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Considering temporal variations in mating opportunities: consequences for sexual selection estimates

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ABTRACT

The strength of precopulatory sexual selection may vary within a breeding season depending on fluctuations in the social environment faced by active individuals. Here, we investigated within-season variations in precopulatory sexual selection in the river lamprey, *Lampetra fluviatilis*. We determined individual lifetime mating success in a group of 20 females and 15 males through constant video recording in an experimental aquarium. Among each sex, we calculated the potential strength of sexual selection and selection on identified traits (body size and temporal spawning patterns) at two different timescales: a fine-grained scale in which we considered mating events at daily time steps and a global scale in which we pooled all mating events that occurred during the breeding season. We found a predictable change in the operational sex ratio that corresponded with a change in the opportunity for sexual selection in males. Selection on both male and female body size was stronger when active competitors were larger. In addition, the timing (onset and span) of both the male's and the female's reproductive activity affected the number of matings performed over the season with a strength dependent on individual body size. Overall, our study documented that river lampreys experience within-season changes in precopulatory sexual selection. Such temporal dynamics are important to consider, and we advocate for longitudinal observation of mating behaviour to complement traditional time-integrated estimation of mating success based on genetic parentage analysis or cross-sectional analysis.

KEYWORDS

intrasexual competition, mate choice, operational sex ratio, opportunity for sexual selection, selection gradient

INTRODUCTION

Phenotypic variation found in natural populations is predicted by the strength and direction of selection pressures acting on heritable traits (Fisher, 1930; Lande & Arnold, 1983). Among these, sexual selection is any selection that is generated by differential access to opposite-sex mates and/or gametes (Andersson, 1994; Kokko et al., 2006; Shuker & Kvarnemo, 2021). In many animal taxa, the intensity of sexual selection varies among spatially isolated populations (e.g. McLain, 1982), demonstrating how ecological variables, such as population density (Prohl, 2002) or habitat complexity (Myhre et al., 2013), may cause variations in sexual selection. Recently, a few studies have also shown that in long-lived animals, populations may experience different levels of sexual selection strength over time (e.g. reviewed by Siepielski et al., 2009), due to demographic or environmental changes between breeding cycles. These studies have highlighted that selection strength can be correlated with the time period over which it is measured, and averaging values over long-term periods may misrepresent or underestimate the effect of selection on phenotypic evolution.

However, less is known about variation in sexual selection over the course of a breeding season and how it may also be driven by smaller-scale changes in environmental or social variables such as operational sex ratio (OSR, i.e. the ratio of sexually receptive males to females; Emlen & Oring, 1977) within a season. In populations with a biased OSR, the mate-limited sex is expected to compete more strongly for access to mates, and therefore to face stronger sexual selection (Emlen & Oring, 1977). The availability of sexually receptive individuals may change throughout the season, potentially affecting selection on phenotype. Indeed, the rate at which males and females become sexually receptive (e.g. due to differential maturation rates) or become sexually unavailable (due to parental care, gamete stock depletion or death; Kokko & Jennions, 2008) generates fluctuations in OSR and heterogeneity in social structure. For instance, Kasumovic et al. (2008) showed within-season variation in OSR and density at different times of the breeding season in the golden orb-web spider, *Nephila plumipes*, leading to significant variation in the strength and direction of sexual selection on male body size between those sampling periods. Similarly, Wacker et al. (2014) highlighted within-season change in sexual selection in male two-spotted gobies, *Gobiusculus flavescens*, that aligns with a change in the OSR.

Nevertheless, most empirical studies, including those mentioned above, estimate mating success via cross-sectional analysis. In this approach, the population is sampled at one or a few particular points in time and individuals are not followed repeatedly during the breeding season. Thus, if mating success varies over the season, single estimates or cross-sectional estimates calculated from a few short time intervals may significantly over- or underestimate overall success. This is particularly true for species that mate several times and for which the duration of individual mating activity is short relative to the length of the breeding season. Although a few studies on hermaphrodites have already inferred mating success via intensive observations of individually tagged individuals (Anthes et al., 2010; Pélissié et al., 2012), none to our knowledge have used longitudinal behavioural data to examine how the selection operates when the social context changes. By ignoring the temporal variations in mating opportunities during a breeding season, traditional selection analysis assumes that small changes in demographics do not alter selection (Kasumovic et al., 2008; Wacker et al., 2014).

In the present study, we estimated precopulatory sexual selection throughout the breeding season of the European river lamprey, *Lampetra fluviatilis,* in an experimental setting. This biological model is relevant for studying within-season variation in sexual selection as the OSR changes between breeding phases, from a majority of females during the nest-building phase, to a prevalence of males during the spawning phase, followed by a return to an excess of females at the end of the season (Jang & Lucas, 2005). Individuals mate frequently (up to 20 matings/h) with different partners on the same nest or on several nests successively, thus constituting a highly polygynandrous system (Docker, 2019). The brevity of the lamprey breeding season (a few weeks; Hardisty & Potter, 1971) as well as their semelparous reproductive strategy (Docker, 2019) makes it possible to observe in a relatively short time the overall mating success of all individuals studied. This point is essential, as it is net selection over an individual's lifetime that ultimately determines the overall response to selection on phenotypic traits (Blanckenhorn, 2000).

To investigate potential changes in sexual selection, we calculated sexual selection metrics at a fine-grained scale that segments mating events at daily time steps. We then estimated overall sexual selection by pooling all mating events that occurred during the breeding season. The potential strength for sexual selection was first estimated by using a fitness variance-based approach (i.e. the opportunity for sexual selection I_s, Crow, 1989; Wade, 1979) and a population-based approach (i.e. OSR, Emlen & Oring, 1977). Although these measures do not reflect the actual strength of selection acting on specific traits, they are essential to consider when the targets of selection are poorly known as in lampreys. We then used direct, trait-based measures of sexual selection (i.e. sexual selection gradients through two Bayesian models) to quantify actual mechanisms of selection on identified traits (body size and temporal spawning pattern). As mating success is usually estimated via genetic data, analysis of sexual selection treats male and female mating success independently and ignores the fact that the success of a male–female sexual interaction can be attributed to the phenotypes of both individuals in a specific demographic context. Using constant behavioural data allowed us to incorporate this aspect as well as disentangle precopulatory components of mating success and investigate the traits that affect each of them. In our study, we considered mating success as a sequence of two interdependent pairwise processes: the number of mating attempts and the probability of successful mating. A first Bayesian model, considering mating interactions at daily time steps, therefore aimed to define how daily variation in social environment (i.e. OSR, size of current competitors) impacted the two processes leading to mating success (i.e. number of mating attempts, rate of successful mating). First, we expected larger males and larger females to mate more successfully as body size generally influences success during intrasexual mate competition (Hunt et al., 2009; Malmqvist, 1983). We also suspected that probability of mating successfully would be higher in pairs of similar size as Hardisty and Potter (1971) predicted that size-assortative mating may occur in lampreys. Then, we predicted interactive effects between individual phenotype and social environment. For instance, we expected that the potentially positive effect of individual body size would be stronger in a more competitive environment. At the scale of the whole breeding season, a second Bayesian model aimed at investigating whether processes leading to mating success were impacted by temporal characteristics of mating activities that may be under selection. We expected that individuals starting early in the season and distributing their mating effort over a long period may experience a higher number of mating attempts. Finally, interaction between body size and temporal distribution of mating activity was tested, since the fitness benefit of adopting a given temporal distribution may depend on individual phenotype. For example, smaller individuals may compensate for a lower competitive ability by concentrating their mating activity in a shorter time.

METHODS

Study Species

The river lamprey is an externally fertilizing fish that spawns in nests hosting groups of two to 50 individuals (Jang & Lucas, 2005; Lasne et al., 2010) and whose mating act consists of a fixed sequence of recognizable behaviours: female positioning, male mounting (i.e. attaching his mouth to the female’s head), tail wrapping and squeezing (Docker, 2019), followed by simultaneous release of gametes, when the pair quivers for approximately 2 s. Such distinctive behaviours allow us to visually discriminate the focal individuals during copulations. This parasitic species is suited for experimental behavioural studies as it can reproduce in captivity (Hagelin, 1959) and tolerates the close presence of observers (Lasne et al., 2010; Yamazaki & Koizumi, 2017).

Field Collection and Maintenance

Individuals were collected in early spring 2019 (between 15 and 26 March) as river lampreys spawn between April and June (Hardisty & Potter, 1971). Thirty-five individuals (20 females, 15 males) were captured during their upstream migration on the Garonne river (southwest France) by professional fishermen, and transferred to the INRAE experimental facilities in Saint-Pée-sur-Nivelle, France (ECP, INRAE, https://doi.org/10.15454/1.5572402068944548E12). Lampreys were acclimated for 1–3 weeks (according to the fishing date) in tanks supplied with Nivelle river water to avoid behavioural changes due to physical and chemical properties of the water during the experiment. Although the chemical composition of Garonne and Nivelle rivers probably differ, our set-up mimicked a situation in which river lampreys migrate from the main stem of a watershed to a small tributary with a different water chemistry. Each individual was tagged with a unique combination of three spots of UV-fluorescent visible implant elastomer (yellow, orange, red or blue) injected in the posterior dorsal fin to allow individual recognition under both white light and UV light (Silver et al., 2009). We measured two morphological traits: total body length (±0.5 mm) and total mass (±0.5 g). The two traits may be targeted by sexual selection (Malmqvist, 1983) and their combination is an indicator of fish condition (i.e. their quality and fecundity). Biometry and tagging were performed after fish were placed in an anaesthesia tank containing benzocaine solution (0.3 ml/litre) for 5 min.

