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Reproducing in hot water: Experimental heatwaves deteriorate multiple reproductive traits in a freshwater ectotherm

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Abstract

1. Heatwaves are occurring at an increasing frequency and intensity under ongoing climate change. As many reproductive traits—including mating behaviour and gamete traits—are sensitive even to small temperature changes, the impact of heatwaves on reproduction and sexual selection processes is likely to be vast. Also, evaluating whether the sexes respond differently to these extreme events is crucial to understand the impact on fecundity and the consequences at the population level. Nonetheless, our knowledge of the effects of heatwaves on these key aspects of animal life is still limited.

2. Here, we expose recently mated male and female guppies *Poecilia reticulata* to an experimental heatwave (32°C, 6°C above the control, for 5 days) to determine its effects on several traits, including sexual behaviour, condition, ornamentation and fertility. Using this design, in contrast to alternative experimental setups, we had the possibility to attribute the effects of the heatwave to males' and females' reproductive traits independently.

3. Overall, our results indicate that heatwaves can drastically affect key reproductive traits and unravel sex-specific responses. In males, there was no effect of the heatwave on survival, but both pre- and postcopulatory reproductive traits were affected. After the heatwave, we detected a decrease in orange colouration (the most important ornament on which female choice is based) and the overall level of sexual activity, and a shift in the preferred mating tactic towards forced copulation attempts. The latter suggests implications in sexual conflict dynamics, as forced copulations override female mate choice. Also, after the heatwave, males had more sperm but of lower quality, and, in addition, an increased variance in sperm number. Overall, heatwaves may thus result in a compromised ability to secure matings and fertilizations. In females, the heatwave significantly affected survival, with increased mortality in the short term, and impaired fecundity, with many females from the heatwave treatment not reproducing at all.

4. The negative effects of heatwaves on key reproductive traits unravelled by our study could have major implications for population dynamics and persistence. It

highlights the need for further studies on how these extreme events affect reproduction, to improve our understanding of the impacts of climate change.

1 | INTRODUCTION

Climate change has resulted in vast changes in the global thermal environment, including an increased frequency and severity of heatwaves (Ummenhofer & Meehl, 2017), affecting the distribution and abundance of species (Buckley & Huey, 2016). Heatwaves are extreme climatic events, characterized as a period of abnormally hot weather (IPCC, 2021). Even though we still lack of an official, worldwide accepted definition of a heatwave, a commonly used one defines heatwaves as a period of 5–7°C higher than the usual temperature for at least 5 days (Sales et al., 2018). Heatwaves are quick and unpredictable in their onset and represent unusually high thermal conditions that can exceed the natural range of temperatures normally experienced by populations (van de Pol et al., 2017). The impact of heatwaves is deemed larger than that of gradual warming over a longer time (Easterling et al., 2000; Jentsch et al., 2007; Sheldon & Dillon, 2016), as organisms do not have the possibility to acclimate to such a short and sudden increase in temperature. Therefore, they are expected to have far-reaching impacts on biodiversity mediated by effects on, for example, movement (or migration), behaviour, physiology, and survival (Stillman, 2019). While a limited, but rising, number of studies investigate the biological impacts of heatwaves, indicating both lethal (Mouthon & Daufresne, 2006; Strydom et al., 2020; Wild et al., 2019) and sublethal impacts (Danner et al., 2021; Huber et al., 2010), only a few studies have focussed on how heatwaves affect reproduction (Hurley et al., 2018; Martinet et al., 2021; Sales et al., 2021; Wild et al., 2019). Yet, the limits that heatwaves, and temperature in general, pose to reproduction may better explain population declines than the limits they pose to survival (Martinet et al., 2021; Parratt et al., 2021; Wild et al., 2019).

Temperature may directly or indirectly affect reproduction, for example by directly jeopardizing gamete production, and hence fertility, or by influencing sexually selected traits associated with pre- and post-mating episodes of sexual selection, including ornaments used in mate choice and mating behaviour (García-Roa et al., 2020; Leith et al., 2021). Regarding the direct effects, high temperatures have already been linked to decreased gamete quantity (e.g. Nguyen et al., 2013; Paxton et al., 2016; Pérez-Crespo et al., 2008; Roux et al., 2010). In fact, the temperature threshold at which fertility deteriorates is often below an organism's lethal thermal limit and populations may face an increased risk of decline, that is a lower rate of offspring production, driven by sub-optimal fertility or complete infertility (of otherwise viable individuals) at high temperatures (Parratt et al., 2021; Walsh et al., 2019). Here, it is important to highlight that male and female reproductive traits may respond very differently to

thermal stress, since the sexes often differ in their thermosensitivity on fertility (Iossa, 2019). Male gametes are often more sensitive to thermal stress than female gametes, in endothermic as well as ectothermic animals (Iossa, 2019). Sperm may be especially thermosensitive due to their intrinsic characteristics, such as a limited repair machinery, that make them particularly susceptible to oxidative stress (Bisht et al., 2017; Reinhardt et al., 2015). Even small changes in temperature have been reported to decrease both the number and quality of sperm (e.g. Binet & Doyle, 2013; Breckels & Neff, 2013; Gasparini et al., 2018; Mehlis & Bakker, 2014). Also, female reproduction can be strongly impacted by heat stress, and negative effects have been observed in the number and quality of oocytes (e.g. Hansen, 2009; Paxton et al., 2016). In general, however, the relatively higher resilience in female fertility to heat stress has been suggested to potentially buffer population persistence under rising temperatures (Iossa, 2019). However, it is worth considering that the effects on reproduction have often been assessed using the number of offspring produced by females after the heat stress, with no possibility to disentangle the effects on females from those on males.

Thermal effects on reproductive traits may not only change reproductive success at the individual level but could also alter sexual selection dynamics (Candolin, 2019; García-Roa et al., 2020; Rosenthal & Elias, 2019). Changes in sperm quality are likely to impair sperm competitiveness (Van Lieshout et al., 2013; Vasudeva et al., 2014) and thus the outcome of sperm competition (where sperm from different males compete to fertilize the same set of eggs; Parker, 1979), and the opportunity for sexual selection at the postmating level. At the same time, temperature can affect premating sexual selection dynamics by changing the expression of sexually selected traits used in mate attraction and mate choice, such as body size, ornamentation, and mating behaviour and interactions (Candolin, 2019; Moore et al., 2021; Rosenthal & Elias, 2019). For example, temperature can affect female choice by disrupting pheromonal communication and thereby the female's ability to assess and choose an appropriate partner (Boullis et al., 2016). It can also alter sexual behaviour maladaptively, for example, when thermal stress—by diverting energy normally dedicated to sexual behaviour to repair or maintenance processes—translates into decreased sexual activity or a shift in mating tactics (e.g. towards sneaky mating), which can in turn increase the intensity of sexual conflict (Candolin & Wong, 2012). On the other hand, adaptive behavioural plasticity may allow organisms to buffer reproductive success under thermal stress and may thus provide resilience to populations and facilitate adaptation to temperature changes (Gómez-Llano et al., 2021; Moritz & Agudo, 2013). For instance, in species with parental care, an increased provisioning rate could allow parents to protect their offspring from

some heat-related costs (Fragueira et al., 2021). By increasing their remating rates, females could protect themselves from compromised male fertility (Sutter et al., 2019; Vasudeva et al., 2021). Altogether, studying the effects of heatwaves on traits related to fertility and sexual selection, and the ability of species to eventually adjust behaviour to counteract the effects on these traits, is crucial to better understand and predict the impacts of climate change on populations.

Here, we study whether and to what extent heatwaves affect reproduction, using a freshwater ectotherm, the guppy *Poecilia reticulata*, as model species. As ectotherms—which are more directly influenced by changes in their thermal environment than endotherms (Angilletta, 2009; Paaijmans et al., 2013)—that live in freshwater ecosystems, where conditions can fluctuate severely under extreme climatic events (Ledger & Milner, 2015; Woodward et al., 2016), guppies may be especially sensitive to heatwaves. In our study, we exposed adult fish to a 5-day experimental heatwave of 6°C—following the definition from Sales et al. (2018)—to comprehensively assess the effects on reproductive traits, including mating behaviour, ornamentation and fertility, and on survival and condition. The guppy is an internal fertilizer, that has become a model species in reproductive and sexual selection studies. Females are highly polyandrous (Hain & Neff, 2007; Liley, 1966), making post-mating selection, through sperm competition and cryptic female choice, an important source of variability in the reproductive success of individuals, with both sperm number and quality having an impact on sperm competitiveness (Boschetto et al., 2011; Devigili, Evans, et al., 2015). An important feature that makes the guppy well suited to study reproductive behaviour is the fact that such behaviour can be assessed both qualitatively, that is, in terms of which of two alternative tactics (with different energy requirements and fitness returns) males adopt to obtain a mating, and quantitatively, for example, as the number of sexual attempts or the time devoted to sexual activities. Specifically, males can either choose to attempt a mating through courtship (more energetically demanding) or through a sneaky mating attempt (less demanding in terms of energy but on average less successful; Devigili et al., 2013; Pilastro & Bisazza, 1999). Finally, the guppy is a relevant model for testing heatwaves, as in the original habitat of the species, small rivers and creeks in Central America, heatwaves are an ecologically relevant problem (Angeles-Malaspina et al., 2018; Stephenson et al., 2014). Moreover, high temperature has been previously shown to affect both pre- and postmating reproductive traits (Breckels & Neff, 2013; Reeve et al., 2014) and reproductive success (Dzikowski et al., 2001) of guppies. Thus, it is likely that also heatwaves may have an impact on reproductive traits.

2 | MATERIALS AND METHODS

2.1 | Fish maintenance

The subjects used in this experiment were descendants of wild guppies from a high predation site of the Tacarigua River in Trinidad. Since 2013, fish are maintained in seminatural conditions in a large pond at the Botanical Garden of the University of Padova. New-born fish were captured from the pond and reared to sexual maturity in the laboratory, under standardized conditions. Water temperature was maintained at $26 \pm 1°C$ and illumination was provided with a 12 L: 12 D cycle. All fish were fed twice a day with a mix of brine shrimp (*Artemia salina* nauplii) and commercial dry food (Duplarin). Upon sexual differentiation, females were separated from males and kept in female-only tanks to ensure the use of virgins for the experiment (necessary to avoid females storing sperm from previous mating; Gasparini & Evans, 2018).

2.2 | Overview of the experimental design

The overall aim was to look at the effects of a heatwave on traits related to reproduction and sexual selection. Specifically, we wanted to examine heatwave effects on sexual behaviour and fertility of adult males and females. To do so, we randomly assigned males and virgin females to either one of the two treatments: heatwave (HT) or control (C). We tested male traits before and after the treatment (sexual behaviour, sperm traits, condition and ornamentation; see below), and female traits (fecundity and sexual interest) only after. A total of 60 males and 60 females were used, at 7 months old. The experimental design consisted of mating males and females and then applying the treatment (heatwave or control) while they were physically separated (see below). This was done to disentangle the effect of the heatwave on the two sexes, and to standardize social and mating history that could affect some traits (e.g. sperm production in males) directly or indirectly, and to avoid any effect of male–female interactions during the heatwave on measured parameters.

