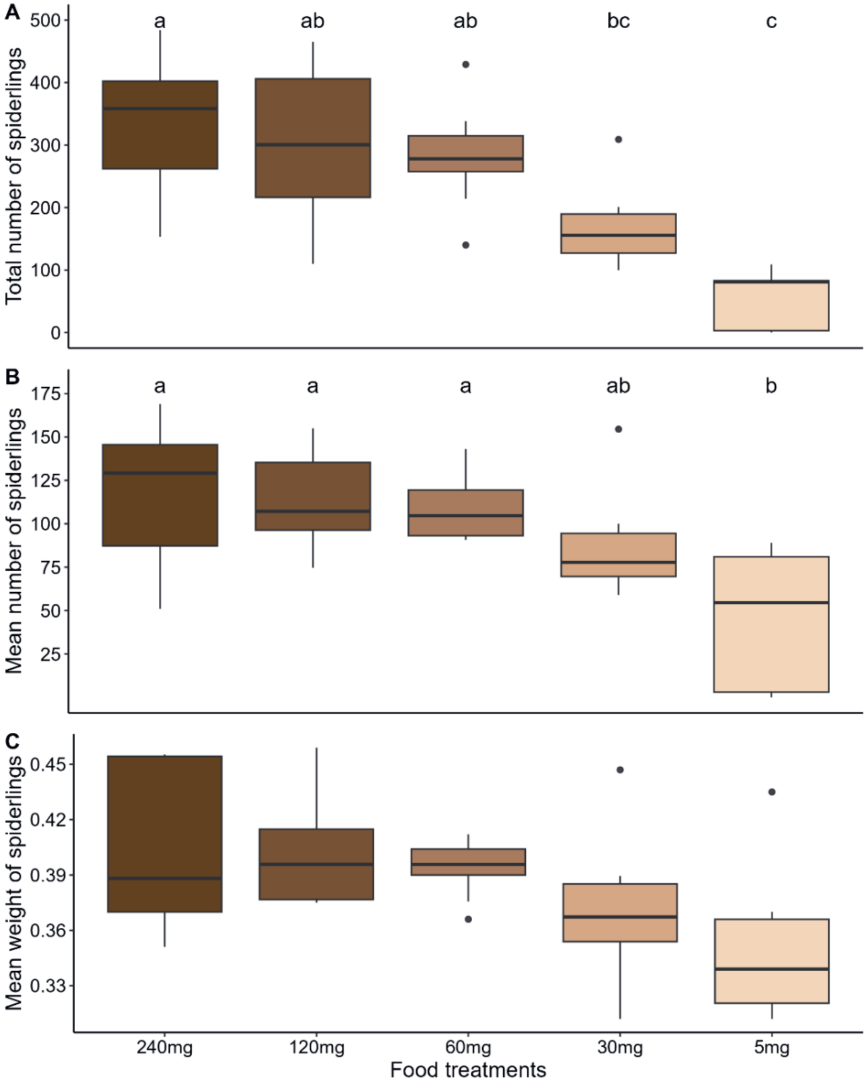

Figure 4. Reproductive success and spiderling body weight in *S. grossa* across different feeding treatments (approx. 240 mg, 120 mg 60 mg, 30 mg, 5 mg food provided weekly to female spiders). (A) cumulative number of progeny, (B) mean number of spiderlings per egg sac (B) mean weight of spiderlings per female (C). Boxplots display a median line, interquartile range (IQR) boxes, 1.5×IQR whiskers and outliners when they are out of the whisker ranges. The significance thresholds are $P < 0.05$ and letters denote significant differences among food treatments.

Females that were provided with 5 mg food per week did not produce egg sacs for several months, but recommenced egg sac production after being fed one large cricket nymph. The egg sac was smaller than the first egg sac in the 120 mg prey treatment (t = 4.10, $p < 0.001$), and the number of neonate spiderlings was also significantly lower (t = 2.56, p = 0.02). However, the mean weight of spiderlings was not different from spiderlings in 120 mg treatment (t = 0.18, p = 0.86).

Discussion

The results of this study show that the food availability (i.e., amount of prey biomass and temporal availability of prey (house crickets, *Acheta domestica*)) significantly affected reproductive parameters in the false widow spider, *S. grossa*. Mated adult female spiders provided with prey weekly (constant prey treatment) produced more progeny over the course of their lifetimes than females fed only every three weeks (intermittent prey treatment). Furthermore, the mean number of spiderlings emerging per egg sac was around 40% higher in spiders that were constantly fed. The time interval between the production of egg sacs was also several days longer, on average, when spiders were only fed intermittently. Body mass of females dropped dramatically after each reproductive event, often by more than 50%, but regained by feeding. However, when the amount of cricket prey biomass offered to reproductive females early in adult life differed, female investment into reproduction was significantly affected, suggesting that they allocate a large part of the limited prey resources to reproduction. If these females were thereafter starved for several months, their physiological condition deteriorated, and no eggs were produced. However, when starved females (i.e., in 5 mg treatment) were then provided with large prey, they laid viable egg sacs within several days, and these in turn produced healthy spiderlings, indicating that their reproductive capacity is retained through harsh and extended periods of resource depletion.

Although *S. grossa* is iteroparous and can produce 10 or more egg sacs over several months if prey is available, females invested remarkable amounts of metabolic resources into each egg sac with a decreasing pattern over time. When provided with large prey immediately after reproduction, females generally regained all (or sometimes even more) body mass lost during reproduction, usually within only 1 or 2 days. With abundant food supply, they started to prepare the next reproductive event, although with a decreasing pattern in reproductive investment as time passes. In nature, however, food availability is clearly unreliable, and immense investment into reproduction may never be fully compensated when prey is scarce or their availability unpredictable. Moreover, the decreasing pattern in egg sac mass and female body mass loss in each reproductive event indicates a decreasing reproductive investment with age, which aligns with other animal, like burying beetles (Cotter et al., 2011). As McNamara et al., (2009) demonstrated individuals balance their resources between reproduction and survival throughout their lives, holding back resources from each reproductive event to extend longevity which in turn increase the number of reproductive opportunities. This suggests that the life-history tradeoff in *S. grossa* between individual longevity, current reproduction and future reproduction under food limitation.

Several studies have examined the effects of food limitation on the biology, behavior and ecology in spiders. Kleinteich et al. (2015) found that lifetime fecundity (egg and hatchling production) and mean clutch size per egg sac in the bridge spider, *Larinioides sclopetarius* (Araneidae) were significantly lower that were fed lower prey numbers (fruit flies) when they were juveniles or adults, or both life stages. However, the total lifespan was extended under food limitation of juveniles and adults, whereas adult food limitation increased adult longevity (defined as the time from maturation until death). By contrast, food limitation of juveniles led to a reduction in adult longevity. Uhl et al. (2004) studied prey availability on growth and reproduction in the cellar spider, *Pholcus phalangioides* (Pholcidae) and revealed that adult male and female size and female reproductive success were all negatively affected when spiders were poorly fed. Other studies with different spider species have similarly found that food limitation leads to a reduction in progeny production (Kreiter & Wise, 2001; Mayntz et al., 2003; Wilder & Rypstra, 2008; Wise, 1979, see also review by Wise, 2006). The effect of prey availability can also vary, depending on the age of the female. This was demonstrated in the Mediterranean tarantula, *Lycosa tarantula* (Lycosidae), where the reproduction of older females was less positively affected by additional prey provisioning than that of younger females (Moya-Laraño, 2002).

Investment into reproduction often comes at the expense of other traits, like longevity. As we showed, female *S. grossa* spiders that received less prey produced smaller clutches at longer time intervals, but ultimately produced slightly more egg sacs over the course of their reproductive life than spiders which were fed constantly. In fact, the most egg sacs produced by any female spider in the lifetime fecundity experiment was 17, and this was by a female that received less prey. When food resources are limited, allocation to metabolic maintenance or internal storage sites may take precedence over allocation to reproduction, resulting in decreased reproductive output (Attisano et al., 2012; Zera & Harshman, 2001). It is also known that some insects can resorb egg proteins for maintenance purposes when food is scarce or absent, and this not only extends longevity but also reduces potential costs of metabolic storage of eggs (Moore & Attisano, 2011). We don't know if this ability is present in *S. grossa* or other spiders. A study with the Australian red-back spider (*Latrodectus hasselti*), which is closely related to *S. grossa* (both are in the subfamily Latrodectinae), prey deprivation caused mated females to shut down egg production completely and to lower resting metabolic rates, thereby extending lifespan significantly, which was longer than that of mated females with unlimited prey (Stoltz et al., 2010). However, once female red-backs were returned to a high-prey diet, their resting metabolic rate increased rapidly and they recommenced the production of egg sacs, ultimately producing as many progeny as well-fed females with shorter lifespans.

Steatoda grossa, like other species in the genus and related species in the Theridiidae (including true widows) exhibit sexual size dimorphism (SSD) where mature females are some 5-10 times heavier than mature males (Harvey, 2022). A previous study showed that prey availability affected the development of male and female *S. grossa* spiderlings in somewhat different ways. Whereas male spiders traded-off development time and body size almost equally under prey limitation, female spiders extended development time up to several months under the same conditions but attained similar body mass at maturity as well-fed females (Harvey, 2022). This reveals that selection favors large size in females, presumably because they invest much more per capita resources into reproduction than males (Shine, 1988).

Our study has reported that constantly-fed false female widow spiders invest considerable amounts of metabolic resources obtained from their prey into reproduction, and that this was sustained as long as prey was readily available. The cost of this immense allocation was demonstrated by the veritable 'roller-coaster' of weight gain and loss that preceded and followed each reproductive event. This must exert profound costs on survival, given the dynamic nature of biomass allocation and utilization. Spiders fed at longer intervals or on very small prey presumably reduced investment into reproduction, thus enabling them to extend their lifespans. Starved females lost some 80% of the potential weight, and yet when provided with prey were all able to produce viable egg sacs after only several weeks.

Synanthropic spiders like *S. grossa*, which inhabit locations where prey may be scarce or unpredictable are clearly able to compensate by shifting metabolic resources towards maintenance under certain conditions. In many web-building spiders, the decision where to build the web is based on such factors as prey availability or regularity, mate attraction or optimal microclimate (Adams, 2000; Harwood & Obrycki, 2007; Lubin et al., 1993; Mcnett & Rypstra, 2000; Riechert & Tracy, 1975; Schuck-Paim & Alonso, 2001). It would be interesting to determine which of these factors plays the most important role in selecting a permanent site to build a web by *S. grossa* and other unrelated synanthropic spiders, and to compare how variable prey availability affects demographic traits in these species.

Supplementary figures and tables

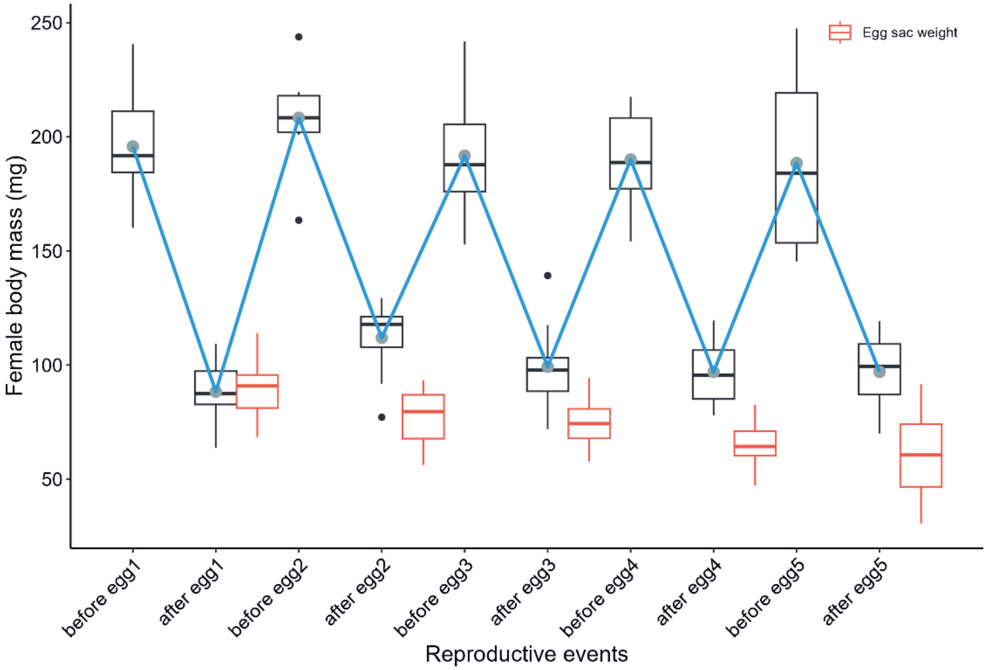

Figure S1. Temporal changes in the mean body mass of females before and after laying egg sacs over the first five consecutive reproductive events. Females regained body mass after each reproductive event by feeding on crickets and lose mass by producing an egg sac. Black lines inside boxes represent the median, and the boxes show the interquartile range (IQR). Grey dots inside the box represents mean and blue lines are connecting means from each events. The whiskers extend from the minimum and maximum values, and data points beyond the whiskers are outliners.The red boxes represents egg sac mass from each reproductive events.

Table S1. Pairwise comparisons of the total number of spiderlings across different feeding treatments.

contrast	estimate	SE	df	t ratio	p value
240 mg - 120 mg	25.2	47.1	34	0.54	0.98
240 mg - 60 mg	47.1	47.1	34	1.00	0.85
240 mg - 30 mg	156.0	50.8	34	3.07	0.03
240 mg - 5 mg	273.8	45.7	34	5.99	<0.01
120 mg - 60 mg	21.9	47.1	34	0.47	0.99
120 mg - 30 mg	130.7	50.8	34	2.57	0.10
120 mg - 5 mg	248.5	45.7	34	5.43	<0.01
60 mg - 30 mg	108.8	50.8	34	2.14	0.23
60 mg - 5 mg	226.7	45.7	34	4.96	<0.01
30 mg - 5 mg	117.8	49.6	34	2.38	0.15

Table S2. Pairwise comparisons of the mean number of spiderlings across different feeding treatments.

contrast	estimate	SE	df	t ratio	p value
240 mg - 120 mg	2.27	17.2	34	0.13	1.00
240 mg - 60 mg	6.62	17.2	34	0.39	1.00
240 mg - 30 mg	27.42	18.6	34	1.48	0.58
240 mg - 5 mg	67.47	16.7	34	4.04	<0.01
120 mg - 60 mg	4.35	17.2	34	0.25	1.00
120 mg - 30 mg	25.15	18.6	34	1.35	0.66
120 mg - 5 mg	65.20	16.7	34	3.90	<0.01
60 mg - 30 mg	20.79	18.6	34	1.12	0.80
60 mg - 5 mg	60.85	16.7	34	3.64	0.01
30 mg - 5 mg	40.06	18.1	34	2.21	0.20

Table S3. Pairwise comparisons of mean weight of spiderlings across different feeding treatments.

contrast	estimate	SE	df	t ratio	p value
240 mg - 120 mg	0.00	0.02	33	-0.04	1.00
240 mg - 60 mg	0.01	0.02	33	0.54	0.98
240 mg - 30 mg	0.03	0.02	33	1.57	0.53
240 mg - 5 mg	0.05	0.02	33	2.75	0.07
120 mg - 60 mg	0.01	0.02	33	0.58	0.98
120 mg - 30 mg	0.03	0.02	33	1.60	0.51
120 mg - 5 mg	0.05	0.02	33	2.78	0.06
60 mg - 30 mg	0.02	0.02	33	1.11	0.80
60 mg - 5 mg	0.04	0.02	33	2.30	0.17
30 mg - 5 mg	0.02	0.02	33	1.03	0.84

Author Contributions

J.A.H., R.G., and Y.D. conceived of the study. J.A.H. and Y.D. performed the experiments and collected data. Y.D. and R.G. analyzed data. Y.D and J.A.H. wrote the manuscript with suggestions from R.G. J.A.H and R.G. reviewed and edited the manuscript, and all authors approved the final version of the manuscript.

