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RESEARCH ARTICLE

Sex-specific transgenerational plasticity: developmental temperatures of mothers and fathers have different effects on sons and daughters

Frank Seebacher*, Stephanie M. Bamford and Amelie Le Roy

ABSTRACT

Each parent can influence offspring phenotype via provisioning of the zygote or sex-specific DNA methylation. Transgenerational plasticity may therefore depend on the environmental conditions experienced by each parent. We tested this hypothesis by conducting a fully factorial experiment across three generations of guppies (*Poecilia reticulata*), determining the effects of warm (28°C) and cold (21°C) thermal backgrounds of mothers and fathers on mass and length, and thermal performance (sustained and sprint swimming speeds, citrate synthase and lactate dehydrogenase activities; 18, 24, 28, 32 and 36°C test temperatures) of sons and daughters. Offspring sex was significant for all traits except for sprint speed. Warmer mothers produced sons and daughters with reduced mass and length, and warmer fathers produced shorter sons. Sustained swimming speed (U_{crit}) of male offspring was greatest when both parents were raised at 28°C, and warmer fathers produced daughters with greater U_{crit}. Similarly, warmer fathers produced sons and daughters with greater metabolic capacity. We show that the thermal variation experienced by parents can modify offspring phenotype, and that predicting the impacts of environmental change on populations would require knowledge of the thermal background of each mother and father, particularly where sexes are spatially segregated.

KEY WORDS: Parental effects, Thermal plasticity, Epigenetics, Performance curve, Metabolism, Locomotor performance, Climate change

INTRODUCTION

Plastic phenotypic responses to environmental change are important in buffering cellular functions from the potentially negative impacts of the environment (Guderley, 2004; Schulte, 2014). For example, changes in external temperature can have a thermodynamic effect on cellular reaction rates in ectotherms that would cause proportional changes in performance (Huey and Kingsolver, 1989; Tattersall et al., 2012). Temperatures that fall outside an optimal range (the performance breadth) (Huey and Kingsolver, 1989) would cause a decrease in performance and fitness and ultimately death (Gunderson and Stillman, 2015). Individuals of many taxa can adjust responses of cellular reaction rates to temperature change by acclimation, and thereby decrease the impact of temperature fluctuations to maintain relatively constant performance in variable environments (James and Tallis, 2019; Schulte et al., 2011; Seebacher et al., 2015a).

However, responses by individuals to their proximate environment can be dependent on conditions experienced in previous generations (Burggren, 2015; LeRoy et al., 2017; Shama and Wegner, 2014). Environmental gradients can act as selection pressures to drive physiological adaptation among populations (Crawford et al., 2020; Guo et al., 2015; Schulte and Healy, 2022). For example, colonisation of freshwater habitats by oceanic stickleback led to genetic changes that produced a distinct freshwater phenotype in as little as 50 years (Lescak et al., 2015). Over an even shorter time frame, epigenetic mechanisms can act across single generations by modifying gametes of parents and thereby altering offspring phenotype (Fellous et al., 2022; Perez and Lehner, 2019). These transgenerational effects are often mediated by DNA methylation (Aliaga et al., 2019; Loughland et al., 2021; Schmitz et al., 2011; Perez and Lehner, 2019). Ultimately, genetic and epigenetic processes occur in parallel, and may interact to modify responses to environmental change (Campbell-Staton et al., 2021) or act independently in response to different aspects of the environment (Aagaard et al., 2022).

Interestingly, transgenerational effects can be sex specific. In guppies (*Poecilia reticulata*), temperatures experienced by previous generations impact male and female offspring phenotypes (metabolism, swimming performance) differently, and these effects persist or are established across more than one generation (LeRoy et al., 2017). For example, in female but not male offspring, the mode (i.e. the temperature at which maximum performance occurs) of swimming performance shifted to coincide with the grandparental rather than the parental or offspring developmental environments (LeRoy et al., 2017). Similarly, there can be differential effects of parental sex on offspring phenotype (Chang et al., 2021; Crean and Bonduriansky, 2014; Hellmann et al., 2020). These effects may be mediated by sex-specific regulation of DNA methylation marks. In zebrafish (*Danio rerio*), the methylation code of fathers serves as the template for all offspring DNA methylation patterns (Potok et al., 2013). In contrast, DNA methyltransferase (DNMT, the enzyme that catalyses DNA methylation) induction was passed on maternally in stickleback, indicating maternal regulation of methylation (Fellous et al., 2022). Both parents can also impact offspring phenotype by passing material to the zygote and embryo via the ovum, placenta, sperm and ejaculate (Crean and Bonduriansky, 2014). Mothers may have greater potential to influence offspring because of the greater volume of material passed to the zygote compared with male gametes (Mousseau and Fox, 1998), and in live-bearing species, material is passed to the embryo via the placenta. Additionally, the effects of temperature can vary between individual traits and between different levels of organisation such as between biochemical reaction rates and locomotor performance (Bozinovic et al., 2020).

