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Population-level food availability affects postcopulatory sexual selection dynamics in the guppy

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Abstract

1. Postcopulatory sexual selection (PCSS), namely sperm competition and cryptic female choice, is typically investigated in benign environments, with a fixed number of partners, which mate at the same time intervals; all conditions that are rarely met in natural populations.

2. Although there is increasing evidence that environmental fluctuations affect sexual selection before mating, whether and to which extent they influence postcopulatory trajectories is still little explored.

3. PCSS was investigated in replicate populations of guppies (*Poecilia reticulata*) in which males and females mated after maintained for 2 weeks on either restricted (RE) or ad libitum (AL) diet and the paternity of the offspring produced by multiply mated females was assigned using microsatellite markers.

4. Compared to AL fish, RE females (i) had fewer mating partners, but the time interval between the first and the last mating was not affected; (ii) produced broods with a lower variance in male fertilization success (a measure of the opportunity for PCSS); and (iii) produced broods with a paternity bias towards the first mate (reversing the last sperm precedence observed in AL populations), and associated more towards males with higher courtship rate.

5. Our results demonstrate that short-term limitation in food availability significantly influence PCSS by modifying both fertilization success variance and sperm precedence pattern. Environmental variation should therefore become part of the research paradigm to improve our understanding of postcopulatory evolutionary dynamics.

KEYWORDS
fertilization success, food restriction, mating interval, opportunity for sexual selection, total sexual selection

1 | INTRODUCTION

In polyandrous species, females mate with more than one male during the same reproductive event. Therefore, the ejaculates from different males compete to fertilize the same set of eggs. Under such circumstances, a male's reproductive success is influenced not only by the number of his mating partners, but also by the proportion of eggs he fertilizes (Parker, 1970). The frequency of polyandry, and hence the relative importance of postcopulatory components in determining male reproductive success can vary extremely among taxa

and mating systems (Taylor et al., 2014) and drive the interspecific variation in the expression of male traits associated with mating and fertilization success (Simmons et al., 2017). However, the relative importance of pre- and postcopulatory success in determining a male's reproductive fitness can also vary within the same species, not only in association with alternative male phenotypes (Gage et al., 1995) but also as a consequence of the fluctuation of environmental conditions, such as, for example, food availability (Cattelan et al., 2020; Janicke et al., 2015; Winkler & Janicke, 2022), temperature (Gómez-Llano et al., 2021; Londoño-Nieto et al., 2023; Moiron et al., 2022; Vasudeva et al., 2014) or predation risk (Glavaschi et al., 2020, 2022). The effect of ecological variation on sexual selection dynamics may vary according to whether the organism is adapted or not to a specific change and may be therefore very specific (Miller & Svensson, 2014). However, organisms under stressful or suboptimal conditions should generally have a reduced amount of resources that can be allocated to reproduction, a condition that can be experimentally mimicked by diet restriction. Indeed, food limitation reduces the expression of male sexually selected traits (Cattelan et al., 2020; Devigili et al., 2013), the strength of female preference for attractive males (Hingle et al., 2001) and female mating rate (Ando et al., 2020; Rowe, 1992). It is widely accepted that food restriction affects mating rate because of a trade-off between foraging and mating behaviour and the relatively larger costs that multiple mating may have when body condition is low (Ando et al., 2020).

The effect of food availability on female mating rate (Ando et al., 2020; Ortigosa & Rowe, 2002; Rowe, 1992) is particularly relevant for postcopulatory sexual selection (PCSS). All else being equal, a lower level of polyandry is expected to decrease the relative importance of fertilization over mating success in determining the variance in male fitness (Collet et al., 2012). This is because, as the level of polyandry decreases, the difference in postcopulatory quality among competing males, and hence the potential for PCSS to influence male reproductive fitness, is expected to decrease. For the above reasons, a reduced level of polyandry, such as that observed in response to temporary stressful environmental conditions (but see Suzaki et al., 2018; Vasudeva et al., 2021 for the effect of extreme temperatures), should generally reduce the relative contribution of fertilization success to male reproductive fitness.

While it seems straightforward to predict that a reduced level of polyandry will be associated with reduced relative contribution of PCSS on male reproductive fitness, whether and how differences in male fertilization success will affect selection on male sexual traits is more difficult to predict. Food limitation is particularly interesting in this respect because it can simultaneously affect female mating rate, the expression of sexually selected traits and trait–fitness correlations (Cattelan et al., 2020). Indeed, since male traits associated with fertilization success, including ejaculate traits, are typically condition dependent (Simmons & Kotiaho, 2002), their expression is usually reduced under food restriction (Gage & Cook, 1994; Godwin et al., 2017; Křemenová et al., 2021; Rahman et al., 2013), although the extent and the direction of this effect can vary according to the species and the ejaculate trait (Macartney et al., 2019; Mehlis

& Bakker, 2014). Furthermore, in some species the expression of precopulatory traits is also associated with fertilization success (Evans, Zane, et al., 2003; Pilastro et al., 2004; Turnell & Shaw, 2015) and they may therefore influence the operation of PCSS (Devigili et al., 2013).