Data Availability

All data including supplementary excel files are deposited in Dryad and will be published once the paper is accepted for publication. Meanwhile, all data are available upon request.

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Chapter 5

Extreme temperatures impact survival and short-term reproductive success in a false widow spider

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Romeyn Vooijs, Simon Pleiter, Joes ten Thij, and Melissah Rowe

This chapter is in preparation for submission

Abstract

Anthropogenic climate change and climate extremes pose a profound threat to biodiversity. In recent years, the duration, frequency, and intensity of heatwaves has increased across most temperate biomes. Ectothermic organisms are highly sensitive to ambient temperature, and exposure to extreme heat can induce a range of lethal and sublethal effects. Spiders are abundant and ubiquitous arthropod predators in most terrestrial ecosystems, and studying their responses to variable temperatures provides valuable insights into effects of extreme heat on this important group of organisms. Here, we exposed different age-cohorts of a false widow spider (*Steatoda grossa*) to varying temperature scenarios, including constant thermal stress conditions and simulated heatwaves, and investigated their impact on survival and reproduction. Under heatwave and constant heat conditions, egg sacs of *S. grossa* perished precociously, whereas survival was high at lower temperatures, indicating a temperature threshold for embryo survival. The growth trajectories of male and female spiderlings that exposed to thermal conditions after hatching were severely depressed, whereas those reared at lower temperatures grew exponentially and attained significantly larger adult mass. Finally, in adults, females exposed to heatwave conditions after mating showed a reduction in reproductive success; producing smaller first egg sacs and fewer spiderlings during the first reproductive bout. However, this effect was transient and overall lifetime reproductive success was not impacted by our single heatwave treatment. Our study shows that the effects of exposure to high temperatures are life stage-specific and that thermal stress more negatively affects the survival and development of eggs and younger spiderlings than adults, although with a short-term reduction in adult reproductive success. This study enhanced the understanding the thermal response of spiders under constant thermal stress and extreme heat events, providing insights of an important component of biodiversity which is largely overlooked under climate change.

Key words

Climate change, climate extremes, egg, heatwave, reproduction, temperature, thermal stress, *Steatoda grossa*,

Introduction

Among a range of anthropogenic threats to biodiversity, climate change is increasingly being seen as one of the most serious (Bellard et al., 2012; Malcolm et al., 2006). At present, global surface temperature has increased by ~1.5°C above the 1850-1900 baseline, and is on track to exceed 2 or even 3 degrees within the coming decades (IPCC, 2023). Climate change is characterized by both deterministic (long-term), and stochastic (short-term) phenomena. Long-term warming is characterized by a slow rise in mean surface temperature, whereas at shorter timescales (days, weeks) there has been an increase in the frequency, duration and intensity of climatic extremes, such as heatwaves (Christidis et al., 2015; Jones et al., 2015). Both facets of climate change generate different kinds of responses in organisms. Long-term incipient warming has led to changes in seasonal phenology or increased numbers of generations per year (Forrest, 2016; Macgregor et al., 2019), as well as in distributional shifts pole-wards and to higher elevations (Chen et al., 2011; Kelly & Goulden, 2008). Climatic extremes, on the other hand, represent virtually instantaneous threats to the survival and fitness of organisms and require organisms to endure or 'ride out' harsh conditions; for example, by seeking cooler microclimates or suspending development via aestivation (Gols et al., 2021; Harvey et al., 2020; Kiritani, 2013). Given that the range of temperatures that organisms can physiologically tolerate plays a critical role in their distributions across space and time (Chown et al., 2004; Sunday et al., 2012), increasing temperatures and extreme heatwave events pose a significant threat to the persistence of natural populations.

Ectothermic organisms, such as insects and other arthropods, are highly sensitive to ambient temperature, and exposure to high temperatures can induce a range of physiological effects. For instance, all species have upper and lower critical thermal limits (CTL) for survival, whereby if they are exposed to temperatures above these critical thresholds for even short periods of time they will perish (Abarca et al., 2024; Hoffmann et al., 2013; Kingsolver & Buckley, 2020). The upper CTL for many organisms is species-specific and depends on such factors as geographic distribution and (micro) habitat occupancy. In many insects, however, this CTL lies between 41-50°C (Heinrich, 1981). Species restricted to warmer climates may have lower thermal tolerances than temperate species due to much lower seasonal and diel temperature variability in these tropical biomes (i.e., the climate variability hypothesis, Dewenter et al., 2024; Gutiérrez-Pesquera et al., 2016), although this depends on factors such as seasonal activity (Johansson et al., 2020). Moreover, early life-stages, such as eggs or juveniles, are often more susceptible to high temperatures than sub-adult and adult stages, as was shown in metamorphic animals (Barnes et al., 2019; Klockmann et al., 2017).

To date, the majority of thermal tolerance studies have focused on how extreme heat impact survival. However, this ignores potentially critical sublethal impacts of extreme temperature that may play an important role in determining species' persistence. For example, exposure to high temperatures can induce sub-lethal effects that reduce fitness. These sub-lethal effects include impacts on mating behaviours, such as courtship and mating stamina, that can reduce mating success (Bessa-Gomes et al., 2003; Carey, 2014; Iossa et al., 2019; Pilakouta & Baillet, 2022). Additionally, in many taxa, individuals exposed to extreme heat conditions survive, but are rendered sterile through the destruction of sperm or eggs inside the bodies of males and females (Janowitz & Fischer, 2011; Sales et al., 2021; Vasudeva et al., 2014; Walsh et al., 2021, 2022). Furthermore, recent studies show that thermal limits to fertility (i.e., critical thermal fertility limits [TFL]) are important and TFL predict many species' distributions better than CTL (Parratt et al., 2021; van Heerwaarden & Sgrò, 2021; Walsh, et al., 2019b). Yet other studies have reported that the growth and development of insects is negatively affected by exposure to heat (Colinet et al., 2015; González-Tokman et al., 2020; Neven, 2000), though these effects can be impacted by several factors, such as the age or life-stage of the insect exposed to heat and the duration of exposure. For example, Zhang et al. (2015) found that temporal variation in exposure to 'hot' days (over 30^{o}C) in the diamondback moth, *Plutella xylostella*, negatively affected adult lifespan, fecundity, and oviposition patterns, but only in some (and not all) stages of the life cycle. These studies highlight a high level of context- and trait-dependency of insect responses to heat.

The effects of thermal stress on reproduction are largely influenced by the timing of the event, especially when thermal exposure is not consistent but rather acute and short (e.g., a heatwave, Leith et al., 2021). Heat exposure during later developmental stages were found to have more negative effects on reproduction than early stages (Ma et al., 2004; Zhang, et al., 2015). As far as we know, only one study has shown that exposing animals, i.e., (burying beetles *Nicrophorus vespilloides*, to a heatwave conditions before, during and after mating generated different reproductive outcomes. Individuals that were exposed to high temperatures during mating experienced significantly lower fecundity than individuals exposed to a heat before or after mating (Pilakouta et al., 2023). Moreover, the transient nature of heatwaves is especially interesting because it also allows animals to recover after it passes. For example, sperm viability in male flour beetles (*Tribolium castaneum*) that experienced five days of exposure to 42°C (extreme heat) was functionally restored after several days of being returned to cooler conditions (Sales et al., 2021).

In this study we investigated the impact of high temperatures on the growth, development, and reproduction in a false widow spider (*Steatoda grossa*). Specifically, we exposed our study system to (1) thermal stress (i.e., high ambient heat conditions), and (2) experimental heatwaves (i.e., an extreme heat event). We investigated three main areas: (i) the effects of thermal exposure on early life stages of spiders (eggs in egg sacs and spiderlings), (ii) the effects of timing of a heatwave before or after mating on female reproduction, (iii) the effects of a heatwave on female reproduction when males or females or both are exposed to heatwave conditions. *S. grossa* is a synanthropic species that likely originated in Eurasia and is now widely distributed around the world. They are commonly found around human residences in urban and rural areas. Here, we compared the survival and development of eggs and spiderlings exposed to temperatures reflecting average, warm, and hot days, as well as heatwave conditions in the Netherlands. Our main hypothesis is that exposure to heat negatively affects survival, growth and reproduction in *S. grossa*, and that these effects are more pronounced in eggs and spiderlings based from previous findings in other arthropods (Jia et al., 2020; Vorhees & Bradley, 2012).

Methods

Spider rearing and maintenance

Egg sacs, spiderlings and adults of *S. grossa* originated from a laboratory population maintained as part of a larger research program at the Netherlands Institute of Ecology (NIOO-KNAW). This population was established from wild-caught individuals collected in various parts of the Netherlands over two years and reared for several generations in the lab (see Dong et al. 2023 for details). The population was maintained under controlled conditions of 22 ± 2°C, 50 ± 5% RH, and a 16 L:8 D photoperiod. Egg sacs laid by females were placed in Petri dishes (8 cm diam.) until hatching, after which spiderlings were placed individually in Petri dishes and fed fruit flies (*Drosophila sp.*). At adulthood, males remained in these same Petri dishes and were fed 2-4 fruit flies (*Drosophila sp.*) every 2-3 weeks, while females were transferred to individual plastic boxes (11 x 11 x 6 cm) fitted with 2 crossed sticks to facilitate web-building and fed individual nymph house crickets (*Acheta domesticus*) weekly thereafter. Sexes of this species can only be distinguished once they undergo several molting stages (c. 5-7 stages in males, 8-9 stages in females) and become sexually dimorphic. Females have a larger abdomen, while males are overall smaller and develop visible pedipalps.

Temperature treatments

Two types of thermal exposure treatments were applied to *S. grossa*: (1) thermal stress (i.e., high ambient heat conditions), and (2) experimental heatwaves (i.e., an extreme heat event). Thermal stress at either 27°C or 32°Cwas applied for a variable number of days (depending upon the specific experiment, see below), creating 5°C and 10°C above the standard experimental population maintenance temperature of 22°C (i.e., control temperature). Nighttime temperatures were set 10°C lower in all groups (22/12, 27/17, and 32/22°C, day/night temperatures) to replicate more natural nighttime conditions and temperature regimes followed the 16 L:8 D photoperiod.

Experimental heatwaves were designed to reflect conditions ecologically relevant to the local source population using information from the Royal Dutch Meteorological Institute (KNMI). The KNMI defines a heatwave as a period of at least five consecutive days with a maximum temperature of 25°C or higher, of which at least three days have a maximum temperature of 30°C or higher (KNMI, 2023). During the last 10 years, heatwaves in the Netherlands have lasted for a period of 6-13 days (mean ± SD: 8.86 ± 3.19), of which between three and nine days exceed 30°C (mean ± SD: 4.57 ± 2.07). In July of 2019, the Netherlands also recorded its first ever recorded day of over 40°C (KNMI, 2023). Here, we created a simulated heatwave lasting 8 days, including 3 days with a daytime temperature of 27°C followed by 5 days with a daytime temperature of 32°C (nighttime temperatures were 10°C lower). Outside of these periods, temperatures were set at 22°C daytime temperature/ 12°C nighttime temperature (16L:8D).

All experimental temperature treatments were applied using incubators from Sanyo, model MLR-350 and Panasonic model MIR-154.

Temperature impacts on egg sacs of Steatoda grossa

Thirty virgin female spiders from the general population were mated and their egg sacs used to examine the impact of thermal stress on spider eggs (Fig 1A). From each female, we collected the first 1-4 egg sacs (within 16 hours after laying) and placed these individually in a Petri dish. Egg sacs were randomly assigned to the control temperature (22°C, n = 21), one of the two thermal stress treatments (27°C, n = 21; and 32°C, n = 19), or the standard experimental heatwave treatment after a 3-day period at control temps (n = 20) (Fig 1A). For this experiment, we exposed egg sacs to the thermal stress treatments continuously until the eggs either hatched or died. Eggs were identified as dead based on their colour and appearance; dead eggs appear shrunken and are dull yellow in colour, whereas healthy eggs are white and round (Fig S1). After hatching, spiderlings were frozen at -20°C and body mass measured (± 1 µg) using a Mettler Toledo Microbalance MT5 (Columbus, OH, U.S.A.). To assess the impact of heat exposure on spider eggs, we quantified latency to hatch (number of days until hatching), the average body mass of spiderlings (total mass of the spiderlings / number of spiderlings), and hatching success (number of spiderlings hatched / total number of eggs laid) for each egg sac and under each of the temperature conditions (22°C, 27°C, 32°C, and the simulated heatwave).

During these experiments, we observed complete mortality of eggs exposed to constant 32°Cconditions (i.e., 100% of eggs failed to hatch). In contrast, eggs exposed to 32°Cfor five days as part of the simulated heatwave successfully hatched (albeit at lower rates than control groups, see results below). We therefore performed two additional heat exposure experiments on egg sacs (using only the first egg sac laid by virgin females) and recorded hatching success under the following conditions: (1) 5 days at 32°Cand then 22°C until hatching (n = 15), and (2) 3 days at 22°C, followed by 5 days 32°C, and finally back to 22°C (n = 16). By qualitatively comparing the results of these additional experiments to those found in our main experiments, we aimed to understand how both the timing of exposure and prior exposure to high, but not extreme, temperatures (i.e., priming) might shape our findings.

Temperature impacts on spiderling growth and survival

Thirty virgin female spiders from the general population were mated to produce spiderlings for this experiment (Fig 1B). From these females, the first egg sacs were collected within 16 hours of laying and placed individually in Petri dishes until hatching. After hatching, 25 spiderlings were randomly selected from each egg sac, placed individually in Petri dishes with a damp cotton ball, and then randomly assigned to one of the temperature treatments: control (22°C, n = 150), one of the two thermal stress treatments (27°C, n = 150; and 32°C, n = 150), or the standard experimental heatwave treatment (1×SHW; n = 80); for spiderlings exposed to the heatwave they were first maintained at control temperatures for 10 days post-hatch and then exposed to heatwave conditions (Fig 2B). In addition, we exposed spiderlings to three additional temperature treatments, including (i) 2x the standard experimental heatwave treatment (2×SHW; n = 80) with a 10-day period between heatwave events, (ii) an extended heatwave (1×LHW; n= 70, exposed at Day 10) consisting of 10 days with temperatures of 27°C/17°C (day/night temperatures), followed by 10 days with temperature of 32°C/22°C (day/night temperatures), and (iii) 2x the extended heatwave (2×LHW; n = 70, exposed at Day 10) with a 10-day period between heatwave events.

Throughout the experiment, spiderlings were fed two frozen fruit flies (*D. hydei*) once a week for the first 6 weeks, then four live fruit flies from weeks 7 to 12, and six live fruit flies from week 13-21. From week 21 onwards, spiders were provided with one late-instar cricket nymph weekly. Female spiders were maintained under these conditions for a maximum period of 17 months, whereas males, because they develop more rapidly, were monitored until maturity (typically 6-8 months). All spiders were checked every three weeks, weighed (if alive), and any mortality recorded. Spiders were weighed under anesthetic using CO2 and body mass measured (± 1 μg) using a Mettler Toledo Microbalance MT5 (Columbus, OH, U.S.A.).