2.3 | Timeline

On day 1, five males (7 months old) were randomly selected and introduced in the experimental tank (18 L) and left to acclimatize for 5 days (Figure S.1.1). On day 6, five non-experimental 'companion' females were released for 10 days in each tank to allow males to have a normal sexual activity. On day 16, the nonexperimental females were confined within the experimental tank in perforated transparent cylinders for 7 successive days. This period of male visual and olfactory, but not physical, contact with the females, was necessary to standardize recent sexual history, to properly assess reproductive traits that can be influenced by recent mating (such as sperm production or sexual behaviour; Bozynski & Liley, 2003; Devigili, Doldán-Martelli, et al., 2015; Gasparini et al., 2009). Males were tested for reproductive behaviour and swimming performance (day 23), and the day after, for sperm production and sperm quality, body condition, and coloration (day 24; see next section for the details). Thereafter, the males were returned to their original experimental tank and left to recover for 2 days before repeating this timeline (as for day 6 to day 24), this time with the experimental females (7-month-old virgins) and the heatwave (or control) treatment (see below). When the temperature treatment was started (day 37) the

females were confined into transparent cylinders. After 7 days the treatment (heatwave or control temperature) ended and males were measured again in the same way as before, for behaviour and swimming performance (day 44), sperm production and sperm quality, body condition, and coloration (day 45). On day 44 females were moved to individual tanks and monitored for fecundity. Once the females gave birth they were tested for sexual interest (assessing their propensity to school with conspecific females versus showing interest in males). However, due to the low number of females producing broods in the heatwave group (only 4 out of 30, see results), postpartum female behaviour could not be compared meaningfully among the two treatments, and we therefore do not describe methods for this assay or show any results on that part. Similarly, we also planned to test transgenerational fitness effects by measuring offspring survival and swimming ability, but due to the low number of broods—and hence offspring—in the heatwave treatment we have not reported the methods or results of this part (see Section 3).

2.4 | Heatwave treatment

In the heatwave treatment, we raised the temperature by 6°C and maintained it for 5 days, following the heatwave definition used in (Sales et al., 2018). The water temperature of the tank was gradually raised from 26 to 32°C on the first day (day 37), using an aquarium heater (100 W, NEWA Therm Pro), left at 32°C for the following 5 days, and gradually lowered back to room temperature (26°C) on the last day (day 43). Tanks of the control treatment were maintained with the same heater at room temperature (26°C). Water temperature in the tanks was checked with a standard aquarium thermometer and a temperature logger (EnvLogger v2.4, ElectricBlueCRL).

2.5 | Measurements

2.5.1 | Male assays

Before and after the temperature treatment, males were tested first for sexual behaviour and swimming performance and the following day for sperm production and sperm quality, body condition, and coloration.

2.5.1.1 | Sexual behaviour

Male's sexual behaviour was assessed in the morning following standard protocol for the species (e.g. Cattelan et al., 2016). One male was placed in an observation tank ($29 \times 39 \times 32$ cm) in which an unreceptive female (randomly chosen from a stock tank) was already present. After a 5-min acclimation period, the male's behaviour was observed for 15 min. The following behaviours were recorded: the total time a male spent following the female (observed when a male was within two body lengths from the female and oriented towards her), as a proxy for *sexual interest*. The total number of courtship displays performed by the male (when a male bends its body, opens the fins and shows himself to the female), and the number of gonopodial thrusts, that is, sneaky mating attempts. We calculated each male's *sexual activity*, defined as the sum of courtship displays and gonopodial thrusts performed by the male. We also determined the male's *preferred tactic*, calculated as the ratio of gonopodial thrusts to courtship displays scaled around zero. A value of 0 indicates that a male performed each tactic at the same frequency, a positive value indicates it performed more gonopodial thrusts, and a negative value indicates a relatively higher rate of courtship displays.

2.5.1.2 | Swimming performance

The swimming performance of males was estimated by measuring its *critical swimming speed* in a flow chamber, using a previously established protocol (Nicoletto, 1991). Each fish was placed into a swimming chamber (a 50 cm long transparent PVC pipe, 1.5 cm in diameter), through which water was pumped at a steady linear flow using an aquarium pump. The water velocity was initially 7 cm s^{-1} and was increased by 3 cm s^{-1} every minute, until the fish fell from the outflow end of the chamber into a water tank below. The total swimming time, the highest water velocity and the time the fish spent swimming at the highest velocity were recorded, from which its *critical swimming speed* was calculated after Brett (1964). Critical swimming speed is defined as the maximum speed that a fish can sustain for a set period (Brett, 1964), that is, how fast a fish can swim, and is a proxy for endurance (Gordon et al., 2015). Swimming performance tests were conducted in the afternoon.

2.5.1.3 | Sperm number and quality

The day after the behavioural assay, each male was anaesthetized to collect the ejaculate. Ejaculate collection followed standard procedure for the species (see, for example Gasparini et al., 2017). Briefly, while the male is sedated, a gentle pressure is applied to the male's abdomen, releasing the sperm. *Sperm number* (the total amount of sperm cells in the ejaculate) and *viability* (the proportion of live and dead sperm), were assessed using an automated cell counter (LUNA Dual Fluorescence Cell Counter). Sperm motility was assessed using computer-assisted sperm analysis (CEROS Sperm Tracker, Hamilton Thorne Research, Beverly, MA, version 12.3), following previously established protocols (e.g. Gasparini et al., 2017). In both sperm viability and sperm motility assays, sperm number was diluted and equalized among males. The following parameters were recorded, based on an average ($\pm$ SE) of 217 ± 6 sperm tracks per sample: average path velocity (VAP), straight line velocity (VSL), curvilinear velocity (VCL), amplitude of the lateral head (ALH), beat cross frequency (BCF), straightness (STR), and linearity (LIN).

2.5.1.4 | Body condition and colouration

While males were sedated for ejaculate collection, they were weighted (to the nearest mg) and photographed using a digital SLR camera (ESOS 450D) under standardized illumination. The photographs included a calibration scale and a colour standard

(GretagMacbeth ColorChecker®). Using ImageJ analysis software (https://imagej.nih.gov/ij/index.html) from those photographs, we measured male's body size (standard length, SL, in mm; distance from the snout to the base of the caudal fin). Body size was used with body weight to quantify a male's body condition (i.e. his fat stores or surplus energy) using *Fulton's body condition index* (Kotrschal et al., 2011). We also measured the area of orange-coloured spots (from here on *orange body area*) with ImageJ and the spectral properties of the orange spots using the software ColourWorker (version 2.0, https://www.colourworker.com/). Each photograph was calibrated with the standard colour reference included in each image (for details see Gasparini et al., 2014).

2.5.2 | Female assays

2.5.2.1 | Fecundity

After the treatment, females were moved to individual 3 L tanks in a recirculating water system (Tecniplast) and monitored every day until giving birth to offspring, which were counted. After producing their first brood, females were maintained individually isolated to record the total number of successive broods produced with the stored sperm. This was done to study the effect of the heatwave on sperm storage, but again, due to the low fecundity in the heatwave group (see below), there were not enough data for a meaningful comparison between the treatments. Because females produce broods approximately every 30 days, when >70 days passed without any offspring produced, the female was considered to not have had more broods (i.e. emptied the sperm storage).

2.5.3 | Male and female survival

Survival of the fish was assessed by daily monitoring the tanks for up to 45 days following the temperature treatment in males, and up to 6 months following the treatment in females. If a male died before the start of the treatment, it was immediately replaced with another male raised and kept under the same laboratory conditions. Males used as replacements ($N = 10$) have only one measure (after treatment). Males that died during the treatment and females were not replaced.

2.6 | Statistical analyses

All analyses were performed in R, version 1.4.1717. The significance of fixed effects in linear mixed models (LMER) was calculated from *F* statistics with the 'LMERTEST' package and Satterthwaite's approximation to calculate the denominator degrees of freedom. The significance of fixed effects in generalized linear mixed models (GLMER) was calculated from chi-square statistics, using Wald chi-square tests from the 'CAR' package. The distribution of residuals from the models was checked to ensure model assumptions were met and corrections for overdispersion were made when needed.

2.6.1 | Males

Male traits were analysed using (G)LMER models including treatment (heatwave or control), time (before or after the temperature treatment), and their interaction, as fixed factors. All models included male identity (ID, 68 levels) as a random factor to account for repeated measures within the same male. The male's experimental tank (tank, 12 levels) was also tested as a random factor but removed when it explained no variance in the model.

We performed a principal component analysis (PCA), using the R package 'PRCOMP', on the seven recorded sperm velocity parameters. We obtained 2 principal components (PCs) with an eigenvalue greater than one (Table S.2.1), which explained 89% of the original variation. The first component ($PC1_{sperm}$), which explained 60% of the variance, represents sperm velocity. The second component ($PC2_{sperm}$), which explained 29% of the variance, represents sperm swimming path straightness. Hereafter, we will refer to these two PCs as *sperm velocity* and *sperm path straightness*. A more detailed description of the components can be found in Supporting Information (Section S.2).

For analysing coloration of the orange spots we performed a PCA on the spectra obtained, where the reflectance values at each wavelength point for each male (from 400 to 700 nm at a 5 nm step) were the original variables. We obtained 3 PCs with an eigenvalue greater than one (see Table S.3.1) which explained 98.8% of total variability. We describe in detail the PC meaning in Supporting Information (Section S.3). In short, the first component ($PC1_{colour}$) represents the brightness of the spot (i.e. how much light is reflected irrespective of the colour). The second component ($PC2_{colour}$) is loaded by wavelengths in the central part of the spectrum and—indirectly and inversely—represents the saturation of the orange coloration of the carotenoid spot (the higher the PC score, the lower the relative orange intensity). The third ($PC3_{colour}$) approximates the relative intensity of the ultraviolet (UV) component of the spot. From here on, we will refer to $PC1_{colour}$ as *brightness*, $PC2_{colour}$ as *saturation*, and $PC3_{colour}$ as *UV saturation*.

LMER models were used for analysing the sexual interest, the male's preferred tactic, critical swimming speed, sperm number (sqrt transformed), sperm velocity, sperm path straightness, body condition, area of the orange spots, orange spot brightness, saturation, and UV saturation. GLMER models were used for analysing sexual activity (model family: Poisson) and sperm viability (family: binomial). The models for critical swimming speed, sperm number, and orange spot area were corrected for male body size by including the male's standard length as a fixed effect. For technical reasons, in three males critical swimming speed could not be estimated, and in three other males sperm quality could not be assessed.

Analysis of variance, to test whether the variance of traits differed between treatment groups or changed across time, was performed using Bartlett's tests (Table S.4.1).