School of Life and Environmental Sciences, Heydon-Laurence Building A08, University of Sydney, Sydney, NSW 2006, Australia.

*Author for correspondence (frank.seebacher@sydney.edu.au)

F.S., 0000-0002-2281-9311; A.L., 0000-0002-7961-566X

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Understanding the interactions between maternal and paternal influences on male and female offspring traits is important, because experiences by each parent can constrain offspring responses to their immediate environment differently. Predicting how changing environments impact individuals and populations may therefore require knowledge of the conditions experienced by each parent. In their natural habitat, guppies experience wide thermal fluctuations spatially between streams and temporally within and between days (~23–32°C) (Reeve et al., 2014), which makes them an ideal study species to test our hypotheses. Our aim was to determine whether the thermal background of mothers and fathers influenced male and female offspring differently. We conducted a fully factorial experiment with the thermal background of mothers and fathers, offspring sex, and acute test temperature as fixed factors to test the hypotheses that (1) mothers have a greater influence on offspring traits because of their greater potential for zygote provisioning; the alternative is that fathers have a greater effect because they have greater influence over DNA methylation; (2) effects of parents differ between male and female offspring; and (3) parental thermal background affects phenotypic traits at different levels of organisation (from enzyme activity to swimming performance and size) differently.

MATERIALS AND METHODS

Study animals and experimental design

All experiments were carried out with the approval of The University of Sydney Animal Ethics Committee (approval number: 2017/1200). Guppies, *Poecilia reticulata* W. Peters 1859, were obtained from a commercial supplier (Aqua Green, Darwin, NT, Australia) who caught fish by dip net from wild populations in the Northern Territory, Australia (12°25′S, 130°50′E). Fish were initially dispersed across three plastic tanks (645×423×276 mm) and kept on a 12 h dark:12 h light cycle. Fish were fed daily to satiety with fish flakes (TetraMin Tropical Flakes, Tetra, Germany), and supplemented with live *Artemia* nauplii 2–3 times per week. There was an air filter (Biochemical sponge filter, Age of Aquariums, Browns Plains, QLD, Australia) connected to an air pump (AC-9908, Resun) in each tank, and submersible heaters (200 W, AquaONE, Kong's Pty Ltd, Ingleburn, Australia) maintained the water temperature within 0.5°C of treatment temperatures. We bred wild-caught fish (generation F_{-1}; Fig. 1) under these conditions.

We collected newly born offspring (F_0 generation) from the F_{-1} generation and dispersed them across five treatment tanks (tanks A–E; Fig. 1) at each of two temperatures: 21°C (cold) and 28°C (warm). We assembled offspring from different clutches in each tank, and fish in different treatment tanks stemmed from different parents. We raised F_0 fish to sexual maturity under these conditions (3–4 months).

We then bred the F_0 fish to produce the F_1 generation. Newly born F_1 offspring were raised under the same conditions as the F_0 generation and by themselves in individual tanks so that we could track tank of origin and temperature treatment for each individual. For our experimental breeding protocol, we also needed to ensure that female fish were virgins when reaching sexual maturity. Female guppies store sperm, so to ascertain the correct experimental paternal background, it was essential to prevent copulation before our experimental breeding.

We then bred F_1 parents to produce the experimental F_2 generation. F_1 parents were chosen based on temperature background to give four different parental developmental temperature combinations: cold fathers+cold mothers; cold fathers+warm mothers; warm fathers+cold mothers; warm fathers+warm mothers (Fig. 1). We bred five parental pairs for each developmental temperature combination, ensuring that each parent within a pair originated from a different tank (at the F_0 generation), and that there were five unique parental pair combinations for each developmental temperature combination; in other words, there were five unique families for each parental (F_1) developmental temperature combination (Fig. 1). F_1 fish were bred as above, and F_2 fish were raised at a common-garden temperature of 24°C to ensure that potential parental (F_1) temperature effects were not confounded by offspring (F_2) developmental temperature. F_1 pairs were kept together for experimental breeding for 24 h. Before the experiments, we exposed experimental (F_1) males to non-experimental females to 'train' them: virgin males are ineffective at copulating.