Temperature impacts on adult reproductive success

Adult virgin males and females from the general population were used to test the effects of experimental heatwaves on adult survival and reproduction (Fig 1C). We used five different experimental pairings: (1) females exposed to a simulated heatwave and subsequently mated with a control (22°C) male (n = 13); (2) males exposed to a simulated heatwave and subsequently mated to a control (22°C) female (n = 11); (3) females mated to control (22°C) males and subsequently exposed to a simulated heatwave (n = 12); (4) males and females exposed to a simulated heatwave and subsequently mated (n = 11); and (5) control males and females mated and maintained at 22°C (n = 10). For all pairings, mating took place in the containers with females at 22°C and males were removed after a full copulation was naturally terminated. Mating success (yes or no) and copulation duration (min) was recorded for each mating trial. Following mating trials, individuals were monitored across their lifetime and any mortality recorded.

Following mating, we collected all egg sacs produced by the female until her death or until she stopped producing egg sacs for a minimum of 6 months. From these, we quantified reproductive success as: (1) the total number of viable egg sacs produced by each female, (2) egg sac mass (for each egg sac produced), (3) total egg sac mass (i.e., sum of all egg sacs produced over the female's lifetime), (4) number of spiderlings (for each egg sac produced), (5) total number of spiderlings (i.e., sum of all spiderlings produced over the female's lifetime), and (6) mean body mass of spiderlings (for each egg sac produced). From these measures, we used (1), (3), and (5) to represent lifetime reproductive success of females (as females were monitored until their death or until no egg sacs were produced for a minimum of six months).

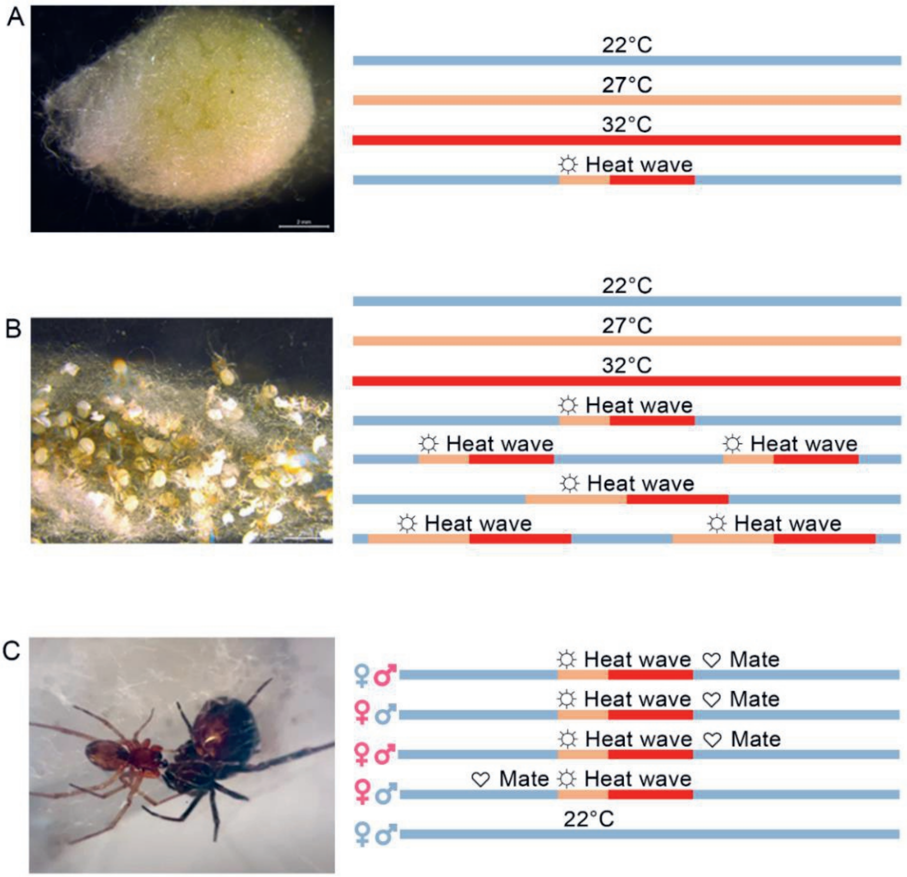

Figure 1. Overview of the temperature treatments used in the study. Two types of thermal exposure were applied: constant thermal stress and experimental heatwaves. Thermal exposure was applied across life stages, including (A) eggs (thermal stress and heatwaves), (B) spiderlings (thermal stress and heatwaves), and (C) adults (heatwaves only). Schematic shows different periods of heat in the different temperature treatments. Blue indicates control temperatures (i.e., 22°C), orange lines indicate 27°C, and red lines indicate 32°C. Across all life stages, animals were exposed to a standard heatwave (i.e., 3 days at 27°C and 5 days at 32°C), while in spiderlings individuals were also exposed to extended heatwaves (i.e., 10 days at 27°C and 10 days at 32°C), as well as repeated standard and extended heatwaves both with 10 days interval between heatwaves. All temperatures shown represent daytime temperature. Nighttime temperatures were set 10°C lower.

Statistical Analysis

All analyses were conducted in R 4.3.1 (R Core Team, 2023),. All linear mixed models were implemented using the R package "lme4" package (Bates et al., 2015) and, where necessary, post hoc contrasts were performed to test for difference among groups using the R package "emmeans" with Tukey HSD p-value adjustment (Lenth et al., 2024). For all models, the interaction term was removed from the final model when it was non-significant (Zuur et al., 2009) and model assumptions were checked with the R package DHARMa (Hartig & Lohse, 2022). All tests were two-tailed and considered significant at $P < 0.05$ and, unless otherwise stated, means are shown ± SD.

Temperature impacts on egg sacs of Steatoda grossa

We used linear mixed models to assess the impact of thermal stress on latency to hatch and average body mass of spiderlings. In these models, temperature treatment (22, 27, 32, and HW) and egg sac number (i.e., first, second, third, or fourth egg sac), as well as their interaction, were included as fixed factors, and female ID was included as a random factor to account for the non-independence of egg sacs (i.e., multiple egg sacs from each female); egg sacs exposed to 32°Cconditions were excluded from the analysis of time to hatching due to complete hatching failure. Next, for hatching success, a logit transformation was applied to the proportion of eggs that hatched. Logit-hatching success was modelled using a GLMM using the "glmmTMB" package (Brooks et al., 2017). Temperature treatment (22, 27, 32, and HW) and egg sac number, as well as their interaction, were included as fixed factors and female ID as a random factor and testing of fixed effects was determined with Type III Wald $\chi 2$ tests in the package "car" (Fox & Weisberg, 2019).

Temperature impacts on spiderling growth and survival

We used body mass data to plot growth trajectories of male and female S. grossa spiderlings over time and compared these trajectories under the different temperature treatments. Growth trajectory curves were fitted by using "ggplot2::geom_smooth" function in package "ggplot2", which is a LOESS method (Wickham, 2016). In addition, we compared the growth rate of spiders (males and females separately) across the different temperature treatments using an ANOVA and post-hoc Tukey HSD pairwise comparisons. Growth rate was estimated as the change in body mass over time. For males, this reflected the change in body mass from neonate hatching until their body mass reached the maximum divided by the time taken to reach maturity (i.e., (maximum body mass - hatching body mass)/ the number of days between hatching and the date when males reached maximum body mass). For females, growth rate reflected the change in body mass from neonate hatching until day 336 divided by time (i.e., (body mass on day 336 - hatching body mass)/336).

Next, we used maturation and death/survival events of spiders to fit Kaplan Meier curves using the R packages "survival" (Therneau et al., 2024) and "survminer" (Kassambara et al., 2021). For males, survivals were based on duration until males reached sexual maturity, while for females, survival was estimated until the termination of the experiment (i.e., at aprox.17 months). We analyzed thermal stress survival (i.e., survival under high ambient temperature regimes) heatwave survival (i.e., survival under the heatwave scenarios) separately, though included the control temperature treatment (22°C) in both analyses. For both analyses, differences among temperature treatments were tested using Log-Rank pairwise comparisons with Bonferroni corrections.

Temperature impacts on adult reproductive success

Logistic regression analysis was used to assess the effects of temperature on mating success (modelled as a binomial response, yes/no) with temperature treatment as the single fixed factor. Next, we used a linear model to assess the impact of temperature on copulation duration, with temperature treatment included as the single fixed factor. We then used linear models to test the effects of heatwave exposure on lifetime reproductive success. In these models, the total number of viable egg sacs produced, the total mass of egg sacs, and the total number of spiderlings produced through a female's lifetime were included as response variables, and temperature treatment as the fixed factor running separate models for each response variable. Finally, we also tested for immediate and short-term impacts of temperature extremes on reproductive output, along with the potential for recovery from heatwave exposure. Such a scenario (i.e., immediate impacts with recovery) are suggested from the literature (Sales et al., 2021), and here we tested for such effects by comparing data from the first and second egg sac produced by each female. More specifically, we used linear mixed models to examine the effect of heatwaves on egg sac mass, number of spiderlings produced, and mean body mass of spiderlings. For the three models (per response variable), we included temperature treatment and egg sac number (egg sac 1 and 2), as well as their interaction, as fixed factors and female ID as a random factor.

Results

Temperature impacts on egg sacs of S. grossa

Temperature had a significant effect on the latency to hatch of spiderlings ($F_{2,46} = 28.85$, $P < 0.001$, Fig 2A). Spiderlings from egg sacs exposed to 27°C hatched significantly faster than those exposed to either the control temperature (c. 15 days earlier, $t_{44.5} = 6.15$, $P < 0.001$) or heatwave conditions (c. 18 days earlier, $t_{46.6} = 6.83$, $P < 0.001$). In contrast, there was no difference in latency to hatch between the control and heatwave conditions ($t_{43.6} = -1.05$, $P = 0.55$). Finally, egg sac number had no effect on time to hatching ($F_{3,46} = 0.17$, $P = 0.91$).

The mean body mass of spiderlings was not influenced by temperature ($F_{2,39}$ = 2.00, P = 0.15), but was significantly influenced by egg sac number ($F_{3,30}$ = 3.23, P = 0.04). More specifically, the mean body mass of spiderlings from the first egg sac (0.31 ± 0.05 mg) was significantly lower than body mass of spiderlings from the second egg sac (0.35 ± 0.05 mg; t_{35} = 2.79, P = 0.04). All other comparisons were not significant (Table S1).

Temperature had a significant impact on hatching success of egg sacs (X^2 = 84.67, df = 3, P < 0.001, Fig 2B). Hatching success was highest when egg sacs were reared at 22°C (i.e., control temperatures). Relative to control temperatures, hatching success was reduced by almost 50% when egg sacs were exposed to simulated heatwave conditions (t_{72} = 3.93, P < 0.001) and by 100% (i.e., all eggs perished) when egg sacs were exposed to constant thermal stress of 32°C(t_{72} = 8.89, P < 0.001). In contrast, egg sac number did not influence hatching success (X^2 = 7.79, df = 3, P = 0.051).

Following from these results, we performed two additional exposure experiments to gain insight into the potential effects of timing of exposure and priming. When egg sacs were immediately exposed to 32°Cfor a 5-day period, all eggs in 15/15 egg sacs failed to hatch (i.e., 100% mortality). When egg sacs were first exposed to 22°C for 3 days and then 32°C for 5 days, only 10/16 egg sacs failed to hatch; although in those egg sacs that did hatch (6/16), hatching success was still extremely low (mean ± SD: 8.0% ± 5.0%, range: 2.4%-14.0%). In contrast, under simulated heatwave conditions (i.e., 3 days at 22°C, then 3 days at 27°C, and 5 days at 32°C) hatching success was moderate (mean ± SD: 37.7% ± 35.5%, range: 0.0%-95.7%, see results above and Fig 2B).

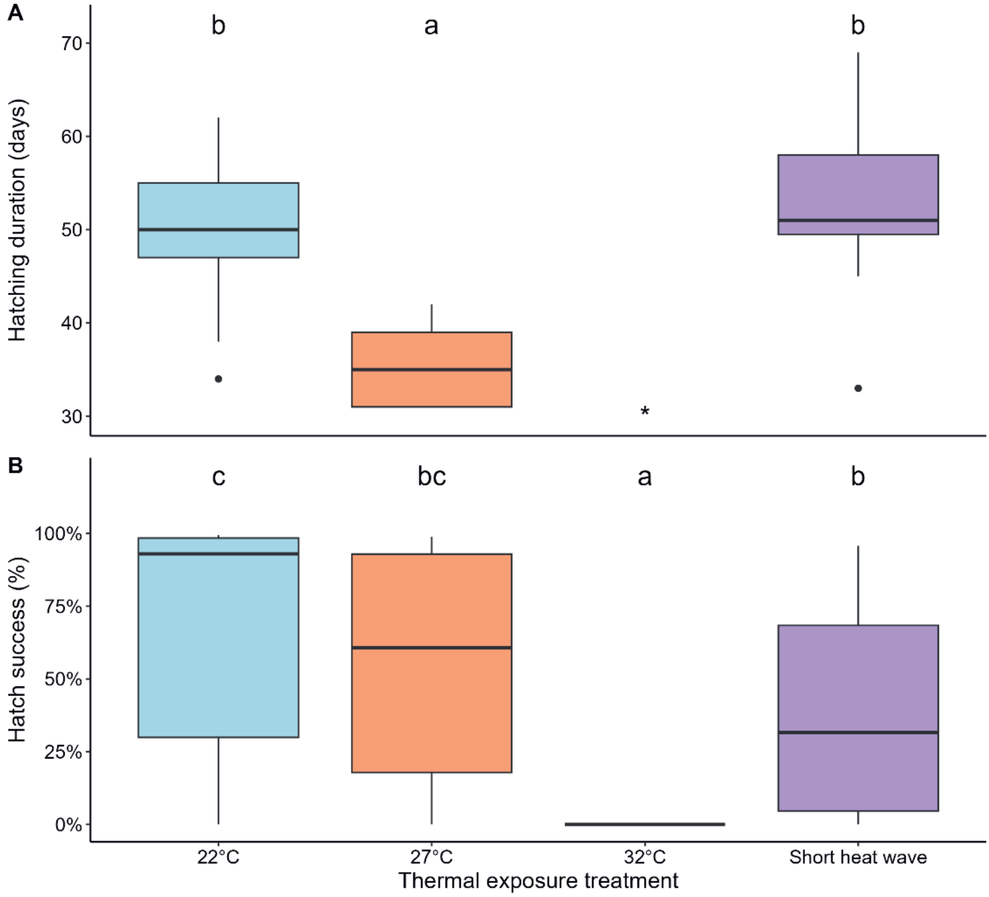

Figure 2. Impact of thermal exposure (22°C, 27°C, 32°C in daytime with 10°C lower in nighttime and a short heatwave condition) on egg sac (A) latency to hatch and (B) hatching success in *Steatoda grossa*. The asterisk at 32°C in (A) denotes that none of the spiderlings hatched and thus data excluded from this analysis. The horizontal line inside boxes represents the median, and the box shows the interquartile range (IQR). The whiskers extend from the minimum and maximum values, and outlines were the dots beyond the whiskers. Letters above boxes denote statistical differences ($P < 0.05$) between treatments. See text for full statistical results.