2.6.2 | Females

The fecundity of females was analysed using GLMER models, including treatment (heatwave or control) as a fixed effect and the

experimental tank (tank) as a random factor. Binomial family was used for the probability of producing at least one brood and Poisson family in the model for brood size. Due to a low number of heatwave females producing broods, analyses to compare the post-partum behaviour, and analyses to compare the offspring's traits and survival in relation to maternal treatment (for determining transgenerational effects), could not be performed.

2.6.3 | Male and female survival

Survival was analysed using the package 'SURVIVAL'. To analyse how survival was affected by treatment (heatwave or control) we carried out survival analyses by fitting Kaplan–Meier survival curves to each of the two groups (estimating survival probability, based on the number of surviving individuals per day), and compared the curves for differences in survival between control and heatwave groups using a log-rank test. Significance of between-group differences in the survival probability until specific time points during the experimental timeline (e.g. survival until the end of the temperature treatment) was calculated from Z statistics.

3 | RESULTS

3.1 | Males

We obtained measures from 68 males (35 control and 33 heatwaves), 40 of which with complete repeated measures. Specific sample sizes are reported for each male trait in Table 1. Mean and variance of raw data traits are reported in Table S.4.1 (part A). Analysis of variance are reported for all the traits in Table S.4.1, across time (part A) and across treatment (part B), and described in the results only when a significant treatment effect was found (Table S.4.1, part B AFTER).

3.1.1 | Sexual behaviour

Sexual interest (time spent actively following the female, in min) was not significantly affected by treatment, time (i.e. before or after the treatment), or their interaction (Table 1, Figure 1a). Male sexual activity (the sum of courtship displays and sneaky mating attempts) was significantly affected by the interaction between treatment and time (Table 1), with males decreasing their total sexual activity after the treatment in the heatwave but not in the control (Figure 1b, Table S.4.1). Throughout all the observations of sexual behaviour, sneaky mating attempts were the overall preferred male tactic, consisting of 72% percent of the total observed sexual activities. The ratio of the two tactics used by each male (i.e. the preferred tactic) was significantly affected by the interaction between treatment and time (Table 1). This interaction indicates the males in the heatwave group (where we found reduced overall sexual activity) decreased predominantly their courtship displays, resulting into a more pronounced use of sneaky mating attempts after the heatwave, while this was not the case in the control group (Figure 1c, Table S.4.1).

3.1.2 | Swimming performance

Critical swimming speed was significantly affected by the interaction between treatment and time (Table 1). The swimming performance of fish increased after the treatment in control males, but not in heatwave males (Table S.4.1).

3.1.3 | Sperm number and quality

Sperm number was significantly affected by treatment and the interaction between treatment and time (Table 1). After the treatment, sperm number was higher in the heatwave group than in the control group (Figure 2a, Table S.4.1), and also the variance was significantly larger in the heatwave group compared to the control group (24.21 vs. 6.24, $p = 0.001$, see Table S.4.1 part B, Figure S.4.1). Sperm viability was not significantly affected by treatment, time or their interaction (Table 1, Figure 2b).

Sperm velocity was significantly affected by treatment and time (Table 1). Sperm velocity was lower in the heatwave treatment, and there was a decline in velocity from before to after the treatment (Figure 2c, Table S.4.1). While the interaction was not significant, visual inspection of the data indicated a greater drop in velocity in the heatwave group (Figure 2c and Figure S.4.2, Table S.4.1), leading us to run two additional separate models for each time (before or after the treatment). These showed that sperm velocity was significantly lower in the heatwave group than in the control group after the treatment (estimate $\pm$ SE $= -1.262 \pm 0.591$, $F = 5.56$, $p = 0.038$), while no between-group difference was observed before the treatment (-0.431 ± 0.457, $F = 0.89$, $p = 0.350$). Sperm path straightness was significantly affected by time (Table 1, Figure 2d, Table S.4.1), but not by the treatment nor the interaction. The straightness of the sperm trajectory increased from pre- to posttreatment (Table 1).

3.1.4 | Body condition and colouration

Male body condition (Fulton's index) was not affected by treatment, time or their interaction (Table 1). There was no effect of treatment or time on the area of orange spots. Interestingly, there was a trend for the interaction between treatment and time (Table 1), with a decrease in orange spot area after the heatwave but not after the control (Table S.4.1).

The brightness and UV saturation of orange spots significantly increased with time (Table 1) in both control and heatwave groups (see Table S.3.2 and Figure S.3.3), but were not affected by treatment or the interaction. However, after the treatment, variance in UV saturation was larger in the heatwave group compared to the control

TABLE 1 Heatwave effects on male traits. Results from (G)LMM models testing each variable in response to the temperature treatment (*control* or *heatwave*), time (*before* or *after* the temperature treatment) and their interaction. *F* statistics for LMM's are given with their corresponding denominator degrees of freedom (dff) and χ^2 statistics for GLMM's are underlined. Significant effects and trends are printed in bold

	Sample size (no. males)	TREATMENT			TIME			TREATMENT×TIME		
		Estimate (SE)	F(ddf)/χ^2	p	Estimate (SE)	F(ddf)/χ^2	p	Estimate (SE)	F(ddf)/χ^2	p
i. Sexual behaviour										
Sexual interest	108 (68)	1.161 (1.236)	0.40 (9.9)	0.540	0.312 (0.991)	0.09 (52.6)	0.770	−1.070 (1.418)	0.57 (52.6)	0.454
Sexual activity	108 (68)	0.168 (0.282)	<u>0.77</u>	0.380	0.204 (0.277)	<u>0.74</u>	0.388	−0.785 (0.399)	<u>3.86</u>	**0.049**
Preferred tactic	108 (68)	−0.401 (0.153)	2.47 (62.2)	0.121	−0.172 (0.149)	0.27 (58.1)	0.609	0.465 (0.213)	4.77 (58.0)	**0.033**
ii. Swimming performance										
Crit. swim speed	105 (67)	0.927 (0.563)	0.06 (64.7)	0.815	1.384 (0.506)	1.96 (55.2)	0.167	−1.828 (0.726)	6.33 (55.6)	**0.015**
iii. Sperm number and quality										
Sperm number	108 (68)	0.035 (0.203)	3.00 (66.6)	**0.088**	−0.123 (0.181)	1.24 (57.4)	0.270	0.553 (0.258)	4.58 (57.6)	**0.037**
Sperm viability	108 (68)	0.187 (0.261)	<u>0.02</u>	0.879	0.015 (0.221)	<u>2.02</u>	0.155	−0.480 (0.317)	<u>2.29</u>	0.131
Sperm velocity	105 (66)	−0.431 (0.503)	4.93 (101)	**0.029**	−1.186 (0.512)	18.53 (101)	**<0.001**	−0.832 (0.737)	1.27 (101)	0.262
Path straightness	105 (66)	0.001 (0.396)	0.54 (13.7)	0.476	1.265 (0.301)	21.44 (51.3)	**<0.001**	−0.547 (0.432)	1.60 (51.2)	0.211
iv. Body condition and colouration										
Fulton's index	108 (68)	0.071 (0.057)	0.99 (62.3)	0.323	0.015 (0.046)	0.07 (48.3)	0.787	−0.049 (0.065)	0.56 (48.2)	0.456
Orange spot area	108 (68)	−0.113 (0.347)	1.18 (10.8)	0.301	0.133 (0.210)	0.63 (46.4)	0.431	−0.522 (0.299)	3.05 (46.8)	**0.087**
Orange brightness	108 (68)	1.495 (1.557)	2.31 (66.3)	0.134	3.084 (1.302)	15.10 (54.2)	**<0.001**	1.082 (1.859)	0.34 (54.1)	0.563
Orange saturation	108 (68)	0.797 (0.935)	5.33 (65.0)	**0.024**	0.763 (0.649)	18.06 (46.6)	**<0.001**	2.467 (0.924)	7.13 (46.5)	**0.010**
UV saturation	108 (68)	0.461 (0.601)	0.04 (64.0)	0.835	1.365 (0.504)	7.59 (51.9)	**0.008**	−0.770 (0.72)	1.15 (51.8)	0.289
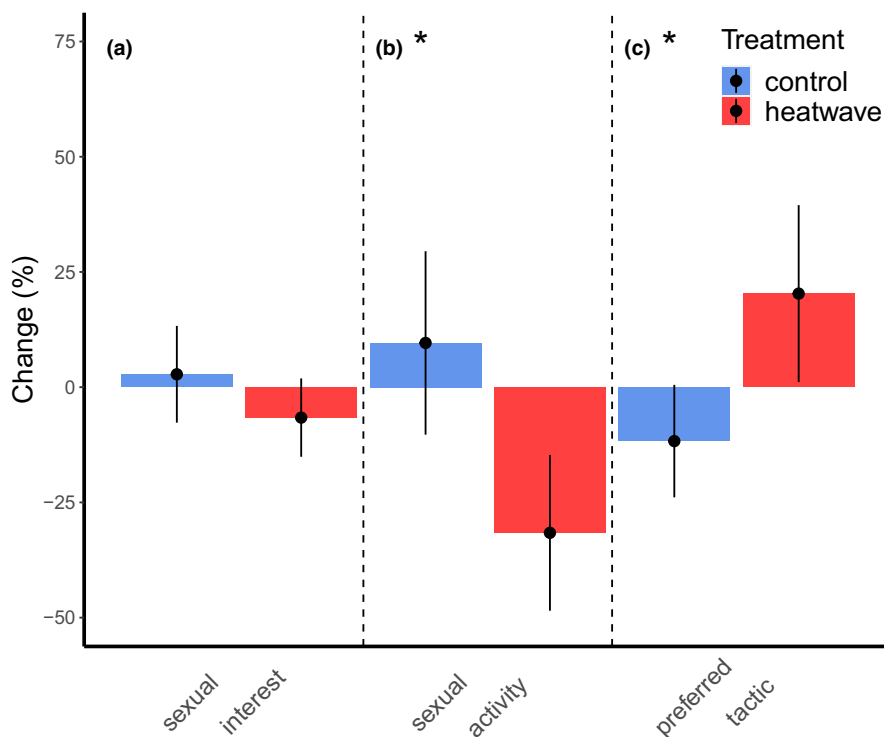
FIGURE 1 The relative change, expressed as a percentage ($\pm$ the relative SE of the difference between the means), in sexual behaviour from before to after a temperature treatment (control or heatwave): (a) sexual interest (i.e. time following a female), (b) sexual activity (i.e. total number of gonopodial thrusts plus sigmoid displays) and (c) preferred tactic (i.e. the ratio of sneaky mating attempts over courtship displays). Asterisks indicate a significant interaction between treatment and time.
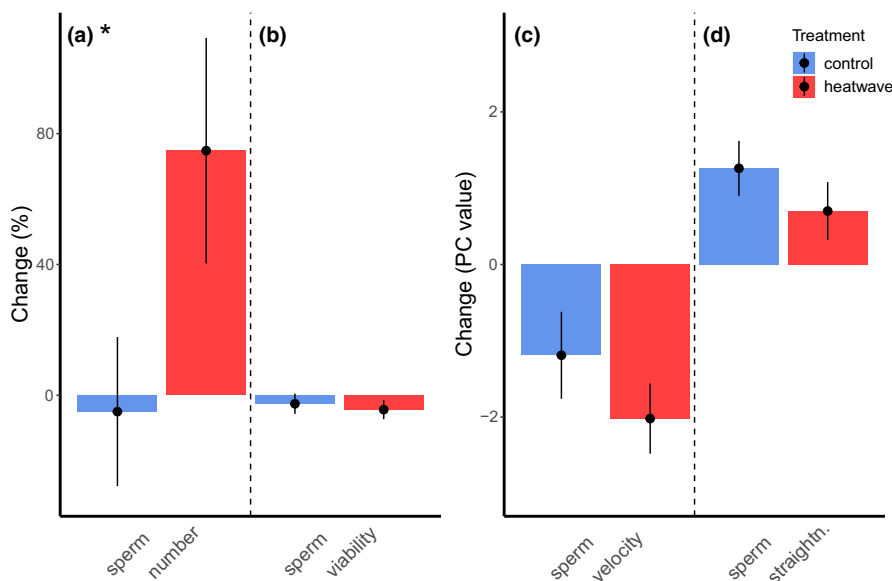
FIGURE 2 The change in male's sperm traits from before to after the temperature treatment in terms of (a) sperm number, (b) sperm viability, (c) sperm velocity and (d) sperm path straightness. (a) and (b) are expressed as a relative change (%), while (c) and (d) as the change in principal component score. A significant treatment by measurement interaction (indicated with an asterisk) existed in the analysis for sperm number (a).

