Short-term water deprivation has widespread effects on mating behaviour in a harvestman

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Individual variation in resource acquisition prior to mating can influence the expression of sexually selected traits and mating dynamics. One important resource that has the potential to affect mating is water; however, little is known about whether variation in individual hydration status impacts mating behaviour. Here, we investigate the effects of short-term water deprivation on mating behaviour in the harvestman *Leiobunum vittatum*, commonly known as daddy longlegs. These harvestmen follow stereotyped stages of mating during which males attempt to clasp females in a 'mating embrace', then attempt to copulate, and may guard females after copulation; females are able to resist male attempts at each stage to end the interaction. During these interactions, males pass fluid nuptial gifts to females for their consumption. We tested how short-term water deprivation affects mating dynamics in harvestmen using a fully factorial, single-choice mating trials between males and females that were deprived or not deprived of water for 4 h. Water deprivation affected behaviour at multiple stages of mating. Females deprived of water were less likely to resist male advances but also less likely to be embraced by males. Most dramatically, the duration of intromission was greatly reduced in trials where both sexes were water-deprived. Together, these results suggest that even short periods of water deprivation impact mating-related behaviour of both sexes and may be an important but neglected factor in sexual selection.
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The condition dependence of many traits and behaviours important for mating is well established (Cotton, Small, & Pomiankowski, 2006; Rowe & Houle, 1996). Resources acquired during adulthood — such as food and nutrients — can significantly influence the expression of sexually selected traits and behaviours, consequently affecting mate acquisition and mating outcomes (Miller & Svensson, 2014). Suboptimal resource acquisition can impact behaviour from all stages of mating, including precopulatory displays and receptivity (Bilde, Tuni, Elsayed, Pekar, & Toft, 2007; Kotiaho, 2000, 2002; Wagner & Hoback, 1999; Yuval, Kaspi, Shloush, & Warburg, 1998), pericopulatory behaviours such as copulation duration and sperm transfer (Field & Yuval, 1999; Fricke, Bretman, & Chapman, 2010; Perry & Rowe, 2010), and post-copulatory behaviours such as intersexual cannibalism and remating rates (Abraham, Goane, Cladera, & Vera, 2011; Andrade, 1998). Many of the mating behaviours impacted by nutritional

status can consequently affect reproductive success (e.g. Andrés & Rivera, 2000; Arnqvist; Danielsson, 1999; Bertram, Whattam, Visanuvimol, Bennett, & Lauzon, 2009; Blay & Yuval, 1997; Mappes, Alatalo, Kotiaho, & Parri, 1996). Thus, resource acquisition during adulthood can play a key role in determining individual reproductive fitness.

The majority of work on resource acquisition and sexual selection has focused on food and nutrients (Cotton, Fowler, & Pomiankowski, 2004). However, another critical but understudied resource — water — has the potential to affect mating behaviour because of its importance in the expression of a wide suite of behavioural traits (e.g. Doi & Toh, 1992; Friedman, Greene, & Gordon, 2019; Krause et al., 2011). Importantly, hydration status affects the expression of a number of behaviours that could consequently affect how animals interact in a mating context, including gregariousness (Cook, 1981; Stamps, 1976), activity levels (Davis & DeNardo, 2009; Lorenzon, Clobert, Oppliger, & John-Alder, 1999), physical performance (Beuchat, Pough, & Stewart, 1984), speed of locomotion (Prates, Angilleta, Wilson, Niehaus, & Navas, 2013) and movement patterns (Fryxell, 2008). Furthermore, many mating behaviours are

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physiologically expensive and may result in water loss (e.g. Franklin, Squires, & Stuart-Fox, 2012; Saeki, Kruse, & Switzer, 2005; Sparkes, Keogh, & Pary, 1996; Watson, Stallmann, & Arnqvist, 1998), posing an additional cost to mating when water is scarce. Animals may thus regulate mating-related behaviour when the risk of dehydration is high: for example, adult male toadlets reduce signalling when occupying dry nests (Mitchell, 2001) and terrestrial gastropods limit the duration of copulation due to the threat of desiccation (Baur, 1992).

Despite the potential for water acquisition to have wide-reaching effects on mating-related behaviours, the effects of direct water acquisition (as opposed to water acquired through food consumption) has been almost entirely ignored in sexual selection research. What we do know about direct water acquisition and mating is limited to the effects of long-term water deprivation on female mating behaviour in two groups of insects that naturally experience long-term water limitation in their environment. In both decorated crickets, *Gryllodes sigillatus*, and seed beetles (*Callosobruchus maculatus*), the hydration status of females affects receptivity and mating rates (e.g. Fox & Moya-Laraño, 2009; Ivy, Johnson, & Sakaluk, 1999). Specifically, female decorated crickets that are water-deprived consume additional spermatophylaxes from males (Ivy et al., 1999), and female seed beetles that are water-deprived mate more often (Fox & Moya-Laraño, 2009; Harano, 2012; Ursprung, den Hollander, & Gwynne, 2009). Obtaining water through mating interactions may allow females to produce more offspring (Ivy et al., 1999), while providing hydration to females may benefit males by reducing female remating rates and thus lowering the risk of future sperm competition (Edvardsson, 2007). These studies on decorated crickets and seed beetles clearly demonstrate that long-term water deprivation (i.e. 24 − 48 h to most of adulthood) can impact female mating behaviour in taxa where long periods without access to water may be common. However, due to wide-ranging effects of hydration status on behaviour discussed above, it would be surprising if water consumption did not affect a broad range of taxa and mating behaviours of both males and females.

In this study, we test, for the first time in any species, the independent and combined effects of short-term water deprivation (4 h) on male and female mating-related behaviour and their consequences for mating outcomes. We do so using the harvestman (commonly known as daddy longlegs) *Leiobunum vittatum* (Arachnida: Opiliones), a species found widely throughout eastern and central North America in deciduous forests (Burns, Hedin, & Shultz, 2013). Reliable access to water is critical for members of this species due to their large surface area/volume ratio (primarily because their legs are long and thin), which makes them susceptible to water loss (Machado & Macías-Ordóñez, 2007). In fact, death by desiccation can occur in less than a day in some members of the genus (Edgar, 1971).

Mating interactions in *L. vittatum* occur over distinct pre-, peri- and postcopulatory stages (Fowler-Finn, Triana, & Miller, 2014; Machado, Requena, Toscano-Gadea, Stanley, & Macías-Ordóñez, 2015); each stage potentially poses unique challenges that require sufficient water stores for optimal performance by either or both sexes. Prior to copulation, males grapple with and embrace females using their legs and pedipalps and females can resist embraces using a number of defensive behaviours (Fowler-Finn et al., 2014). Once embraced, males evert their genitalia using hydraulic pressure, sometimes everting and then withdrawing the penis over 100 times prior to mating (Fowler-Finn et al., 2014). Additionally, males orally pass glandular secretions containing essential amino acids as nuptial gifts to females prior to or during copulation (Burns et al., 2013; Kahn, Cao, Burns, & Boyer, 2018;

Machado & Macías-Ordóñez, 2007). If these nuptial gifts are composed of a significant amount of water, hydration status could potentially affect the composition or quantity of this nuptial gift. Once copulation has ended, some males remain with and guard the female by clasping her leg(s) (Fowler-Finn et al., 2014). Low water stores may impede the ability of males to perform any of these actions, reduce the ability of females to resist or control interactions and hamper the assessment of mate quality for both sexes. Furthermore, mating interactions in *L. vittatum* are long − sometimes exceeding 3 h (Fowler-Finn et al., 2019) − and these extended interactions may lead to significant water loss for both sexes. For example, locomotion in males has been observed in multiple field and laboratory situations to be severely hampered following copulation, with males in the laboratory regaining the ability to walk properly only after access to water (K. D. Fowler-Finn, personal observation).