Experimental Set-up

The experiment took place in a 4 m^3 (10 ×1 m and 0.4 m water depth) longitudinal section of a large 25 m^3 circular flume, supplied with water from the Nivelle river in a semiopen circuit, with water replacement of 6 litres/min. To mimic natural spawning conditions, current speed was set to 0.3 m/s, spawning substrate and shelters (tiles, woody debris) were provided, and water temperature (known to affect spawning activity, Hardisty & Potter, 1971) was monitored daily and followed that of the river (between 14 and 18 °C). To facilitate observation, spawning substrate was limited to two 0.48 m^2 (0.6 x 0.8 m) boxes placed 5 m from each other, and filled (0.1 m depth) with a mixture of sand, gravel and pebbles corresponding to the spawning habitat selected by river lampreys in natural environments (Jang & Lucas, 2005). The aquarium was lit with white neon bulbs following the natural photoperiod (12:12 h with 30 min of dawn and dusk), but as river lampreys are active 24 h per day during the spawning period (Sjoberg, 1977), we placed a UV light above the substrate boxes to identify individuals at night. Two video cameras (Basler acA1920-40gc) continuously recorded lampreys' activity in each spawning patch throughout the experiment. The flume section was inspected three times a day (morning, noon, evening) to ensure that no spawning occurred outside the spawning substrate, and to collect dead individuals. All individuals were placed in the experimental tank on 3 April and the experiment ended on 3 May, after the death of the last individual.

Mating Characterisation

All video footage was analysed with BORIS software (Friard and Gamba, 2016) to note the exact timing of each behaviour and the identity of the individuals involved. Numerous attempts to copulate were interrupted before the male could squeeze the female's abdomen. We therefore discriminated successful mating acts (i.e. those ending with squeezing and quivering) from failed mating attempts. Each successful mating act usually involved one male and one female but sometimes two or more males copulated simultaneously with the same female, as highlighted by previous studies(Case, 1970; Docker, 2015; Huggins & Thompson, 1970; Malmqvist, 1983). In that case, the identity of each male involved was noted, so that the female was considered to have successfully mated with each of them.

Definition of Mating Success

Considering lampreys' mating system (external fertilization, high polygynandry, very frequent matings), the classic genetic view of mating success (i.e. number of different individuals with which the focal individual produced at least one offspring) may show little interindividual variation, while a behavioural view that quantifies the number of mating events may represent more accurately the link between an individual's phenotype, its social environment and its ability to secure matings. Thus, we first calculated MS_P, defined as the number of partners with which the focal individual successfully mated to fit the genetic definition of mating success. Then, we calculated MS_M as the number of successful mating acts performed by the focal individual with all its mates to match the behavioural definition of mating success.

Estimation of Potential Strength of Sexual Selection

We first characterized the potential strength of sexual selection by calculating the opportunity for sexual selection (I_s) at the scale of the whole season. We measured I_s for each sex as the variance in mating success (i.e. both as the number of mates, MS_P, and the number of matings, MS_M) divided by the square of the mean mating success (Wade, 1979; Wade & Arnold, 1980). Recent research showed that I_s is connected to OSR, even in the absence of sexual selection as a result of a decreased mean mating success of the dominant sex (Jennions et al., 2012; Klug et al., 2010). To consider this source of bias, we therefore evaluated the expected I_s under random mating given the observed OSR and mean mating success, as recommended by Janicke and Morrow (2018). For this purpose, we randomly distributed the total number of mating acts among all individuals in each sex. We iterated this distribution 10 000 times in R and we subtracted the median I_s of these simulations from the observed I_s. The number obtained was defined as the bias-corrected I_s (hereafter Is_{bc}). We also report 95% confidence intervals for all estimates of Is_{bc} obtained from the simulations. When simulating MS_P, care was taken to ensure that no individual was assigned a higher mating success than the actual total number of individuals of the opposite sex. Sex-specific differences in variances were assessed using Levene’s *F* test.

We then split the breeding season into 22 daily periods, which allowed us to assess both among-days variation in the social environment and a large number of mating events within each day. In the same way as above, we analysed potential variation in the strength of sexual selection by calculating Is_{bc} each day. We then calculated OSR for each day as the ratio of sexually receptive males to females. Individuals were considered sexually receptive from their first mating attempt until their death, as there is no postmating refractory period in male and female lampreys (L. Daupagne, personal observation). We also calculated what we called the functional sex ratio (FSR) on each day as the ratio of sexually active males to females (i.e. individuals that actually attempted to copulate on that day). We then calculated correlations between Is_{bc} and OSR using a Spearman rank correlation test. Indeed, as stated by Emlen and Oring (1977), a biased OSR should induce a greater variance in mating success of the dominant sex due to a higher degree of mate monopolization, and therefore a greater Is_{bc}.

Quantitative Estimation of Sexual Selection on Identified Traits

Instead of the classic multiple regression approach (e.g. Lande & Arnold, 1983), which separately models the mating success (number of mates) of either males or females as a linear combination of individual phenotypic traits and possibly social environment (Okasha, 2004), we considered each mating act as a statistical unit, following the approach of Gauthey et al., (2017). In our first Bayesian model, we considered parameters reflecting changes in social environment each day that may affect sexual selection estimates: (1) individual body size of both interacting partners, (2) size difference between partners, (3) OSR, (4) average competitors' size relative to the size of the focal individual, and (5) the time since the start of the individual's reproductive period. Competitors were defined as individuals of the same sex as the focal individual and were sexually receptive on the focal day (i.e. those contributing to the OSR). The last parameter (5) may reflect variation in mating effort over an individual's reproductive period. We first checked for correlation between morphological traits (body size and body mass) with a Spearman rank correlation test. As there was a significant correlation (□ = 0.76), only total body size was included in our analysis.

From daily behavioural observations, we constructed two different matrices confronting all possible pairs of sexually receptive individuals of each sex each day: a first three-dimensional array of the total number of mating attempts between each male *m* and female *f* on day *t* ($A_{m,f,t}$), and a second three-dimensional array of the total number of successful matings ($C_{m,f,t}$) between each male *m* and female *f* on day *t*. The number of mating attempts was modelled by a Poisson distribution and the number of successful matings was modelled by a binomial distribution.

$$A_{m,f,t} \sim \text{Poisson}(T_{m,f,t})$$

$$C_{m,f,t} \sim \text{binomial}(A_{m,f,t}, \theta_{m,f,t})$$

We tested the effects of body size of the focal male and female (S_m and S_f, respectively), the absolute difference in partner size ($\text{abs}(S_m - S_f)$), the average competitors' size relative to the size of the focal individual on day *t* (($\text{CSM}_t - S_m$) and $\text{CSF}_t - S_f$)), the OSR on day *t* (OSR_t) and time (days) since the start of the individual's reproductive period ($\text{RP}_{m,t}$ and $\text{RP}_{f,t}$) on the number of mating attempts ($T_{m,f,t}$) (equation 1) and on the probability that a mating attempt was successful ($\theta_{m,f,t}$) (equation 2). Because our goal was to test whether selection on an individual trait depended on the social context, we also included interaction terms as follows:

$$T_{m,f,t} = \exp\begin{pmatrix} \alpha_1 + \beta_1\, S_m + \beta_2\, S_f + \beta_3\, \text{abs}(S_m - S_f) + \beta_4\, \text{OSR}_t + \beta_5\, (S_m * \text{OSR}_t) \\ + \beta_6\, (S_f * \text{OSR}_t) + \beta_7 (\text{CSM}_t - S_m) + \beta_8\, (\text{CSF}_t - S_f) + \beta_9\, \text{RP}_{m,t} + \beta_{10}\, \text{RP}_{f,t} \\ + r_{1,m} + r_{1,f} \end{pmatrix}$$

$$\theta_{m,f,t} = \text{inv.logit}\begin{pmatrix} \alpha_2 + \beta_{11}\, S_m + \beta_{12}\, S_f + \beta_{13}\, \text{abs}(S_m - S_f) + \beta_{14}\, \text{OSR}_t + \beta_{15}\, (S_m * \text{OSR}_t) \\ + \beta_{16}\, (S_f * \text{OSR}_t) + \beta_{17} (\text{CSM}_t - S_m) + \beta_{18}\, (\text{CSF}_t - S_f) + \beta_{19}\, \text{RP}_{m,t} + \beta_{20}\, \text{RP}_{f,t} \\ + r_{2,m} + r_{2,f} \end{pmatrix}$$

where $r_{.,m}$ and $r_{.,f}$ are random male and female effects (individuals' effects) to account for uncontrolled sources of variation.