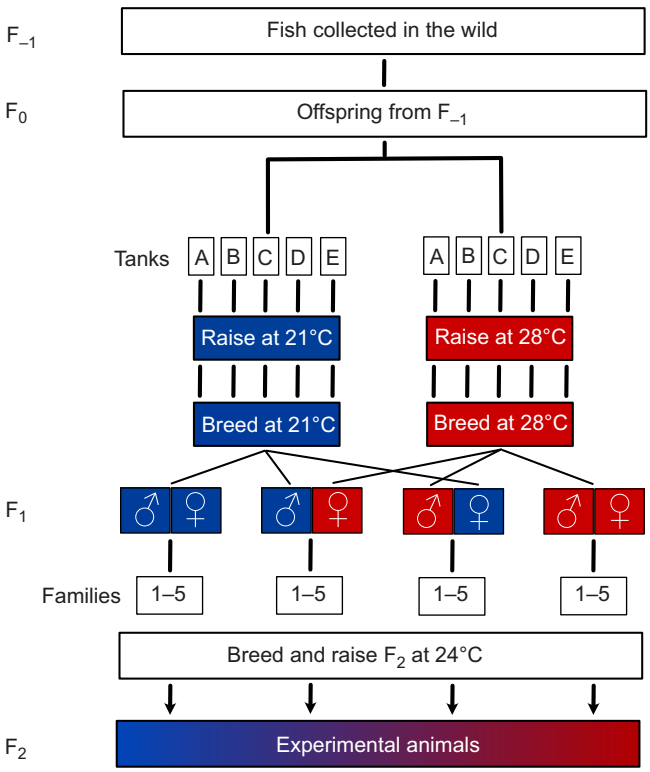

Fig. 1. Outline of the experimental design. Guppies (*Poecilia reticulata*) were obtained from wild populations (F_{-1}), and bred to produce the F_0 generation. F_0 fish were raised at 21°C (cold, blue boxes) and at 28°C (warm, red boxes), with fish dispersed across five tanks in each treatment. F_0 fish were bred to produce the F_1 generation, which became the parents of the experimental fish ('offspring', F_2). F_1 fish from different developmental temperature treatments were raised in isolation and upon reaching sexual maturity were bred so that cold and warm males and females were matched in all possible combinations (cold–cold, cold–warm, warm–cold, cold–cold). F_1 parents were matched in unique combinations based on their tank of origin at F_0, and there were five unique families for each parental developmental temperature treatment. F_2 fish were bred and raised in common garden conditions at 24°C for approximately 100 days, at which point they were phenotyped.

We collected four male and four female offspring (F_2) from each of the five parental families within each parental developmental temperature treatment to give 20 male and 20 female experimental animals per parental developmental temperature combination. We phenotyped F_2 fish after they reached sexual maturity. At the end of phenotyping, we measured the standard length and mass of each offspring fish. Length was determined from photos (analysed in ImageJ) taken side-on from fish placed into narrow transparent containers, and mass was determined on an electronic balance (Sartorius) after placing fish in a tared weighing dish containing sufficient water to submerge the fish. At the time of measurement,

mean (±s.e.m.) age of the fish was 99.5±0.5 days, and there was no mortality of fish throughout the experiment.

Swimming performance

We measured maximal sustained swimming capacity as critical sustained swimming speed (U_{crit}) according to published protocols (Seebacher et al., 2015b). Briefly, U_{crit} was measured in a cylindrical, clear plastic (Perspex) flume (150 mm length, 32 mm diameter) tightly fitted over the intake end of a submersible inline pump (12 V DC, iL500, Rule). A plastic grid separated the flume from the pump, and a bundle of hollow straws at the inlet helped to maintain laminar flow; the flume was submerged in a plastic tank (645×423×276 mm). We used a variable DC power source (NP9615; Manson Engineering Industrial, Hong Kong, China) to adjust the flow speed, which we measured in real time during swimming trials using a flow meter (DigiFlow 6710 M, Savant Electronics, Taichung, Taiwan). Fish were transferred immediately to the desired acute test temperature, and swam at an initial flow rate of 0.06 m s^{-1} for 20 min followed by an increase in flow speed of 0.02 m s^{-1} every 5 min until the fish could no longer hold their position in the water column. The first time fish fell back to the plastic grid, flow was stopped for 10 s, after which the previous flow rate was resumed. The trial was stopped when fish fell back to the grid a second time.

Sprint speed was determined as a startle response (Oufiero and Garland, 2009; Simmonds et al., 2019) of individual fish in an arena (405×600 mm, 40 mm water depth). Startle responses were induced by dropping a metal ball (9 g, 8 mm diameter) through a hollow tube (1 m length) from a pre-determined height (1.1 m) (Oufiero and Garland, 2009). We filmed the startle response from above at 120 frames s^{-1} (with a GoPro Hero 6 camera, San Mateo, CA, USA), and a submerged 300 mm ruler served as a scale. We analysed videos (using Tracker Video Analysis and Modelling software, 4.01, Open Source Physics, www.opensourcephysics.org) using the fish centre of mass as the tracking point. Centre of mass was defined as the location at 0.35 body lengths from the tip to the snout (Turesson et al., 2009). Four startle responses were measured for each fish and the fastest speed was used in the analysis (Oufiero and Garland, 2009). Startle responses were only used in the analysis when the movement of the fish was not impeded by the edge of the tank. We measured U_{crit} and sprint speed in each of 18–20 fish per treatment and sex at five acute test temperatures (18, 24, 28, 32 and 36°C).