Based on the behavioural information presented above, we tested the hypothesis that short-term water deprivation has sex-specific effects on mating dynamics because water deprivation will affect motivation and/or performance in the sexes in different ways. Broadly, we predicted that dehydrated males would be less willing or able to mate, have a harder time securing females and be less likely to mate-guard following copulation compared to hydrated males. The predictions for the effects of water deprivation on females are less straightforward. Water-deprived females may be more likely to mate and show less resistance if they can recoup water through the nuptial gift, but less likely to mate if nuptial gifts are not typically hydrating. Finally, given that predictions for dehydration in males oppose predictions for females, we made no specific predictions regarding the effects of hydration status when both sexes are water-deprived. To test the effects of water deprivation on male and female behaviour, we withheld water from males, females, both, or neither sex for 4 h prior to single-choice mating trials. We then examined mating behaviour and outcomes across pre-, peri- and postcopulatory stages of mating. We found that the effects of water deprivation depended both on the stage of the mating interaction and on which sex was deprived of water. Given the extensive consequences of short-term dehydration on mating behaviour in *L. vittatum*, we suggest that hydration status may be an overlooked, but important, factor in mating dynamics in many taxa.

METHODS

Husbandry

We collected adult *L. vittatum* from vegetation, leaf litter and a dry creek bed found in a forested area near St Louis, Missouri, U.S.A. (38°33′1″N, 90°32′35″W) in September 2016 and in September and October 2017 and brought them to the laboratory at Saint Louis University. We determined the sex of each individual, gave each individual a unique identity number and housed them in individual plastic deli cups (10 cm diameter × 7.6 cm depth). Males can be distinguished from females by their smaller bodies and larger pedipalps (Burns et al., 2013). We covered the deli cups with mosquito netting and a lid with a hole cut out to allow airflow. The mosquito netting on the top of the container and a popsicle stick provided surfaces on which the harvestmen could climb (Appendix, Fig. A1). We provided water and food, consisting of crushed fish flakes, ad libitum, and changed cages as needed to keep them free of mould. All harvestmen were kept in the laboratory at room temperature with ~65% relative humidity. All animals were handled and kept in concordance with the ASAB/ABS Guidelines for Use of Animals in Research.

Water Deprivation Treatments

Males and females were randomly assigned to one of two treatments: (1) deprived of water ($N = 30$ for both males and females) or (2) not deprived of water ($N = 43$ for both males and females). To deprive individuals, we placed them in a new enclosure with a popsicle stick but no water or food for 4 h. Those assigned to the no deprivation treatment were placed in a similar enclosure for 4 h, but with a dish of water. Thus, the only difference between treatments was whether or not an individual had direct access to water. Many mating interactions can exceed 3 h, and preliminary qualitative observations showed that 4 h of water deprivation results in normal feeding and locomotion but slowed reaction times (K. D. Fowler-Finn, personal observation).

Mating Arenas

Mating trial arenas were set up similarly to Fowler-Finn et al. (2014, 2018, 2019). In brief, we made a circular arena measuring 25.5 cm in diameter with 11.5 cm high walls constructed from transparent acetate to prevent harvestmen from climbing. The floor of the arena consisted of white printer paper. We wiped down the arena with ethanol and replaced the paper floor in between trials to remove any potential chemical cues.

Mating Trials

We used a fully factorial design to randomly pair individuals for mating trials, in which the male, female, neither or both individuals were deprived of water prior to a trial. After 4 h in their respective treatments, we transferred each harvestman into individual 8 cm acetate holding pens within the mating arena (Appendix, Fig. A2). After 2 min of acclimation, or when the individuals were settled (which never took longer than 5 min), we lifted the holding pens to allow the harvestmen to freely interact until the end of the trial. Trials ended once an embraced pair broke apart and ended contact (whether or not intromission occurred), when a female rejected the male's attempt to embrace three times, or after 3 h, whichever condition came first (following Fowler-Finn et al., 2014, 2018, 2019). We recorded all trials on a Canon digital camera (model: Vixia HF R52) for later behavioural analyses. We ran 40 trials in September and October 2016 and 33 trials in September and October 2017. All mating trials were started between 0900 and 1700 hours.

Overall Trial Outcome

We recorded the overall outcome of the mating trials as follows: (1) a failure to embrace if the male never successfully embraced the female; (2) an embrace with no intromission if the male embraced the female but never copulated; or (3) a successful mating if the male both embraced and copulated with the female (identified by when the male inserts his penis into the female's genital opening and inflates his haematodocha; Fowler-Finn et al., 2014). These three possibilities represent the stages at which mating interactions can break down and may allow us to distinguish the causes of failed mating attempts.

To test the role of water deprivation on overall trial outcome, we ran a nominal logistic regression model with mating outcome as the dependent variable. The model contained male water deprivation treatment (yes/no), female water deprivation treatment (yes/no) and a male treatment by female treatment interaction term as independent variables. The model also included male body size, female body size, a male treatment by male body size interaction and a female treatment by female body size interaction as additional independent variables. Given that mating dynamics can vary seasonally (K. D. Fowler-Finn, personal observation), we also included Julian day (days from 1 January) as a covariate in the model. Two trials ended prematurely and so were excluded from the analysis of overall outcome.

Behavioural Analyses

In addition to the overall outcome of the trial, we scored several mating behaviours within each of the three stages of *L. vittatum* mating (precopulation, pericopulation and postcopulation).

(1) Precopulatory behaviours involve the male's attempts to position himself to mate (and sometimes female resistance to these attempts). Once contact occurs, the male almost always grapples with the female in an effort to embrace the female in a position that limits her movements. This position consists of the male and female oriented towards each other, with the male's pedipalps hooking the female behind the coxae of her second legs (Fig. 1; Fowler-Finn et al., 2014, 2018, 2019). Males that are not able to embrace the female on their first attempt usually try again once contact with the female is re-established. During the precopulatory phase of mating, we scored three behaviours (all previously described in Fowler-Finn et al., 2014): (a) whether females resisted male embraces (identified when a female fled, bobbed violently and/or dipped her frontal end in a downward position to seemingly make herself difficult to grasp); (b) whether males successfully embraced females on their first attempt; and (c) whether males successfully embraced a female in any of his first three attempts.

(2) Pericopulatory behaviours occur when pairs are in intromission. During this phase of mating, we scored whether embraced pairs copulated and, if so, the duration of intromission.

(3) Postcopulatory behaviours occur after intromission has finished. During this phase, we scored whether males that copulated subsequently guarded the females (i.e. whether pairs stayed in contact for > 15 s after intromission ended, usually with the male gripping the female's leg with his own second legs and following her around the arena, which has also been observed in the field; K. D. Fowler-Finn, personal observation) and the duration of postcopulatory contact.

