



Effects of inbreeding and elevated rearing temperatures on strategic sperm investment

Meng-Han Joseph Chung^{1,*}, Md Mahmud-Al-Hasan¹, Michael D. Jennions^{1,2} and Megan L. Head¹

¹Division of Ecology and Evolution, Research School of Biology, Australian National University, Canberra, Australian Capital Territory 2601, Australia

²Stellenbosch Institute for Advanced Study (STIAS), Wallenberg Centre, Stellenbosch University, Stellenbosch 7600, South Africa

*Corresponding author: Division of Ecology and Evolution, Research School of Biology, Australian National University, Canberra, Australian Capital Territory 2601, Australia. Email: chungmenghan@gmail.com

Handling Editor: Emily DuVal

Males often strategically adjust the number of available sperm based on the social context (i.e. sperm priming response), but it remains unclear how environmental and genetic factors shape this adjustment. In freshwater ecosystems, high ambient temperatures often lead to isolated pools of hotter water in which inbreeding occurs. Higher water temperatures and inbreeding can impair fish development, potentially disrupting sperm production. We used guppies (*Poecilia reticulata*) to investigate how developmental temperature (26 °C, 30 °C) and male inbreeding status (inbred, outbred) influence their sperm priming response. We also tested if sperm priming was affected by whether the female was a relative (sister) and whether she was inbred or outbred. There was no effect of rearing temperature; male inbreeding status alone determined the number of available sperm in response to female presence, her inbreeding status, and her relatedness. Inbred males produced significantly more sperm in the presence of an unrelated, outbred female than when no female was present. Conversely, outbred males did not alter the number of sperm available in response to female presence or relatedness. Moreover, inbred males produced marginally more sperm when exposed to an unrelated female that was outbred rather than inbred, but there was no difference when exposed to an inbred female that was unrelated versus related. Together, a sperm priming response was only observed in inbred males when exposed to an outbred female. Outbred females in our study were larger than inbred females, suggesting that inbred males strategically allocated ejaculate resources toward females in better condition.

Key words: climate warming; developmental stress; inbreeding; poor start in life; sperm investment.

Introduction

Males often plastically adjust the rate of sperm production, ejaculate size and/or ejaculate composition based on the social context (Kelly and Jennions 2011; Bartlett et al. 2017). This adaptive plasticity is favored by selection for several reasons. First, sperm production and germline maintenance are energetically expensive (Maklakov and Immler 2016). Evidence for condition-dependence of ejaculate traits (Macartney et al. 2019) suggests that sperm production is costly and trades off with investment in somatic traits (Dowling and Simmons 2012). Second, sperm are vulnerable to oxidative stress due to their high metabolic activity and limited DNA repair (Reinhardt 2007; Helfenstein et al. 2010). This vulnerability results in the deterioration of stored sperm, manifest as slower swimming speed and decreased longevity, which lowers fertilization success (review: Monaghan and Metcalfe 2019). In combination, costly sperm production discourages constant high investment in sperm, while post-meiotic damage to sperm favors a shorter interval between its production and ejaculation (Pizzari et al. 2008). These factors select for males that adjust the number of available sperm in response to the social context (i.e. sperm priming response; Aspbury and Gabor 2004). Indeed, strategic sperm investment in response to mate availability or quality and to the perceived level of sperm competition occurs in many taxa (review: Magris 2021).

Female availability naturally varies because of variation in the environment. Changes in abiotic factors often moderate habitat complexity, resource availability, and movement between populations (Ferber et al. 2014; van der Hoek et al. 2022), altering mate encounter rates and female availability. For example, the temperature can shape mate availability in ectotherms by changing the adult sex ratio (Edmands 2021), and through sex differences in behavioral thermoregulation (Ortega et al. 2016). However, despite strong correlations between the physical environment and mating opportunities, studies of sperm priming responses typically manipulate the social context within a single, constant environment (e.g. Moatt et al. 2014; Cattelan and Pilastro 2018; Firman et al. 2018). To date, little attention has been paid to how abiotic factors interact with the social context to moderate strategic sperm investment.

Temperature is an important abiotic factor that can influence sperm production, with the effect contingent on the life stages (Pilakouta and Ålund 2021). During the juvenile stage, warmer temperatures can reduce optimal body size and increase developmental costs (Baudron et al. 2014; Marshall et al. 2020), potentially diverting resources away from the onset of spermatogenesis. On the other hand, the temperature experienced as an adult can affect ejaculation and sperm performance (review: Wang and Gunderson 2022). For example, in the European bullhead, adult

Received: 15 November 2023; **Revised:** 24 March 2024; **Editorial decision:** 1 June 2024; **Accepted:** 4 June 2024.

© The Author(s) 2024. Published by Oxford University Press on behalf of the International Society for Behavioral Ecology.

This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted reuse, distribution, and reproduction in any medium, provided the original work is properly cited.

males living in warmer water show a decline in relative testis size (Dorts et al. 2012); and corals in warmer waters produce fewer sperm (Paxton et al. 2016). Likewise, males that experience heat stress often exhibit lower sperm quality (e.g. motility, viability) and an increase in the proportion of abnormal sperm (Hurley et al. 2018; Küçük and Aksoy 2020; Breedveld et al. 2023). To date, however, how elevated temperatures during development (independent of adult temperatures) affect sperm investment and its plasticity in different social environments is unclear because males in most studies are kept at the same temperature during rearing and adulthood (Zeh et al. 2012; Breckels and Neff 2013; Vasudeva et al. 2014).

Clarifying the effect of temperature on plastic sperm investment becomes even more challenging when temperature also affects the occurrence of inbreeding in a population. In freshwater ecosystems, warmer temperatures often decrease water flow and increase the likelihood of isolated bodies of water forming, constraining how conspecifics interact and elevating the risk of inbreeding (e.g. snail; Jarne et al. 2000). While higher temperatures and inbreeding can individually depress sperm performance (meta-analysis: Losdat et al. 2014), there is also evidence that stressful environments (e.g. low food availability, high temperatures) exacerbate the negative effect of inbreeding on traits related to fitness (Crnokrak and Roff 1999).

When living in isolated pools, not only are males more likely to be inbred themselves, but the chance of encountering related females is greater. Animals often exhibit behaviors that reduce the likelihood of mating with kin, such as sex-biased dispersal and mate choice based on kin recognition (review: Nichols 2017). As such, males should invest less in sperm when mating with more closely related females to avoid producing inbred offspring with lower fitness (Lewis and Wedell 2009; Patterson and Pilakouta 2024; but see Kokko and Ots 2006 for kin selection benefit). Here, we designed an experiment using the guppy (*Poecilia reticulata*) to test the effects of rearing temperature and changes in inbreeding status on sperm priming responses. We aim to address the following questions: (1) Do males produce more sperm in the presence of a female than in her absence (i.e. sperm priming response)? (2) Do higher rearing temperatures lower the sperm priming response? (3) Do inbred males show less plasticity in sperm investment (i.e. inbreeding depression for sperm priming response)? (4) Do males produce fewer sperm when a related female is present (i.e. inbreeding avoidance)?

Guppies in tropical streams are often restricted to isolated pools during the dry season (Griffiths and Magurran 1997), leading to higher encounter rates between relatives and naturally elevated levels of inbreeding (Johnson et al. 2010). Inbreeding in guppies has been shown to lower sperm production (Zajitschek and Brooks 2010; Gasparini et al. 2013), fertility (Pitcher et al. 2008; Johnson et al. 2010) and offspring survival (Nakadate et al. 2003). There is some evidence that females actively avoid inbreeding and prefer to mate with unrelated males (Daniel and Rodd 2016) and that multiply mating females bias fertilization toward less closely related males (Gasparini and Pilastro 2011; Fitzpatrick and Evans 2014; but see Evans et al. 2008; Pitcher et al. 2008).

