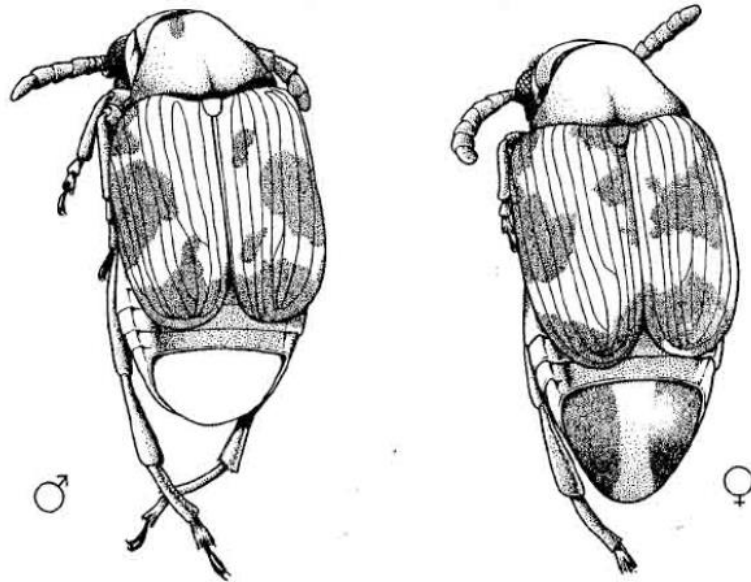


**Effect of supplemented water on fecundity and longevity of female bean beetles, *Callosobruchus maculatus*, in sex ratio manipulated environments**



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**June 2019**

Front page:

Drawing of male (left) and female (right) *Callosobruchus maculatus*

Figure: Beck, C. W., & Blumer, L. S. (2011).

## **Acknowledgments**

I would like to express my very great appreciation to my supervisor Sigrunn Eliassen for her guidance, valuable time and constructive suggestions during the development of this research and master-thesis. As well as for the great freedom to explore how to be a researcher. I wish to express my gratitude to various people for their contribution to this thesis; Richard Telford, for the statistical guidance; Julie Skadal, for the use of the laboratory and equipment; Andreas N. Holme, for his advice and encouragement during this thesis; Elise Doddema, for helping collecting data. I am grateful for my fellow students, every single one has been part of making these years unforgettable. As well as, express my gratitude towards my always supporting family and friends, which have been here for me during the process of writing this thesis.

## **Abstract**

Polyandry is an important evolutionary phenomenon, despite matings being associated with considerable costs for females. In insects, these costs are often related to time and energy expenditures, physical injuries and increased risk of predation. Female remating can also result in benefits, such as the acquisition of water or nutrients from the spermatophore, increasing fecundity and longevity. Therefore, there is a trade-off between the cost and benefits of multiple matings, in which females adjust their mating behavior. Female *Callosobruchus maculatus* (F.), bean beetles, are polyandrous capital breeders that receive a relatively large spermatophore from males during copulations. These spermatophores contain water and nutrients that females can utilize, and which influence fecundity and longevity. This suggests that if females are given access to water it may be less profitable for her to remate. The effect of supplemented water on female mating behavior was experimentally tested in different sex ratio environments. Results showed that the availability of water increases female fecundity and longevity. However, this hydration benefit on longevity is reduced when females are exposed to a male-biased environment. This indicates that the availability of supplemented water does not offset the costs of male harassment for females *C. maculatus*.

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## **Introduction**

Early publications on reproductive behavior in insect species indicated that polygyny was the dominant mating system, with males competing for mates and females being choosy (Gwynne & Bailey, 1988). However, further research revealed evidence of polyandry as a more prevailing mating strategy, where females copulates and is inseminated by more than one male for fertilization of a given litter of progeny (Archer & Elgar, 1999; Bateman, 1998; Gwynne & Bailey, 1988; Parker & Birkhead, 2013; Zeh, Newcomer, & Zeh, 2002). Arnqvist, Nilsson and Katvala (2005) discuss whether our understanding of ultimate causes of polyandry is complete, and highlights that it is not clear if and in what sense females have optimal mating rates. Therefore, the level of polyandry is said to be dependent on the cost and benefits associated with multiple mating (Arnqvist, Nilsson, & Katvala, 2005). The cost associated with polyandry is higher disease risk, greater susceptibility to predation when mating or searching for mate (Arnqvist, 1989; Wing, 2002), reduced offspring production and chemical or physical damage (Arnqvist et al., 2005; C. J. Wilson & Tomkins, 2015). However, since polyandry is an important evolutionary phenomenon that affects the strength of pre- and post-copulatory sexual selection (Kvarnemo & Simmons, 2013; Pizzari & Wedell, 2013; Shuster, Briggs, & Dennis, 2013), it should be explained either by the direct or indirect fitness benefits (Arnqvist & Nilsson, 2000; Arnqvist et al., 2005; Holman & Kokko, 2013; Torres-Vila, 2013; C. J. Wilson & Tomkins, 2015; Yasui, 1998). Direct benefits females receive from remating could be increased egg production (Arnqvist & Nilsson, 2000; Ronkainen, Kaitala, & Kivelä, 2010), elevated post-birth offspring survival (Firman & Simmons, 2008), offspring quality (Torres-Vila, 2013) or increased longevity (Snook, 2014). Whereas, indirect benefits include the “next-generation” benefits that increase the fitness of the individual’s offspring (Yasui, 1998) such as inbreeding avoidance (Tregenza & Wedell, 2002), decreasing the chance of genetic incompatibility (Zeh & Zeh, 1996) and increasing the chance of acquiring good genes from superior males (Kempnaers, Verheyen, & Dhondt, 1997; Yasui, 1997).