Enzyme activity

We determined metabolic capacity of fish as the maximum catalytic velocity (V_{max}) of lactate dehydrogenase (LDH) and citrate synthase (CS) (Guderley, 2004). LDH catalyses the reaction converting pyruvate to lactate that results in the production of ATP in the absence of oxygen, and the V_{max} of LDH is therefore representative of the capacity for anaerobic ATP production. CS is an enzyme of the tricarboxylic acid cycle that is closely correlated with tissue mitochondrial density and thereby represents oxidative metabolic capacity (Dalziel et al., 2005). Assays were conducted according to published protocols (Thibault et al., 1997). Fish were euthanised by immersion in a buffered MS222 solution (0.4 g l^{-1}; Sigma-Aldrich, Castle Hill, NSW, Australia) and tail muscle was dissected on ice and stored at −80°C for later assays. Muscle tissue samples (25–45 mg) were homogenised (in a TissueLyser LT, Qiagen) in nine volumes of extraction buffer (50 mmol l^{-1} imidazole, 2 mmol l^{-1} $MgCl_2$, 5 mmol l^{-1} EDTA, 1 mmol l^{-1} reduced glutathione and 0.1% Triton, pH 7.5). Samples were centrifuged at 200 rpm (180 ***g***) for 45 s, and the supernatant was used for assays. For LDH assays, tissue homogenates were further diluted by a factor of 10, and we followed the absorbance of NADH at 340 nm (in a temperature-controlled Ultrospec UV/Vis Spectrophotometer, GE Healthcare) in an assay medium containing 100 mmol l^{-1} potassium phosphate, pH 7.0, 0.16 mmol l^{-1} NADH and 0.4 mmol l^{-1} pyruvate.

To determine V_{max} of CS, we followed the reduction of 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) at 412 nm in an assay medium containing 100 mmol l^{-1} Tris-HCl, pH 8.0, 0.1 mmol l^{-1} DTNB, 0.1 mmol l^{-1} acetyl Co-A and 0.15 mmol l^{-1} oxaloacetate to start the reaction, which was omitted in control assays testing for the reduction of DTNB in the absence of CS. All assays were conducted in duplicate. We measured enzyme activity in 9–11 fish per treatment and sex at five acute test temperatures (18, 24, 28, 32 and 36°C) in random order.

Statistical analysis

We conducted permutational analyses of all response variables (mass, length, U_{crit}, sprint speed, CS and LDH activities) because these analyses use data per se for analyses and are free from assumptions about underlying distributions (Drummond and Vowler, 2012; Ludbrook and Dudley, 1998). Fixed factors were offspring sex, mother developmental temperature (mother T), father developmental temperature (father T), and test temperature (except for mass and length). We included test temperature as a quadratic term ($TT+TT^2$) in the model to account for its non-linear effects.

We initially conducted analyses of the full model (but excluding the four-way interaction). However, offspring sex was significant for most response variables, so we analysed responses of male and female offspring separately, but present the full models in Tables S1 and S2.

We used parent family (parent id) as a random factor and determined its effect and variance component in the R package *minque*. We analysed the effects of fixed factors and their interactions in the R package *lmPerm*, including offspring id as a random factor to account for repeated measures at different test temperatures. In the analyses of mass and length, we included the age of each fish at the time of measurement as a covariate. We analysed U_{crit} and sprint speed in m s^{-1} with length as a covariate; however, we present data in the figures as body lengths (BL) s^{-1} to facilitate comparisons between treatments. In the main text, we present marginal means, averaging across levels of non-significant factors to simplify data presentation of several factors. However, we present the full dataset in Figs S1–S3.

To provide estimates of the magnitude of the effects of mothers and fathers on male and female offspring, we calculated effect sizes as standardised mean differences [(mean 1−mean 2)/pooled s.d.] and their 95% confidence intervals (CI) (Nakagawa and Cuthill, 2007). Hypothesis testing using *P*-values is a useful tool to help interpret experimental results. However, *P*-values in themselves do not contain biological information, whereas effect sizes and 95% CI provide information about the magnitude and precision of observed effects (Nakagawa and Cuthill, 2007). We calculated effect sizes so that positive effects indicate higher trait values at 28°C parental developmental temperature. We determined 95% CI by bootstrapping with replacement (Calmettes et al., 2012) for all effect sizes using the R package *boot*.

RESULTS

Size

There were significant differences in standard length and mass between female and male offspring (Table S1). Standard length of male offspring from fathers raised at 28°C was significantly lower than standard length of those from fathers raised at 21°C (Table 1,

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Table 1. Results of permutational analyses of offspring length and mass

Source	Length	Mass
Male offspring		
mother T	0.29	0.10
father T	<0.0001	0.55
mother T×father T	0.57	0.53
Parent id	0.01	<0.0001
	30.0%	54.1%
Female offspring		
mother T	<0.0001	0.0004
father T	0.86	0.38
mother T×father T	0.032	0.92
Parent id	<0.0001	<0.0001
	41.9%	51.5%

Probabilities from analyses of independent factors (mother T and father T, temperature at which mothers and fathers were raised, respectively), and the random factor parent family (parent id; *P*-values and % of total variance) are shown. Residual d.f.=73.

