Winner-loser effects on life history traits

Lauren M. Harrison*[1,2], Oliver P. Stuart[1,3] & Michael D. Jennions[1,4]

1. Division of Ecology & Evolution, Research School of Biology, The Australian National University, 46 Sullivans Creek Road, Acton, ACT, 2600, Australia.
2. School of Biological Sciences, University of East Anglia, Norwich Research Park, Norwich NR4 7TJ, UK.
3. School of Biological, Earth and Environmental Sciences, Distillery Fields, North Mall, University College Cork T23 N73K, Ireland.
4. Stellenbosch Institute for Advanced Study (STIAS), Wallenberg Research Centre at Stellenbosch University, Stellenbosch 7600, South Africa.

* Corresponding author: Lauren.M.Harrison@uea.ac.uk

ORCIDs:
L.M.H.: 0000-0002-6690-5035
O.P.S.: 0000-0002-8815-5829
M.D.J.: 0000-0001-9221-2788

Keywords: male-male competition, ageing, mating costs, telomeres, oxidative stress, mosquitofish

Abstract

Ageing of adult males could be accelerated by both high mating/reproductive effort and fighting for mates. Testing the relative importance of these factors is challenging, however, because males that win fights also tend to have more mates. We used a 2 x 2 experimental design to test how a prolonged (9 week) period of either winning or losing fights, and either high or low reproductive effort (manipulating by varying access to females) interact to affect male ageing and future reproduction allocation in the mosquitofish, *Gambusia holbrooki*. We measured telomere length and several life-history traits, including mating effort and ejaculates (sperm count and velocity). After 9 weeks there were significant differences between winners and losers in their mating effort, but not in their ejaculates. Males with a higher past reproductive effort (i.e. access to females) had significantly lower current mating effort and grew more slowly. Males with a higher past reproductive effort also had slower swimming sperm, but only if they were smaller than average in body size. Surprisingly, neither males with a higher past reproductive effort nor males that repeatedly lost fights had shorter telomeres. Our findings show that past social dynamics affect how males allocate resources to reproduction and somatic maintenance.

Introduction

Life history theory is based on organisms having limited available resources that create trade-offs in investment among growth, reproduction and somatic maintenance (Stearns, 1989; Lemaître *et al.*, 2024). Sexual selection on males, due to intense male-male competition for mating opportunities and sperm competition to fertilise eggs, tends to favour greater investment into reproduction than somatic maintenance than occurs in females (Harshman & Zera, 2007; Lemaître *et al.*, 2020a). For instance, males in polygynous species that face intense competition for females tend to have relatively lower lifetime breeding success than monogamous species (Lukas & Clutton-Brock, 2014) because of costs associated with mating competition that reduce male lifespan (Bonduriansky *et al.*, 2008; Lemaître *et al.*, 2020b). Males that monopolise access to females also face more frequent challenges from rivals. These challenges increase the risk of injuries, elevate energetic costs (Goymann & Wingfield, 2004), and select for greater investment into sexual traits over somatic maintenance (e.g., Hunt et al., 2004). These trade-offs between life-history traits for males can therefore increase ageing and shorten lifespan when males are in environments that favour greater investment into sexually selected traits (Bonduriansky *et al.*, 2008).

Social interactions can modulate life-history trade-offs for males. Being socially dominant has been shown to accelerate ageing in some mammals (Snyder-Mackler *et al*., 2020; Anderson *et al.*, 2021). Faster ageing of high-ranking males might be partly due to increased oxidative stress during the mating season as dominant males monopolise mating opportunities and breed more often (Beaulieu *et al.*, 2014). Increased levels of courtship, higher mating rates, more frequent fighting for access to mates, and, presumably, the stress associated with maintaining social dominance, could all hasten ageing because of the costs of reproduction (Bonduriansky *et al*., 2008).

One way to test for life-history trade-offs among reproduction and somatic maintenance is to determine how the outcomes of male-male contests influence telomeric attrition. Telomeres are repeating sequences of noncoding DNA at the end of chromosomes (Blackburn, 1984). Telomeres shorten when somatic cells divide (Haussmann & Marchetto, 2010). Stress can accelerate telomere loss when reactive oxygen species (e.g., free radicals; ROS), produced by metabolic processes and immune cells, exceeds the capacity of antioxidant defences to mitigate or repair damage (Houben *et al.*, 2008; Monaghan *et al*., 2009). Consequently, rates of telomeric attrition are linked to the levels of physiological stress experienced by animals (Angelier *et al.*, 2018), and telomere length is often used as an indicator of ageing and potential lifespan (Heidinger *et al.*, 2012; Dantzer & Fletcher, 2015). It should be noted, however, that some studies do not find a link between telomere length and lifespan (e.g. Eastwood *et al*., 2023).

