Inbreeding and high developmental temperatures affect cognition and boldness in guppies (*Poecilia reticulata*)

Vinogradov IM*[1], Zang C[1], Mahmud-Al-Hasan M[1], Head ML[1], Jennions MD[1,2]

1. Division of Ecology and Evolution, Research School of Biology, Australian National University, 46 Sullivans Creek Road, ACT, 2600, Australia
2. Stellenbosch Institute for Advanced Study (STIAS), Wallenberg Research Centre at Stellenbosch University, Stellenbosch 7600, South Africa

* corresponding author, email: ivan.vinogradov@anu.edu.au

Keywords

Fish cognition, inhibitory control, inbreeding depression, thermal stress, *Poecilia reticulata*

Acknowledgements

We thank the staff of ANU Animal Services and M. Chung, for assistance with fish husbandry.

Author Contributions

I.M.V., C.Z., M.L.H. and M.D.J. conceived and designed the study. I.M.V., C.Z., and M.A.H.M. collected the data and analysed the data. All authors interpreted the data, cowrote the manuscript and gave permission for publication.

Funding

The study was funded by the Australian Research Council (DP190100279 to M.D.J.).

Ethical note

All experimental procedures were carried out under approval from ANU Animal Ethics Committee (Approval #A2018/27) and complied with existing laws regulating the treatment of vertebrates in Australia.

Abstract

Inbreeding impairs the cognitive abilities of humans, but its impact on cognition in other animals is poorly studied. For example, environmental stress (e.g. food limitation, extreme temperatures) often amplifies inbreeding depression in morphological traits, but whether cognition is similarly affected is unclear. We therefore tested if a higher temperature (30 vs 26°C) during development exacerbates any difference in inhibitory control between inbred (f =0.25) and outbred guppies (*Poecilia reticulata*). Inhibitory control is an aspect of cognition that is often measured in vertebrates using a detour test (animals have to navigate around a transparent barrier to reach a reward). We also tested if inbreeding and temperature affect 'boldness', which is a putative personality trait in guppies. Inbreeding did not lower inhibitory control, but inbred fish were significantly less bold than outbred fish. There was no effect of temperature on the boldness of either sex. However, males, but not females, raised at the higher temperature had significantly lower inhibitory control. Inbreeding depression was not greater for either inhibitory control or boldness for fish that developed at the higher temperature. Our study is among the first to test if experimentally induced inbreeding impairs cognition in a non-domesticated vertebrate. We show that both inbreeding and higher temperatures during development can affect the behaviour and cognitive abilities of fish. These findings are noteworthy given the twin threats of rising global temperatures and more frequent inbreeding as habitat fragmentation reduces population sizes.

Introduction

The offspring produced when close relatives breed tend to be less fit ("inbreeding depression"). This occurs because inbreeding increases homozygosity, which unmasks deleterious recessive alleles and sometimes reduces advantageous heterozygosity (Charlesworth and Charlesworth, 1987). Inbreeding depression has been reported for fecundity, survival, and many other life-history traits (e.g. Fraimout et al., 2023; Giontella et al., 2020; Opatová et al., 2016; Smallbone et al., 2016; Vega-Trejo et al., 2015).

Until recently, few researchers have asked how inbreeding affects the behaviour of wild animals. Some notable exceptions include mating behaviour (Ala-Honkola et al., 2009; Mariette et al., 2006; Pilakouta and Smiseth, 2017; Reid et al., 2004), risk-taking (Richardson and Smiseth, 2017), and boldness (Müller and Juškauskas, but see Herdegen-Radwan, 2019a). Crucially, however, only a handful of studies have used experimentally controlled breeding to compare inbred and outbred individuals. This oversight is problematic as inbreeding is more likely when population density is low. Inbreeding and factors that lower population density and fitness (e.g. low quality habitat) might be confounded in wild populations, and difficult to separate statistically, hence the need for experiments (Keller and Waller, 2002; Vega-Trejo et al., 2018). As available natural habitat shrinks due to urbanization, agriculture and climate change, wild populations of animals have become smaller, and inbreeding has become more common. This has prompted increased interest in how inbreeding affects the performance, and ultimately the reproductive success of wild animals.

The effect of inbreeding on animal cognition - the ability to acquire, store, analyse, and use information from the environment - is barely studied in non-human animals (Shettleworth, 2010). The main exception is work on laboratory rodents (e.g. Yuen et al., 2017; Kim et al., 2019; Harker and Whishaw, 2002). In addition, there are reports of a moderate decrease in learning ability of inbred *Drosophila melanogaster* Nepoux et al (2010); and inbred burying beetles (*Nicrophorus vespilloides*) make poorer breeding decisions (Richardson et al (2018). Impaired cognition may be especially costly in stressful environment that require fast, effective decision making (Price, 1999; Richardson et al., 2018). However, it remains unknown if greater stress during development amplifies inbreeding depression for cognitive abilities in wild animals.

Inbreeding depression is likely to vary in its magnitude across environments because it is often greater in more stressful environments (e.g. high temperatures, less food) (Armbruster and Reed, 2005; Reed et al., 2012). One meta-analysis reported that 66% of variation in inbreeding depression is explained by the level of environmentally-induced stress (Fox and Reed, 2011). However, a more recent-meta analysis showed no inbreeding-environment interaction when different types of stressors (e.g. heat, food availability) were pooled (Vega-Trejo et al., 2022). Interestingly, females tend to experience slightly, but significantly, greater inbreeding depression than males (Vega-Trejo et al., 2022). Any sex difference in responses to environmental stress could further complicate attempts to determine what factors explain population or species differences in inbreeding depression (Fox and Stillwell, 2009).

Environmental stress during development can have life-long fitness consequences for adults (Monaghan, 2008). As organisms develop they undergo metabolically costly growth, generating reactive oxygen species, and suffer oxidative stress (Metcalfe and Alonso-Alvarez, 2010; Monaghan, 2008). Some studies report that environmental stress early in life amplifies oxidative stress and lowers fitness (e.g. Haussmann et al., 2012; Marasco et al., 2013). But other studies report that early life stress can improve fitness if individuals adapt physiologically to cope better with unpredictable environmental conditions as adults (Garratt et al., 2015;

Losdat et al., 2018; Rubenstein et al., 2016). Developmental plasticity is often a crucial factor that allows animals to adjust to environmental heterogeneity (Taborsky, 2017). Relatively few studies have tested how stress in early-life (versus life-long or adult only) affects inbreeding depression. To date, there is mixed evidence that thermal stress or poor nutrition in early life has a more harmful effect on inbred than outbred animals (minimal effect: Goodrich et al., 2013; Schou et al., 2015; Valtonen et al., 2011; Vega-Trejo et al., 2016; effect in some treatment groups: Dahlgaard and Loeschcke, 1997; Freitak et al., 2014; Nakadate et al., 2003; Syukri et al., 2020).

Here we used guppies (*Poecilia reticulata*) to test how a high temperature during development, which is assumed to be stressful, affects adult cognition. Guppies are live-bearing fish (Order: Poeciliidae) that possess three characteristics relevant for our study. First, guppies often inbreed in nature (Griffiths and Magurran, 1997; Hain and Neff, 2007). Inbreeding is linked to reduced survival (Nakadate et al., 2003), altered mating behaviour (van Oosterhout et al., 2003; Zajitschek et al., 2009; Zajitschek and Brooks, 2008, but see Deacon et al., 2014), and smaller brain size (Burns et al., 2009; Vila-Pouca et al., 2022). Reduced brain size in inbred guppies is likely to lower their cognitive abilities (e.g. response to predators: van der Bijl et al., 2015). Second, guppies have well-studied response to elevated temperatures, with negative effects on body size, sperm traits, mating behaviour, and survival (Breckels and Neff, 2013; Rahman et al., 2020) when temperatures exceed 30 °C (compared to 24-27 °C where guppies thrive, Gibson and Hirst, 1955; Johansen and Cross, 1980; Reeve et al., 2014). This suggests that high temperatures are stressful. There is also limited observational evidence that inbreeding lowers thermal tolerance in adult guppies (e.g. Nakadate et al., 2003; Syukri et al., 2020). Third, poecilid fishes are a common model system to study cognition (review: Salena et al., 2021) and boldness, a repeatable personality trait in guppies (Harris et al., 2010; Kemp et al., 2022).

In our study, we used an established cognitive assay, namely a detour test, to measure inhibitory control (i.e., exert self-control to obtain a delayed reward (Diamond, 2013; Savaşçı et al., 2021)). Inhibitory control is a fitness-enhancing executive cognitive function that has been extensively studied in mammals, birds, and other vertebrates (e.g. Coomes et al., 2022; Johnson-Ulrich and Holekamp, 2020; Szabo et al., 2020; review: Kabadayi et al., 2018; for a critique see van Horik et al., 2018). Many studies have investigated inhibitory control in fish (Jungwirth et al., 2024; Macario et al., 2021;; Triki et al., 2022), including in guppies (Prentice et al., 2023), and some studies show that it differs between the sexes (e.g. Savaşçı et al., 2021; Vinogradov et al., 2022, reviews: Lucon-Xicatto and Bisazza, 2017b; Cummings, 2018; Bshary and Triki, 2022) and might influence reproductive success in poecilid (Vinogradov, 2024).

We designed a study to test how a high temperature during development affects the inhibitory control of experimentally created inbred and outbred guppies. We had a balanced 2×2×2 (temperature × inbreeding × sex) design, with equal numbers of each sex. Half the fish were raised to maturity at 27 °C and half at 30 °C; half were outbred offspring of unrelated parents and half were the inbred offspring of two full siblings. We measured inhibitory control and boldness (see Herdegen-Radwan, 2019b) over four consecutive trials. We made four predictions. First, inhibitory control ability is lower in inbred fish. Second, fish raised at the higher temperature will have worse inhibitory control; and this effect will be greater for inbred fish. Third, females have greater inhibitory control than males, but both sexes will learn (i.e. improved inhibitory control) over consecutive trials. Four, males will be bolder than females, as this is often reported for poecilid fishes (Harris et al., 2010; Vinogradov et al., 2022).

Methods

Origin and Maintenance of Fish

Test fish were from a stock established at the Australian National University in 2018 from two independent laboratory stocks originally collected from Alligator Creek, Townsville. These fish resembles guppies from Trinidad based on their mating behaviour and coloration (Brooks and Endler, 2001). Our stock population was reared under a 14:10 h light cycle at 26 °C and fed *ad libitum* with *Artemia* nauplii twice daily. Juveniles from this stock were collected in March 2023. To ensure virginity, we sexed fish before they matured and then kept them in single sex tanks.

Inbreeding and Temperature Treatment

A 150 male-female pairs of virgins were randomly set up in individual 3-L plastic tanks for two weeks. The males were then removed and the females left undisturbed for another week. Three weeks after the initial pairing, we checked daily for newborn fry. Females that did not give birth within 6 weeks were reintroduced to their original partner for another week. We obtained 80 outbred, full sib families that produced at least 4 offspring. We then had a fully balanced breeding design. In each block, fish from two families (e.g., block 1: A and B; block 2: C and D) were mated to create inbred and outbred fish. Inbred offspring were produced by pairing a brother and sister from the same family (e.g., AA and BB), and outbred offspring were produced by pairing, for example, an A male with a B female and a B male with an A female (i.e., AB and BA, CD and DC). In total we used 60 families to set up 30 blocks.