Females of several insect species receive benefits from multiple matings measured as increased fecundity and longevity (Snook, 2014; Torres-Vila, 2013) under conditions of dehydration or starvation (Edvardsson, 2007; Fox, 1993; Tseng, Yang, Lin, & Horng, 2007; C. J. Wilson & Tomkins, 2015). Most of these benefits are related to the spermatophore received from males during mating (Arnqvist et al., 2005; Edvardsson, 2007; Fox & Moya-Laraño, 2009; Rankin & Arnqvist, 2008). For instance, a comparative analyses on butterflies

indicate that females reproductive success increases with the degree of polyandry (Larsdotter Mellström & Wiklund, 2010), which is most likely due to the utilization of water and/or nutrition from the spermatophore (Gwynne & Bailey, 1988). Another research shows that female brush crickets metabolize carbohydrates in spermatophore rather quickly (Voigt, Kretzschmar, Speakman, & Lehmann, 2008). Thus, spermatophores can represent an important resource for females (Gwynne & Winston, 1988; Larsdotter Mellström & Wiklund, 2010). Males, on the other hand, can enhance their reproductive success by producing large spermatophores which may increase female refractory period, hence the proportion of eggs that are fertilized by him (Moya-Laraño & Fox, 2006; Savalli & Fox, 1999b; Takakura, 1999), or function as parental investment (Gwynne, 2007). There is also evidence from field studies that offspring of polyandrous females often have lower juvenile mortality (Fisher, Double, Blomberg, Jennions, & Cockburn, 2006). Laboratory experiments confirm that this is directly due to polyandry (Simmons, 2005; Tregenza & Wedell, 1998). However, these studies are to some degree restricted since it is unclear whether polyandry has the same effect in the field as in the laboratory, as juvenile survival depends on interactions between environmental conditions and investment in growth (Fisher et al., 2006).

The key to fully understand the mechanisms of sexual selection and social behavior (Fox, 1993) relies on understanding the evolution of female polyandry (Arnqvist et al., 2005). An ideal model species for investigating the trade-off between costs and benefits of male and female interactions, sexual conflict and sperm competition (Arnqvist et al., 2005; Edvardsson & Tregenza, 2005; Iglesias-Carrasco, Bilgin, Jennions, & Head, 2018) hence polyandry, is the seed beetle *Callosobruchus maculatus* (Fabricius) (Coleoptera: Bruchidae). The seed beetle *C. maculatus* are capital breeders; the larvae of this species feeds and develops in seeds of beans and spend their short adult lifespan (14 days) remating and laying eggs (Beck & Blumer, 2011; Harano, 2012; Hudaib, Hayes, Brown, & Eady, 2010). The females of *C. maculatus* often mate with multiple males even when a single mating is sufficient to fertilize their eggs (Eady, 1994, 1995; Larsdotter Mellström et al., 2010; C. J. Wilson et al., 2015; but see: Fox, 1993). Polyandry can lead to both direct and indirect benefits for females (Arnqvist & Nilsson, 2000; Holman & Kokko, 2013; C. J. Wilson & Tomkins, 2015), however there is a trade-off between potential costs and benefits associated with multiple mating (Edvardsson, 2007). Female *C. maculatus* risk physical damage through male harassment, injury to the genital tract during copulations and costs associated with male ejaculates (Eady, Hamilton, & Lyons, 2007). Injury to the female genital tract is associated with spines on males intromittent



organs that puncture female's reproductive tract during mating (Eady et al., 2007). In order to reduce possible injuries, females frequently attempt to kick their mates off during copulations (Edvardsson & Tregenza, 2005; Martinossi-Allibert, Arnqvist, & Berger, 2017; Rönn & Hotzy, 2012; but see: C. J. Wilson & Tomkins, 2014). If they are prevented from kicking, copulations last longer and the injuries females sustain are more severe (Crudginton & Siva-Jothy, 2000). Similarly, Edvardsson and Tregenza (2005) found that kicking-deprived females suffer a reduced lifetime fecundity and that injured females are less likely to remate as to avoid further damage (Edvardsson & Tregenza, 2005). Additionally, they found that females may respond by increasing their rate of oviposition if they perceive the injuries as a threat to their survival (Edvardsson & Tregenza, 2005). However, prolonged copulations could result in the transfer of larger ejaculates (Edvardsson & Canal, 2006; Van Lieshout, McNamara, & Simmons, 2014, but see: Brown et al, 2009), thereof larger amounts of water and/or nutrients which females can benefit from (Arnqvist et al., 2005). There is evidence that males do not gain benefits through harming their mates during copulations. Harm caused by spines on the intromittent organs is a pleiotropic by-product, suggesting that the spines have evolved for other reasons, such as serving as an anchor during copulation or function in sperm competition (Edvardsson & Canal, 2006; Edvardsson & Tregenza, 2005; Hotzy & Arnqvist, 2009; Rönn & Hotzy, 2012; Rönn, Katvala, & Arnqvist, 2007). Therefore, the trade-offs associated with polyandry (Edvardsson, 2007; Harano, 2012; Simmons, 2001) seems to cause a complex relationship between female's fitness and their mating rate (Edvardsson, 2007; Rönn, Katvala, & Arnqvist, 2006).