Fig. 2A,B). There were no other significant effects of parental temperature on male offspring length or mass (Table 1). Parental family had a significant effect on length and mass (Table 1).

Female offspring length and mass were influenced significantly by the mother's temperature, and both were lower in offspring from mothers raised at 28°C than in offspring from mothers raised at 21°C (Fig. 2; Table 1). A significant interaction between mother and father temperature indicates that fathers raised at 28°C decreased the length of female offspring born to mothers raised at 21°C, but increased the length of offspring from mothers raised at 28°C (Table 1, Fig. 2A,B). Parental family had a significant effect (Table 1).

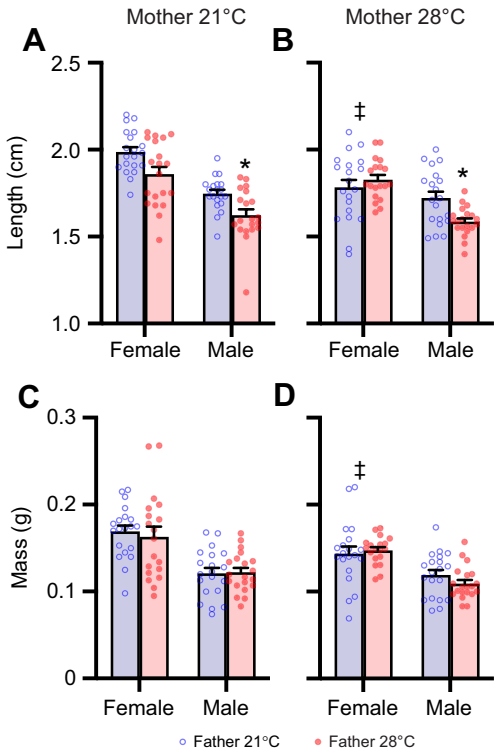

Fig. 2. Offspring size. Standard length (A,B) and mass (C,D) of male and female offspring born to mothers raised at 21°C (left) or 28°C (right), matched with fathers raised either at 21°C (blue) or at 28°C (red). Overall, warmer mothers produced smaller female offspring (‡) and warmer fathers produced shorter male offspring (*), and there was a weak but significant interaction between mother and father developmental temperature in determining female offspring length. Means±s.e.m. and data points from individual fish are shown (*n*=18–20 fish per treatment).

Swimming performance

U_{crit} differed significantly between offspring sexes, but sprint speed did not (Table S2). U_{crit} and sprint performance in male and female offspring were significantly influenced by test temperature. The effect of test temperature manifests as an inverted 'U'-shaped thermal performance curve where performance increases with increasing test temperature up to a maximum beyond which it decreases (Table 2, Fig. 3; Fig. S1). Parental family influenced U_{crit} in both offspring sexes, and sprint performance in males but not in females (Table 2).

In female offspring, U_{crit} was significantly influenced only by the developmental temperature of fathers, and female offspring from fathers raised at 28°C had higher U_{crit} (Table 2, Fig. 3A). In contrast, sprint performance in females was determined by a significant interaction between developmental temperature of mothers and acute test temperature (Table 2, Fig. 3D); counterintuitively, sprint speed was somewhat higher in offspring from warm mothers at lower test temperatures (18 and 24°C), and vice versa at higher test temperatures (32 and 36°C).

U_{crit} in male offspring was affected by a significant three-way interaction between mother and father developmental temperature, and test temperature (Table 2, Fig. 3B,C) indicating that maximal U_{crit} was highest when mother and father developmental temperature were both 28°C. Sprint performance in males depended on a significant interaction between mother and father developmental temperature, which manifested in particular in increased sprint performance in offspring from mothers and fathers raised at 28°C (Fig. 3E,F).

Table 2. Results of permutational analysis of offspring swimming performance and enzyme activity

Source	U_{crit}	Sprint	CS	LDH
Male offspring				
mother T	0.032	0.23	0.90	0.33
father T	0.0024	0.15	<0.0001	0.27
test T	<0.0001	<0.0001	<0.0001	<0.0001
mother T×father T	0.033	0.017	0.20	0.19
mother T×test T	0.38	0.12	1	0.13
father T×test T	0.031	1	0.29	0.97
mother T×father T×test T	0.0004	0.45	0.27	0.14
Parent id	<0.0001	<0.0001	<0.0001	<0.0001
	35.5%	16.8%	39.3%	26.9%
Female offspring				
mother T	0.86	0.92	0.18	0.94
father T	0.015	0.20	<0.0001	<0.0001
test T	<0.0001	<0.0001	<0.0001	<0.0001
mother T×father T	0.18	0.96	0.12	0.49
mother T×test T	0.66	0.022	1	1
father T×test T	0.39	0.33	0.20	1
mother T×father T×test T	0.11	0.12	0.38	0.45
Parent id	<0.0001	0.11	<0.0001	<0.0001
	42.3%	6.0%	56.1%	48.4%

Probabilities from analyses of the effects of independent factors (mother T and father T, temperature at which mothers and fathers were raised, respectively; test T, acute test temperature), and the random factor parent family (parent id; *P*-values and % of total variance) on offspring traits (U_{crit}, critical sustained swimming performance; Sprint, sprint speed, CS, citrate synthase activity, LDH, lactate dehydrogenase activity) are shown. Individual id was used as a random factor; within residual d.f.=314 and 145 for swimming performance and enzyme activity, respectively.