Then, we constructed a second Bayesian model to test how mating success could be affected by two variables pertaining to the temporal distribution of mating effort: date of first mating attempt and delay between first and last mating attempt (i.e. duration of mating activity). From behavioural observations, we constructed two different matrices confronting all possible pairs of individuals of each sex: a first matrix of the total number of mating attempts over the whole breeding season ($A_{m,f}$) and a second matrix of the total number of successful matings ($C_{m,f}$). The number of mating attempts between a pair was modelled by a Poisson distribution while the number of successful matings was modelled by a binomial distribution.

$$A_{m,f} \sim \text{Poisson}(T_{m,f})$$

$$C_{m,f} \sim \text{binomial}(A_{m,f}, \theta_{m,f})$$

We tested the effects of body size of focal male and female (respectively S_m and S_f), absolute difference in partner size ($\text{abs}(S_m - S_f)$), mating activity first date (I_m and I_f) and duration of mating activity (D_m and D_f) on both the number of mating attempts ($T_{m,f}$) (equation 3) and the probability of mating success ($\theta_{m,f}$) (equation 4) as follows:

$$T_{m,f} = \exp\begin{pmatrix} \alpha_1 + \beta_1\, S_m + \beta_2\, S_f + \beta_3\, \text{abs}(S_m - S_f) + \beta_4\, I_m + \beta_5\, I_f + \beta_6\, D_m + \beta_7\, D_f + \\ \beta_8\, (S_m * I_m) + \beta_9\, (S_f * I_f) + \beta_{10}\, (S_m * D_m) + \beta_{11}\, (S_f * D_f) + r_{1,m} + r_{1,f} \end{pmatrix} \tag{3}$$

$$\theta_{m,f} = \text{inv.logit}\begin{pmatrix} \alpha_2 + \beta_{12}\, S_m + \beta_{13}\, S_f + \beta_{14}\, \text{abs}(S_m - S_f) + \beta_{15}\, I_m + \beta_{16}\, I_f + \beta_{17}\, D_m + \beta_{18}\, D_f \\ + \beta_{19}\, (S_m * I_m) + \beta_{20}\, (S_f * I_f) + \beta_{21}\, (S_m * D_m) + \beta_{22}\, (S_f * D_f) + r_{2,m} + r_{2,f} \end{pmatrix} \tag{4}$$

where $r_{.,m}$ and $r_{.,f}$ are random male and female effects (individuals' effects) to account for uncontrolled sources of variation. All explanatory variables were centred (minus their mean) and standardized (divided by their standard deviation).

Statistical Analysis

All classical statistical analyses were performed using R software version 4.0.4 (R Core Team, 2022). Average values presented throughout the paper correspond to the mean. The two statistical models in this study were fitted under a Bayesian framework that allows the handling of a large number of predictor variables using HMC sampling applied by STAN through the R package RStan (Carpenter et al., 2017). For each model, three independent chains were run to save 5000 iterations after a warmup of 1000 iterations and with a thinning of 5. In each chain, we used a noninformative Cauchy distribution for all parameters ($C(0,5)$) (Gelman et al., 2008). Chain convergence was visually checked and parameter convergence was assessed with the Gelman and Rubin's convergence diagnostic (gelman.diag function, Brooks & Gelman, 1998; Gelman & Rubin, 1992). Each parameter was considered as significant if 0 was not included in its highest density interval at 95% ($HDI_{95\%}$). Information on the validation of the models is provided in the Appendix and Table A1.

Ethical Note

The use of experimental animals complied with the French environmental and animal welfare laws, guidelines and policies as evidenced by the authorization issued by the ethical committee for birds and fishes in the French region Nouvelle Aquitaine (authorization #2019021009248986). The potential harm to the animals is very limited and mainly concerns exposure to electricity during fishing, tagging under anaesthesia and keeping the individuals in an aquarium during reproduction. Lampreys are semelparous and die shortly after reproduction.

Thus, the condition of the individuals deteriorates very rapidly during reproduction, even in the natural environment. We did not observe any premating mortality (the dead individuals had all participated in reproduction and contained no or very few gametes).

RESULTS

General Description of the Mating System

Body size and weight did not differ between the sexes: males measured on average (±SD, minimum:maximum) 282.9 mm (±21.7, 237:315) while females measured on average 284.2 mm (±16.9, 250:316). The mating season, calculated as the time between the first and last day on which matings were observed, lasted 22 days. The first mating attempts occurred on 9 April while the last one occurred on 30 April. Differences existed in terms of temporal distribution of mating effort: females took part in the spawning during a much shorter period than males in terms of number of days between the first and last mating attempt (Fig. 1), on average 6.5 days in females and 14 days in males (ANOVA: $F_{1,33} = 42.88$, $P < 0.001$). The females started to mate on average later than males (ANOVA: $F_{1,33} = 4.74$, $P = 0.03$) and more gradually. Differences also appeared in terms of number of days between the last mating attempt observed and the day of death: females died much faster than males (Fig. 1), on average 1.5 days in females and 3.5 in males (Kruskal–Wallis test: $H_{11} = 9.16$, $P < 0.001$). This sex-specific temporal distribution of mating effort led to fluctuations in size distribution of active individuals (Appendix Fig. A1). A total of 8582 mating attempts were observed, among which 6815 were successful matings. The mating system clearly can be qualified as polygynandrous, as the 15 males successfully mated on average (±SD, minimum:maximum) 454.33 (±355.82, 17:1088) times with on average 14.73 (±2.91, 9:20) females, and the 20 females mated on average 340.75 (±222.52, 8:897) times with on average 11.05 (±3.22, 4:15) males. The number of successful matings was correlated with the number of mates for males (Spearman rank correlation: r_S = 0.7, N = 15, P < 0.005) but not for females (r_S = 0.29, N = 20, P = 0.21).

Estimation of Potential Strength of Sexual Selection

At daily time steps

The OSR and FSR were on average (±SD, minimum:maximum) 1.97 (±0.90, 1.08:3. 67) and 1.7 (±0.69, 0.75:3), respectively (Appendix Fig. A2). The indices were highly correlated (Spearman rank correlation: r_S = 0.76, N = 20, P < 0.001), so only OSR was used in further analysis.

The Is_{bc} with MS$_\mathrm{P}$ (i.e. the number of partners) was on average (±SD, minimum:maximum) 0.38 (±0.59, -0.49:1.85) per day in males and 0.54 (±0.52, -0.33:1.48) per day in females (Appendix Fig. A3a). The Is_{bc} with MS$_\mathrm{M}$ (i.e. the number of matings) was on average (±SD, minimum:maximum) 1.92 (±1.24, 0.46:5.57) per day in males and 2.07 (±1.05, 0.93:5.96) per day in females (Appendix Fig. A3b).

A Spearman rank correlation was computed to assess the relationship between Is_{bc} and OSR in each sex (Appendix Fig. A4). There was a positive correlation between the two variables in males when Is_{bc} was calculated with MS$_\mathrm{M}$ (r_S = 0.53, N = 20, P = 0.01) but not with MS$_\mathrm{P}$ (r_S = -0.12, N = 20, P = 0.60). There was no significant correlation in females with Is_{bc} calculated with either MS$_\mathrm{M}$ (r_S = 0.38, N = 20, P = 0.07) or MS$_\mathrm{P}$ (r_S = 0.15, N = 20, P = 0.49).

At the scale of the whole breeding season

The Is_{bc} with MS$_\mathrm{P}$ was slightly lower for males than for females while the Is_{bc} with MS$_\mathrm{M}$ was higher for males (Table 1). Variances in relative mating success were not significantly different as a function of sex, either for MS$_\mathrm{P}$ or MS$_\mathrm{M}$ (F = 2.08, P = 0.16 and F = 0.76, P = 0.39, respectively; Table 1).

Quantitative Measurement of Sexual Selection

At daily time steps: variation in the social environment

Female body size had a negative effect on the number of mating attempts but a positive effect on the probability of mating success, while male body size had a positive effect on the number of mating attempts but a negative effect on the probability of success (Fig. 2a). Difference in partner size had a negative effect on both the number of mating attempts and the probability of mating success (Fig. 2b). The OSR had a negative effect on the number of mating attempts but had no effect on the probability of success (Fig. 2c). In both sexes, the interaction between body size and OSR had a positive effect on the number of mating attempts (Figs 2d, 3a, c). In males, the interaction had a negative effect on the probability of mating success (Fig. 3d) while the interaction had a positive effect in females (Figs 2d, 3b). In males, the average size difference between the focal individual and its competitors had a positive effect on the number of mating attempts but a negative effect on the probability of mating success, while in females, it had a negative effect on the number of mating attempts but a positive effect on the probability of success (Fig. 2e). Finally, in males, the time (days) since the start of the individual's reproductive period had a negative effect on the number of mating attempts, while in females, it had a positive effect on the number of mating attempts but a negative effect on the probability of success.

At the scale of the whole season: temporal spawning patterns

In either sex, body size had overall no effect on either the number of mating attempts or the probability of mating success (Fig.4a). The difference in partner size had a negative effect on both the number of mating attempts and the probability of mating success (Fig. 4b). The first date of mating activity had a positive effect on the number of attempts and a negative effect on the probability of mating success in females, but it had no effect on both metrics in males (Fig. 4c). The total duration of mating activity had no significant effect on either the number of mating attempts or the probability of success in either sex (Fig. 4d). The interaction between body size and the first date of mating activity had a negative effect on the number of mating attempts in females and a positive effect on the probability of success, while it had no effect on both metrics in males (Fig. 4e). Finally, the interaction between body size and duration of mating activity had no significant effect on either the number of mating attempts or the probability of success in either sex (Fig. 4f).

DISCUSSION

Our results showed that (1) the social environment (OSR, body size distribution of active individuals) fluctuated throughout the breeding season of a group of river lampreys in controlled conditions, (2) the number of matings on a given day depended on individual body size, social environment on that day and their interaction, and (3) the timing (onset and span) of breeding activity could affect the number of matings performed over the season with a strength dependent on individual body size. We discuss the implications of these findings in terms of both sexual selection in lampreys and broader meaning for sexual selection estimation in polygynandrous external fertilizers.