We tested whether water deprivation affected the presence/absence of mating behaviours scored during each stage of mating by running nominal logistic regressions. The dependent variables in these models were female resistance (yes/no), embrace on male's first attempt (yes/no), any successful embrace over the course of the trial (yes/no), intromission for pairs that embraced (yes/no) and postcopulatory guarding for pairs that copulated (yes/no). The models included male water deprivation treatment (deprived/not deprived), female water deprivation treatment (deprived/not deprived), and a male treatment by female treatment interaction
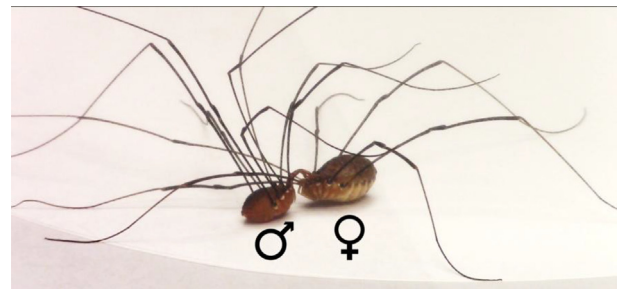

Figure 1. A male (left) and female (right) *Leiobunum vittatum* in a stereotypical embrace.

term as independent variables. We additionally included the following independent variables in the model: male body size, female body size, a male treatment by male body size interaction, a female treatment by female body size interaction, and Julian day as a covariate. Due to a smaller sample size and the issue of statistical separation (Allison, 2008), the model for postcopulatory guarding did not include male by female treatment interaction as a fixed effect. We have incomplete data for two trials where precopulatory behaviours could not be scored because of incomplete filming; we include these trials in all analyses except for the analyses of female resistance and embrace on first attempt.

We tested whether water deprivation affected the duration of intromission and the length of postcopulatory guarding using parametric survival models with either intromission duration or postcopulatory guarding duration as the dependent variable. Per Akaike's information criterion (AIC) scores, a Weibull distribution fit best for the intromission duration model while an exponential distribution fit best for the postcopulatory guarding model. Thus, we used these distributions for their respective models. In each model, we included male water deprivation treatment (deprived/not deprived), female water deprivation treatment (deprived/not deprived) and a male treatment by female treatment interaction term as independent variables. Additionally, we included in the models male and female body size, a male treatment by male body size interaction and a female treatment by female body size interaction as independent variables. Julian day also was included as a covariate. Six trials ended with mated pairs still in postcopulatory contact (some pairs stayed in contact for > 160 min); we used the censor function of the survival analysis to account for these trials in the postcopulatory guarding analysis (SAS Institute, 2018).

Body Size Measurements

Both male and female body size impact male–female interactions in *L. vittatum* (Fowler-Finn et al., 2014, 2018) and are therefore important to measure and statistically control for when quantifying the effects of water deprivation on mating behaviours and outcomes. Thus, once trials were complete, we preserved all individuals in 70% ethanol for morphological analyses. We took two photographs of the dorsal surface of each harvestman — remounting the individual in between photos — using a Leica camera (MC170 HD) attached to a Leica microscope (M205 C). We oriented every individual in a standardized position with the pedipalps facing forward. We used these pictures to measure cephalothorax width using Leica's LAS X software (v.3.0.4). Cephalothorax width is a good proxy for static body size measurements commonly used for arachnids (e.g. Fowler-Finn & Hebets, 2006; Hebets, Gering, Bingman, & Wiegmann, 2014; Rittschof, 2010; Sasson, Johnson, & Brockmann, 2012) and is measured in harvestmen as the width from the end points of the carapace between the right and left second and third pairs of legs (Fowler-Finn et al., 2014, 2018). For every individual, we measured each of the two pictures twice and then took the mean of means for the cephalothorax width measurement.

The Effect of Water Deprivation on Body Mass

In 2017, we weighed a subset of individuals at three time points: just prior to placing them in their assigned treatment for 4 h, immediately prior to the start of the behavioural trial and immediately after the mating trial ended ($N = 20$ for each sex). We weighed individuals to the nearest 0.1 mg using a balance (Mettler Toledo XS104). We used the difference in mass before and immediately after the treatment to assess the impact of the water deprivation treatment on mass. We used the difference in mass

before and after the mating trial to test for the possibility that intromission and/or hydration status affected the change in mass during a mating interaction. Changes in mass could be due to energetic expenditures during the mating interaction or the passing of a nuptial gift and/or ejaculate from the male to the female, all of which could be affected by the hydration status of each sex. One male and three females excreted faeces and/or fluids during the mating trial and were thus excluded from this analysis.

We first tested whether individuals from the two treatments differed in mass prior to undergoing the treatment using t tests run separately for each sex. Next, to determine the effect of water deprivation treatment on body mass, we compared mass lost/gained across the two treatments using a separate t test for each sex.

We used general linear models to test whether water deprivation and intromission affected mass loss over the course of the trial for pairs that embraced, as might be expected if water deprivation limits the amount of fluid males pass to the female during copulation. In these models, the dependent variable was the amount of mass lost while the independent variables were deprivation treatment for both the male and the female, whether intromission occurred, trial duration (log transformed) and the mass of both the male and the female at the start of the trial. We ran separate models for mass lost by males and mass lost by females. Additionally, we tested whether male and female mass loss within a trial were correlated by running a Pearson product-moment correlation.

We used general linear models to test whether water deprivation affected mass loss for pairs that copulated. Because only individuals that copulated were included, we reduced the number of variables to accommodate the smaller sample sizes in these models ($N = 9$ for males, $N = 8$ for females). In these models, the dependent variable was the amount of mass lost during the trial while the independent variables were water deprivation treatment (coded as neither deprived, male deprived, female deprived, or both deprived), trial duration (log transformed) and the mass of both the male and the female at the start of the trial. We ran separate models for mass lost by males and mass lost by females. Additionally, we tested whether male and female mass loss within a trial were correlated by running a Pearson product-moment correlation.

All statistics were conducted using JMP 14.0 (SAS Institute, Cary, NC, U.S.A.).

RESULTS

The Effects of Water Deprivation on Overall Outcomes

Sixteen per cent of trials ended without an embrace, 42% of trials ended with an embrace but no intromission, and the remaining 42% of trials ended in an embrace with intromission ($N = 71$ trials). Mating trial outcome was affected by female, but not male, water deprivation: mating trials with water-deprived females were more likely to end without a successful embrace, although the difference was marginally nonsignificant ($P = 0.052$; Table 1, Fig. 2). Additionally, trials were somewhat more likely to result in intromission following a successful embrace when females were water-deprived (Fig. 2). The only other factor influencing trial outcome was female size: trials were more likely to end without an embrace when females were small (Table 1).

The Effects of Water Deprivation on Presence/Absence of Mating Behaviours

Females that were not water-deprived were more likely to resist male attempts to embrace (Table 1) but also more likely to eventually be secured in an embrace, although this latter result was

marginally nonsignificant ($P = 0.051$; Table 1). Additionally, female size affected resistance and successful embraces: smaller females were less likely to resist but also less likely to be embraced on a male's first attempt or at any point during the trial. Male water deprivation by itself did not influence the likelihood of any of the mating behaviours measured, but it did interact with male body size to affect two behaviours (Table 1): water-deprived males that were large were more likely to face female resistance and less likely to secure a female.

The Effects of Water Deprivation on Intromission and Postcopulatory Guarding

While neither male nor female water deprivation affected the likelihood of intromission (Table 1), both male and female water deprivation significantly affected the duration of intromission (Table 2). The duration of intromission was especially short when both sexes were water-deprived (Table 2, Fig. 3). We found no effect of water deprivation of either sex on the likelihood or duration of postcopulatory guarding (Tables 1, 2, Appendix, Fig. A4).