Recent studies suggest that water, rather than nutrients, in the spermatophore is the most important substance that is transferred during copulations for female *C. maculatus* (Edvardsson, 2007; Ursprung, den Hollander, & Gwynne, 2009; but see: Fox & Moya-Laraño, 2009). This might imply that females suffer from dehydration (Arnqvist et al., 2005) since females often increase the frequency of mating when they are maintained without access to water, as is the norm in *Callosobruchus* experiments (Edvardsson, 2007; Paukku & Kotiaho, 2005). Except for the hydration benefit, females that receive larger spermatophores obtain other benefits such as increased fecundity and longevity (Fox, 1993; Savalli & Fox, 1999a; Snook, 2014; Tseng et al., 2007) due to the larger quantity of water (Paukku & Kotiaho, 2005), which is especially important for insects such as *C. maculatus* that inhabit areas where water is scarce (Harano, 2012; Paukku & Kotiaho, 2005). The hydration benefits females receive through spermatophores have also been shown in other species of insects, like

the cricket *Gryllus sigillatus* (Johnson, Ivy, & Sakaluk, 1999), but it appears that females here allocate water from the spermatophore towards reproduction as opposed to survival (Arnqvist et al., 2005). Further, Edvardsson (2007) showed that dehydrated female *C. maculatus* did not have the strength to fend off harassing males during mating, which often limits female reproductive output and lifespan. However, if the females are able to derive water from large ejaculates, they may be able to change their mating behavior in response to the trade-off between the need for additional water and the cost of mating (Edvardsson, 2007; Harano, 2012).

There is a lack of research done on the combined effects of supplemented water and sex ratio manipulation on mating behavior, fecundity and longevity of female bean beetles, *C. maculatus*. The aim of this thesis is to investigate whether the availability of supplemented water has an impact on male harassment and reproductive success of female *C. maculatus*. This has been done by measuring weight loss, number of eggs laid, and longevity of beetles with and without supplemented water. To investigate the impact of male harassment on female mating behavior, the sex ratio was manipulated. The following predictions were made: 1) both female longevity and fecundity will increase when water is available, 2) female longevity will decrease when sex ratio is male biased 3) water availability in a male biased environment will offset the cost of male harassment.

## **Materials and Methods**

### *Study species*

*C. maculatus*, also known as southern cowpea weevil, is an agricultural pest insect of stored *Vigna* legumes in tropical regions (Beck & Blumer, 2011; Paukku & Kotiaho, 2005). Females of this species lay eggs on the coat of *Vigna radiata*, mung beans (Eady et al., 2007), and the first instar larvae burrow themselves inside the beans 4 -5 days later (Beck & Blumer, 2011). Here they complete their development, pupation and eclosion in about 25 - 40 days (Daud & Taha, 2008; Eady et al., 2007). The development time is affected by the environmental factors such as temperature and relative humidity (Beck & Blumer, 2011). Both sexes are fully mature and ready to copulate 24 to 36 hours after eclosion (Beck & Blumer, 2011). In laboratory setting with a sex ratio 1:1, a single female is capable of producing more than 100 eggs in her lifetime (Beck & Blumer, 2011). While other published data suggest that a female, when having access to continuous mating, on average lay about 15 eggs every 24 hour (Utida, 1972; Wagner & Bakare, 2017). The eggs are approximately 0.75mm long and oval shaped,

they appear transparent when newly laid, and gradually turns white (Beck & Blumer, 2011). The adults of this species require no food or water to complete their life cycle, they rely on metabolic water and nutrients acquired during larval development. However, females may utilize supplemented water or nutrients if available (Ursprung et al., 2009), and several studies have demonstrated benefits from supplementary feeding (Adebayo & Oke, 2017)

### *General implementation*

The population used in present study was derived from a laboratory culture from Carolina Biological Supply, USA, and has been cultured on *Vigna* legumes at the Department of Biological Sciences, UiB, since February 2016. The same host bean was used throughout the experiment to eliminate the impact of host change (Messina, 2004). A total of 50 adult males and 50 adult females were used to start a new culture using a single layer of beans *V. radiata* in a petri dish. The culture was maintained in an incubator with a temperature of  $28 \pm 0.5$  °C and a 24-hour light cycle for three-four weeks. A few days before eclosion, beans with a single egg was separated into 24-well plates, to assure that individuals used in the experiment were virgins.

### *Weighing routines*

The initial weight of males and females was measured 24-48 hours after eclosion, using a Sartorius® M3P micro balance, a six-decimal balance. Each individual was placed in a tiny container with plastic film on top, to prevent escaping. Since the micro balance is sensitive to environmental factors such as air, humidity and clothing, two minutes passed before reading the final weight results. However, if the weight fluctuated too much either up or down i.e.  $>0.010$  mg within 20 seconds, it was calibrated. To calculate the measurement uncertainty of the weighing instrument in use, the following formula was used:  $1,7\mu\text{g} + 0,0409 \mu\text{g}/\text{mg} \times R$ , R is the measured weight of each individual. The weight of females the day they emerged, initial weight, had an uncertainty range of 0.03% - 0.05 % and ranged 0.04 % - 0.13 % on the day they died, final weight.

### *Experimental setup*

Six petri dishes, 100 x 15 mm, containing 15 grams of *V. radiata* (approximately 300 beans) were prepared; three of which contained a piece of cotton soaked in osmotic water kept in a small container (experimental group) and the rest without (control group). Each petri dish in both experimental and control group had 10 individuals with varying male to female ratio 1:1, 1:4 or 4:1. The dishes were kept in an incubator at  $28 \pm 0.5$  °C during the entire experiment.

Each dish was controlled daily and any fatalities were registered. Additionally, the cotton container was refilled with water if found too dry. Within 24 hours of death, the final body weight was measured. The experimental setup was conducted four times, consisting of 120 individual measurements of females in total. However, the beetles were not individually marked; hence average female weight loss for each group was used in statistical models. The total number of eggs laid on a sample of 100 beans for each dish was counted to calculate the average female oviposition in each petri dish. For analysis on weight loss and fecundity  $n$  represents the number of petri dishes, while in analysis of longevity  $n$  represents number of females.