Food availability may also affect temporal mating pattern of females, which is important in determining male fertilization success (Carleial et al., 2020; Pélissié et al., 2014; Sorci et al., 2023). In internal fertilizers, sperm competition success depends on the male mating order, an effect often referred to as sperm precedence (Parker, 1970) and on the temporal interval between successive female matings (Birkhead & Møller, 1993). Strong sperm precedence (Pischedda & Rice, 2012) and prolonged mating intervals (Gasparini et al., 2018) are associated with an increased within-female variance in male fertilization success, although the strength of these associations seems to be contingent on the number of competing males. For example, in several arthropods the sperm precedence, and hence the variance in fertilization success decreases as the number of mates increases (Laturney et al., 2018; Matzke et al., 2022; Zeh & Zeh, 1994). Whether food availability affects female temporal mating pattern (e.g. the temporal distance between the first and the last mating), the number of mating partners, sperm precedence and hence PCSS has not been investigated so far.

We explored the effect of food availability on PCSS in the guppy, a freshwater livebearing fish characterized by intense PCSS (Neff & Wahl, 2004). Guppies have become a model species in precopulatory (Houde, 1997) and postcopulatory (Evans & Pilastro, 2011) sexual selection studies because they fully express their mating behaviour in the laboratory under experimental conditions that realistically mimic the fluctuations of environmental factors observed in nature (Magurran, 2005). In this species, competitive fertilization success of males is influenced by both pre- and postcopulatory traits and the components of male reproductive success associated with mating and fertilization success are positively correlated (Devigili et al., 2015). For this reason, with the term PCSS we refer here to any difference in male fertilization success that it is associated with male sexual traits, including precopulatory and postcopulatory traits. A previous study (Cattelan et al., 2020) demonstrated that food restriction reduced the level of polyandry, increased the variance in male mating and reproductive success, but did not significantly affect the variance in male fertilization success across females (Cattelan et al., 2020). The variance in fertilization success represents an estimate of the opportunity for PCSS: the larger the variance the stronger can potentially be the selection on sexual traits (Arnold & Wade, 1984). The across-female variance in male fertilization analysed by Cattelan et al. (2020), however, does not allow to investigate the effect that the temporal mating pattern (e.g. being the first or last male to mate with a given female) may have on male fertilization success. Following the approach of Carleial et al. (2020) we analysed the within-female variance in male fertilization success from the data obtained by Cattelan et al. (2020). We considered the brood of each polyandrous female as a competitive fertilization event and related the fertilization success of the males that mated with the

female to the temporal succession of their copulations and the expression of their sexually selected traits. Cattelan et al. (2020), used two sets of experimental populations each of which consisted of six males and six females that were exposed to different food availability regimes (restricted [RE] and ad libitum [AL]) for 2 weeks. At the end of the diet treatment, the six males and six females of each replicate population were allowed to mate for 1.5 h per day, over 5 consecutive days. The identity of the mating males and females and the day on which they mated were recorded. The paternity of the offspring produced by the females was subsequently assigned using microsatellite markers. We considered here only the broods produced by females that were observed to mate with at least two different males during the 5-day mating sessions. We first compared the within-female variance in male fertilization success in the two diet treatments and tested whether it was affected by the number of competitors (number of males each female mated with) and by the temporal interval between the first and the last mating of the female. Second, we explored, in the two diet treatments, the association between a male's fertilization success, the expression of his sexually selected traits (Devigili et al., 2015, 2016) and his mating day relative to that of the other competitors. The two approaches provide complementary estimations of strength and shape of PCSS (Marie-Orleach et al., 2021). The variance-based approach estimates the opportunity for PCSS by comparing the within-female variance in individual male fertilization success in the two diet groups; the trait-based approach explores, in the two diet groups, the association between sexual traits expressed by males that are considered to be important and their fertilization success.