The Effects of Water Deprivation on Body Mass

Prior to the experimental treatment, the mass of individuals did not significantly differ between the two treatments (Appendix, Table A1). Both males and females assigned to the water deprivation treatment lost significantly more mass during the 4 h of water deprivation than individuals not deprived of water (Fig. 4, Appendix, Table A1).

All individuals lost mass over the course of the trial (Appendix, Fig. A3). For pairs that embraced during a trial, neither treatment nor intromission affected the mass lost during the trial for males or females (Appendix, Table A2). In fact, none of the factors tested affected mass lost by females over the course of the trial, but both the trial duration and the starting mass of the female significantly affected mass lost by males over the course of the trial: males lost more mass if paired with heavier females and if the trial was long (Appendix, Table A2). We also found that the amount of mass lost by males and females within a trial were positively correlated (Pearson correlation: $r_{14} = 0.61$, $P = 0.01$).

When we examined just those pairs that copulated, we again found that none of the factors tested affected the amount of mass lost by females over the course of the trial, but that the amount of mass lost by males was affected by trial length: heavier males and those in longer trials lost more mass (Appendix, Table A2). Males also tended to lose more mass when paired to heavier females,
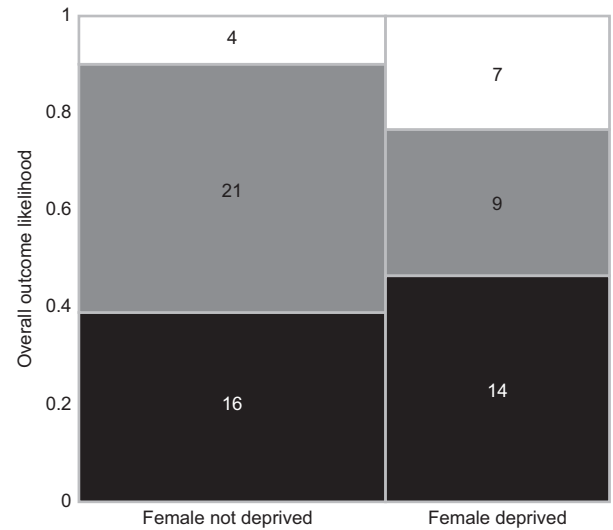

Figure 2. The likelihood that *Leiobunum vittatum* mating trials ended without an embrace (□), ended with an embrace but no intromission (▓), or ended in intromission if an embrace was achieved (■) when females were water-deprived ($N = 41$) or not deprived of water ($N = 30$). Numbers within each panel indicate the number of trials with that outcome.

Table 2
Statistical results from our models on the effects of water deprivation, male and female carapace width (CW) and their interactions on the duration of intromission and postcopulatory guarding

	Duration of intromission ($N = 30$)		Duration of postcopulatory guarding ($N = 22$)	
	χ^2	P	χ^2	P
♂ Deprivation	4.2	**0.042**	0.37	0.544
♂ CW	0	0.977	0.69	0.405
♂ Deprivation × ♂ CW	0.58	0.447	1.4	0.231
♀ Deprivation	14.2	**<0.001**	0.00	0.965
♀ CW	0.09	0.763	0.04	0.832
♀ Deprivation × ♀ CW	0.09	0.767	0.60	0.438
♂ Deprivation × ♀ deprivation	10.8	**0.001**	1.0	0.310
Julian day	2.3	0.133	1.2	0.271

Bolded P values indicate significant effects.

although this result was marginally not significant. The amount of mass lost by males and females within a trial was not significantly correlated ($r_6 = 0.59$, $N = 8$, $P = 0.13$).

Table 1
Statistical results from our models on the effects of water deprivation, male and female carapace width (CW), the interaction of water deprivation and CW, and Julian day on overall mating outcome and the likelihood of exhibiting each mating behaviour

	Overall outcome ($N = 71$)		Precopulatory behaviours						Peri- and postcopulatory behaviours			
			Female resistance ($N = 71$)		Embrace on 1st attempt ($N = 71$)		Any successful embrace ($N = 71$)		Intromission ($N = 60$)		Guarding ($N = 30$)	
	χ^2	P	χ^2	P	χ^2	P	χ^2	P	χ^2	P	χ^2	P
♂ Deprivation	1.1	0.587	0.64	0.423	0.73	0.393	0.18	0.668	0.86	0.353	1.4	0.238
♂ CW	0.18	0.915	3.9	**0.049**	0.31	0.578	0.03	0.873	0.04	0.836	0.67	0.413
♂ Deprivation × ♂ CW	4.7	0.097	4.4	**0.036**	0.9	0.333	3.5	*0.060*	1	0.324	0.5	0.464
♀ Deprivation	5.9	*0.052*	6.2	**0.013**	2.5	0.117	3.8	*0.051*	2	0.156	0.8	0.379
♀ CW	11	**0.004**	12	**<0.001**	4.5	**0.034**	11	**0.001**	0.4	0.512	0.3	0.585
♀ Deprivation × ♀ CW	3.4	0.182	0.4	0.53	2.8	0.096	3	0.084	0.4	0.554	0.1	0.789
♂ Deprivation × ♀ deprivation	0.5	0.783	0.1	0.758	1.1	0.298	0.1	0.757	0.3	0.590		
Julian day	2.9	0.233	1.3	0.256	2.4	0.122	2.6	0.108	0.4	0.513	0.2	0.655

Bolded P values indicate significant effects and italicized P values indicate marginally nonsignificant effects.
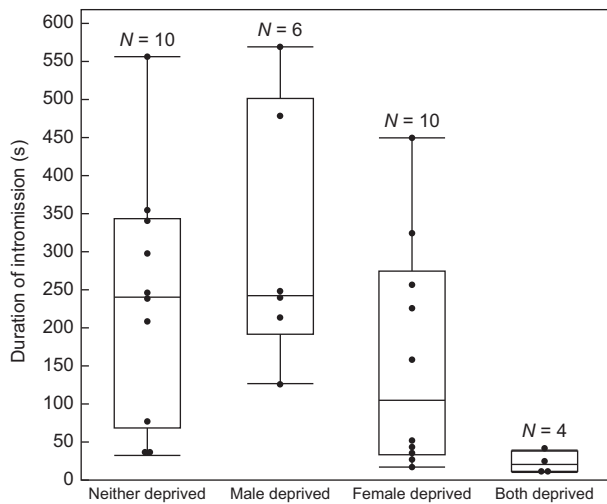
Figure 3. Intromission duration in *Leiobunum vittatum* when neither sex, both sexes or only one sex was deprived of water. Box plots show median intromission duration with first and third quartile ranges. Dots represent observed data points. Sample sizes for each treatment are given above the box plots.

Figure 4. Effect of water deprivation treatment on mass in male and female *Leiobunum vittatum*. □: Deprived; ▪ : Not deprived. Box plots show median mass with first and third quartile ranges. Dots represent observed data points. Sample sizes for each treatment are given above the box plots.

