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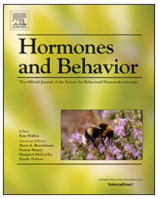

Corticosterone mediated mate choice affects female mating reluctance and reproductive success

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ABSTRACT

The study of stress-related hormones as mediators of sexual selection has traditionally focused on the effect of glucocorticoids on male quality and competing ability. However, environmental stressors are expected to affect both males and females, and the strength of sexual selection might be affected by changes in female mating decisions, a hypothesis that has rarely been tested. Here, we investigated whether female common lizard (*Zootoca vivipara*) mating behaviour and mating preferences are affected by different levels of administered corticosterone and conditioned by the familiarity of their partners, which is known to influence *Z. vivipara* social behaviour. To this end, two females, one corticosterone-treated and one control female, were simultaneously presented with an unfamiliar male and the following day with either a familiar or an unfamiliar male. Females treated with corticosterone (Cort) were more aggressive towards males and mated less. Furthermore, copulation probability in Cort females, but not in control females, increased with body size. On the second day, Cort females only mated with familiar partners. In contrast, male behaviour towards females was not affected by treatment and only bigger males successfully copulated with Cort females. This shows that corticosterone directly affected female mating behaviour and mating preferences, while male mating behaviour was unaffected by the female's level of corticosterone. Environmental and social stressors may affect reproductive strategies of females, the strength of sexual selection, and sexual conflict through their effects on female glucocorticoid levels, potentially in a wide range of species.

1. Introduction

Mate choice is a key component of sexual selection, affecting reproductive success and thereby individual fitness (Darwin, 1874). Changes in mating behaviour can alter the strength and direction of sexual selection and thus influence secondary sexual trait evolution (Andersson, 1994; Kirkpatrick, 1982; Lande, 1981) and population dynamics (Schindler et al., 2013). However, the physiological basis of such behavioural changes is far from being understood.

In the last decades, a case has been made for the idea that stress-related hormones may play an important role in sexual selection, based on the association between environmental stressors and mate choice (Husak and Moore, 2008; Moore and Jessop, 2003). For example, courtship and mating behaviour, which are energetically costly, are associated with increased corticosterone levels (Leary et al., 2004; Reedy et al., 2014; Romero, 2002). Corticosterone is the main glucocorticoid involved in the stress response of amphibians, reptiles, birds and small mammals, and many of the underlying mechanisms of the vertebrate stress response are highly conserved (Wingfield, 2003), indicating that the effects of corticosterone on mating behaviour may be similar across these taxa. In contrast to the detrimental effects of chronic stress conditions, acute corticosterone elevations have been suggested to be beneficial (Wingfield and Kitaysky, 2002). Acute elevation of corticosterone produces a variety of behavioural and physiological effects involved in the acquisition, reallocation, and utilization of resources that have implications on several aspects of reproduction, including fecundity, reproductive investment, offspring phenotype (Breuner et al., 2008; Tokarz and Summers, 2011; Wingfield and Sapolsky, 2003), and mating decisions (Cotton et al., 2006).

The effects of corticosterone on individual performance and individual quality of the chosen sex (i.e. the male, assuming conventional sex-roles) have been widely studied (John-Alder et al., 2009; Leary and Knapp, 2014; Roberts et al., 2007a; Roberts et al., 2007b). However, in a stressful environment (e.g. decreased food availability, high predator

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pressure), stressors are expected to affect both males and females and thus sexual selection exerted by both sexes. Stressors may affect sexual selection indirectly; for example, if corticosterone affects a male's quality and thereby his probability of being selected as a mate, or directly, by modifying the mating preferences of a choosing female.

Earlier studies investigating the effects of blood corticosterone levels on males have reported a variety of effects. Increased blood corticosterone levels suppressed mating behaviour (Moore and Miller, 1984; Moore and Mason, 2001), affected a male's ability to compete with other males (DeNardo and Licht, 1993; Lindsay et al., 2016), changed performance traits (Miles et al., 2007; Moore and Hopkins, 2009) and the quality of sexually selected traits (e.g. call in toads, Leary et al., 2006; birdsong and plumage ornamentation in birds, Honarmand et al., 2015; Roulin et al., 2008; Schmidt et al., 2014; Spencer et al., 2005; body condition, Moore and Jessop, 2003). In contrast, the effects of increased blood corticosterone levels on females have received much less attention, even though females frequently exhibit higher blood corticosterone levels than males (e.g. Klose et al., 2006; Romero, 2002; Taylor et al., 2004). Previous observations from various taxa suggest that in females, differences in blood corticosterone levels lead to changes in mating preferences (Davis and Leary, 2015; DeVries et al., 1995; LaPlante et al., 2014). However, whether these changes translate into differential pairing/mating or reproductive outcome, and thus affect sexual selection, has rarely been tested. Concurrently, mating behaviour may depend on the level of familiarity (e.g. "Coolidge effect"), suggesting that the effects of corticosterone on female mating preferences may depend on the familiarity of the mating partner.

Here, we tested whether corticosterone affects mating behaviour, mating preferences, and reproductive success of female common lizards (*Zootoca vivipara*) and whether these effects depend on partner familiarity. *Z. vivipara* is a small polygynandrous lizard and males and females mate sequentially with several partners (Fitze et al., 2010). To initiate copulation, a male grips the female on the posterior abdomen with its mouth and then tries to twist his body around hers in order to introduce his hemipenis. This pre-copulatory behaviour may immediately result in copulation, although frequently, females try to fend off males' copulation attempts by biting and fleeing, which may lead to long-lasting struggle (Fitze et al., 2010). Access to mating is mainly controlled by females (Fitze et al., 2010; Fitze et al., 2014; San-Jose et al., 2014), but depending on the situation, males, and especially large males, can force copulation (Fitze and Le Galliard, 2008; Fitze et al., 2005), potentially imposing high costs for females, including death (Le Galliard et al., 2008; Le Galliard et al., 2005a).

An experiment in male common lizards showed that exogenous administration of corticosterone affects male mating behaviour: it increased male interest for females and the number of mating attempts initiated by males (Gonzalez-Jimena and Fitze, 2012). In contrast, the effects of corticosterone on female common lizards have mainly been studied during pregnancy and in relation to post-natal effects on the offspring (Belliure et al., 2004; De Fraipont et al., 2000; Ganesh and Yajurvedi, 2002; Meylan et al., 2002; Meylan and Clobert, 2004; Meylan et al., 2010; Vercken et al., 2007). Effects of stress and elevated blood corticosterone levels on female (pre-copulatory) mating behaviour have rarely been studied, and more generally, very little information exists on this subject, especially in amphibians and reptiles (Moore and Jessop, 2003). Common lizards can distinguish familiar from unfamiliar individuals by olfactory cues and familiarity affects their social behaviour (Léna and De Fraipont, 1998; Léna et al., 2000).

Here we assigned female *Z. vivipara* to either a corticosterone or a control group. Pairs of females, consisting of a corticosterone and a control female of similar body size and body condition, were presented to a single unfamiliar male and, on the next day, to a single familiar or unfamiliar male. Treatment effects on male and female behaviour were quantified. We predicted (1) that corticosterone would affect mating behaviour, such that corticosterone-treated females would reduce intersexual social interactions (e.g. less interest towards males). As a consequence of this altered behaviour, we predicted (2) that corticosterone-treated females would have reduced reproductive success/mating probability compared to control females. We also predicted (3) that these effects on mating behaviour, mating probability, or both may depend on the familiarity with the male partner (i.e. a significant corticosterone × familiarity interaction), because corticosterone-treated females may be more inclined to avoid the costs of being courted by multiple unfamiliar males. Finally, according to general sexual selection theory (Bateman, 1948; Cluttonbrock and Parker, 1992; Trivers, 1972), we expected (4) that males would try to copulate regardless of corticosterone treatment and might show more interest in unfamiliar females.