group (9.48 vs 3.93, $p = 0.034$, see Table S.4.2, part B). Orange saturation decreased in both treatments but significantly more in the heatwave males compared to the control males (Table S.3.2/S.4.1, Figure S.3.3 panel B).
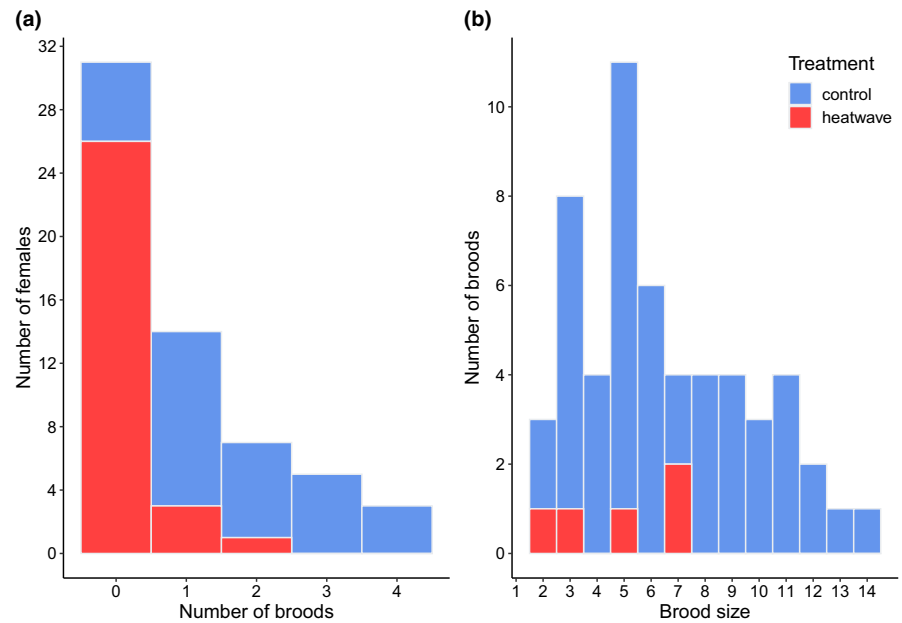
3.2 | Females

3.2.1 | Fecundity

Four out of 30 (13.3%) heatwave females produced at least one brood, versus 25 out of 30 (83.3%) control females (Figure 3a). The probability

of producing a brood was significantly reduced in the heatwave females ($\chi^2 = 32.517$, $p < 0.001$). When considering only those females that survived long enough to give birth to their first potential brood (survival >40 days since the first day of being released with males, $N = 39$), the probability of producing a brood was still significantly reduced in the heatwave females ($\chi^2 = 16.25$, $p < 0.001$). The average brood size for broods from heatwave females was 4.80 ± 1.02, versus an average of 6.68 ± 0.44 for the control females (Figure 3b), but this difference was not significant between treatment groups ($\chi^2 = 2.693$, $p = 0.101$). However, the number of females giving birth in the heatwave group was only $n = 4$, so the analysis on the number of offspring produced (brood size) needs to be interpreted with caution.

FIGURE 3 The number of females producing broods (from zero broods up to four broods) (a), and the number of broods produced at each brood size (b).

3.3 | Male and female survival

3.3.1 | Male survival

Male survival showed no consistent differences between the two treatments, as evidenced by the log rank test for differences in survival probability ($n = 70$, $\chi^2 = 1.7$, $p = 0.2$) as well as the crossing survival curves (Figure 4a). Also, performing survival analysis from the start until different time points throughout the experiment confirmed this result. No differences were found in survival until the day before the start of the temperature treatment (until day 36; $Z = -1.316$, $p = 0.188$), until the end of the temperature treatment (day 43; $Z = 0.260$, $p = 0.795$), and until the end of the week following the temperature treatment (day 50; $Z = 0.052$, $p = 0.958$, see Figure 4a for the different time points).

3.3.2 | Female survival

After 6 months since the start of the treatment there were 12/60 females still alive (6 in the control and 6 in the heatwave group). On average, females in the control group had a longevity of 305.1 ± 10.0 days, while the females in the heatwave group of 277.5 ± 11.9 days (difference not significant). The ratio of surviving females (Figure 4b) throughout the entire monitoring period was not significantly different between the two groups ($\chi^2 = 2.6$, $p = 0.100$). However, important differences in survival throughout the course of the experiment emerged when considering survival until different time points within the experimental timeline. Specifically, we estimated differences in survival from the beginning of the treatment until: (1) the end of the treatment (i.e. short-term survival; until day 43 in the timeline, see Figure 4b), (2) 1 week after the treatment (day 50), (3) 1 month after the treatment (day 73) and (4) the end of the experiment (day 103). The probability of survival for females that

experienced a heatwave was significantly lower in the first cut-off (1) ($Z = 2.450$, $p = 0.014$), second cut-off (2) ($Z = 4.157$, $p < 0.001$), and third cut-off (3) ($Z = 3.533$, $p < 0.001$), but not in the last cut-off (4) ($Z = 1.615$, $p = 0.106$). This indicates that heatwave females died more likely during and shortly after the heatwave, but if they survived, longevity in the long term was not affected.

4 | DISCUSSION

Our findings show detrimental and sex-specific effects of heatwaves on reproduction and survival. In males, the experimental heatwave had negative consequences on several reproductive traits, including sexual behaviour, ornamentation, and sperm traits, that translate into decreased mating and fertilization success. It had little to no impact on male survival. In females, the heatwave greatly impaired fecundity as well as survival, with surviving females mostly failing to produce a brood. Such strong effects on females indicate unpredicted high sensitivity to thermal stress in females, which were gravid during the heatwave in our experiment. Our study provides one of the few examples thus far of multiple sublethal reproductive costs imposed by heatwaves, as well as of intra-specific variation in thermal tolerance (i.e. depending on the sex or life-history stage of an animal). Through their effects on reproductive traits and reproductive success, heatwaves can have strong implications for population demography, sexual conflict, and sexual selection, highlighting the need to further investigate the effects of heatwaves to predict evolutionary pathways and species vulnerability under rapid climate change.

4.1 | Heatwaves at population level

Heatwaves had sex-specific effects on survival, fecundity, condition, and fertility. This provides evidence that extreme climatic
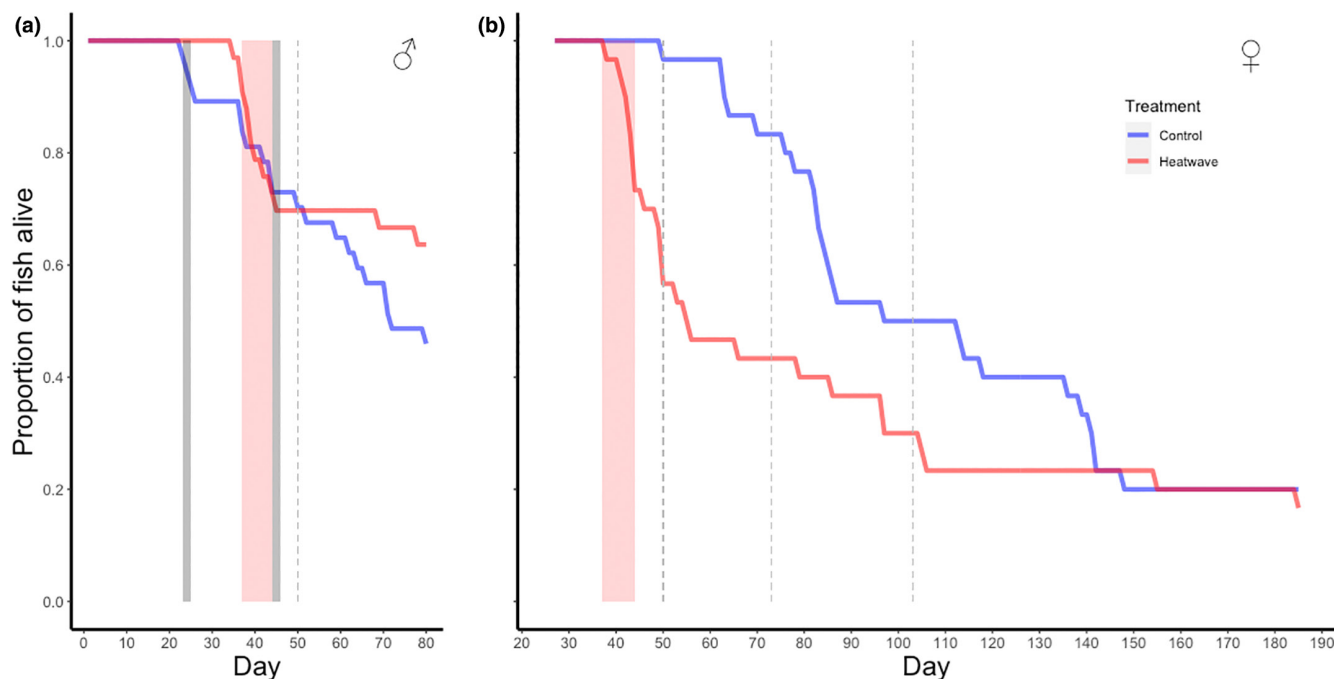
FIGURE 4 Relative survival of (a) males and (b) females throughout the experiment. The red vertical bars indicate the period during which the fish were exposed to either treatment (control or heatwave) and the grey vertical bars indicate the days on which males were measured for condition and pre- and postcopulatory reproductive traits. Males were monitored for survival for up to 45 days following the temperature treatment. Females were introduced on day 27 and monitored for up to 6 months following the treatment. Dashed vertical lines indicate time points of 1 week (day 50), 1 month (day 73) and 2 months (day 103) following the temperature treatment (the cut-offs used in the survival analyses).

events can have severe and sublethal consequences on individuals, potentially altering population dynamics. Females undergoing a heatwave showed reduced survival and lower fecundity—only 13% or heatwave females produced a brood compared to 83% of control females. This demonstrates that extreme heat events can impose not only survival costs but also large reproductive costs which can result in population decline, corroborating results from other taxa, including, for example, recent findings in a marine ectotherm (Siegle et al., 2022). Moreover, such detrimental effects can lead to events with strong potential effects on population structure such as bottle necks, founder events and genetic drift or modification in population's age and sex ratio with temporal and spatial genetic changes in metapopulations (Coleman et al., 2020; Gurgel et al., 2020; Shama et al., 2011).