DISCUSSION

We found widespread effects of male and female short-term water deprivation on mating dynamics in the harvestman *L. vittatum* during pre- and peri- (but not post-) copulatory stages. While female water deprivation had direct effects on mating behaviour, the effects of male water deprivation were primarily mediated by male size. Furthermore, we found that male and female deprivation had sometimes similar, and other times opposing, effects. Notably, intromission length was dramatically reduced when both sexes were water-deprived. We also found that female deprivation increased female resistance and decreased the likelihood of a successful embrace. While we found no evidence for the transfer of a substantial nuptial gift, greater male weight loss in longer trials suggests that mating interactions are hydraulically costly. While food acquisition is well known to affect sexually selected traits and behaviours (e.g. Abraham et al., 2011; Alujua et al., 2001; Bertram et al., 2009; Fricke et al., 2010; Wagner & Hoback, 1999; Yuval et al., 1998), the considerable effects of short-term water deprivation that we found here indicate that the acquisition of water by itself may affect mating and reproductive success and could be an important but overlooked resource to consider in sexual selection research.

One dramatic effect of water deprivation on mating interactions that we found was a nearly order of magnitude reduction in intromission duration when both sexes were water-deprived (mean of 22 s with both sexes deprived versus 180 s for all other trials). While nutritional status affects copulation duration in some taxa (Aluja, Rull, Sivinski, Trujillo, & Pérez-Staples, 2009; Field & Yuval, 1999), this study is the first to our knowledge to show similar effects due to hydration status. If copulation duration in *L. vittatum* correlates with sperm transfer and/or reproductive fitness as it does in many taxa (Andrés & Rivera, 2000; Arnqvist & Danielsson, 1999; Edvardsson & Canal, 2006), water deprivation could directly reduce reproductive fitness for both male and female harvestmen. The cost of reduced intromission length could be particularly costly to water-deprived males if sperm competition is present, which seems likely given that both male and female *L. vittatum* are promiscuous (Macías-Ordóñez, 1997). Males that mate for brief periods may transfer few sperm and thus have a low chance at fertilization if sperm competition occurs in the female reproductive tract. Females that mate for
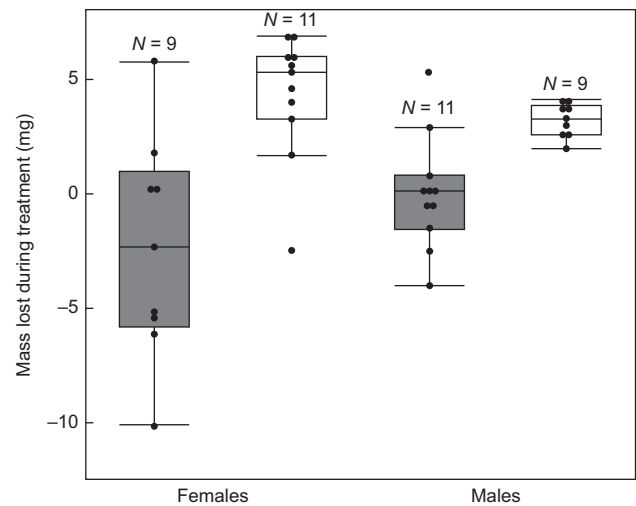
brief periods may be sperm-limited and unable to fertilize all their eggs, which would be especially costly if future mating attempts are limited. More broadly, the result of dramatically reduced intromission durations when both sexes were water-deprived underscores the importance of quantifying the interactive effects of male and female status, whether hydration or otherwise, on reproductive outcomes, particularly for animals with limited opportunities to mate.

Water deprivation in females directly affected the progression of mating interactions: water-deprived *L. vittatum* females were less likely to resist male attempts but less likely to embrace. While this may at first appear paradoxical, two nonmutually exclusive possibilities may explain the pattern. First, females may be able to indicate their unwillingness to mate to reduce male attempts. In support of this idea, we found that in most trials that ended without an embrace, the females showed no resistance (8 of 11 trials), a pattern found in multiple species in the genus (Fowler-Finn et al., 2018). Second, males may assess female hydration status and avoid water-deprived females if hydration status correlates with a measure of female quality such as egg production (e.g. Benoit et al., 2010; Broufas, Pappas, & Koveos, 2009). Male choice is well known in many taxa (e.g. Jones, Monaghan, & Nager, 2001; Lüpold, Manier, Ala-Honkola, Belote, & Pitnick, 2010; Sargent, Gross, & Van Den Berghe, 1986), and, in at least one harvestman species exhibiting exclusive paternal care, males seem to evaluate female quality based on cues present on the female's tegument (Requena & Machado, 2014). Furthermore, male *L. vittatum* sometimes show reluctance to mate, perhaps after some assessment of female quality (Machado et al., 2015), and show discrimination against females of other species in the genus (K. D. Fowler-Finn, personal observation). Thus, hydration status may affect not only an individual's behaviours but also the assessment of that individual by others. Further testing to discriminate between these two possibilities could prove insightful.

Even though water-deprived females were less likely to embrace, they tended to be more likely to copulate once embraced. This pattern may result from increased female receptivity when water-stressed (e.g. Fox & Moya-Laraño, 2009; Harano, 2012), which could be beneficial if mating allows females to recoup lost water, possibly through male nuptial gifts or male ejaculate (Ivy et al., 1999; Ursprung et al., 2009). Like other members of the

genus, *L. vittatum* males pass fluid nuptial gifts to females during mating (Burns et al., 2013; Kahn et al., 2018; Machado & Macías-Ordóñez, 2007). However, we found no effect of intromission or of female water deprivation on changes in mass for either sex, suggesting that males are not passing large amounts of fluid to the females. Given that mating behaviours are often energetically expensive (Franklin et al., 2012; Sparkes et al., 1996; Watson et al., 1998), females may be more likely to mate if it reduces the energetic or hydraulic costs of mating interactions. We found a positive correlation between male and female mass loss, and male mass loss increased with longer interactions, which suggests some interactions may be more costly than others. However, females that mated did not lose less mass than females that did not mate, as would be expected if copulating were a strategy to reduce energetic costs. Thus, it remains unclear whether increased female mating receptivity in *L. vittatum* buffers the effects of dehydration, as has been proposed for other taxa (Edvardsson, 2007; Harano, 2012; Ivy et al., 1999).

In contrast to females, male hydration status did not have any direct effects on the progression of mating interactions. Despite our prediction that water-deprived males would be less likely to pursue mating, we found that water-deprived males were equally likely as their hydrated male counterparts to attempt to mate, embrace females on their first attempt or at any point in the trial, and progress to intromission once embraced. Given the evidence that mating interactions are costly (males lost more mass in trials that were longer and when paired with heavier females), it appears that even water-deprived males are willing to incur the costs of any increased stress due to water lost during mating interactions. Furthermore, water-deprived males were also just as likely to mate-guard. Given the prevalence and significant benefits of mate guarding in this species (Fowler-Finn et al., 2019; Macías-Ordóñez, 1997), the willingness to guard, even when dehydrated, could have significant reproductive benefits that outweigh any costs associated with extended contact with females after intromission.