2. Material and methods

2.1. Species description

The common lizard, *Zootoca vivipara*, is a small polygynandrous Lacertid (snout-to-vent length (SVL) of adults: 45–70 mm) that inhabits peat bogs and moist heathland across Eurasia (Massot et al., 1992). The species presents sexual size dimorphism, with females being longer than males (Braña, 1996). In the Pyrenean populations, male lizards emerge from hibernation in February-early March, on average one month prior to the females, who emerge between late March and the beginning of April (Bauwens, 1981). Mating takes place from the first day after female emergence onwards (Breedveld and Fitze, 2015; Fitze et al., 2010). Males can father offspring of up to 14 different females, while females give birth to offspring of up to 5 different males (Fitze et al., 2005; Laloi et al., 2004). Overall, qualitatively better/bigger males are more likely to father offspring (Fitze et al., 2008; Heulin, 1988). Female lizards ovulate spontaneously in absence of mating (Bleu et al., 2011) and show facultative multiple breeding (Breedveld et al., 2017). Experimental evidence supports that context-dependent female mate choice underlies frequency-dependent sexual selection in this species (San-Jose et al., 2014).

2.2. Experimental procedures

In 2009, we released adult male and female common lizards (from the North-East Spain subclade; Horreo et al., 2018) in enclosures located at the Instituto Pirenaico de Ecología (CSIC, Jaca, Spain), each sex in a separate enclosure to avoid any intersexual contact. Enclosures ($10\,m^2$) were delimited by galvanised metal plates and consisted of a patch of natural vegetation, two water ponds, and four stone piles providing natural food and water as well as basking sites and shelters. Forty adult males and 40 adult females (≥ 2 years old) were captured in the two enclosures in March/April 2010, right after female emergence. Following capture, all lizards were individually identified, weighted to the nearest mg, and measured (SVL) to the nearest mm. During the experiment lizards were housed under standardised conditions in individual terraria ($25 \times 15 \times 15$ cm) containing a water pool and shelter. Light and heat were provided by a 40 W bulb from 7 a.m. to 6 p.m. and a UV lamp from 12 a.m. to 2 p.m. Water was available ad libitum and all animals were fed every other day with moth larvae (*Galleria mellonella,* Pyralidae). Males and females were placed in separate shelves and all individuals were isolated from neighbours. Within sexes, individuals of the same treatment group were randomly distributed among shelves and floors (all $P > 0.05$).

Since female emergence from hibernation occurs over several weeks (Breedveld and Fitze, 2015; pers. obs.), we conducted the experiment in three blocks. Blocks 1 and 2 consisted of 32 (16 males, 16 females), and block 3 of 16 (8 males, 8 females) lizards, respectively. In each block, emerging females were captured during 10 ± 2 days and once the number of females required for a block was collected, males were captured. As a result of this procedure, the time from emergence and the time spent in captivity did not differ for lizards among blocks (all

$P > 0.56$). All mating assays took place during April 2010. The experiment complied with Spanish laws and local regulations. All procedures were in accordance with ABS/ASAB guidelines for the ethical treatment of animals and under licenses from the governments of Aragón and Navarra (permits LC/ehv 24/2010/105 & 106).

2.3. Corticosterone treatment

Effects of corticosterone on mate choice and intra- and intersexual behaviour were investigated by experimentally manipulating blood corticosterone levels. Females of similar SVL (average range (biggest-smallest) = 0.7 mm; minimum = 0 mm; and maximum = 2 mm) and body condition (average range (biggest-smallest) = 0.35; minimum = 0.04; and maximum = 0.67) were paired. One female of the pair was randomly assigned to a corticosterone group ("Cort") and the other to a control group ("control"). There were no differences in SVL nor body condition between treatment groups (all $F_{1,\ 38} \leq 0.01$, $P \geq 0.92$). Cort females were submitted to a daily application of a dilution of 4.5 μl of commercial sesame oil mixed with corticosterone (1.5 μg of corticosterone per μl of oil; Corticosterone Sigma C2505), a dose that raises blood corticosterone levels to an average of 194.5 ± 7.9 ng/ml and thus by 94.7% (Gonzalez-Jimena and Fitze, 2012; Meylan et al., 2003). In female common lizards, increases of similar magnitude occur during the mating period, when baseline glucocorticoid levels reach the yearly maxima (Dauphin-Villemant et al., 1990; Romero, 2002), and thus the here experienced corticosterone increase falls within the species' naturally occurring range. Control females were treated with 4.5 μl of sesame oil only. Before the beginning of the mating assays, treatments were applied twice a day (i.e. at 6 a.m. and 6 p.m.) for four days. The duration and the magnitude of the blood corticosterone increase correspond to a corticosterone elevation observed during the acute stress response, during which females maintain reproductive behaviours (Meylan et al., 2003; Moore and Jessop, 2003; Tokarz and Summers, 2011). Besides the applied corticosterone treatment, the handling and conditions of confinement were exactly the same for all groups of lizards.

2.4. Set-up of mating assays

Mating assays were conducted in 50 × 50 cm escape-proof wooden boxes containing a heat rock for thermoregulation (substrate temperature 30 ± 2 °C) and two roofless cardboard refuges, allowing lizards to hide from conspecifics and be watched continuously by an observer. Natural daylight and two halogen lamps illuminated the mating boxes and surrounding dark cloth provided a standardised environment. The day before use, all boxes and heat rocks were cleaned with water-diluted bleach.

Each block was subjected to two mating trials: "Trial A" followed by "Trial B". Both trials consisted of two sets of mating assays carried out on consecutive days (namely "day 1"/"day 2"), hereafter referred to as "day of presentation". Female pairs (one Cort, one control) thus had the opportunity to copulate on four consecutive days. On each day, they were presented with a single male (Fig. 1), allowing to unequivocally distinguish between effects of corticosterone on intersexual, intrasexual, or sex-unspecific behaviour, while controlling for potential variance stemming from the use of a different male. In each trial, half of the female pairs were presented twice with the same male (the familiar-partner group, "FP" henceforth) and the other half with two different males (the unfamiliar-partner group, "UP"). Each male was presented to the same female pair twice (in FP) or to two different female pairs (in UP) and either used in Trial A or Trial B, while female pairs were used in both trials (Fig. 1). Consequently, females and males had the opportunity to mate with 2 or 4 different partners, in the FP and the UP group, respectively.

The within trial design allowed testing if corticosterone and familiarity affect 1) the probability of mating and 2) mate preferences. Two trials (Trial A and B) were conducted because multiple paternity is common in the wild and the number of sires in polyandrous clutches is most frequently two (Romero-Diaz unpub. data; Fitze et al., 2005; Hofmann and Henle, 2006; Laloi et al., 2004).

In blocks 1 and 2, two simultaneous mating assays were conducted in each of four time intervals (between 9 and 11 h, 11 and 13 h, 13 and 15 h, and 15 and 17 h), i.e. eight mating assays per day, whereas in block 3 one mating assay was conducted at each time interval, i.e. four mating assays per day. All experiments were conducted within the daily activity period described for this species (House et al., 1980) and the number of potential partners was within the natural range (Laloi et al., 2004). Because this species exhibits sexual size dimorphism and size assortative mating (Heulin, 1988), pairs of females were matched with males of slightly smaller SVL (on average 4.3 mm smaller). Male and female SVL and body condition did not differ among treatments in any of the blocks (corticosterone treatment, $P > 0.9$; partner familiarity, $P > 0.5$). In each mating assay, the male was introduced first into the box and the two females of a pair were thereafter released simultaneously to avoid the pre-setting of a dominance hierarchy (Aragón et al., 2006).

Lizard behaviour was recorded using digital camcorders (JVC Everio GZ-MG730) placed above the boxes at a standard distance. Videos were watched blind with respect to treatments and all intra- (female to female) and intersexual (female to male and vice versa) behaviour was quantified. The following general behaviours were recorded: number of (No.) approaches, No. tongue extrusions, No. chases, No. escapes, No. bites, No. fights (i.e. "rolling"), and No. appeasement displays (tail and forelimb rapid movements; Gonzalez-Jimena and Fitze, 2012), and the intersexual mating behaviours: No. pre-copulations, average duration of pre-copulation, and probability of copulation (1 = successful copulation; 0 = no copulation). In addition, we recorded average time spent walking and scratching/climbing as a general measure of individual activity. All behavioural variables are described in detail in Table 1 and, with the exception of average duration of pre-copulation and probability of copulation, they correspond to counts per assay. For principal component analyses, we split the abovementioned behaviour into "pre-copulatory" and "post-copulatory" behaviour, depending on whether it occurred before or after copulation. Pre-copulatory behaviour was analysed per time elapsed between introduction of the females into the box and the start of the copulation, and post-copulatory behaviour between the end of the copulation and the end of the mating assay, both measures corresponding to counts per unit time.