The strong impact on survival and fecundity of females, which were subjected to the heatwave during pregnancy, point to an increased sensitivity to heat stress of this sex, that can be exacerbated by the costs associated with pregnancy. In this species, females are almost always pregnant, as they reproduce constantly, passing from one reproductive cycle to the next one with no breaks, and have only a few days of receptivity after giving birth. In our experiment, females were at the beginning of pregnancy (roughly 1 week after fertilization) when subjected to the heatwave. It remains to be further investigated if the effects we found in this stage are also found during the rest of the pregnancy, and with what magnitude, but it is likely that a heatwave will impose

even greater costs later in the pregnancy, based on recent studies on decreased thermal tolerance during pregnancy in this species (Auer et al., 2021). Ectotherm females change thermal preferences during pregnancy and, under normal conditions, will select their optimum body temperature within the available thermal gradient through behavioural thermoregulation (e.g. Dayananda et al., 2017; Le Galliard et al., 2003; Mathies & Andrews, 1997). During heatwaves, extreme temperatures and a reduced thermal gradient could mean that the optimal temperature for embryogenesis (or for any other specific life-stage; Truebano et al., 2018) is no longer available, thus obstructing effective thermoregulation and the ability of an individual to avoid thermal stress. Under heat stress, energy production may be reduced and energy reserves normally available for reproduction may be unavailable or re-allocated to basal physiological functions or stress responses (Portner & Knust, 2007; Sokolova et al., 2012; Somero, 2002). Even though the thermal limitations that heatwaves impose on effective behavioural thermoregulation (through which individuals may avoid thermal stress) could have been exacerbated under experimental conditions, we believe our results are relevant to natural scenarios, where heatwave intensity and duration are expected to be even higher (Angeles-Malaspina et al., 2018; Ledger & Milner, 2015).

A decreased tolerance to heatwaves during pregnancy could be widespread, also in endothermic organisms. Indeed, in several species of mammals, including humans, exposure to gestational heat

adversely impacts pregnancy, by altering, among others, gestation length, birth weight and the rate of stillbirth (Boddicker et al., 2014; Dado-Senn et al., 2020; Kuehn & McCormick, 2017; Marco-Jiménez et al., 2017), highlighting the sensitivity of this life stage to heat stress. Further studies are needed to understand the mechanisms underlying this sensitivity, as well as the (likely) cumulative costs of heatwaves on female fecundity when coupled with effects on male reproductive traits, like those found on sperm number and quality (see discussion below). Indeed, since our experiment was designed to disentangle effects on females and males (sexes were separated during the heatwave), we can safely attribute our findings of decreased fecundity solely to direct effects of heatwaves on already pregnant females, and not to indirect effects mediated by the effects on males, such as the shift in male mating strategies or detrimental effects on sperm.

In males, the experimental heatwave affected both pre- and postcopulatory reproductive traits. Among the precopulatory traits, the decrease in male mating attempts (sexual activity), the shift in the preferred mating tactic used, and the decrease in the orange colouration were the most notable ones. In the guppy, female mate choice accounts for an important portion of the variation in paternity (Gasparini & Evans, 2018) and male orange colouration is one of the most important traits on which female choice is based (Houde, 1997; Magurran, 2005). Heatwave-induced reductions in the area and saturation of orange spots will thus translate in a decreased male attractiveness and will potentially affect male mating success through female mate choice (also see the next section). In addition, the decrease in total mating attempts made by males that experienced a heatwave, along with the shift in their preferred tactic, is likely to decrease their total reproductive success. Compared to sneaky copulations, consensual mating—obtained by male after courtship—results in a higher amount of sperm transferred to females and hence in males siring more offspring in a sperm competition scenario (Boschetto et al., 2011; Pilastro & Bisazza, 1999). Together, the findings on males' precopulatory traits indicate that heatwaves can impose serious costs on the reproductive fitness of males. While we did not find an effect of the heatwave on the males' body condition index (a proxy for energy reserves), their swimming endurance was affected, indicating that heatwaves do decrease condition. This reduction in condition may explain the fact that males relied less on courtship displays, as these are energetically costly compared to the less demanding sneaky mating attempts (Houde, 1997).

Concerning postcopulatory traits of males, we found sublethal heatwave effects on sperm quantity and quality, in line with previous findings in insects (Sales et al., 2018, 2021). Strikingly, heatwave males showed an increase in sperm number after the treatment, which was not the case in control males. The increase in number of spermatozoa produced likely resulted from an elevated rate of metabolism and associated accelerated spermatogenesis under the higher temperature, as observed in some other fishes (Nóbrega et al., 2009; Postingel Quirino et al., 2021). However, the heatwave had negative effects on sperm quality, since sperm velocity was

lower in the heatwave males compared to the control males after the treatment. This suggests that the heatwave decreased the velocity of sperm directly or, indirectly, through a potential trade-off between sperm production rate and sperm quality (Snook, 2005). While many studies and theoretical models point to sperm quantity (i.e. sperm number) as the ejaculate trait that best predicts male fertilization success under sperm competition (sensu Parker, 1998), evidence is growing that sperm qualitative traits, including swimming velocity and viability, also influence male fertilization success in many species (Gage et al., 2004; Gasparini et al., 2010; Gomendio & Roldan, 2004; Laskemoen et al., 2010; Pizzari, 2009; Snook, 2005), including the guppy (Boschetto et al., 2011; Cardozo et al., 2020). In addition, it is worth noting that an increase in sperm number does not necessarily translate into more sperm transferred to the female, as in this species it is the female that controls the number of sperm she receives from the male, independent of his sperm reserve (Pilastro et al., 2007). Therefore, sperm quality may be as important as the relative quantity in determining the outcome of sperm competition (Boschetto et al., 2011; Cardozo et al., 2020). In addition, temperature-induced changes in sperm quality could have potential cross-generational implications, that is, effects on offspring traits, mediated by paternal effects (Crean & Bonduriansky, 2014; Evans et al., 2019; Gasparini et al., 2018). While the magnitude of the consequences of heatwaves on male reproductive fitness needs further investigation, our results clearly show that heatwaves drive changes in male mating behaviour, male attractiveness, and sperm traits. This, together with the effects on female fecundity and survival, provides strong evidence that heatwaves have important consequences for reproduction and population demography, and thus are an important component in shaping the impact of climate change at population level.

4.2 | Heatwaves, sexual conflict and sexual selection

In our study, we also considered whether heatwaves affect trait variance, as this can alter the relative differences in individual competitiveness or attractiveness and thereby influence sexual selection dynamics (Rosenthal & Elias, 2019). The variance of some of the males' reproductive traits changed. Males' sperm number increased in the heatwave but not in the control group, not only in terms of mean number but also in terms of variance, that is, increasing the relative differences among males (Table S.4.1 part B, Figure S.4.1, but note one outlier). On the other hand, sperm velocity, which decreased in both groups and to a greater extent in heatwave males, showed a lower degree of variance after the heatwave than after the control treatment, though this difference was not significant (Table S.4.1 part B, Figure S.4.2). Despite the heatwave induced reductions in mean orange saturation and sexual activity in males, the variance in neither of these traits was affected (Table S.4.1 part B). We can additionally examine how variance changed over time (Table S.4.1

part A). Over time (from before to after the treatment), all males' orange spots became brighter and increased in UV intensity, independent of their treatment (Table 1). The increase in mean UV intensity was accompanied by an increase in variance in the heatwave, but not in the control group (Table S.4.1 part A, Figure S.3.3.C, but note one outlier). Instead, the increase in mean orange brightness was accompanied by a significant increase in brightness variation only in the control group (heatwave fish showed a non-significant increase in variation, Table S.4.1 part A, Figure S.3.3.A). By changing the variance in reproductive traits, heatwaves may thus alter the relative differences among males, for example in terms of attractiveness, and thereby change the opportunities for sexual selection. However, how environmental effects impact sexual selection via changes in trait variance needs to be further evaluated.

The effects of heatwaves on female survival and fecundity can also affect sexual selection dynamics, by leading to changes in population structure and the level of sexual conflict. For instance, effects on female survival could shape patterns of mate competition through density dependent processes (Kokko & Rankin, 2006) and by altering operational sex ratios (Weir et al., 2011). Operational sex ratios could be further shifted, but cryptically, by the sublethal effects of heatwaves on female fecundity, especially in combination with sublethal effects on male fertility, that is, non-observable temperature-driven sex ratio shifts due to cryptic sterility (Walsh et al., 2021). Finally, shifts in population density and sex ratios can change the level of sexual conflict, which in turn can affect sexual selection (Rowe et al., 1994). Similarly, our finding that after a heatwave males rely more on sneaky matings compared to control males, a tactic that undermines female choice, indicates that there are more opportunities for sexual conflict under heat stress. Altogether, by affecting survival and traits underlying pre- and postcopulatory selection, heatwaves are likely to play an important role in the evolutionary feedbacks between thermal ecology and sexual selection (Leith et al., 2022).

5 | CONCLUSIONS

We found that heatwaves affect a range of fitness-related traits, important for reproduction and sexual selection and that responses were different in males and females. This confirms that accounting for sex-specific differences in thermosensitivity is crucial to model realistic scenarios for natural populations under heat stress (Iossa, 2019). Changes in individual reproductive traits and reproductive success, and hence individual fitness, could translate into demographic changes in populations as a whole and thereby alter population viability (Kokko & López-Sepulcre, 2007; Oppel et al., 2014). The findings therefore support the notion that sublethal reproductive costs imposed by heatwaves could lead to changes in offspring production and population demography (Siegle et al., 2022). Demographic changes could also have carry-over effects on ecosystems, by altering community composition through cascading effects across the

trophic levels, for example due to altered predator–prey densities and interactions (Woodward et al., 2016).

The effects of heatwaves on trait variance, especially in terms of male coloration and sperm production, could translate into altered dynamics of pre- and postcopulatory reproductive success among competing males, or lead to reduced opportunities for effective mate choice. Thereby, heatwave-driven changes in individual reproductive traits could lead to altered sexual selection dynamics. Changes in (natural and sexual) selection regime can alter the gene pool, for instance, through bottleneck effects resulting from restricted pools of reproducing animals (Dahlke et al., 2020).