In each block we randomly collected 4 newborns from each of the two inbred and two outbred families (N = 16 offspring/block). These offspring were photographed and then isolated in individual 1-litre tanks. We assigned eight tanks to a stable control temperature (26°C) and eight to a stable high temperature treatment (30°C). All fish were fed *ad libitum* with *Artemia* nauplii twice daily. Fish were photographed at 4 weeks of age, and again on the day they became sexually mature. To record the time to maturity, we inspected the fish daily from four weeks of age onward. Males were considered mature once their 'hood', a sensory protrusion, grew beyond the tip of the gonopodium (Houde, 1997). Females were considered mature once speckling coloration appeared at their anal region, or yoked eggs were visible through the body wall (Houde, 1997). Standard length was quantified using *ImageJ* (Schneider et al., 2012). All measurements were made blind to treatment type. Death before maturity was noted.

Two weeks after maturation, all fish were transferred to tanks at 26°C to experience a common garden temperature prior to running cognitive trials. Four weeks after maturation, fish were ready to be used in cognition trials.

Cognitive Trials

We set up four identical trial tanks (60×42x40cm) with white plastic walls. A focal fish was placed in a start zone with a transparent U-shaped barrier blocking the direct route to a 4-litre plastic tank containing three male and three female conspecifics (henceforth, “social reward”). Initially, we used a removable opaque wall to block the focal fish inside the start zone. After 1 minute we lifted the wall, allowing the fish to leave the start zone. The time the fish took to leave the start zone and enter the open area was our measure of boldness. To reach the social reward, the focal fish had to resist the urge to try to swim through the transparent barrier, and instead had to detour around it. If successful, the focal fish was blocked in the goal zone to interact with the social reward fish for five minutes, and then returned to the start zone for the next trial. If the focal fish did not reach the goal zone in five minutes, it was gently guided there with a net. This act controlled for the amount of social interaction each individual had per trial. If a focal fish did not reach the goal zone in 5 minutes *and* failed to approach the transparent barrier, we classified it as unmotivated, and the trial was discarded. Each fish underwent four consecutive trials.

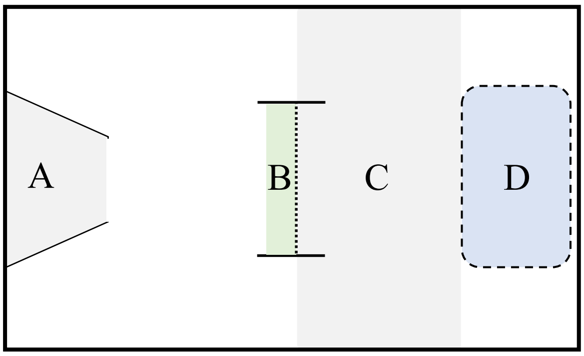

Figure 1. Diagram of the inhibitory control assay apparatus inside a glass tank (60 x 42 x 42 cm). Solid and dashed lines indicate opaque and transparent walls, respectively. Each focal fish began its trial in the 'start zone' (A) where it was held for one minute. The time taken to leave the 'start zone' (i.e., fully cross the border) was our measure of 'boldness'. A smaller transparent tank (21 x 14 x 14 cm) contained three male and three female conspecifics and was located opposite the start zone (D). A transparent 12 cm wide plastic barrier (B) was located directly between the 'start zone' and the stimulus tank (D). The focal fish had to detour around the barrier to enter the 'goal zone' (C; the fish's whole body had to enter the zone for the attempt to be defined as successful). The total time a fish spent within 1.5 cm of the barrier (B) (zone coloured green) was our measure of inhibitory control. The time it took a fish to reach the 'goal zone' (C) after leaving the 'start zone' (A) was the measure of 'solving time'.

All trials were video recorded, and data was independently collected from the videos by two observers blind to the identity of the focal fish (except its sex). We recorded three response variables: (1) time taken to leave the start zone, hereafter 'boldness' (White et al., 2013); (2) time spent trying to swim through the transparent barrier as a measure of 'inhibitory control' (includes 0 values for fish that never approached the barrier); and (3) total time taken to reach the goal zone (excluding the time spent in the start zone) as a measure of 'solving time'. In total, we sampled 239 fish. Only two fish were excluded from the analysis because they were never motivated to complete the task (see *Methods*). We ran $n = 27\text{-}31$ fish for each of the eight treatment groups.

Statistical Analysis

Our analysis plan was pre-registered on the Open Science Framework (https://osf.io/2e8s4) and any deviations from this plan are explicitly stated here. We used R v4.2.2 (R Core Team, 2022) to run our analysis. RMarkdown files with the original code and the accompanying data tables are provided with the manuscript.

We ran hurdle lognormal mixed models to quantify the effects of three fixed factors: sex, inbreeding and temperature. We included all two- and three-way interactions between these factors. We also included standard length (zero-centred for each sex) and trial number (1 to 4) as fixed covariates; and fish ID as a random factor. We examined three response variables: boldness, inhibitory control, and total solving time. Hurdle models were used to distinguish trials where boldness, inhibitory control, or solving time were set to zero if a fish did not, respectively: (a) leave the start zone within three minutes; (b) attempt to swim through the transparent barrier; (c) solve the trial within 5 minutes of leaving the start zone. We included boldness as a fixed covariate in the models for inhibitory control and solving time. Models excluding boldness, produced similar findings (see *Supplementary Materials*).

During our initial data exploration, we noted a bimodal distribution of inhibitory control values (Figure S1), seemingly because a substantial proportion of fish that did not solve the task within the allotted 5 minutes had very low inhibitory control (i.e., spent a long time at the barrier). That is, some fish were still trying to swim through the barrier at the 5-minute cut-off, so their true inhibitory control could not be measured accurately. We therefore deviated from our initial pre-registered analysis plan and fit models using only the subset of trials that fish solved within the allotted time. Consequently, the time taken to solve a trial, which was analysed separately using hurdle binomial mixed models, became the most useful indicator of high or low inhibitory control ability.

If any model showed a significant interaction involving sex, we ran separate models for males and females. If the three-way interaction was not significant it was dropped, provided that the fit of the original and reduced models was not significantly different. Similarly, non-significant two-way interaction were dropped to test for main effects of sex, temperature, and inbreeding. Model performance was analysed using the package *DHARMa* (Hartig, 2022).

Better fish performance is always indicated by a smaller value of the response variable: bolder fish take *less* time to leave the start zone, fish with greater inhibitory control spend *less* time trying to swim through the transparent barrier, and smarter fish take *less* time to reach the goal zone. Statistical significance was evaluated at alpha = 0.05 (two-tailed). Summary statistics are presented as means ± standard error (SE).

Results

Differences in Boldness

We measured boldness in 730 of 948 trials for the 237 fish tested. Boldness was not recorded in the other 218 trials because in 144 trials the fish failed to leave the start zone within three minutes; and in 74 trials the fish was deemed unmotivated (see *Methods*).

There was no significant effect of sex on the interaction between developmental temperature and inbreeding affecting boldness ($\chi2 = 0.071$, $P = 0.40$) (Table S1). There were also no two-way interactions between sex, temperature, or inbreeding (all $P > 0.05$, Table S2). We therefore removed all interactions from our final model (for initial models see the *Supplementary Materials*).

Outbred guppies were significantly bolder than inbred ones ($\chi2 = 5.14$, $P = 0.02$, Table 1), but developmental temperature did not affect boldness ($\chi2 = 1.38$, $P = 0.24$). Males were significantly bolder than females, and also more likely to leave the start zone within three minutes (henceforth, “likelihood to emerge”) ($\chi2 = 10.18$, $P = 0.001$; $\chi2 = 0.02$, $P = 0.005$). Smaller guppies were marginally bolder ($\chi2 = 4.02$, $P = 0.05$), but body size had no effect on the likelihood to emerge ($\chi2 = 1.15$, $P = 0.28$). Finally, in later trials guppies were bolder ($\chi2 = 12.68$, $P = <0.001$) and more likely to emerge ($\chi2 = 6.03$, $P = 0.01$).

Table 1. Parameter estimates from a hurdle negative lognormal mixed model predicting boldness in male and female guppies *Poecilia reticulata*. The output is shown for the fixed effects in the conditional model (positive estimates indicate slower emergence time, hence less bold) and in the zero-inflation model (positive estimates indicated higher likelihood of boldness being 0, indicating that a fish failed to emerge from the start zone within 3 minutes). Reference

level for the categorical predictors are shown in square brackets. Standard errors (SE) and *P* values were obtained from the model summary, while chi-square values and their *P* values were calculated with likelihood ratio tests. Significant results are emboldened.

Predictors of Boldness	Estimate	SE	Z	*P*	χ^2	*P* (χ^2)
Conditional model						
Sex [m]	-0.26	0.08	-3.19	**0.001**	10.18	**0.001**
Temp [26 °C]	0.10	0.08	1.17	0.24	1.38	0.24
Lineage [outbred]	-0.19	0.08	-2.27	**0.02**	5.14	**0.02**
Trial	-0.09	0.02	-3.56	**< 0.001**	12.68	**< 0.001**
Body size (scaled)	0.08	0.04	2.01	**0.05**	4.02	**0.05**
Zero-inflation						
Sex [m]	-0.86	0.38	-2.25	**0.02**	7.84	**0.005**
Temp [26 °C]	-0.15	0.38	-0.41	0.68	0.25	0.62
Lineage [outbred]	-0.05	0.38	-0.12	0.90	0.00	0.95
Trial	-0.35	0.14	-2.57	**0.01**	6.03	**0.01**
Body size (scaled)	-0.18	0.19	-0.96	0.34	1.15	0.28

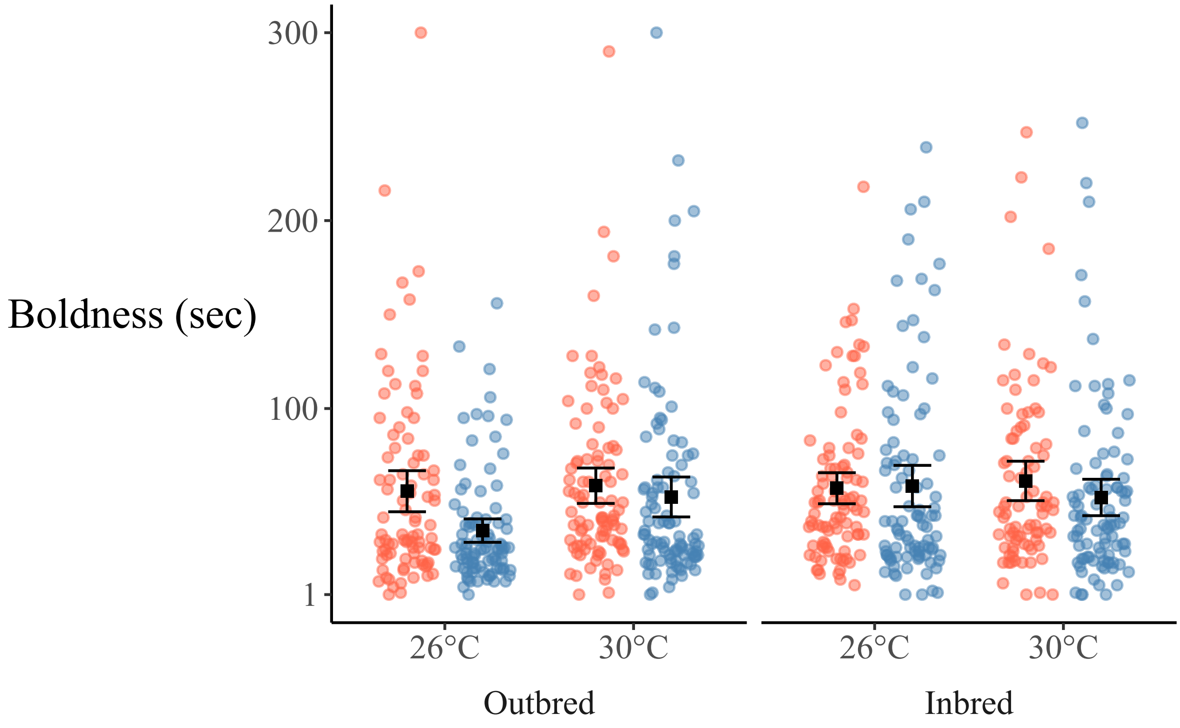

Figure 2. Boldness (time to emerge from the start zone) of inbred and outbred female (red) and male (blue) guppies *Poecilia reticulata* raised to maturity at either 26 °C or 30 °C. Group means and 95% confidence intervals are in black. Each point represents boldness measure in one of four consecutive trials, excluding trials when a fish failed to emerge from the start zone within three minutes (n = 144) and trials where boldness could not be measured (n = 74, see text).