Warm temperatures are common in the tropics, where guppies are abundant (Le Roy et al. 2017), but higher temperatures (30 °C) can lower sperm performance (Breckels and Neff 2013). A higher sperm count increases fertilization success in guppies (Boschetto et al. 2011), but sperm production is energetically costly (Rahman et al. 2013; Evans et al. 2023). Consequently, male guppies prudently invest in sperm in response to female availability (Bozynski and Liley 2003; Cattelan et al. 2016;

Cattelan and Pilastro 2018). This study investigated how inbred and outbred males from different rearing temperatures (26 °C vs 30 °C) adjusted the number of available sperm across various social environments, including differences in female availability (present or absent), female relatedness to the male (related or unrelated), and the female's inbreeding status (inbred or outbred).

Materials and methods

Fish origin and maintenance

Guppies used in our experiment were descendants of fish from 2 independent laboratory stocks that were collected from Alligator Creek near Townsville (Australia) in April 2002 (Lindholm et al. 2014) and September 2010 (Kranz et al. 2018), respectively. Our stock population has been kept at the Australian National University since 2019. Laboratory-born juveniles were raised in mixed-sex groups until their sex could be determined prior to maturation (an elongated anal fin for males and a visible gravid spot for females). To ensure virginity, males and females were separated into single-sex 90 L tanks (~50 individuals/tank) before being used for the treatments after 2–3 mo.

All stock fish were maintained under a 14:10-h photoperiod at 26 °C and fed twice daily with *Artemia* nauplii ad libitum and commercial fish flakes. Experimental fish in individual tanks were only fed *Artemia* ad libitum. The project received approval from the Animal Ethics Committee (A2021/04).

Establishing the inbreeding treatment

To start, we housed a virgin female with a virgin male in a 3 L tank. Both individuals were randomly selected from single-sex stock tanks ($n = 150$ pairs). After 2 wk, the males were removed, leaving the females in the tanks. We inspected tanks daily for newborn fry starting 3 wk after the initial pairing. If a female did not give birth within 6 wk, or produced fewer than 4 offspring, she was re-paired with the same male for another wk. A total of 80 outbred, full-sibling families were generated. Siblings of the same sex from the same family were housed in communal tanks until they reached maturity. Afterwards, we randomly selected and paired 2 families to create “blocks” of inbred and outbred fish (e.g. families A and B for block 1, families C and D for block 2; Fig. 1). Within each block, a male and a female from either the same family (AA or BB) or different families (AB or BA) were paired as above. In total, we generated 60 unique inbred broods and 57 outbred broods spread across 38 blocks.

Manipulating rearing temperature

For each inbred and outbred brood, we randomly assigned half the newborn offspring to either a warm (30 °C) or control (26 °C) temperature treatment. Each thermal environment, therefore, contained siblings with similar genetic backgrounds. Offspring were individually housed in 1 L tanks. Starting from 4 wk after birth, we inspected the males daily to determine maturation (via visual inspection of an apical hood extending beyond the tip of his gonopodium). 2 wk after reaching maturation, all fish were placed at 26 °C to create a common garden setting for adults. After males had spent 2 to 5 months at 26 °C, we examined their sperm priming response to variation in female availability, female relatedness to the male, and the female's inbreeding status. This long delay minimized any short-term influence of adult thermal acclimation when the warm-reared (30 °C) males were moved to the common garden temperature (Guderley 1990; Seebacher et al. 2014; Little et al. 2021).

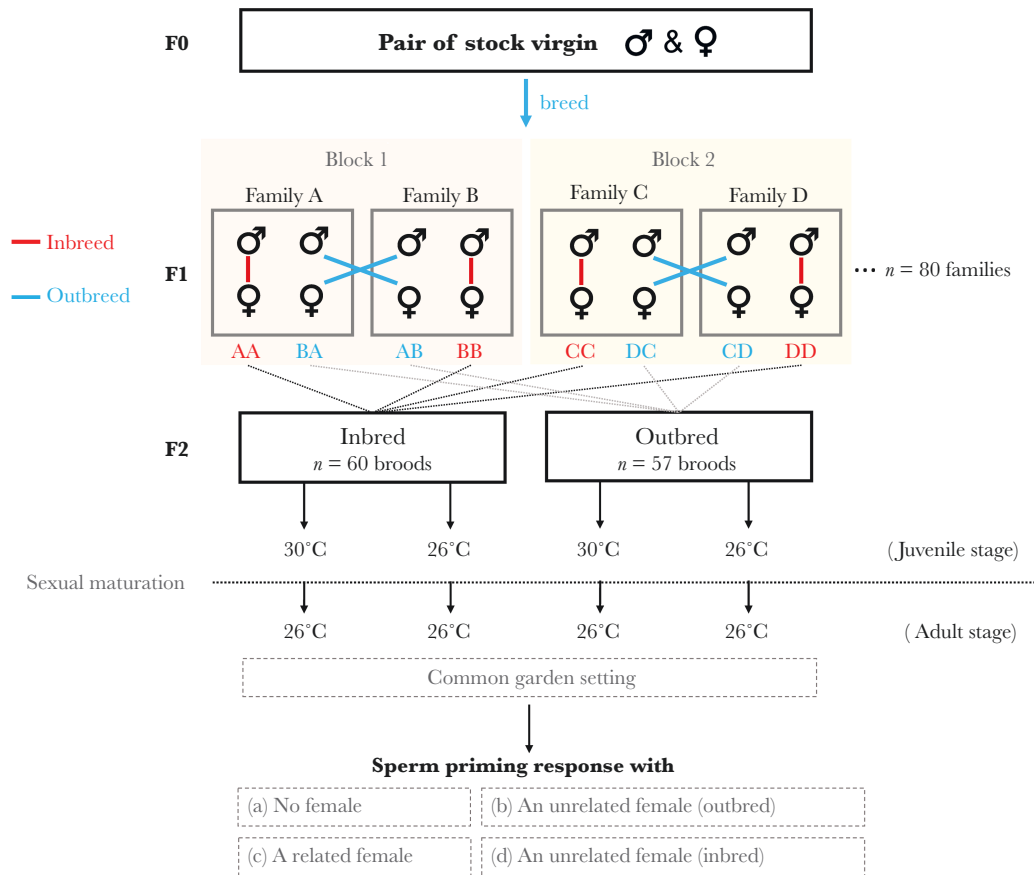


Fig. 1. Schematic of the experimental design, showing the block design used to generate inbred and outbred focal fish. Within each block (e.g. Family A \times Family B), reciprocal crosses occur between the families (AA, BA, AB, BB). Offspring from each cross-type were assigned alternately to each of the 2 temperature groups. 2 wk after reaching maturity, adult males were transferred to the common garden temperature and 2 to 5 months later exposed to different social environments (a–d) to measure their sperm priming response. Note: only inbred males were exposed to environment (d). A related female was either inbred or outbred based on the male's inbreeding status.

Manipulating female availability and genetic relatedness

To quantify strategic sperm investment in response to different social environments, we first emptied a male's sperm reserves prior to a potential sperm priming phase. Males were anesthetized with Aqui-S (0.0075% v/v) for 30 s before being placed under a dissecting microscope on a glass slide covered by 1% polyvinyl alcohol solution. The gonopodium (intromittent organ) was swung forwards, and we gently pressed on the male's abdomen to expel sperm bundles. Following 1 h for recovery in their individual 1 L tanks, males from each of the 4 treatment groups (inbred-warm, inbred-control, outbred-warm, outbred-control) ($n = 85\text{--}107$ per group; see [Supplementary Material](#)) were randomly introduced into one of 3 social environments: a 3 L tank with either: (a) no female behind a mesh barrier ($n = 112$), (b) an unrelated female (i.e. an outbred, non-sibling) behind a mesh barrier ($n = 110$), or (c) a related female (i.e. his sister, either inbred or outbred based on the male's own inbreeding status) behind a mesh barrier ($n = 109$) for 7 d. We only used stimulus females that were raised at the control temperature to eliminate any temperature-induced variation in female fecundity that might subsequently affect the male's response.