### *Statistical analyses*

All statistical analyses were carried out in RStudio (1.2.1268). The backward elimination method was used, followed by two-way ANOVA for predictors such as weight loss and fecundity. This method was used to find out whether sex ratio manipulation and/or design had significant effect on weight loss and fecundity. Backward elimination is a stepwise regression method, normally used when there are few predictor variables. The analysis started with both variables, sex ratio and design, and subsequently simplified by eliminating one variable at a time (Brusco & Steinley, 2015); removing non-significant terms. A two-way ANOVA, an analysis of variance, was used to for further statistical analysis. The main purpose of a two-way ANOVA was to compare the mean differences between the groups that have been split into two independent variables (sex ratio and design), as well as to recognize any effects of an interaction between the two variables on the dependent variable, weight loss or fecundity (Laerd Statistics, 2018) (see Appendix A for coding of backward elimination method). To make sure that the backward elimination was suitable for the statistical analysis, diagnostics of the linear regression models were run on the models, the four plots: 1) residual versus fitted values, 2) normal Q-Q plot, 3) scale Location and 4) residuals versus leverage was as well adapted as possible with the collected data. An outlier was removed from the fecundity data due to a high Cook's distance; influence of individual observations on the estimated coefficients in a linear regression's analysis (Cook, 2011), since ANOVA is sensitive to outliers.

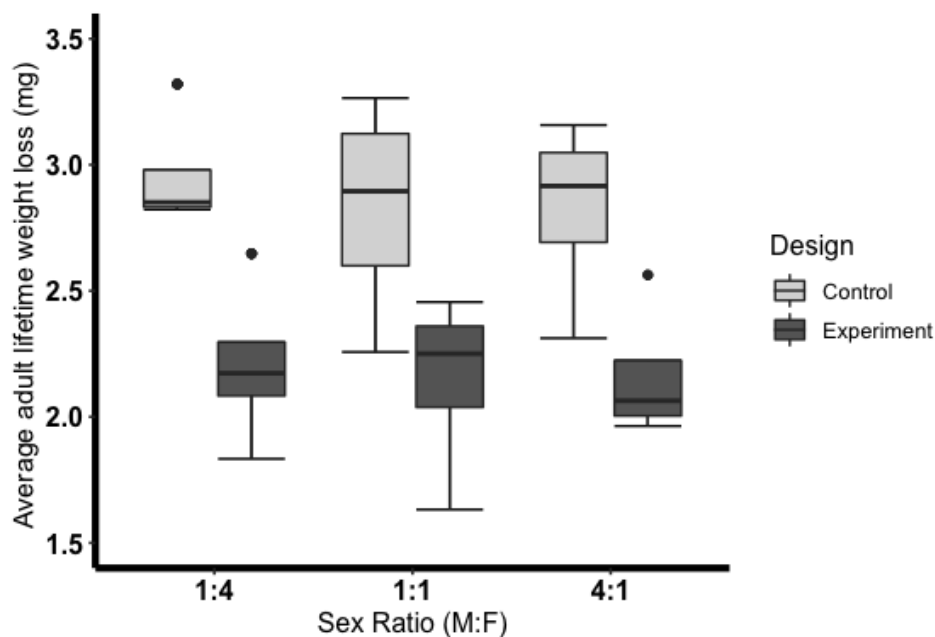
To test the effect of water availability and sex ratio on female longevity, a survival analysis was used. This function in Rstudio uses the Kaplan–Meier formula to create survival curves (Therneau & Grambsch, 2000) and compares the survival distributions of the sample with a log-rank test. This method estimates fraction of subjects living for a certain amount of

time after an event: the probability of surviving in a given length of time (Kishore, Goel, & Khanna, 2011). This type of analysis was chosen to handle the “time to event data” and to avoid any bias caused by censoring, if the total survival time for a subject cannot be exactly determined (Rich et al., 2010). Several survival models with different error distributions were made and further compared to each other through an ANOVA. The type of error distribution was chosen based on the model with the lowest unexplained variability (see Appendix B for coding of survival plot). All data are presented as mean  $\pm$  SE unless otherwise stated.

## Results

### *Female weight loss*

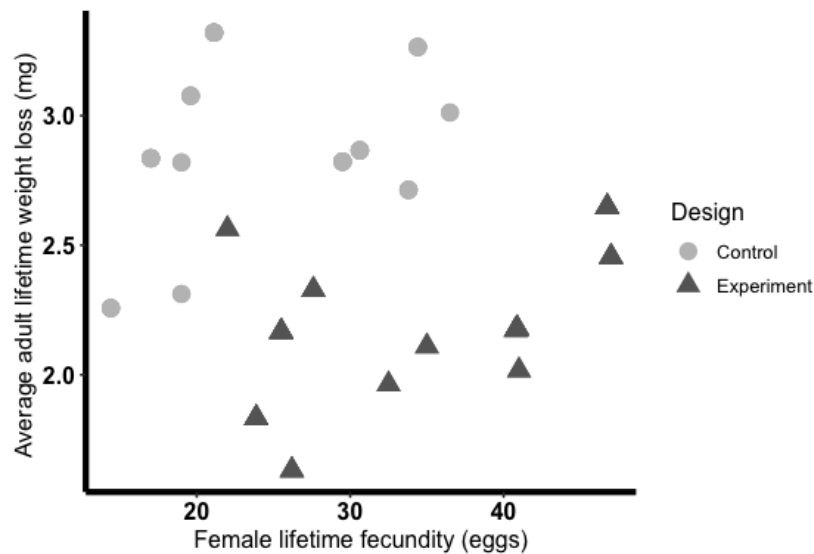
Average adult lifetime weight loss differed significantly between females that had access to water ( $2.2 \pm 0.1$  mg;  $n = 24$ ) and those that did not ( $2.9 \pm 0.1$  mg;  $n = 24$ ) (Linear model,  $F_{1,22} = 20.55$ ,  $p < 0.001$ ). Sex ratio manipulation did not have an effect on female weight loss within the experimental and control groups (Linear model,  $F_{1,22} = 20.55$ ,  $p = 0.82$ ; Figure 1).