Differences in Inhibitory Control: measured as time spent at the barrier

We obtained inhibitory control measures for 874 trials, in 270 of which the fish reached the goal zone without approaching the barrier. Guppies reached the goal zone within 5 minutes (‘solved trial’) in 647 trials (74%). Guppies that reached the goal zone spent far less time (31.2 ± 51.3 s, n = 647) trying to swim through the transparent barrier than those that failed (212.2 ± 86.8 s, n = 227) (Figure S1). We ran our inhibitory control models using only trials where fish solved the task, since our measure of inhibitory control could not be obtained from fish still at the barrier after 5 minutes. To address this limitation, we separately tested which factors affected the likelihood of reaching the goal zone (see *Differences in Solving Ability*).

There was no significant effect of sex on the interaction between developmental temperature and inbreeding ($\chi2 = 0.14$, $P = 0.71$, Figure S3), nor were any two-way interactions between sex, temperature and inbreeding significant (all $P > 0.1$, Figure S4). We therefore removed all interactions from the final model.

In trials when fish reached the goal zone, their sex, inbreeding status, and developmental temperature had no effect on their inhibitory control or their likelihood of detouring around the barrier without approaching it (Table 2, Figure 3). In later trials, fish were more likely to detour around the barrier without approaching it ($P = 0.01$), which is indicative of learning. However, the time spent trying to swim through the barrier if it was approached did not decrease in later trials. Body size had no significant effect on inhibitory control ($\chi2 = 3.64$, $P = 0.06$) or the likelihood of detouring around the barrier without approaching it ($\chi2 = 0.64$, $P = 0.43$).

Table 2. Parameter estimates from a hurdle negative lognormal mixed model predicting inhibitory control in male and female guppies *Poecilia reticulata*. The model only includes data for trials where a fish reached the goal zone within the allotted 5 minutes (see *Methods*). The output is shown for the fixed effects in the conditional model and in the zero-inflation model (i.e., likelihood of inhibitory control being 0 in trials where a fish did not attempt to swim through the transparent barrier). Reference levels for the categorical predictors are shown in square brackets. Standard errors (SE) and *P* values were obtained from the model summary, while chi-square values and their *P* values were calculated with likelihood ratio tests. Significant results are emboldened.

Predictors of Inhibitory Control	Estimate	SE	Z	*P*	χ^2	*P* (χ^2)
Conditional model						
Sex [m]	0.06	0.12	0.52	0.60	0.27	0.60
Temp [26 °C]	0.09	0.13	0.71	0.48	0.50	0.48
Lineage [outbred]	-0.03	0.12	-0.23	0.82	0.05	0.82
Trial	-0.02	0.06	-0.40	0.69	0.16	0.69
Body size (scaled)	0.11	0.06	1.91	0.06	3.64	0.06
Zero-inflation						
Sex [m]	0.20	0.22	0.91	0.36	0.84	0.36
Temp [26 °C]	-0.14	0.22	-0.63	0.53	0.66	0.42
Lineage [outbred]	-0.12	0.22	-0.55	0.59	0.18	0.67
Trial	0.23	0.09	2.68	**0.01**	6.13	**0.01**
Body size (scaled)	0.10	0.11	0.90	0.37	0.64	0.43

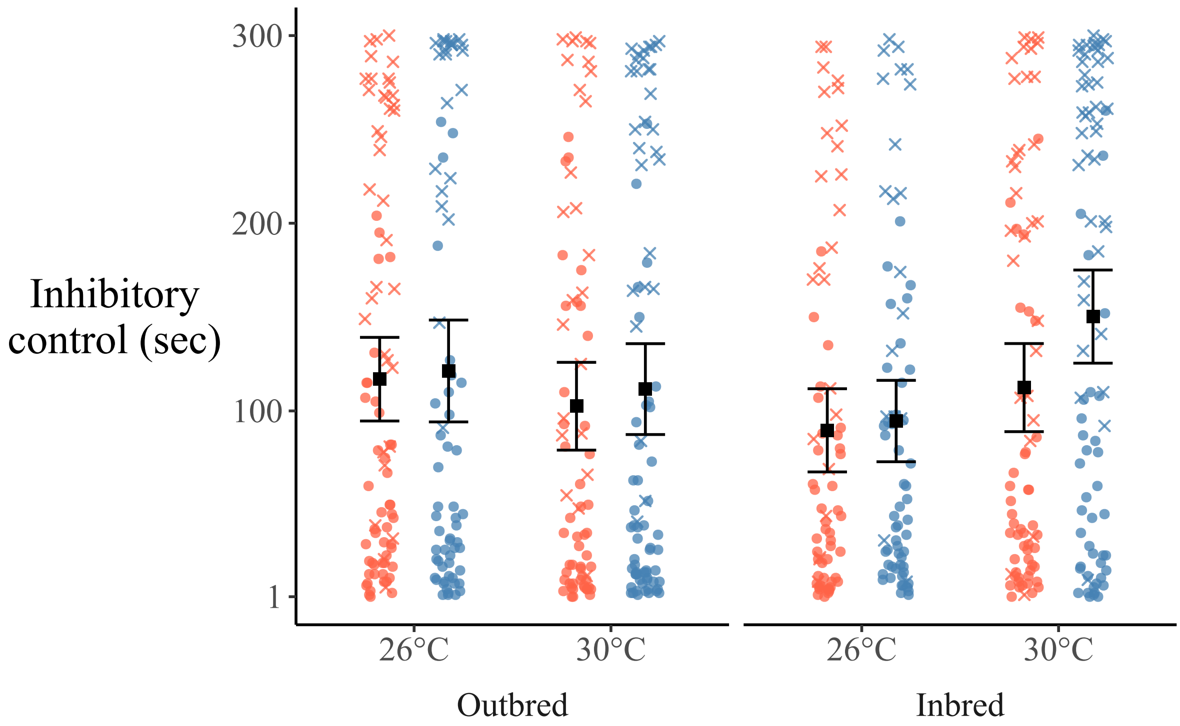

Figure 3. Inhibitory control (time spent trying to swim through a transparent barrier) of inbred and outbred female (red) and male (blue) guppies *Poecilia reticulata* reared at two temperatures. Group means and 95% confidence intervals are in black. Crosses refer to trials terminated at 5 minutes because a fish had not reached the goal zone, and circles refer to trials where a fish reached the goal zone within 5 minutes. We excluded trials where a guppy did not approach the transparent barrier (i.e., where inhibitory control = 0) from this graph.

Differences in Inhibitory Control: measured as solving time

Guppies failed to reach the goal zone in 227 of the 604 trials where they first approached the transparent barrier. Failure to solve the task is a strong indicator of poor inhibitory control, i.e., these fish spent a long time at the barrier (on average 181.0 seconds longer than in solved trials, Fig. 3). Over 90% of guppies who failed a trial spent >25% of their trial time trying to swim through the barrier.

There was no significant effect of sex on how the interaction between developmental temperature and inbreeding affected solving time (three-way interaction: $\chi 2 = 0.65$, $P = 0.42$). There were also no significant two-way interactions between sex, temperature, and inbreeding (all $P > 0.1$, Table S6), except for a significant interaction between temperature and sex in the zero-inflated model ($\chi 2 = 9.87$, $P = 0.002$, Table S6). We therefore dropped all interactions from the final conditional model to report main effects, but we kept the temperature by sex interaction in the zero-inflated model (Table 3).

Males reached the goal zone less often when they developed at the higher temperature ($t = 3.11$, $P = 0.01$, pairwise comparison, Figure S4), but developmental temperature did not affect the likelihood that females reached the goal zone ($t = 0.58$, $P = 0.94$). Inbreeding had no effect on the likelihood on reaching the goal zone ($\chi 2 = 0.39$, $P = 0.53$, Table 3).

In trials where fish reached the goal zone, their sex, developmental temperature, and inbreeding status had no effect on their solving time (all P > 0.1, Table 3). Body size also had no effect on the time taken to reach the goal zone, or the likelihood of so doing (both $P > 0.1$).

In later trials fish reached the goal zone more often ($P = 0.005$) and sooner ($P < 0.001$), indicative of learning. Bolder fish reached the goal zone sooner ($P = 0.03$), but were not significantly more likely to reach it within the allotted 5 minutes ($P = 0.10$).

Table 3. Parameter estimates from a hurdle negative binomial mixed model predicting solving time in male and female guppies *Poecilia reticulata*. The output is shown for the fixed effects in the conditional model and fixed effects and their interactions in the zero-inflation model (i.e., likelihood of solving time being 0, which refers to trials that were not solved within 5 minutes of leaving the start zone). Reference level for the categorical predictors are shown in square brackets. Standard errors (SE), and *P* values were obtained from the model summary, while chi-square values and their *P* values were calculated with likelihood ratio test. Significant results are emboldened.

