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# Combined effects of rearing and testing temperatures on sperm traits

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## Abstract

Temperature experienced during early development can affect a range of adult life-history traits. Animals often show seemingly adaptive developmental plasticity—with animals reared at certain temperatures performing better as adults at those temperatures. The extent to which this type of adaptive response occurs in gonadal tissue that affects sperm traits is, however, poorly studied. We initially reared male mosquito fish (*Gambusia holbrooki*) at either 18°C or 30°C, and then measured their sperm reserves as adults. We also looked at the velocity of their sperm, at both the matched and mismatched temperatures. Although males reared at 30°C were larger than those initially reared at 18°C, there was no detectable effect of rearing temperature on absolute sperm number. Sperm swam faster at 30°C than 18°C regardless of the male's rearing temperature. Therefore, we found no evidence of adaptive developmental plasticity. Rearing temperature did, however, significantly influence the relationship between male body size and sperm velocity. Larger males had faster sperm when reared at the warmer temperature and slower sperm when reared at the cooler temperature. This suggests that rearing temperature could alter the relationship between pre-copulatory sexual selection and post-copulatory sexual selection as male size affects mating success. Finally, there was a positive correlation between velocities at the two test temperatures, suggesting that temperature experienced during sperm competition is unlikely to affect a male's relative fertilization success.

KEYWORDS

*Gambusia holbrooki*, sperm number, sperm velocity, temperature

## 1 | INTRODUCTION

The environmental conditions that an individual experiences during its development and early life can have a lasting effect on its adult traits and performance. Abiotic factors, such as nutrition or humidity, and biotic factors, such as competition or predation risk, experienced during key periods of development are often key in determining the development of life-history traits (e.g. Metcalfe & Monaghan, 2001; Relyea, 2001). How individuals respond to their environment during development is evolutionarily important because it can influence how they interact with their environment as adults, hence their relative fitness. In general, most responses to early life environments are either adaptive or nonadaptive. Adaptive responses increase an individual's fitness because they

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perform better as adults (reviewed in Ghalambor et al., 2007). This can occur when the adult environment matches that experienced during development (or, in rare cases, when the adult and developmental environments are predictably in opposition; Mainwaring et al., 2017; Reed et al., 2010; Sgrò et al., 2016; Stockley & Seal, 2001; Vitasse et al., 2010). For example, male dung flies, *Scatophaga stercolaria*, raised at high larval densities mature as small males with relatively larger testes, which seems to increase their daily reproductive effort in response to an anticipated short lifespan (Stockley & Seal, 2001). Nonadaptive responses, on the other hand, are expected when individuals develop in poor or stressful environments and are unable to fully develop fitness-enhancing traits (reviewed in Ghalambor et al., 2007). For example, seedlings in an environment with low moisture and lacking essential minerals do not grow to full adult height and produce fewer seeds (van Keunen & Fischer, 2005). Here, regardless of whether the developmental and adult environments are matched, individuals reared in certain environments (i.e. stressful ones) consistently perform worse than individuals developing in other environments.

Due to its profound effects on physiological and biochemical processes, temperature is a key abiotic factor shaping the development of ectotherms. Rearing temperatures exert changes in life-history traits such as development time, size at maturity and lifespan (Ciota et al., 2014; Fox & Czesak, 2000; Lee et al., 2003), often with major consequences for individual fitness and population viability. However, despite the known effects of temperature on life-history traits, how it affects the development of reproductive traits under sexual selection has received little attention. Even so, there is some evidence that rearing temperature affects the development of male secondary sexual characters that females use to choose males (e.g. Breckels & Neff, 2013; Brian et al., 2011). For example, in three-spined stickleback, *Gasterosteus aculeatus*, a natural reduction in nuptial colouration during the summer is accelerated by increased temperatures (Borg, 1982). Given the role of sexually selected traits in determining male reproductive success in many species, exploring how developmental temperature influences the expression of these traits will increase our understanding of how species respond to climate change.