2 | METHODS

Scale of inference	Scale at which the factor of interest is applied	Number of replicates at the appropriate scale
Population	Population	AL 10
		RE 8

2.1 | Study species

Guppies are freshwater, livebearing, internally fertilizing fish native to Trinidad and Venezuela which reproduce throughout the year (Magurran, 2005). In Trinidad, natural populations undergo fluctuations in the food availability between the wet and the dry season, which in turn affect their reproductive investment (Reznick, 1989). Females preferably mate with more colourful males and males that have a higher courtship rate (Houde, 1997). Virgin females are sexually receptive for several days after the first mating and during this receptivity window they engage in consensual copulations with several males (Evans & Gasparini, 2013; Liley, 1966). In this species, competitive fertilization success is associated with the number of sperm

inseminated and their swimming velocity (Boschetto et al., 2011; Devigili et al., 2016), male ornaments, namely the area of the body colour spots (Evans, Zane, et al., 2003) and courtship rate (Evans & Magurran, 2001; Pitcher et al., 2003). This fertilization advantage derives from the positive correlation between male ornaments (colours and courtship rate) and ejaculate quality (Locatello et al., 2006; but see Evans, 2010), and from the cryptic female preference for colourful males (Pilastro et al., 2004). Furthermore, fertilization success is influenced by the time elapsed between successive matings: when two males mate with the same female 24 h apart, the last male to mate is favoured, that is, last male sperm precedence (Pitcher et al., 2003). Note that when females are artificially inseminated 24 h apart with equal number of sperm from two different males, the first male has a fertilization advantage (Magris et al., 2017), indicating that the last male sperm precedence after natural matings likely derives from cryptic female choice (Gasparini & Evans, 2018; Pitcher et al., 2003). At longer mating intervals, such as 1 month or more, a strong last male precedence is observed both after both natural copulations (Schmidt, 1920) and artificial inseminations (Gasparini, Daymond, et al., 2018), probably due to the senescence of female stored sperm. Sperm precedence at the time interval during which females are sexually receptive and mate with multiple partners (typically 5 days, Houde, 1997), is unknown.

2.2 | Experimental design

The fish were descendants of wild-caught guppies collected in 2002 from the Lower Tacarigua River in Trinidad, a high predation site where guppies coexist with several predator species. We used the fertilization success data collected in a previous study (Cattelan et al., 2020): we analysed data from 18 replicate populations of adult, 6-month-old guppies, each consisting of six males and six virgin females that were randomly assigned either to an ad-libitum diet treatment (AL, $n = 10$) or a restricted diet treatment (RE, $n = 8$) for 15 days. Sexes were housed in separated tanks during the diet treatment. AL populations were fed twice a day with dry and fresh (ca. 150 *Artemia salina*) food, while RE were fed only once a day with ca. 60 *A. salina* to females and 40 to males (further details are provided in the Supporting Information). These food amounts mimic the variation in food availability experienced by natural Trinidadian guppy populations in the dry and wet seasons known to affect female fecundity (Reznick, 1989). Furthermore, the same diet treatment was previously used in other studies that confirmed that it reduces sperm production and courtship rate but does not suppress reproduction (Devigili et al., 2013; Grether et al., 2008; Kolluru et al., 2009). After the 15 days of diet treatment, all fish were fed AL during the mating trials and until a brood was produced. For each mating trial the six males and six females of each experimental population (hereafter referred to as a block) were allowed to freely interact in the same tank for 1.5 h over 5 consecutive days, which represent the typical female sexual receptivity window in this species (Liley, 1966). During each mating session we recorded the identity of the mating fish and male

sexual behaviour (number of sigmoid displays). Five days after the end of the mating sessions, we measured male body size (body area), male colour spots (orange area), sperm number (sperm reserves at rest) and sperm velocity (VAP = average path velocity) (Devigili et al., 2015). At the end of the mating sessions females were individually housed until parturition, and the offspring were collected for molecular paternity assignment. For the present study, we considered only broods produced by females that were observed to mate with at least two partners and excluded 17 monogamous females. The sample size consisted of 35 AL females and 20 RE females, their offspring (N = 262 AL; N = 121 RE) and their putative fathers (N = 52 AL; N = 31 RE). Further details of the experimental design, diet protocol, methods for measuring male traits, recording mating behaviour and assigning paternity are summarized in the Supporting Information. We have complied with our institution's ethical regulations, and our study protocol was approved by the University of Padova Institutional Ethical Committee (permit no. CEASA 178739, 23/09/2014 Tit. III Cl. 13 Fasc. 55).