Predictor of Solving Time	Estimate	SE	*P*	χ^2	*P* (χ^2)
Conditional model					
Sex [male]	-0.02	0.08	0.29	2.17	0.14
Temperature [26°C]	0.06	0.08	0.14	0.60	0.43
Breeding [outbred]	-0.06	0.08	0.44	0.49	0.48
Trial	-0.15	0.03	**< 0.001**	20.64	**< 0.001**
Body size (scaled)	0.02	0.04	0.62	0.24	0.62
Boldness	0.002	0.001	**0.03**	4.46	**0.03**
Zero-inflation model					
Sex [m]	-0.71	0.29	**0.02**	0.51	0.47
Temperature [26°C]	-0.15	0.26	0.56	3.78	0.052
Breeding [outbred]	-0.11	0.19	0.57	0.39	0.53
Trial	-0.22	0.08	**0.004**	7.95	**0.005**
Body size (scaled)	0.02	0.10	0.83	0.07	0.80
Boldness	0.002	0.001	0.24	1.61	0.20
Sex [male]: Temperature [26°C]	1.07	0.40	**0.007**	8.49	**0.004**

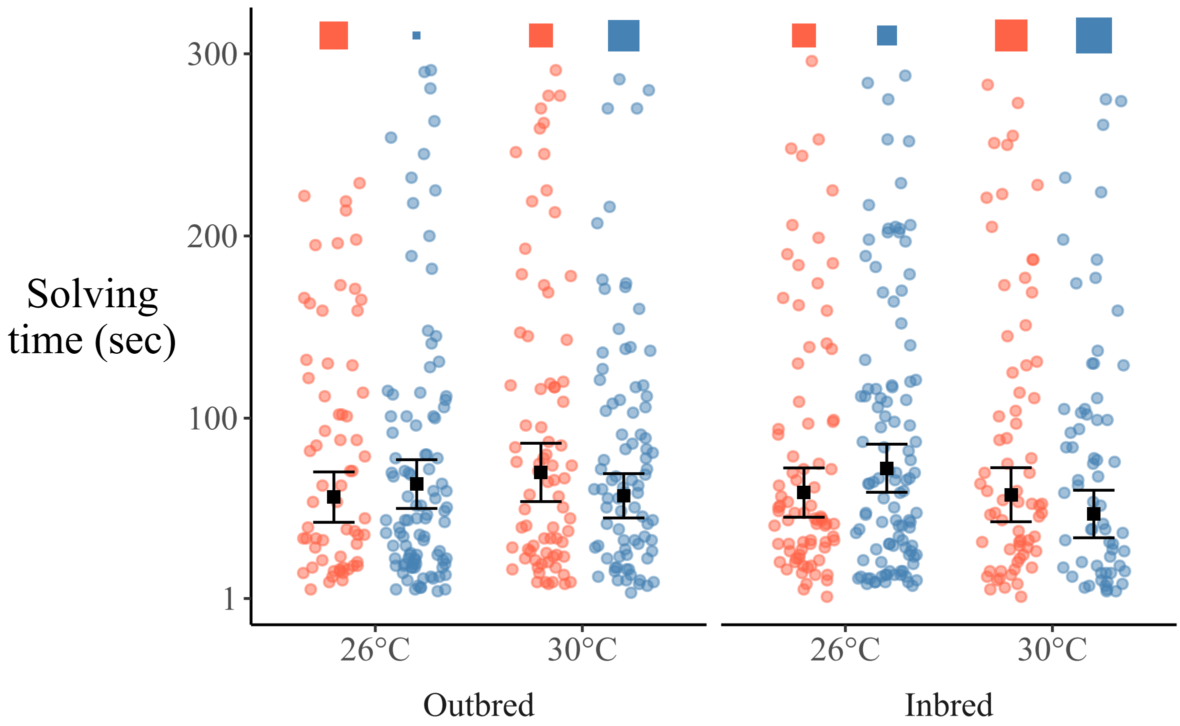

Figure 4. Time to reach the goal zone of inbred and outbred female (red) and male (blue) guppies *Poecilia reticulata* reared at two different temperatures. Group means and 95% confidence intervals are in black. Each circle represents solving time in a single trial. The size of the squares at the top of the plot is proportional to how many trials were unsolved within the allocated 5 minutes.

Discussion

There was a significant sex difference in how developmental temperature affected cognition, but no effect of inbreeding for either sex in *P. reticulata*. A higher temperature did not affect female inhibitory control, but it significantly decreased the likelihood that males solved the task. We attribute failure to solve the task to poor inhibitory control based on the very strong relationship between time spent trying to swim through the transparent barrier and whether the task was solved (Figs. 3 and S1). Both sexes were better at solving the task in later trials, which is indicative of learning. Males were significantly bolder than females, and both sexes were less bold when inbred. Fish also became bolder in later trials, as seen in other studies (Ólafsdóttir and Magellan, 2016; Vinogradov et al., 2022; but see Kemp et al., 2022). Finally, there was no evidence for either sex to support the key prediction that inbreeding depression for cognition or boldness is amplified by a higher temperature during development, which is assumed to be stressful.

Effects of Inbreeding

Inbreeding did not reduce the inhibitory control ability of guppies reared at either normal or elevated temperatures. One explanation is that one generation of inbreeding is insufficient to affect cognition. Repeated inbreeding increases homozygosity causing deleterious recessive alleles to be expressed more often, and fewer loci to have advantageous heterozygosity (Charlesworth and Charlesworth, 1987; Keller and Waller, 2002). Past studies on poecilid fishes suggest, however, the inbreeding effects are highly variable and trait specific. Some studies report behavioural changes after one generation of inbreeding (e.g. Mariette et al., 2006; Nakadate et al., 2003). In contrast, body size and colour were unaltered after a generation of inbreeding (e.g. Mariette et al., 2006; Vega-Trejo et al., 2016), but affected after 2-4 generations of inbreeding (e.g. van Oosterhout et al., 2003; Zajitschek and Brooks, 2008). It is possible that greater inbreeding decreases brain size in guppies (Burns et al., 2009) and, consequently, lowers their cognitive abilities (van der Bijl et al., 2015).

A second explanation for no detectable inbreeding depression is stronger selection on brain function than other traits. Stronger selection will more often remove deleterious recessive alleles, lowering standing directional dominance variation which drives inbreeding depression. Effective brain function is central to fitness-enhancing behaviours, so it might be more resistant to inbreeding, especially as mating with relatives is common in wild guppies (Griffiths and Magurran, 1997; Hain and Neff, 2007). These fitness-enhancing behaviours include inhibitory control, which is under sexual selection in a related poecilid (Vinogradov, 2024). This 'selection on brain function' explanation is problematic though as: (a) many traits that show inbreeding depression are under strong selection (e.g., fecundity; male attractiveness); (b) traits that are strongly selected are often costly, hence condition-dependent and affected by many loci (Houle & Rowe 1996). Such traits are highly susceptible to inbreeding unmasking deleterious alleles (Charlesworth and Charlesworth, 1987).

In our study, outbred guppies were generally bolder than inbred ones (Figure S3), which contrasts with another study that found no inbreeding depression (Herdegen-Radwan, 2019a). Reduced boldness may be an adaptive response to inbreeding depression in other traits that increases predation risk (e.g., swimming speed; Nettle and Bateson, 2015). There are also claims that boldness is a costly fitness-enhancing behaviour that is therefore more pronounced in individuals in good condition (Lewis, 2015; Luttbeg and Sih, 2010). If inbreeding lowers body condition this alone could reduce boldness (Herdegen-Radwan, 2019a; Lewis, 2015). Inbreeding can also decrease sexual ornamentation and sexual motivation in male guppies, and the latter effect could explain their decreased boldness (i.e. less motivated to approach reward females). This would not, however, explain the reduced boldness of females. Boldness itself did not affect inhibitory control (Table S4), which is consistent with other studies on fish (Savaşçı et al., 2021; Vinogradov et al., 2022) and, more broadly, with no correlation between personality traits and cognition (multi-taxa meta-analysis: Dougherty and Guillette, 2018).

Effect of Temperature

We interpreted a failure to reach the goal zone (solve the task) as arising from a poor ability to inhibit the impulse to try to swim through the transparent barrier (i.e., lower inhibitory control) (Fig. 3). Using this criterion there was a strong sex difference in how an elevated developmental temperature affected inhibitory control: males, but not females, had lower inhibitory control at the higher temperature (Figure S4). In fish that solved the task there was, however, no effect of developmental temperature on either the time spent at the barrier or the likelihood of initially approaching it (Table 3). In sum, our results suggest that a high temperature during development lowers the inhibitory control ability of males, but not that of females.

Developmental stress retards neural development, hence cognitive abilities, in many vertebrates (e.g. Buchanan et al., 2013; Dayananda and Webb, 2017; Jones et al., 2005). High temperatures in particular has been shown to lower cognitive abilities in several taxa (review: Soravia et al., 2021). However, several studies have also reported that a higher developmental temperature improves cognition (e.g., Vila Pouca et al., 2019; Amiel and Shine, 2012; Beltrán et al., 2020; Clark et al., 2014). It is unclear why only the inhibitory control of male guppies was affected by a high temperature in our study. Inhibitory control might be more developmental stability in females if it has a greater adaptive value for females than males. Indeed, strongly selected traits are expected to be less responsive to environmental perturbations (Buchanan et al., 2013; Siegal and Bergman, 2002; Waddington, 1942). In Poeciliid, females often have greater inhibitory control than males (Lucon-Xiccato and Bisazza, 2017; Vinogradov et al., 2022), which might indicate it has higher adaptive value to females. It is also possible that thermal stress affects cognition indirectly through sex-specific changes in other condition-dependent traits (Reznick et al., 2000).

Inbreeding and a higher developmental temperature

It has been suggested that inbreeding depression is worse in stressful environments, but the evidence from experiments is mixed (Goodrich et al., 2013; Schou et al., 2015; Valtonen et al., 2011; Vega-Trejo et al., 2016, Dahlgaard and Loeschcke, 1997; Freitak et al., 2014). We found no evidence that a higher temperature during development, which is assumed to impose thermal stress, increased inbreeding depression for inhibitory control. In both sexes, however, the lowest inhibitory control was shown by inbred fish reared at a high temperature (Figure 3, Figure 4). It is possible that the elevated temperature we used (30 °C) was insufficient to cause a detectable rise in inbreeding depression. In other guppy studies, temperatures of up to 35 °C have been used but this caused high mortality, suggesting that 35 °C is unnaturally high (Kanda, 1991; Nakajima et al., 2009). It is worth noting that a higher temperature could result in selective mortality thereby biasing subsequent assays of cognition. Insufficient statistical power is, of course, always a concern especially as measures of inhibitory control can be highly variable (Beran, 2015; Macario et al., 2021; Savaşçı et al., 2021). However, we had sufficient power to detect sex-differences in inhibitory control and improvements in task solving over successive trials, suggesting that our sample sizes sufficed to detect biologically important effects.

Conclusion

Here, we present one of the first experimental tests of the effect of inbreeding on cognition in a non-domesticated vertebrate. Inbreeding has no effect on inhibitory control in guppies, but it did reduce their boldness. Male, but not female, guppies that experienced a high temperature during development had lower inhibitory control. Most importantly, there was no evidence to support the key prediction that thermal stress amplifies inbreeding depression of cognition. In unpredictable environments, survival often depends on cognitive plasticity (Ducatez et al., 2020). This makes increased temperature fluctuations due to climate change concerning, especially if higher temperatures lower cognitive performance. Our results highlight the potential vulnerability of fish to rising temperatures, and the need to pay more attention to behavioural traits, such as cognition and personality, that affect survival in wild animals.

References

Ala-Honkola, O., Uddström, A., Pauli, B.D., Lindström, K., 2009. Strong inbreeding depression in male mating behaviour in a poeciliid fish. J. Evol. Biol. 22, 1396–1406. https://doi.org/10.1111/j.1420-9101.2009.01765.x

Amiel, J.J., Shine, R., 2012. Hotter nests produce smarter young lizards. Biol. Lett. 8, 372–374. https://doi.org/10.1098/rsbl.2011.1161

Armbruster, P., Reed, D.H., 2005. Inbreeding depression in benign and stressful environments. Heredity 95, 235–242. https://doi.org/10.1038/sj.hdy.6800721

Beltrán, I., Loiseleur, R., Durand, V., Whiting, M.J., 2020. Effects of early thermal environment on the behavior and learning of a lizard with bimodal reproduction. Behav. Ecol. Sociobiol. 74, 73. https://doi.org/10.1007/s00265-020-02849-6

Beran, M.J., 2015. The comparative science of “self-control”: what are we talking about? Front. Psychol. 6. https://doi.org/10.3389/fpsyg.2015.00051

Bshary, R., & Triki, Z. 2022. Fish ecology and cognition: insights from studies on wild and wild-caught teleost fishes. Curr Opin in Beh Sci, 46, 101174. https://doi.org/10.1016/j.cobeha.2022.101174).