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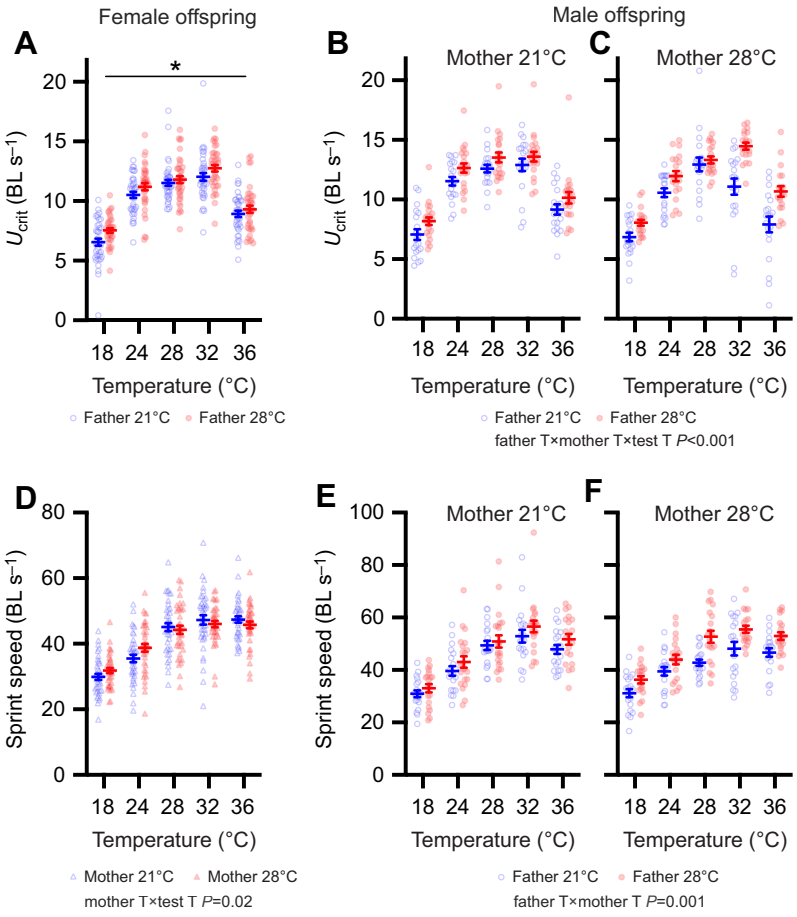

Fig. 3. Critical sustained swimming speed (U_{crit}) and sprint performance of offspring. (A) U_{crit} of female offspring (marginal means±s.e.) was affected by test temperature and developmental temperature of fathers (*). (B,C) U_{crit} of male offspring from mothers raised at 21°C (B) or 28°C (C), and matched with fathers raised at 21°C (blue) or 28°C (red) was affected by a significant three-way interaction of father×mother×test temperature T (as indicated). (D) Sprint speed in female offspring was affected by a significant interaction between developmental temperature of mothers and test temperature (as indicated; marginal means±s.e.). (E,F) Sprint in male offspring was affected by a significant interaction between the developmental temperature of mothers (E, 21°C; F, 28°C) and fathers (blue, 21°C; red, 28°C) (as indicated). For B,C and E,F, means±s.e.m. and data points from individual fish are shown (sample sizes are n=18–20 fish per treatment group).

Enzyme activity

CS and LDH activity differed between offspring sexes (Table S2), and activities in both female and male offspring were significantly influenced by test temperature and parental family; all enzyme activities increased with increasing test temperatures (Table 2, Fig. 4; Figs S2 and S3). Enzyme activities in both offspring sexes, except for LDH in males, were influenced significantly by the developmental temperature of fathers, and fathers raised at 28°C produced offspring with higher enzyme activities compared with fathers raised at 21°C (Table 2, Fig. 4A–C). LDH activity in males was not affected by any of the fixed factors (Table 2, Fig. 4).

Effect sizes

Warm mothers produced smaller offspring, and this effect was particularly pronounced in daughters (greater magnitude of effect sizes with no overlap of 95% CI with zero; Fig. 5). Warmer fathers produced shorter sons but otherwise had no effect on offspring size. However, fathers raised at the warmer temperature (28°C) tended to produce offspring with higher performance, particularly swimming performance in both offspring sexes, and LDH activity in daughters. Warmer developmental temperatures of mothers also increased U_{crit} in females and LDH activity in sons, but decreased CS activity in daughters.