2.3 | Statistical analysis

We determined the within-female standardized variance in male fertilization success (I_{fs}) for each female in the two diet treatments (Jones, 2009). The within-female variance in male fertilization success contains both deterministic (i.e. fertilization biases associated with the effect of diet on postcopulatory processes) and stochastic components (i.e. the binomial error under expected constant fertilization success) (Marie-Orleach et al., 2021). The binomial error is expected to increase with the number of competitors and to decrease with brood size, which are both larger in the AL females as compared to their RE counterparts. In order to estimate the stochastic component in the two diet groups, we first estimated the I_{fs} solely due to the binomial error using a simulation in which, within female, fertilization success was expected to be the same for all males that mated with that female, whereas brood size and number of competitors were those observed in the two diet treatments. Using PopTools (Hood, 2011), we implemented a Monte Carlo procedure in which we iterated (10,000 iterations) the within-female variance in paternity share under equal fertilization success and compared the distribution of the simulated differences in I_{fs} between treatments with the observed I_{fs} difference in the two treatments. Second, we investigated the association between a female's mating interval (days between her first and last copulation) and her I_{fs}. Third, we used a linear mixed model (LMM) to compare I_{fs} between the two diet treatments, in relation with mating interval, brood size and number of sexual partners (competitors), including block as a random factor. Finally, we used a GLMM with binomial error distribution and logit link function, to measure the contribution of a set of predictors to individual male fertilization success across diet treatments. We considered the following factors: (i) the relative position of each male in a female's temporal mating sequence expressed in days since a female's first mating

(hereafter mating day); within each female, the male that mated on her first mating day (which may or may not occur during the first mating trial) had a value set to 1, and the males that mated in the subsequent days had a value corresponding to the number of days elapsed since the females mated first; (ii) male sexually selected traits known to be associated with fertilization success in this species, namely body area (as a proxy of male body size), orange area and courtship rate (number of sigmoid displays) among precopulatory traits (Evans & Magurran, 2001; Evans, Zane, et al., 2003) and sperm number and sperm velocity at rest, among postcopulatory traits (Boschetto et al., 2011). In the GLMM we entered female identity as the repeated measure subject, the number of offspring sired by each male as the dependent variable, and brood size as the binomial total. The fixed factors (mating day, body size, orange spots, courtship rate, sperm number and velocity) were entered as continuous linear predictors, and male identity, nested within block, was entered as random factor. In order to attain a positive definite Hessian matrix and denominator degrees of freedom that approximate the number of females (Arnqvist, 2020), we entered as random factor the interaction between female identity and mating day. Since the probability of siring an offspring did not depend on the diet (all males and females underwent the same diet treatment within each female and block), we tested the effect of each predictor and its interaction with the treatment. Note that including the treatment as factor, as expected, did not change the results (not shown). We first ran a model that included all fixed factors and their interaction with the diet and progressively removed nonsignificant interactions, starting from interaction terms with the highest *p* value. The final model revealed a significant interaction between diet and two covariates, mating day and courtship rate. We therefore further tested the effect of these covariates in the two treatments separately. For three females (two ALs and one RE), brood size was equal to one and were excluded from the analyses of I_{fs}. These three broods were instead included in the analysis of male fertilization success and for this reason the sample size differed across different analyses. The analyses were conducted using PopTools in Excel (Hood, 2011), SPSS version 28.0 and Matlab version R2018a.

3 | RESULTS

3.1 | Food availability and female mating pattern

Most of the females mated on the first day (63% and 75% in the AL and RE group respectively). The rest of the females had their first mating during the second mating day, with the exception of four females in the AL group that mated for the first time on day 3 (Figure S1). Latency to the first mating did not differ between RE and AL females (Mann–Whitney *U*-test, z = 0.81, p = 0.45). On average the female mating interval (days between the first and the last mating, including the first and the last day) was 3.07 days (SD = 1.07, n = 55) and did not significantly differ between treatments (Table 1, Figure S2), despite the

TABLE 1 Mean±standard deviation of brood size, number of individual males that mated with a female (competitors), number of competitors that sired at least one offspring per female (sires), number of days between the first and the last mating for each female (mating interval) and within-female standardized variance in fertilization success (I_{fs}). Differences between means were tested using a Student's t-test. Significant differences between diet treatments are in bold. Similar results were obtained using a linear mixed model in which block was entered as random factor.

	Ad libitum diet (AL)	Restricted diet (RE)	Student's t-test	p
Brood size	7.486±5.02	6.050±3.086	$t_{53}=1.16$	0.25
Competitors	**3.371±1.03**	**2.650±0.81**	**$t_{53}=2.68$**	**0.0098**
Sires	1.818±0.81	1.842±0.60	$t_{50}=0.11$	0.91
Mating interval	3.057±1.13	3.300±0.925	$t_{53}=0.81$	0.42
I_{fs}	**2.031±1.19**	**1.175±0.961**	**$t_{50}=2.83$[a]**	**0.004**

[a]After square-root transformation.

higher number of male partners per female in the AL diet as compared to the RE females (Table 1). These results collectively indicate that the females did not differ between diet treatments in their latency to mate (day of first mating) nor in the duration in their receptive time (mating interval), but in the number of mating partners (competitors).