Breckels, R.D., Neff, B.D., 2013. The effects of elevated temperature on the sexual traits, immunology and survivorship of a tropical ectotherm. J. Exp. Biol. 216, 2658–2664. https://doi.org/10.1242/jeb.084962

Brooks, R., Endler, J.A., 2001. Direct and indirect sexual selection and quantitative genetics of male traits in guppies (*Poecilia reticulata*). Evolution 55, 1002–1015. https://doi.org/10.1111/j.0014-3820.2001.tb00617.x

Buchanan, K.L., Grindstaff, J.L., Pravosudov, V.V., 2013. Condition-dependence, developmental plasticity, and cognition: implications for ecology and evolution. Trends Ecol. Evol. 28, 290–296. https://doi.org/10.1016/j.tree.2013.02.004

Burns, J.G., Saravanan, A., Helen Rodd, F., 2009. Rearing environment affects the brain size of guppies: lab-reared guppies have smaller brains than wild-caught guppies. Ethology 115, 122–133. https://doi.org/10.1111/j.1439-0310.2008.01585.x

C Yuen, R.K., Merico, D., Bookman, M., L Howe, J., Thiruvahindrapuram, B., Patel, R.V., Whitney, J., Deflaux, N., Bingham, J., Wang, Z., Pellecchia, G., Buchanan, J.A., Walker, S., Marshall, C.R., Uddin, M., Zarrei, M., Deneault, E., D’Abate, L., Chan, A.J.S., Koyanagi, S., Paton, T., Pereira, S.L., Hoang, N., Engchuan, W., Higginbotham, E.J., Ho, K., Lamoureux, S., Li, W., MacDonald, J.R., Nalpathamkalam, T., Sung, W.W.L., Tsoi, F.J., Wei, J., Xu, L., Tasse, A.-M., Kirby, E., Van Etten, W., Twigger, S., Roberts, W., Drmic, I., Jilderda, S., Modi, B.M., Kellam, B., Szego, M., Cytrynbaum, C., Weksberg, R., Zwaigenbaum, L., Woodbury-Smith, M., Brian, J., Senman, L., Iaboni, A., Doyle-Thomas, K., Thompson, A., Chrysler, C., Leef, J., Savion-Lemieux, T., Smith, I.M., Liu, X., Nicolson, R., Seifer, V., Fedele, A., Cook, E.H., Dager, S., Estes, A., Gallagher, L., Malow, B.A., Parr, J.R., Spence, S.J., Vorstman, J., Frey, B.J., Robinson, J.T., Strug, L.J., Fernandez, B.A., Elsabbagh, M., Carter, M.T., Hallmayer, J., Knoppers, B.M., Anagnostou, E., Szatmari, P., Ring, R.H., Glazer, D., Pletcher, M.T., Scherer, S.W., 2017. Whole genome sequencing resource identifies 18 new candidate genes for autism spectrum disorder. Nat. Neurosci. 20, 602–611. https://doi.org/10.1038/nn.4524

Charlesworth, D., Charlesworth, B., 1987. Inbreeding depression and its evolutionary consequences. Annu. Rev. Ecol. Syst. 18, 237–268. https://doi.org/10.1146/annurev.es.18.110187.001321

Clark, B.F., Amiel, J.J., Shine, R., Noble, D.W.A., Whiting, M.J., 2014. Colour discrimination and associative learning in hatchling lizards incubated at ‘hot’ and ‘cold’ temperatures. Behav. Ecol. Sociobiol. 68, 239–247. https://doi.org/10.1007/s00265-013-1639-x

Coomes, J.R., Davidson, G.L., Reichert, M.S., Kulahci, I.G., Troisi, C.A., Quinn, J.L., 2022. Inhibitory control, exploration behaviour and manipulated ecological context are associated with foraging flexibility in the great tit. J. Anim. Ecol. 91, 320–333. https://doi.org/10.1111/1365-2656.13600

Cummings, M.E., 2018. Sexual conflict and sexually dimorphic cognition—reviewing their relationship in poeciliid fishes. Behav. Ecol. Sociobiol. 72, 73. https://doi.org/10.1007/s00265-018-2483-9

Dahlgaard, J., Loeschcke, V., 1997. Effects of inbreeding in three life stages of *Drosophila buzzatii* after embryos were exposed to a high temperature stress. Heredity 78 (Pt 4), 410–416. https://doi.org/10.1038/hdy.1997.64

Dayananda, B., Webb, J.K., 2017. Incubation under climate warming affects learning ability and survival in hatchling lizards. Biol. Lett. 13, 20170002. https://doi.org/10.1098/rsbl.2017.0002

Deacon, A.E., Barbosa, M., Magurran, A.E., 2014. Forced monogamy in a multiply mating species does not impede colonisation success. BMC Ecol. 14, 18. https://doi.org/10.1186/1472-6785-14-18

Diamond, A., 2013. Executive functions. Annu. Rev. Psychol. 64, 135–168. https://doi.org/10.1146/annurev-psych-113011-143750

Dougherty, L.R., Guillette, L.M., 2018. Linking personality and cognition: a meta-analysis. Philos. Trans. R. Soc. B Biol. Sci. 373, 20170282. https://doi.org/10.1098/rstb.2017.0282

Ducatez, S., Sol, D., Sayol, F., Lefebvre, L., 2020. Behavioural plasticity is associated with reduced extinction risk in birds. Nat. Ecol. Evol. 4, 788–793. https://doi.org/10.1038/s41559-020-1168-8

Fox, C.W., Reed, D.H., 2011. Inbreeding depression increases with environmental stress: an experimental study and meta-analysis. Evolution 65, 246–258. https://doi.org/10.1111/j.1558-5646.2010.01108.x

Fox, C.W., Stillwell, R.C., 2009. Environmental effects on sex differences in the genetic load for adult lifespan in a seed-feeding beetle. Heredity 103, 62–72. https://doi.org/10.1038/hdy.2009.31

Fraimout, A., Rastas, P., Lv, L., Merilä, J., 2023. Inbreeding depression in an outbred stickleback population. Mol. Ecol. 32, 3440–3449. https://doi.org/10.1111/mec.16946

Freitak, D., Bos, N., Stucki, D., Sundström, L., 2014. Inbreeding-related trade-offs in stress resistance in the ant *Formica exsecta*. Biol. Lett. 10, 20140805. https://doi.org/10.1098/rsbl.2014.0805

Garratt, M., Lemaître, J.-F., Douhard, M., Bonenfant, C., Capron, G., Warnant, C., Klein, F., Brooks, R.C., Gaillard, J.-M., 2015. High juvenile mortality is associated with sex-specific adult survival and lifespan in wild roe deer. Curr. Biol. 25, 759–763. https://doi.org/10.1016/j.cub.2014.11.071

Gibson, M.B., Hirst, B., 1955. The effect of salinity and temperature on the pre-adult growth of guppies. Copeia 1955, 241–243. https://doi.org/10.2307/1440474

Giontella, A., Sarti, F.M., Biggio, G.P., Giovannini, S., Cherchi, R., Pieramati, C., Silvestrelli, M., 2020. Genetic parameters and inbreeding effect of morphological traits in Sardinian Anglo Arab horse. Animals 10, 791. https://doi.org/10.3390/ani10050791

Goodrich, S.H., Beans, C.M., Roach, D.A., 2013. Environmental conditions during early life determine the consequences of inbreeding in *Agrostemma githago* (Caryophyllaceae). J. Evol. Biol. 26, 499–508. https://doi.org/10.1111/jeb.12065

Griffiths, S.W., Magurran, A.E., 1997. Schooling preferences for familiar fish vary with group size in a wild guppy population. Proc. R. Soc. Lond. B Biol. Sci. 264, 547–551. https://doi.org/10.1098/rspb.1997.0078

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

Harris, S., Ramnarine, I.W., Smith, H.G., Pettersson, L.B., 2010. Picking personalities apart: estimating the influence of predation, sex and body size on boldness in the guppy Poecilia reticulata. Oikos 119, 1711–1718. https://doi.org/10.1111/j.1600-0706.2010.18028.x

Hartig, F., 2022. DHARMa: residual diagnostics for hierarchical (multi-level/mixed) regression models. URL https://cran.r-project.org/web/packages/DHARMa/vignettes/DHARMa.html.

Haussmann, M.F., Longenecker, A.S., Marchetto, N.M., Juliano, S.A., Bowden, R.M., 2012. Embryonic exposure to corticosterone modifies the juvenile stress response, oxidative stress and telomere length. Proc. Biol. Sci. 279, 1447–1456. https://doi.org/10.1098/rspb.2011.1913

Herdegen-Radwan, M., 2019a. Does inbreeding affect personality traits? Ecol. Evol. 9, 10929–10937. https://doi.org/10.1002/ece3.5487

Herdegen-Radwan, M., 2019b. Bolder guppies do not have more mating partners, yet sire more offspring. BMC Evol. Biol. 19, 211. https://doi.org/10.1186/s12862-019-1539-4

Johansen, P.H., Cross, J.A., 1980. Effects of sexual maturation and sex steroid hormone treatment on the temperature preference of the guppy, *Poecilia reticulata* (Peters). Can. J. Zool. 58, 586–588. https://doi.org/10.1139/z80-082

Johnson-Ulrich, L., Holekamp, K.E., 2020. Group size and social rank predict inhibitory control in spotted hyaenas. Anim. Behav. 160, 157–168. https://doi.org/10.1016/j.anbehav.2019.11.020

Jones, J.C., Helliwell, P., Beekman, M., Maleszka, R., Oldroyd, B.P., 2005. The effects of rearing temperature on developmental stability and learning and memory in the honey bee, Apis mellifera. J. Comp. Physiol. A 191, 1121–1129. https://doi.org/10.1007/s00359-005-0035-z

Jungwirth, A., Horsfield, A., Nührenberg, P., Fischer, S., 2024. Estimating cognitive ability in the wild: validation of a detour test paradigm using a cichlid fish (*Neolamprologus pulcher*). Fishes 9, 50. https://doi.org/10.3390/fishes9020050

Kabadayi, C., Bobrowicz, K., & Osvath, M. 2018. The detour paradigm in animal cognition. Animal cognition, 21, 21-35. https://doi.org/10.1007/s10071-017-1152-0

Kanda, N., 1991. Strain differences at thermal resistance in the guppy, *Poecilia reticulata*. Tohoku J. Agric. Res. 42, 25–31.