**Figure 1.** Average weight loss (mg) of female *C. maculatus* in the experimental group (presence of water) or control group (absence of water) for different sex ratio manipulations. Sex ratio is given as the male to female ratio, 1:4 = 2M:8F, 1:1 = 5M:5F, 4:1 = 8 M:2 F.

### *Female fecundity*

Number of eggs laid differed significantly between females with access to water ( $34.1 \pm 2.6$  eggs/female;  $n = 23$ ) and those without ( $25.0 \pm 2.4$  eggs/female;  $n = 23$ ) (Linear model,  $F_{1,21} = 6.47$ ,  $p = 0.02$ ;  $n = 23$ ) (Figure 2). There was no effect of sex ratio manipulation on numbers of eggs laid (Linear model,  $F_{1,21} = 6.47$ ,  $p = 0.96$ ;  $n = 23$ ).

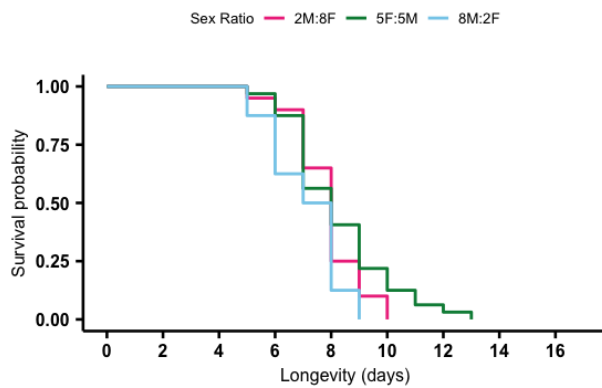


**Figure 2.** Average adult lifetime weight loss (mg) as a function of female lifetime fecundity (eggs). Female lifetime fecundity measured as average number of eggs laid per female *C. maculatus* in either experimental group (presence of water) or control group (absence of water). Both transparent and white colored eggs were counted on 100 beans.

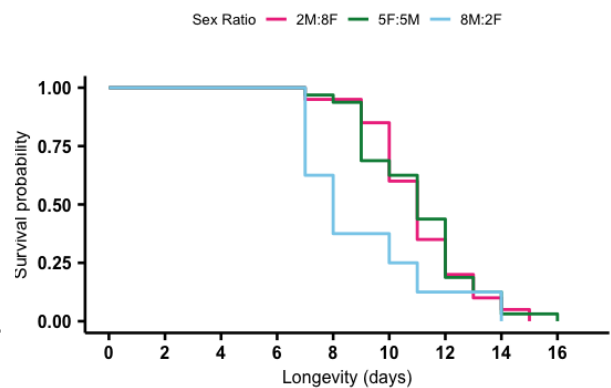
### *Female longevity*

Longevity differed significantly between females that had access to water ( $10.90 \pm 0.25$  days;  $n = 120$ ) compared to females without water ( $6.67 \pm 0.33$  days;  $n = 120$ ) (Log-rank test, *distribution* = "lognormal",  $p < 0.001$ ). Sex ratio manipulation had a significant effect on female adult lifespan (Log-rank test, *distribution* = "lognormal",  $p = 0.03$ ). In the control group, sex ratio of 1:1 did not differ from the female- nor the male-biased manipulation ( $p = 0.49$ ;  $p = 0.29$ ). Between the female-biased (1:4) and the male-biased (4:1) sex ratio, there was a small, but non-significant difference ( $p = 0.07$ ; Figure 3a). In the experimental group, male-biased sex ratio differed significantly from both 1:1 sex ratio ( $p = 0.005$ ) and the female-biased sex ratio ( $p = 0.003$ ) (Figure 3b).

a)



b)



**Figure 3.** (a) control group; (b) experimental group. Both plots represent survival probability of female *C. maculatus* as a function of time (days since emergence from bean). The longevity was measured from the day of eclosion until they died. The different survival curves represent male to female sex ratios from 1:4=2M:8F, 1:1=5M:5F and 4:1=8M:2F. Survival probability is the likelihood of individual survival at different stages of time.

## Discussion

Female *C. maculatus* that had access to water had lower weight loss, lived longer and had higher fecundity than females without water supplement. Male-biased sex ratio had a negative effect on longevity when females had access to water. This may indicate that the benefit of supplemented water does not offset the cost of male harassment in the male-biased populations as predicted.

The results of the present study suggest that female *C. maculatus* that had access to water, engaged in activities leading to the intake of water, such as sitting on the wet cotton. In terms of weight loss, females that had access to water lost less weight than those without. Despite the adults being able to reproduce without feeding or drinking, females chose to drink when water was available. Fox and Moya-Laraño (2009) similarly found a small but significant effect of water intake on weight loss. Several similar diet manipulation studies have been conducted, but with different types of solutions such as water, sugar- and yeast-water. Various results have been obtained, leading to mixed conclusions on how water and/or nutrients affect adult *C. maculatus*. Fox and Moya-Laraño (2009) found that access to water had little or no effect on female fecundity nor longevity, while access to sugar-water (10 % sucrose solution) i.e. calories did. The intake of sugar-water can be explained as a substitute to floral nectar, which function as an adult nutrient resource (Clement, 1992). In contrast,

Ursprung *et al.* (2009) and Edvardsson (2007) concluded that access to water alone did increase fecundity and longevity in female *C. maculatus*, consistent with the results in this study.