There is some evidence that telomere length declines with greater male investment into reproduction (Bauch *et al.*, 2016; Parolini *et al.*, 2017; Taff & Freeman-Gallant, 2017; but see Morbiato *et al.*, 2023). Moreover, males that vary in how much they invest into different reproductive traits, as is the case when males pursue alternative mating strategies, might also differ in their rate of telomere shortening if some traits are more costly than others. For example, the red and yellow headed colour morphs of male painted dragons (*Ctenophorus pictus*) have different reproductive tactics which seems to affect their telomere length (Rollings *et al.*, 2017). Red-headed males that invest heavily into winning fights have shorter telomeres than yellow-headed males that invest more into ejaculates (Rollings *et al.*, 2017). The interpretation of this finding is challenging, however, as it is unclear whether a male's initial telomere length is linked to which morph it develops into, hence its reproductive strategy, or whether the strategies directly cause a change in the rate of telomere loss. Either process could generate the observed morph differences in telomere length. Collectively, however, these findings suggest that male-male competition and sperm competition could differ in their effect on rates of ageing and telomere loss. This does not, however, mean that sperm production is cheap (e.g., Morbiato *et al.*, 2023; Chung *et al.*, 2024), only that increased fighting appears to be more costly than increased sperm production for males. Determining causality is also challenging. Social dominance is often determined by factors like physical condition, body size or fighting ability (Hardy & Briffa, 2013). These factors might also influence telomere length due to correlations with early life environments or experiences (Lewin *et al.*, 2015; but see Wood *et al.*, 2021). Social dominance is also likely to be correlated with mating rates, and associated mating costs, because the winners of fights tend to have greater access to potential mates. The effects of fighting and mating rate on telomeres are therefore likely to be conflated.

Here, we test how the outcome of male-male contests (winning/losing), and variation in reproductive effort influence seven male life history traits, namely, three mating behaviours, sperm count and sperm velocity, body growth and relative telomere length. Prior contest experiences can influence future fight outcomes whereby winners are more likely to win subsequent fights, and *vice versa* for losers ("winner-loser effect") (Hsu *et al.*, 2006). Winning males often have a brief elevation in testosterone levels (Carré *et al.*, 2013), while losers have decreased expression of testosterone, or elevated levels of so-called 'stress hormones', such as glucocorticoids (Earley *et al.*, 2013). While the outcome of a single contest is unlikely to shorten telomeres, consistent differences in hormonal profiles from a history of consistently either winning or losing contests could exacerbate oxidative damage and accelerate telomeric attrition (Casagrande & Hau, 2019). We already know that prior contest experience can influence a male's current reproductive success (Harrison *et al.*, 2018, 2023; Filice & Dukas, 2019). Therefore, males that consistently win contests might adaptively increase their investment into reproductive rather than somatic traits to exploit their winning status (Harrison *et al.*, 2023). This leads to the prediction that somatic maintenance, ageing and telomere length will be affected by the winner-loser effect.

We conducted a fully factorial experiment with a poecilid fish to test whether the prolonged experience of always winning or always losing male-male contests (hereafter 'contest experience'), and either being with or without access to mating opportunities during this period ('reproductive treatment)' affect male life history traits. It should be noted that the main effect of the reproductive treatment is to allow or prevent focal males from mating/ejaculating. Consequently, males with full access to females ('contest and mating' group) should have greater reproductive effort than those without ('contest only' group) because they must invest in sperm replenishment after each mating. In addition, males with full access to females can also spend more time chasing the female which increases their investment in this activity. Males who encounter a female through the barrier tend to swim up and down the barrier 'tracking' the female, but they are unable to invest in the darting behaviour associated with an actual mating attempt, nor can they move towards the female if she moves away from the barrier.

We then tested how contest experience and reproductive treatment subsequently affect: a) investment in sexually selected reproduction traits, namely mating effort and ejaculates (sperm count and velocity); b) growth and mortality; and c) telomere length. We predict that: (i) winners will invest more heavily than losers into reproduction (based on Harrison *et al.*, 2018); (ii) the costs of increased reproductive effort due to greater past access to females will accelerate ageing and shorten telomeres (based on patterns observed in many taxa; reviewed in Sudyka, 2019).