The effect of developmental environment on sexually selected traits is not limited to those traits under precopulatory selection. The developmental environment might also affect traits under post-copulatory sexual selection. Developmental temperature is, for example, known to affect traits related to sperm competitiveness, such as sperm number (Zeh et al., 2012), size (Blanckenhorn & Hellriegel, 2002), velocity (Breckels & Neff, 2014) and mobility (Dadras et al., 2017) and, as such, influence male fertilization success. When determining the effect of developmental temperature on male fitness, it is, however, important to consider the environment in which males or their sperm compete. This is particularly relevant in species with high temporal variation in temperature relative to their adult lifespan. In such species, competing individuals often experience different developmental temperatures and compete at a range of temperatures. Therefore, testing for an interaction between the developmental and adult temperatures might be integral for understanding how environmental change affects the strength of sexual selection, the congruence between selection on different traits (e.g. those under pre- and post-copulatory selection), phenotypic variation in traits, and how traits are phenotypically or genetically correlated.

To explore how temperature affects sperm traits in the eastern mosquito fish, *Gambusia holbrooki*, we manipulated both the rearing temperature of males and the temperature at which sperm velocity was measured. In addition to quantifying the main effects of rearing and testing temperature, our factorial experimental design allowed us to test whether the sperm of males reared at one temperature were faster at the same temperature compared with that of males that developed at a different temperature (i.e. an adaptive response), or whether a cooler (or hotter) developmental temperature is simply associated with a general decline in sperm velocity. It is important to note that in mosquito fish spermatogenesis does not begin till later in development (e.g. around 90 days at 25°C, Koya et al., 2003), and thus, our experiment does not test adaptive plasticity of the sperm themselves. Mosquito fish are a suitable study species to explore the effects of developmental temperature as (a) the temperature during development can vary greatly both spatially (within and between waterways) and temporally (autumn versus spring) (Kahn et al., 2013); (b) temperature variation across seasons is predictable; (c) when there is spatial variation associated with depth or shade, males can choose their habitat to modify the temperature they experience (Pyke, 2005); (d) there are high levels of sperm competition, as males spend most of their time chasing females (Bisazza & Marin, 1991) and make frequent copulation attempts (up to one attempt/minute, Wilson, 2005); females mate multiply and store sperm from several males for long periods (Pyke, 2005). This suggests that, as has shown in a related poeciliid species (Boschetto et al., 2011), sperm number and velocity are likely to play a key role in sperm competition and determining male reproductive success. Further indirect evidence that sperm quantity might be important for sperm competition in mosquito fish is the fact that males exposed to competitors increase their general investment in sperm count (Evans et al., 2003; but lower their velocity, see Spagopoulou et al., 2020).

There is considerable research looking at targets of precopulatory sexual selection in *G. holbrooki*. Several studies report that females prefer to associate with larger males (Aich et al., 2020; Bisazza & Marin, 1991; Hughes, 1985) which implies a large male mating advantage. On the other hand, some studies report that smaller males have a greater insemination success per mating attempt (Pilastro et al., 1997), whereas others find that larger males are more effective (Booksmythe et al., 2013). Furthermore, larger males tend to produce more sperm (e.g. Vega-Trejo et al., 2019, but see e.g. Locatello et al., 2008) which could further enhance their reproductive success. Studies looking directly at male paternity under different environmental and social conditions have shown both an advantage accruing to smaller males (Head et al., 2017) and no effect of male size on their share of paternity (Booksmythe et al., 2016; Vega-Trejo et al., 2017). If the developmental environment affects

the relationship between body size and sperm traits, it could thus influence the outcome of sexual selection.

## 2 | METHODS

Focal male mosquito fish were the laboratory-reared offspring of wild-caught fish from Canberra (Australia). Parents were paired for 1 week to allow mating, and females were then isolated in 1-L aquaria and checked twice daily until they gave birth. Fry were separated into individual 1-L aquaria and placed in control temperature rooms set at either 18°C (cold) or 30°C (hot) and a 14:10 L:D photoperiod. Fish were fed *Artemia sp*. nauplii twice a day for the duration of the experiment.