Temperature impacts on spiderling growth and survival

Newly hatched male and female spiderlings exhibited different growth trajectories when exposed to different temperature regimes (Fig 3). Under heatwave scenarios, females exhibit higher variation in growth rate than males (Fig 3A, B). Growth rate differed significantly among temperature treatments for both females ($F_{6,81} = 5.23$, $P < 0.001$) and males ($F_{6,146} = 6.57$, $P < 0.001$). More specifically, female growth rate showed some variations among the different heatwave scenarios (1×SHW-1×LHW, $P = 0.001$; 2×SHW-1×LHW, $P = 0.001$; 1×SHW-2×LHW, $P = 0.08$; 2×SHW-2×LHW, $P = 0.08$, see details in Table S2), but did not differ among the constant thermal stress conditions the high ambient temperatures treatment (all $P > 0.78$; Table S2). When female spiderlings were exposed to constant daytime temperatures of 32°C, growth rate was severely impacted, with females declining in body mass over time, whereas at both 27°C and the control (22°C) temperature, female growth trajectories were similar (Fig 3C). Conversely, male growth rate at 22°C was higher than at either 27°C or 32°C (22-27°C, $P < 0.001$; 22-32°C, $P = 0.004$; 27-32°C, $P = 0.6$); males held at high/extreme temperatures showed declines in body mass over time (i.e., males lost weigh with increasing duration of exposure to high/extreme temperatures). In contrast, male growth rates did not differ under the various heatwave scenarios (all $P > 0.38$; Table S2).

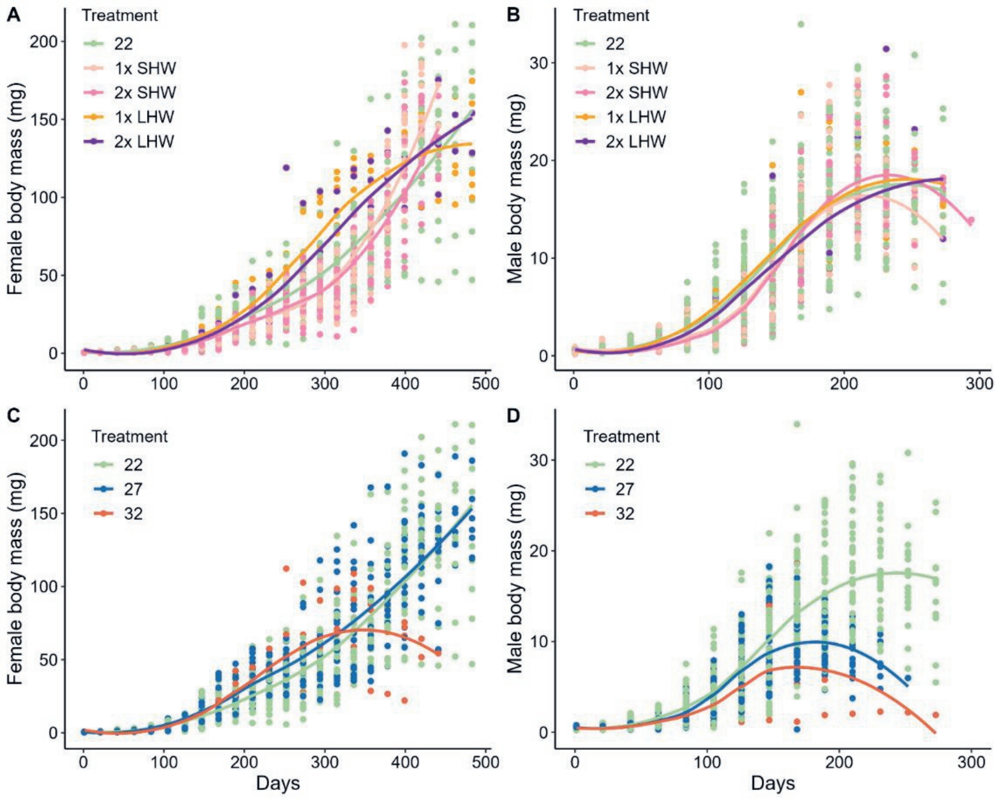

Figure 3. Growth trajectories of *Steatoda grossa* spiderlings under different temperature treatments. Growth of females (A) and males (B) under simulated heatwave scenarios (SHW = standard heatwave, LHW = the extended heatwave), and growth of females (C) and males (D) under different thermal stress scenarios (i.e., 22°C, 27°C, 32°C. Note: axes scales of the graphs differ for males and females.

Spiderling survival rate was impacted by temperature exposure (Fig 4); spiderling survival was lowest at 32°Cwith most individuals dying within the first 50 days (survival percentage for each treatment at day 50: 22°C, 81.2 %; 27°C, 54.67 %; 32°C, 12.26 %; 1×SHW, 52.5 %; 2×SHW, 52.5 %; LHW, 44.29 %; 2×LHW, 43.08 %). Interestingly, after 50 days, most deaths occurred during molting (personal observations). More specifically, spiderling survival was significantly influenced by heatwave conditions ($X^2 = 25.5$, $df = 4$, $P < 0.001$, Fig 4A), although this depended upon the duration of heatwaves. Spiderling survival rate did not differ between spiderlings exposed to control temperatures and those exposed to either the standard heatwave or repeated standard heatwave ($P = 0.58$ and 0.21 respectively). In contrast, exposure to a single (1×LHW) or repeated extended heatwave (2×LHW) significantly reduced spiderling survival probability compared to control temperatures (both $P < 0.001$). Notably, the frequency of heatwaves did not influence the survival ($P = 1.00$ for 1×SHW-2×SHW and 1×LHW-2×LHW, Fig 4A). Spiderling survival also differed significantly across different constant temperature treatment (i.e., 22, 27 and 32°C; $X^2 = 183$, $df = 2$, $P < 0.001$, and $P < 0.001$ for all pairwise comparisons, Fig 4B).

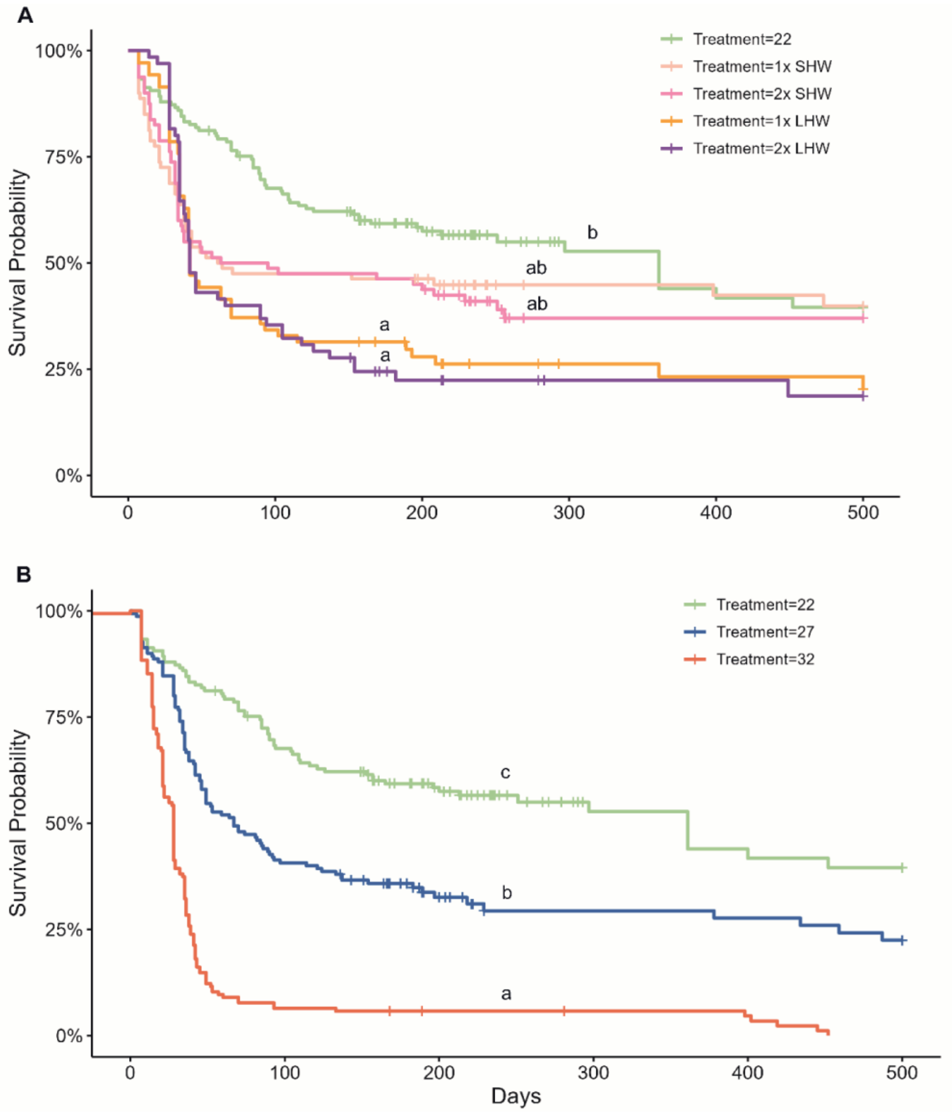

Figure 4. Survival probabilities of *Steatoda grossa* spiderlings exposed to (A) different simulated heatwave scenarios (22= 22°C, SHW = standard heatwave, LHW = the extended heatwave), or (B) different constant temperatures (22, 27 and 32 refer to 22°C, 27°C, and 32°C, all with 10°C lower in nighttime). For comparison, the 22°C survival curve is shown in both panels (note that temperatures are 10°C lower during the night). Letters above the lines denote statistical differences between treatments.

Heatwave impacts on adult reproductive success

Mating success was not influenced by exposure to heatwave conditions (*LR* $X^2 = 5.59$, $df = 4$, $P = 0.23$). In contrast, copulation duration was significantly influenced by exposure to heatwaves ($F_{4,47} = 3.57$, $P = 0.01$; Fig 5A), although these effects varied among the experimental pairings. Specifically, relative to the control group, copulation duration was significantly reduced when females were exposed to a simulated heatwave before mating ($t_{47} = 3.34$, $P = 0.01$) and when both sexes were exposed to a simulated heatwave before mating ($t_{47} = 3.13$, $P = 0.02$; Other comparisons were not significantly different, Table S3). However, despite the effects of heatwave exposure on copulation duration, we found no effect of exposure to experimental heatwaves on the lifetime reproductive success of females (total egg sac mass: $F_{4,47} = 0.44$, $P = 0.78$, Fig 5B; total number of viable egg sacs: $F_{4,47} = 0.83$, $P = 0.51$, Fig 5C; total number of spiderlings: $F_{4,47} = 0.71$, $P = 0.59$, Fig 5D). Finally, we observed high rates of female survival following heatwave exposure; 100% of females were alive 86 days after heatwave exposure, while 71% (41/58 females) were still alive > 300 days after mating.

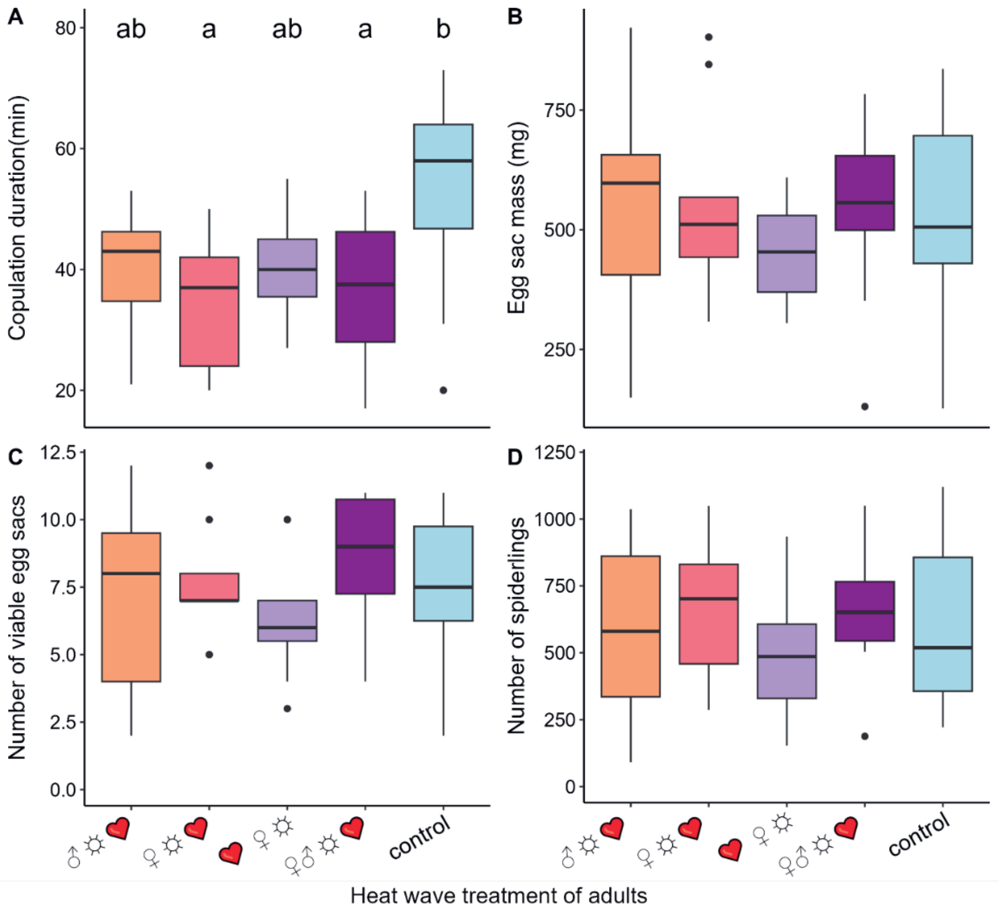

Figure 5. Impacts of simulated heatwaves on copulation duration and female lifetime reprodutive success of *S. grossa*. (A) copulation duration, (B) total egg sac mass, (C) total number of viable egg sacs produced, (D) total number of spiderlings. The hearts on X-axis denote mating and male/female symbols in front of the heart denote which sex was exposed to a heatwave before mating. Letters denote significant differences among groups.

We also studied short-term impacts of heatwave conditions on female reproductive success. A significant interaction between heatwave treatment and egg sac number had an effect on egg sac mass (X^2= 21.23, *df* = 4, *P* < 0.001, Fig 6A). Post-hoc testing showed that females exposed to a heatwave after mating (i.e., experimental pairing #3) produced a significantly lighter first egg sac than the females in other treatment groups (*t* range: 3.40-3.60, *df* = 73.2, *P* range: 0.005-0.01; Table S4), and tended to be lighter comparing to treatment that both female and male exposed to a heatwave ($t_{73.2}$= 2.53, *P* = 0.096), but that this difference was not observed for the second egg sac (*t* range: 0.33-1.15, *df* = 73.2, *P* range: 0.78-1; Table S4). Furthermore, in these same females (i.e., those exposed to heatwaves after mating), we found that the first egg sac tended to be smaller than the second egg sac (t_{47}= 2.00, *P* = 0.052; Table S5), whereas in all other groups, the first egg sac is significantly heavier than the second egg sac (*t* range: 2.09-3.43, *df* = 47, *P* range: 0.001-0.04; Table S5).

In addition, we found a significant effect from the interaction between heatwave treatment and egg sac number on number of spiderlings (X^2 =16.42, *df* =4, *P* = 0.003, Fig 6B). Females exposed to a heatwave after mating (i.e., experimental pairing #3) produced significantly fewer spiderlings in their first egg sac compared to all other treatment groups (*t* range: 2.45-4.41, *df* = 92.2, *P* range: <0.001-0.11; Table S6), and again tended to have fewer spiderlings compared to treatment that both female and male exposed to a heatwave ($t_{92.2}$ = 2.45, *P* = 0.11), whereas there was no difference among experimental pairings in terms of mass of the second egg sac (*t* range: 0.01-0.65, *P* range: 0.97-1; Table S6). Furthermore, in females exposed to heatwave conditions after mating, the number of spiderlings produced in an egg sac was significantly fewer spiderlings in the first compared to the second egg sac (t_{47} = 2.81, *P* = 0.01), whereas there was no difference between the number of spiderlings produced between first and second egg sacs in other treatment (*t* range: 0.5-1.62, *P* range: 0.11-0.62; Table S7), except in the control treatment. In this treatment, where neither parent was exposed to a simulated heatwave, the first egg sac contained more spiderlings than the second (t_{47} = 2.41, *P* = 0.02). In contrast, we found no effect of either heatwave scenario (X^2 = 6.65, *df* = 4, *P* = 0.16) or egg sac number (X^2= 3.16, *df* =1, *P* = 0.08) on the mean body mass of spiderlings at hatching.