In summary, heatwaves have important consequences for populations not only by affecting survival, but also through sublethal impacts on reproduction and mating system dynamics. The effects of heatwaves on reproduction could be widespread across taxa (Andreasson et al., 2020; Leach et al., 2021; Sales et al., 2021; Siegle et al., 2022), and thus require further study to improve our ability to predict how populations will respond and evolve under rapid climate change.

AUTHOR CONTRIBUTIONS

Merel C. Breedveld and Clelia Gasparini conceived and designed the experiment. Merel C. Breedveld, Alessandro Devigili and Oliviero Borgheresi collected the data. Merel C. Breedveld and Alessandro Devigili analysed the data. Merel C. Breedveld led the writing of the manuscript, supported by Clelia Gasparini and Alessandro Devigili. All authors contributed critically to the development of the drafts and gave final approval for publication.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest. Clelia Gasparini is an Associate Editor of Functional Ecology, but took no part in the peer review and decision-making processes for this paper.

DATA AVAILABILITY STATEMENT

Data deposited in the Dryad Digital Repository https://doi.org/10.5061/dryad.dv41ns22x (Breedveld et al., 2023).

ETHICS STATEMENT

All experiments were carried out with the approval of the University of Padova's Animal Ethics Committee and the national authorities; Organismo Preposto al Benessere degli Animali (OPBA; approval no: 313/2022-PR).

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REFERENCES

Andreasson, F., Nilsson, J.-Å., & Nord, A. (2020). Avian reproduction in a warming world. *Frontiers in Ecology and Evolution*, 8, 576331. https://doi.org/10.3389/fevo.2020.576331

Angeles-Malaspina, M., González-Cruz, J. E., & Ramírez-Beltran, N. (2018). Projections of heat waves events in the intra-americas region using multimodel ensemble. *Advances in Meteorology*, *2018*, 7827984. https://doi.org/10.1155/2018/7827984

Angilletta, M. J. (2009). *Thermal adaptation: A theoretical and empirical synthesis.* Oxford University Press.

Auer, S. K., Agreda, E., Chen, A. H., Irshad, M., & Solowey, J. (2021). Late-stage pregnancy reduces upper thermal tolerance in a live-bearing fish. *Journal of Thermal Biology*, *99*, 103022. https://doi.org/10.1016/j.jtherbio.2021.103022

Binet, M. T., & Doyle, C. J. (2013). Effect of near-future seawater temperature rises on sea urchin sperm longevity. *Marine and Freshwater Research*, *64*, 1–9. https://doi.org/10.1071/mf12121

Bisht, S., Faiq, M., Tolahunase, M., & Dada, R. (2017). Oxidative stress and male infertility. *Nature Reviews Urology*, *14*, 470–485. https://doi.org/10.1038/nrurol.2017.69

Boddicker, R. L., Seibert, J. T., Johnson, J. S., Pearce, S. C., Selsby, J. T., Gabler, N. K., Lucy, M. C., Safranski, T. J., Rhoads, R. P., Baumgard, L. H., & Ross, J. W. (2014). Gestational heat stress alters postnatal offspring body composition indices and metabolic parameters in pigs. *PLoS ONE*, *9*, e110859. https://doi.org/10.1371/journal.pone.0110859

Boschetto, C., Gasparini, C., & Pilastro, A. (2011). Sperm number and velocity affect sperm competition success in the guppy (*Poecilia reticulata*). *Behavioral Ecology and Sociobiology*, *65*, 813–821. https://doi.org/10.1007/s00265-010-1085-y

Boullis, A., Detrain, C., Francis, F., & Verheggen, F. J. (2016). Will climate change affect insect pheromonal communication? *Current Opinion in Insect Science*, *17*, 87–91. https://doi.org/10.1016/j.cois.2016.08.006

Bozynski, C. C., & Liley, N. R. (2003). The effect of female presence on spermiation, and of male sexual activity on 'ready' sperm in the male guppy. *Animal Behaviour*, *65*, 53–58. https://doi.org/10.1006/anbe.2002.2024

Breckels, R. D., & Neff, B. D. (2013). The effects of elevated temperature on the sexual traits, immunology and survivorship of a tropical ectotherm. *Journal of Experimental Biology*, *216*, 2658–2664. https://doi.org/10.1242/jeb.084962

Breedveld, M. C., Devigili, A., Borgheresi, O., & Gasparini, C. (2023). Data from: Reproducing in hot water: Experimental heatwaves deteriorate multiple reproductive traits in a freshwater ectotherm. *Dryad Digital Repository*, https://doi.org/10.5061/dryad.dv41ns22x

Brett, J. R. (1964). The respiratory metabolism and swimming performance of young sockeye salmon. *Journal of the Fisheries Board of Canada*, *21*, 1183–1226. https://doi.org/10.1139/f64-103

Buckley, L. B., & Huey, R. B. (2016). Temperature extremes: Geographic patterns, recent changes, and implications for organismal vulnerabilities. *Global Change Biology*, *22*, 3829–3842. https://doi.org/10.1111/gcb.13313

Candolin, U. (2019). Mate choice in a changing world. *Biological Reviews*, *94*, 1246–1260. https://doi.org/10.1111/brv.12501

Candolin, U., & Wong, B. B. (2012). *Behavioural responses to a changing world: Mechanisms and consequences.* Oxford University Press.

Cardozo, G., Devigili, A., Antonelli, P., & Pilastro, A. (2020). Female sperm storage mediates post-copulatory costs and benefits of ejaculate

anticipatory plasticity in the guppy. *Journal of Evolutionary Biology*, *33*, 1294–1305. https://doi.org/10.1111/jeb.13673

Cattelan, S., Evans, J. P., Pilastro, A., & Gasparini, C. (2016). The effect of sperm production and mate availability on patterns of alternative mating tactics in the guppy. *Animal Behaviour*, *112*, 105–110. https://doi.org/10.1016/j.anbehav.2015.11.024

Coleman, M. A., Minne, A. J. P., Vranken, S., & Wernberg, T. (2020). Genetic tropicalisation following a marine heatwave. *Scientific Reports*, *10*, 12726. https://doi.org/10.1038/s41598-020-69665-w

Crean, A. J., & Bonduriansky, R. (2014). What is a paternal effect? *Trends in Ecology & Evolution*, *29*, 554–559. https://doi.org/10.1016/j.tree.2014.07.009

Dado-Senn, B., Laporta, J., & Dahl, G. E. (2020). Carry over effects of late-gestational heat stress on dairy cattle progeny. *Theriogenology*, *154*, 17–23. https://doi.org/10.1016/j.theriogenology.2020.05.012

Dahlke, F. T., Wohlrab, S., Butzin, M., & Pörtner, H.-O. (2020). Thermal bottlenecks in the life cycle define climate vulnerability of fish. *Science*, *369*, 65–70. https://doi.org/10.1126/science.aaz3658

Danner, R. M., Coomes, C. M., & Derryberry, E. P. (2021). Simulated heat waves reduce cognitive and motor performance of an endotherm. *Ecology and Evolution*, *11*, 2261–2272. https://doi.org/10.1002/ece3.7194

Dayananda, B., Ibargüengoytía, N., Whiting, M. J., & Webb, J. K. (2017). Effects of pregnancy on body temperature and locomotor performance of velvet geckos. *Journal of Thermal Biology*, *65*, 64–68. https://doi.org/10.1016/j.jtherbio.2017.02.005

Devigili, A., Doldán-Martelli, V., & Pilastro, A. (2015). Exploring simultaneous allocation to mating effort, sperm production, and body growth in male guppies. *Behavioral Ecology*, *26*, 1203–1211. https://doi.org/10.1093/beheco/arv067

Devigili, A., Evans, J. P., Di Nisio, A., & Pilastro, A. (2015). Multivariate selection drives concordant patterns of pre- and postcopulatory sexual selection in a livebearing fish. *Nature Communications*, *6*, 8291. https://doi.org/10.1038/ncomms9291

Devigili, A., Kelley, J. L., Pilastro, A., & Evans, J. P. (2013). Expression of pre- and postcopulatory traits under different dietary conditions in guppies. *Behavioral Ecology*, *24*, 740–749. https://doi.org/10.1093/beheco/ars204

Dzikowski, R., Hulata, G., Karplus, I., & Harpaz, S. (2001). Effect of temperature and dietary L-carnitine supplementation on reproductive performance of female guppy (*Poecilia reticulata*). *Aquaculture*, *199*, 323–332. https://doi.org/10.1016/s0044-8486(01)00561-0

Easterling, D. R., Meehl, G. A., Parmesan, C., Changnon, S. A., Karl, T. R., & Mearns, L. O. (2000). Climate extremes: Observations, modeling, and impacts. *Science*, *289*, 2068–2074. https://doi.org/10.1126/science.289.5487.2068

Evans, J. P., Wilson, A. J., Pilastro, A., & Garcia-Gonzalez, F. (2019). Ejaculate-mediated paternal effects: Evidence, mechanisms and evolutionary implications. *Reproduction*, *157*, R109–R126. https://doi.org/10.1530/rep-18-0524

Fragueira, R., Helfenstein, F., Fischer, K., & Beaulieu, M. (2021). Birds of different morphs use slightly different strategies to achieve similar reproductive performance following heatwave exposure. *Journal of Animal Ecology*, *90*, 2594–2608. https://doi.org/10.1111/1365-2656.13564

Gage, M. J. G., Macfarlane, C. P., Yeates, S., Ward, R. G., Searle, J. B., & Parker, G. A. (2004). Spermatozoal traits and sperm competition in Atlantic salmon: Relative sperm velocity is the primary determinant of fertilization success. *Current Biology*, *14*, 44–47. https://doi.org/10.1016/j.cub.2003.12.028

García-Roa, R., Garcia-Gonzalez, F., Noble, D. W. A., & Carazo, P. (2020). Temperature as a modulator of sexual selection. *Biological Reviews*, *95*, 1607–1629. https://doi.org/10.1111/brv.12632

Gasparini, C., Dosselli, R., & Evans, J. P. (2017). Sperm storage by males causes changes in sperm phenotype and influences the

reproductive fitness of males and their sons. *Evolution Letters*, *1*, 16–25. https://doi.org/10.1002/evl3.2

Gasparini, C., & Evans, J. P. (2018). Female control over multiple matings increases the opportunity for postcopulatory sexual selection. *Proceedings of the Royal Society B: Biological Sciences*, *285*, 20181505. https://doi.org/10.1098/rspb.2018.1505

Gasparini, C., Kelley, J. L., & Evans, J. P. (2014). Male sperm storage compromises sperm motility in guppies. *Biology Letters*, *10*, 20140681. https://doi.org/10.1098/rsbl.2014.0681

Gasparini, C., Lu, C., Dingemanse, N. J., Tuni, C., & Sinclair, B. (2018). Paternal-effects in a terrestrial ectotherm are temperature dependent but no evidence for adaptive effects. *Functional Ecology*, *32*, 1011–1021. https://doi.org/10.1111/1365-2435.13022