We began to monitor fish for signs of sexual maturity from 28 days after birth. Males were determined to be sexually mature when their gonopodium was translucent, with a spine visible at the tip (Zulian et al., 1993). At 3 months of age, no fish reared at 18°C had matured, nor did they show any signs of approaching maturation. Because we were interested in the effects of temperature experienced during rearing, the temperature for these males was elevated to 30°C at 112 days of age, to induce maturation (which took 39 ± 3 days (mean ± *SE*) after being moved). It is important to note that we are interested in the effects of developmental environment on adult traits and not adaptive plasticity of sperm themselves. As such, increasing the temperature to induce maturation does not detract from our ability to address our core aim of investigating the effect of temperature differences during development. In addition, by homogenizing the temperature of all individuals before spermatogenesis we rule out any potential plastic responses during the production of the sperm. Similar changes in temperature happen in the wild for individuals that are born in winter and reach maturity during the spring when the temperature increases (Kahn et al., 2013). Upon maturation, we anaesthetized males in an ice slurry, lay them on their side next to a scale and took a digital photograph to measure their standard length using *ImageJ* (Abràmoff et al., 2004).

We analysed the sperm count and sperm velocity of males 22–66 days after sexual maturity. This limited time frame was chosen to reduce variation in sperm velocity associated with time since maturity that has been found previously for a broader age range (Vega-Trejo et al., 2016). Logistical constraints prevented us from using a narrower time frame, but we ensured that males from both the 18°C and 30°C treatments were equally represented across the time frame (mean ± SE: 18°C = 45.92 ± 9.69, 30°C = 47.72 ± 10.97). To collect sperm, we followed the methods of Vega-Trejo et al.(2016). Briefly, males were anaesthetized in ice water, patted dry and placed on a glass slide coated with 1% polyvinyl alcohol, under a dissecting microscope. We swung the gonopodium forward and placed 100 μl of saline solution at its base. We then gently applied pressure to the male's abdomen so that his full sperm reserve was released into the saline solution. We took 4 samples of 3 sperm bundles for velocity measures. These samples were each placed in 2 μl of extender medium. We then transferred the remainder of the ejaculate to an Eppendorf tube with 100–900 μl of extender medium (depending on the amount of ejaculate stripped) for sperm counts.

To estimate the size of a male's sperm reserve, we thoroughly mixed the sample and then placed 3 μl on a 20-micron capillary slide (Leja) and counted the sperm using a CEROS Sperm Tracker (Hamilton Thorne Research, Beverly, MA, USA) under 100× magnification. We counted five subsamples per male (repeatability: $r \pm SE = 0.932 \pm 0.017$, $p < .001$) and used each count to estimate the total amount of sperm (excluding that in the bundles used for velocity measures) that each male had in their ejaculate, calculated as follows:

$$\frac{\text{Total sample volume}}{100} \times \text{count} \times \frac{0.1}{\text{field of view volume}}.$$

Sperm velocity was measured at two temperatures: 18°C (cold) and 30°C (hot) to match the rearing temperatures. We analysed two samples per ejaculate at each temperature. The order of the temperatures was randomized across males. To measure sperm velocity, we placed 4 μl of activator medium (150 mM KCl, 2 mg/ml bovine serum albumin) in the centre of a cell of a 12-cell multitest slide (MP Biomedicals, Aurora, OH, USA), added 2 μl of the sperm sample and covered this with a coverslip. The slide was then placed on a temperature-controlled microscope stage (Linkam) set at either 18°C or 30°C. We analysed sperm velocity, using CEROS sperm tracker software (Hamilton Thorne), within 30 s of activation. We measured 28.63 ± 2.52 *SE* sperm tracks per sperm sample. We recorded three standard measures of sperm velocity: (1) average path velocity (VAP), which estimates the average velocity of sperm cells over a smoothed cell path; (2) curvilinear velocity (VCL), the actual velocity along the trajectory; and (3) straight-line velocity (VSL). Here, we only analyse VCL, because these measures are all highly correlated (VCL-VAP: $r = 0.91$, VCL-VSL: $r = 0.89$, VAP-VSL: 0.99, $N = 131$) and previous studies have found VCL is most closely related to paternity under sperm competition in other poeciliid fishes (Boschetto et al., 2011).

### 2.1 | Data analysis

We obtained sperm number measures for 41 males (*N*: 30°C = 15; 18°C = 26) and sperm velocity measures for 40 males (*N*: 30°C = 15; 18°C = 25). There was one male for whom we were unable to obtain sperm velocity measures.