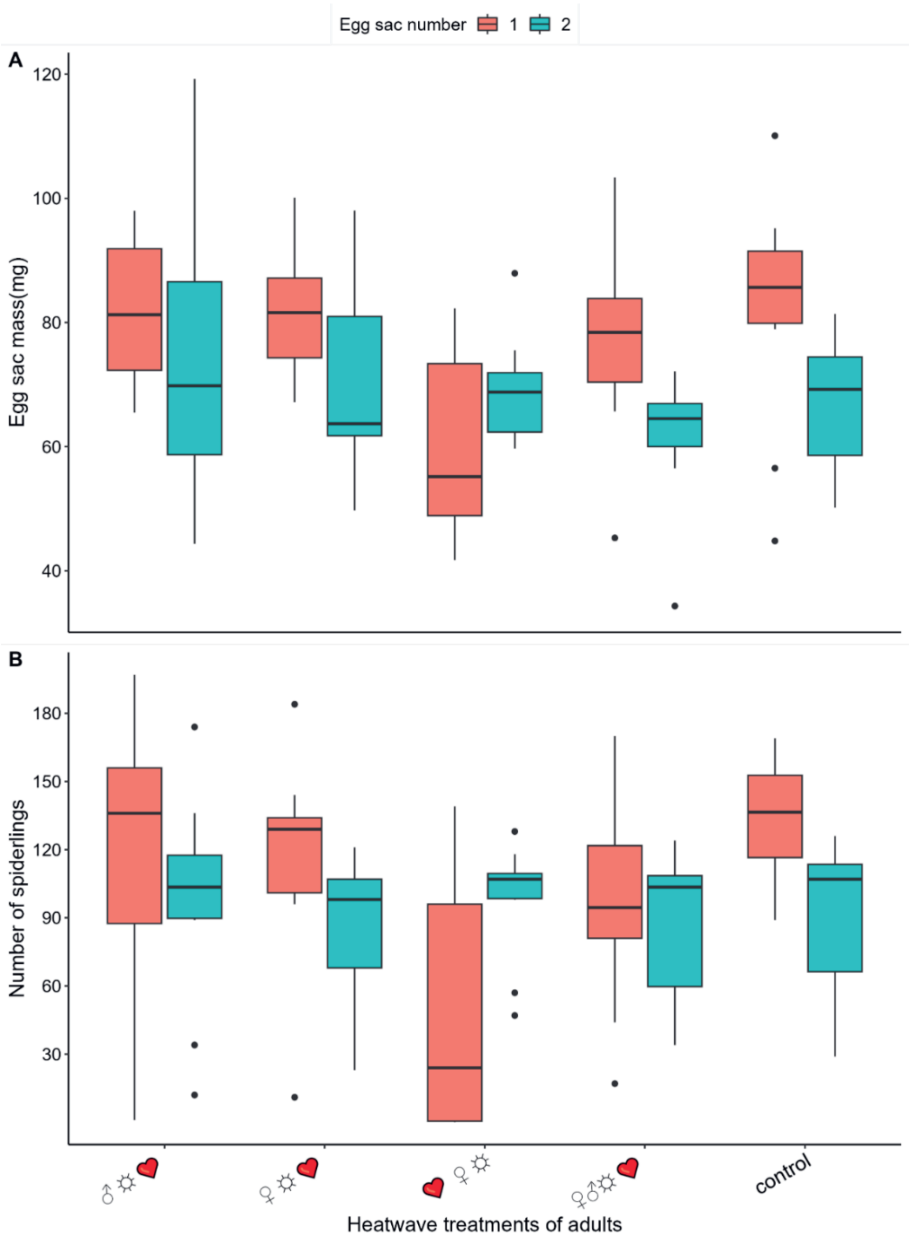

Figure 6. Comparison between the first egg sac (in red) and the second egg sac (in blue) of *S. grossa* females in (A) mean egg sac mass; (B) mean number of spiderlings. The heart symbols on X-axis denote mating and male/female symbols in front of the heart denote which sex was exposed to a heatwave before mating.

Discussion

The results of this study reveal that exposure to extreme temperatures, including both constant thermal stress and simulated heatwaves, strongly impact spider development, growth, and reproduction, although impacts were variable across life stages and, in some cases, depended upon the type of exposure (i.e., constant thermal stress versus heatwave conditions), duration of exposure, and exposure timing. For egg sacs, we found that all egg sacs maintained at under constant thermal stress conditions of 32°C failed to hatch, whereas hatching success increased dramatically at a slightly lower temperature (27°C). Embryonic survival was highest at 22°C, with almost all egg sacs successfully hatching, although spiderlings took longer to hatch at this temperature than at 27°C. Importantly, this finding reveals that the critical thermal limit (CTL) for embryonic development in *S. grossa* is > 27°C and ≤ 32°C. Furthermore, hatching success was significantly impacted by our experimental heatwave conditions (almost 70% eggs failed to develop), demonstrating that exposure to just a single heatwave event during the egg sac stage can have significant consequences for spider reproduction and survival. Indeed, we found that even a short (5-day) exposure to high temperatures immediately after an egg sac is laid results in 100% mortality of eggs, while an exposure delay of just 3-days (i.e., 3 days at control temperatures prior to exposure to 32°C) reduces mortality. Moreover, when eggs are exposed to extreme temperatures later in development (at day 6) and temperatures ramp up over time (i.e., our simulated heatwave conditions), hatching success increases dramatically. Given this, we suggest that both the timing of exposure and priming effects play an important role in determining the extent of the negative impacts of extreme heat exposure.

Most female spiders deposit their eggs in silk ‘cocoons’ (Turnbull, 1973) that protect them from predators (Hieber, 1992a) as well as desiccation (Chiavazzo et al., 2015; Ewunkem & Agee, 2022; Hieber, 1992b). Dead eggs of *S. grossa* at 32°C were visibly ‘shrunken’, suggesting that the silken egg sac is only able to offer protection against up to a point, beyond which egg mortality is complete. Physiological responses to thermal stress include the rapid expression of heat shock proteins (HSP) (Feder & Hofmann, 1999). HSP enhances the resilience of organisms, facilitating some level of thermal tolerance in spiders and other arthropods (King & MacRae, 2015; Xiao et al., 2016), and may underlie the priming effects we observed here. However, our results also suggest that HSP levels or their production in spider embryos may be significantly more limiting than in later stages (i.e., within several days) r. Early embryonic production of HSP70 has been reported in insects (Graziosi et al., 1980; Kalosaka et al., 2009; Welte et al., 1993), which showed that HSP existed in 6 hours old embryos. Another potential mechanism to minimize the impact of extreme heat on embryonic development is habitat selection. Specifically, females may benefit from selecting cooler microclimates for web building in order to optimize the success of embryonic development. In *S. grossa*, eggs sacs are attached to the female's web, which is frequently placed in locations such as under rocks, logs, sheds and in human-constructed structures (Levy & Amitai, 1982), potentially minimizing their exposure to high temperatures. However, heatwaves can potentially transform a cool shelter to an "oven", which may make the selection of suitable web-building locations unpredictable. Regardless, our findings clearly demonstrate a negative effect of extreme heat on the eggs of *S. grossa*, with consequences for the reproductive success of adults.

Spiderling growth was impacted by extreme heat conditions, although these effects differed between the sexes and upon both the type of exposure (constant thermal stress vs. heatwaves) and duration of heat exposure. Notably, although males and females had similar body masses at hatching, exposure to heatwaves of varying duration affected growth rates of females more strongly than males. Not surprisingly, the impact of constant exposure to extreme temperatures was more severe. Growth of female spiderlings was severely depressed under constant 32°C with individuals losing body mass over time such that body mass at maturity was considerably lower than that of females held under control and warm conditions. In contrast, male growth trajectories, were depressed at both 27°C and 32°C, suggesting that they are more sensitive to high temperatures than females. The mechanism behind the different responses in two sexes and different types of heat exposure is intricate and remains largely unknown. Since body size are similar at the early stage of development, I speculate the differences showed up when body size difference in sex started to be apparent. The different growth curves in females and males suggests an adaptive growing strategy under high temperature pressure. Females aim to acquire a large body size to the largest capability, while males appear to tradeoff body size with a faster maturation.

Spiderling survival was also negatively impacted by high temperatures, which is consistent with findings in other spider species (e.g., western black widow spider, *Latrodectus hesperus*, Johnson et al., 2019). Under heatwave conditions, we found that spiderling survival was only impacted when heatwaves continued for extended periods, suggesting that juvenile spiders face more challenges when extreme conditions have a longer duration, which is highly likely to occur in future climate change scenario. Interestingly, regardless of the duration of heatwaves, spiderlings exposed to repeated heatwaves did not suffer higher mortality than when exposed to a single heatwave. One explanation for this finding, is that spiderlings gain thermal tolerance during the initial heatwave exposure, possibly due to the upregulation of HSPs. An alternative, but not necessarily mutually exclusive explanation for our finding, is that the first heatwave exposure selects for individuals with high thermal tolerance and thus individuals still alive at the time of the second exposure are simply more resistant to thermal stress. In the western black widow spider, high temperature dramatically reduces ecdysteroid concentrations before molting, delaying molting and increasing mortality (Moen et al., 2022). Ecdysteroids are key hormones that regulate molting, metamorphosis and preproduction by arthropods (Chang, 1993; Krishnakumaran & Schneiderman, 1970). Changes in growth rate and high mortality under thermal exposure could be caused by an imbalance of growth hormone production, such as ecdysteroids. Ecdysteroids are correlated with inductions of HSP, as has been shown in lobsters and fruit flies (Chang et al., 1999; Thomas & Lengyel, 1986).

In adults, we found that heatwave exposure influenced mating behaviour. Specifically, in two of our experimental pairings we observed significant reductions in copulation duration. In both cases, the heatwave exposure involved exposing females prior to mating, suggesting the reduction in copulation duration may be a female-driven process. Sex differences in responses to thermal adaption have been discussed, but differences in mating behaviours and the mechanisms underlying the differential responses of the sexes remains rarely studied (but see Liao et al., 2014; Zhang et al., 2016). In *S. grossa*, we suggest body size difference may help explain our findings. Specifically, in this species, body mass of males is approx. 7 times lower than that of females (Harvey, 2022). Climate change and global warming is driving many organisms smaller, especially ectotherms (Horne et al., 2015; Ohlberger, 2013; Peralta-Maraver & Rezende, 2021), although the reasons are unclear. Smaller individual might be more adaptive to thermal exposure whereas larger individuals bearing more metabolic burdens with behaviours. It is possible that our results are explained by the small body size of males. Interestingly, despite impacts on copulation duration, we found no impact of heatwave exposure on female lifetime reproductive success. However, in a previous study (Dong et al. *submitted*), we found that reproductive success was decreased only when copulations were shorter than 10-minutes. In this study, all copulations exceed 20 minutes, even when females were exposed to heatwaves prior to mating. Thus, our finding of reduced copulation duration without impacts on reproductive success are not surprising and in line with our understanding of copulation and sperm transfer dynamics in this species.

In stark contrast to the lack of effect on lifetime reproductive success, we observed critical short-term impacts on the reproductive success of *S. grossa*, but only for females that were exposed to heatwave conditions after mating. These females produced smaller first egg sacs, which hatched fewer spiderlings. We interpret these results as an impact of extreme heat on sperm stored in the female, as has been observed in some species of *Drosophila* (Walsh et al. 2021). Spider sperm is coiled and encapsulated in a protein-rich sheath prior to mating (i.e. when stored in the male) and during transfer (Foelix, 1982; Herberstein et al., 2011; Vöcking et al., 2013), with decapsulation and activation of sperm cells occurring post-mating inside the female (Herberstein et al., 2011). Females exposed to heatwaves after mating may therefore experience reduced reproductive success due to the effects of extreme heat on decapsulated sperm. However, we also found that female reproductive output recovered by the time of the second egg sac, suggesting that only a subpopulation of stored sperm was impacted. In some spiders, sperm decapsulation appears to occur shortly after insemination (Berendonck & Greven, 2005), whereas in others the process is not an instantaneous, but occurs over time (Vöcking et al., 2013). Although the sperm biology of *S. grossa* is currently undescribed, we have observed motile sperm in the spermathecae of females shortly after mating (*pers. obs.*). Thus, we suggest that the short-term reproductive decline and subsequent recovery we observed in this study might be explained by heat-induced damage to decapsulated sperm subpopulations, while additional encapsulated subpopulations remain protected from the effects of extreme heat.

Taken together, our findings suggest that survival and reproduction can be negatively impacted by extreme heat conditions in *S. grossa*; eggs exposed to extreme temperatures fail to hatch, spiderling die, and females exposed to heat after mating show reduced, albeit temporarily, offspring production. It is important to note, however, that our findings reflect the impact of highly controlled and often discrete periods of heat exposure, which may not reflect the conditions faced by individuals in natural populations. For example, although we found that adult female *S. grossa* individuals were able to recover from the negative effects of extreme heat when returned to cooler ambient temperatures, it is possible that under natural conditions individuals would face multiple heatwaves during their reproductive lifespan. Indeed, given that extreme heat events are predicted to increase in intensity, duration, and frequency (Christidis et al., 2015; Perkins et al., 2012), we suggest individuals will likely face multiple heat events and will be impacted across multiple stages in the reproductive process. Given this, an individual female may experience reduced reproductive success due to the impact of heat on stored sperm (and these impacts may repeat over time) and reduced hatching success of egg sacs laid during or shortly prior to extreme heat conditions. Thus, an important next step is to gain an understanding of the potential cumulative effects of heat exposure for individual fitness and population persistence. Moreover, as heatwaves in the Netherlands are now seeing temperatures approach and even exceed 40°C (van Tilburg & Hudson, 2022), spiders and other ectotherms are likely to be exposed to conditions approaching or even exceeding their CTL for survival.

Our experiments also suggest that different life-stages are more or less vulnerable to the effects of extreme heat. Variation in thermal tolerance across life history stages has been reported in a number of insect taxa, with eggs frequently showing greater susceptibility to heat exposure than later juvenile stages, and adults showing the least susceptibility (Bowler & Terblanche, 2008; Jia et al., 2020; Klockmann et al., 2017; Vorhees & Bradley, 2012). Intriguingly, Barnes et al., (2019) showed that critical thermal maximum (CT_{Max}, the upper thermal threshold) for adults were lower than that for 1-3 months old spiderlings in two widow spider species (*L. mactans*, *L. geometricus*). In that instance, the authors suggested that heat tolerance diminishes with increasing age. However, our findings contradict this, possibly because we exposed eggs and spiderlings at the very early stages of their life cycle, when they may still have limitations on HSPs and related hormones, such as ecdysteroids.