Keller, L.F., Waller, D.M., 2002. Inbreeding effects in wild populations. Trends Ecol. Evol. 17, 230–241. https://doi.org/10.1016/S0169-5347(02)02489-8

Kemp, D.J., Lynch, K.E., Samantha, S.J., 2022. Variation in boldness behaviour across individuals, sexes and strains of the guppy (*Poecilia reticulata*). Mar. Freshw. Res. 73, 441–453. https://doi.org/10.1071/MF21129

Kim, D.-H., Jang, Y.-S., Jeon, W.K., Han, J.-S., 2019. Assessment of cognitive phenotyping in inbred, genetically modified mice, and transgenic mouse models of Alzheimer’s disease. Exp. Neurobiol. 28, 146–157. https://doi.org/10.5607/en.2019.28.2.146

Lewis, D.M.G., 2015. Evolved individual differences: advancing a condition-dependent model of personality. Personal. Individ. Differ., Theory and Measurement in Personality and Individual Differences 84, 63–72. https://doi.org/10.1016/j.paid.2014.10.013

Losdat, S., Blount, J.D., Marri, V., Maronde, L., Richner, H., Helfenstein, F., 2018. Effects of an early-life paraquat exposure on adult resistance to oxidative stress, plumage colour and sperm performance in a wild bird. J. Anim. Ecol. 87, 1137–1148. https://doi.org/10.1111/1365-2656.12822

Lucon-Xiccato, T., Bisazza, A., 2017. Sex differences in spatial abilities and cognitive flexibility in the guppy. Anim. Behav. 123, 53–60. https://doi.org/10.1016/j.anbehav.2016.10.026

Lucon-Xiccato, T., & Bisazza, A. 2017b. Individual differences in cognition among teleost fishes. Behavioural Processes, 141, 184-195. https://doi.org/10.1016/j.beproc.2017.01.015

Luttbeg, B., Sih, A., 2010. Risk, resources and state-dependent adaptive behavioural syndromes. Philos. Trans. R. Soc. B Biol. Sci. 365, 3977–3990. https://doi.org/10.1098/rstb.2010.0207

Macario, A., Darden, S.K., Verbruggen, F., Croft, D.P., 2021. Intraspecific variation in inhibitory motor control in guppies, *Poecilia reticulata*. J. Fish Biol. 98, 317–328. https://doi.org/10.1111/jfb.14608

Marasco, V., Spencer, K.A., Robinson, J., Herzyk, P., Costantini, D., 2013. Developmental post-natal stress can alter the effects of pre-natal stress on the adult redox balance. Gen. Comp. Endocrinol. 191, 239–246. https://doi.org/10.1016/j.ygcen.2013.07.003

Mariette, M., Kelley, J.L., Brooks, R., Evans, J.P., 2006. The effects of inbreeding on male courtship behaviour and coloration in guppies. Ethology 112, 807–814. https://doi.org/10.1111/j.1439-0310.2006.01236.x

Metcalfe, N.B., Alonso-Alvarez, C., 2010. Oxidative stress as a life-history constraint: the role of reactive oxygen species in shaping phenotypes from conception to death. Funct. Ecol. 24, 984–996. https://doi.org/10.1111/j.1365-2435.2010.01750.x

Monaghan, P., 2008. Early growth conditions, phenotypic development and environmental change. Philos. Trans. R. Soc. B Biol. Sci. 363, 1635–1645. https://doi.org/10.1098/rstb.2007.0011

Müller, T., Juškauskas, A., 2018. Inbreeding affects personality and fitness of a leaf beetle. Anim. Behav. 138, 29–37. https://doi.org/10.1016/j.anbehav.2018.02.002

Nakadate, M., Shikano, T., Taniguchi, N., 2003. Inbreeding depression and heterosis in various quantitative traits of the guppy, *Poecilia reticulata*. Aquaculture 220, 219–226. https://doi.org/10.1016/S0044-8486(02)00432-5

Nakajima, M., Fujisawa, K., Taniguchi, N., 2009. Estimation of the mode of inheritance of thermal tolerance in the guppy *Poecilia reticulata*. Fish. Sci. 75, 683–687. https://doi.org/10.1007/s12562-009-0089-x

Nepoux, V., Haag, C.R., Kawecki, T.J., 2010. Effects of inbreeding on aversive learning in Drosophila. J. Evol. Biol. 23, 2333–2345. https://doi.org/10.1111/j.1420-9101.2010.02094.x

Nettle, D., Bateson, M., 2015. Adaptive developmental plasticity: what is it, how can we recognize it and when can it evolve? Proc. R. Soc. B Biol. Sci. 282, 20151005. https://doi.org/10.1098/rspb.2015.1005

Ólafsdóttir, G.Á., Magellan, K., 2016. Interactions between boldness, foraging performance and behavioural plasticity across social contexts. Behav Ecol Sociobiol 70, 1879–1889. https://doi.org/10.1007/s00265-016-2193-0

Opatová, P., Ihle, M., Albrechtová, J., Tomášek, O., Kempenaers, B., Forstmeier, W., Albrecht, T., 2016. Inbreeding depression of sperm traits in the zebra finch *Taeniopygia guttata.* Ecol. Evol. 6, 295–304. https://doi.org/10.1002/ece3.1868

Pilakouta, N., Smiseth, P.T., 2017. Female mating preferences for outbred versus inbred males are conditional upon the female's own inbreeding status. Anim. Behav. 123, 369–374. https://doi.org/10.1016/j.anbehav.2016.11.023

Prentice, P.M., Thornton, A., Kolm, N., Wilson, A.J., 2023. Genetic and context-specific effects on individual inhibitory control performance in the guppy (*Poecilia reticulata*). J. Evol. Biol. 36, 1796–1810. https://doi.org/10.1111/jeb.14241

Price, E.O., 1999. Behavioral development in animals undergoing domestication. Appl. Anim. Behav. Sci. 65, 245–271. https://doi.org/10.1016/S0168-1591(99)00087-8

R Core Team, 2022. R: The R Project for Statistical Computing. URL https://www.r-project.org/.

Rahman, Md. Moshiur, Pinkey, I.A., Ferthous, J., Arafat, S.T., Rahman, S.M., Asaduzzaman, Md., Rahman, Md. Mostafizur, Rouf, M.A., 2020. Modulation of phenotypic traits under different rearing temperatures: Experimental evidence in male guppy (*Poecilia reticulata*). Int. J. Aquat. Biol. 8. https://doi.org/10.22034/ijab.v8i5.856

Reed, D.H., Fox, C.W., Enders, L.S., Kristensen, T.N., 2012. Inbreeding–stress interactions: evolutionary and conservation consequences. Ann. N. Y. Acad. Sci. 1256, 33–48. https://doi.org/10.1111/j.1749-6632.2012.06548.x

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. Biol. J. Linn. Soc. 111, 511–520. https://doi.org/10.1111/bij.12217

Reid, J.M., Arcese, P., Cassidy, A.L.E.V., Hiebert, S.M., Smith, J.N.M., Stoddard, P.K., Marr, A.B., Keller, L.F., 2004. Song repertoire size predicts initial mating success in male song sparrows, *Melospiza melodia*. Anim. Behav. 68, 1055–1063. https://doi.org/10.1016/j.anbehav.2004.07.003

Reznick, D., Nunney, L., Tessier, A., 2000. Big houses, big cars, superfleas and the costs of reproduction. Trends Ecol. Evol. 15, 421–425. https://doi.org/10.1016/S0169-5347(00)01941-8

Richardson, J., Comin, P., Smiseth, P.T., 2018. Inbred burying beetles suffer fitness costs from making poor decisions. Proc. R. Soc. B Biol. Sci. 285, 20180419. https://doi.org/10.1098/rspb.2018.0419

Richardson, J., Smiseth, P.T., 2017. Intraspecific competition and inbreeding depression: increased competitive effort by inbred males is costly to outbred opponents. Am. Nat. 189, 539–548. https://doi.org/10.1086/691328

Rubenstein, D.R., Skolnik, H., Berrio, A., Champagne, F.A., Phelps, S., Solomon, J., 2016. Sex-specific fitness effects of unpredictable early life conditions are associated with DNA methylation in the avian glucocorticoid receptor. Mol. Ecol. 25, 1714–1728. https://doi.org/10.1111/mec.13483

Savaşçı, B.B., Lucon-Xiccato, T., Bisazza, A., 2021. Ontogeny and personality affect inhibitory control in guppies, *Poecilia reticulata*. Anim. Behav. 180, 111–121. https://doi.org/10.1016/j.anbehav.2021.08.013

Schneider, C.A., Rasband, W.S., Eliceiri, K.W., 2012. NIH Image to ImageJ: 25 years of image analysis. Nat. Methods 9, 671–675. https://doi.org/10.1038/nmeth.2089

Schou, M.F., Loeschcke, V., Kristensen, T.N., 2015. Inbreeding depression across a nutritional stress continuum. Heredity 115, 56–62. https://doi.org/10.1038/hdy.2015.16

Shettleworth, S.J., 2009. Cognition, evolution, and behavior, Oxford University Press, Oxford, U.K

Siegal, M.L., Bergman, A., 2002. Waddington's canalization revisited: Developmental stability and evolution. Proc. Natl. Acad. Sci. 99, 10528–10532. https://doi.org/10.1073/pnas.102303999

Smallbone, W., van Oosterhout, C., Cable, J., 2016. The effects of inbreeding on disease susceptibility: *Gyrodactylus turnbulli* infection of guppies, *Poecilia reticulata*. Exp. Parasitol. 167, 32–37. https://doi.org/10.1016/j.exppara.2016.04.018

Soravia, C., Ashton, B.J., Thornton, A., Ridley, A.R., 2021. The impacts of heat stress on animal cognition: Implications for adaptation to a changing climate. WIREs Clim. Change 12, e713. https://doi.org/10.1002/wcc.713

Syukri, F., Nadiatul, H.H., Nakajima, T., Nakajima, M., 2020. Inbreeding effect towards genetic markers and thermal tolerance in guppy *Poecilia reticulate*. J. Environ. Biol. 41, 1194–1203. https://doi.org/10.22438/jeb/41/5(SI)/MS_11

Szabo, B., Hoefer, S., Whiting, M.J., 2020. Are lizards capable of inhibitory control? Performance on a semi-transparent version of the cylinder task in five species of Australian skinks. Behav. Ecol. Sociobiol. 74, 118. https://doi.org/10.1007/s00265-020-02897-y

Taborsky, B., 2017. Chapter Three - Developmental plasticity: preparing for life in a complex world, in: Naguib, M., Podos, J., Simmons, L.W., Barrett, L., Healy, S.D., Zuk, M. (Eds.), Adv Stud Beh. Academic Press, pp. 49–99. https://doi.org/10.1016/bs.asb.2016.12.002

Triki, Z., Fong, S., Amcoff, M., Kolm, N., 2022. Artificial mosaic brain evolution of relative telencephalon size improves inhibitory control abilities in the guppy (*Poecilia reticulata*). Evolution 76, 128–138. https://doi.org/10.1111/evo.14405

Troy Harker, K., Whishaw, I.Q., 2002. Place and matching-to-place spatial learning affected by rat inbreeding (Dark–Agouti, Fischer 344) and albinism (Wistar, Sprague–Dawley) but not domestication (wild rat vs. Long–Evans, Fischer–Norway). Behav. Brain Res. 134, 467–477. https://doi.org/10.1016/S0166-4328(02)00083-9

Valtonen, T.M., Roff, D.A., Rantala, M.J., 2011. Analysis of the effects of early nutritional environment on inbreeding depression in *Drosophila melanogaster*. J. Evol. Biol. 24, 196–205. https://doi.org/10.1111/j.1420-9101.2010.02157.x

van der Bijl, W., Thyselius, M., Kotrschal, A., Kolm, N., 2015. Brain size affects the behavioural response to predators in female guppies (*Poecilia reticulata*). Proc. R. Soc. B Biol. Sci. 282, 20151132. https://doi.org/10.1098/rspb.2015.1132

van Horik, J. O., Langley, E. J., Whiteside, M. A., Laker, P. R., Beardsworth, C. E., Madden, J. R. 2018. Do detour tasks provide accurate assays of inhibitory control? P R SOC B, 285(1875), 20180150. https://doi.org/10.1098/rspb.2018.0150

van Oosterhout, C., Trigg, R.E., Carvalho, G.R., Magurran, A.E., Hauser, L., Shaw, P.W., 2003. Inbreeding depression and genetic load of sexually selected traits: how the guppy lost its spots. J. Evol. Biol. 16, 273–281. https://doi.org/10.1046/j.1420-9101.2003.00511.x