Inbred males in environment (c) (related female) were unavoidably exposed to an *inbred* sister. Hence, a decreased number of available sperm could result from either a response to high genetic relatedness to the female and/or a response to her being

a lower-quality female (if inbreeding itself lowers female quality; [White et al. 2015](#)). To untangle these confounding explanations, we established an additional environment (d) exclusively for inbred males. Specifically, inbred males experienced the presence of (d) an inbred but unrelated female (i.e. an inbred, non-sibling). Males were assigned alternately to the 4 environments (a–d) ($n = 26\text{--}31$; see [Supplementary Material](#)). The standard length (SL: the snout tip to the base of caudal fin) of the stimulus females ranged from 23.96 to 34.10 mm. Outbred females (27.73 ± 0.13 mm SL) were significantly larger than inbred females (27.15 ± 0.15 mm) (LM, $F_{1,269} = 7.973$, $P = 0.005$). There was, however, no significant size difference between the inbred females that were used in environments (c) and (d) (LM, $F_{1,102} = 1.141$, $P = 0.288$). We were able to control for differences in female inbreeding status and test for an effect of female relatedness on sperm priming for inbred males by comparing how they responded in environments (c) (inbred, related female) and (d) (inbred, unrelated female). For outbred males, the effect of relatedness on sperm priming involved comparing how they responded in environments (b) (outbred, unrelated female) and (c) (outbred, related female).

Males in the no-female treatment did not receive any female cues, while the other males experienced visual and olfactory stimuli from a female. Tanks were separated by white paper to prevent visual contact. Exposing males to a female (or no female) for 7 d is a widely used time period in studies of sperm priming in guppies ([Bozynski and Liley 2003](#); [Cattelan et al. 2016](#);

Cattelan and Pilastro 2018). It reflects natural conditions as males can be confined to isolated ponds for days to weeks, causing variation in both the availability and genetic relatedness of potential mates (Houde 1997; Magurran 2005). Virgin females show cyclical changes in sexual responsiveness (Liley 1966, 1968), so we mated each stimulus female with a stock male a week before the experiment.

After 7 d in the assigned social context, males were anesthetized and re-stripped to count their sperm (see below). They were also photographed to measure their SL using ImageJ (Abramoff et al. 2004). We failed to extract sperm from 8 out of 383 males, which was irrelevant to their assigned group (Supplementary Material). These males were excluded from the analyses.

Sperm count

The sperm stripped after 7 d was collected into a known volume (400–800 μL) of saline solution (0.9% NaCl) using a 100 μL pipette. We vortexed the sperm solution for 30 s and mixed it several times using a 20 μL pipette. Next, 3 μL of the solution was placed on a 20-micron capillary slide (Leja). We used a CEROS Sperm Tracker (Hamilton Thorne Research, Beverly, MA, USA) to determine the sperm count per view (i.e. concentration) under 100 \times magnification. The samples were collected blind to a male's treatment (inbreeding status, rearing temperature, social context) to eliminate observer bias. We calculated the mean sperm count under 5 randomly selected views per male (repeatability $r \pm \text{SE} = 0.886 \pm 0.009$, $P < 0.001$, $n = 375$ males). We then divided the mean by the volume of each field of view (0.00468 μL) and multiplied the value by the volume of added saline solution (i.e. 400–800 μL) to calculate the total sperm number.

Statistical analyses

We ran 2 separate analyses to address our research questions. First, we ran a linear mixed model (LMM) to investigate the effects of male inbreeding status (inbred, outbred), rearing temperature (warm, control), and social environment (no female, unrelated outbred female, related female) and all three 2-way interactions on sperm priming response (i.e. the total number of sperm produced in 7 d). Notably, we did not test for a 3-way interaction due to the complexity of interpretation and the need for a larger sample size to maintain adequate statistical power.

Second, we noted that inbred males might produce fewer sperm than outbred males in the presence of a related female due to their sister being inbred and, therefore, of lower quality (e.g. less fecund; White et al. 2015). To test whether the observed effect of male inbreeding status (see Results) was confounded by the related female's inbreeding status, we ran an additional LMM exclusively for inbred males. We separated the effects of the inbreeding status of the female and her genetic relatedness to the male by considering 3 types of female (b,c,d) that inbred males encountered. We compared the response to females that were unrelated and either (b) outbred or (d) inbred to test for an effect of female inbreeding status. We then compared the response to females that were inbred and either (c) related or (d) unrelated to the male to test for an effect of female relatedness. We treated male rearing temperature, female type, and their interaction as fixed factors in the model.

In all models, nonsignificant 2-way interactions were removed to test for the main effects of fixed factors (Engqvist 2005). For transparency, all initial and final model outputs are presented in the Supplementary Material. We ran Tukey's post-hoc pairwise test (*emmeans* package) for any significant main effect involving

factors with 3 levels. In all models, brood identity was included as a random factor to account for measurements of several males from the same brood, then assigned to different temperatures and social environments. Sperm production is strongly dependent on male size and age (Pitcher and Evans 2001; Gasparini et al. 2010; Kamaszewski et al. 2020), so their SL and adult age at testing were standardized (mean = 0, SD = 1) and included as separate covariates in all analyses. Sperm data was power-transformed to fulfill the homogeneity of variances and the normality of residual assumptions using Levene's test and Shapiro-Wilks test, respectively.

The significance level was set at $\alpha = 0.05$ (2-tailed). We conducted Wald chi-square tests (*Anova* function in the *car* package) to determine P values. Type III sums of squares were used for models with interaction terms, and type II sum of squares for models without interactions. Summary statistics are presented as mean \pm SE. Models were run using R v4.0.5 in R studio v1.3.1093.

Results

Larger males produced significantly more sperm ($\chi^2_1 = 20.138$; $P < 0.001$; Supplementary Fig. S1), while older males produced significantly less sperm ($\chi^2_1 = 33.986$; $P < 0.001$; Supplementary Fig. S2). Controlling for male size and age at testing, male inbreeding status affected the total number of sperm, but the effect depended on the social environment (i.e. female presence and her relatedness) (an interaction: $\chi^2_2 = 7.195$; $P = 0.027$; Fig. 2). Neither of the other interactions were significant (inbreeding status \times temperature: $\chi^2_1 = 2.030$; $P = 0.154$; temperature \times social environment: $\chi^2_2 = 1.792$; $P = 0.408$).

Inbred and outbred males produced a similar number of sperm when no female was present ($P = 0.962$), but they adjusted the sperm count differently when the social environment changed. For inbred males, those in the presence of an unrelated female produced significantly more sperm than males without a female ($P = 0.027$). Likewise, inbred males with an unrelated female produced more sperm than those in the presence of a related female (i.e. sister), but this was not significant ($P = 0.066$). The sperm count of inbred males did not differ between the no female or related female treatments ($P = 0.959$) (all Tukey's tests; Fig. 2a). In contrast, the sperm count of outbred males did not significantly differ among the 3 social environments (Tukey's tests, all $P > 0.472$) (Fig. 2b). These results indicate a sperm priming response by inbred males, when presented with an unrelated female, but not for outbred males. Finally, there was no significant difference in sperm count between males reared at the control temperature and those reared at the warmer temperature ($\chi^2_1 = 3.062$; $P = 0.080$) (Fig. 3).