Most spiders are generalist predators and are important natural enemies in many terrestrial ecosystems (Khudr et al., 2020; Nyffeler & Birkhofer, 2017), including agricultural systems (Michalko et al., 2019; Nyffeler & Birkhofer, 2017; Sunderland & Samu, 2000). Spiders also act as vital prey for many birds and small mammals (Gajdos & Kristin, 1997; Nyffeler & Knörnschild, 2013). *S. grossa* is at least partially synanthropic, and may be somewhat 'buffered' against climatic extremes by seeking out cooler microclimates in urban habitats (Cabon, et al., 2024a; Cabon, et al., 2024b; Pincebourde et al., 2016). Future studies should investigate sex-specific responses of many more spider species across a range of families and habitats to determine the effects of climate change on demographic parameters in this fascinating predator group. Thus, while there is mounting evidence suggests that well-studied arthropods such as insects are being negatively affected by climatic extremes (Harvey et al., 2020; Harvey, et al., 2023b), the understanding of how climatic extremes will affect a wide range of spider taxa remains less clear (Harvey & Dong, 2023).Understanding these impacts on spiders, a key and ubiquitous predator in almost all ecosystems, is important for biodiversity and climate change.

Supplementary tables and figures

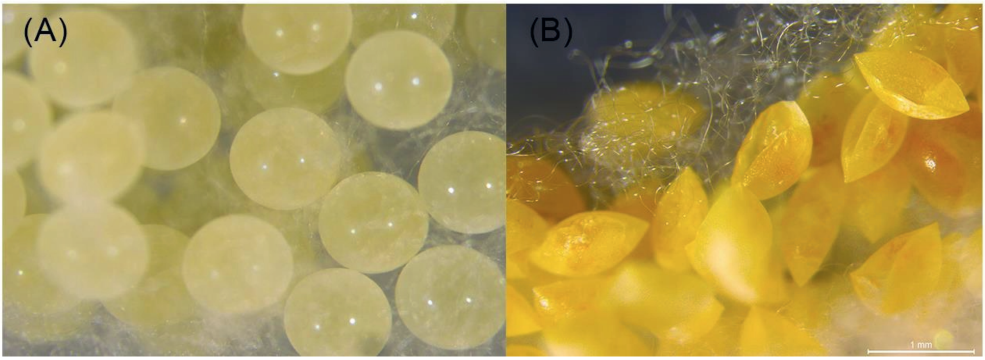

Figure S1. Eggs from a healthy egg sac (A) and an egg sac in 32°C (B) at day 10 after the egg sac was laid. Photo Credit: Rosalinde Keijzer.

Table S1. Post hoc result for comparison between egg sac numbers in *Temperature impacts on egg sacs of Steatoda grossa*.

Contrast	Estimate	SE	df	t ratio	p value
eggsac1 - eggsac2	-0.043	0.016	35	-2.792	0.040
eggsac1 - eggsac3	-0.037	0.019	43.2	-1.918	0.236
eggsac1 - eggsac4	-0.045	0.020	36.8	-2.256	0.127
eggsac2 - eggsac3	0.006	0.018	38.5	0.336	0.987
eggsac2 - eggsac4	-0.002	0.019	37.1	-0.111	1.000
eggsac3 - eggsac4	-0.008	0.022	40.8	-0.368	0.983

Table S2. Post hoc comparison results for female spiderlings growth rate on day 336 and male spiderlings growth rate when they reached maximum body mass in *Temperature impacts on spiderling growth and survival*. In this table, column diff refers to difference in means between the two groups; columns lwr and upr refers to the lower or upper bound of the confidence interval for the difference in means; column p adj stands for the adjusted *p* value.

Comparison	**Female spiderlings**				**Male spiderlings**			
	diff	**lwr**	**upr**	**p adj**	**diff**	**lwr**	**upr**	**p adj**
1x SHW-1x LHW	-0.149	-0.251	-0.047	0.001	0.000	-0.030	0.030	1.000
22-1x LHW	-0.122	-0.221	-0.024	0.006	0.004	-0.022	0.029	1.000
27-1x LHW	-0.094	-0.197	0.009	0.097	-0.022	-0.050	0.005	0.179
2x LHW-1x LHW	-0.036	-0.175	0.104	0.987	-0.015	-0.050	0.020	0.850
2x SHW-1x LHW	-0.151	-0.257	-0.046	0.001	0.006	-0.024	0.036	0.996
32-1x LHW	-0.072	-0.203	0.059	0.642	-0.045	-0.090	-0.001	0.043
22-1x SHW	0.026	-0.041	0.094	0.898	0.004	-0.016	0.024	0.997
27-1x SHW	0.055	-0.018	0.128	0.269	-0.022	-0.044	0.000	0.047
2x LHW-1x SHW	0.113	-0.006	0.233	0.075	-0.015	-0.046	0.016	0.776
2x SHW-1x SHW	-0.002	-0.079	0.075	1.000	0.006	-0.019	0.032	0.988
32-1x SHW	0.077	-0.033	0.186	0.349	-0.045	-0.087	-0.004	0.024
27-22	0.029	-0.040	0.097	0.867	-0.026	-0.042	-0.010	<0.001
2x LHW-22	0.087	-0.030	0.204	0.282	-0.019	-0.046	0.008	0.367
2x SHW-22	-0.029	-0.101	0.044	0.896	0.003	-0.018	0.023	1.000
32-22	0.050	-0.056	0.157	0.783	-0.049	-0.088	-0.010	0.004
2x LHW-27	0.058	-0.062	0.179	0.764	0.007	-0.021	0.036	0.987
2x SHW-27	-0.057	-0.135	0.021	0.298	0.029	0.007	0.051	0.003
32-27	0.022	-0.088	0.132	0.997	-0.023	-0.063	0.017	0.603
2x SHW-2x LHW	-0.116	-0.238	0.007	0.078	0.021	-0.009	0.052	0.375
32-2x LHW	-0.037	-0.182	0.109	0.988	-0.030	-0.076	0.015	0.417
32-2x SHW	0.079	-0.034	0.192	0.352	-0.052	-0.093	-0.010	0.005

Table S3. Post hoc result for comparison for copulation duration in heatwave experiment for adults in *Heatwave impacts on adult reproductive success*.

Contrast	**Estimate**	**SE**	**df**	**t ratio**	***p* value**
♂ ☼ ❤ - ♀ ☼ ❤	5.417	5.210	47	1.040	0.835
♂ ☼ ❤ - ❤ ♀ ☼	0.144	4.930	47	0.029	1.000
♂ ☼ ❤ - ♀♂☼ ❤	3.817	5.060	47	0.755	0.942
♂ ☼ ❤ - control	-12.683	5.060	47	-2.509	0.106
♀ ☼ ❤ - ❤ ♀ ☼	-5.273	5.310	47	-0.994	0.857
♀ ☼ ❤ - ♀♂☼ ❤	-1.600	5.430	47	-0.295	0.998
♀ ☼ ❤ - control	-18.100	5.430	47	-3.336	0.014
❤ ♀ ☼ - ♀♂☼ ❤	3.673	5.160	47	0.712	0.953
❤ ♀ ☼ - control	-12.827	5.160	47	-2.486	0.111
♀♂☼ ❤ - control	-16.500	5.280	47	-3.125	0.024

Table S4. Post hoc results of comparisons for the first and second egg sac mass in heatwave experiment for adults in *Heatwave impacts on adult reproductive success*. The bolded *t ratio* values and *p* values are for comparisons that involved the treatment that females exposed to heatwaves after mating (i.e., ❤️ ♀ ☼, experimental pairing #3).

Contrast	**The first egg sac**					**The second egg sac**				
	Estimate	**SE**	**df**	**t ratio**	**p value**	**Estimate**	**SE**	**df**	**t ratio**	**p value**
♂ ☼ ❤️ **-** ♀ ☼ ❤️	-0.435	6.41	73.2	-0.068	1.000	3.961	6.41	73.2	0.618	0.972
♂ ☼ ❤️ **-** ❤️ ♀ ☼	21.849	6.07	73.2	**3.600**	**0.005**	4.934	6.07	73.2	**0.813**	**0.926**
♂ ☼ ❤️ **-** ♀♂☼ ❤️	5.791	6.23	73.2	0.930	0.884	12.229	6.23	73.2	1.964	0.294
♂ ☼ ❤️ **- control**	0.277	6.23	73.2	0.045	1.000	7.031	6.23	73.2	1.129	0.791
♀ ☼ ❤️ **-** ❤️ ♀ ☼	22.284	6.53	73.2	**3.410**	**0.009**	0.973	6.53	73.2	**0.149**	**1.000**
♀ ☼ ❤️ **-** ♀♂☼ ❤️	6.226	6.68	73.2	0.932	0.884	8.268	6.68	73.2	1.238	0.730
♀ ☼ ❤️ **- control**	0.712	6.68	73.2	0.107	1.000	3.070	6.68	73.2	0.460	0.991
❤️ ♀ ☼ **-** ♀♂☼ ❤️	-16.058	6.35	73.2	**-2.528**	**0.096**	7.295	6.35	73.2	**1.148**	**0.780**
❤️ ♀ ☼ **- control**	-21.572	6.35	73.2	**-3.396**	**0.010**	2.097	6.35	73.2	**0.330**	**0.997**
♀♂☼ ❤️ **- control**	-5.514	6.5	73.2	-0.848	0.915	-5.198	6.5	73.2	-0.799	0.930

Table S5. Post hoc comparison between the first egg sac mass and the second egg sac mass in heatwave experiment for adults in *Heatwave impacts on adult reproductive success*.

Treatment	**Contrast**	**Estimate**	**SE**	**df**	**t ratio**	**p value**
♂ ☼ ❤	eggsac1 - eggsac2	8.46	4.05	47	2.086	0.042
♀ ☼ ❤	eggsac1 - eggsac2	12.86	4.68	47	2.746	0.009
❤ ♀ ☼	eggsac1 - eggsac2	-8.46	4.23	47	-1.997	0.052
♀♂☼ ❤	eggsac1 - eggsac2	14.90	4.44	47	3.354	0.002
control	eggsac1 - eggsac2	15.21	4.44	47	3.425	0.001

Table S6. Post hoc results of comparisons for number of spiderlings from the first and second egg sac in heatwave experiment for adults in *Heatwave impacts on adult reproductive success*. The bolded *t ratio* values and *p* values are for comparisons that involved the treatment that females exposed to heatwaves after mating (i.e., ❤ ♀ ☼, experimental pairing #3).

Contrast	Number of spiderlings (1st egg sac)					Number of spiderlings (2nd egg sac)				
	Estimate	SE	df	t ratio	p value	Estimate	SE	df	t ratio	p value
♂ ☼ ❤ - ♀ ☼ ❤	0.056	18.5	92.2	0.003	1.000	12.056	18.5	92.2	0.652	0.966
♂ ☼ ❤ - ❤ ♀ ☼	64.106	17.5	92.2	**3.665**	**0.004**	-0.197	17.5	92.2	**-0.011**	**1.000**
♂ ☼ ❤ - ♀♂☼ ❤	19.333	17.9	92.2	1.077	0.818	10.367	17.9	92.2	0.578	0.978
♂ ☼ ❤ - control	-16.567	17.9	92.2	-0.923	0.887	7.767	17.9	92.2	0.433	0.993
♀ ☼ ❤ - ❤ ♀ ☼	64.051	18.8	92.2	**3.400**	**0.009**	-12.253	18.8	92.2	**-0.650**	**0.966**
♀ ☼ ❤ - ♀♂☼ ❤	19.278	19.3	92.2	1.001	0.854	-1.689	19.3	92.2	-0.088	1.000
♀ ☼ ❤ - control	-16.622	19.3	92.2	-0.863	0.910	-4.289	19.3	92.2	-0.223	0.999
❤ ♀ ☼ - ♀♂☼ ❤	-44.773	18.3	92.2	**-2.445**	**0.113**	10.564	18.3	92.2	**0.577**	**0.978**
❤ ♀ ☼ - control	-80.673	18.3	92.2	**-4.406**	**<0.001**	7.964	18.3	92.2	**0.435**	**0.992**
♀♂☼ ❤ - control	-35.900	18.7	92.2	-1.916	0.317	-2.600	18.7	92.2	-0.139	1.000

Table S7. Post hoc comparison between the number of spiderlings in the first and the second egg sac in heatwave experiment for adults in *Heatwave impacts on adult reproductive success*.

Treatment	Contrast	Estimate	SE	df	t ratio	p value
♂ ☼ ❤	eggsac1 - eggsac2	17.7	15.9	47	1.112	0.272
♀ ☼ ❤	eggsac1 - eggsac2	29.7	18.3	47	1.618	0.112
❤ ♀ ☼	eggsac1 - eggsac2	-46.6	16.6	47	-2.812	0.007
♀♂☼ ❤	eggsac1 - eggsac2	8.7	17.4	47	0.500	0.619
control	eggsac1 - eggsac2	42.0	17.4	47	2.414	0.020

Author Contributions

Y.D., J.A.H., R.G., and M.R. conceived the study; Y.D., R.V., X.W., S.P., and J.T. performed the experiments and collected data with suggestions from J.A.H., M.R., and R.G.; Y.D., and R.G., and M.R. analyzed data; Y.D., J.A.H., and M.R. wrote early drafts of the manuscript with suggestions from R.G.; All authors reviewed and approved the final version of the manuscript.

Data Availability

Data is deposited in Dryad and being private for review. Meanwhile, all data are available upon request.

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Widow Spiders

Chapter 6

General discussion

Steatoda grossa, a synanthropic web-building spider with a widespread global distribution (World Spider Catalog, 2023) is by now a model species in the study of spider biology. False widow spiders in the genus *Steatoda* are closely related to true widow spiders in the genus *Latrodectus* (both are in the subfamily Latrodectinae), and they share similarity in appearance and toxin composition but with significantly lower potency to humans (Atakuziev et al., 2014; Graudins et al., 2002). Although *Steatoda* spiders are not aggressive, bites are still occasionally reported in cases where adult spiders were unintentionally squeezed or threatened (Dunbar et al., 2022; Isbister & Gray, 2003). Similar to widow spiders, almost all *Steatoda* spiders exhibit female-biased sexual size dimorphism (SSD; Blanckenhorn et al., 1995; Corcobado et al., 2010; Kuntner & Coddington, 2020). Females of synanthropic species like *S. grossa, S. nobilis* and *S. bipunctata* are frequently found staying in webs around human residence while males have a more wandering lifestyle (Bauer et al., 2019). Despite its relevance, the reproduction of spiders in this genus has been little studied. In this thesis, I explored reproduction of *S. grossa* in response to several biotic and abiotic factors. Understanding these responses provides useful information in comparison with other spider species and helps to place relevant aspects of spider life-history into the context of broader evolutionary theory. My main aim was to better understand reproductive strategies of *S. grossa*, contributing to a broader comprehension of spider ecology and evolution, and to provide insights on how to appreciate the life-histories of this fascinating organism.

In this general discussion, I firstly summarize all the major results of this thesis and demonstrate the influence of abiotic and biotic factors mentioned in this thesis on reproductive success both directly and indirectly. I then discuss the role of body size on both female and male reproductive success along with the influence of food availability and thermal stress on both sexes. Following this, I discuss the evolutionary forces underpinning SSD in this spider species and how this affects reproductive behaviour. Then, I discuss the thermal responses of *S. grossa*, with particular emphasis on differences in the thermal vulnerability among various life stages and thermal impacts on reproduction during adult stage. Finally, I propose ideas for future studies based on my studies here. Compared to other animal taxa, spiders are relatively well-represented in studies on sexual selection, whereas their ecology and ecological impacts have received much less attention. It is important to emphasize the importance of understanding the crucial role that spiders play as predators in communities and ecosystems.