3.2 | Food availability and within-female variance in fertilization success

RE females produced slightly smaller broods than AL females, although the difference was not significant (Table 1). Despite the higher number of mates per female, the number of sires per brood did not differ between treatments (Table 1). This was because a higher proportion of a female's sexual partners failed to sire any offspring in the AL diet (0.45±0.22, mean±SD, $n=33$) than in the RE diet (0.29±023, $n=19$; logistic regression, dependent variable=fertilization failures, binomial total=number of sexual partners, factor=diet; $t_{1,49}=2.88$, $p=0.006$; brood size, $t_{1,49}=2.59$, $p=0.013$). The within-female variance in fertilization success, I_{fs}, was significantly higher in the AL populations than in the RE group (Table 1). The observed difference in mean I_{fs} between AL and RE (delta$_{obs(AL-RE)}$ $I_{fs}=0.857$) was significantly larger than that expected from binomial error only (delta$_{sim(AL-RE)}$ $I_{fs}=0.156$, $p<0.001$, Monte Carlo simulation). We found that I_{fs} increased significantly with the mating interval in both treatments and there was no significant interaction between diet and mating interval (Table 2, Figure 1). In contrast, brood size and the number of competitors were not significantly associated with I_{fs} after statistically controlling for the mating interval. Treatment and mating interval remained significant ($p=0.03$ and $p<0.001$ respectively) after removing nonsignificant terms from the model (brood size and competitors).

3.3 | Food availability and postcopulatory sexual selection on male traits

Finally, we investigated whether and how a male's fertilization success was associated with the day he mated with the female (mating

TABLE 2 Within-female standardized variance in fertilization success (I_{fs}) in relation to diet, mating interval, brood size and number of competitors (linear mixed model; variance component: block=0.023±0.15 SE).

Fixed factors	b	SE	t	p
Diet	0.928	0.341	2.72	**0.012**
Brood size	−0.038	0.033	1.16	0.252
Mating interval	0.360	0.149	2.42	**0.019**
Competitors	0.248	0.164	1.51	0.139

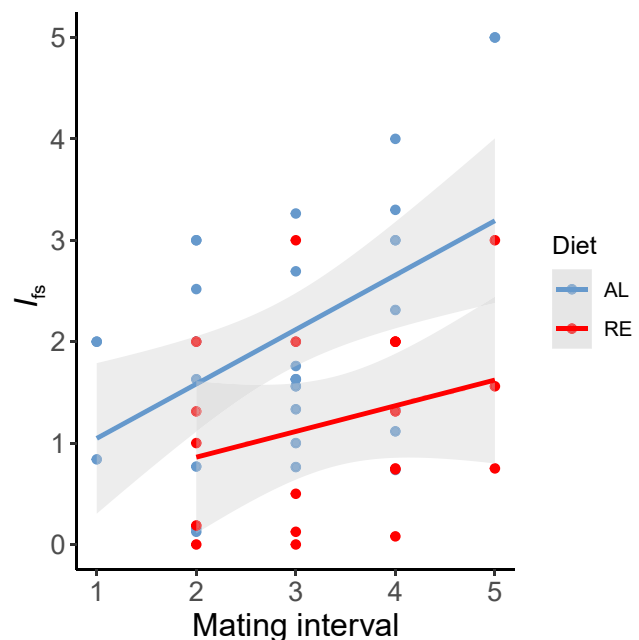*Note*: Significant values have $p \leq 0.05$ and are reported in bold.

FIGURE 1 Relationship between mating interval (days elapsed between the first and the last mating of each female, including the first day) and the within-female variance in fertilization success (I_{fs}). Low I_{fs} values indicate that paternity, within each female, was more equally shared among competing males, whereas high I_{fs} values indicate that paternity was biased towards one or more individual competing males. Lines represent the least square regression to the points. Ad libitum (AL) females are in blue, restricted (RE) females in red.

TABLE 3 Results from the GLMM in which male fertilization success (number of offspring sired) was the dependent variable, brood size was the binomial total, and mating day and male traits were the covariates. Significant terms in bold. Regression coefficients of the interactions are referred to the ad libitum group where the restricted was set to 0. Mating day=day on which a male mated with the female relative to her temporal mating sequence; sigmoid displays=number of sigmoid display performed; body area=area of the body (mm^2); orange area=area of the orange spot (mm^2); sperm number=number of sperm bundles (log$_{10}$ transformed) stripped at sexual rest; sperm velocity=average path velocity (VAP) µm/s; ddf=denominator degrees of freedom.