2.5. Statistics

Data analyses were performed using JMP© (SAS Institute Inc.) and R (R Development Core Team, 2012), with packages geepack (Yan, 2002) and lme4 (Bates et al., 2011). Male and female intra- and intersexual interactive behaviours were analysed with Generalised Estimating Equations (GEE; Tables A1 and A2). Thereafter, four separate principal component analysis (PCA) were run on male and female behaviour, before and after copulation, and principal components (PC) with eigenvalues > 1 were analysed (Tables 3 and 5). Since in the FP group the male presented on day 1 was unknown to the female (Fig. 1), familiarity and day of presentation were parametrised as one factor with 3 levels: "day 1, UP" (i.e. day 1, UP + day1, FP), "day 2, FP", and "day 2, UP"; hereafter referred to as "day & familiarity. GEEs included corticosterone treatment of the actor (when analysing female behaviour) or of the receiver (when analysing male behaviour), day & familiarity, trial, and their interactions as factors, as well as individual ID to account for repeated measures, using the *id* argument of the geeglm function. To test whether differences existed between presentation days or familiarity, post-hoc comparisons between factor levels day 1, UP and day 2, UP, and between day 2, UP and day 2, FP were conducted, respectively. In the analyses of female behaviour, the sex of the receiver

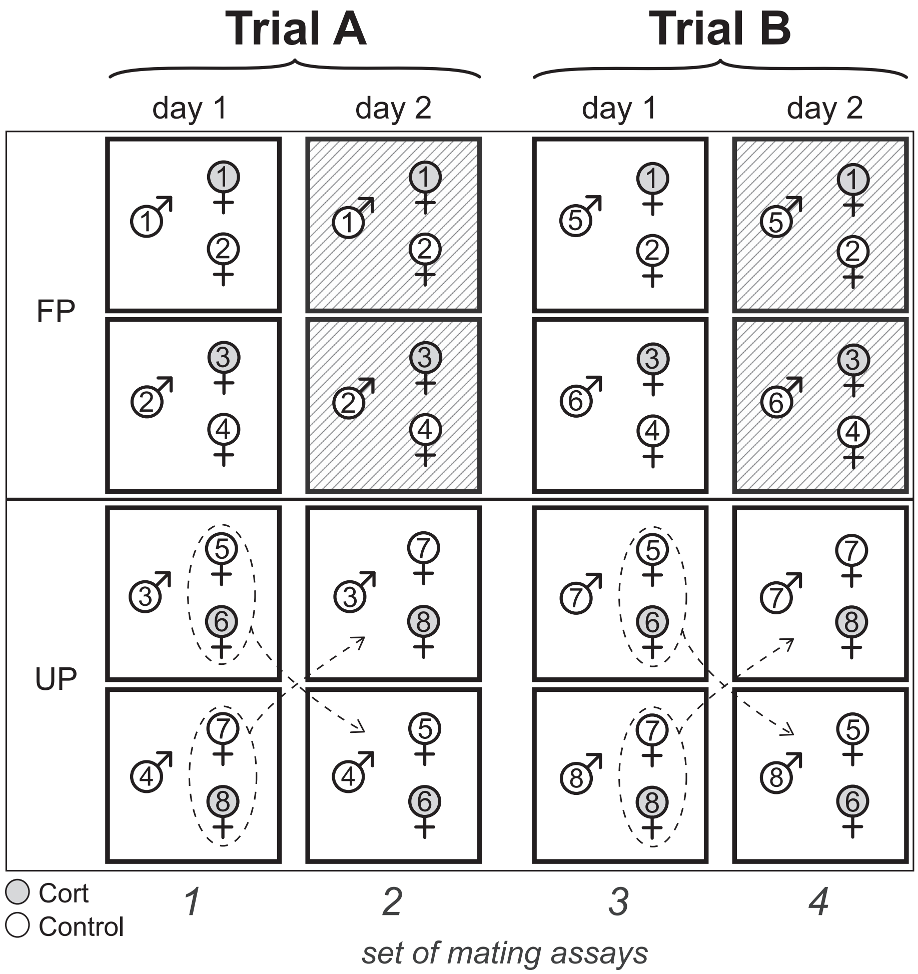

Fig. 1. Experimental design. A full treatment-combination consisted of four sets of mating assays (rows), two trials (Trial A and Trial B, see columns), and a total of 16 mating assays. Blocks 1 and 2 consisted of twice the here shown mating assays, thus eight sets of mating assays and 32 mating assays in total, and block 3 consisted of the 16 here shown mating assays. In each mating assay, one Cort (●) and one control (○) female were presented with one male. Half of the female pairs were presented with the same partner on two consecutive days, i.e. on day 2 with a familiar partner (FP, shaded area), while the other half were presented with two unfamiliar partners (UP). Each female pair was used in two consecutive trials (A and B) following the same experimental design, while males were only used in one trial. See text for additional details.

was additionally included as a factor, and a female's average time spent walking and scratching/climbing was added as a covariate to control for enhanced activity of Cort lizards. Moreover, actor SVL was included as a covariate in all analyses. The probability of copulation was analysed using a binomial logistic mixed model, with corticosterone treatment and day & familiarity as fixed factors and female SVL as a covariate. Day & familiarity was nested within mating assay, which was modelled as a random factor. Average pre-copulation duration was analysed using GLMs with negative binomial error to account for overdispersion.

Initial models included all parameters and their interactions and non-significant terms were backward eliminated. Weighted GEEs were used in the presence of heteroscedasticity. Bonferroni-holm corrections were applied to post-hoc contrasts (Holm, 1979) and effect sizes were calculated for pairwise comparisons using Cohen's *d*. Two-tailed tests with significance level $P \leq 0.05$ were used throughout. Model assumptions were tested (Zuur et al., 2009) and results of minimal adequate models are shown.

3. Results

3.1. Treatment effects on female behaviour

The number of approaches and tongue extrusions were affected by a significant interaction between corticosterone treatment and female SVL (all $\chi^2_1 \geq 4.66$, $P \leq 0.031$; Table A1). Both behaviours significantly increased with body size in corticosterone-treated (Cort), but not in control females (Fig. A1, Cort: $\chi^2_1 = 7.65$, $P < 0.01$; control: $\chi^2_1 = 4.66$, $P = 0.460$; and Fig. A2, Cort: $\chi^2_1 = 52.3$, $P < 0.001$; control: $\chi^2_1 = 0.60$, $P = 0.440$, respectively). Cort females tended to bite more than control females ($\chi^2_1 = 3.40$, $P = 0.065$, $d = 0.23$; Table A1, Fig. 2a). The number of tongue extrusions ($\chi^2_2 = 10.30$, $P < 0.01$), appeasement displays ($\chi^2_2 = 8.52$, $P = 0.014$), and bites ($\chi^2_2 = 15.40$, $P < 0.001$) were affected by an interaction between day & familiarity and sex (Table A1). On day 2, females in the UP group directed more tongue extrusions towards the other female than towards the male ($\chi^2_1 = 12.87$, $P = 0.01$, $d = 0.28$; Fig. A3) and unfamiliar males (UP treatment) received more tongue extrusions on day 1 compared to day 2 ($\chi^2_1 = 10.46$, $P = 0.007$, $d = 0.46$; Fig. A3b). On day 1, females exhibited significantly more appeasement displays towards the male than the female ($\chi^2_1 = 17.68$, $P < 0.001$, $d = 0.45$; Fig. A3), and less appeasement displays towards the female on day 1 than towards the female of the familiar partner group on day 2 ($\chi^2_1 = 12.14$, $P = 0.003$, $d = 0.15$; Fig. A3a). Females bit unfamiliar males more than they bit the female, both on day 1 and on day 2 ($\chi^2_1 = 14.61$, $P < 0.001$, $d = 0.42$; $\chi^2_1 = 40.66$, $P < 0.001$, $d = 0.66$, respectively; Fig. A3) and familiar males received significantly less bites than unfamiliar males (all $\chi^2_1 \geq 6.41$, $P \leq 0.05$, $d = 0.34$; Fig. A3b). The number of appeasement displays significantly decreased with female body size in the UP group on day 1 ($\chi^2_1 = 5.64$, $P = 0.035$) and day 2 ($\chi^2_1 = 10.34$, $P = 0.003$), and no significant decrease existed in the FP group ($\chi^2_1 = 1.51$, $P = 0.220$; Table A1, Fig. A4). Moreover, females exhibited significantly more escapes from the male than the female ($\chi^2_1 = 11.08$, $P < 0.001$, $d = 0.30$; Fig. A3), the number of escapes significantly decreased with female body size (Table A1, Fig. A5), and the number of chases did not differ among treatments nor receiver sex. Finally, females exhibited less tongue extrusions and escapes during Trial B ($\chi^2_1 = 17.45$, $P < 0.001$, $d = 0.46$; and $\chi^2_1 = 5.03$, $P = 0.025$,