Given that changes in the social environment resulted in a sperm priming response by inbred males (Fig. 2a), we also tested how changes in female type (i.e. female relatedness and her inbreeding status) affected the number of available sperm for inbred males (see Materials and methods). Controlling for male size ($\chi^2_1 = 7.573$; $P = 0.006$) and age at testing ($\chi^2_1 = 13.661$; $P < 0.001$), female type significantly affected sperm count ($\chi^2_2 = 6.239$; $P = 0.044$), but the effect was not moderated by male rearing temperature (i.e., no interaction: $\chi^2_2 = 3.795$; $P = 0.150$). Despite the significant overall effect of female type, however, we did not find any significant differences in the pairwise comparisons among the 3 female types (Fig. 4). Nevertheless, there was a trend for inbred males exposed to an outbred, unrelated female to produce more sperm when compared to inbred males exposed to an

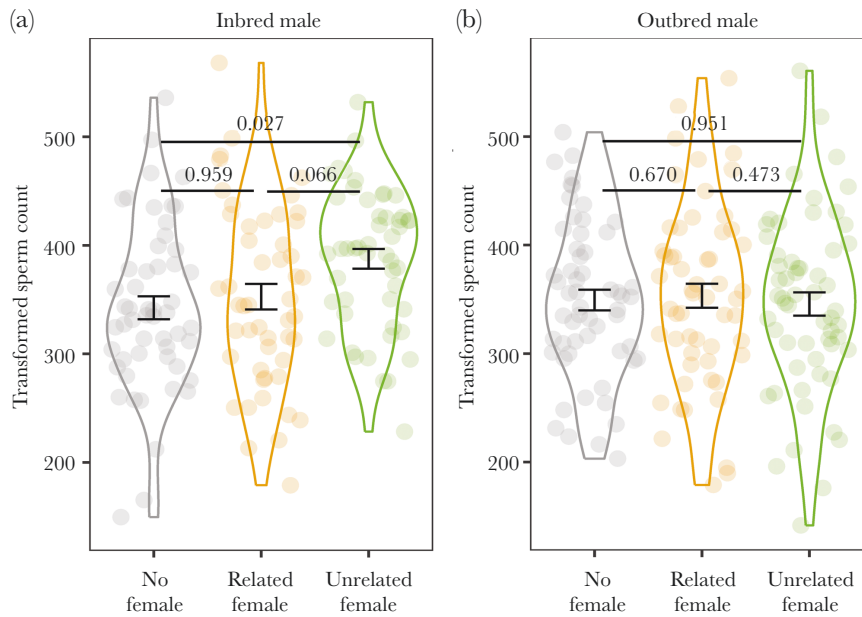


Fig. 2. Effect of social environment on sperm count for (a) inbred and (b) outbred males. Related females were either inbred or outbred, depending on the male's inbreeding status. Unrelated females were all outbred. Data is shown as mean \pm SE. *P* values generated using Tukey's tests are shown.

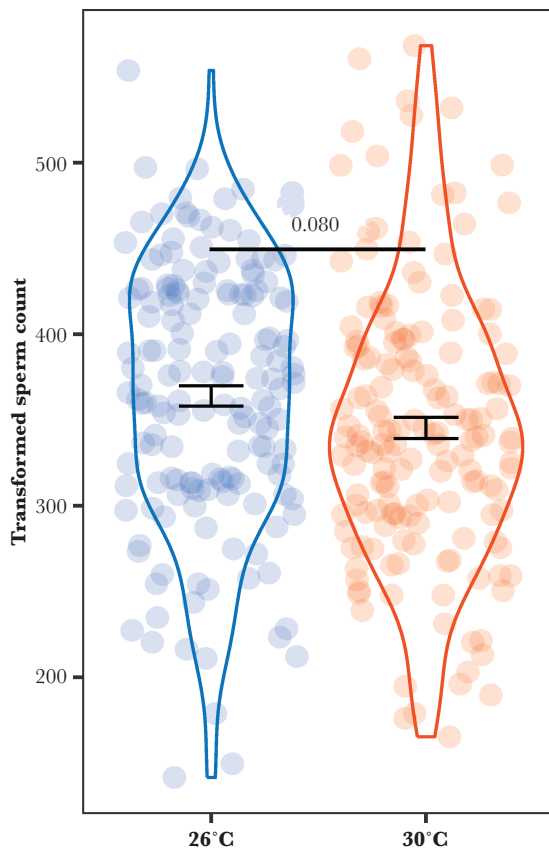


Fig. 3. Effect of male rearing temperature on sperm count. Data is shown as mean \pm SE. *P* value shows the significance level of the main effect based on a GLMM (main text).

inbred, unrelated female (Tukey's tests, $P = 0.085$) (i.e. an effect of female inbreeding status), as well as compared to inbred males exposed to an inbred, related female (Tukey's tests, $P = 0.084$) (i.e. a combined effect of female inbreeding status and relatedness).

In contrast, the amount of sperm produced by inbred males was similar for those experiencing the inbred *unrelated* and inbred *related* female treatments (Tukey's test, $P = 0.995$) (i.e. no effect of female relatedness). Sperm count for inbred males was unaffected by male rearing temperature ($\chi^2_1 = 3.264$; $P = 0.071$).

Discussion

We investigated strategic sperm investment in response to 4 covarying factors that male guppies in isolated ponds experience, namely—thermal stress, inbreeding, mate availability, and mate quality. We found that a higher rearing temperature did not affect strategic sperm investment, but male inbreeding status determined how he adjusted the number of available sperm in response to variation in mate availability and mate quality. Variation in female availability and her quality often results from changes in abiotic factors (Banks et al. 2005) and inbreeding levels (Kyriazis et al. 2021), but our results suggest that inbreeding alone could alter sexually selective pressures on sperm priming response, regardless of rearing temperature.

Inbred males adjusted sperm investment based on female inbreeding status, but outbred males did not

A sperm priming response was only observed in inbred males, and our results suggest that these responses by inbred males are driven by *female inbreeding status* (i.e. unrelated females that were inbred or outbred) rather than *female relatedness* (i.e. inbred females that were related or unrelated). In a similar study on burying beetles, inbred females preferred to mate with outbred males, while outbred females showed no preference (Pilakouta and Smiseth 2017). In that study, the authors suggest that this pattern could occur if a decline in offspring fitness is greater when inbred males fertilize the eggs of inbred rather than outbred females (Pilakouta and Smiseth 2017), as this would favor inbred females that actively avoid inbred males. In our experiment, inbred female guppies were significantly smaller than outbred

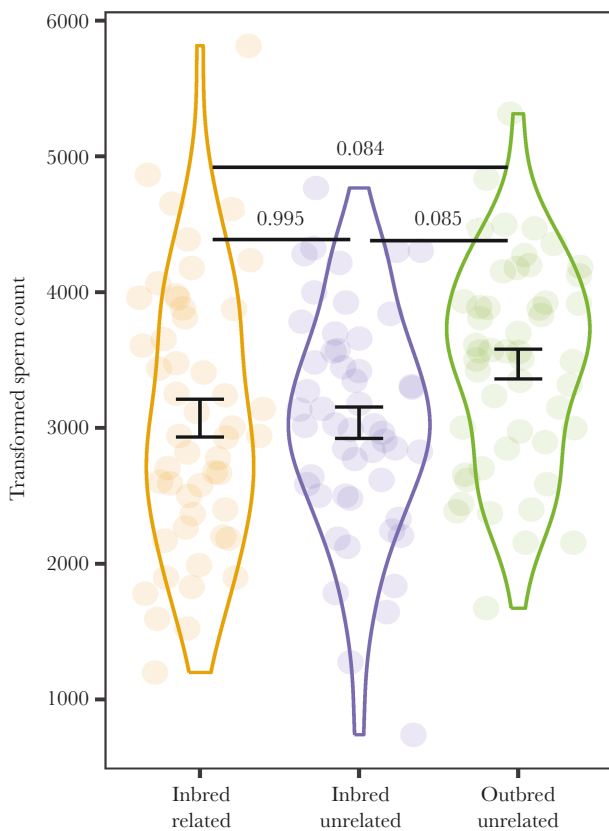


Fig. 4. Effect of female type (inbred related, inbred unrelated, outbred unrelated) on sperm count by inbred males. Data are shown as mean \pm SE. P values generated using Tukey's tests are shown. Note: there is a significant main effect of female type (main text).

females, suggesting that they are less fecund (Reznick and Endler 1982; Auer et al. 2010). As a result, inbred males might benefit by producing more sperm when mating with outbred (or simply larger) females as they have more eggs. Our finding for inbred males aligns with past studies in *Drosophila littoralis*, where males prefer outbred females because reproductive output was higher for outbred females (Ala-Honkola et al. 2015).