We cannot predict the effect of winning/losing on telomere length because, while losing is stressful, it may also reduce a male's mating rate, lowering the associated costs of reproduction (e.g. sperm replenishment). Previous studies show that stressful abiotic environments accelerate telomere shortening (Chatelain *et al.*, 2020), but it remains unknown if consistently losing social contests has a similar effect. How the outcome of male-male contests, *independent* of an individual's condition, phenotype, or prior life experience (i.e., confounding factors that affect fight outcome but might be correlated with initial telomere length), affect telomere shortening is untested. In general, however, we expected any differences between winners and losers (whatever the direction of this difference might be) to be magnified if males previously had the opportunity to mate (i.e., were in the 'contest and mating' group) because this should increase their total past reproductive investment. We therefore predict that there will be a significant interaction between contest experience and reproductive treatment *if* contest experience has an effect on the measured trait.

Materials and methods

This study was pre-registered at the Open Science Foundation (OSF; https://osf.io/saj46/). We used a fully factorial 2x2 experimental design to test for winner-loser effects (contest experience), effects of past reproductive investment (reproductive treatment), and their interaction, on seven male life history traits, namely, three mating behaviours, sperm count and sperm velocity, body growth and relative telomere length (Figure 1).

Study species

In eastern mosquitofish, *Gambusia holbrooki*, males are aggressive toward each other, and larger males are socially dominant (Caldwell & Caldwell, 1962; McPeek, 1992). Contests between similarly sized males begin with lateral displays where males circle each other, then escalate to fin nipping, and end with the winner chasing the loser (McPeek, 1992). Both sexes mate multiply, and there is intense male-male competition for mates followed by sperm competition to fertilise eggs (Zane *et al.*, 1999). We have previously taken pairs of size matched males and experimentally made one of them a winner and the other a loser (the same approach we use here). We then allowed each pair of males to compete for a female. On average, prior winners spent significantly more time than prior losers near the female (Harrison *et al.*, 2018, 2023). Males invest heavily into both fighting and pursuing females and survive for only one breeding season (Kahn *et al.*, 2013). It is likely that male allocation of resources to reproduction and somatic maintenance over the breeding season depends on their prior social experiences.

Animal collection and maintenance

Mosquitofish were caught in streams in Canberra, Australia in December-January 2021-22. We collected recently matured fish. This was determined by the presence of a fully formed gonopodium (modified anal fin), and by the fact that we had monitored the field population to detect when males were starting to mature (i.e. no mature males were detected shortly before collections started). By collecting males at the start of the breeding season we minimized variation in adult age among our focal experimental males. Fish were housed in 90 L same-sex stock tanks (~50 fish/tank) at 28 ± 1 °C under 14 L:10 D light regime and fed fish flakes *ad libitum* twice daily. All experiments were conducted under protocol A2021/04 (ANU Animal Ethics Committee).

Creating winners and losers

We randomly selected focal males to assign to experimental treatments and followed them individually throughout the study (*n* = 176). We used males that spanned the natural size range (17.3-23.8 mm standard length (SL); *n* = 142; Table 1) as our previous work has shown that male mating responses to winning or losing contests are size-dependent: small males are more affected by winning, and large males by losing (Harrison *et al.*, 2018, 2023). Focal males were anaesthetized briefly to measure their standard body length (SL) and then marked with a subcutaneous elastomer tag (NorthWest Marine Technology, WA, USA). Tagged focal males were then kept in individual 1 L aquaria for a week to minimize the influence of recent social interactions prior to assigning males to a 'context experience' (Kasumovic *et al.*, 2010). Exact male ages were unknown, but males were randomly assigned to treatments so that, on average, pre-experimental telomere length should not differ across the four treatment groups.