Gasparini, C., Peretti, A. V., & Pilastro, A. (2009). Female presence influences sperm velocity in the guppy. *Biology Letters*, *5*, 792–794. https://doi.org/10.1098/rsbl.2009.0413

Gasparini, C., Simmons, L. W., Beveridge, M., & Evans, J. P. (2010). Sperm wwimming velocity predicts competitive fertilization success in the green swordtail *Xiphophorus helleri*. *PLoS ONE*, *5*, e12146. https://doi.org/10.1371/journal.pone.0012146

Gomendio, M., & Roldan, E. R. S. (2004). Implications of diversity in sperm size and function for sperm competition and fertility. *International Journal of Developmental Biology*, *52*, 439–447. https://doi.org/10.1387/ijdb.082595mg

Gómez-Llano, M., Scott, E., & Svensson, E. I. (2021). The importance of pre-and postcopulatory sexual selection promoting adaptation to increasing temperatures. *Current Zoology*, *67*, 321–327. https://doi.org/10.1093/cz/zoaa059

Gordon, S. P., Chen, Y. Y., Yamashita, K., Bejar, C., Wilshire, A., & Cheung, V. (2015). Sex-specific genetic differences in endurance swimming of Trinidadian guppies. *Ecology and Evolution*, *5*, 5318–5328. https://doi.org/10.1002/ece3.1789

Gurgel, C. F. D., Camacho, O., Minne, A. J. P., Wernberg, T., & Coleman, M. A. (2020). Marine heatwave drives cryptic loss of genetic diversity in underwater forests. *Current Biology*, *30*, 1199–1206.e1192. https://doi.org/10.1016/j.cub.2020.01.051

Hain, T. J. A., & Neff, B. D. (2007). Multiple paternity and kin recognition mechanisms in a guppy population. *Molecular Ecology*, *16*, 3938–3946. https://doi.org/10.1111/j.1365-294x.2007.03443.x

Hansen, P. J. (2009). Effects of heat stress on mammalian reproduction. *Philosophical Transactions of the Royal Society, B: Biological Sciences*, *364*, 3341–3350. https://doi.org/10.1098/rstb.2009.0131

Houde, A. (1997). *Sex, color, and mate choice in guppies*. Princeton University Press.

Huber, V., Adrian, R., & Gerten, D. (2010). A matter of timing: Heat wave impact on crustacean zooplankton. *Freshwater Biology*, *55*, 1769–1779. https://doi.org/10.1111/j.1365-2427.2010.02411.x

Hurley, L. L., McDiarmid, C. S., Friesen, C. R., Griffith, S. C., & Rowe, M. (2018). Experimental heatwaves negatively impact sperm quality in the zebra finch. *Proceedings of the Royal Society B: Biological Sciences*, *285*, 20172547. https://doi.org/10.1098/rspb.2017.2547

Iossa, G. (2019). Sex-specific differences in thermal fertility limits. *Trends in Ecology & Evolution*, *34*, 490–492. https://doi.org/10.1016/j.tree.2019.02.016

IPCC. (2021). Annex VII: Glossary. In J. B. R. Matthews, V. Möller, R. van Diemen, J. S. Fuglestvedt, V. Masson-Delmotte, C. Méndez, S. Semenov, & A. Reisinger (Eds.), *Climate change 2021: The physical science basis. Contribution of working group I to the sixth assessment report of the intergovernmental panel on climate change [Masson-Delmotte, V., P. Zhai, A. Pirani, S.L. Connors, C. Péan, S. Berger, N. Caud, Y. Chen, L. Goldfarb, M.I. Gomis, M. Huang, K. Leitzell, E. Lonnoy, J.B.R. Matthews, T.K. Maycock, T. Waterfield, O. Yelekçi, R. Yu, and B. Zhou (eds.)]* (pp. 2215–2256). Cambridge University Press.

Jentsch, A., Kreyling, J., & Beierkuhnlein, C. (2007). A new generation of climate-change experiments: Events, not trends. *Frontiers in Ecology

and the Environment*, *5*, 365–374. https://doi.org/10.1890/1540-9295(2007)5[365:ANGOCE]2.0.CO;2

Kokko, H., & López-Sepulcre, A. (2007). The ecogenetic link between demography and evolution: Can we bridge the gap between theory and data? *Ecology Letters*, *10*, 773–782. https://doi.org/10.1111/j.1461-0248.2007.01086.x

Kokko, H., & Rankin, D. J. (2006). Lonely hearts or sex in the city? Density-dependent effects in mating systems. *Philosophical Transactions of the Royal Society, B: Biological Sciences*, *361*, 319–334. https://doi.org/10.1098/rstb.2005.1784

Kotrschal, A., Fischer, B., & Taborsky, B. (2011). A noninvasive method to determine fat content in small fish based on swim bladder size estimation. *Journal of Experimental Zoology Part A: Ecological Genetics and Physiology*, *315A*, 408–415. https://doi.org/10.1002/jez.686

Kuehn, L., & McCormick, S. (2017). Heat exposure and maternal health in the face of climate change. *International Journal of Environmental Research and Public Health*, *14*, 853. https://doi.org/10.3390/ijerph14080853

Laskemoen, T., Kleven, O., Fossøy, F., Robertson, R. J., Rudolfsen, G., & Lifjeld, J. T. (2010). Sperm quantity and quality effects on fertilization success in a highly promiscuous passerine, the tree swallow *Tachycineta bicolor*. *Behavioral Ecology and Sociobiology*, *64*, 1473–1483. https://doi.org/10.1007/s00265-010-0962-8

Le Galliard, J.-F., Le Bris, M., & Clobert, J. (2003). Timing of locomotor impairment and shift in thermal preferences during gravidity in a viviparous lizard. *Functional Ecology*, *17*, 877–885. https://doi.org/10.1046/j.0269-8463.2003.00800.x

Leach, T. S., Buyanurt, B., & Hofmann, G. E. (2021). Exploring impacts of marine heatwaves: Paternal heat exposure diminishes fertilization success in the purple sea urchin (*Strongylocentrotus purpuratus*). *Marine Biology*, *168*, 103. https://doi.org/10.1007/s00227-021-03915-x

Ledger, M. E., & Milner, A. M. (2015). Extreme events in running waters. *Freshwater Biology*, *60*, 2455–2460. https://doi.org/10.1111/fwb.12673

Leith, N. T., Fowler-Finn, K. D., & Moore, M. P. (2022). Evolutionary interactions between thermal ecology and sexual selection. *Ecology Letters*, *25*, 1919–1936. https://doi.org/10.1111/ele.14072

Leith, N. T., Macchiano, A., Moore, M. P., & Fowler-Finn, K. D. (2021). Temperature impacts all behavioral interactions during insect and arachnid reproduction. *Current Opinion in Insect Science*, *45*, 106–114. https://doi.org/10.1016/j.cois.2021.03.005

Liley, N. R. (1966). Ethological isolating mechanisms in four sympatric species of poeciliid fishes. *Behaviour, Supplement, No. 14*, III–VII, 1–197. https://doi.org/10.2307/30039158

Magurran, A. E. (2005). *Evolutionary ecology: The Trinidadian guppy*. Oxford University Press.

Marco-Jiménez, F., García-Diego, F. J., & Vicente, J. S. (2017). Effect of gestational and lactational exposure to heat stress on performance in rabbits. *World Rabbit Science*, *25*, 17. https://doi.org/10.4995/wrs.2017.5728

Martinet, B., Zambra, E., Przybyla, K., Lecocq, T., Anselmo, A., Nonclercq, D., Rasmont, P., Michez, D., & Hennebert, E. (2021). Mating under climate change: Impact of simulated heatwaves on the reproduction of model pollinators. *Functional Ecology*, *35*, 739–752. https://doi.org/10.1111/1365-2435.13738

Mathies, T., & Andrews, R. M. (1997). Influence of pregnancy on the thermal biology of the lizard, *Sceloporus jarrovi*: Why do pregnant females exhibit low body temperatures? *Functional Ecology*, *11*, 498–507. https://doi.org/10.1046/j.1365-2435.1997.00119.x

Mehlis, M., & Bakker, T. C. M. (2014). The influence of ambient water temperature on sperm performance and fertilization success in three-spined sticklebacks (*Gasterosteus aculeatus*). *Evolutionary Ecology*, *28*, 655–667. https://doi.org/10.1007/s10682-014-9707-x

Moore, M. P., Hersch, K., Sricharoen, C., Lee, S., Reice, C., Rice, P., Kronick, S., Medley, K. A., & Fowler-Finn, K. D. (2021). Sex-specific ornament

evolution is a consistent feature of climatic adaptation across space and time in dragonflies. *Proceedings of the National Academy of Sciences of the United States of America*, 118, e2101458118. https://doi.org/10.1073/pnas.2101458118

Moritz, C., & Agudo, R. (2013). The future of species under climate change: Resilience or decline? *Science*, 341, 504–508. https://doi.org/10.1126/science.1237190

Mouthon, J., & Daufresne, M. (2006). Effects of the 2003 heatwave and climatic warming on mollusc communities of the Saône: A large lowland river and of its two main tributaries (France). *Global Change Biology*, 12, 441–449. https://doi.org/10.1111/j.1365-2486.2006.01095.x

Nguyen, T. M., Bressac, C., & Chevrier, C. (2013). Heat stress affects male reproduction in a parasitoid wasp. *Journal of Insect Physiology*, 59, 248–254. https://doi.org/10.1016/j.jinsphys.2012.12.001

Nicoletto, P. F. (1991). The relationship between male ornamentation and swimming performance in the guppy, *Poecilia reticulata*. *Behavioral Ecology and Sociobiology*, 28, 365–370. https://doi.org/10.1007/bf00164386

Nóbrega, R. H., Batlouni, S. R., & França, L. R. (2009). An overview of functional and stereological evaluation of spermatogenesis and germ cell transplantation in fish. *Fish Physiology and Biochemistry*, 35, 197–206. https://doi.org/10.1007/s10695-008-9252-z

Oppel, S., Hilton, G., Ratcliffe, N., Fenton, C., Daley, J., Gray, G., Vickery, J., & Gibbons, D. (2014). Assessing population viability while accounting for demographic and environmental uncertainty. *Ecology*, 95, 1809–1818. https://doi.org/10.1890/13-0733.1

Paaijmans, K. P., Heinig, R. L., Seliga, R. A., Blanford, J. I., Blanford, S., Murdock, C. C., & Thomas, M. B. (2013). Temperature variation makes ectotherms more sensitive to climate change. *Global Change Biology*, 19, 2373–2380. https://doi.org/10.1111/gcb.12240

Parker, G. A. (1979). Sexual selection and sexual conflict. In M. S. Blum & N. A. Blum (Eds.), *Sexual selection and reproductive competition in insects* (pp. 123–166). Academic Press.

Parker, G. A. (1998). Sperm competition and the evolution of ejaculates: Towards a theory base. In T. R. Birkhead & A. P. Møller (Eds.), *Sperm competition and sexual selection* (pp. 3–49). Academic Press.