Unlike inbred males, outbred males did not adjust the number of sperm in response to the social environment. As such, female availability did not increase the number of available sperm (i.e. no sperm priming; Evans 2009; but see Bozynski and Liley 2003; Cattelan et al. 2016; Cattelan and Pilastro 2018). Outbred male guppies in our experiment only encountered outbred females (either related or unrelated). Unlike inbred males, however, outbred males did not increase the number of sperm when in the presence of an outbred female (irrespective of her relatedness). This difference in strategic sperm investment between inbred and outbred males might arise if inbred males gain greater marginal benefits than outbred males by upregulating sperm investment in the presence of outbred females. For example, inbred males might compensate for their reduced sperm competitiveness when competing for high-quality (i.e. outbred or larger) mates (Zajitschek et al. 2009; Michalczyk et al. 2010).

Why does female relatedness not affect sperm priming by male guppies?

Given lower fertility (Pitcher et al. 2008; Johnson et al. 2010) and reduced offspring fitness (Nakadate et al. 2003) caused by inbreeding in guppies, we expected males to reduce sperm investment when

encountering their sister. We offer several possible reasons for the absence of any effect of female relatedness on a male's sperm priming. First, males may use other mechanisms to lower the risk of inbreeding, including male-biased dispersal (Croft et al. 2003; Borges et al. 2022), changes in mating effort (Dougherty et al. 2022) and strategic ejaculation (Wedell et al. 2002; but see Simmons and Thomas 2008). For example, male guppies reduce the intensity of their courtship when directed towards sisters (Fitzpatrick et al. 2014). Second, the males in our study were socially isolated with no interactions with conspecifics prior to testing. Studies of sperm priming often use males reared in mixed-sex groups (Cattelan et al. 2016; Cattelan and Pilastro 2018) or collected from the wild (Aspbury and Gabor 2004; Chung et al. 2019). Prior social experience might be critical to acquire the phenotypic information required for kin discrimination (Penn and Frommen 2010; de Boer et al. 2021). However, this may not be the case for guppies. In an elegant experiment, Daniel and Rodd (2021) showed that male guppies born and reared in isolation could readily discriminate between full and half siblings from different broods. This suggests that early-life exposure to phenotypic cues of kinship is not a prerequisite for kin recognition in guppies. Third, male guppies may not benefit from post-copulatory mechanisms that reduce inbreeding (Zajitschek et al. 2006; Pitcher et al. 2008) because females show strong mate preferences for unrelated males (Daniel and Rodd 2016) or because there are kin-selected benefits to fertilizing sisters, despite inbred offspring being less fit (Kokko and Ots 2006).

Interestingly, instead of exhibiting inbreeding avoidance, there is evidence that male guppies upregulate sperm velocity in the presence of related females (Fitzpatrick et al. 2014), potentially offsetting cryptic female choice against their sperm (Gasparini and Pilastro 2011; Fitzpatrick and Evans 2014). Costly sperm investment should discourage inefficient insemination, but mating with sisters can be advantageous when mating opportunities are scarce, and there is a low opportunity cost for males (Waser et al. 1986; Kokko and Ots 2006). In this light, it is noteworthy that male guppies in our study and in Fitzpatrick et al. (2014) were virgins held in sexual isolation prior to testing. This means that the stimulus female was their only apparent mating opportunity, with no alternative options (i.e. no opportunity costs). This might explain why there was no effect of female relatedness on the number of sperm. By way of analogy, female guppies biased paternity towards unrelated males only when they received sperm from related and unrelated males simultaneously (Gasparini and Pilastro 2011; Fitzpatrick and Evans 2014). There was no differential usage when females were inseminated by a single related or unrelated male (Gasparini and Pilastro 2011). Our findings raise several questions for further research: Does the mating status of male guppies affect how they respond to females that differ in their relatedness? And what role do opportunity costs play? For example, male guppies discriminate among females that vary in size less pronouncedly when they had previously encountered females consecutively rather than simultaneously (Jordan and Brooks 2012). It would be interesting to test how sperm investment differs for sequential and simultaneous encounters with related and unrelated females (Barrett et al. 2014).

Warmer rearing temperatures neither affect sperm investment nor modify the effect of inbreeding or social context

We found no interaction between male inbreeding status and rearing temperature. This is unexpected as inbreeding depression is exacerbated under stressful environments in many taxa (Armbruster

and Reed 2005; Reed et al. 2012). In addition, inbreeding has been proposed as one reason why stocks of captive guppies have a narrower thermal tolerance than wild-caught individuals (Karayu cel et al. 2008; Breckels and Neff 2013). Unlike previous studies (Breckels and Neff 2013; Rahman et al. 2020), we found no significant effect of rearing temperature on guppy ejaculates. It is worth noting that males in these earlier studies were maintained at 30 °C or even greater temperatures (32 °C) during sperm measurement, so their findings may result from the effects of the adult rather than the developmental environment. It is possible that higher temperatures during development reduced sperm production in newly mature males, but this decline was reversed after adults were returned to control temperatures for several months. Our result is in line with a recent meta-analysis of fishes that reported greater sensitivity of ejaculates to environmental challenges in adulthood than those during the juvenile stage (Macartney et al. 2019).

Finally, we found no interaction between rearing temperature and social environments, suggesting that an elevated developmental temperature does not reduce the ability to plastically adjust the number of available sperm (Billard 1986). Given that we found no overall effect of rearing temperature on sperm production, this result is perhaps not surprising. However, it would be interesting to know whether the same results would occur if adults were also tested at 30 °C since previous studies imply that adult temperature affects sperm production (Rahman et al. 2020). Ultimately, fertilization success is determined by the overall performance of ejaculate traits under sperm competition (Boschetto et al. 2011). Future research should examine sperm competitiveness and share of paternity when inbred and outbred males compete and test for any moderating effect of rearing temperature.

Conclusion

We found that a male's inbreeding status affected his plasticity in sperm investment. This implies stronger sexual selection on inbred males to strategically allocate ejaculate resources. Further, we show plasticity in sperm investment in response to a female's inbreeding status and/or body size, rather than whether she was a relative (i.e. risk of inbreeding). Together, these results suggest that inbred males seem to face greater demands when it comes to identifying outbred (or larger) females that might provide greater direct (e.g. more eggs) and/or indirect benefits (e.g. enhanced offspring heterozygosity) (Fromhage et al. 2009). This may be due to differences in selection favoring inbreeding-dependent plasticity because the proportion of inbred males and inbred females usually covaries across populations (e.g. inbred males and females are more common in isolated pools than in rivers). Our findings suggest that the negative impact of inbreeding on male fertility, at least sperm quantity, might be less than expected if inbred males are better at fertilizing eggs from outbred (or larger) females that are more fecund. Finally, it is worth noting that this study did not explore the 3-way interaction of rearing temperature, male inbreeding status, and social context. However, addressing questions, such as "does sperm priming by inbred males in response to outbred females remain unchanged in higher versus control temperatures?" would be an important next step to unravel the complex interplay between genetic and environmental factors that might affect male reproductive success.

Supplementary material

Supplementary material is available at *Behavioral Ecology* online.

Acknowledgments

We thank the ANU Animal Services staff for help with fish maintenance.

Author contributions

All authors conceived and designed the study. M-HJC and MM-A-H conducted the experiment. M-HJC analyzed the data and drafted the manuscript, with MDJ and MLH providing critical revisions. All authors approved the final manuscript.

Funding

We were funded by an Australian Research Council grant to MDJ (DP190100279).

Ethics approval

The project received approval from the ANU Animal Ethics Committee (A2021/04).

Conflict of interest

We declare no competing interests.

Data availability

Analyses reported in this article can be reproduced using the data and code provided by Chung et al. (2024).