Table 1
Ethogram of recorded behaviours in this study.

Behaviour	Description	Interpretation
Tongue extrusion	A synonym of tongue flicking. A rapid extrusion of the tongue directed towards another individual.	Tongue extrusion is associated with exploratory activity and interest[a,b].
Approach	When a lizard moves closer to another individual or passes by at a distance smaller than the body length (SVL) of one lizard.	The number of approaches are a measure of interest[c,d].
Escape	When a lizard moves away from another lizard by accelerating explosively.	Escaping reflects avoidance and thus it is interpreted as a submissive behaviour[e].
Chase	When a lizard pursues another lizard to catch it.	Unlike approaches, chases usually end up in biting or fighting and thus are considered an aggressive behaviour[f].
Appeasement display	A series of rapid side-to-side movements of the tail or tail tip, and/or rapid up-and-down movements of the forelimbs.	Appeasement displays are exhibited in order to avoid costly interactions and reflect submissive behaviour[g,h].
Bite	When a lizard bites or snaps at another individual. This behaviour can be in response to aggression by another individual or initiated by the focal individual. Note that there exists a difference between biting and gripping with the mouth (in the context of pre-copulation and copulation).	The number of bites is an indicator of the level of aggressiveness[e].
Fight	When two lizards engage in an exchange of bites, this may escalate into a 'fight'. During fights, one lizard bites the other, and the latter tries to escape the former's grip by intensely turning and twisting its body. This behaviour can result into the rivals rolling over each other considerable distances.	Fighting is positively correlated with chasing and biting and is interpreted as a more intense form of aggression.
Pre-copulation	When a male grips the female on the posterior abdomen with his mouth and then tries to twist his body around hers to introduce his hemipenis into the female's cloaca.	Mating behaviour.
Copulation	Copulation corresponds to the successful introduction of the male's hemipenis into the female's cloaca.	Mating behaviour.
Walking and/or climbing	When a lizard exhibits directional movement within the wooden box for longer than 1 s. Climbing was considered "walking" but on vertical surfaces, namely refuges or walls.	Walking/climbing are general measures of activity.

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$d = 0.24$, respectively; Table A1).

3.2. Pre-copulatory female behaviour

PCA rendered three principal components with eigenvalues > 1 (Table 2). The first principal component ($PC1_{fb}$; "females before" copulation) accounted for 26.88% of the variance in behaviour. It described "submissive behaviour" since the number of appeasement displays and escapes loaded highest. The second principal component ($PC2_{fb}$) explained 19.42% of the variance and mainly described the number of approaches and tongue extrusions, hence "interest" in other lizards, and the third component ($PC3_{fb}$) explained 14.99% of the variance and represented the number of fights and bites, hence "aggressive behaviour".

3.2.1. Submissive behaviour ($PC1_{fb}$)

Corticosterone treatment, day & familiarity, and trial did not significantly affect submissive behaviour (Table 3). We found a significant interaction between the receiver's sex and SVL (Table 3, Fig. 3a). Overall, females were more submissive towards the male than towards the female, and this behaviour was negatively correlated with female SVL only when directed towards the male (male: $\chi^2_1 = 14.20$, $P < 0.001$; female: $\chi^2_1 = 4.30$, $P = 0.153$). No other interactions were significant (all $P > 0.05$).

3.2.2. Interest ($PC2_{fb}$)

We found a significant interaction between corticosterone treatment and SVL on female interest in other lizards (Table 3, Fig. 3b). In Cort females interest increased with SVL ($\chi^2_1 = 18.65$, $P < 0.001$) while it was not size-dependent in control females ($\chi^2_1 = 0.01$, $P = 1.000$). Female interest was significantly lower during Trial B (Table 3, $d = 0.30$), and was not affected by day & familiarity (all $P > 0.05$). There was also a significant interaction between the sex of the receiver and female SVL (Table 3, Fig. 3c). Interest towards other females increased with female SVL ($\chi^2_1 = 9.44$, $P = 0.008$) and no significant relationship existed towards males ($\chi^2_1 = 1.28$, $P = 1.000$). All other interactions were not significant (all $P > 0.05$).

3.2.3. Aggressive behaviour ($PC3_{fb}$)

A significant interaction between corticosterone treatment and sex (Table 3, Fig. 4) revealed that Cort females were more aggressive towards males than towards females ($\chi^2_1 = 6.45$, $P = 0.022$, $d = 0.24$), while no significant differences existed between Cort and control females in aggressive behaviour directed towards females ($P = 0.480$). Aggressive behaviour was also conditioned by day & familiarity (Table 3). On day 1, females were significantly more aggressive than when presented with a familiar male on day 2 (post-hoc comparison: $t_1 = 2.34$, $P = 0.05$, $d = 0.29$). On day 2, there were no significant differences in aggressive behaviour between females exposed to either a familiar or an unfamiliar male ($P > 0.05$). Additionally, aggression increased with female body size, regardless of treatment (Table 3). Trial