Over the course of my research, I found that reproduction in *S. grossa* is influenced by copulation duration and food availability of females which is highly related to female body size, the number of reproductive events, and thermal stress. In **Chapter 2**, male body size did not influence the mating success but did highly influence the outcome of male-male competition. Under male-male competition scenarios, copulation duration was significantly reduced compared to non-competition scenarios. Following this result, in **Chapter 3**, I found that when copulation duration is shorter than 10 minutes, lifetime reproductive success is hampered. From an undisturbed copulation, females reproduce on average 8 times with a mean 15-day interval between reproductive events. Surprisingly, some females can continuously reproduce egg sacs more than 10 times over the course of a year. A major prerequisite for successful reproduction is the provisioning of adequate prey. In **Chapter 4,** prey availability influences a female's body mass after maturation and thus influences reproductive success. Meanwhile, when females more gradually produce egg sacs, their egg sacs decrease in mass as the number of reproductive events increases. Finally, in **Chapter 5**, I showed that thermal stress influences the survival, development and reproduction in *S. grossa*. Eggs are the most vulnerable to heat stress, followed by spiderlings and then adults. Juvenile female and male spiderlings exhibited different body size development strategies under thermal stress. The simulated heatwave only influenced reproductive success when it was experienced for females after mating. It only hampered the first reproductive event, which is occurred soon after being exposed to the simulated heatwave. These adults recovered their reproductive capacity thereafter. Furthermore, lifetime reproduction was not influenced by exposure to simulated heatwave conditions.

Factors influencing body size and sexual size dimorphism in spiders

Body size plays a crucial role in the reproductive success of both female and male organisms. Although it incurs significant costs to obtain a large body size, for example, the requirement for greater nutritional intake, a prolonged developmental period and higher risks of predation and parasitism (Blanckenhorn, 2000), being large for both females and males has apparent advantages on reproduction. Especially for animals that do not exhibit parental care, female body size has a direct and substantial impact on their reproductive success (Shine, 1988). Larger females generally have a higher energy storage and can allocate more resources to offspring production, such as a larger brood size which was observed in insects and spiders (Bartlett & Ashworth, 1988; Skow & Jakob, 2003). This intuitively aligns with my results in **Chapter 4**, where females having greater food availability maintained larger body sizes post-maturation and subsequently produced egg sacs with more spiderlings that successfully hatched. However, it is not a linear relationship, since female body size does not correlate with food availability beyond an upper threshold limit. Under conditions of extreme food scarcity, females experience significant weight loss, which dramatically impedes their reproductive capabilities. These results reinforce the importance of females having obtaining sufficient prey (beyond a critical lower threshold) in order to optimize reproduction and survival.

The strategies of resource allocation to various fitness-related functions in *S. grossa* differ significantly with sex. In a previous study, it was shown that females extend their development time in order to optimize body size at adult under variable prey availability, whereas males trade-off their body size and development time under food variability in order to obtain the optimal phenotype (Harvey, 2022). This means that males tend to mature faster in order to achieve mating success as quickly as possible under unfavorable conditions, but at a cost of reduced size. Similarly, in **Chapter 5**, females prioritized body size more than males under thermal stress, provided they did not perish when exposed to high temperatures, whereas males compromised body size and matured more quickly under constant maximum daily temperatures of 27°C and 32°C. Males seemed to follow the temperature-size rule (TSR), in which individuals mature at a smaller size under a warmer environment (Atkinson, 1994; Verberk et al., 2021). Faster development to adulthood is advantageous for finding mates earlier, and even mating success if a male is fortunate to encounter a virgin female with no other males presenting. For example, in Australian redback spiders (*Latrodectus hasselti,* which exhibits extreme SSD, or 'eSSD'), small males had 10 times higher fitness than large males because of their significantly faster development time to adulthood (Kasumovic & Andrade, 2009), which supports the 'first come, first served' hypothesis.

The advantage of larger size in males is mainly for access to females in competitive scenarios and resources sequestration (Chown & Gaston, 2010), although larger males may also be more prone to sexual cannibalism in taxa exhibiting eSSD. This is largely consistent with my findings in *S. grossa*. It has been shown in **Chapter 2**, where male body size itself does not influence mating behavior and success. Differences in male body size do not influence the occurrence of competition, which means a disadvantaged male (i.e., a small individual) does not avoid conflicts against larger rivals. Nevertheless, large males dominated the outcome of male-male competition and thus achieved higher mating success. When the "loser" (i.e., the smaller male) was unable to prevail in competition with a larger male, and did not secure a mating success, it still affected copulation duration by physically disrupting mating behaviour between the larger male and the female. In **Chapter 3**, I demonstrated that disrupted copulation results in a significantly lower reproductive success when it occurs fairly early during the mating sequence. This may be related to a reduced amount of sperm transferred from the male to the female, which has been also reported in some insects (Shandilya et al., 2021; Tong et al., 2021), and other spiders (Albo et al., 2013; Snow & Andrade, 2004). However, combining the results from these two chapters, the decrease in copulation duration due to physical disruption by small males does not necessarily reduce reproductive success. Therefore, the reproductive fitness of large males remains mostly unhindered, demonstrating the evolutionary advantages of larger male body size in this species.

Female-biased SSD commonly exists in many spider species and reaches its zenith in families such as the Araneidae and Theridiidae. For instance, the body mass ratio of females to males in the genera *Trichonephila* and *Latrodectus* may be 50 to 1 or even greater (Cheng & Kuntner, 2014; Kleinteich & Schneider, 2010; Kuntner & Coddington, 2020). The SSD in *S. grossa* (body mass ratio 5-10:1; Harvey, 2022) is not as pronounced as those taxa mentioned above. Females *S. grossa* attain a larger body size at maturation and retain after maturation if provided with sufficient prey (as shown in **Chapters 4 & 5**). In contrast, males exhibit a wider range of variation in body size than females. Large males had a competitive advantage in mating success with reduced copulation duration only due to disruptions from small rivals (as found in **Chapter 2**), but this did not result in the decreased reproductive success of large males (as found in **Chapter 3**). As with the redback spider *L. hasselti*, where small males also have high fitness in terms of female reproductive output, my results with *S. grossa* suggest that the selective pressure on male size is not very strong. Other factors, such as selection for morphological or behavioral traits that influence mate location (e.g., climbing ability and dispersal, Corcobado et al., 2010; Grossi & Canals, 2015; Moya-Laraño, 2002), low levels of sexual cannibalism (Elgar & Fahey, 1996) and lower mortality of immature spiders in urban habitats with low predation risk, could all contribute to a less exaggerated SSD in this species, compared with close relatives in the Latrodectinae.

Thermal stress, mating behaviour, survival and reproduction

As described in **Chapters 2 & 3**, physical disruptions, such as those occurring during competitive interactions between males on the web, or those caused by other perceived threats like the presence of a predator, sudden wind or heavy rainfall, can lead to an early termination of copulation. In **Chapter 5**, exposure of females to high temperatures resulted in a decrease the copulation duration. Specifically, exposure to an 8-day heatwave for females decreased mean copulation duration by around 20 minutes. However, the decrease in copulation was not enough to render a reduction in reproductive success based on results from **Chapter 3**, which showed that the reproductive success was negatively affected when copulation duration was shorter than 10 minutes. In other spider species, extreme heat stress influenced web building, foraging and copulatory behaviors in spiders (Barghusen et al., 1997; Brown & Houghton, 2020; Jiao et al., 2009; Johnson et al., 2020; Lubin & Henschel, 1990), which subsequently influenced mating behavior and reproductive success. It was also reported in insects that high temperatures reduced mating success, and thus hampered reproductive success (Iossa et al., 2019; Mak et al., 2023; Zeng et al., 2018). Considering the growing intensity, duration and frequency of heatwave events in the current climate change scenario (Brown, 2020; Christidis et al., 2015; IPCC, 2023; Jones et al., 2015; Russo et al., 2015), I speculate that the effects of heatwaves on mating behaviors in this species and closely related species will become more pronounced in the future.

Heat stress not only impacts mating behavior but also significantly influences mortality across different life stages in spiders, with eggs being the most vulnerable, followed by spiderlings, and then adults (as found in **Chapter 5**). One hypothesis argues that immobile stages, such as eggs and pupae of insects have a higher heat tolerance because researchers expected that a decline in thermal tolerance coupled with increasing behavioral adaptation to temperature extremes (Bowler & Terblanche, 2008). In *S. grossa*, and other spiders, this theory does not hold true. The vulnerability of eggs might be due to the lack of heat shock protein (HSP) expression during the embryonic stage (King & MacRae, 2015), insufficient HSP levels in early juveniles (Feder et al., 1996; Feder & Hofmann, 1999; Kregel, 2002), and the release of ecdysteroids during a later juvenile stage for molting (Moen et al., 2022; S. R. Thomas & Lengyel, 1986). Notably, eggs are particularly susceptible, even if adults did not directly experience heatwave conditions. This means that females should seek out cooler microclimates in which to place their egg sacs under the perceived risk of heat exposure during their reproductive cycle. Moreover, young spiderlings face the same challenges. Even if reproduction occurs during a transient period of optimal weather, unpredictable temperature extremes in future can hinder the survival of eggs and young spiderlings which are clearly more prone to heat stress. Since egg sacs are attached with the web of the mother, and offspring are constrained early in development in their ability to seek optimal microclimates, the web-site selection by the mother is of crucial importance.

Many studies have reported that fertility is especially sensitive to high temperatures. Walsh, et al., (2019b) highlighted the definition of thermal fertility limits (TFLs), which suggest that individual organisms have a narrower range of upper and lower thermal limits for fertility than the critical thermal limits (CTLs) for survival. Typically, individuals exposed to heat survive but become infertile and this can jeopardize population persistence. Sexual specificity in TFLs is common in most organisms including animals and plants, with many studies showing that sperm of males is especially vulnerable (Hurley et al., 2018; Iossa, 2019; Sales et al., 2018; Walsh, et al., 2019a). The introduction of TFLs and its sexual specificity is a milestone in understanding species distributions and extinction risks under changing climatic regimes. However, it is better to consider taxonomic-group specificity within the broader context of biodiversity. In Chapter 5, my findings did not indicate that sperm and eggs are necessarily vulnerable to thermal stress, except in cases where sperm had already been transferred into from the male palp into the female reproductive tract. Eggs inside females were not influenced, while sperm stored inside the palps of male spiders are usually encapsulated and coiled (Eberhard, 2004; Herberstein et al., 2011), potentially providing a shield against thermal stress. Although this thesis did not directly investigate the protective mechanisms provided by sperm encapsulation and coiling, it highlights a promising direction for future research. Understanding how traits in different taxonomic groups help organisms respond to climate change could offer valuable insights into TFLs, and thus better predict the risks of biodiversity decline in a broader context.

What factors have enabled *S. grossa* to attain a cosmopolitan distribution?

Even though the reproduction in *S. grossa* was hampered under unfavorable biotic and abiotic conditions, the results of my research in this thesis still reveal that this species exhibits strong resilience. Firstly, in **Chapter 2**, females are not choosy for males for mating in terms of body size. They almost always accept any males that exhibit a sequence of courtship behaviors. **Chapter 3** showed that as long as the copulation lasts for 10 minutes or longer, males were able to transfer sufficient sperm for females to produce 500 or more spiderlings. Moreover, in **Chapter 4**, the food starvation treatment revealed the resilience of female reproduction. When prey was scarce or absent, female spiders ceased reproduction until the prey availability improved, demonstrating a strategic pause for future reproductive success. Some starved females remarkably lost significant body mass, but recovered when provided with large prey and immediately recommenced the production of viable egg sacs. In **Chapter 5**, adults were only briefly affected by heatwaves and soon recovered, although eggs and spiderlings fared less well. Be mindful that *S. grossa* is a highly synanthropic spider, and exploits habitats around human dwellings such as cupboards, sheds, storage cellars, and greenhouses. These environments often provide shelter from extreme weather conditions, but pose significant challenges in terms of prey availability. If these sites contain food or waste that attracts insects, spiders may find an abundant food supply. On the contrary, in enclosed spaces, prey may be scarce, meaning the females need to be able to adapt to variable and often unpredictable conditions. Even when food is temporarily scarce, *S. grossa* exhibits adaptive behaviour such as sibling cannibalism to cope with food limitations (Harvey, et al., 2023a).

The traits that demonstrate resilience have probably contributed to the success of *S. grossa* in maintaining and expanding their distribution globally (Faúndez et al., 2017; Hann, 1994; Levi, 1962; Levy & Amitai, 1982). Some traits observed in *S. grossa*, such as extended copulation which is longer than necessary for sperm transfer (Snow & Andrade, 2004), thermal resistance (Araújo et al., 2013; Barnes et al., 2019; Malmos et al., 2021), and sibling cannibalism under food limitation (Wise, 2006), may be general across many spider species. Other "notorious" invasive spiders, which are closely related to *S. grossa*, like *L. geometricus* (Mowery, et al., 2022b; Vincent et al., 2009) and *S. nobilis* (Bauer et al., 2019) share some similar traits, and in some cases, these may be even more exaggerated. For example, *L. geometricus* produces a fairly unique and thick sac for protecting eggs against heat and natural enemies, such as parasitoids (Hayasaka et al., 2021; Mowery, et al., 2022a). A recent study suggests that higher trophic level organisms are more sensitive to climate change compared to those in lower trophic levels (da Silva et al., 2023). However, the resilience of spiders to climate change and climatic extremes is relatively understudied and remains controversial (Blandenier et al., 2014; Fürst et al., 2023; Harvey & Dong, 2023; Leroy et al., 2013; Mammola et al., 2018; Webber et al., 2011), partly due to variations in traits such as thermal tolerance and dispersal ability (Bonte et al., 2008, 2012). What is evident is the specificity within spider taxa, meaning that while some species may be susceptible to climate change, others with more resilient traits – such as thermophilic species - may actually benefit under some global warming scenarios.

This resilience could potentially be advantageous for agricultural systems regarding the role of spiders in pest control, but it could also lead to increased challenges when some synanthropic spiders, and especially species venomous or biting species, become urban pests.

Significance of this thesis and future directions

Body size is a pivotal life-history trait in ecology, significantly influencing an organism's survival, reproductive success, and overall fitness. Larger body size often correlates with greater competitive ability and increased reproductive capacity, but it also demands higher resource intake, longer development times, higher risks of predation and parasitism, leading to potential trade-offs in life-history strategies. These trade-offs become particularly evident in fluctuating prey availability scenarios and other unfavorable conditions. In this thesis, the advantage of large size in both female and male *S. grossa* were demonstrated, enhancing the understanding of body size evolution and SSD in this species. Despite the disruptive effects of competitive interactions between larger and smaller males reducing copulation duration, female reproductive success remains mostly unaffected because the reduction is not substantial enough to hinder reproduction. Moreover, even if copulation duration is reduced, a female may encounter other males that will mate with her, because females show no reticence to mating, regardless of previous sexual experience. This offers insights into the relationship between reproduction and mating behaviour in *S. grossa*. Additionally, reproductive strategies in *S. grossa* under prey limitation reveal a life-history tradeoff between current and future reproductive success. For example, females may produce fewer eggs per egg sac but increase the number of egg sacs over time, or they may delay reproduction until abundant or large prey is available. These results provide key insights into the biology and ecology of *S. grossa*, enhancing our understanding of the factors driving body size evolution and sexual size dimorphism in this species, which is also useful in comparison with related species. They also reveal how this spider's life-history strategies are optimized to maximize reproductive success, offering a broader perspective on the ecological and evolutionary dynamics that shape life-history traits.