References

- Abràmoff MD, Magalhães JP, Ram SJ. 2004. Image processing with ImageJ. *Biophoton Int.* 11(7):36–42.
- Ala-Honkola O, Laine L, Pekkala N, Kotiaho JS, Honkola T, Puurtinen M. 2015. Males benefit from mating with outbred females in *Drosophila littoralis*: male choice for female genetic quality? *Ethology.* 121(6):577–585. <https://doi.org/10.1111/eth.12369>
- Armbruster P, Reed DH. 2005. Inbreeding depression in benign and stressful environments. *Heredity.* 95(3):235–242. <https://doi.org/10.1038/sj.hdy.6800721>
- Aspbury AS, Gabor CR. 2004. Differential sperm priming by male sailfin mollies (*Poecilia latipinna*): effects of female and male size. *Ethology.* 110(3):193–202. <https://doi.org/10.1111/j.1439-0310.2003.00963.x>
- Auer SK, Arendt JD, Chandramouli R, Reznick DN. 2010. Juvenile compensatory growth has negative consequences for reproduction in Trinidadian guppies (*Poecilia reticulata*). *Ecol Lett.* 13(8):998–1007. <https://doi.org/10.1111/j.1461-0248.2010.01491.x>
- Banks SC, Ward SJ, Lindenmayer DB, Finlayson GR, Lawson SJ, Taylor AC. 2005. The effects of habitat fragmentation on the social kin structure and mating system of the agile antechinus, *Antechinus agilis*. *Mol Ecol.* 14(6):1789–1801. <https://doi.org/10.1111/j.1365-294X.2005.02535.x>
- Barrett LT, Evans JP, Gasparini C. 2014. The effects of perceived mating opportunities on patterns of reproductive investment by male guppies. *PLoS One.* 9(4):e93780. <https://doi.org/10.1371/journal.pone.0093780>
- Bartlett MJ, Steeves TE, Gemmill NJ, Rosengrave PC. 2017. Sperm competition risk drives rapid ejaculate adjustments mediated by seminal fluid. *eLife.* 6:e28811. <https://doi.org/10.7554/eLife.28811>

- Baudron AR, Needle CL, Rijnsdorp AD, Tara Marshall C. 2014. Warming temperatures and smaller body sizes: synchronous changes in growth of North Sea fishes. *Global Change Biol.* 20(4):1023–1031. <https://doi.org/10.1111/gcb.12514>
- Billard R. 1986. Spermatogenesis and spermatology of some teleost fish species. *Reprod Nutr Dév.* 26(4):877–920. <https://doi.org/10.1051/rnd:19860601>
- Borges IL, Dangerfield JC, Angeloni LM, Funk WC, Fitzpatrick SW. 2022. Reproductive benefits associated with dispersal in head-water populations of Trinidadian guppies (*Poecilia reticulata*). *Ecol Lett.* 25(2):344–354. <https://doi.org/10.1111/ele.13929>
- Boschetto C, Gasparini C, Pilastro A. 2011. Sperm number and velocity affect sperm competition success in the guppy (*Poecilia reticulata*). *Behav Ecol Sociobiol.* 65(4):813–821. <https://doi.org/10.1007/s00265-010-1085-y>
- Bozynski CC, Liley NR. 2003. The effect of female presence on spermiation, and of male sexual activity on ‘ready’ sperm in the male guppy. *Anim Behav.* 65(1):53–58. <https://doi.org/10.1006/anbe.2002.2024>
- Breckels RD, Neff BD. 2013. The effects of elevated temperature on the sexual traits, immunology and survivorship of a tropical ectotherm. *J Exp Biol.* 216(14):2658–2664. <https://doi.org/10.1242/jeb.084962>
- Breedveld MC, Devigili A, Borgheresi O, Gasparini C. 2023. Reproducing in hot water: experimental heatwaves deteriorate multiple reproductive traits in a freshwater ectotherm. *Funct Ecol.* 37(4):989–1004. <https://doi.org/10.1111/1365-2435.14279>
- Cattelan S, Evans JP, Pilastro A, Gasparini C. 2016. The effect of sperm production and mate availability on patterns of alternative mating tactics in the guppy. *Anim Behav.* 112:105–110. <https://doi.org/10.1016/j.anbehav.2015.11.024>
- Cattelan S, Pilastro A. 2018. Sperm priming response to perceived mating opportunities is reduced in male guppies with high baseline sperm production. *Curr Zool.* 64(2):205–211. <https://doi.org/10.1093/cz/zoy008>
- Chung MHJ, Jennions MD, Fox RJ. 2019. Novel ablation technique shows no sperm priming response by male eastern mosquitofish to cues of female availability. *Behav Ecol Sociobiol.* 73(12):167. <https://doi.org/10.1007/s00265-019-2779-4>
- Chung MHJ, Mahmud-Al-Hasan M, Jennions MD, Head ML. 2024. Data from: Effects of inbreeding and elevated rearing temperatures on strategic sperm investment. *Behav Ecol.* <https://doi.org/10.5061/dryad.wstqjq2vt>
- Cmokrak P, Roff DA. 1999. Inbreeding depression in the wild. *Heredity.* 83(3):260–270. <https://doi.org/10.1038/sj.hdy.6885530>
- Croft DP, Albanese B, Arrowsmith BJ, Botham M, Webster M, Krause J. 2003. Sex-biased movement in the guppy (*Poecilia reticulata*). *Oecologia.* 137(1):62–68. <https://doi.org/10.1007/s00442-003-1268-6>
- Daniel MJ, Rodd FH. 2016. Female guppies can recognize kin but only avoid incest when previously mated. *Behav Ecol.* 27(1):55–61. <https://doi.org/10.1093/beheco/arv122>
- Daniel MJ, Rodd FH. 2021. Kin recognition in guppies uses self-referencing based on olfactory cues. *Am Nat.* 197(2):176–189. <https://doi.org/10.1086/712352>
- de Boer RA, Vega-Trejo R, Kotrschal A, Fitzpatrick JL. 2021. Meta-analytic evidence that animals rarely avoid inbreeding. *Nat Ecol Evol.* 5(7):949–964. <https://doi.org/10.1038/s41559-021-01453-9>
- Dorts J, Grenouillet G, Douxfils J, Mandiki SNM, Milla S, Silvestre F, Kestemont P. 2012. Evidence that elevated water temperature affects the reproductive physiology of the European bullhead *Cottus gobio*. *Fish Physiol Biochem.* 38(2):389–399. <https://doi.org/10.1007/s10695-011-9515-y>
- Dougherty LR, Skirrow MJA, Jennions MD, Simmons LW. 2022. Male alternative reproductive tactics and sperm competition: a meta-analysis. *Biol Rev.* 97(4):1365–1388. <https://doi.org/10.1111/brv.12846>
- Dowling DK, Simmons LW. 2012. Ejaculate economics: testing the effects of male sexual history on the trade-off between sperm and immune function in Australian crickets. *PLoS One.* 7(1):e30172. <https://doi.org/10.1371/journal.pone.0030172>
- Edmands S. 2021. Sex ratios in a warming world: thermal effects on sex-biased survival, sex determination, and sex reversal. *J Hered.* 112(2):155–164. <https://doi.org/10.1093/jhered/esab006>
- Engqvist L. 2005. The mistreatment of covariate interaction terms in linear model analyses of behavioural and evolutionary ecology studies. *Anim Behav.* 70(4):967–971. <https://doi.org/10.1016/j.anbehav.2005.01.016>
- Evans JP. 2009. No evidence for sperm priming responses under varying sperm competition risk or intensity in guppies. *Naturwissenschaften.* 96(7):771–779. <https://doi.org/10.1007/s00114-009-0529-6>
- Evans JP, Brooks RC, Zajitschek SRK, Griffith SC. 2008. Does genetic relatedness of mates influence competitive fertilization success in guppies? *Evolution.* 62(11):2929–2935. <https://doi.org/10.1111/j.1558-5646.2008.00496.x>
- Evans JP, Turnbull EJ, Lymbery RA. 2023. Testing for age-dependent effects of dietary restriction on the strength of condition dependence in ejaculate traits in the guppy (*Poecilia reticulata*). *R Soc Open Sci.* 10(8):230805. <https://doi.org/10.1098/rsos.230805>
- Ferger SW, Schleuning M, Hemp A, Howell KM, Böhning-Gaese K. 2014. Food resources and vegetation structure mediate climatic effects on species richness of birds. *Glob Ecol Biogeogr.* 23(5):541–549. <https://doi.org/10.1111/geb.12151>
- Firman RC, Garcia-Gonzalez F, Simmons LW, André GI. 2018. A competitive environment influences sperm production, but not testes tissue composition, in house mice. *J Evol Biol.* 31(11):1647–1654. <https://doi.org/10.1111/jeb.13360>
- Fitzpatrick JL, Evans JP. 2014. Postcopulatory inbreeding avoidance in guppies. *J Evol Biol.* 27(12):2585–2594. <https://doi.org/10.1111/jeb.12545>
- Fitzpatrick LJ, Gasparini C, Fitzpatrick JL, Evans JP. 2014. Male-female relatedness and patterns of male reproductive investment in guppies. *Biol Lett.* 10(5):20140166. <https://doi.org/10.1098/rsbl.2014.0166>
- Fromhage L, Kokko H, Reid JM. 2009. Evolution of mate choice for genome-wide heterozygosity. *Evolution.* 63(3):684–694. <https://doi.org/10.1111/j.1558-5646.2008.00575.x>
- Gasparini C, Devigili A, Dosselli R, Pilastro A. 2013. Pattern of inbreeding depression, condition dependence, and additive genetic variance in Trinidadian guppy ejaculate traits. *Ecol Evol.* 3(15):4940–4953. <https://doi.org/10.1002/ece3.870>
- Gasparini C, Marino IAM, Boschetto C, Pilastro A. 2010. Effect of male age on sperm traits and sperm competition success in the guppy (*Poecilia reticulata*). *J Evol Biol.* 23(1):124–135. <https://doi.org/10.1111/j.1420-9101.2009.01889.x>
- Gasparini C, Pilastro A. 2011. Cryptic female preference for genetically unrelated males is mediated by ovarian fluid in the guppy. *Proc R Soc B.* 278(1717):2495–2501. <https://doi.org/10.1098/rspb.2010.2369>
- Griffiths SW, Magurran AE. 1997. Schooling preferences for familiar fish vary with group size in a wild guppy population. *Proc R Soc B.* 264(1381):547–551. <https://doi.org/10.1098/rspb.1997.0078>
- Guderley H. 1990. Functional significance of metabolic responses to thermal acclimation in fish muscle. *Am J Physiol.* 259(2):R245–R252. <https://doi.org/10.1152/ajpregu.1990.259.2.r245>