Fixed factors	Coefficient	SE	ddf	t	p
Full model					
Mating day	−0.569	0.213	45	2.669	**0.010**
Sigmoid displays	0.073	0.046	40	1.567	0.125
Body area	0.016	0.072	38	0.225	0.823
Orange area	0.118	0.142	41	0.830	0.412
Sperm number	−1.528	1.697	42	0.900	0.373
Sperm velocity (VAP)	0.026	0.028	36	0.943	0.352
Mating day×diet	0.889	0.266	45	3.345	**0.002**
Sigmoid displays×diet	−0.081	0.056	40	1.456	0.153
Body area×diet	0.004	0.072	37	0.055	0.956
Orange area×diet	−0.056	0.167	41	0.334	0.740
Sperm number×diet	0.535	2.098	43	0.255	0.800
Sperm velocity×diet	−0.011	0.031	38	0.351	0.728
Final model					
Mating day	−1.212	0.454	50	2.326	**0.024**
Sigmoid displays	−0.422	0.182	45	2.897	**0.006**
Mating interval×diet	0.098	0.034	41	3.128	**0.003**
Sigmoid displays×diet	0.689	0.220	56	3.337	**0.002**

day), relative to the other males that mated with the female, and with his sexual traits. Results of the full (all predictors and their interactions with the treatment) and the reduced (significant terms only) models are presented in Table 3 (intermediate models with their command syntax are listed in the Supporting Information, Appendix S1). (1) We found a significant interaction between mating day and treatment on fertilization success. Specifically, the fertilization success of RE males decreased as the mating day increased, meaning that the first male had a fertilization advantage, whereas the opposite pattern (i.e. the last male had a fertilization advantage, Figure 2) was observed in AL males. Furthermore, (2) we found a significant interaction between diet and courtship rate (number of sigmoid displays), indicating that the association between courtship rate and fertilization success differed in the two diet treatments. The effect of mating day and courtship on fertilization success were confirmed also when the two treatments were analysed separately (Table 4). Specifically, the mating day predicted fertilization success in opposite directions in the two treatments (first male advantage in the RE group and last male advantage in the AL group). In contrast, the number of sigmoid displays was positively associated with fertilization success only in the RE treatment (Table 4). In all the analyses we found a significant effect of the random term associated with male identity (initial model, $z=3.67$, $p<0.001$; final model, $z=3.77$, $p<0.001$), suggesting that some individual males consistently had higher paternity share than others (Supporting Information, Appendix S1).
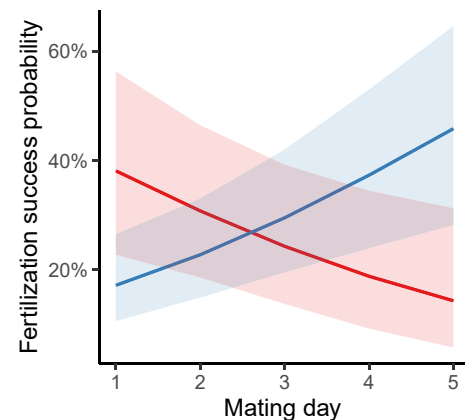

FIGURE 2 Relationships between male mating day (the day a male mated with the female relative to the day on which the female did her first mating) and fertilization success in the two diet treatments (blue=ad libitum males; red=restricted males). Shaded areas represent the 95% confidence intervals.

4 | DISCUSSION

Food availability is often variable within and across different reproductive seasons, strongly affecting adults' reproductive strategies (Kitaysky et al., 2010; Lebl et al., 2011; Molleman et al., 2022) and sexual selection (Cornwallis & Uller, 2010; Evans

TABLE 4 Results of the GLMMs predicting male fertilization success in relation to the mating day and the number of sigmoid displays in the two diet treatments analysed separately final models.

	Coefficient	SE	ddf	t	p
Ad libitum					
Mating day	0.373	0.172	24	2.161	**0.041**
Restricted					
Mating day	−0.331	0.149	279	2.223	**0.034**
Sigmoid displays	0.068	0.029	18	2.379	**0.029**

Note: Significant values have $p \leq 0.05$ and are reported in bold.

& Garcia-Gonzalez, 2016; Ingleby et al., 2010) in many animal species. According to theoretical predictions (Evans & Garcia-Gonzalez, 2016), our results confirm that PCSS dynamics are significantly influenced by food availability, revealing complex, and to some extent, unexpected effects. In particular, we found that a temporary restriction of food availability at the population level (RE populations) determined (1) a reduction of the variance in fertilization success, (2) a tighter association between a male's fertilization success and his attractiveness (courtship rate) and (3) a first male fertilization advantage in contrast with the last male advantage observed in the AL populations. Food availability had therefore a profound effect on PCSS dynamics, despite apparently minor behavioural differences in female mating pattern.