The effects of male water deprivation on mating interactions — apart from intromission duration — were mediated by male size. For example, large males that had access to water were less likely to embrace the female, a pattern we might expect if large males are in better condition, since males in better condition are often pickier in mate selection (e.g. Amundsen & Forsgren, 2003; Preston, Stevenson, Pemberton, Coltman, & Wilson, 2005; Shine, Phillips, Waye, LeMaster, & Mason, 2003). However, all of these large hydrated males attempted, but failed, to embrace the females. This pattern suggests that females may control the progression of mating (Fowler-Finn et al., 2014). We found that females more often resisted when paired with large males, especially when the males were water-deprived. Interestingly, females did not resist in the majority of trials that ended without an embrace, which occurred more often when males were large and not deprived of water. This result could be evidence that females are signalling their willingness to mate upon contact and that these large and hydrated males are heeding those signals. Conversely, large dehydrated males may be ignoring these female signals/cues and thus experience more female resistance. Males in poor condition or those with low life expectancy often increase reproductive effort (Sadd et al., 2006; Thanda Win, Kojima, & Ishikawa, 2013); water-deprived *L. vittatum* males may be more likely to ignore female cues if these males assess that their future reproductive success is low due to dehydration. Finally, we did not find any effects of water deprivation or intromission on mass loss in males, suggesting that nuptial gifts in *L. vittatum* are not substantial. Thus, whether water deprivation affects nuptial gifts remains an intriguing question that may be better answered in taxa where the quantification of nuptial gifts is more tenable.

Conclusions

The role of water deprivation on mating behaviour remains a relatively unexplored topic in sexual selection research, with this study being the first (to our knowledge) to examine the effects of short-term water deprivation of both sexes on mating behaviours. We found that even short periods of water deprivation have significant sex-specific and interactive effects. Short pulses of dehydration may be common for many taxa, either due to environmental availability or trade-offs with other activities. Thus, we suspect that hydration status is likely to have broad effects on mating across diverse taxa and should extend beyond systems living in arid environments. Furthermore, since many animals acquire water from food they ingest, our results suggest that future research investigating the impacts of food acquisition on sexually selected traits and behaviours may benefit from disentangling the specific effects of the nutrients and water acquired from the food. With the occurrence of drought and the decreased reliability of water sources on the rise globally (Dai, 2013; van Aalst, 2006), the impact of water in mating interactions should only increase, and thus, we encourage more widespread tests of water deprivation on mating behaviour and reproductive success.

References

Abraham, S., Goane, L., Cladera, J., & Vera, M. T. (2011). Effects of male nutrition on sperm storage and remating behavior in wild and laboratory *Anastrepha fraterculus* (Diptera: Tephritidae) females. *Journal of Insect Physiology, 57*(11), 1501–1509.

Allison, P. D. (2008). Convergence failures in logistic regression. *Proceedings of the SAS Global Forum: Statistics and Data Analysis, 2008*(360), 1–11. http://www2.sas.com/proceedings/forum2008/360-2008.pdf.

Aluja, M., Jácome, I., & Macías-Ordóñez, R. (2001). Effect of adult nutrition on male sexual performance in four neotropical fruit fly species of the genus *Anastrepha* (Diptera: Tephritidae). *Journal of Insect Behavior, 14*(6), 759–775.

Aluja, M., Rull, J., Sivinski, J., Trujillo, G., & Pérez-Staples, D. (2009). Male and female condition influence mating performance and sexual receptivity in two tropical fruit flies (Diptera: Tephritidae) with contrasting life histories. *Journal of Insect Physiology, 55*(12), 1091–1098.

Amundsen, T., & Forsgren, E. (2003). Male preference for colourful females affected by male size in a marine fish. *Behavioral Ecology and Sociobiology, 54*(1), 55–64.

Andrade, M. C. (1998). Female hunger can explain variation in cannibalistic behavior despite male sacrifice in redback spiders. *Behavioral Ecology, 9*(1), 33–42.

Andrés, J. A., & Rivera, A. C. (2000). Copulation duration and fertilization success in a damselfly: An example of cryptic female choice? *Animal Behaviour, 59*(4), 695–703.

Arnqvist, G., & Danielsson, I. (1999). Copulatory behavior, genital morphology, and male fertilization success in water striders. *Evolution, 53*(1), 147–156.

Baur, B. (1992). Random mating by size in the simultaneously hermaphroditic land snail *Arianta arbustorum*: Experiments and an explanation. *Animal Behaviour, 43*(3), 511–518.

Benoit, J. B., Patrick, K. R., Desai, K., Hardesty, J. J., Krause, T. B., & Denlinger, D. L. (2010). Repeated bouts of dehydration deplete nutrient reserves and reduce egg production in the mosquito *Culex pipiens. Journal of Experimental Biology, 213*(16), 2763–2769.

Bertram, S. M., Whattam, E. M., Visanuvimol, L., Bennett, R., & Lauzon, C. (2009). Phosphorus availability influences cricket mate attraction displays. *Animal Behaviour, 77*(2), 525–530.

Beuchat, C. A., Pough, F. H., & Stewart, M. M. (1984). Response to simultaneous dehydration and thermal stress in three species of Puerto Rican frogs. *Journal of Comparative Physiology B, 154*(6), 579–585.

Bilde, T., Tuni, C., Elsayed, R., Pekar, S., & Toft, S. (2007). Nuptial gifts of male spiders: Sensory exploitation of the female's maternal care instinct or foraging motivation? *Animal Behaviour, 73*(2), 267–273.

Blay, S., & Yuval, B. (1997). Nutritional correlates of reproductive success of male Mediterranean fruit flies (Diptera: Tephritidae). *Animal Behaviour, 54*(1), 59–66.

Broufas, G., Pappas, M., & Koveos, D. (2009). Effect of relative humidity on longevity, ovarian maturation, and egg production in the olive fruit fly (Diptera: Tephritidae). *Annals of the Entomological Society of America, 102*(1), 70–75.

Burns, M. M., Hedin, M., & Shultz, J. W. (2013). Comparative analyses of reproductive structures in harvestmen (Opiliones) reveal multiple transitions from courtship to precopulatory antagonism. *PloS One, 8*(6), e66767.

Cook, A. (1981). Huddling and the control of water loss by the slug *Limax pseudoflavus* Evans. *Animal Behaviour, 29*(1), 289–298.

Cotton, S., Fowler, K., & Pomiankowski, A. (2004). Do sexual ornaments demonstrate heightened condition-dependent expression as predicted by the handicap hypothesis? *Proceedings of the Royal Society of London, Series B: Biological Sciences, 271*(1541), 771–783.

Cotton, S., Small, J., & Pomiankowski, A. (2006). Sexual selection and condition-dependent mate preferences. *Current Biology, 16*(17), R755–R765.

Dai, A. (2013). Increasing drought under global warming in observations and models. *Nature Climate Change, 3*(1), 52.

Davis, J. R., & DeNardo, D. F. (2009). Water supplementation affects the behavioral and physiological ecology of Gila monsters (*Heloderma suspectum*) in the Sonoran Desert. *Physiological and Biochemical Zoology, 82*(6), 739–748.

Doi, N., & Toh, Y. (1992). Modification of cockroach behavior to environmental humidity change by dehydration (Dictyoptera: Blattidae). *Journal of Insect Behavior, 5*(4), 479–490.

Edgar, A. L. (1971). *Studies on the biology and ecology of Michigan Phalangida (Opiliones)*.

Edvardsson, M. (2007). Female *Callosobruchus maculatus* mate when they are thirsty: Resource-rich ejaculates as mating effort in a beetle. *Animal Behaviour, 74*(2), 183–188.

Edvardsson, M., & Canal, D. (2006). The effects of copulation duration in the bruchid beetle *Callosobruchus maculatus*. *Behavioral Ecology, 17*(3), 430–434.

Field, S., & Yuval, B. (1999). Nutritional status affects copula duration in the Mediterranean fruit fly, *Ceratitis capitata* (Insecta Tephritidae). *Ethology Ecology & Evolution, 11*(1), 61–70.