Parratt, S. R., Walsh, B. S., Metelmann, S., White, N., Manser, A., Bretman, A. J., Hoffmann, A. A., Snook, R. R., & Price, T. A. R. (2021). Temperatures that sterilize males better match global species distributions than lethal temperatures. *Nature Climate Change*, 11, 481–484. https://doi.org/10.1038/s41558-021-01047-0

Paxton, C. W., Baria, M. V. B., Weis, V. M., & Harii, S. (2016). Effect of elevated temperature on fecundity and reproductive timing in the coral *Acropora digitifera*. *Zygote*, 24, 511–516. https://doi.org/10.1017/s0967199415000477

Pérez-Crespo, M., Pintado, B., & Gutiérrez-Adán, A. (2008). Scrotal heat stress effects on sperm viability, sperm DNA integrity, and the offspring sex ratio in mice. *Molecular Reproduction and Development*, 75, 40–47. https://doi.org/10.1002/mrd.20759

Pilastro, A., & Bisazza, A. (1999). Insemination efficiency of two alternative male mating tactics in the guppy *Poecilia reticulata*. *Proceedings of the Royal Society B: Biological Sciences*, 266, 1887–1891. https://doi.org/10.1098/rspb.1999.0862

Pilastro, A., Mandelli, M., Gasparini, C., Dadda, M., & Bisazza, A. (2007). Copulation duration, insemination efficiency and male attractiveness in guppies. *Animal Behaviour*, 74, 321–328. https://doi.org/10.1016/j.anbehav.2006.09.016

Pizzari, T. (2009). Sexual selection: Sperm in the fast lane. *Current Biology*, 19, R292–R294. https://doi.org/10.1016/j.cub.2009.02.026

Portner, H. O., & Knust, R. (2007). Climate change affects marine fishes through the oxygen limitation of thermal tolerance. *Science*, 315, 95–97. https://doi.org/10.1126/science.1135471

Postingel Quirino, P., Da Silva Rodrigues, M., Da Silva Cabral, E. M., De Siqueira-Silva, D. H., Mori, R. H., Butzge, A. J., Nóbrega, R. H., Ninhaus-Silveira, A., & Veríssimo-Silveira, R. (2021). The influence of increased water temperature on the duration of spermatogenesis in a neotropical fish, *Astyanax altiparanae* (Characiformes, Characidae). *Fish Physiology and Biochemistry*, 47, 747–755. https://doi.org/10.1007/s10695-020-00869-7

Reeve, A. J., Ojanguren, A. F., Deacon, A. E., Shimadzu, H., Ramnarine, I. W., & Magurran, A. E. (2014). Interplay of temperature and light influences wild guppy (*Poecilia reticulata*) daily reproductive activity. *Biological Journal of the Linnean Society*, 111, 511–520. https://doi.org/10.1111/bij.12217

Reinhardt, K., Dobler, R., & Abbott, J. (2015). An ecology of sperm: Sperm diversification by natural selection. *Annual Review of Ecology, Evolution, and Systematics*, 46, 435–459. https://doi.org/10.1146/annurev-ecolsys-120213-091611

Rosenthal, M. F., & Elias, D. O. (2019). Nonlinear changes in selection on a mating display across a continuous thermal gradient. *Proceedings of the Royal Society B: Biological Sciences*, 286, 20191450. https://doi.org/10.1098/rspb.2019.1450

Roux, O., Le Lann, C., Van Alphen, J. J. M., & Van Baaren, J. (2010). How does heat shock affect the life history traits of adults and progeny of the aphid parasitoid *Aphidius avenae* (Hymenoptera: Aphidiidae)? *Bulletin of Entomological Research*, 100, 543–549. https://doi.org/10.1017/s0007485309990575

Rowe, L., Arnqvist, G., Sih, A., & Krupa, J. J. (1994). Sexual conflict and the evolutionary ecology of mating patterns: Water striders as a model system. *Trends in Ecology & Evolution*, 9, 289–293. https://doi.org/10.1016/0169-5347(94)90032-9

Sales, K., Vasudeva, R., Dickinson, M. E., Godwin, J. L., Lumley, A. J., Michalczyk, Ł., Hebberecht, L., Thomas, P., Franco, A., & Gage, M. J. G. (2018). Experimental heatwaves compromise sperm function and cause transgenerational damage in a model insect. *Nature Communications*, 9, 4771. https://doi.org/10.1038/s41467-018-07273-z

Sales, K., Vasudeva, R., & Gage, M. J. G. (2021). Fertility and mortality impacts of thermal stress from experimental heatwaves on different life stages and their recovery in a model insect. *Royal Society Open Science*, 8, 201717. https://doi.org/10.1098/rsos.201717

Shama, L. N., Kubow, K. B., Jokela, J., & Robinson, C. T. (2011). Bottlenecks drive temporal and spatial genetic changes in alpine caddisfly metapopulations. *BMC Evolutionary Biology*, 11, 1–11. https://doi.org/10.1186/1471-2148-11-278

Sheldon, K. S., & Dillon, M. E. (2016). Beyond the mean: Biological impacts of cryptic temperature change. *Integrative and Comparative Biology*, 56, 110–119. https://doi.org/10.1093/icb/icw005

Siegle, M. R., Taylor, E. B., & O'Connor, M. I. (2022). Heat wave intensity drives sublethal reproductive costs in a tidepool copepod. *Integrative Organismal Biology*, 4, 1–10. https://doi.org/10.1093/iob/obac005

Snook, R. R. (2005). Sperm in competition: Not playing by the numbers. *Trends in Ecology & Evolution*, 20, 46–53. https://doi.org/10.1016/j.tree.2004.10.011

Sokolova, I. M., Frederich, M., Bagwe, R., Lannig, G., & Sukhotin, A. A. (2012). Energy homeostasis as an integrative tool for assessing limits of environmental stress tolerance in aquatic invertebrates. *Marine Environmental Research*, 79, 1–15. https://doi.org/10.1016/j.marenvres.2012.04.003

Somero, G. N. (2002). Thermal physiology and vertical zonation of intertidal animals: Optima, limits, and costs of living. *Integrative and Comparative Biology*, 42, 780–789. https://doi.org/10.1093/icb/42.4.780

Stephenson, T. S., Vincent, L. A., Allen, T., Van Meerbeeck, C. J., McLean, N., Peterson, T. C., Taylor, M. A., Aaron-Morrison, A. P., Auguste, T., Bernard, D., Boekhoudt, J. R. I., Blenman, R. C., Braithwaite, G. C., Brown, G., Butler, M., Cumberbatch, C. J. M., Etienne-Leblanc, S., Lake, D. E., Martin, D. E., … Trotman, A. R. (2014). Changes in extreme temperature and precipitation in the Caribbean region, 1961–2010. *International Journal of Climatology*, 34, 2957–2971. https://doi.org/10.1002/joc.3889

Stillman, J. H. (2019). Heat waves, the new normal: Summertime temperature extremes will impact animals, ecosystems, and human communities. *Physiology*, *34*, 86–100. https://doi.org/10.1152/physiol.00040.2018

Strydom, S., Murray, K., Wilson, S., Huntley, B., Rule, M., Heithaus, M., Bessey, C., Kendrick, G. A., Burkholder, D., Fraser, M. W., & Zdunic, K. (2020). Too hot to handle: Unprecedented seagrass death driven by marine heatwave in a world heritage area. *Global Change Biology*, *26*, 3525–3538. https://doi.org/10.1111/gcb.15065

Sutter, A., Travers, L. M., Oku, K., Delaney, K. L., Store, S. J., Price, T. A. R., & Wedell, N. (2019). Flexible polyandry in female flies is an adaptive response to infertile males. *Behavioral Ecology*, *30*, 1715–1724. https://doi.org/10.1093/beheco/arz140

Truebano, M., Fenner, P., Tills, O., Rundle, S. D., & Rezende, E. L. (2018). Thermal strategies vary with life history stage. *Journal of Experimental Biology*, *221*, jeb171629. https://doi.org/10.1242/jeb.171629

Ummenhofer, C. C., & Meehl, G. A. (2017). Extreme weather and climate events with ecological relevance: A review. *Philosophical Transactions of the Royal Society, B: Biological Sciences*, *372*, 20160135. https://doi.org/10.1098/rstb.2016.0135

van de Pol, M., Jenouvrier, S., Cornelissen, J. H. C., & Visser, M. E. (2017). Behavioural, ecological and evolutionary responses to extreme climatic events: Challenges and directions. *Philosophical Transactions of the Royal Society, B: Biological Sciences*, *372*, 20160134. https://doi.org/10.1098/rstb.2016.0134

Van Lieshout, E., Tomkins, J. L., & Simmons, L. W. (2013). Heat stress but not inbreeding affects offensive sperm competitiveness in *Callosobruchus maculatus*. *Ecology and Evolution*, *3*, 2859–2866. https://doi.org/10.1002/ece3.667

Vasudeva, R., Deeming, D. C., & Eady, P. E. (2014). Developmental temperature affects the expression of ejaculatory traits and the outcome of sperm competition in *Callosobruchus maculatus*. *Journal of Evolutionary Biology*, *27*, 1811–1818. https://doi.org/10.1111/jeb.12431

Vasudeva, R., Dickinson, M., Sutter, A., Powell, S., Sales, K., & Gage, M. J. G. (2021). Facultative polyandry protects females from compromised male fertility caused by heatwave conditions. *Animal Behaviour*, *178*, 37–48. https://doi.org/10.1016/j.anbehav.2021.05.016

Walsh, B. S., Mannion, N. L. M., Price, T. A. R., & Parratt, S. R. (2021). Sex-specific sterility caused by extreme temperatures is likely to create cryptic changes to the operational sex ratio in *Drosophila virilis*. *Current Zoology*, *67*, 341–343. https://doi.org/10.1093/cz/zoaa067

Walsh, B. S., Parratt, S. R., Hoffmann, A. A., Atkinson, D., Snook, R. R., Bretman, A., & Price, T. A. R. (2019). The impact of climate change on fertility. *Trends in Ecology & Evolution*, *34*, 249–259. https://doi.org/10.1016/j.tree.2018.12.002

Weir, L. K., James, W., & Hutchings, J. A. (2011). The influence of operational sex ratio on the intensity of competition for mates. *The American Naturalist*, *177*, 167–176. https://doi.org/10.1086/657918

Wild, S., Krützen, M., Rankin, R. W., Hoppitt, W. J. E., Gerber, L., & Allen, S. J. (2019). Long-term decline in survival and reproduction of dolphins following a marine heatwave. *Current Biology*, *29*, R239–R240. https://doi.org/10.1016/j.cub.2019.02.047

Woodward, G., Bonada, N., Brown, L. E., Death, R. G., Durance, I., Gray, C., Hladyz, S., Ledger, M. E., Milner, A. M., Ormerod, S. J., Thompson, R. M., & Pawar, S. (2016). The effects of climatic fluctuations and extreme events on running water ecosystems. *Philosophical Transactions of the Royal Society, B: Biological Sciences*, *371*, 20150274. https://doi.org/10.1098/rstb.2015.0274

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