Vega-Trejo, R., de Boer, R.A., Fitzpatrick, J.L., Kotrschal, A., 2022. Sex-specific inbreeding depression: A meta-analysis. Ecol. Lett. 25, 1009–1026. https://doi.org/10.1111/ele.13961

Vega-Trejo, R., Head, M.L., Jennions, M.D., 2016. Inbreeding depression does not increase after exposure to a stressful environment: a test using compensatory growth. BMC Evol. Biol. 16, 68. https://doi.org/10.1186/s12862-016-0640-1

Vega-Trejo, R., Head, M.L., Jennions, M.D., 2015. Evidence for inbreeding depression in a species with limited opportunity for maternal effects. Ecol. Evol. 5, 1398–1404. https://doi.org/10.1002/ece3.1445

Vega-Trejo, R., Kruuk, L.E.B., Jennions, M.D., Head, M.L., 2018. What happens to offspring when parents are inbred, old or had a poor start in life? Evidence for sex-specific parental effects. J. Evol. Biol. 31, 1138–1151. https://doi.org/10.1111/jeb.13292

Vila Pouca, C., Gervais, C., Reed, J., Michard, J., Brown, C., 2019. Quantity discrimination in Port Jackson sharks incubated under elevated temperatures. Behav. Ecol. Sociobiol. 73, 93. https://doi.org/10.1007/s00265-019-2706-8

Vila-Pouca, C., Vedder, S., Kotrschal, A., 2022. Hybridization may promote variation in cognitive phenotypes in experimental guppy hybrids. Am. Nat. 200, 607–619. https://doi.org/10.1086/720731

Vinogradov, I.M, 2024. Cognitive abilities in fish: biological predictors and impact on reproductive success. Doctoral dissertation, Australian National University. https://doi.org/10.25911/RYJ7-6W44

Vinogradov, I.M., Jennions, M.D., van Veen, E., Fichtel, C., Kappeler, Peter.M., Fox, R.J., 2022. The effect of sex, age and boldness on inhibitory control. Anim. Behav. 193, 133–143. https://doi.org/10.1016/j.anbehav.2022.09.011

Waddington, C.H., 1942. Canalization of development and the inheritance of acquired characters. Nature 150, 563–565. https://doi.org/10.1038/150563a0

Zajitschek, S.R.K., Brooks, R.C., 2008. Distinguishing the effects of familiarity, relatedness, and color pattern rarity on attractiveness and measuring their effects on sexual selection in guppies (*Poecilia reticulata*). Am. Nat. 172, 843–854. https://doi.org/10.1086/593001

Zajitschek, S.R.K., Lindholm, A.K., Evans, J.P., Brooks, R.C., 2009. Experimental evidence that high levels of inbreeding depress sperm competitiveness. J. Evol. Biol. 22, 1338–1345. https://doi.org/10.1111/j.1420-9101.2009.01738.x

Supplementary Materials

Supplementary Figures

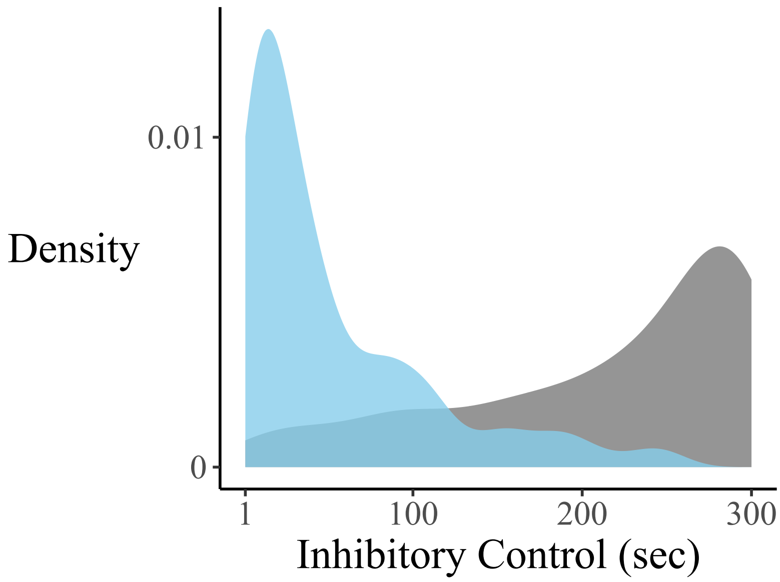

Figure S1. Density plots of inhibitory control (time spent trying to swim through the transparent barrier) for guppies that either solved the task within the allocated 5 minutes (in blue) or failed (in grey). Data is pooled across all trials and treatments.

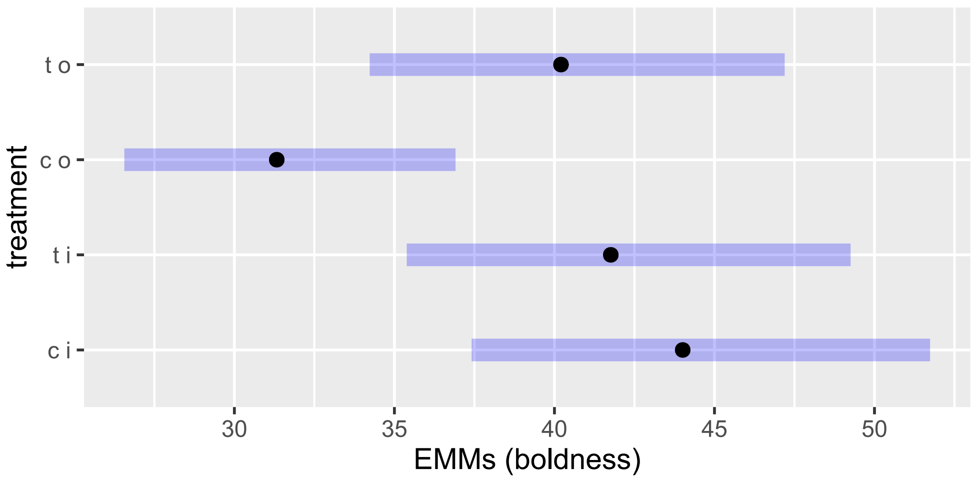

Figure S2. Estimated marginal means and 95% confidence intervals for the mixed effect model predicting boldness of guppies. The model includes an interaction of two factors (abbreviated): temperature (c/t = control 26°C /treatment 30°C); inbreeding status (o/i = outbred/inbred). Treatments with non-overlapping confidence intervals have significantly different marginal means.

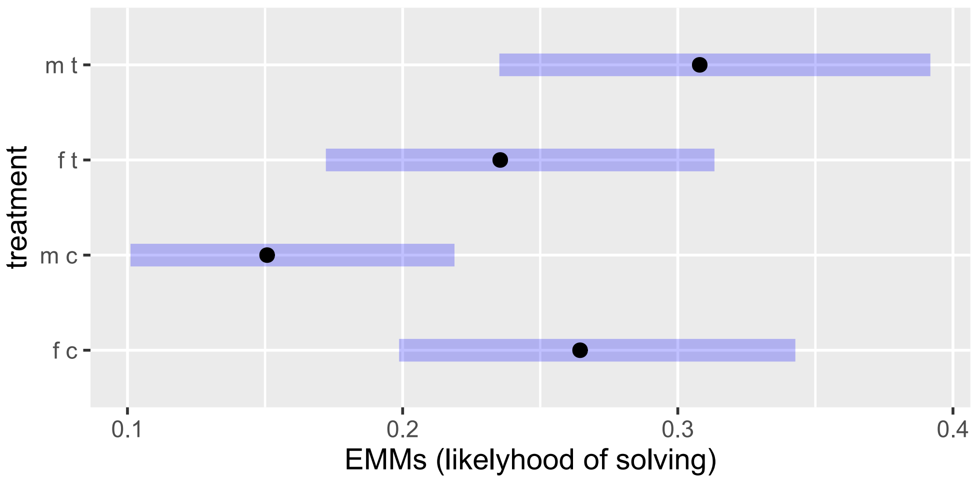

Figure S3. Estimated marginal means and 95% confidence intervals for the zero-inflated mixed effects model predicting likelihood of guppies to solve the task in the allotted 5 minutes. The model includes an interaction of two factors (abbreviated): sex (m/f = male/female); temperature (c/t = control 26°C /treatment 30°C). The only significant difference between the marginal means is between the males from the control and the high temperature treatment (*P* = 0.027).

Supplementary Tables for Linear Models

Boldness models

Table S1. Parameter estimates from an initial hurdle negative binomial mixed model with 3-way interactions predicting boldness in male and female guppies *Poecilia reticulata*. The output is shown for the fixed effects in the conditional model (positive estimates indicate slower emergence time, hence less bold) and in the zero-inflation model (positive estimates indicated higher likelihood of boldness being 0, meaning that a fish failed to emerge from the start zone within 3 minutes). Reference level for the categorical predictors are shown in square brackets. Standard errors (SE) and *P* values were obtained from the model summary, while chi-square values and their *P* values were calculated with likelihood ratio tests. Significant results are emboldened.

Predictor	Estimate	SE	Z	*P*	χ^2	*P* (χ^2)
Conditional model						
Sex [m]	-0.19	0.16	-1.20	**0.23**	10.13	**0.001**
Temp [26 °C]	-0.03	0.16	-0.18	0.85	1.38	0.24
Breeding [outbred]	-0.22	0.16	-1.38	0.17	5.25	**0.02**
Trial	-0.09	0.02	-3.61	**< 0.001**	13.00	**< 0.001**
Body size (scaled)	0.09	0.04	2.07	**0.04**	4.30	**0.03**
Sex [m]: Temp [26 °C]	-0.04	0.23	-0.17	0.86	0.35	0.56
Sex [m]: Breeding [outbred]	-0.22	0.23	-0.96	0.34	0.26	0.61
Temp [26°C]: Breeding [outbred]	0.16	0.23	0.68	0.49	3.35	0.07
Sex [m]: Temp [26 °C]: Breeding [outbred]	0.27	0.32	0.84	0.40	0.71	0.40
Zero-inflation						
Sex [m]	0.01	0.74	0.02	0.99	7.88	**0.005**
Temp [26 °C]	0.36	0.71	0.51	0.61	0.30	0.58
Breeding [outbred]	0.72	0.68	1.06	0.29	0.005	0.94
Trial	-0.35	0.14	-2.56	**0.01**	6.02	**0.01**
Body size (scaled)	-0.22	0.19	-1.17	0.24	1.84	0.17
Sex [m]: Temp [26 °C]	-0.74	1.05	-0.70	0.48	0.60	0.44
Sex [m]: Breeding [outbred]	-1.34	1.06	-1.26	0.21	3.42	0.06
Temp [26 °C]: Breeding [outbred]	-0.66	0.96	-0.69	0.49	0.37	0.54
Sex [m]: Temp [26 °C]: Breeding [outbred]	0.54	1.53	0.35	0.72	0.08	0.78

Table S2. Parameter estimates from an initial hurdle negative binomial mixed model with 2-way interactions predicting boldness in male and female guppies *Poecilia reticulata*. The output is shown for the fixed effects in the conditional model (positive estimates indicate slower emergence time, hence less bold) and in the zero-inflation model (positive estimates indicated higher likelihood of boldness being 0, meaning that a fish failed to emerge from the start zone within 3 minutes). Reference level for the categorical predictors are shown in square brackets. Standard errors (SE) and *P* values were obtained from the model summary, while chi-square values and their *P* values were calculated with likelihood ratio tests. Significant results are emboldened.