First, to test for a difference in body size between males reared at 18°C and 30°C, we ran a linear model with male size as the response variable and rearing temperature as a fixed factor. Then, to determine whether rearing temperature influenced maximal sperm reserves we ran a linear mixed model with the number of sperm stripped from a male as the response variable. Rearing temperature and male body size (standardized; mean = 0, *SD* = 1) were fixed factors, and we included male ID as a random effect because we had 5 subsamples per male. We initially included the day of testing as a random effect,

but it explained no variation and was excluded from the final model. We then ran two models: the first included all two-way interactions between the fixed factors, because we were interested in whether rearing temperature changed the relationship between sperm number and male size; and the second contained only main effects, as main effects cannot be interpreted with interactions in the model (Engqvist, 2005). We ran models with and without male size as a covariate, since male size is often correlated with sperm number, and mean male size differed between the rearing temperatures (see Results). The models gave similar results, and we only present those from the model including male size (see Appendix S1: Table S1 for the other model).

To test whether rearing or testing temperature influenced sperm velocity and, more interestingly, whether sperm swam relatively faster at the temperature in which the male was reared, we analysed sperm velocity (VCL) using a linear mixed model. Rearing temperature, testing temperature and standardized male size were included as fixed factors. The day the sample was tested was included as a random effect. We included a random intercept and random slopes (with relation to sperm testing temperature) for male ID, so that we could determine whether some males had consistently faster sperm than others (random intercept) and whether the sperm of all males responded to testing temperature in the same way (random slopes). To test whether adding random slopes improved the model fit, we conducted a log-likelihood ratio test comparing models with and without the random slopes term. Once we established whether or not to include a random slope term, we ran two models to test how the fixed effects influenced sperm velocity. The first model included all two-way interactions between the fixed factors, and the second contained only main effects.

Initially, our models included 'days since maturity', as previous studies have shown that it can influence both sperm number and velocity (e.g. Vega-Trejo et al., 2016). However, we excluded this term from our final models as it had no effect. Including it did not alter the interpretation of the effects of the other terms in the models (see Appendix S1: Tables S2 and S3). All models had residuals that met the assumptions of normality and homoscedasticity. P-values were from type III Wald chi-square tests.

To further explore how the sperm of each male responded to the testing temperature, we calculated the correlation ($r$) between mean sperm velocities at 18°C and 30°C. We also tested whether the variation in sperm velocity differed depending on sperm testing temperature using Levene's test for homogeneity of variances. All analyses were conducted in R (version 3.5.1).

## 3 | RESULTS

### 3.1 | Male size

Males reared at 30°C matured at a significantly larger size than those reared at 18°C ($F_{(1,37)} = 8.040$, $p = .007$, cold: mean ± $SE$ = 21.041 ± 0.319; hot: mean ± $SE$ = 22.727 ± 0.510).

### 3.2 | Sperm number

The number of sperm in a male's sperm reserves was independent of the temperature experienced during development, or his body size (Table 1).

**TABLE 1** The effects of rearing temperature and male body size on male sperm number

| **Full model (Including interactions)** | **Estimate** | ***SE*** | $\chi^2$ | ***p*** |
|---|---|---|---|---|
| Fixed factors | | | | |
| (intercept) | 10,863,936 | 1,450,074 | **56.130** | **<.001** |
| Rearing temperature (30°C) | −1328373 | 2,485,278 | | |
| Male size | −1248258 | 3,234,903 | | |
| Rearing temp*Male Size | −1671712 | 4,709,399 | 0.126 | .723 |
| Random factors | Variance | *SD* | | |
| Male ID | 4.635e + 13 | 6,807,876 | | |
| Residual | 5.021e + 12 | 2,240,747 | | |
| **Reduced model (main effects only)** | **Estimate** | ***SE*** | $\chi^2$ | ***p*** |
| Fixed factors | | | | |
| (intercept) | 10,735,072 | 1,387,397 | **59.870** | **<.001** |
| Rearing temperature (30°C) | −1447404 | 2,434,708 | 0.353 | .552 |
| Male size | −2034039 | 2,324,629 | 0.766 | .382 |
| Random factors | Variance | *SD* | | |
| Male ID | 4.526e + 13 | 6,727,418 | | |
| Residual | 5.021e + 12 | 2,240,751 | | |

Bold text indicates signifcant effects.