Despite a female-biased adult sex ratio (0.75) in the breeding group, the OSR was always male biased and varied throughout the reproductive season (Fig. A2), because of sex differences in the onset and time span of sexual activity (Fig. 1). While most males were active from the beginning of the season and stayed active on average for 15 of the 22 days during which the group spawned, individual females started their activity in a more gradual way and for on average 6.5 days. Such patterns have also been documented in the brook lamprey, *Lampetra planeri*, in an experimental approach (Malmqvist, 1983) and in the sea lamprey, *Petromyzon marinus*, in the field (Dhamelincourt et al., 2021). Although the water recirculation in our experimental set-up may have increased the synchrony of sexual maturation due to an accumulation of sex pheromones throughout the experiment, this result suggests this is not the case or that such an acceleration of maturation also occurs in the wild, for example when spawning densities upstream are high (Wang et al., 2013). Besides the span of reproductive activity, the time between last mating and death was shorter in females than in males, further suggesting that the rapid senescence triggered by reproduction was more acute in females than in males. This sex difference in the timing of reproduction therefore led to the observed biased OSR (Fig. A2), causing fluctuations in the social environment in which sexual selection operates. We then found a positive correlation between OSR and bias-corrected opportunity for sexual selection (Is_{bc}) with MS_M in males but not females (Fig. A4a), as originally suspected by Emlen and Oring (1977) and recently observed by Janicke and Morrow (2018) in a meta-analysis performed on 82 estimates of Is_{bc} in 58 animal species. Additionally, female Is_{bc} with MS_M was insensitive to OSR and remained sufficiently high to equal male Is_{bc} when cumulated over the whole season (Table 1). This indicates that there was as much scope for sexual selection in females as in males in this polygynandrous system, but that the availability of males was not the factor that constrained the variability of access to mates and matings among females. Outcompeting rivals probably do not bring significant direct or indirect benefits, leading to an overall less pronounced mate monopolization in females. Interestingly, no correlation between OSR and Is_{bc} with MS_P was found in males (Fig. A4b), showing that the chosen quantitative approach to measure mating success does affect the inference of sexual selection. By approximating the number of mating events with the number of mates, the ‘genetic definition’ of mating success cannot detect potential multiple inseminations and thus the degree of mate monopolization. This first analysis, by using a population and a fitness-based approach, highlighted that changes in social environment affect both males’ access to reproduction in terms of the number of matings achieved and, consequently, the potential strength of sexual selection in this sex.

With a statistical framework that models each mating as a two-step process (attempt and success; Gauthey et al., 2017), we then investigated how these changes in social environment directly modulate the strength of sexual selection on individual phenotypic traits. The distinction between the number of mating attempts and the probability of success sheds light on the part of the mating process that is affected by individual and social characteristics. We showed that social context affected each part of the process both directly and through interactions with individual phenotype. In our model, larger males appeared to attempt more matings, but smaller males had a higher probability of mating successfully. This pattern was even stronger on days with higher OSR, as large males performed better in terms of mating attempts, but smaller individuals had an even higher probability of mating successfully (Figs 2, 3). The first result was in line with our predictions, as we initially expected that larger males would have an overall advantage in intrasexual mate competition and thus have easier access to females, especially at a male-biased OSR. On the other hand, the higher probability of mating success in small males was more surprising as we initially thought that females would overall favour large males. This pattern may reflect either a female preference towards smaller males that can be expressed more freely under high OSR, or the choice of small males to mate with lower quality females that might accept them more easily, especially when the competition is high (see below). Our model also highlighted that size-assortative mating does occur in lampreys, as suggested by previous work (Malmqvist, 1983), and concerns both processes leading to mating (attempt and success). The higher probability of success in pairs with similar size may be due to a physical constraint imposed by size differences between partners while the higher number of attempts in pairs with similar size may result from a mate choice in relation to one's own size. This latter may explain the higher probability of mating success in small males, especially at a high OSR. If male–male competition is sufficiently costly, the benefits conferred by mating with large females are outweighed, which may ultimately lead low-competitive males (i.e. small males) to attempt to mate with small low-quality females (i.e. small females) that would accept them more easily. The higher probability of mating successfully in small males is therefore the result of a size-assortative mating pattern based on mate choice.

The social context affected the number of matings in females too. High OSR increased both the number of mating attempts and the probability of success for large females. This suggests that under stronger male–male competition, larger females are more often solicited by males and accept mating more readily. Lamprey females parcel their large egg stock (around 26 000 in the anadromous river lamprey; Docker, 2019) in many clutches (on average 341 in this study). Larger females bear more eggs and may therefore mate with more males, which could benefit them directly and indirectly. A direct benefit of female multiple mating in lampreys may be an insurance against incomplete fertilization due to sperm limitation. On the other hand, indirect genetic benefits may include (1) improved viability or competitiveness of their offspring or (2) higher genetic diversity of offspring which may increase the possibility that some individuals can survive in a changing environment (Fox & Rauter, 2003; Garcia-Gonzalez et al., 2015; Yasui, 2001). Finally, compared to their competitors the smaller females seemed to attempt fewer matings but had a higher probability of mating successfully. This suggests that small females are solicited even less by males when competition is high and therefore more readily accept mating.

Given the interactions between individual phenotype and fluctuating social environment, and the sex difference in the temporal distribution of reproductive activity, one could expect to see sexual selection on the timing of reproductive activity, possibly in relation to sex and phenotype.

In males, there was a tendency for long-lasting males to perform more mating attempts. This was in accordance with our initial thought that males mating over a long period may maximize the number of mating opportunities by encountering more females during the mating season. Interestingly, long-lasting males also appeared to have a slightly lower probability of mating successfully. This may suggest that such males have more time to seek out matings with high-quality females that have a higher probability of rejecting them. The duration of an individual's mating season could also be an adjustment to its ability to successfully mate with members of the opposite sex. If one individual repeatedly fails to reproduce, it will remain in the breeding pool for longer to deplete its gamete stock.

The starting date had no effect on either the number of mating attempts or the probability of mating success in males. This result was all the more surprising as in polygynandrous species such as lampreys, males arrive at the breeding grounds before females, suggesting an advantage of early males in terms of access to females (Morbey & Ydenberg, 2001). Females are, however, relatively asynchronous (Fig. 1) and the OSR was lower from day 8 to day 13 than from day 1 to day 7; males starting early therefore did not have access to a higher number of receptive females. However, we cannot exclude the possibility that this may be an artefact of captivity. The early arrival of females in the wild may be more synchronous or may be selected for reasons other than the mating parameters investigated here.

In females, early onset of mating activity decreased the number of mating attempts but increased the probability of success, and the interactions with body size imply that these effects of early activity were stronger in smaller females. This may suggest that late-arriving females attempt more matings with males active at the end of the season but are more selective towards them. A recent experiment using sea lampreys showed that ovulatory females use spermine, a pheromone that originates in the seminal plasma, to identify males actively releasing sperm (Scott et al., 2019). As late-active males have a higher probability of being sperm depleted, females are probably more discriminating towards them to prevent costly matings. Small females appeared to be even more selective which is consistent with their lower fecundity. Such results suggest that females are selected to start early, especially if they are small. Interestingly, estimates values (β_5/β_{16}) were similar to those corresponding to the effect of the time since the start of the female's reproductive period in our daily model (β_{10}/β_{20}). This suggests that females that had been mating for a long time and were more likely to have few eggs left, attempted more matings but were more selective to avoid costly matings with low-quality males.

Conclusions

Overall, our study highlighted the seasonal dynamics of precopulatory sexual selection in lampreys and how it is directly related to characteristics of the sexual environment. From a methodological perspective, our results illustrate the complexity of measuring sexual selection and the necessity to increase studies using fine-grained behavioural data sets. For most studies, quantification of sexual selection remains generally based on one of a few samplings per season that may therefore lead to biased or even incorrect estimation of sexual selection, depending on which day the metrics are calculated. For instance, if we had only calculated metrics on days when the OSR was high, we would have overestimated the actual strength of sexual selection on female body size. Ongoing methodological advances in telemetry monitoring (Tentelier et al., 2016; Whitford & Klimley, 2019) or video recording systems (Yang et al., 2018), for instance, are overcoming previous difficulties in obtaining such high-resolution behavioural data. From a more theoretical perspective, showing that the benefits of exhibiting specific sexual traits are highly context dependent has important implications. Based on our results, we can hypothesize that conditional temporal strategies, in which individuals adapt the timing of sexual activity according to their own phenotype, may exist in lampreys. In swordtails, *Xiphophorus helleri*, it has been shown that the age at sexual maturity shifts in developing individuals in accordance with the perceived quality of competitors and potential mates. After maturity, environmental visual cues might also lead to behavioural plasticity (e.g. longer or shorter mating seasons) depending on the competitive challenges faced by individuals.

Author Contributions

L.D. was involved in the video analysis, statistical analysis and in writing and editing the manuscript. C.B. helped with statistical analysis and critically revised the manuscript. A.M. and M.D. collected the data and helped with the video analysis. E.L. helped with data analysis and critically revised the manuscript. C.T. contributed to the initial conception and the design of the study, helped with data analysis and critically revised the manuscript.

Declaration of Interest

None.

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Appendix

Bayesian Model Validation

At daily time steps

Random effects were slightly more variable for males than for females for the number of mating attempts (mean of 0.61 and 0.47, respectively) while they were more variable for females for the probability of mating success (mean of 0.11 and 0.31 respectively; Fig. A5). The correlations between random individual effects of two processes were calculated in each sex but none were statistically significant (Fig. A6).

Spearman rank correlations were computed to assess the pairwise relationship between random individual effects on the number of mating attempts and probability of success in both sexes. In males, there was no correlation between the number of mating attempts and probability of mating success (r_{13}= -0.09, P = 0.75). Similarly, there was no correlation in females (r_{18} = 0.26, P = 0.27; Fig. A6).