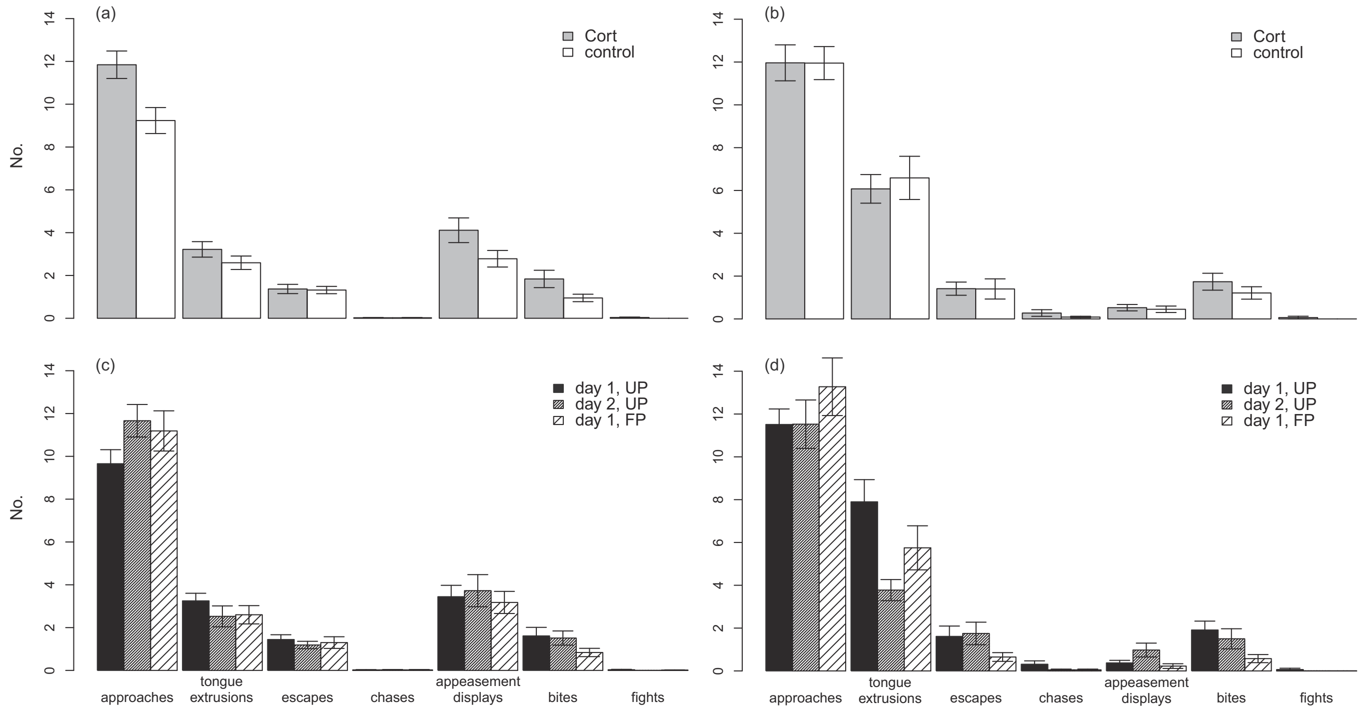

Fig. 2. Mean ± SE observed counts of female (left panels: a, c) and male (right panels: b, d) behaviour by corticosterone treatment (above panels: a, b) and day & familiarity (below panels: c, d). See Table 1 for a description of each behaviour and the Results section and Tables A1 and A2 for statistical tests.

Table 2

Principal component analysis of pre- ($_{\text{fb; females before}}$) and post-copulatory ($_{\text{fa; females after}}$) female intra- and intersexual behaviour. Shown are component loadings for rotated components with eigenvalues ≥1. Main explanatory variables are depicted in bold (cut-off = 0.6; the difference between highest and lowest loading above the cut-off is smaller than between the lowest loading above and the highest loading below the cut-off). PC1$_{\text{fb}}$ and PC1$_{\text{fa}}$ describe "submissive behaviour", PC2$_{\text{fb}}$ and PC2$_{\text{fa}}$ "interest" in other lizards, PC3$_{\text{fb}}$ "aggressive behaviour" and No. indicates "number".

Behaviour	Component loadings				
	Pre-copulatory			Post-copulatory	
	PC1$_{\text{fb}}$	PC2$_{\text{fb}}$	PC3$_{\text{fb}}$	PC1$_{\text{fa}}$	PC2$_{\text{fa}}$
No. tongue extrusions	−0.213	**0.764**	0.311	−0.149	**0.771**
No. approaches	0.101	**0.755**	−0.188	−0.032	**0.816**
No. escapes	**0.759**	−0.112	0.051	**0.897**	0. 199
No. chases	0.425	0.418	−0.409	−0.096	−0.208
No. appeasement displays	**0.739**	0.063	0.138	**0.918**	0.078
No. bites	0.578	0.090	**0.605**	0.545	−0.271
No. fights	0.123	−0.018	**0.738**		

and the other interactions were not significant (all $P > 0.05$).

3.3. Post-copulatory female behaviour

No fights took place after copulation. We obtained two PCs with eigenvalues > 1. PC1$_{\text{fa (“females after” copulation)}}$ explained 32.94% of the variance and represented "submissive behaviour" since escapes and appeasement displays loaded highest, whereas PC2$_{\text{fa}}$ accounted for 23.69% of the variance and described "interest" in other lizards, because the number of approaches and tongue extrusions loaded highest (Table 2).

3.3.1. Submissive behaviour (PC1$_{fa}$)

We found a significant interaction between day & familiarity and female SVL on submissive behaviour of females (Table 3). Submissive behaviour significantly declined with increasing female SVL on day 1 and day 2, in the presence of an unfamiliar male, while no significant correlation existed in females exposed to a familiar male on day 2 (Table 3, Fig. B1). Females were more submissive towards males than females ($d = 0.57$), and submissive behaviour towards the male, compared to the female, decreased significantly more with increasing female SVL (Table 3, Fig. B2). Corticosterone treatment and the other interactions were not significant (all $P > 0.05$).

3.3.2. Interest (PC2$_{fa}$)

There was a significant interaction between corticosterone treatment and SVL. Interest in other lizards decreased with SVL in control females and increased with SVL in Cort females (Table 3, Fig. 3d). Female interest was significantly lower during Trial B (Table 3, $d = 0.99$) and day & familiarity was not significant.

3.4. Treatment effects on male behaviour

Males exhibited significantly more chases towards Cort, compared to control females ($\chi^2_1 = 6.28$, $P = 0.012$, $d = 0.19$; Table A2, Fig. 2b) and the number of escapes from control ($\chi^2_1 = 10.58$, $P = 0.002$), but not from Cort females ($\chi^2_1 = 0.82$, $P = 0.366$), significantly decreased with male body size (Table A2, Fig. A6). In addition, day & familiarity affected the number of tongue extrusions, appeasement displays, and chases (all $\chi^2_2 > 8.04$, all $P \leq 0.018$; Table A2). Males performed more tongue extrusions on day 1 compared to either familiarity group on day 2 (UP: $t_{(1)} = 3.26$, $P = 0.004$, $d = 0.60$; FP: $t_{(1)} = 3.82$, $P < 0.001$, $d = 0.27$; Fig. 2d). On day 2, males also exhibited a significantly higher number of appeasement displays towards unfamiliar (UP) females compared to familiar (FP) females ($t_{(1)} = 2.67$, $P = 0.023$, $d = 0.49$; Fig. 2d). Similarly, the number of chases tended to be higher on day 1 than in the familiar group on day 2 ($t_{(1)} = 2.18$, $P = 0.073$, $d = 0.26$; Fig. 2d). Moreover, we found a significant interaction between day & familiarity and male SVL (Table A2), showing that on day 2 the number of bites decreased with male body size in the UP group compared to the FP group (post-hoc comparison: $\chi^2_1 = 6.69$, $P = 0.029$, $d = 0.41$; Fig. A7).

Table 3

Treatment effects on female pre- ($_{\text{fb; females before}}$) and post-copulatory ($_{\text{fa; females after}}$) submissive behaviour (PC1), interest (PC2), and aggressive behaviour (PC3). Shown are estimates ± SE, test statistics, degrees of freedom (d. f.), and *P* values of the parameters included in the minimal adequate model derived from GEEs. Significance is depicted in bold. Estimates ± SE correspond to the factor level given in brackets. CORT: Corticosterone treatment; DF: day & familiarity; UP: unfamiliar partner; FP: familiar partner.