**TABLE 2** The effects of rearing temperature, testing temperature and male body size on sperm velocity (VCL)

| Full model (including interactions) | Estimate | *SE* | $\chi^2$ | *p* |
|---|---|---|---|---|
| Fixed factors | | | | |
| (intercept) | 50.926 | 2.166 | **552.918** | **<.001** |
| Rearing temperature (30°C) | −5.172 | 3.684 | | |
| Testing temperature (30°C) | 14.849 | 3.499 | | |
| Male size | −8.853 | 4.589 | | |
| Rearing temp*Testing temp | −1.120 | 6.049 | 0.034 | .853 |
| Rearing temp*Male Size | 14.989 | 6.823 | **4.826** | **.028** |
| Testing temp*Male size | 7.913 | 5.887 | 1.807 | .179 |
| Random factors | Variance | *SD* | | |
| Male ID (intercept) | 8.65 | 2.942 | | |
| Testing temperature\|male ID | 89.92 | 9.483 | | |
| Day | 0.000 | 0.000 | | |
| Residual | 155.42 | 12.467 | | |
| **Reduced model (main effects only)** | **Estimate** | ***SE*** | $\chi^2$ | ***p*** |
| Fixed factors | | | | |
| intercept) | 51.810 | 2.404 | **464.333** | **<.001** |
| Rearing temperature (30°C) | −3.567 | 3.842 | 0.862 | .353 |
| Testing temperature (30°C) | 14.559 | 2.663 | **29.900** | **<.001** |
| Male size | −0.366 | 3.645 | 0.010 | .920 |
| Random factors | Variance | *SD* | | |
| Male ID | 9.38 | 3.063 | | |
| Testing temperature\|male ID | 88.07 | 9.385 | | |
| Day | 10.58 | 3.252 | | |
| Residual | 155.84 | 12.484 | | |

Bold text indicates signifcant effects.

## 3.3 | Sperm velocity

The random slope term for male ID within testing temperature accounted for around a third of the variance in our full model (Table 2). Removing the random slope term from the model significantly reduced the model fit ($x^2$ = 10.667, $p$ = .0048), suggesting that the sperm of individual males differ in how testing temperature affected their velocity. Even so, the swimming velocity of a male's sperm at 18°C and 30°C was significantly positively correlated ($r$ = 0.41, $t$ = 2.70, $df$ = 36, $p$ = .010; Figure 1), and the variance in males' sperm swimming velocity was significantly greater at 30°C than at 18°C ($F_{(1,75)}$ = 12.631, $p$ < .001). Thus, a higher testing temperature increased variation in sperm velocity among males, but did not substantively change the rank performance of males (Figure 2).

Sperm swam significantly faster at 30°C than at 18°C ($p$ < .001; Table 2, Figure 3), but rearing temperature did not influence sperm velocity at different testing temperatures (interaction: $p$ = .853; Table 2). Rearing temperature did, however, significantly influence the relationship between body size and sperm velocity (rearing temp*male size: $p$ = .028; Table 2). When reared at 30°C, larger