After one week of isolation, focal males were randomly assigned to be winners or losers by being paired with either a smaller or larger rival. This method controls for intrinsic differences in fighting ability between males that might otherwise determine contest outcomes or affect investment into reproduction and somatic maintenance (Harrison *et al.*, 2018, 2023). In this way, males of all sizes can be made to consistently win or lose contests (two sample *t*-test of winner vs loser body size (week 0): $t = -0.11$, df = 140, $P = 0.91$; Table 1). Contests took place in 6 L aquaria that contained gravel, plastic plants for refugia, and had black plastic on three sides to minimize disturbance. Focal and rival males freely interacted for one week, after which a female was introduced to the tank to apply the reproductive treatment for another 8 weeks. In half the tanks the two males could freely interact with the female and therefore the focal male could fully invest in reproduction (i.e. chase the female and mate). In the other half of the tanks there was also a female present, but she was kept behind a mesh barrier to prevent the males from mating with her (Figure 1). Equal numbers of winner and loser focal males therefore either had full access to a female ('contests and mating') or only interacted with a female through the barrier ('contest only') (NB: 'contest' refers to the fact that all males competed with a rival for the full 9 weeks). Male mosquitofish with and without full access to females have previously been shown to differ in their subsequent reproductive investment (see naïve vs intact males with a female in Chung *et al.*, 2021, 2024). Rival males and stimulus females were rotated every 3-5 days so that focal males continued to fight to establish dominance, and to approach females (Vega-Trejo *et al.*, 2014). The total experimental period of 9 weeks is approximately half the lifespan of *G. holbrooki* at our field site (Kahn *et al.*, 2013; Chung *et al.*, 2024).

For logistic reasons the experiment was run in eleven blocks over time. Block identity is therefore included as a random factor in the statistical analyses.

Male mating behaviour

After 9 weeks, each focal male was placed in a new 6 L aquaria with a random stock female. Each female was used once. Male mating behaviour was observed for 20 mins. We recorded: (a) time spent near the female (<5 cm and facing her); (b) the number of mating attempts; and (c) the number of successful attempts (i.e., those with the potential to transfer sperm). Once the trial ended, we measured the female's body length using digital calipers (mean ± s.d.: 30.29 ± 3.34 mm, *n* = 142).

Ejaculates

Immediately following the mating behaviour trials, focal males were anaesthetised in ice slurry to measure their body length (SL) and to strip their sperm (O'Dea *et al.*, 2014; Vega-Trejo *et al.*, 2016). Males were then isolated for 5 days in 1 L aquaria to replenish their sperm reserves (O'Dea *et al.*, 2014), after which we again stripped them. We measured replenished sperm rather than sperm collected immediately after the mating trial as males might have varied in whether they inseminated females during their mating behaviour trial. Using replenished sperm allows us to test for the rate of sperm production (over 5 days) and swimming speed of sperm standardised for its age. Sperm collection and subsequent measurements were made blind to treatment by one of the authors (LMH). We had two ejaculate measures: total sperm count and sperm velocity.

We followed established protocols to measure sperm count and sperm velocity (O'Dea *et al.*, 2014; Harrison *et al.*, 2023). For sperm counts, we vortexed the sample to disperse sperm then pipetted 3 µL onto a 20 µm capillary slide (Leja), and used a CEROS Sperm Tracker (Hamilton Thorne Research, Beverly, MA, USA) to count sperm under x100 magnification. We randomly counted five subsamples per sample. These subsample counts were highly repeatable (R = 0.90; 95% CI: 0.86-0.93; *P* <0.001; from the R package *rptR* (Stoffel *et al.*, 2017)), so we used the average value per male for further analyses. We estimated total sperm counts by adding the average sperm number per bundle to account for the six bundles removed for sperm velocity analyses.

To measure sperm velocity, we took two samples of three sperm bundles from each male's ejaculate and pipetted the bundles into two separate PCR tubes containing 2 µL extender medium (Harrison *et al.*, 2023). We then pipetted each sample onto a cell of a 12-cell multi-test slide (MP Biomedicals, Aurora, OH, USA) coated with 1% polyvinyl alcohol solution (PVA). Sperm was 'activated' with 3 µL of solution (125 mM KCL and 2 mg/mL bovine serum albumin). We used a CEROS Sperm Tracker to record two measures of sperm velocity: VAP (average path velocity) and VCL (curvilinear velocity). VAP and VCL were highly correlated (*r* = 0.97; 95% CIs = 0.96, 0.98; *P* <0.0001; *n* = 97 males), so we only used VCL in our analyses as it is more biologically relevant. We measured sperm velocity immediately after sperm activation. The two measures per male of average sperm velocity were significantly repeatable (R = 0.29; 95% CI: 0.11-0.47; *P* = 0.003), and we used the weighted average in our analyses.