Parameter	Estimate ± SE	$\chi^2_{(d.\ f.)}$	*P*	Estimate ± SE	$\chi^2_{(d.\ f.)}$	*P*
Submissive behaviour	$PC1_{fb}$			$PC1_{fa}$		
DF [day1, UP]				**−7.31 ± 3.10**	**$18.30_{(2)}$**	**< 0.001**
DF [day2, FP]				**−12.38 ± 2.96**		
Female SVL	**−0.01 ± 0.00**	**$4.29_{(1)}$**	**0.038**	**−0.16 ± 0.04**	**$14.51_{(1)}$**	**< 0.001**
Receiver's sex [males]	**0.94 ± 0.32**	**$8.83_{(1)}$**	**0.003**	**4.20 ± 1.86**	**$5.06_{(1)}$**	**0.024**
DF × female SVL [day1, UP]				**0.12 ± 0.05**	**$18.80_{(2)}$**	**< 0.001**
DF × female SVL [day2, FP]				**0.21 ± 0.05**		
Receiver's sex × female SVL [males]	**−0.01 ± 0.00**	**$7.04_{(1)}$**	**0.008**	**−0.06 ± 0.03**	**$4.25_{(1)}$**	**0.039**
Interest	$PC2_{fb}$			$PC2_{fa}$		
CORT [control]	**2.85 ± 1.00**	**$8.13_{(1)}$**	**0.004**	**3.58 ± 1.52**	**$5.52_{(1)}$**	**0.019**
Female SVL	**0.06 ± 0.01**	**$24.75_{(1)}$**	**< 0.001**	0.03 ± 0.02	$2.65_{(1)}$	0.103
Receiver's sex [male]	**1.36 ± 0.56**	**$5.93_{(1)}$**	**0.015**			
Trial [trial B]	**−0.15 ± 0.06**	**$5.28_{(1)}$**	**0.021**	**−0.44 ± 0.10**	**$23.43_{(1)}$**	**< 0.001**
CORT × female SVL [control]	**−0.05 ± 0.02**	**$8.80_{(1)}$**	**0.003**	**−0.06 ± 0.02**	**$6.21_{(1)}$**	**0.013**
Receiver's sex × female SVL [male]	**−0.02 ± 0.01**	**$5.72_{(1)}$**	**0.017**			
Aggressive behaviour	$PC3_{fb}$					
CORT	0.01 ± 0.02	$0.49_{(1)}$	0.484			
DF [day1, UP]	**0.05 ± 0.02**	**$6.17_{(2)}$**	**0.046**			
DF [day2, FP]	**−0.00 ± 0.02**					
Female SVL	**0.01 ± 0.00**	**$8.70_{(1)}$**	**0.003**			
Receiver's sex [male]	**0.09 ± 0.03**	**$6.45_{(1)}$**	**0.011**			
CORT × receiver's sex [control, male]	**−0.09 ± 0.04**	**$4.80_{(1)}$**	**0.028**			

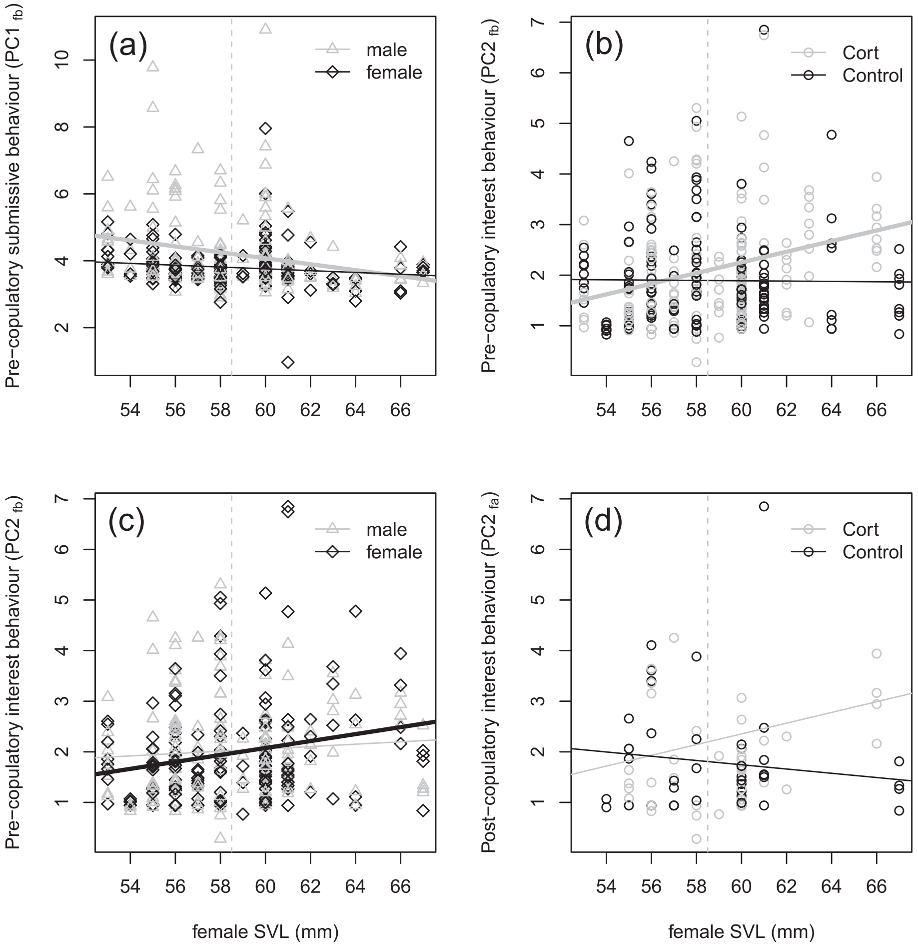

Fig. 3. Relationship of pre- and post-copulatory female behaviour and body size (SVL) depending on the sex of the receiver (a, c) or the corticosterone treatment (b, d). Shown are pre-copulatory submissive behaviour ($PC1_{fb}$) and interest ($PC2_{fb}$), and post-copulatory interest ($PC2_{fa}$). The vertical dashed line depicts average female SVL and solid lines represent predictions from GEE models. Thicker lines denote significant slopes ($P < 0.05$).

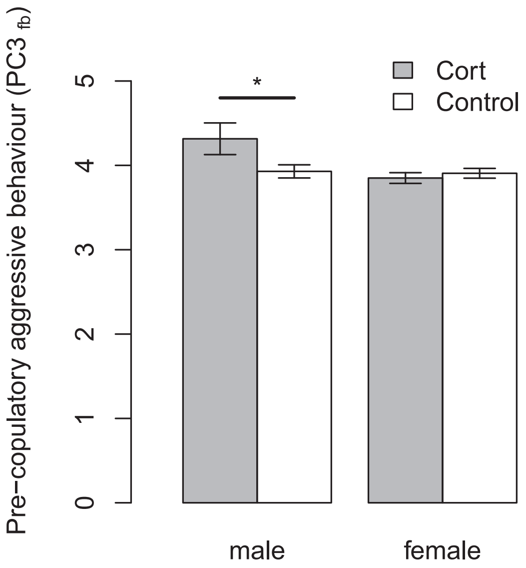

Fig. 4. Female pre-copulatory aggressive behaviour ($PC3_{fb}$) in relation to corticosterone treatment and sex of the receiver. Shown are means ± SE. Significant differences from post-hoc contrasts between treatment groups are depicted by an asterisk (*$P < 0.05$).

3.5. Pre-copulatory male behaviour

PCA rendered three principal components with eigenvalues > 1. $PC1_{mb}$ explained 28.43% of the variance and represented “aggressive behaviour” since the number of chases and fights loaded highest. $PC2_{mb}$ explained 28.71% of the variance and escapes and appeasement displays loaded highest, representing “submissive behaviour”. $PC3_{mb}$ accounted for 17.23% of variance and described approaches and tongue extrusions, both representing “interest” in other lizards (Table 4).

Corticosterone treatment did not affect male behaviour (Table 5). However, we found a significant effect of day & familiarity on aggressive behaviour before copulation ($PC1_{mb,}$ Table 5). On day 1, males were significantly more aggressive than familiar or unfamiliar males on day 2 (UP: $t_{(1)} = 2.35$, $P = 0.043$, $d = 0.32$; FP: $t_{(1)} = 2.57$, $P = 0.024$, $d = 0.35$) and there were no significant differences among treatments on day 2. Moreover, submissive behaviour of males decreased with increasing male body size ($PC2_{mb,}$ Table 5). No other effects or interactions were significant (all $P > 0.05$).

Table 4
Principal component analysis of pre- ($_{mb;\ males\ before}$) and post-copulatory ($_{ma;\ males\ after}$) male intersexual behaviour. Shown are component loadings for rotated components with eigenvalues ≥1. Main explanatory variables (cut-off = 0.6; the difference between highest and lowest loading above the cut-off is smaller than between the lowest loading above and the highest loading below the cut-off) are depicted in bold. $PC1_{mb}$ and $PC1_{ma}$ describe “aggressive behaviour”, $PC2_{mb}$ and $PC3_{ma}$ “submissive behaviour”, $PC3_{mb}$ and $PC2_{ma}$ “interest” in other lizards, and No. indicates “number”.