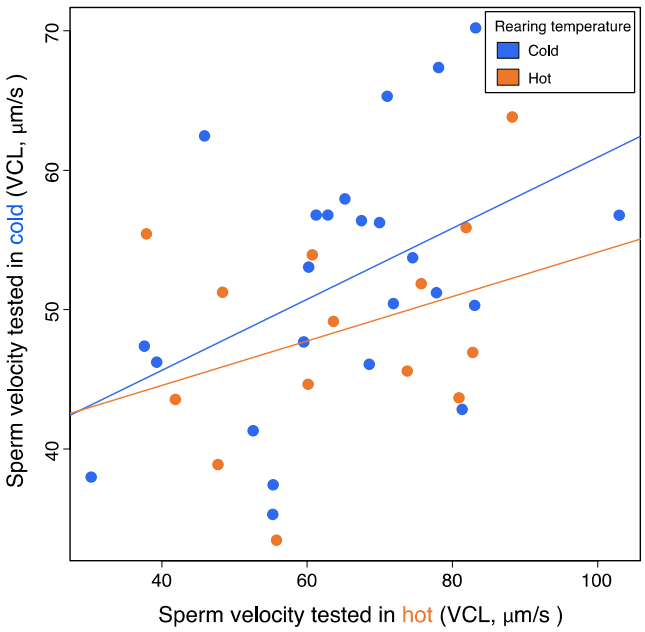


**FIGURE 1** The relationship between male sperm swimming velocities in both hot and cold testing temperatures

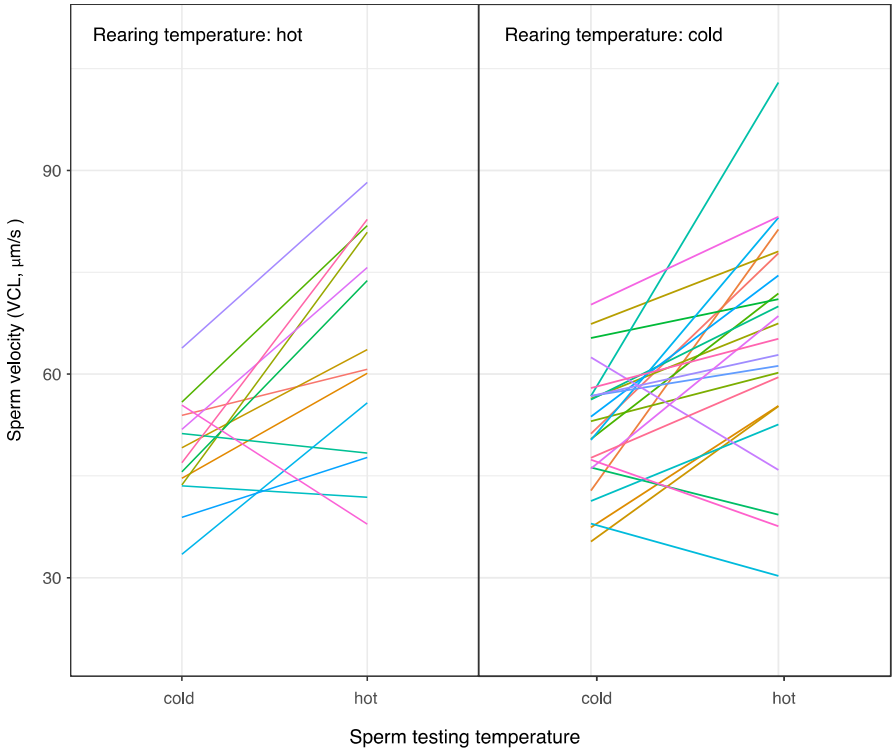


**FIGURE 2** Reaction norm of sperm swimming speed of each male in different testing temperatures. Coloured lines link the sperm velocities of individual males in the hot and cold testing temperatures

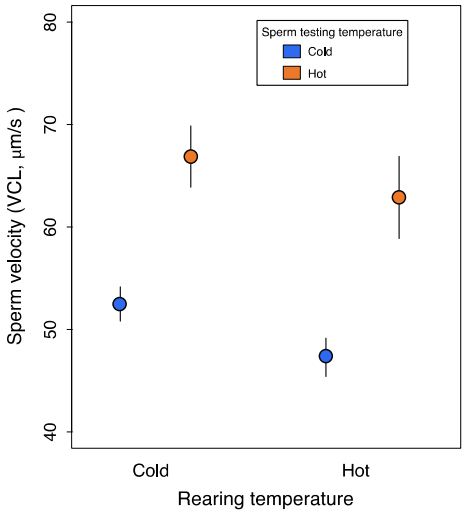


**FIGURE 3** Effects of rearing temperature and testing temperature on sperm swimming velocity (VCL, mean ± *SE*)

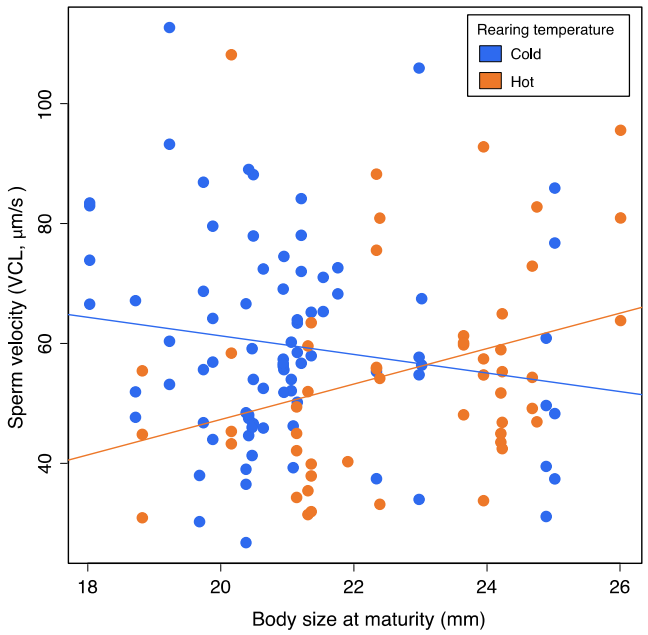


**FIGURE 4** Relationship between male size at maturity and sperm swimming velocity for males reared in hot and cold temperatures