DISCUSSION

We have shown that the sex of both parents and offspring is an important modifier of transgenerational thermal effects, and that these effects differ among traits. We accept our first hypothesis with respect to offspring size, as mothers had a greater effect on the size of offspring than fathers, and increased temperature experienced by mothers led to reduced size of offspring. However, we accept the alternative to the first hypothesis for physiological traits, where fathers had a greater effect on locomotor performance and metabolic capacity than mothers, and warmer fathers produced sons and daughters with greater performance. We accept our second hypothesis, as there were differences in parental effects between sons and daughter in most traits. We also accept our third hypothesis that parental thermal background had different effects on phenotypic traits at different levels of organisation. We acknowledge that our reference to 'parental' effect may represent combined effects of grandparental (F_0) and parental (F_1) generations. Parental family of origin had a relatively large effect on most traits, indicating a genetic component in determining offspring traits. These results are significant by showing that variable environments can have sex-specific effects, and that predictions of environmental impacts on wildlife may require knowledge of maternal and paternal thermal history.

Mothers had a greater effect on the body size of daughters, whereas fathers influenced the length of sons. These sex-specific effects are interesting because they indicate that preferential transmission of the paternal methylome to offspring (Potok et al., 2013) is not the only mechanism altering offspring phenotype. The overall negative effect of warm mothers on the size of sons and daughters may reflect provisioning of the zygote by mothers (Crean and Bonduriansky, 2014; Mousseau and Fox, 1998), which should affect both offspring sexes, although differences in maternal effects between offspring sexes persisted (Crean and Bonduriansky, 2014; Mousseau and Fox, 1998). Genomic imprinting may have produced

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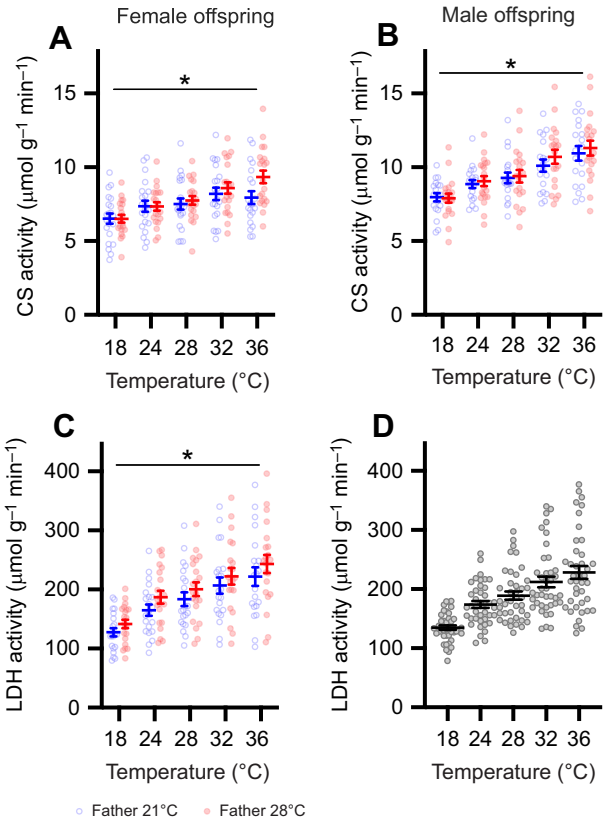

Fig. 4. Metabolic enzyme activity of offspring. Citrate synthase (CS) activity in female (A) and male (B) offspring, and lactate dehydrogenase (LDH) activity in female offspring (C) were affected significantly (*) by test temperature and the developmental temperature of fathers (21°C, blue; 28°C, red). LDH activity in male offspring (D) was affected only by test temperature. Marginal means±s.e. and data from individual fish are shown (n = 9–10).

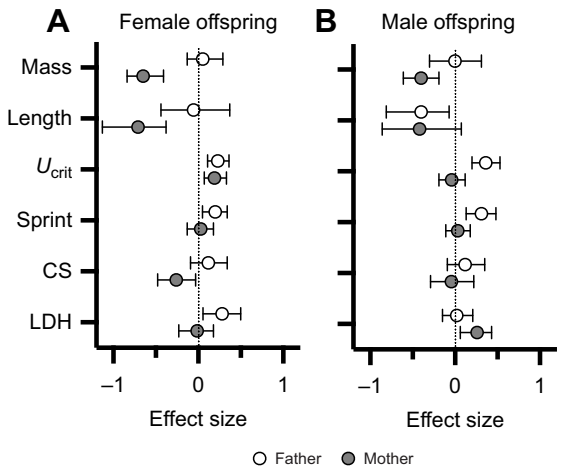

Fig. 5. Effect size of parental developmental temperature on offspring traits. Positive effect sizes indicate that the warmer (28°C) parental developmental temperature increased trait values, and paternal (open circles) and maternal (filled circles) effect sizes (±95% confidence intervals) are shown separately for female (A) and male (B) offspring.