Predictor	Estimate	SE	Z	*P*	χ^2	*P* (χ^2)
Conditional model						
Sex [m]	-0.26	0.14	-1.85	0.06	10.10	**0.002**
Temp [26 °C]	-0.10	0.14	-0.71	0.48	1.37	0.24
Breeding [outbred]	-0.29	0.14	-2.07	**0.04**	5.24	**0.02**
Trial	-0.09	0.02	-3.60	**0.0003**	12.93	**< 0.001**
Body size (scaled)	0.09	0.04	2.05	**0.04**	4.22	**0.04**
Sex [m]: Temp [26 °C]	0.10	0.17	0.59	0.56	0.34	0.55
Sex [m]: Breeding [outbred]	-0.08	0.16	-0.51	0.61	0.26	0.61
Temp [26°C]: Breeding [outbred]	0.29	0.16	1.83	0.07	3.33	0.07
Zero-inflation						
Sex [m]	-0.11	0.65	-0.17	0.87	7.88	**0.005**
Temp [26 °C]	0.24	0.63	0.39	0.70	0.30	0.58
Breeding [outbred]	0.61	0.61	1.01	0.31	0.01	0.94
Trial	-0.35	0.14	-2.56	**0.01**	6.01	**0.01**
Body size (scaled)	-0.23	0.19	-1.18	0.24	1.85	0.17
Sex [m]: Temp [26 °C]	-0.49	0.78	-0.63	0.53	0.60	0.44
Sex [m]: Breeding [outbred]	-1.08	0.77	-1.41	0.16	3.42	0.07
Temp [26 °C]: Breeding [outbred]	-0.45	0.75	-0.60	0.55	0.37	0.54

Inhibitory control models

Table S3. Parameter estimates from an initial hurdle negative binomial mixed model with 3-way interactions predicting inhibitory control in male and female guppies *Poecilia reticulata*. The model includes data only for a subset of fish that reached the goal zone within allocated 5 minutes (see *Methods*). The output is shown for the fixed effects in the conditional model and in the zero-inflation model (i.e., likelihood of inhibitory control being 0 in trials where a fish did not attempt to swim through the transparent barrier). Reference level for the categorical predictors are shown in square brackets. Standard errors (SE), and *P* values were obtained from the model summary, while chi-square values and their *P* values were calculated with likelihood ratio tests. Significant results are emboldened.

Predictor	Estimate	SE	Z	*P*	χ^2	*P* (χ^2)
Conditional model						
Sex [m]	0.12	0.26	0.48	0.63	0.01	0.98
Temp [26 °C]	0.18	0.26	0.69	0.49	0.81	0.37
Breeding [outbred]	0.15	0.26	0.57	0.57	0.86	0.35
Trial	-0.02	0.05	-0.45	0.65	0.20	0.65
Body size (scaled)	0.16	0.07	2.35	**0.02**	5.51	**0.02**
Boldness	0.00	0.00	1.43	0.15	2.06	0.15
Sex [m]: Temp [26 °C]	0.04	0.38	0.11	0.91	0.27	0.60
Sex [m]: Breeding [outbred]	-0.36	0.35	-1.02	0.31	1.16	0.28
Temp [26 °C]: Breeding [outbred]	-0.25	0.35	-0.70	0.48	0.38	0.54
Sex [m]: Temp [26 °C]: Breeding [outbred]	0.18	0.49	0.37	0.71	0.14	0.71
Zero-inflation						
Sex [m]	-0.20	0.43	-0.45	0.65	0.01	0.91
Temp [26 °C]	-0.69	0.46	-1.49	0.14	2.18	0.14
Breeding [outbred]	-0.60	0.46	-1.31	0.19	0.08	0.78
Trial	0.27	0.09	2.92	**0.003**	7.46	**0.006**
Body size (scaled)	0.04	0.12	0.34	0.74	0.01	0.91
Boldness	-0.00	0.00	-0.77	0.44	1.28	0.26
Sex [m]: Temp [26 °C]	0.55	0.66	0.84	0.40	0.34	0.56
Sex [m]: Breeding [outbred]	0.77	0.63	1.22	0.22	0.01	0.93
Temp [26 °C]: Breeding [outbred]	1.06	0.65	1.64	0.10	0.45	0.50
Sex [m]: Temp [26 °C]: Breeding [outbred]	-1.59	0.92	-1.72	0.09	3.08	0.08

Table S4. Parameter estimates from an initial hurdle negative binomial mixed model with 2-way interactions predicting inhibitory control in male and female guppies *Poecilia reticulata*. The model includes data only for a subset of fish that reached the goal zone within allocated 5 minutes (see *Methods*). The output is shown for the fixed effects in the conditional model and in the zero-inflation model (i.e., likelihood of inhibitory control being 0 in trials where a fish did not attempt to swim through the transparent barrier). Reference level for the categorical predictors are shown in square brackets. Standard errors (SE), and *P* values were obtained from the model summary, while chi-square values and their *P* values were calculated with likelihood ratio tests. Significant results are emboldened.

Predictor	Estimate	SE	Z	*P*	χ^2	*P* (χ^2)
Conditional model						
Sex [m]	0.07	0.22	0.33	0.74	0.01	0.98
Temp [26 °C]	0.13	0.22	0.58	0.56	0.82	0.37
Breeding [outbred]	0.10	0.22	0.44	0.66	0.85	0.36
Trial	-0.03	0.05	-0.46	0.64	0.21	0.64
Body size (scaled)	0.16	0.07	2.37	**0.02**	5.62	**0.02**
Boldness	0.00	0.00	1.42	0.16	2.02	0.16
Sex [m]: Temp [26 °C]	0.14	0.27	0.53	0.60	0.28	0.60
Sex [m]: Breeding [outbred]	-0.27	0.25	-1.07	0.28	1.15	0.28
Temp [26 °C]: Breeding [outbred]	-0.15	0.25	-0.62	0.54	0.38	0.54
Zero-inflation						
Sex [m]	0.15	0.38	0.40	0.69	0.01	0.91
Temp [26 °C]	-0.29	0.39	-0.73	0.47	2.18	0.14
Breeding [outbred]	-0.21	0.39	-0.54	0.59	0.08	0.78
Trial	0.27	0.09	2.93	**0.003**	7.52	**0.006**
Body size (scaled)	0.05	0.12	0.40	0.69	0.03	0.86
Boldness	-0.00	0.00	-0.84	0.40	1.41	0.23
Sex [m]: Temp [26 °C]	-0.24	0.47	-0.52	0.60	0.34	0.56
Sex [m]: Breeding [outbred]	0.03	0.46	0.06	0.96	0.01	0.93
Temp [26 °C]: Breeding [outbred]	0.28	0.46	0.61	0.55	0.45	0.50

Solving time models

Table S5. Parameter estimates from an initial hurdle negative binomial mixed model with 3-way interactions predicting solving time in male and female guppies *Poecilia reticulata*. The output is shown for the fixed effects in the conditional model and fixed effects and their interactions in the zero-inflation model (i.e. likelihood of solving time being 0, which refers to trials that were not solved within 5 minutes of leaving the start zone). Reference level for the categorical predictors are shown in square brackets. Standard errors (SE), and P values were obtained from the model summary, while chi-square values and their *P* values were calculated with likelihood ratio test. Significant results are emboldened.

Predictor	Estimate	SE	Z	*P*	χ^2	*P* (χ^2)
Conditional model						
Sex [m]	0.12	0.15	0.82	0.41	0.10	0.76
Temp [26 °C]	0.09	0.16	0.55	0.58	1.05	0.30
Breeding [outbred]	0.03	0.16	0.17	0.86	0.62	0.43
Trial	-0.14	0.03	-4.43	**< 0.001**	19.65	**< 0.001**
Body size (scaled)	0.03	0.04	0.74	0.46	0.54	0.46
Boldness	0.002	0.001	2.00	**0.05**	4.01	**0.05**
Sex [m]: Temp [26 °C]	-0.15	0.23	-0.66	0.51	0.02	0.89
Sex [m]: Breeding [outbred]	-0.28	0.21	-1.30	0.19	1.05	0.31
Temp [26°C]: Breeding [outbred]	0.00	0.23	0.00	0.99	0.67	0.41
Sex [m]: Temp [26 °C]: Breeding [outbred]	0.25	0.31	0.81	0.42	0.65	0.42
Zero-inflation						
Sex [m]	-1.24	1.01	-1.23	0.22	1.69	0.19
Temp [26 °C]	0.37	1.00	0.37	0.71	11.95	**< 0.001**
Breeding [outbred]	0.71	1.00	0.71	0.48	2.24	0.13
Trial	-0.32	0.11	-2.76	**0.01**	6.68	**0.01**
Body size (scaled)	0.11	0.26	0.44	0.66	0.06	0.81
Boldness	0.002	0.002	0.79	0.43	2.96	0.08
Sex [m]: Temp [26 °C]	1.99	1.41	1.42	0.16	9.87	**0.002**
Sex [m]: Breeding [outbred]	-1.21	1.42	-0.85	0.39	0.97	0.32
Temp [26 °C]: Breeding [outbred]	-1.30	1.41	-0.92	0.36	2.18	0.14
Sex [m]: Temp [26 °C]: Breeding [outbred]	0.67	1.97	0.34	0.73	0.25	0.62

Table S6. Parameter estimates from an initial hurdle negative binomial mixed model with 2-way interactions predicting solving time in male and female guppies *Poecilia reticulata*. The output is shown for the fixed effects in the conditional model and fixed effects and their interactions in the zero-inflation model (i.e. likelihood of solving time being 0, which refers to trials that were not solved within 5 minutes of leaving the start zone). Reference level for the categorical predictors are shown in square brackets. Standard errors (SE), and P values were obtained from the model summary, while chi-square values and their *P* values were calculated with likelihood ratio test. Significant results are emboldened.

Predictor	Estimate	SE	Z	*P*	χ^2	*P* (χ^2)
Conditional model						
Sex [m]	0.04	0.15	0.26	0.80	0.37	0.54
Temp [26 °C]	-0.04	0.15	-0.24	0.81	0.66	0.42
Breeding [outbred]	-0.07	0.16	-0.43	0.67	0.30	0.58
Trial	-0.19	0.03	-5.61	**< 0.001**	31.50	**< 0.001**
Body size (scaled)	-0.00	0.05	-0.05	0.96	0.00	0.96
Boldness	0.00	0.00	2.11	**0.04**	4.43	**0.04**
Sex [m]: Temp [26 °C]	-0.01	0.18	-0.07	0.94	0.01	0.94
Sex [m]: Breeding [outbred]	-0.17	0.17	-0.97	0.33	0.94	0.33
Temp [26°C]: Breeding [outbred]	0.23	0.17	1.31	0.19	1.72	0.19
Zero-inflation						
Sex [m]	-1.41	0.88	-1.61	0.11	1.69	0.19
Temp [26 °C]	0.18	0.86	0.21	0.84	11.95	**< 0.001**
Breeding [outbred]	0.53	0.87	0.61	0.54	2.24	0.13
Trial	-0.30	0.11	-2.72	**0.01**	6.60	**0.01**
Body size (scaled)	0.11	0.26	0.44	0.66	0.06	0.80
Boldness	0.00	0.00	0.79	0.43	3.12	0.08
Sex [m]: Temp [26 °C]	2.33	1.04	2.25	**0.02**	9.87	**0.002**
Sex [m]: Breeding [outbred]	-0.86	1.00	-0.87	0.39	0.97	0.32
Temp [26 °C]: Breeding [outbred]	-0.93	0.98	-0.94	0.35	2.18	0.14