males tended to have faster-swimming sperm ($x^2 = 2.930$, $p = .087$), but when reared at 18°C larger males tended to have slower-swimming sperm ($x^2 = 2.621$, $p = .105$) (P-values are from analyses split by rearing temperature; Figure 4).

## 4 | DISCUSSION

Differences in temperature experienced during development can strongly influence adult traits, including those under sexual selection, such as body size or ejaculate quality. We experimentally manipulated the rearing temperature of male eastern mosquito fish, *G. holbrooki*, and then tested for effects on sperm number, and sperm velocity at two temperatures. We found that the rearing temperature had no effect on the number of sperm produced, nor did it affect sperm velocity. There was no evidence that males produce sperm that swim faster in a thermal environment that matched the one experienced during development. Instead, irrespective of rearing temperature, males with faster-swimming sperm at a cooler temperature also tended to

have faster-swimming sperm at a warmer temperature. This suggests that variation in temperature during development is unlikely to be a major source of variation in ejaculate quality (sperm number or velocity), hence male success under sperm competition. Intriguingly, however, the temperature during development altered the relationship between traits under precopulatory sexual selection (i.e. body size) and post-copulatory sexual selection: larger males reared at a warmer temperature tended to have faster-swimming sperm, whereas large males reared at a cooler temperature tended to have slower-swimming sperm. In mosquito fish, although the relationship seems to vary depending on the social conditions (e.g. number and/or density of males), male body size is often under precopulatory sexual selection based on insemination success (Booksmythe et al., 2013; Pilastro et al., 1997), female association patterns (Bisazza & Marin, 1991; Hughes, 1985; Kahn et al., 2010) and actual paternity (Head et al., 2017; but see Booksmythe et al., 2016; Vega-Trejo et al., 2017). Thus, our results suggest that low developmental temperatures might disrupt the positive phenotypic correlation between body size and sperm traits.

We found no effect of developmental temperature on male sperm reserves or sperm velocity. This contrasts with studies in other taxa which have found that variation in the rearing temperature affects sperm production (e.g. wasps: Lacoume et al., 2007; pseudoscorpions: Zeh et al., 2012; seed beetles: Vasudeva et al., 2014) and velocity (fish: Alavi & Cosson, 2005). One thing that is worth noting about ours and previous studies is that they vary in whether rearing temperature manipulations overlapped with the period of spermatogenesis. In our study, manipulations were conducted prior to spermatogenesis, but in some of the previous studies (e.g. Vasudeva et al., 2014; Zeh et al., 2012) manipulations are likely to have overlapped with spermatogenesis, and so their results may be influenced not only by developmental plasticity, but also by sperm plasticity itself. The timing of manipulations may cause variation in results between studies. Another possible explanation for the difference between our results and previous studies is that previous studies exposed animals to more extreme temperatures (e.g. extreme lows and highs, or cold shocks). For example, male seed beetles, *Callosobruchus maculatus*, reared at an intermediate temperatures of 20–25°C transferred larger ejaculates than those reared at higher (35°C, Fox et al., 2006) or lower (17°C, Vasudeva et al., 2014) stressful temperatures. Although exposure to extreme temperatures is interesting to understand how animals respond to stressful conditions, to understand how they respond to changing environments (for instance climate change) it is also important to explore how less extreme environmental variation affects phenotypes.