Water and nutrient supplements also influence females mating frequency. The willingness to remate has been found to decrease when water is available, suggesting that females who are water-deprived most likely remate due to water contained in the male's spermatophore (Edvardsson, 2007; Hudaib *et al.*, 2010). This is consistent with Harano (2012) research on *Callosobruchus chinensis*, another seed beetle. By experimentally manipulating water availability, he found that females were more likely to remate when deprived of water than when given. Based on this, he concluded that female receptivity to remating is plastic in response to water availability in *C. chinensis*, which may apply for *C. maculatus* as well (Harano, 2012). This is consistent with Edvardsson (2007) and Ursprung's *et al.* (2009) research, which conclude that females remate for ejaculate water rather than nutrients. Fox and Moya-Laraño (2009) came to the same conclusion but regarding nutrient-deprived *C. maculatus* females, indicating that females are capable of changing their remating receptivity in response to the need for nutrition. Similarly, katydids (Gwynne, 1990; Simmons & Gwynne, 1991), sagebrush crickets (Johnson *et al.*, 1999) and nursery web spiders (Bilde, Tuni, Elsayed, Pekar, & Toft, 2007) increase their mating frequency when they are nutrition-deprived as well. Further, Fox and Moya-Laraño (2009) and Fox (2006) distinguish between water-deprived and nutrient-deprived effects in their research and found that females were less likely to remate when sugar was available compared to water (Fox & Moya-Laraño, 2009). This suggests that the nutritional component of the spermatophore is more important for female mating behavior than the hydration benefit. For instance, female bush crickets, *Isophya kraussi*, obtain their energy by metabolizing carbohydrates in male spermatophore (Voigt *et al.*, 2008), which covers 2 or 3 days of metabolic requirements (Voigt, Michener, & Kunz, 2005). A distinction between hydration and nutrition effects is needed, which is supported by Arnqvist *et al.* (2005). Arnqvist and colleagues (2005) recommend doing further research on female mating while manipulating the environmental factors such as humidity, water, and nutrients availability.

However, in the present study, females that had access to water had higher fecundity compared to those without. The experimental setup, however, did not include measurements of mating frequency, therefore we cannot conclude whether females were able to change their remating receptivity in the response of the need of water. Based on the obtained results, water availability provides benefits in terms of less weight loss and higher fecundity. Nevertheless, there is conflicting results regarding the effects of multiple mating on fecundity and longevity in *C. maculatus*, indicating



that the understanding of this relationship is incomplete (Arnqvist et al., 2005; Torres-Vila, 2013). For instance, several studies have discovered that multiple mating increase female's oviposition (Savalli & Fox, 1999a; N. Wilson, Tufton, & Eady, 1999), whereas Fox (1993) did not found such effect. Although, Fox (1993) found that multiple mating increases females' lifespan, others have found the opposite (Crudginton, 2001). Savalli and Fox (1999a) found that females after receiving large spermatophores lay higher number of eggs, while Eady and Brown (2000) did not.

Further, Arnqvist et al. (2005) suggest that multiple mating can result in two distinct and viable mating strategies, since it is associated with interacting costs and benefits. When females *C. maculatus* do not have access to water nor nutrients, multiple mating is likely to increase their lifespan (Arnqvist et al., 2005; Fox, 1993). This is referred to as *benefit maximizers*, where females mate at a high rate. These females will experience more direct costs such as increased injury from male genitalia. However, at the same time, they will also receive larger amounts of water or nutritional substances from the ejaculates which will keep them hydrated. If water or nutrients is available, females mate at low rates, a strategy referred to as *cost minimizers* (Arnqvist et al., 2005). By accepting only one mating, females will minimize the direct cost of copulation and still maintain a high egg production rate early in life. Normally this mating strategy will involve more incompletely developed eggs and a higher risk of dehydration. This suggests that female *C. maculatus* alter their mating behavior to reduce the cost of polyandry when water is supplemented (Arnqvist et al., 2005). Although female *C. maculatus* have a higher mating frequency when they are deprived of resources, other insect species where females do not receive nuptial gifts, such as the water strider *Gerris buenoi*, females are more reluctant to mate under starvation. This is presumably because mating conflicts with the time spent foraging (Edvardsson, 2007; Rowe, 1992), which seems to effect frequency and duration of mating (Ortigosa & Rowe, 2002; Rowe, 1992). Therefore, Edvardsson (2007) concluded that female *C. maculatus* need to be more independent of the manipulative substances that males transfer through spermatophore if they should have a chance on changing their mating propensity in response to their need of resources. One solution to the independence from the male spermatophore is that female *C. maculatus* are expected to choose seeds with high moisture content to oviposit on (Hudaib et al., 2010). By doing so, the offspring will encounter less hydration stress, which is based on the assumption of the overall importance of water to *C. maculatus* (Hudaib et al., 2010). However, in a study done by Hudaib et al. (2010), this notion was not supported, as females preferred seeds with low moisture content. They suggested that a possible explanation for this was related to physical characteristics or chemical attributes of the host bean. For instance, the analysis of the micro-environment surrounding beans with high moisture revealed emissions of D-

limonene, which is an oil that function as oviposition deterrent (Hudaib et al., 2010). On the other hand, females respond much greater to the environmental cues associated with dry beans since females have evolved to exploit post-harvest storage conditions (Hudaib et al., 2010).