4.1 | Food availability and within-female variance in fertilization success

Within-female variance in fertilization success (I_{fs}) was significantly higher in the AL than in the RE group. This difference was determined by the higher frequency of AL males that mated with a female but failed to sire any offspring, as compared to RE males. As result of the higher rate of fertilization failure, the two groups did not differ in the number of sires per brood despite the different number of mating partners. To the best of our knowledge, this is the first experimental evidence that an environmental factor can determine a systematic bias in the relationship between the number of mating partners and the number of sires. Inferring polyandry and the opportunity for PCSS from paternity data may therefore be misleading when comparing populations that may be exposed to different environmental conditions. For example, in socially monogamous birds, polyandry is typically estimated from extrapair paternity and compared across and within populations. However, the possibility that environmental conditions may affect how multiple mating is translated into multiple paternity is rarely acknowledged (Brouwer & Griffith, 2019; Valcu et al., 2021).

We also found that I_{fs} significantly increased with the mating interval. It has been postulated in reptiles, birds and mammals (Birkhead & Møller, 1993; Orr & Brennan, 2015), that a prolonged female sperm storage may increase the opportunity for PCSS through several, not mutually exclusive mechanisms. For example, the longer the fertilization window the higher the probability that the sperm of several males will compete to fertilize the eggs (Carleial et al., 2020). Furthermore, the interaction between sperm and female reproductive organs, associated with prolonged female sperm storage, may increase the opportunity for sperm selection by the female (cryptic female choice) and for sperm to compete for prolonged viability (sperm competition). Our results indirectly support both hypotheses. On one hand, the female time interval was positively correlated with the number of partners in our study (Pearson correlation, $r = 0.37$, $p = 0.006$, $n = 55$), suggesting that the longer the period of female sexual receptivity, the greater the probability females mate multiply (Carleial et al., 2023). On the other hand, I_{fs} remained correlated with mating interval after controlling for the number of competitors (Table 2), indirectly suggesting that the opportunity for PCSS may increase with the duration of female sperm storage.

The positive correlation between I_{fs} and the number of competitors we observed contrasts with the results obtained in other studies on arthropods, which found that an increasing number of competitors was associated with a decreased variance in fertilization success (Laturney et al., 2018; Matzke et al., 2022; Zeh & Zeh, 1994). Arthropods usually have a stronger sperm precedence pattern than vertebrates (Simmons, 2001), possibly because females have multiple sperm storage organs and a greater control over fertilization (Laturney et al., 2018) or because males are better able to displace the sperm from previous matings (Cordoba et al., 2003). Both mechanisms are expected to be less relevant in the guppy, where fertilization success is largely depending on the number of sperm transferred during copulation (Boschetto et al., 2011). Clearly, further studies will be necessary to understand the mechanisms behind the association between the number of competitors and the variance in fertilization success in different species.

4.2 | Food availability and postcopulatory selection on male traits

The analysis of within-female males' fertilization success in relation to male sexual traits and temporal mating pattern revealed some unexpected effects of diet treatment on the pattern of PCSS. We found that within-female male order (mating day) was the stronger predictor of fertilization success in both treatments, although in opposite directions. Ejaculate traits (sperm number and velocity) and male colours did not predict fertilization success, whereas courtship rate was positively correlated with fertilization success only in the RE group.

Sperm precedence is usually estimated from the success of two males mating at a fixed interval. Studies in which the number of competing males is larger than two and mating interval is variable have demonstrated that the number of competitors (Laturney et al., 2018; Matzke et al., 2022; Zeh & Zeh, 1994) and the time interval between successive matings (Carleial et al., 2020; Friesen et al., 2020; Magris et al., 2017; but see Carleial et al., 2023; Rodrigues et al., 2020), influence the link between male traits and fertilization success. We demonstrated that food availability reverses the sperm precedence pattern typically observed in guppies. Furthermore, we found that courtship rate was positively associated with fertilization success in the RE group, but not in the AL group. This latter result fits with the prediction that sexual selection is stronger on those sexual traits whose condition dependence is heightened under stress (David et al., 2000; Rowe & Houle, 1996).