- Helfenstein F, Losdat S, Møller AP, Blount JD, Richner H. 2010. Sperm of colourful males are better protected against oxidative stress. *Ecol Lett.* 13(2):213–222. <https://doi.org/10.1111/j.1461-0248.2009.01419.x>
- Houde AE. 1997. Sex, color, and mate choice in guppies. Princeton: Princeton University Press.
- Hurley LL, McDiarmi CS, Friesen CR, Griffith SC, Rowe M. 2018. Experimental heatwaves negatively impact sperm quality in the zebra finch. *Proc R Soc B.* 285(1871):20172547. <https://doi.org/10.1098/rspb.2017.2547>
- Jarne P, Perdieu MA, Pernot AF, Delay B, David P. 2000. The influence of self-fertilization and grouping on fitness attributes in the freshwater snail *Physa acuta*: population and individual inbreeding depression. *J Evol Biol.* 13(4):645–655. <https://doi.org/10.1046/j.1420-9101.2000.00204.x>
- Johnson AM, Chappell G, Price AC, Helen Rodd F, Olendorf R, Hughes KA. 2010. Inbreeding depression and inbreeding avoidance in a natural population of guppies (*Poecilia reticulata*). *Ethology.* 116(5):448–457. <https://doi.org/10.1111/j.1439-0310.2010.01763.x>
- Jordan LA, Brooks RC. 2012. Recent social history alters male courtship preferences. *Evolution.* 66(1):280–287. <https://doi.org/10.1111/j.1558-5646.2011.01421.x>
- Kamaszewski M, Skrobisz M, Wójcik M, Kawalski K, Szczepanowski A, Bujarski P, Szudrowicz H, Herman AP, Martynow J. 2020. The role of transcription factors in gonad development and sex differentiation of a teleost model fish – guppy (*Poecilia reticulata*). *Animals.* 10(12):2401. <https://doi.org/10.3390/ani10122401>
- Karayucel I, Orhan AK, Karayucel S. 2008. Effect of temperature on some reproductive parameters of gravid females and growth of newly hatched fry in guppy, *Poecilia reticulata* (Peters, 1860). *J Anim Vet Adv.* 7(10):1261–1266.
- Kelly CD, Jennions MD. 2011. Sexual selection and sperm quantity: meta-analyses of strategic ejaculation. *Biol Rev.* 86(4):863–884. <https://doi.org/10.1111/j.1469-185X.2011.00175.x>
- Kokko H, Ots I. 2006. When not to avoid inbreeding. *Evolution.* 60(3):467–475. <https://doi.org/10.1111/j.0014-3820.2006.tb01128.x>
- Kranz AM, Forgan LG, Cole GL, Endler JA. 2018. Light environment change induces differential expression of guppy opsins in a multi-generational evolution experiment. *Evolution.* 72(8):1656–1676. <https://doi.org/10.1111/evo.13519>
- Küçük N, Aksoy M. 2020. Effect of environmental heat stress on Kivırcık ram sperm parameters. *J Hell Vet Med Soc.* 71(1):2073–2080. <https://doi.org/10.12681/jhvms.22968>
- Kyriazis CC, Wayne RK, Lohmueller KE. 2021. Strongly deleterious mutations are a primary determinant of extinction risk due to inbreeding depression. *Evol Lett.* 5(1):33–47. <https://doi.org/10.1002/evl3.209>
- Le Roy A, Loughland I, Seebacher F. 2017. Differential effects of developmental thermal plasticity across three generations of guppies (*Poecilia reticulata*): canalization and anticipatory matching. *Sci Rep.* 7(1):4313. <https://doi.org/10.1038/s41598-017-03300-z>
- Lewis Z, Wedell N. 2009. Male moths reduce sperm investment in relatives. *Anim Behav.* 77(6):1547–1550. <https://doi.org/10.1016/j.anbehav.2009.03.013>
- Liley NR. 1966. Ethological isolating mechanisms in four sympatric species of poeciliid fishes. *Behav Suppl.* 13:1–197. <https://doi.org/10.1163/156853966X00020>
- Liley NR. 1968. The endocrine control of reproductive behavior in the female guppy *Pocilia reticulata* peters. *Anim Behav.* 16(2):318–331. [https://doi.org/10.1016/0003-3472\(68\)90016-x](https://doi.org/10.1016/0003-3472(68)90016-x)
- Lindholm AK, Head ML, Brooks RC, Rollins LA, Ingleby FC, Zajitschek SRK. 2014. Causes of male sexual trait divergence in introduced populations of guppies. *J Evol Biol.* 27(2):437–448. <https://doi.org/10.1111/jeb.12313>
- Little AG, Loughland I, Seebacher F. 2021. What do warming waters mean for fish physiology and fisheries? *J Fish Biol.* 97(2):328–340. <https://doi.org/10.1111/jfb.14402>
- Losdat S, Chang SM, Reid JM. 2014. Inbreeding depression in male gametic performance. *J Evol Biol.* 27(6):992–1011. <https://doi.org/10.1111/jeb.12403>
- Macartney EL, Crean AJ, Nakagawa S, Bonduriansky R. 2019. Effects of nutrient limitation on sperm and seminal fluid: a systematic review and meta-analysis. *Biol Rev.* 94(5):1722–1739. <https://doi.org/10.1111/brv.12524>
- Magris M. 2021. Strategic adjustment of ejaculate quality in response to variation of the socio-sexual environment. *Behav Ecol Sociobiol.* 75(6):91. <https://doi.org/10.1007/s00265-021-03032-1>
- Magurran AE. 2005. Evolutionary ecology: the trinidadian guppy. Oxford: Oxford University Press.
- Maklakov AA, Immler S. 2016. The expensive germline and the evolution of ageing. *Curr Biol.* 26(13):577–586. <https://doi.org/10.1016/j.cub.2016.04.012>
- Marshall DJ, Pettersen AK, Bode M, White CR. 2020. Developmental cost theory predicts thermal environment and vulnerability to global warming. *Nat Ecol Evol.* 4(3):406–411. <https://doi.org/10.1038/s41559-020-1114-9>
- Michalczuk L, Martin OY, Millard AL, Emerson BC, Gage MJG. 2010. Inbreeding depresses sperm competitiveness, but not fertilization or mating success in male. *Proc R Soc B.* 277(1699):3483–3491. <https://doi.org/10.1098/rspb.2010.0514>
- Moatt JP, Dytham C, Thom MDF. 2014. Sperm production responds to perceived sperm competition risk in male *Drosophila melanogaster*. *Physiol Behav.* 131:111–114. <https://doi.org/10.1016/j.physbeh.2014.04.027>
- Monaghan P, Metcalfe NB. 2019. The deteriorating soma and the indispensable germline: gamete senescence and offspring fitness. *Proc R Soc B.* 286(1917):20192187. <https://doi.org/10.1098/rspb.2019.2187>
- Nakadate M, Shikano T, Taniguchi N. 2003. Inbreeding depression and heterosis in various quantitative traits of the guppy, *Poecilia reticulata*. *Aquaculture.* 220(1-4):219–226. [https://doi.org/10.1016/s0044-8486\(02\)00432-5](https://doi.org/10.1016/s0044-8486(02)00432-5)
- Nichols HJ. 2017. The causes and consequences of inbreeding avoidance and tolerance in cooperatively breeding vertebrates. *J Zool.* 303(1):1–14. <https://doi.org/10.1111/jzo.12466>
- Ortega Z, Mencia A, Pérez-Mellado V. 2016. Sexual differences in behavioral thermoregulation of the lizard *Scelarcis perspicillata*. *J Therm Biol.* 61:44–49. <https://doi.org/10.1016/j.jtherbio.2016.08.006>
- Patterson C, Pilakouta N. 2024. Effects of parental care on the magnitude of inbreeding depression: a meta-analysis in fishes. *Am Nat.* 203(2):50–62. <https://doi.org/10.1086/728001>
- Paxton CW, Baria MVB, Weis VM, Harii S. 2016. Effect of elevated temperature on fecundity and reproductive timing in the coral *Acropora digitifera*. *Zygote.* 24(4):511–516. <https://doi.org/10.1017/S0967199415000477>
- Penn DJ, Frommen JG. 2010. Kin recognition: an overview of conceptual issues, mechanisms and evolutionary theory. In: Kappeler P, editor. *Animal behaviour: evolution and mechanisms*. New York: Springer Heidelberg Dordrecht. p. 55–85.
- Pilakouta N, Ålund M. 2021. Sexual selection and environmental change: what do we know and what comes next? *Curr Zool.* 67(3):293–298. <https://doi.org/10.1093/cz/zoab021>
- Pilakouta N, Smiseth PT. 2017. Female mating preferences for outbred versus inbred males are conditional upon the female's own inbreeding status. *Anim Behav.* 123:369–374. <https://doi.org/10.1016/j.anbehav.2016.11.023>