Fowler-Finn, K., Boyer, S., Ikagawa, R., Jeffries, T., Kahn, P., Larsen, E., et al. (2018). Variation in mating dynamics across five species of leiobunine harvestmen (Arachnida: Opiliones). *Biology, 7*(2), 36.

Fowler-Finn, K., Boyer, S. L., Ikagawa, R., Jeffries, T., Kahn, P. C., Larsen, E. M., et al. (2019). Qualitative and quantitative comparisons of mating behaviour across multiple populations and six species of leiobunine harvestmen (Arachnida: Opiliones). *Behaviour, 156*, 363–390.

Fowler-Finn, K. D., & Hebets, E. A. (2006). An examination of agonistic interactions in the whip spider *Phrynus marginemaculatus* (Arachnida, Amblypygi). *Journal of Arachnology, 34*(1), 62–77.

Fowler-Finn, K. D., Triana, E., & Miller, O. G. (2014). Mating in the harvestman *Leiobunum vittatum* (Arachnida: Opiliones): From premating struggles to solicitous tactile engagement. *Behaviour, 151*(12–13), 1663–1686.

Fox, C. W., & Moya-Laraño, J. (2009). Diet affects female mating behaviour in a seed-feeding beetle. *Physiological Entomology, 34*(4), 370–378.

Franklin, A. M., Squires, Z. E., & Stuart-Fox, D. (2012). The energetic cost of mating in a promiscuous cephalopod. *Biology Letters, 8*(5), 754–756.

Fricke, C., Bretman, A., & Chapman, T. (2010). Female nutritional status determines the magnitude and sign of responses to a male ejaculate signal in *Drosophila melanogaster*. *Journal of Evolutionary Biology, 23*(1), 157–165.

Friedman, D. A., Greene, M. J., & Gordon, D. M. (2019). The physiology of forager hydration and variation among harvester ant (*Pogonomyrmex barbatus*) colonies in collective foraging behavior. *Scientific Reports, 9*(1), 1–9.

Fryxell, J. M. (2008). Predictive modelling of patch use by terrestrial herbivores. In H. H. T. Prins, & F. Van Langevelde (Eds.), *Resource ecology* (Vol. 23, pp. 105–123). Dordrecht, The Netherlands: Springer.

Harano, T. (2012). Water availability affects female remating in the seed beetle, *Callosobruchus chinensis*. *Ethology, 118*(10), 925–931.

Hebets, E. A., Gering, E. J., Bingman, V. P., & Wiegmann, D. D. (2014). Nocturnal homing in the tropical amblypygid *Phrynus pseudoparvulus* (class Arachnida, order Amblypygi). *Animal Cognition, 17*(4), 1013–1018.

Ivy, T. M., Johnson, J. C., & Sakaluk, S. K. (1999). Hydration benefits to courtship feeding in crickets. *Proceedings of the Royal Society B: Biological Sciences, 266*(1428), 1523–1527.

Jones, K. M., Monaghan, P., & Nager, R. G. (2001). Male mate choice and female fecundity in zebra finches. *Animal Behaviour, 62*(6), 1021–1026.

Kahn, P. C., Cao, D. D., Burns, M., & Boyer, S. L. (2018). Nuptial gift chemistry reveals convergent evolution correlated with antagonism in mating systems of harvestmen (Arachnida, Opiliones). *Ecology and Evolution, 8*(14), 7103–7110.

Kotiaho, J. S. (2000). Testing the assumptions of conditional handicap theory: Costs and condition dependence of a sexually selected trait. *Behavioral Ecology and Sociobiology, 48*(3), 188–194.

Kotiaho, J. S. (2002). Sexual selection and condition dependence of courtship display in three species of horned dung beetles. *Behavioral Ecology, 13*(6), 791–799. https://doi.org/10.1093/beheco/13.6.791.

Krause, E. G., de Kloet, A. D., Flak, J. N., Smeltzer, M. D., Solomon, M. B., Evanson, N. K., et al. (2011). Hydration state controls stress responsiveness and social behavior. *Journal of Neuroscience, 31*(14), 5470–5476.

Lorenzon, P., Clobert, J., Oppliger, A., & John-Alder, H. (1999). Effect of water constraint on growth rate, activity and body temperature of yearling common lizard (*Lacerta vivipara*). *Oecologia, 118*(4), 423–430.

Lüpold, S., Manier, M. K., Ala-Honkola, O., Belote, J. M., & Pitnick, S. (2010). Male *Drosophila melanogaster* adjust ejaculate size based on female mating status, fecundity, and age. *Behavioral Ecology, 22*(1), 184–191.

Machado, G., & Macías-Ordóñez, R. (2007). Reproduction. In R. Pinto-da-Rocha, G. Machado, & G. Giribet (Eds.), *Harvestmen: The biology of Opiliones* (pp. 414–454). Cambridge, MA: Harvard University Press.

Machado, G., Requena, G. S., Toscano-Gadea, C., Stanley, E., & Macías-Ordóñez, R. (2015). Male and female mate choice in harvestmen: General patterns and inferences on the underlying processes. In A. V. Peretti, & A. Aisenberg (Eds.), *Cryptic female choice in arthropods: Patterns, mechanisms and prospects* (pp. 169–201). Cham, Switzerland: Springer.

Macías-Ordóñez, R. (1997). *The mating system of Leiobunum vittatum Say 1821 (Arachnida: Opiliones: Palpatores): Resource defense polygyny in the striped harvestman* (Ph.D. thesis). Bethlehem, PA: Lehigh University.

Mappes, J., Alatalo, R. V., Kotiaho, J., & Parri, S. (1996). Viability costs of condition-dependent sexual male display in a drumming wolf spider. *Proceedings of the Royal Society of London, Series B: Biological Sciences, 263*(1371), 785–789.

Miller, C. W., & Svensson, E. I. (2014). Sexual selection in complex environments. *Annual Review of Entomology, 59*, 427–445.

Mitchell, N. J. (2001). Males call more from wetter nests: Effects of substrate water potential on reproductive behaviours of terrestrial toadlets. *Proceedings of the Royal Society of London, Series B: Biological Sciences, 268*(1462), 87–93.

Perry, J. C., & Rowe, L. (2010). Condition-dependent ejaculate size and composition in a ladybird beetle. *Proceedings of the Royal Society B: Biological Sciences, 277*(1700), 3639–3647.

Prates, I., Angilleta, M. J., Jr., Wilson, R. S., Niehaus, A. C., & Navas, C. A. (2013). Dehydration hardly slows hopping toads (*Rhinella granulosa*) from xeric and mesic environments. *Physiological and Biochemical Zoology, 86*(4), 451–457.

Preston, B., Stevenson, I., Pemberton, J., Coltman, D., & Wilson, K. (2005). Male mate choice influences female promiscuity in Soay sheep. *Proceedings of the Royal Society of London, Series B: Biological Sciences, 272*(1561), 365–373.

Requena, G. S., & Machado, G. (2014). Mating behavior of a Neotropical arachnid with exclusive paternal care. *Acta Ethologica, 17*(1), 23–30.

Rittschof, C. C. (2010). Male density affects large-male advantage in the golden silk spider, *Nephila clavipes*. *Behavioral Ecology, 21*(5), 979–985.

Rowe, L., & Houle, D. (1996). The lek paradox and the capture of genetic variance by condition dependent traits. *Proceedings of the Royal Society of London, Series B: Biological Sciences, 263*(1375), 1415–1421. https://doi.org/10.1098/rspb.1996.0207.