sex-specific results in offspring. At least in mammals (mice), maternally expressed genes tend to suppress offspring growth (Iwasa, 2020; Latchney et al., 2022), which may explain the negative effects of mothers on female offspring size, but not their lack of effect on sons. Paternally expressed genes such as insulin-like growth factor 2 tend to promote metabolism and growth (Thorvaldsen et al., 1998), which supports our data showing that fathers had positive effects on metabolic capacity and locomotor performance, but is at variance with our finding that fathers had a negative effect on the length of sons. The varied effects of mothers and fathers on the metabolic capacity of offspring also do not support genomic imprinting as the only underlying mechanisms. However, in mice, imprinted genes may also be differentially methylated (Latchney et al., 2022), which introduces another regulatory layer that could produce interactive effects.

Interestingly, zebrafish showed the same patterns of genomic imprinting as mice, where methylation marks regulated imprinted genes (Martin and McGowan, 1995). However, the signal was not a simple on–off switch but a quantitative relationship where increased levels of methylation produced decreased expression of the gene, leading to variegated expression of imprinted genes. The degree of methylation was temperature sensitive in zebrafish (lower temperatures increased methylation), and it was modified by the sex of the offspring, where males showed lower levels of methylation (Martin and McGowan, 1995). Hence, it may be expected that imprinted genes of males at higher temperatures are less suppressed, which reflects our finding that warmer fathers produced offspring with greater performance. DNA methylation and RNAi signalling, another mechanism involved in gene silencing, can be temperature sensitive (Campos et al., 2012; Kameda et al., 2004; Loughland et al., 2021). Differential imprinting between sexes may therefore occur via germline differentially methylated regions (DMRs) when male and female gonads were at different temperatures during gamete differentiation (Prontera and Donti, 2014), as was the case in our guppy F_1 parents. Together, these different effects of temperature on mothers and fathers, and sex-specific methylation of offspring could produce sex- and temperature-specific imprinted genomes that may at least partly explain our results, although this would need to be confirmed experimentally. Swimming performance has a relatively high heritability in guppies and in stickleback (*Gasterosteus aculeatus*) (Garenc et al., 1998; Nicoletto, 1995). Moreover, there can be paternal epigenetic effects on muscle performance, and exercise training in male mice can influence muscle function and exercise capacity in their offspring via DMRs in skeletal muscle (Costa-Júnior et al., 2021; Freitas-Dias et al., 2022). These dynamics concur with our findings that both family and temperature exposure of parents contributed to phenotypes, and it may explain why fathers contributed more to offspring swimming performance than mothers.

The differential effects of maternal and paternal thermal experience on sons and daughters can have repercussions for responses to environmental temperature changes such as climate change. Temperatures experienced by previous generations can influence offspring phenotype via epigenetic modification of gametes (LeRoy et al., 2017; Shama and Wegner, 2014; Shama et al., 2014). Guppies experience wide thermal fluctuations in their natural habitat (Reeve et al., 2014), and climate change will continue to increase thermal variation globally (Vasseur et al., 2014; Wedler et al., 2023). The differential effects of the thermal experience of mothers and fathers therefore present a new level of complexity. For example, in our guppies, warm mothers produced smaller daughters, and reductions in size reduce reproductive fitness in fish (Barneche et al., 2018). Hence, temperature increases in the maternal environment in particular may influence population dynamics in the next generation. Similarly, warmer fathers produced smaller sons, which may reduce the fitness of sons by increasing vulnerability to

predation (Sogard, 1997). Hence, male offspring may experience increased predation pressure, which could lead to changes in sex ratio. However, offspring performance increased when parental temperatures coincided at 28°C, which implies that smaller size may trade-off with increased performance at particular parental temperature combinations.

Predictions of the effects of changes in the thermal environment would require knowledge of microhabitat use and thermal conditions experienced by mothers and fathers. In some fish, including guppies, males and females may segregate spatially as a result of sexual harassment, predation pressure or differences in performance with respect to water velocity profiles, and they could thereby experience different thermal conditions (Croft et al., 2006; Hockley et al., 2014; Matley et al., 2020). Hence, a secondary cause (e.g. predation pressure) could result in temperature-dependent transgenerational impacts on offspring phenotypes. Environmental variability, either natural or anthropogenic, could increase stratification of thermal habitats and thereby exacerbate transgenerational effects of spatial segregation between sexes.

Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: F.S., A.L.; Methodology: F.S., S.M.B.; Formal analysis: F.S.; Investigation: S.M.B., A.L.; Resources: F.S.; Data curation: F.S.; Writing - original draft: F.S.; Writing - review & editing: S.M.B., A.L.; Visualization: F.S.; Supervision: F.S.; Project administration: F.S., S.M.B.; Funding acquisition: F.S.

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Data availability

All data associated with this study are available from Dryad (Seebacher et al., 2023): https://doi.org/10.5061/dryad.8sf7m0ctt

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