Relative telomere length

Focal males were euthanized following their final sperm stripping and their tails removed and stored in 80% ethanol at -20°C. We used a commercial tissue DNA extraction kit (Monarch® Genomic DNA Purification Kit, New England BioLabs, Australia) to extract and purify genomic DNA from the tail muscle tissue. Prior to extraction, tail muscle was left in the lysis buffer mixture overnight (~18 hours) on an Eppendorf ThermoMixer set at 56°C and maximum mixing speed. Genomic DNA was concentrated with 70 µL elution buffer and quantitated with a Qubit fluorometer prior to dilution to 20 ng/µL with 10 mM Tris.

Relative telomere length (rTL) was measured using real-time quantitative PCR (Cawthon, 2002), determined as the ratio (T/S) of telomere repeat length (T) to a single-copy reference gene length (S). We used standard telomere primers Tel1b (5′-CGGTTTGTTTGGGTTTGGGTTTGGGTTTGGGTTTGGGTT-3′) and Tel2b (5′-GGCTTGCCTTACCCTTACCCTTACCCTTACCCTTACCCT-3′) (Criscuolo *et al.*, 2009). Following previous studies that have measured teleost fish telomeres (Gao & Munch, 2015; Monteforte *et al.*, 2020; Morbiato *et al.*, 2023), we used a *Gambusia*-specific region of the melanocortin 1 receptor (MC1R) as our control single-copy reference gene with the primers MC1R.F (5'-CCTGTAGGCGTAGATGAGCG-3') and MC1R.R (5'-CACCAGTCCCTTCTGCAACT-3') (see Supplementary Material for full details).

We ran qPCRs for each sample in triplicate on 96-well plates. Telomere and MC1R amplifications were run concurrently on separate plates using QuantStudio3 (Thermo Fisher Scientific, Waltham, USA). For a given male, we first ran telomere qPCRs before immediately running MC1R qPCRs with each sample in the corresponding well position across plates to minimise variation. We used 5 µL PowerUp^{TM} SYBR^{TM} Green Master Mix with 300 nM of both forward and reverse primers (9 µL total volume) and 1 µL of 20 ng/µL DNA extract. The qPCR cycling profile for MC1R started at 95°C for 3 min for denaturation, followed by 40 cycles of 95°C for 15 s, 60°C for 30 s, and 72°C for 20 s for amplification. For telomeres, denaturation started at 95°C for 10 min, followed by 40 cycles of 95°C for 15 s, 60°C for 15 s, then 72°C for 15 s. Both profiles had a final cycle (15 s at 95°C, 1 min at 60°C, and 15 sec at 95°C) that generated melt curves to confirm qPCR specificity.

Each plate had three negative controls (9 µL reagent mix and 1 µL MilliQ purified water), two inter-plate control samples (run in triplicate, the same two individuals across all plates), and a golden sample at five DNA concentrations (0.05, 0.2, 1, 5 and 20 ng/µL) to generate the standard curve and determine the amplification efficiency of each plate (telomere: 1.99-2.11; MC1R: 1.93-2.01). The telomeres and MC1R cq (the number of PCR cycles taken to reach fluorescence threshold) were highly repeatable across the triplicate samples (telomere: $R = 0.77$, $SE = 0.03$, $P < 0.0001$; MC1R: $R = 0.95$, $SE = 0.01$, $P < 0.0001$). Relative telomere length was calculated using the equation in (Pfaffl, 2001, see Supplementary Material). Where possible, we follow the MIQE guidelines (Bustin *et al.*, 2009) for reporting telomere measurements and qPCR results (see Supplementary Material for data on amplification efficiency, repeatability of measures and more detailed information on how we calculated relative telomere length).

Statistical analyses

To analyse sources of variation in the three mating behaviours that we measured (number of mating attempts, number of successful attempts, time spent with the female), we ran separate generalised linear mixed models (GLMMs) with negative binomial error distributions (log-link functions). Our full models had contest experience (winner/loser), reproductive treatment ('contests only'/'contests and mating'), male size (SL) (week 0), and all three-way and two-way interactions as fixed factors. It should be noted that while males grow as adults, growth is minimal and size at week 0 and week 9 are highly correlated across males. Female size was also included as a covariate (no interaction terms) and experimental block (Block ID) as a random effect.

To analyse sources of variation in sperm count, sperm velocity, relative telomere length and growth, we first fit GLMMs with Gaussian error distributions (identity-link functions) with contest experience, reproductive treatment, male size, and all three-way and two-way interactions as fixed factors, and block identity as a random effect. As stated above, for relative telomere length, there was moderately low repeatability of telomere Cq across the triplicate samples. To account for this, we calculated rTL separately for each individual sample (three rTL measures per male; see Supplementary Materials). Relative telomere length models therefore included Male ID as a random effect to account for the use of three measures per male. For growth between weeks 0 and 9, models included male size (SL) at week 9 as the response variable and male size (SL) at week 0 (standardised and centred) as a covariate.