Previous studies have shown that female behavior and reproductivity are affected by male investment i.e. inseminating various amounts of spermatophore during copulations (McNamara et al., 2016). For instance, male *C. maculatus* lose about 5-10% of their body mass during transmission of the spermatophore (Fox, Hickman, Raleigh, & Mousseau, 1995; Savalli, Czesak, & Fox, 2000; Savalli & Fox, 1998), approximately 200  $\mu\text{g}$  (Katvala, Rönn, & Arnqvist, 2008). However, the amount of spermatophore transferred is reduced by each mating (Edvardsson, 2007; Wagner & Bakare, 2017). Wagner & Bakare (2017) propose that males after the first mating loose about 5.3 % of their body mass compared by the third mating 1.4%. While females either gain weight or have their weight loss offset by the transmission of the spermatophore. Thus, some of the female weight loss might have been offset by ejaculation transfer due to access to multiple males in the present study. Further research suggest that spermatophore size is correlated to male body size (Fox, Stillwell, Wallin, & Hitchcock, 2006), which appears to be influenced by diet and environmental conditions, such as temperature (Fox & Moya-Laraño, 2009). There is also evidence that temperature affects female body mass and fecundity (Fox et al., 2006). In insects, morphological and physiological plasticity often tends to represent an adaptive respond to environmental conditions (Salifou et al., 2016). Salifou and colleagues (2016) found that body size and life history traits in *C. maculatus* vary among the different strains, despite an apparent homogeneity. The morphological and biological diversity among the strains seems to correlated with their origin, where main climatic factors such as temperature and relative humidity is responsible (Ofuya & Credland, 1995; Salifou et al., 2016). For instance, a strain from Senegal is usually smaller and has a lower intrinsic rate of natural increase, which thought to correlate with the decreased lifespan and fecundity, compared to the strains from Burkina Faso and Niger (Salifou et al., 2016). The strains experience different climatic conditions which might not only affect body size but also the necessity to remate to utilize the water from spermatophore (Salifou et al., 2016). There are significant differences among biotypes, such as how beetles utilize water, tolerance of water stress as well as biological parameters such fecundity and longevity (Fox & Moya-Laraño, 2009; Yoder, Christensen, & Keeney, 2010). This might indicate that water deprivation alone is unlikely to be the only reason why polyandry has evolved as a mating strategy in *C. maculatus* (Fox & Moya-Laraño, 2009). However, the origin of the population used in the present study is unknown. Furthermore, beetles have been maintained in a laboratory setting for several generations, it is possible that this population has been adapted to these artificial conditions.

Even if environmental conditions might affect the spermatophore size, there must be an explanation to why males have evolved to transfer large spermatophores during copulations (Edvardsson, 2007). A virgin male *C. maculatus* can through a single insemination transfer tens of thousands of sperm, whereas females only manage to store efficiently about 15 % in their spermathecae, primary site of sperm storage (Eady, 1994, 1995), causing sperm to be lost at a constant rate (Eady, 1994). Both sexes benefit from the transfer of such large spermatophores. The “excess” sperm can either function to delay or prevent female’s receptivity for a new partner and/or function as resources for females to utilize. Based on this, during intense sperm competition males would benefit from producing large spermatophores to ensure fertilization since the amount of sperm will either prevent or delay female receptivity. Once females spermathecae are saturated i.e. the necessary sperm for fertilization are transferred, without reducing fertility, the remaining spermatophore can then be metabolized by the female (Eady, 1994). For instance, butterflies (Lepidoptera) follow a nutrient-routing strategy where they allocate essential amino acids to egg production and non-essential material to metabolism (O’Brien, Fogel, & Boggs, 2002; Voigt et al., 2008). This might explain why females benefit from polyandry, when other resources such as water or nutrients are not available. An interesting question is whether the excess sperm in insects also constitute a form of parental investment or as a mating effort (Edvardsson, 2007; Vahed, 1998; Wright, 1998). Regardless the different effort, the two alternatives can be traded off against each other since increased investment in one of them leads to less resources being available for the other one (Wright, 1998). However, Vahed (1998) concluded that mating effort is the most prevalent function of the excess sperm. This is based upon most female insects being promiscuous and sperm competition between males is high, leading to much of the male’s investment to be wasted on the production of offspring fathered by other males (Edvardsson, 2007). Additionally, there is no evidence which suggests that large ejaculates function as a parental investment.

Access to multiple males or high density of males can either impose a cost or a benefit to females due to the elevated mating frequency and/or male harassment (Iglesias-Carrasco et al., 2018). The degree to which females increase their mating rate when more males are present is presumably dependent on the cost of resisting males (Harano, 2012; Iglesias-Carrasco et al., 2018). Therefore, female’s optimal mating strategy is expected to be density dependent in order to acquire direct and indirect benefits (Iglesias-Carrasco et al., 2018). When male density is low, females experience reduced mating opportunities and are predicted to be less selective towards males, as there are costs associated with locating mates (Iglesias-Carrasco et al., 2018). In contrast, being exposed to a high male density, females may be more

selective and more reluctant to remate due to the costs of excess mating and/or increased male harassment (Iglesias-Carrasco et al., 2018). Manipulation of female mating frequency by either enforcing monandry or polyandry or by modification of adult sex ration will typically increase the strength of sexual selection. Although sex ratio manipulation has been widely used to alter sexual selection in a wide range of species, this approach has not commonly been used in *C. maculatus* studies (McNamara et al., 2016). This notion is supported by Iglesias-Carrasco et al. (2018), who propose that the effect of male density on female fitness is still unknown. However, in the present study, the sex ratio manipulation was altered to determine whether supplemented water could offset the cost of male harassment in a male-biased environment. The results suggest that male-biased sex ratio environments had a negative effect on female longevity. Findings from other studies revealed that females exposed to a high density of males had lower fecundity over their lifetime, whereas females with access to water lived longer (Edvardsson, 2007; Iglesias-Carrasco et al., 2018). Compared to results obtained in the present study 1) there was no significant effect of male-biased environment on fecundity, 2) females lived longer with access to water compared to a without, but a high density of males had a negative effect on longevity. A proposed explanation might be that females will have lower fitness due to increased male harassment and injuries through multiple matings. In addition to male density and presence of water and/or nutrients, environmental conditions might influence female mating behavior.