Behaviour	Component loadings					
	Pre-copulatory			Post-copulatory		
	$PC1_{mb}$	$PC2_{mb}$	$PC3_{mb}$	$PC1_{ma}$	$PC2_{ma}$	$PC3_{ma}$
No. tongue extrusions	0.057	−0.193	**0.827**	−0.028	**0.948**	−0.086
No. approaches	0.107	0.243	**0.785**	−0.050	**0.948**	0.072
No. escapes	0.035	**0.819**	−0.013	−0.022	0.011	**0.927**
No. chases	**0.904**	−0.069	0.045	**0.961**	−0.054	0.121
No. appeasement displays	−0.020	**0.805**	0.040	**0.911**	−0.011	−0.146
No. bites	0.518	0.097	0.085	**0.621**	−0.065	0.524
No. fights	**0.891**	−0.051	0.042			

3.6. Post-copulatory male behaviour

No fights occurred after copulation. Three principal components had eigenvalues > 1 (Table 4). In $PC1_{ma}$ (38.16% of variance), the number of bites, chases, and appeasement displays loaded highest, representing “aggressive behaviour”. $PC2_{ma}$ (28.95% of variance) described “interest” in other lizards, because approaches and tongue extrusions loaded highest, whereas in $PC3_{ma}$ (18.38%) only escapes loaded high, which corresponded to “submissive behaviour”.

Males showed no significant differences in post-copulatory behaviour towards Cort or control females (Table 5). There was a significant interaction between day & familiarity and male SVL on aggressive ($PC1_{ma}$) and submissive ($PC3_{ma}$) behaviour (Table 5). Aggression and submission significantly decreased with increasing male body size in UP assays on day 2 ($PC1_{ma}$: $\chi^2_1 = 51.09$, $P < 0.001$; $PC3_{ma}$: $\chi^2_1 = 32.84$, $P < 0.001$) and in FP assays (on day 2) ($PC1_{ma}$: $\chi^2_1 = 173.69$, $P < 0.001$; $PC3_{ma}$: $\chi^2_1 = 3554.77$, $P < 0.001$; Figs. B3 and B4, respectively), while no significant correlation existed on day 1 ($P > 0.05$).

3.7. Mating behaviour

Average pre-copulation duration did not depend on corticosterone treatment, day & familiarity, or trial (all $P > 0.05$). However, male SVL positively predicted pre-copulation duration (estimate = 0.24 ± 0.10, $\chi^2_1 = 6.22$, $P = 0.012$).

Twenty-two of the 80 mating assays (27.5%) resulted in successful copulation. In none of the assays did a male copulate with more than one female. Of the 22 copulations, 17 occurred with control (9 UP, 8 FP) and 5 with Cort (0 UP, 5 FP) females. Of these 5 copulations, 3 occurred on day 1, and 2 on day 2, while all copulations of control females were on day 1. The probability of copulation was significantly affected by an interaction between corticosterone treatment and female SVL ($\chi^2_1 = 5.39$, $P = 0.020$). The probability of copulation increased with female SVL in Cort females and it was not size-dependent in control females (Fig. 5). Smaller Cort females did not copulate at all, while smaller females of the control group copulated with the same probability as larger control females. In addition, the probability of copulating was significantly higher in control than in Cort females ($\chi^2_1 = 9.30$, $P < 0.001$). Day & familiarity and its interaction with Corticosterone treatment or SVL were not significant (all $P > 0.05$).

4. Discussion

The study of stress hormones as mediators of sexual selection has traditionally focused on glucocorticoid effects on male quality and competitive ability (Hill, 2014; Husak and Moore, 2008), neglecting the potential, independent impact of stressors on female mating decisions. By experimentally manipulating the level of blood corticosterone of female common lizards, we showed that this stress hormone significantly affects female mating behaviour. Corticosterone-treated females (Cort) were significantly more aggressive towards males than were females of the control group (Control; Fig. 3); they bit and fought more and they exhibited less tongue extrusions and approaches (Table A1), which points to increased female mating reluctance. As a consequence, Cort females copulated significantly less than control females.

In addition, corticosterone affected female mating behaviour in a size-dependent manner. For example, interest increased with body size in Cort females before copulation (Fig. 2b), as well as the total number of approaches and tongue extrusions (Table A1). Similarly, the probability of copulation increased with body size in Cort but was size-independent in control females (Fig. 5). This pattern is consistent with the effects on female interest, and previous experiments by Le Galliard et al.

Table 5

Treatment effects on pre- ($_{\text{mb; males before}}$) and post-copulatory ($_{\text{ma; males after}}$) aggressive behaviour ($PC1_{mb}$, $PC1_{ma}$), submissive behaviour ($PC2_{mb}$, $PC3_{ma}$), and interest ($PC3_{mb}$, $PC2_{ma}$) of males. Shown are estimates ± SE, test statistics, degrees of freedom (d. f.), and *P* values of the parameters included in the minimal adequate model derived from GEEs. Significance is depicted in bold. Estimates ± SE correspond to the factor level given in brackets. CORT: Corticosterone treatment; DF: day & familiarity; UP: unfamiliar partner; FP: familiar partner.

Parameter	Estimate ± SE	$\chi^2_{(d.\ f.)}$	*P*	Estimate ± SE	$\chi^2_{(d.\ f.)}$	*P*
Submissive behaviour	$PC2_{mb}$			$PC3_{ma}$		
DF [day1, UP]				**−25.23 ± 5.2**	**$25.8_{(2)}$**	**< 0.001**
DF [day2, FP]				**−19.84 ± 4.14**		
Male SVL	**−0.01 ± 0.00**	**$4.88_{(1)}$**	**0.027**	**−0.44 ± 0.08**	**$32.8_{(1)}$**	**< 0.001**
DF × male SVL [day1, UP]				**0.46 ± 0.10**	**$24.6_{(2)}$**	**< 0.001**
DF × male SVL [day2, FP]				**0.36 ± 0.08**		
Interest	$PC3_{mb}$			$PC2_{ma}$		
DF [day1,UP]				0.33 ± 0.16	$5.86_{(2)}$	0.053
DF [day2,FP]				−0.07 ± 0.13		
Aggressive behaviour	$PC1_{mb}$			$PC1_{ma}$		
DF [day1,UP]	**0.32 ± 0.14**	**$7.42_{(2)}$**	**0.024**	**−28.02 ± 4.08**	**$47.60_{(2)}$**	**< 0.001**
DF [day2, FP]	**−0.03 ± 0.03**			**−23.89 ± 3.85**		
Male SVL				**−0.51 ± 0.07**	**$51.10_{(1)}$**	**< 0.001**
DF × male SVL [day1, UP]				**0.51 ± 0.08**	**$44.76_{(2)}$**	**< 0.001**
DF × male SVL [day2, FP]				**0.43 ± 0.07**		

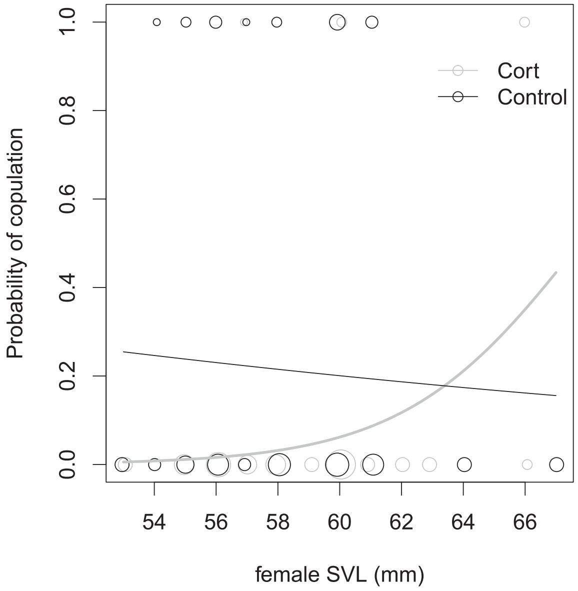

Fig. 5. Probability of copulation in relation to corticosterone treatment and female SVL. Model predictions for Cort (grey) and control (black) groups are depicted with solid lines. Larger Cort females copulated with higher probability than small Cort females, while the probability of copulation was size-independent in control females. Overall, control females were more likely to copulate than Cort females. Point size corresponds to the number of females, with the smallest dot representing $n = 1$ and the biggest dot $n = 18$.