We performed a Gelman–Rubin diagnostic to analyse the difference between the three Markov chains. For each parameter, the scale reduction factor $\hat{r}$ was equal to 1, highlighting that all chains had converged (see Table S1 on the online Supplementary Material). Based on the posterior distribution of all parameters of our model, the numbers of mating attempts and successful matings were calculated for each individual and these predictions were plotted against the numbers of mating attempts and successful matings observed in our experiment (Fig. A7). Spearman rank correlations were computed to assess the pairwise relationship between the number of mating attempts and successful matings predicted in our model and those observed in our experiment. In both sexes, there were strong positive correlations between predicted and observed numbers of mating attempts ($r_{33} = 0.99$, $P < 0.001$; Fig. A7a), and predicted and observed numbers of successful matings ($r_{33} = 0.99$, $P < 0.001$; Fig. A7b). For most individuals, numbers predicted by the model were equivalent to the number of interactions observed. The model predicted on average (±SD, [$\text{HDI}_{95\%}$]) 616 (±482, [46:1388]) mating attempts in males, 462 (±312, [64:890]) mating attempts in females, 477 (±382, [37:1117]) successful matings in males and 358 (±244, [46:758]) successful matings in females.

At the scale of the whole season

Random effects were slightly more variable for males than for females for the number of mating attempts (mean of 0.67 and 0.41, respectively) and were similar for the probability of mating success (mean of 0.13 and 0.12, respectively; Fig. A8) The correlations between random effects of both processes were calculated in each sex but none were statistically significant (Fig. A9).

Spearman rank correlations were computed to assess the pairwise relationship between random individual effects on the number of mating attempts and probability of success in both sexes. In both males and females, there was no correlation between number of mating attempts and probability of mating success (males: $r_{13} = -0.32$, $P = 0.24$; females: $r_{18} = -0.12$, $P = 0.61$; Fig. A9).

We performed a Gelman–Rubin diagnostic to analyse the difference between the three Markov chains. For each parameter, the scale reduction factor $\hat{r}$ was equal to 1, highlighting that all chains had converged (see Table S2 on the online Supplementary Material). Based on the posterior distribution of all parameters of our model, the numbers of mating attempts and successful matings were calculated for each individual and these predictions were plotted against the numbers of mating attempts and successful matings observed in our experiment (Fig. A10). Spearman rank correlations were computed to assess the pairwise relationship between the numbers of mating attempts and successful matings predicted in our model and those observed in our experiment. In both sexes, there were positive correlations between predicted and observed numbers of mating attempts ($r_{33} = 0.48$, $P < 0.05$; Fig. A10a), and predicted and observed numbers of mating attempts ($r_{33} = 0.46$, $P < 0.05$; Fig. A10b). However, numbers predicted by the model exceeded the number of observations in most cases. Indeed, the model predicted on average (±SD, [$\mathrm{HDI}_{95\%}$]) 1296 (±1242, [66:3360]) mating attempts in males, 972 (±738, [133:2547]) mating attempts in females, 1038 (±996, [53:2662]) successful matings in males and 778 (±615, [121:2134]) successful matings in females.

TABLES

Table 1. Estimates of mating success and the potential strength of sexual selection in *Lampetra fluviatilis* males and females at the scale of the whole breeding season

	Males					**Females**					**Levene's test**		
	N	**Mean**	**SD**	**Obs I_s**	**$Is_{bc[95\% C.I.]}$**	***N***	**Mean**	**SD**	**Obs I_s**	**$Is_{bc[95\% C.I.]}$**	**F**	**Df**	***P***
MS_P	15	14.7	2.9	0.04	**-0.01** [-0.04:0.01]	20	11	3.2	0.08	**0.03** [-0.01:0.05]	2.1	1	0.16
MS_M		454	356	0.61	**0.60** [0.59:0.61]		341	223	0.43	**0.41** [0.41:0.42]	0.8	1	0.39

Number of individuals (*N*), mean mating success, standard deviation (SD), observed opportunity for sexual selection (Obs I_s), and bias-corrected I_s (Is_{bc}) with 95% confidence intervals are presented with mating success calculated either as the number of mates (MS_P) or the number of mating acts (MS_M). Differences in variances for mating success were estimated via Levene's test.

Table S1. Summary of estimated parameters distributions and derived quantities using the posterior draws of the model at daily time steps (see equation 1 and 2 for details). The summary includes: means, Monte Carlo standard errors (se_mean), standard deviations (sd), quantiles, effective sample sizes (n_eff), and split Rhats (i.e. the potential scale reduction derived from all chains after splitting each chain in half and treating the halves as chains). m{x,1:15} and f_{x,1;20} are random male and female effects. σm[x] and σf[x] are the standard deviation of random individual effects for males and females, respectively. x refers to the process concerned, either the number of mating attempts (1) or the probability of successful mating (2). The summary comprises all chains merged.

Table S2. Summary of estimated parameters distributions and derived quantities using the posterior draws of the model at the scale of the whole season (see equation 3 and 4 for details). The summary includes: means, Monte Carlo standard errors (se_mean), standard deviations (sd), quantiles, effective sample sizes (n_eff), and split Rhats (i.e. the potential scale reduction derived from all chains after splitting each chain in half and treating the halves as chains). m{x,1:15} and f_{x,1;20} are random male and female effects. σm[x] and σf[x] are the standard deviation of random individual effects for males and females, respectively. x refers to the process concerned, either the number of mating attempts (1) or the probability of successful mating (2). The summary comprises all chains merged.

FIGURES

Figure 1. Delay between the start of the experiment and the first mating attempt, duration of mating activity and delay between the last mating attempt and death (days) in 15 male (blue) and 20 female (orange) *Lampetra fluviatilis*. The box plots show the median (horizontal line), mean (cross) and 25th and 75th percentiles; the whiskers indicate the values within 1.5 times the interquartile range and the circles are the jittered data points. Tests: one-way ANOVA/Kruskal–Wallis: **P* < 0.05; ****P* < 0.005.

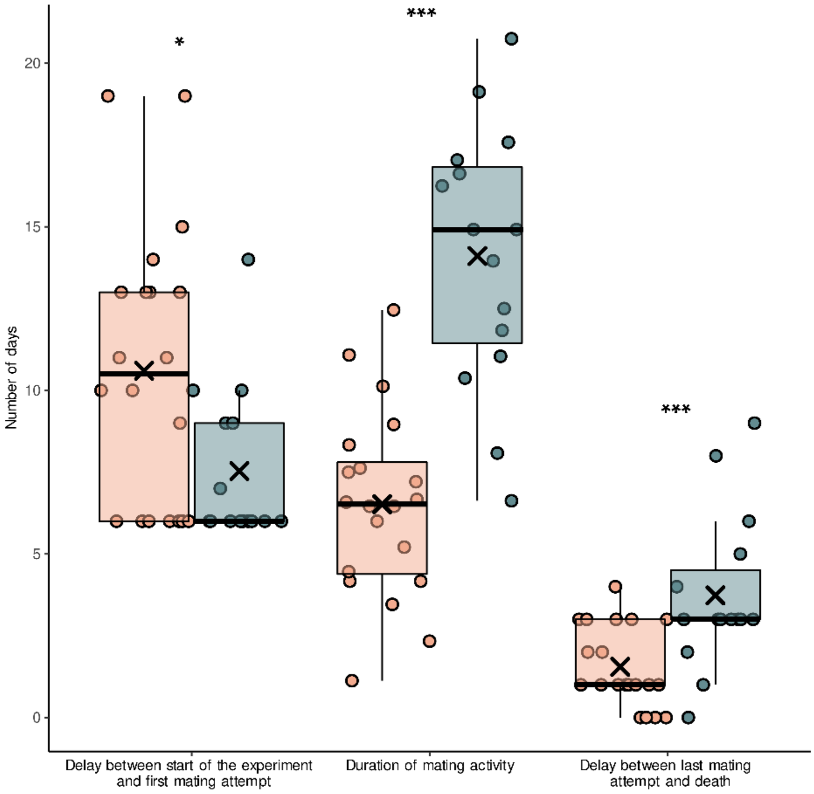

Figure 2. Posterior distribution for the parameters of the model inferring the effect of (a) individual body size, (b) difference in partner size, (c) operational sex ratio (OSR), (d) interaction between individual body size and OSR, (e) difference in competitors' size and individual body size and (f) time since the start of an individual's reproductive period in males (blue) and females (orange) on the number of mating attempts and on the probability of mating success. Labels on the x-axes refer to parameters presented in equations 1 and 2. Density plots show the distribution of the parameter values sampled from 12 000 iterations. The coloured

portions inside the density plots represent the 95% credible intervals. The dashed red vertical lines correspond to a null effect.