A model study done by Yasui (2001) suggests that monogamy may evolve under stable environmental conditions, but only when females do not receive benefits, except for bet-hedging and experience low costs from multiple mating (Miyatake & Matsumura, 2004; Yasui, 2001). However, laboratory environments may also impose a more promiscuous mating strategy than in the field, such as in the medfly, *Ceratitis capitata* (Miyatake & Matsumura, 2004). It has been reported that up to 40 % more remating in the laboratory set up compared in the field 3.8 % - 21 % (Bonizzoni et al., 2002; Chapman, Miyatake, Smith, & Partridge, 1998). Remating intervals may also be affected by laboratory settings, for instance in melon fly species *Bactrocera cucurbitae*, females tend to have shorter remating intervals in the laboratory than in the wild populations (Kuba & Soemori, 1988; Miyatake & Matsumura, 2004). Male harassment is often neglected in laboratory experiments of *C. maculatus*; females are often enclosed in small spaces with high male density, which might eliminate the natural response of avoidance of male that reduces the cost of harassment (den Hollander & Gwynne, 2009). In wild populations female *C. maculatus* may tend to avoid areas with high male

density and thereof excess mating (den Hollander & Gwynne, 2009). Therefore, laboratory experiments may poorly reflect natural conditions (Rönn et al., 2006). Additionally, a high male density in natural populations may also favor convenience polyandry (Huchard et al., 2012; Krupa & Sih, 1993). For instance, high male density would impose cost of rejections for females, therefore it tends to be more economical for females to increase mating frequency rather than continue rejecting mating attempts. Further, Iglesias-Carrasco et al. (2018) concluded that relatively few studies have looked into how different environments affect male-female interactions. This is probably because it tends to correlate with changes in both male harassment and benefits of increased mating frequency, where females mating decisions is most likely depend on the environment (Iglesias-Carrasco et al., 2018). Further research of the combined effect of supplemented water and sex ratio manipulation is necessary to establish to what extent it affects female *C. maculatus* fecundity and longevity. The same applies to whether supplemented water can offset the cost of male harassments. Future studies should include more biased sex ratio manipulations, experiments with the removal of female's hind legs preventing them from resisting males, and measurements of mating frequencies to investigate the effect of male harassment on female fitness.

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## Appendix

### *Appendix A*

A copy of the script from RStudio that was used to create models and run test regarding weight loss and fecundity.

```
#Model: weight loss
```

```
mod1 <-lm(Mean_Mass_Loss_Female_Absolute ~Prop_Male*Design, data=BeanB1)
```

```
anova(mod1)
```

```
mod2 <-lm(Mean_Mass_Loss_Female_Absolute ~Design, data=BeanB1)
```

```
anova(mod2)
```

```
summary(mod2)
```

```
par(mfrow=c(2,2))
```

```
plot(mod2)
```

```
#Model: Fecundity
```

```
mod5 <-lm(NO_Eggs_Female~Prop_Male*Design, data=BeanB2)
```

```
anova(mod5
```

```
  prop_male term
```

```
mod6 <-lm(NO_Eggs_Female~Design,data=BeanB2)
```

```
anova(mod6)
```

```
summary(mod6)
```

```
par(mfrow=c(2,2))
```

```
plot(mod6)
```

## Appendix B

A copy of the script from RStudio that was used to create models and run test regarding longevity

```
#Longevity - Sex Ratio
```

```
fit1.surv <- survreg(Surv(Life_Length)~Prop_Male, dist="logistic", data=BeanBE)
fit2.surv <- survreg(Surv(Life_Length)~Prop_Male, dist="extreme", data=BeanBE)
fit3.surv <- survreg(Surv(Life_Length)~Prop_Male, dist="gaussian", data=BeanBE)
fit4.surv <- survreg(Surv(Life_Length)~Prop_Male, dist="weibull", data=BeanBE)
fit5.surv <- survreg(Surv(Life_Length)~Prop_Male, dist="exponential", data=BeanBE)
fit6.surv <- survreg(Surv(Life_Length)~Prop_Male, dist="loglogistic", data=BeanBE)
fit7.surv <- survreg(Surv(Life_Length)~Prop_Male, dist="lognormal", data=BeanBE)
anova(fit1.surv, fit2.surv, fit3.surv, fit4.surv, fit5.surv, fit6.surv, fit7.surv)
```

```
#fit7.serv gir lavest unexplained variability--"lognormal"
```

```
fit2.surv <- survreg(Surv(Life_Length)~+1, dist="lognormal", data=BeanBE)
fit2.1.surv <- survreg(Surv(Life_Length)~Prop_Male, dist="lognormal", data=BeanBE)
anova(fit2.surv, fit2.1.surv, test="Chi")
summary(fit2.1.surv)
```

```
# Longevity: Design
```

```
fit1.surv <- survreg(Surv(Life_Length)~Design, dist="logistic", data=BeanBD)
fit2.surv <- survreg(Surv(Life_Length)~Design, dist="extreme", data=BeanBD)
fit3.surv <- survreg(Surv(Life_Length)~Design, dist="gaussian", data=BeanBD)
fit4.surv <- survreg(Surv(Life_Length)~Design, dist="weibull", data=BeanBD)
fit5.surv <- survreg(Surv(Life_Length)~Design, dist="exponential", data=BeanBD)
fit6.surv <- survreg(Surv(Life_Length)~Design, dist="loglogistic", data=BeanBD)
fit7.surv <- survreg(Surv(Life_Length)~Design, dist="lognormal", data=BeanBD)
anova(fit1.surv, fit2.surv, fit3.surv, fit4.surv, fit5.surv, fit6.surv, fit7.surv)
```

```
#fit7.serv gir lavest unexplained variability--"lognormal"
```

```
fit0.surv <- survreg(Surv(Life_Length)~+1, dist="lognormal", data=BeanBD)
```

```
fit1.surv <- survreg(Surv(Life_Length)~Design, dist="lognormal", data=BeanBD)
anova(fit0.surv, fit1.surv, test="Chi")
summar(fit1.surv)
```