We found no interaction between the developmental and test temperatures affecting sperm velocity. A male's share of paternity under sperm competition is often positively related to sperm velocity (Birkhead et al., 1999; Gage et al., 2004; Gasparini et al., 2010; Malo et al., 2005). Previous studies have shown that sperm traits in many species can be adjusted to match previous or prevailing environmental or social conditions (e.g. Bretman et al., 2011; Hopkins et al., 2019; Hosken & Ward, 2001; Parker & Pizzari, 2010; Ramm & Stockley, 2009) to increase a male's reproductive success during sperm competition. It is therefore plausible that a similar 'anticipatory' response could occur if developmental and adult temperatures tend to match in the wild. However, this was not the case in our study, suggesting that optimal sperm velocity does not vary between temperatures, or that sperm traits in mosquito fish do not change in response to developmental environments. The lack of any detectable effect of rearing temperature (either on its own or in combination with testing temperature) on sperm number or velocity suggests that variation in developmental temperature is unlikely to affect variance in reproductive success among males due to sperm competitiveness. An interesting avenue for further research in this area would be to explore the potential for adaptive plasticity of sperm themselves by manipulating the temperature during spermatogenesis.

We found that, irrespective of the developmental temperature, sperm velocity was higher at the warmer test temperature. This supports the general claim that an increase in the environmental temperature elevates sperm motility, especially in ectotherms (reviewed in Dadras et al., 2017). Furthermore, we found a positive phenotypic correlation among males for sperm velocity at the two test temperatures. This suggests that temperature during sperm competition is unlikely to affect a male's relative fertilization success. How temperature may affect a males' ability to fertilize eggs over a longer time frame is, however, unknown. In some species, sperm velocity trades off with the duration of motility (reviewed in Dadras et al., 2017), which could over time reduce the competitive ability of males that initially have fast-swimming sperm. This trade-off might be of critical importance for species with sperm limitation and for which the storage of long-lived sperm might be advantageous (Levitan, 2000). In poeciliids, like mosquito fish, although sperm can be stored in the female reproductive tract for long periods it is kept inactive in sperm bundles. The trade-off between sperm motility and sperm longevity is more likely to play out once sperm are activated, since once active the distance needed to travel is more important than storage time in influencing relative male reproductive success. This potential trade-off between distance travelled and velocity illustrates that the outcome of sperm competition is determined not only by a male's own sperm traits, but potentially also those of competing males (Tab orsky et al., 2018), and by how they interact with the female reproductive tract (Berger et al., 2011; Miller & Pitnick, 2002; Vasudeva et al., 2014). Improving our knowledge of sperm biology, and how ejaculates interact within the reproductive tract is critical to understand the effect of the environment in driving male paternity under sperm competition.

Intriguingly, we found that rearing temperature altered the phenotypic correlation between body size and sperm velocity: when reared at the warmer temperature larger males had faster-swimming sperm, but the reverse was the case for males reared at the cooler temperatures. Larger male mosquito fish are generally more attractive to females (Aich et al., 2020; Bisazza et al., 2001; Kahn et al., 2010) and have greater insemination success per mating attempt (Head et al., 2015). However, precopulatory sexual selection on male size in mosquito fish is complex and there is also evidence that smaller males are better at sneaking copulations with females (Pilastro et al., 1997) and, as a consequence, sometimes have greater reproductive success than larger males (Head et al., 2017). Our results

suggest that some thermal environments might alter the extent to which pre-copulatory sexual selection and post-copulatory sexual selection favour the same males (Evans & Garcia-Gonzalez, 2016). For example, at lower temperatures smaller males might partially compensate for being less attractive by gaining a greater share of paternity under sperm competition due to their fast-swimming sperm. If so, this might partly explain why males at the cooler rearing temperatures reached maturity at a smaller size than those reared at higher temperatures. This is not an inevitable consequence of a stressful environment. It has been previously shown in studies manipulating food availability that males on a low intake diet will delay maturation to reach the same adult size (Livingston et al., 2014). In the wild, the sign and magnitude of the correlation between male body size and sperm velocity in *G. holbrooki* could strongly influence male reproductive success when males that mature at different sites compete with each other (Pyke, 2005). However, temporal variation in temperature is less likely to matter since competitors are likely to have experienced similar developmental temperatures (e.g. winter versus spring temperatures, Kahn et al., 2013). An interesting extension of our study would be to conduct experiments that formally test whether net selection on body size resulting from size-dependent mating success varies due to the effect of developmental temperature on ejaculate traits. If so, temperature variation might partly account for the maintenance of very high variation in male body size in *G. holbrooki* in the wild (Kahn et al., 2010).

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## CONFLICT OF INTEREST

We declare no conflict of interest.

## PEER REVIEW

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## DATA AVAILABILITY STATEMENT

The data will be published in DRYAD upon acceptance.

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## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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