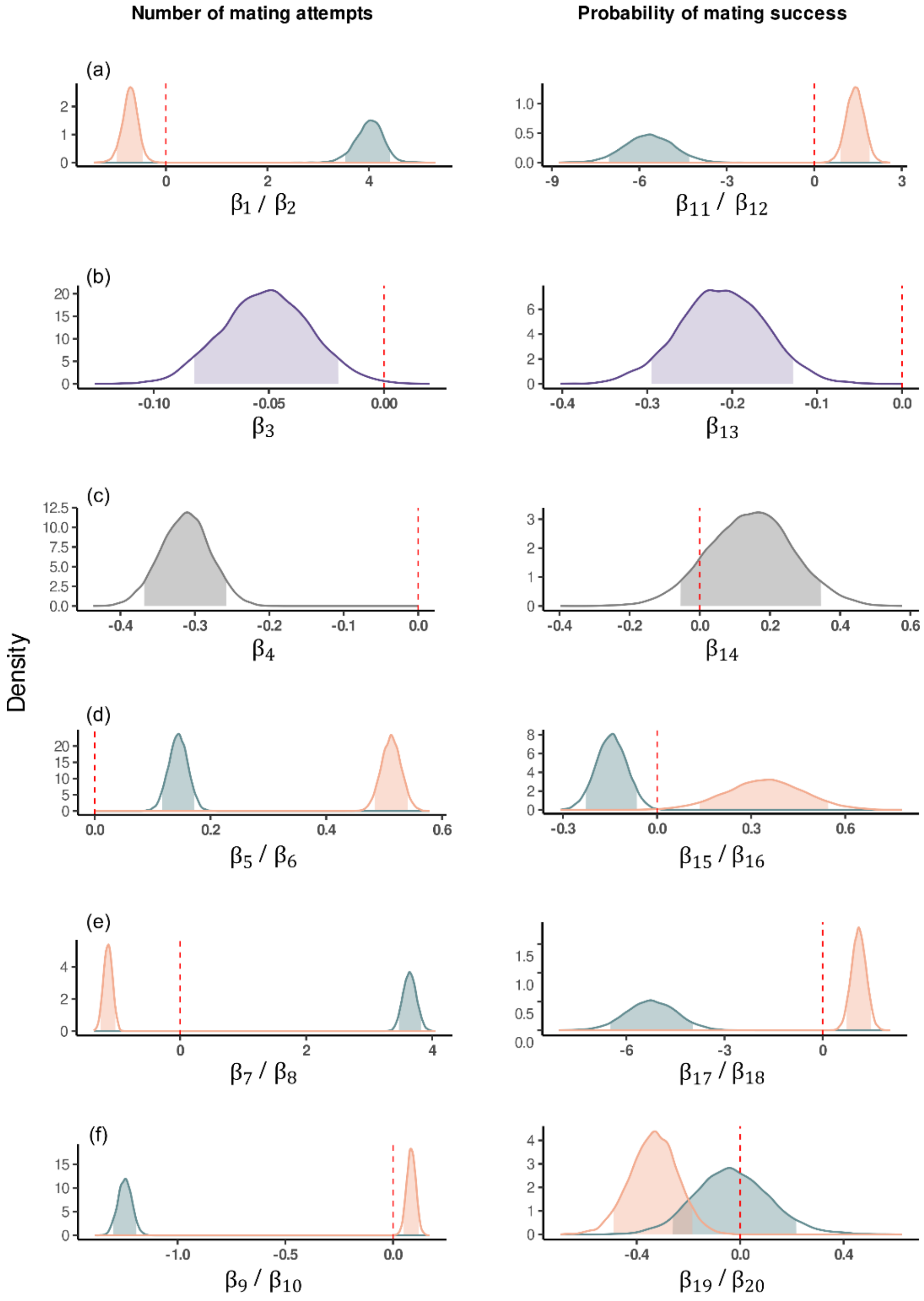

Figure 3. Predicted number of mating attempts and probability of mating success in males (blue) and females (orange) at low, even and high operational sex ratio (OSR). The grey areas represent the 95% confidence level interval for predictions from each linear model.

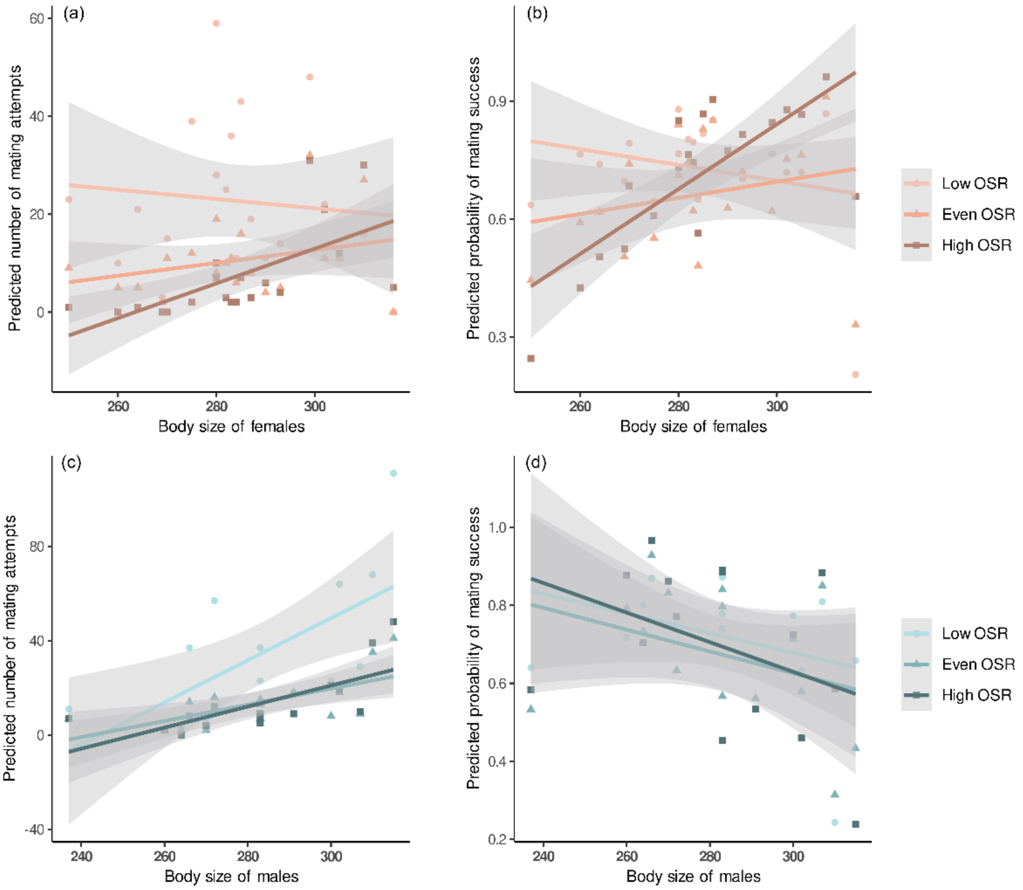

Figure 4. Posterior distribution for the parameters of the model inferring the effect of (a) individual body size, (b) difference in partner size, (c) first date of mating activity, (d) total duration of mating activity, (e) interaction effect between body size and first date of mating activity and (f) interaction between body size and duration of mating activity in males (blue) and females (orange) on the number of mating attempts and the probability of mating success at the scale of the whole breeding season. Labels on the x-axes refer to parameters presented in equations 3 and 4. Density plots show the distribution of the parameter values sampled from 12 000 iterations. The coloured portions inside the density plots represent the 95% credible intervals. The dashed red vertical lines correspond to a null effect.

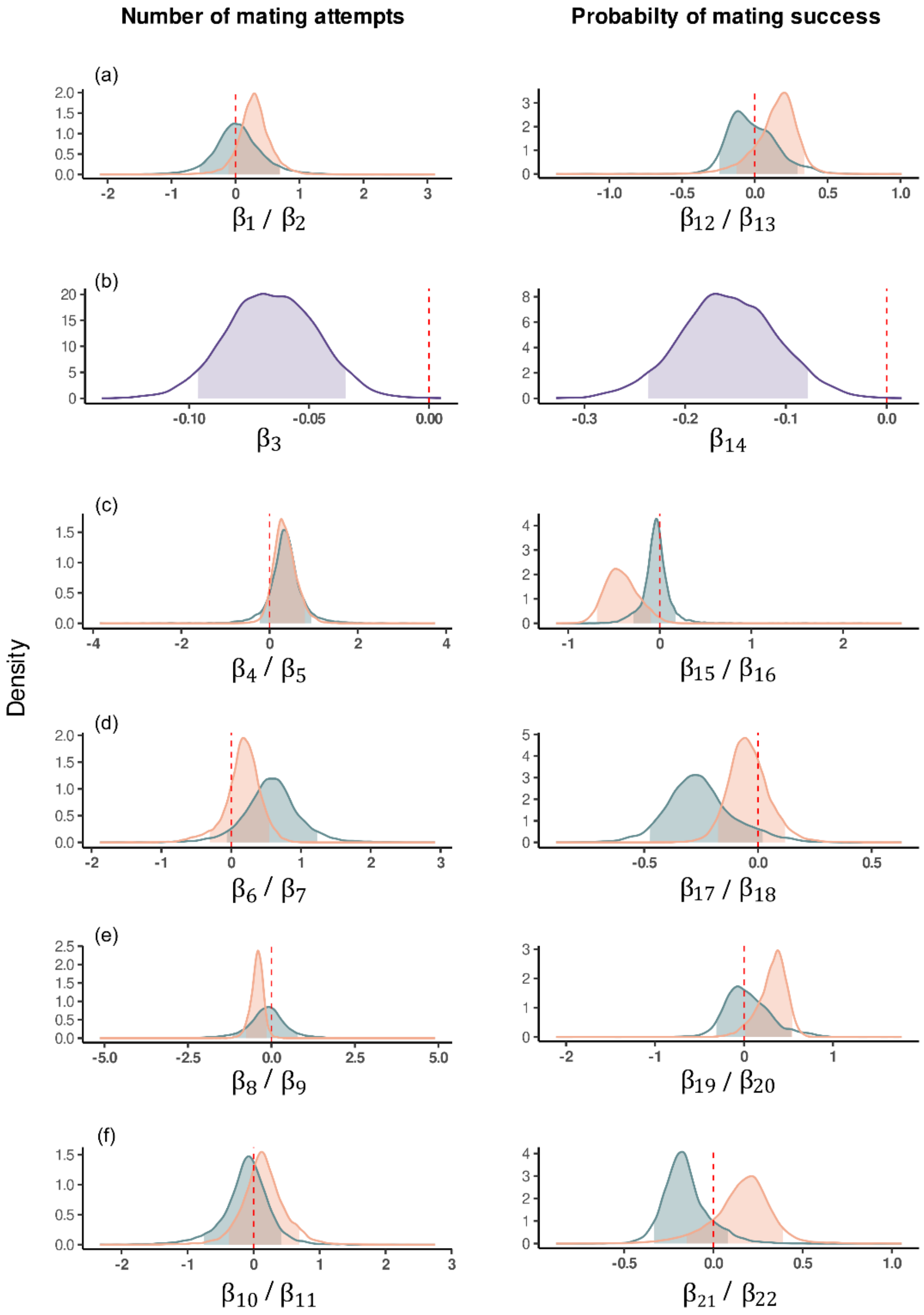

Figure A1. Size distribution (total length in mm) of active individuals each day of the breeding season (males: blue; females: orange). The box plots show the median and 25th and 75th percentiles; the whiskers indicate the values within 1.5 times the interquartile range and the circles are the jittered data points.