Climate change is one of the most serious threats to biodiversity and ecosystem functioning of our time and it has attracted significant scientific and societal attention (Bellard et al., 2012; Thomas et al., 2004). Recently, insect decline has become a focal point of concern (Harvey, et al., 2023b; Wagner et al., 2021), and climate change has been highlighted as a prominent factor, given that insects are ectotherms and represent a substantial portion of Earth's biodiversity (Chapman, 2009; May, 1988; Stork, 2018). This trend alarms ecologists because insects play critical roles as prey and serve numerous ecological functions (Wilson, 1987). However, spiders were not widely mentioned in this discussion. The vast majority of spiders are wholly predatory and consume between 400 and 800 million tons of prey every year (Nyffeler & Birkhofer, 2017) and are therefore important pest control agents in agroecosystems (Riechert & Lockley, 1984). However, compared to most other animals, spiders are profoundly underrepresented in conservation policies (Milano et al., 2021; Troudet et al., 2017). Despite their ecological importance, spiders' responses to climate change have not received adequate attention (Harvey & Dong, 2023). My research which examined responses in *S. grossa* to thermal stress across all life stages and sexes and on development and reproduction adds important data that helps to fill this knowledge gap. Understanding these aspects across many more species is crucial for determining how climate change affects spiders and for developing strategies for broader biodiversity conservation.

Building on the findings from this thesis, future research might explore the mechanisms underlying mating behaviour in *S. grossa*. For example, the factors that affect sperm transfer pattern during copulation, and the function of extended copulation duration, which I suggest might be related to sperm competition. Moreover, adaptive mechanisms in spiders for coping with the climate change challenges are fascinating and of great importance, for instance, examining mechanisms that affect reproductive success under heatwave conditions. From a broader biodiversity perspective, it is important to monitor the population dynamics of spiders and their prey in natural and agricultural environments over longer periods of time (Kleijn & Sutherland, 2003; Mei et al., 2023). Detailed, long-term field studies on predator-prey interactions involving invertebrates are still fairly limited (Damien & Tougeron, 2019; Van der Putten et al., 2010). Moreover, it is not really understood what actual contribution spiders make in the field to the abundance of prey populations (Sunderland, 1999), which makes their role as natural enemies more difficult to predict. However, exploring the population dynamics of spiders and their prey will better help us to understand and predict the consequences a suite of global environmental changes for biodiversity and ecosystem functioning.

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Summary

If individuals fail to reproduce, populations can face extinction. Many biotic and abiotic factors, such as competition, predation, food availability, and environmental conditions, can substantially influence reproductive success. Despite facing all of these factors, spiders are rarely studied in the context of biotic and abiotic impacts on reproduction. Across the globe, spiders are the most abundant and ubiquitous predators of invertebrates. Thus, it is important to gain a more comprehensive understanding of their reproduction and the factors that shape population viability and persistence. *Steatoda grossa*, a false black widow spider, is a globally distributed species, commonly found around human residences in both urban and rural areas. The species has been spreading throughout most temperate areas and is potentially one of the most successful invasive spiders; as are the closely related *S. nobilis* and *Latrodectus geometricus* (same subfamily, Latrodectinae). *S. grossa* exhibits female-biased sexual dimorphism, and males show a set of ritualized courtship behaviours that are critical for male mating success. Here, I explore the fascinating mating and reproductive behaviour of *S. grossa* and then investigate how both biotic and abiotic factors influence the outcome of reproductive processes. In summary, in this thesis, I examine how male-male competition, mating behaviour, food availability and thermal stress affect reproduction in *S. grossa*.

In Chapter 2, I tested the role of male body size in mating success and male-male competition in *S. grossa*. Male body size is important for mating success in many species. In a female-biased sexually dimorphic animal, the benefit of a male having a large body size remains unclear. I designed the experiment which created encounters with a female using either two size-mismatched males (thus creating conditions of male-male competition for mating success) or a single male. I found that all males obtained a mating, regardless of body size when rivals were absent (i.e., non-competitive conditions). However, under competitive conditions, larger males achieved greater mating success; thus demonstrating an important role for male body

size in pre-copulatory mating success in *S. grossa*. At the same time, I also found that copulation duration was significantly reduced when multiple males compete for copulations. Combined, this work sheds novel insight on male body size evolution in this species and provides a potential explanation for the extent of sexual size dimorphism observed in this species; the benefits of large male body size shown in this thesis help explain why male dwarfism (i.e., extremely small male size) has not evolved in *S. grossa*. Finally, my finding that male-male competition reduces copulation duration resulting from male-male competition may have potential ramifications for male and female reproductive success.

Next, in Chapter 3, I found that mating disruption (i.e., interruption of mating resulting in reduced copulation duration) decreases female spiders' reproductive success. In sexually reproducing taxa, copulation is critical for the transfer of sperm from the male to the female. In many taxa, including spiders, with relatively long copulation duration, various biotic and abiotic factors have the potential to disrupt mating before it would be naturally terminated. This includes, for example, the male-male competition conditions investigated in Chapter 2, but also predation, and environmental conditions such as rain and wind. Here, I simulated the physical disruption of copulation (removing the male from the mating arena to prevent re-mating) at a range of time points (i.e., 2, 5, 10, 20 minutes compared to natural matings, lasting 40-60 minutes) and recorded female reproductive success. My results show that early disruption of copulation leads to significantly lower reproductive success in the false widow spider. Specifically, while copulations longer than 10 minutes resulted in equivalent reproductive success to natural matings, copulations less than 10 minutes resulted in fewer offspring. This suggests that a copulation of just 10-minutes allows the male to inseminate enough sperm for the female to fertilize most or all of her eggs. Following from this result, I also conducted an experiment to explore if an extended copulation functions as mate-guarding. I introduced virgin fresh males to females who either mated for a long duration or a short one and checked if female accepted another mating. Extended copulation duration did not influence female receptivity to fresh males 24 h later, but might influence oviposition and fertilization processes. Finally, I suggest that extended copulation in this species might play a role in sperm competition.

In Chapter 4, I tested the effects of food availability on female body mass changes and reproductive success in *S. grossa*. All organisms need food in order to grow, survive and reproduce. In most web-building spiders, females require more food to reach sexual maturity and are relatively larger than males. I provided females with different numbers and sizes of prey (house crickets) and measured their subsequent reproductive investment and success. I found that females provided with intermittent prey produced fewer progeny per egg sac and the interval between sequential egg sacs increased. Additionally, female spiders continuously invested almost half of their body mass in each reproductive event, but this investment showed a decreasing pattern over time, which also resulted in a decreasing pattern in egg sac mass. Moreover, starved females stopped reproducing for several months, but recommenced egg sacs production when fresh prey was provided. Indeed, females laid viable egg sacs within several days of feeding, and these in turn produced healthy spiderlings, indicating that reproductive capacity is only temporarily suspended during harsh conditions and extended periods of resource depletion. The synanthropic lifestyle of *S. grossa* might induce them to periodic prey scarcity in many human-related habitats, such as cupboards and sheds. This chapter shows the general pattern of spider reproduction over time and strategies to cope with food limitations.

Finally, in Chapter 5, I explored the impact of thermal stress on survival, development and reproduction across different life stages of *S. grossa*. Human-induced climate change is having a dramatic effect on the global thermal environment. In addition to increases in average local temperatures, climate change is predicted to drive increases in climatic extremes, including heatwaves. Importantly, these changes can pose a significant threat to the persistence of natural populations, and these threats might be especially critical for ectotherms given that body temperature regulation depends on external environmental conditions in ‘cold-blooded’ animals. In this chapter, I exposed different age-cohorts of the false widow spider to different temperatures and studied the effects of both constant thermal stress and extreme temperature events (i.e., heatwaves) on reproduction. Egg sacs of *S. grossa* perished precociously under both thermal conditions, whereas survival was high at lower temperatures, indicating a temperature threshold on neonate survival to hatching. For spiderlings, the growth trajectories were impacted by extreme ambient temperatures; spiderlings exposed to high temperatures exhibited severely depressed growth rates, whereas those reared at lower temperatures grew exponentially and attained significantly larger adult weights. However, female and male spiderlings showed a different growth rate under thermal exposure, suggesting a sex-specific thermal resistance. Finally, in adults, females exposed to a heatwave after mating suffered short-term impacts on reproductive success; females laid significantly smaller first egg sacs and produced significantly fewer spiderlings from these egg sacs. However, these negative impacts were only apparent in the first egg sac, and by the second egg sac female reproductive success appeared to recover. Combined, the findings in this chapter demonstrate that the effects of exposure to high temperatures are life stage-specific, sex specific and that exposure to heat more negatively affects the survival and development of eggs and younger spiderlings than adults.

In this thesis, I revealed size-dependent advantages in males *S. grossa* during competition for access to females. I argue that the benefits of larger size in males provide insights into male body size evolution and sexual size dimorphism in this species. Moreover, I show that male-male competition (i.e., mating disruption) leads to reductions in copulation duration. Following this, the impact of mating disruption on female reproductive success was analyzed and my findings suggest that sperm transfer takes place early during mating. The effects of food availability on female body mass changes and reproductive success were also investigated. That work highlighted significance of a large female body mass and the reproductive strategies in *S. grossa* under food limitation for trade-offing current and future reproductive success, for example producing fewer eggs per egg sac but more egg sacs over time; holding back on reproduction until abundant food is available. Additionally, my thesis evaluates the consequences of thermal stress on survival, development and reproduction across different life stages, providing insights into the species' potential adaptability to current climate change scenarios. Combined, this thesis enhances our understanding of spider reproductive ecology. Building on these findings, future research could explore the mechanisms underlying sperm transfer, extended copulation duration. Moreover, adaptive mechanisms in spiders for coping with the climate change challenges are interesting, for instance, the mechanisms for the transient reduced reproductive success. Climate change and climate extremes is posing threats to many organisms, leading to detrimental impacts on biodiversity. As the most abundant predator of invertebrates, spiders are often overlooked in biodiversity conservation efforts. It is important to study the impacts of thermal stress on a broader range of high-trophic-level animals, which provides a more comprehensive understanding of ecological resilience and the effects of climate change on biodiversity.

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About the author

Yuting Dong was born in 1995 in Shandong (China). In 2013, she finished the bachelor study in Forestry (Ecology) in Nanjing Forestry University. During the bachelor study, she researched the maternal effect from light on the morphology of maple seeds. In 2017, she obtained a master's degree from Nanjing Normal University (Nanjing, China) where she specialised in infectious disease ecology. During her studies, she took part in two projects, one is about the effect of habitat fragmentation on the immunity of Drosophila and the other is about the landscape and climate effects on the Lyme Risk in the United States. After that, she fled across the globe and arrived in the Netherlands to conduct this Ph.D. research at Netherlands Institute of Ecology and Wageningen University & Research. Within her 4-year contract, she successfully completed her Ph.D. thesis on time.

List of publications

Dong, Y., Harvey, J. A., Steegh, R., Gols, R., & Rowe, M. (2023). The role of male body size in mating success and male–male competition in a false widow spider. *Animal Behaviour*, *206*, 53–59. https://doi.org/10.1016/j.anbehav.2023.09.011

Dong, Y., Harvey, J. A., Gols R., Wang, X., Vooijs, R., Pleiter, S., ten Thij, J., Rowe, M., (2024). Extreme temperatures impact survival and short-term reproductive success in a false widow spider. *In preparation.*

Dong, Y., Gols R., Rowe, M., Harvey, J. A., (2024). The effects of food availability on female reproductive success. *In preparation.*

Dong, Y., Harvey, J. A., van den Hengel, M., Zwanenburg, A., Rowe, M., Gols R., (2024). Copulation interruption decreases female reproductive success in a false widow spider. *In submission.*

Harvey, J. A., Steegh, R., **Dong, Y.**, & Gols, R. (2023). Sibling cannibalism in the false widow spider is dependent on spiderling density and the reliable availability of fresh prey. *Ethology*, *129*(11), 630–639. https://doi.org/10.1111/eth.13399

Harvey, J. A., & **Dong, Y.** (2023). Climate change, extreme temperatures and sex-related responses in spiders. *Biology*, *12*(4), 615.

Chen, B. J., Wang, X., **Dong, Y.**, During, H. J., Xu, X., & Anten, N. P. (2021). Maternal environmental light conditions affect the morphological allometry and dispersal potential of *Acer palmatum samaras. Forests*, *12*(10), 1313.

Dong, Y., Huang, Z., Zhang, Y., Wang, Y. X., & La, Y. (2020). Comparing the climatic and landscape risk factors for Lyme disease cases in the Upper Midwest and Northeast United States. *International Journal of Environmental Research and Public Health*, *17*(5), 1548.

Ma, Y., He, G., Yang, R., Wang, Y. X., Huang, Z. Y., & **Dong, Y.** (2022). Effect of land-use change on the changes in human Lyme risk in the United States. *Sustainability*, *14*(10), 5802.

PE&RC Training and Education Statement

With the training and education activities listed below the PhD candidate has complied with the requirements set by the C.T. de Wit Graduate School for Production Ecology and Resource Conservation (PE&RC) which comprises of a minimum total of 30 ECTS (= 20 weeks of activities)

Review/project proposal (6 ECTS)

- Reproduction in the false widow spider under conditions of male-male competition and exposure to extreme heat

Post-graduate courses (11.4 ECTS)

- Resilience in living system, PE&RC (2023)
- Life history theory, PE&RC (2024)
- Ecological methods 1, WUR (2020)
- Tidy R and data transformation , PE&RC (2022)
- Bayesian Data analysis, PE&RC (2023)

Laboratory training and working visits (0.3 ECTS)

- Spider biology, University of Toronto (2023)

Invited review of journal manuscripts (1 ECTS)

- Functional Ecology, Spider Waste Enhances Soil Nutrient Content, Soil Respiration, and Plant Growth (2024)

Competence, skills and career-oriented activities (3 ECTS)

- Scientific writing, Wageningen in'to Languages (2023)
- Fair data management, PE&RC (2022)
- Mindful productivity, PE&RC (2022)

Scientific Integrity/Ethics in science activities (0.3 ECTS)

- Scientific integrity, WUR (2023)

PE&RC Annual meetings, seminars and PE&RC weekend/retreat (2.1 ECTS)

- PE&RC first year retreat (2021)
- PE&RC last year retreat (2023)
- PE&RC day (2022, 2023)

National scientific meetings, local seminars, and discussion groups (3 ECTS)

- WEES seminars (2022-2023)
- KNAW PhD&Post doc career day seminar (2021)
- WUR Entomology group colloquium (2022)
- Netherlands Annual Ecology Meeting (2023)
- International Symposium on Microbial Ecology (2022)
- NIOO Institutional seminar (2023)

International symposia, workshops and conferences (5.6 ECTS)

- International Society of Behavioral Ecology, Stockholm, Sweden (2022)
- International Congress of Entomology, Kyoto, Japan (2024)

Societally relevant exposure (1 ECTS)

- Collaboration with local film company for making spider documentary (2024)

Committee work (1 ECTS)

- Work for PE&RC PhD Council (2023)

BSc/MSc thesis supervision (6 ECTS)

- Research topic 1: Courtship behaviour and copulation duration in *Steatoda grossa*
- Research topic 2: The effects of heatwaves on egg sacs of false widow spiders
- Research topic 3: The effects of heatwaves on adult reproduction in *Steatoda grossa*
- Research topic 4: The effects of heatwaves on egg sacs of brown widow spiders
- Research topic 5: Long term effects of copulation duration on reproductive success in *Steatoda grossa*.
- Research topic 6: Thermal effects on development of false widow spiders
- Research topic 7: Thermal effects on development and cannibalism behaviours of false widow spiders and brown widow spiders
- Research topic 8: The long-term thermal effects on reproductive success in *Steatoda grossa*
- Research topic 9: The thermal effects on egg survival and development in *Steatoda grossa*
- Research topic 10: The effect of food availability on the growth curve of two Steatoda species; S. *bipunctata* and S. *triangulosa*.
- Research topic 11: The effect of variable temperature on the development of *Steotoda grossa*

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