Sadd, B., Holman, L., Armitage, H., Lock, F., Marland, R., & Siva-Jothy, M. (2006). Modulation of sexual signalling by immune challenged male mealworm beetles (*Tenebrio molitor*, L.): Evidence for terminal investment and dishonesty. *Journal of Evolutionary Biology, 19*(2), 321–325.

Saeki, Y., Kruse, K. C., & Switzer, P. V. (2005). Physiological costs of mate guarding in the Japanese beetle (*Popillia japonica* Newman). *Ethology, 111*(9), 863–877.

Sargent, R. C., Gross, M. R., & Van Den Berghe, E. P. (1986). Male mate choice in fishes. *Animal Behaviour, 34*(2), 545–550.

Sas Institute. (2018). *JMP 14 reliability and survival methods*. Cary, NC: SAS Institute.

Sasson, D. A., Johnson, S. L., & Brockmann, H. J. (2012). The role of age on sperm traits in the American horseshoe crab, *Limulus polyphemus*. *Animal Behaviour, 84*(4), 975–981. https://doi.org/10.1016/j.anbehav.2012.07.023.

Shine, R., Phillips, B., Waye, H., LeMaster, M., & Mason, R. (2003). The lexicon of love: What cues cause size-assortative courtship by male garter snakes? *Behavioral Ecology and Sociobiology, 53*(4), 234–237.

Sparkes, T. C., Keogh, D. P., & Pary, R. A. (1996). Energetic costs of mate guarding behavior in male stream-dwelling isopods. *Oecologia, 106*(2), 166–171.

Stamps, J. (1976). Rainfall, activity and social behaviour in the lizard, *Anolis aeneus*. *Animal Behaviour, 24*(3), 603–608.

Thanda Win, A., Kojima, W., & Ishikawa, Y. (2013). Age-related male reproductive investment in courtship display and nuptial gifts in a moth, *Ostrinia scapulalis*. *Ethology, 119*(4), 325–334.

Ursprung, C., den Hollander, M., & Gwynne, D. T. (2009). Female seed beetles, *Callosobruchus maculatus*, remate for male-supplied water rather than ejaculate nutrition. *Behavioral Ecology and Sociobiology, 63*(6), 781–788.

van Aalst, M. K. (2006). The impacts of climate change on the risk of natural disasters. *Disasters, 30*(1), 5–18.

Wagner, W. E., & Hoback, W. W. (1999). Nutritional effects on male calling behaviour in the variable field cricket. *Animal Behaviour, 57*(1), 89–95.

Watson, P. J., Stallmann, R. R., & Arnqvist, G. (1998). Sexual conflict and the energetic costs of mating and mate choice in water striders. *American Naturalist, 151*(1), 46–58.

Yuval, B., Kaspi, R., Shloush, S., & Warburg, M. S. (1998). Nutritional reserves regulate male participation in Mediterranean fruit fly leks. *Ecological Entomology, 23*(2), 211–215.

Appendix

Table A1
Effect of water deprivation treatment on mass in *Leiobunum vittatum*

	Mean ± SE mass (mg)			Mean % mass lost
	Before treatment	After treatment	Lost	
Males				
Deprived	83.1 ± 3.6	79.8 ± 3.6	3.2 ± 0.2	3.8
Not deprived	84.3 ± 3.7	84.3 ± 3.5	0.0 ± 0.8	0.0
Females				
Deprived	181.2 ± 9.3	176.8 ± 9.0	4.3 ± 0.8	2.4
Not deprived	182.4 ± 16.1	184.8 ± 15.4	-2.4 ± 1.6	-1.3

Table A2
The effect of intromission, water deprivation, starting mass and trial duration on mass lost during trials for pairs that embraced, and the effect of treatment, starting mass and trial duration on mass lost during trials where intromission occurred

Factor	Males			Females		
	F ratio	N	P	F ratio	N	P
Trials with embrace		17			15	
Intromission (Y/N)	1.8		0.212	0.53		0.487
Male deprivation	1.5		0.253	0.44		0.524
Female deprivation	0.23		0.639	0.51		0.494
Male mass	0.08		0.788	0.45		0.521
Female mass	7.0		**0.024**	0.51		0.494
Trial duration	5.8		**0.037**	2.0		0.192
Trials with intromission		9			8	
Treatment	6.1		0.144	7.3		0.264
Male mass	24.8		**0.038**	9.7		0.198
Female mass	10.7		*0.08*	12.7		0.174
Trial duration	40.6		**0.024**	5.4		0.259

Trial duration and intromission duration were log-transformed prior to analysis. Bolded P values indicate significant effects while italicized P values indicate almost significant effects.

Figure A2. Male (left) and female (right) in the mating arena prior to being allowed to interact.

Figure A1. Cages used to house harvestmen. Photo credit: Leticia Classen-Rodriguez.
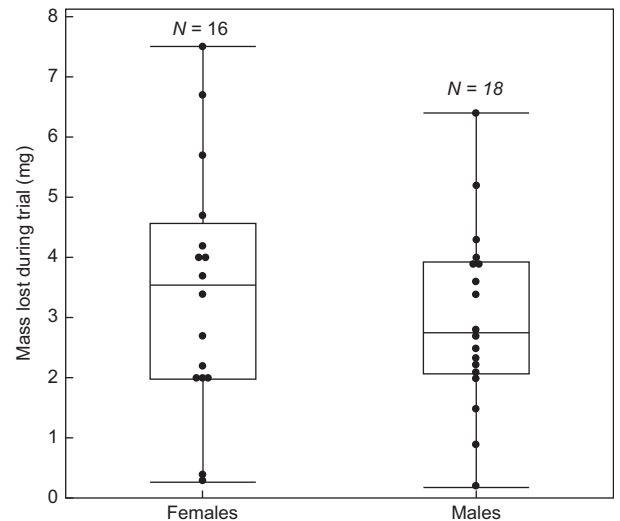

Figure A3. Mass lost during trial by sex. All individuals lost weight during the mating trials. Box plots show median mass with first and third quartile ranges. Dots represent observed data points. Sample sizes are given above box plots.
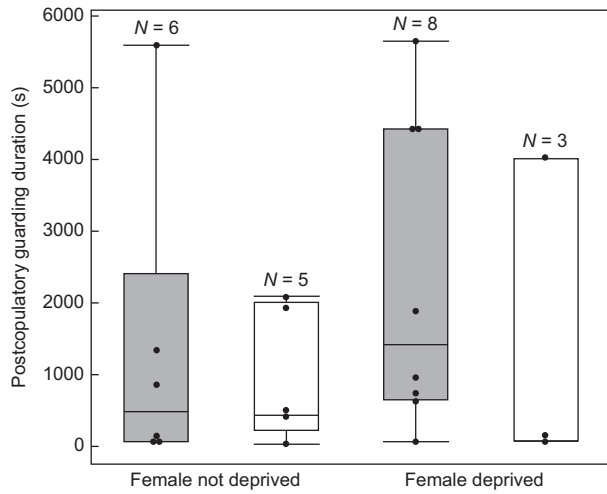
Figure A4. Duration of postcopulatory guarding by male *Leiobunum vittatum* that were deprived (☐) or not deprived (▣) of water when paired with females that were or were not deprived of water. Box plots show median postcopulatory guarding duration with first and third quartile ranges. Dots represent observed data points. Sample sizes for each treatment are given above the box plots.