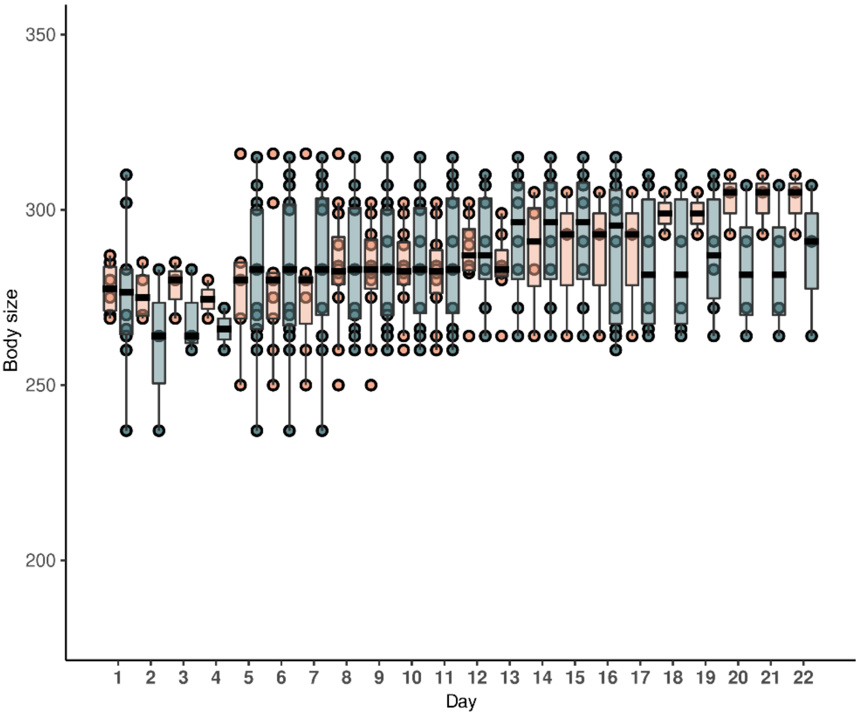

Figure A2. The operational sex ratio (OSR) and the functional sex ratio (FSR) each day of the breeding season. The OSR corresponds to the ratio of males to females that are ready to mate on a given day while FSR corresponds to the ratio of males to females that attempt to mate on a given day. The dashed line corresponds to an unbiased sex ratio (1) while the dotted line represents the adult sex ratio in the breeding group (0.75).

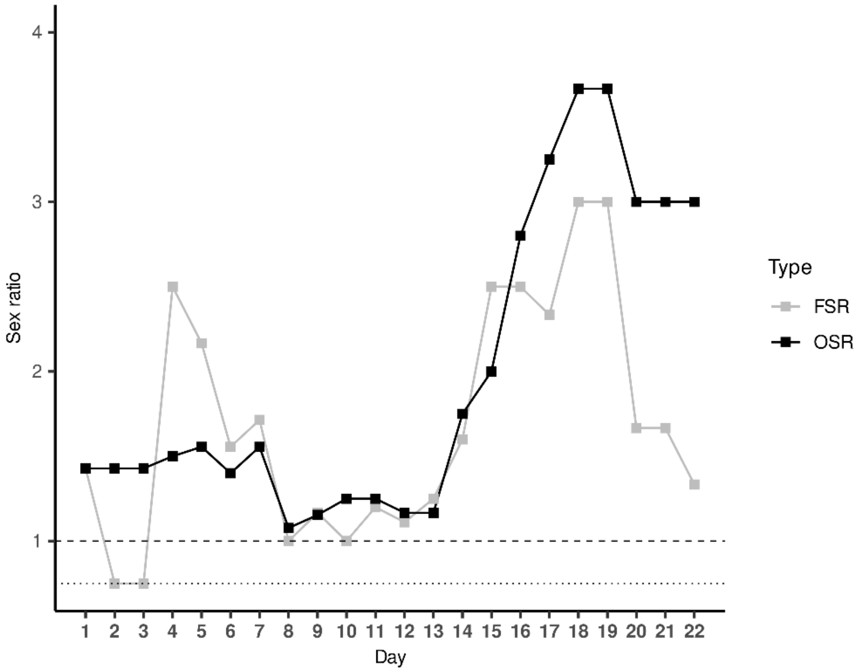

Figure A3. Bias-corrected opportunity for sexual selection (Is_{bc}) with 95% confidence intervals are presented (vertical whiskers) with mating success calculated either (a) as the number of partners (MS_P) or (b) as the number of matings (MS_M) in males (blue) and females (orange) on each day of the breeding season.

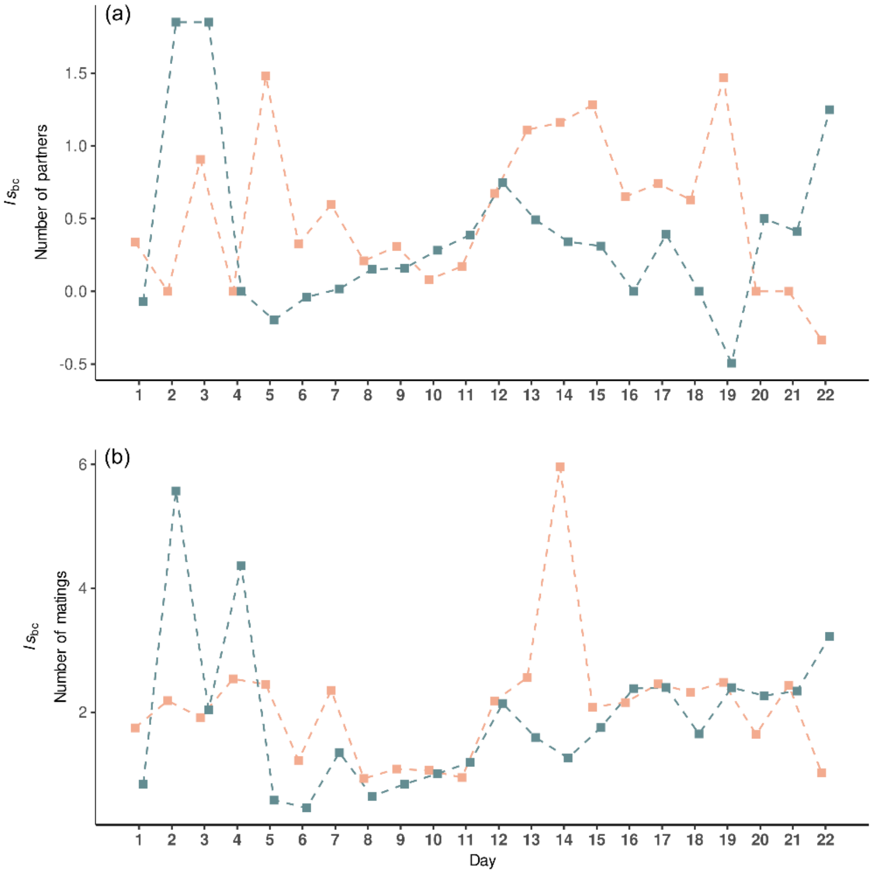

Figure A4. Relationship between the opportunity for sexual selection (Is_{bc}) and the operational sex ratio (OSR) with mating success calculated either (a) as the number of matings (MS_M) or (b) as the number of partners (MS_P) in males (blue) and females (orange).

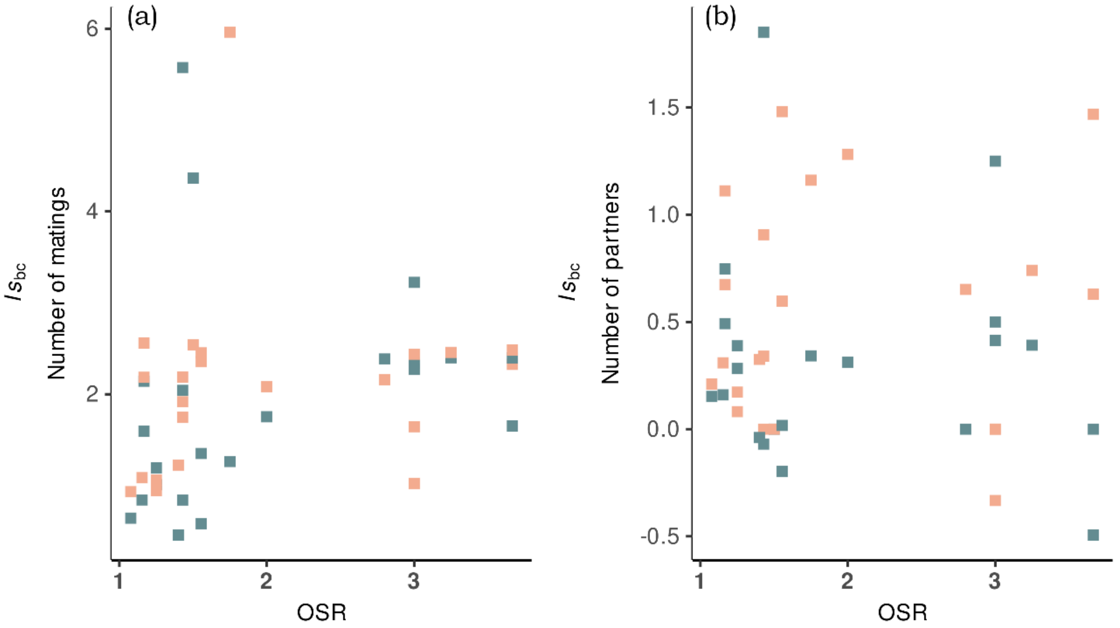

Figure A5. Posterior probability distribution of the standard deviation of random individual effects on (a) the number of mating attempts and (b) the probability of mating success on each day for males (blue) and females (orange) at daily time steps. The dashed red vertical lines correspond to a null effect. Density plots show the distribution of the parameter values sampled from 12 000 iterations. The coloured portions inside the density plots represent the 95% credible intervals.