- Pitcher TE, Evans JP. 2001. Male phenotype and sperm number in the guppy (*Poecilia reticulata*). *Can J Zool.* 79(10):1891–1896. <https://doi.org/10.1139/z01-142>
- Pitcher TE, Rodd FH, Rowe L. 2008. Female choice and the relatedness of mates in the guppy (*Poecilia reticulata*). *Genetica.* 134(1):137–146. <https://doi.org/10.1007/s10709-008-9246-x>
- Pizzari T, Dean R, Pacey A, Moore H, Bonsall MB. 2008. The evolutionary ecology of pre- and post-meiotic sperm senescence. *Trends Ecol Evol.* 23(3):131–140. <https://doi.org/10.1016/j.tree.2007.12.003>
- Rahman MM, Kelley JL, Evans JP. 2013. Condition-dependent expression of pre- and postcopulatory sexual traits in guppies. *Ecol Evol.* 3(7):2197–2213. <https://doi.org/10.1002/ece3.632>
- Rahman MM, Pinkey IA, Ferthous J, Arafat ST, Rahman SM, Asaduzzaman M, Rahman MM, Rouf MA. 2020. Modulation of phenotypic traits under different rearing temperatures: experimental evidence in male guppy (*Poecilia reticulata*). *Int J Aquat Biol.* 8(5):344–364. <https://doi.org/10.22034/ijab.v8i5.856>
- Reed DH, Fox CW, Enders LS, Kristensen TN. 2012. Inbreeding-stress interactions: evolutionary and conservation consequences. *Ann N Y Acad Sci.* 1256(1):33–48. <https://doi.org/10.1111/j.1749-6632.2012.06548.x>
- Reinhardt K. 2007. Evolutionary consequences of sperm cell aging. *Q Rev Biol.* 82(4):375–393. <https://doi.org/10.1086/522811>
- Reznick D, Endler JA. 1982. The impact of predation on life history evolution in Trinidadian guppies (*Poecilia reticulata*). *Evolution.* 36(1):160–177. <https://doi.org/10.1111/j.1558-5646.1982.tb05021.x>
- Seebacher F, Beaman J, Little AG. 2014. Regulation of thermal acclimation varies between generations of the short-lived mosquitofish that developed in different environmental conditions. *Funct Ecol.* 28(1):137–148. <https://doi.org/10.1111/1365-2435.12156>
- Simmons LW, Thomas ML. 2008. No postcopulatory response to inbreeding by male crickets. *Biol Lett.* 4(2):183–185. <https://doi.org/10.1098/rsbl.2007.0578>
- van der Hoek Y, Sirami C, Faida E, Musemakweli V, Tuyisingize D. 2022. Elevational distribution of birds in an Eastern African montane environment as governed by temperature, precipitation, and habitat availability. *Biotropica.* 54(2):334–345. <https://doi.org/10.1111/btp.13051>
- Vasudeva R, Deeming DC, Eady PE. 2014. Developmental temperature affects the expression of ejaculatory traits and the outcome of sperm competition in *Callosobruchus maculatus*. *J Evol Biol.* 27(9):1811–1818. <https://doi.org/10.1111/jeb.12431>
- Wang WW-Y, Gunderson AR, Gunderson AR. 2022. The physiological and evolutionary ecology of sperm thermal performance. *Front Physiol.* 13:754830. <https://doi.org/10.3389/fphys.2022.754830>
- Waser PM, Austad SN, Keane B. 1986. When should animals tolerate inbreeding? *Am Nat.* 128(4):529–537. <https://doi.org/10.1086/284585>
- Wedell N, Gage MJG, Parker GA. 2002. Sperm competition, male prudence and sperm-limited females. *Trends Ecol Evol.* 17(7):313–320. [https://doi.org/10.1016/S0169-5347\(02\)02533-8](https://doi.org/10.1016/S0169-5347(02)02533-8)
- White KL, Eason DK, Jamieson IG, Robertson BC. 2015. Evidence of inbreeding depression in the critically endangered parrot, the kakapo. *Anim Conserv.* 18(4):341–347. <https://doi.org/10.1111/acv.12177>
- Zajitschek SRK, Brooks RC. 2010. Inbreeding depression in male traits and preference for outbred males in *Poecilia reticulata*. *Behav Ecol.* 21(4):884–891. <https://doi.org/10.1093/beheco/arq077>
- Zajitschek SRK, Evans JP, Brooks R. 2006. Independent effects of familiarity and mating preferences for ornamental traits on mating decisions in guppies. *Behav Ecol.* 17(6):911–916. <https://doi.org/10.1093/beheco/arl026>
- Zajitschek SRK, Lindholm AK, Evans JP, Brooks RC. 2009. Experimental evidence that high levels of inbreeding depress sperm competitiveness. *J Evol Biol.* 22(6):1338–1345. <https://doi.org/10.1111/j.1420-9101.2009.01738.x>
- Zeh JA, Bonilla MM, Su EJ, Padua MV, Anderson RV, Kaur D, Yang DS, Zeh DW. 2012. Degrees of disruption: projected temperature increase has catastrophic consequences for reproduction in a tropical ectotherm. *Global Change Biol.* 18(6):1833–1842. <https://doi.org/10.1111/j.1365-2486.2012.02640.x>