(2008) and Fitze et al. (2005) showing that in populations with male-biased sex ratios (populations with higher male harassment to females) the number of mating partners increased with female body size. This size-dependent corticosterone effect on a female's probability of copulation mirrors the effect of corticosterone on the number of male copulation attempts (see Fig. 1 in Gonzalez-Jimena and Fitze, 2012), suggesting that corticosterone affects male and female copulatory behaviour similarly.

Several hypotheses could explain the existence of size-dependent effects on copulation probability. First, the motivation to mate may differ between individuals of different sizes. Larger females generally produce larger clutches (Bauwens, 1999) and thus larger females—relative to smaller females—may experience higher benefits from a single reproductive event (and higher costs if no reproduction occurs). Moreover, *Z. vivipara* exhibits indeterminate growth and thus body size is correlated with age, suggesting that age-specific reproductive strategies, such as terminal investment (Massot et al., 2011; Richard et al., 2005) may also play a role. Consequently, larger females may exhibit more interest and a higher motivation to copulate, and thus corticosterone effects may mainly manifest negatively in smaller females. Second, mate choice requires time and energy, potentially jeopardizing survival and fecundity (Andersson, 1994), and male coercion can entail high costs, including injuries and even death (Le Galliard et al., 2008; Le Galliard et al., 2005b), suggesting that the cost/benefit balance of mating is size-dependent. In this study, all females were sexually mature (SVL ≥ 53 mm), but size-dependent effects on interest and copulation probability were limited to Cort females. If corticosterone increases the costs of reproduction, all Cort females would have copulated less than control females. Moreover, there were no significant differences among Cort and control females in the size-dependent effect on aggression towards males (i.e. the interactions Cort treatment × receiver's sex × female SVL and Cort treatment × female SVL, $P > 0.05$) and Cort females were more aggressive than control females. Thus, it is unlikely that size-dependency of female interest and copulation probability in Cort females arose due to differences in the cost/benefit balance between smaller and larger females as a whole.

Third, larger and shorter females may exhibit different physiological responses to raises in corticosterone levels. For example, Summers (1995) found that, in *Anolis carolinensis,* an increase in blood corticosterone inhibits ovarian growth only in small females. This is congruent with the observed pattern of mating probability, where small control females mated and Cort females did not (Fig. 5), but it does not explain why interest of small females was similar in Cort and control females nor why size-dependent treatment differences in behaviour mainly manifested in large females (e.g. Fig. 4b, d). Fourth, differential blood corticosterone levels between smaller and larger females could lead to the observed differences in interest and copulation probability between different-sized females. However, at least in *Z. vivipara,* basal and post-treatment corticosterone levels are independent of female body size and corpulence (Meylan et al., 2003; Summers, 1995) and circulating corticosterone levels do not differ among adult females of different ages (Massot et al., 2011). Moreover, there is no reason to believe that larger females were healthier than smaller females given that they did not differ in body condition, and survival of adult female *Z. vivipara* is not necessarily body size-dependent (e.g. Le Galliard et al., 2010). This suggests that size- or age-specific strategies that benefit larger/older females (see first hypothesis), rather than the latter hypotheses, may explain the observed patterns.

Unpredictable extrinsic events can increase corticosterone levels within minutes or hours (Wingfield et al., 1998). Such events include the presence of predators, conspecifics, parasites, infection, and wounding, and at the population scale, parameters like population density (e.g. crowding), food-predator balance, and weather conditions (Le Galliard et al., 2008; Summers and Norman, 1988; Svensson et al., 2001; Wingfield, 2003; Wingfield and Kitaysky, 2002). The here observed body size-dependent effects of increased corticosterone levels on females thus suggest that many other intrinsic or extrinsic sources of stress, through their effects on glucocorticoid levels, may affect a female's mating behaviour, reproductive success and thereby the strength of sexual selection. However, we emphasize that glucocorticoid release is only one of the multiple aspects of the physiological stress response, and thus more information about other concurrent processes and how these may interact is needed for a complete understanding of the overall effects of stress on mating and reproduction.

We predicted that familiarity may affect corticosterone effects on mating behaviour or mating probability; however, we found no evidence of either. Here, partner familiarity mainly affected post-copulatory behaviour or males, and thus familiarity had limited and no direct impact on female mating probability. Interestingly, the effects of day & familiarity on female appeasement displays and male aggressive and submissive behaviour were also size-dependent. For instance, smaller unfamiliar males were more aggressive (e.g. they bit more) on day 2 (Table A2, Fig. B3). Since male and female body sizes were matched, this suggests that post-copulatory submissive behaviour (e.g. appeasement displays) of smaller females (Table A1, Fig. B1) may have been in response to the male's behaviour, rather than directly induced by the treatment. The submissive behaviour of females, their overall reduced interest during Trial B (i.e. when presented with either a second [FP group], or with a third and fourth [UP group] male) and the virtual absence of multiple copulations is in line with female reluctance to remate, female choosiness and previous findings showing that females copulate on average with 2 males (Fitze et al., 2010; Fitze et al., 2005). Thus, our results show a lack of support for familiarity-dependent intersexual female mating behaviour, including familiarity-dependent effects of corticosterone.

Importantly, male pre-copulatory behaviour was independent of a female's corticosterone treatment and familiarity (Table 5), which agrees with classical sex roles, where females optimize mate quality whereas males optimize the number of mating partners (Andersson, 1994; Bateman, 1948; Trivers, 1972). On day 1, males were significantly more aggressive before copulation, suggesting that they were more motivated to mate. On day 2, males exhibited reduced post-copulatory interest and significantly less interest in the FP treatment (Fig. B5), in line with a preference for unfamiliar females (Pizzari et al., 2003). Because male pre-copulatory behaviour was not affected by corticosterone treatment nor familiarity, increased aggression of Cort females towards males cannot be explained by increased interest or harassment of males towards Cort, compared to control females. This indicates that male behaviour during sexual selection is independent of a female's corticosterone level and familiarity. Given that males and females can distinguish familiar from unfamiliar conspecifics (Léna and De Fraipont, 1998), this also suggests that, prior to copulation, males are less choosy than females (Gonzalez-Jimena and Fitze, 2012).

Our findings agree with a large body of literature that indicates a strong association between corticosterone (or cortisol) and reproduction in many species, including fish, amphibians, reptiles, birds and mammals (Crespi et al., 2013; Moore and Jessop, 2003). They support the idea that in stress-inducing situations, where glucocorticoids are released, intrinsic factors can importantly influence the magnitude of this association. For example, individual size or age (present study), body mass, immune state (Hau et al., 2016), or sex (Vitousek et al., 2018), are some of the factors that have been shown to influence the effects of corticosterone on reproduction. This intrinsic context-dependency may in part be responsible for the lack of a consistent functional relationship between corticosterone and reproductive success, both across and within species (Bonier et al., 2009; Breuner et al., 2008; Crespi et al., 2013).

In conclusion, here we show that exogenous stressors, simulated by the administration of corticosterone, led to higher female aggression towards males and reduced interest, suggesting increased female reluctance to mate, which manifested in significantly fewer copulations. These results, stemming from PCAs of behavioural data, were supported by independent analyses on the observed frequencies. Moreover, the copulation probability of corticosterone-treated females increased with body size, suggesting that an acute increase of corticosterone may only negatively affect smaller females. Because male reproductive success is not affected by blood corticosterone differences (Gonzalez-Jimena and Fitze, 2012), our results suggest that exogenous stressors may modify the intensity of sexual selection and female reproductive strategy by altering female glucocorticoid levels and thus impose direct selection on mating biases (Kokko et al., 2003; Kokko and Monaghan, 2001). Given the phylogenetic conservation of the stress response in vertebrates, these results further suggest that factors affecting glucocorticoid levels (and possibly other physiological components) may directly affect the opportunity and intensity of sexual selection and sexual conflict over mating rate in a large number of organisms.

Acknowledgements

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.yhbeh.2019.04.011.

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