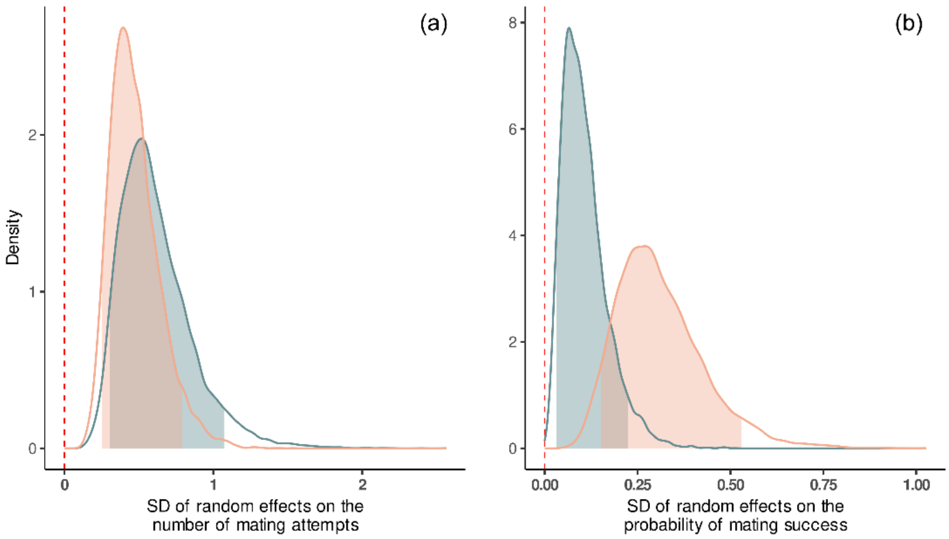

Figure A6. Pairwise relationship between random individual effects on the number of mating attempts and the probability of success for males (blue) and females (orange) at daily time steps.

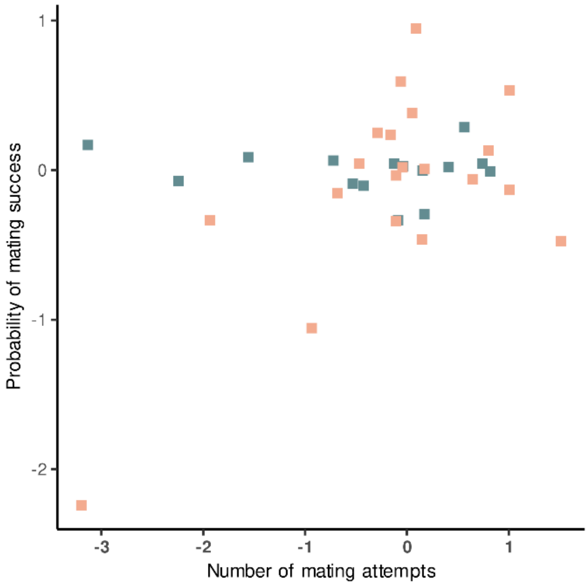

Figure A7. Pairwise relationship between the number of (a) mating attempts and (b) successful matings predicted in our model and those observed in our experiment for males (blue) and females (orange) at daily time steps. The dashed line has intercept zero and slope one, which corresponds to a perfect fit between observed and predicted values.

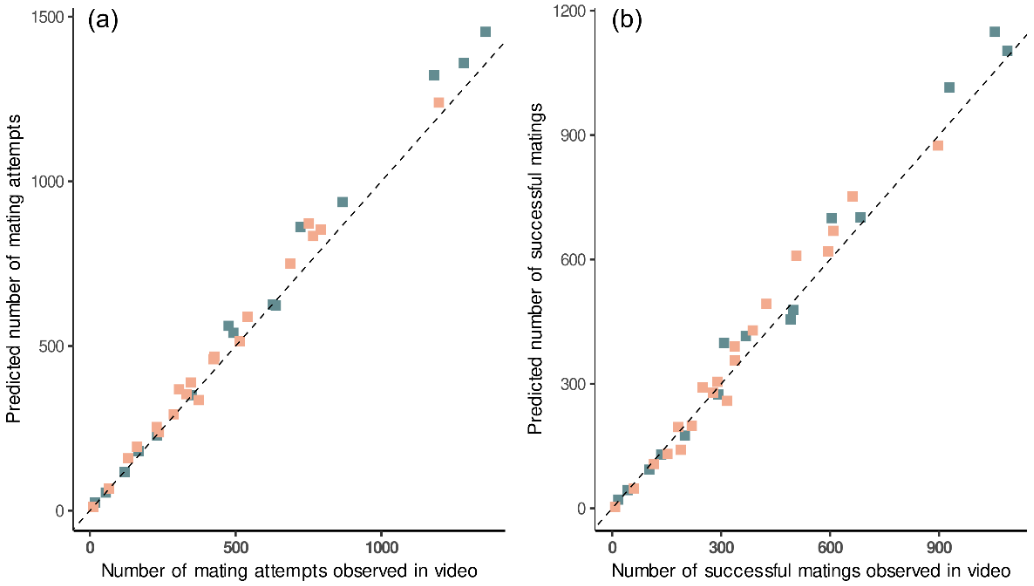

Figure A8. Posterior probability distribution of the standard deviation of random individual effects on (a) the number of mating attempts and (b) the probability of mating success for males (blue) and females (orange) at the scale of the whole breeding season. The dashed red vertical line corresponds to a null effect. Density plots show the distribution of the parameter values sampled from 12 000 iterations. The coloured portions inside the density plots represent the 95% credible intervals.

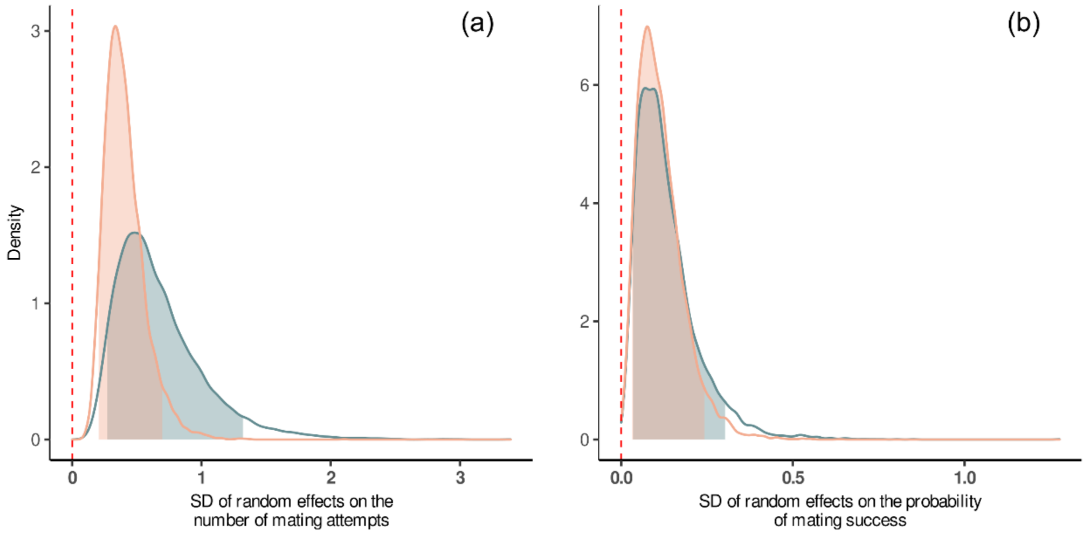

Figure A9. Pairwise relationship between random individual effects on the number of mating attempts and the probability of success for males (blue) and females (orange) at the scale of the whole breeding season.

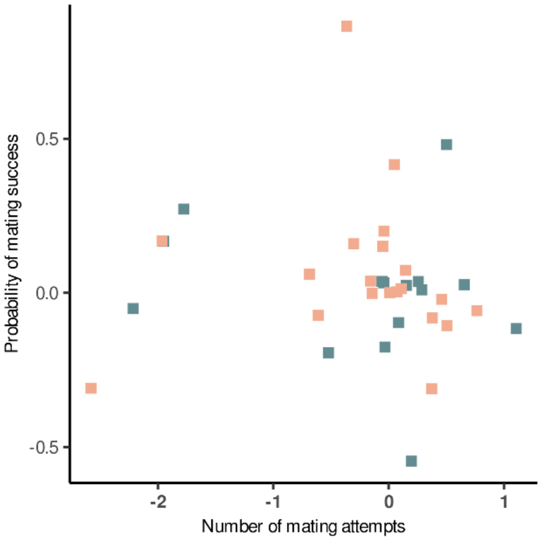

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Figure A10. Pairwise relationship between the number of (a) mating attempts and (b) successful matings predicted in our model and those observed in our experiment for males (blue) and females (orange) at the scale of the whole breeding season. The dashed line has intercept zero and slope one, which corresponds to a perfect fit between observed and predicted values.