While these results seem straightforward, we can only speculate about the underlying proximate mechanisms that may be responsible for the observed effects. This is because a full factorial design, where males and females from each diet treatment are paired with same or different mates, would be needed to disentangle male and female effects. For example, the stronger link between courtship rate and fertilization success observed in the RE group may be directly driven (i) by a male effect, if food limitation determines a stronger positive correlation between courtship rate and ejaculate quality (Evans et al., 2015), (ii) by a female effect, if diet affects the strength of female cryptic preference for males which court more (Evans & Magurran, 2001), or (iii) by their interaction, if, for example, a stronger cryptic preference arises because RE males have a higher variance in courtship rate (Cattelan et al., 2020).

Opposite sperm precedence patterns in the two diet treatments may also result from male and female effects. Diet restriction negatively impacts male sperm reserves (Cattelan et al., 2020; Godwin et al., 2017; Rahman et al., 2014). RE males may therefore become more rapidly sperm depleted than AL males during the mating trial and suffer a lower fertilization success when mated last. While this explanation cannot be ruled out, a male's fertilization success was not affected by the number of females he mated with in the previous days (Supporting Information, Appendix S2), as expected if sperm depletion determined the sperm precedence pattern. Alternatively, males may change their sperm allocation strategy across subsequent matings in response to their nutritional condition, as it has been shown in the neriid fly *Telostylinus angusticollis*, where males in good conditions allocate more sperm to the last matings (Wylde et al., 2020). Adaptive sperm allocation has been reported in other poeciliid species (Evans, Pierotti, et al., 2003; Schlupp & Plath, 2005), but evidence that male guppies can strategically allocate their sperm reserves among different matings has not been found so far (Evans, 2009; Magris et al., 2020). Nutritional conditions may also affect post-meiotic sperm senescence (Pizzari et al., 2008). Male-stored sperm show a rapid senescence rate in guppies (Gasparini et al., 2014). An accelerated post-meiotic senescence rate of sperm of males in poor nutritional conditions, either before mating or after mating before fertilization, may result in a reduced fertilization success of the last male. Female nutritional condition may also affect sperm senescence (Cardozo & Pilastro, 2018; Cardozo et al., 2020; Evans & Gasparini, 2013).

The positive correlation between courtship rate and fertilization success, observed in the RE group, confirmed previous results obtained in this guppy population, in which precopulatory traits are associated with fertilization success (Devigili et al., 2015; Evans, Zane, et al., 2003). Diet restriction increased the phenotypic variance in courtship rate but not that of the other male sexual traits (Cattelan et al., 2020), increasing the opportunity for sexual selection on this trait. Our results demonstrated that the increased variance in courtship rate also male fertilization success. Although this was the only male sexual trait associated with fertilization success, male identity explained a significant component of the variance in fertilization success. This suggests that individual male guppies vary in their postcopulatory competitiveness (Evans & Rutstein, 2008) and that there may be other traits associated with male postcopulatory success. For example, we did not consider the rarity of the male colour pattern which strongly affects male mating success in guppies (Hughes et al., 2013). Among postcopulatory traits, sperm longevity (Pizzari & Parker, 2009) and seminal fluid composition (Poiani, 2006) have been shown to affect fertilization success in several other species.

In conclusion, the results of our study have two broad implications for our comprehension of the interaction between environmental conditions and the operation of PCSS. Theoretical and empirical studies have highlighted the sensitivity of precopulatory sexual selection dynamics to environmental fluctuations (Candolin & Wong Bob, 2019; Cornwallis & Uller, 2010; Evans & Garcia-Gonzalez, 2016) and anthropogenic changes such as urbanization (Cronin et al., 2022) and climate warming (García-Roa et al., 2020). Human-induced environmental changes are expected to affect food resources available to natural populations. For example, climate change is increasing the frequency and the magnitude of extreme weather events, which likely determine temporary reductions in food availability (Edwards & Richardson, 2004; Pörtner & Farrell, 2008) and urbanization alters the quality and the abundance of available food (reviewed in Cronin et al., 2022). Our results demonstrate that food availability, and possibly most anthropogenic environmental factors can extend their influence on sexual selection dynamics occurring after mating.

AUTHOR CONTRIBUTIONS

Andrea Pilastro conceived and designed the study. Silvia Cattelan and Elisa Morbiato conducted the experiment. Elisa Morbiato and Andrea Pilastro analysed the data with contribution by Silvia Cattelan. Andrea Pilastro wrote the paper with contributions from all the authors.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Figure S1. Histograms of the frequency of the first day of mating (ad libitum, blue; restricted, red).

Figure S2. Relationship between the variance in male fertilization success, mating interval, number of competitors and brood size in the two diet groups. Lines are the linear regression lines fitted to the diet groups. Each dot represents a female (ad libitum, blue; restricted, green).

Table S1. Microsatellite for paternity assignments.

Data S1. Fertilization success and variance dataset.