All statistical analyses were conducted using R version 4.0.2 (R Development Core Team, 2020). We used the *glmmTMB* package (Brooks *et al.*, 2017) to first fit GLMMs with different error distributions and link functions. We then used the *DHARMa* package (Hartig, 2020) to run model diagnostics and identify the best-fitting model. To quantify main effects, we removed non-significant interaction terms from the final model. We obtained significance of fixed effects, as reported in the text, from ANOVA type II Wald Chi-squared (χ^2) tests, or type III tests for models with interactions. We set $\alpha = 0.05$, except for three-way interaction terms where α was set at 0.01. All tests were two-tailed. Descriptive statistics are shown in Table 1 and full model outputs are shown in Supplementary Table S1.

Results

Male mating behaviour

On average, there was no difference between winners and losers in the number of mating attempts that males made, and no difference in mating attempts when males had or had not previously had full access to a female and could mate (Figure 2A).

Winners spent significantly more time than losers associating with the female ($\chi^2 = 28.45$, df = 1, $P < 0.0001$; Table 2), but they were not more successful at mating ($\chi^2 = 1.97$, df = 1, $P = 0.160$; Table 2; Figure 2B & C). Males that had previously been able to mate made significantly fewer successful mating attempts ($\chi^2 = 5.99$, df = 1, $P = 0.014$), and spent far less time associating with females ($\chi^2 = 27.10$, df = 1, $P < 0.0001$), than males previously without full access to females (Table 2).

Only one of the three mating behaviours we measured showed an interaction between past contest experience and reproductive treatment: there was a significant three-way interaction between male size, contest experience and reproductive treatment that affected the number of mating attempts (χ^2 = 10.44, df = 1, P = 0.001; Table 2). The number of mating attempts increased with male body size for winners when males had previously been able to mate but decreased if they had not; and the reverse pattern occurred for losers (Figure 3A). Neither male nor female body size significantly affected the number of successful mating attempts (male size: χ^2 = 2.37, df = 1, P = 0.124; female size: χ^2 = 1.69, df = 1, P = 0.194; Figure S1), nor the time spent near the female (male size: χ^2 = 0.45, df = 1, P = 0.501; female size: χ^2 = 0.92, df = 1, P = 0.338; Figure S1).

Sperm count and velocity

Contrary to our expectations, there were no significant interactions between past contest experience and reproductive treatment that affected either sperm count or velocity (Table 2; Figure S2). Winners and losers did not significantly differ in either sperm count (χ^2 = 0.06, df = 1, P = 0.804; Figure 3B) or sperm velocity (χ^2 = 0.94, df = 1, P = 0.333; Figure 3C). As expected, however, larger males had a higher sperm count (χ^2 = 4.00, df = 1, P = 0.047; Figure 3B).

Males that previously had or had not been able to mate did not differ in their sperm count (χ^2 = 0.30, df = 1, P = 0.582; Figure 3B), but there was a significant interaction with male size that affected sperm velocity (χ^2 = 7.97, df = 1, P = 0.005). Larger males had faster swimming sperm when they had previously been able to mate, but there was no effect of body size when males had not previously had full access to females (Figure 3C).

Growth

Adult male growth was negligible. Nevertheless, males that had previously been able to mate grew significantly slower than males that had not had full access to females (χ^2 = 5.62, df = 1, P = 0.018). Winning or losing did not affect male growth (χ^2 = 1.51, df = 1, P = 0.219) (Table 2; Figure 3D).

Relative telomere length

There was no significant interaction between past contest experience and the past opportunity to mate affecting relative telomere length (Table 2). Neither past contest experience (χ^2 = 1.63, df = 1, P = 0.201) nor the past opportunity to mate (χ^2 = 0.00, df = 1, P = 0.995) affected relative telomere length (Fig 4A). Larger males had significantly longer telomeres (χ^2 = 5.89, df = 1, P = 0.015; Figure 4B).

Mortality

Of the initial 176 focal males, 34 died during the 9-week mating treatment (~83% survival; Figure S3). In the contests only treatment, 3 winners and 13 losers died. In the contest and mating treatment, 7 winners and 11 losers died. We ran a *post hoc* Cox proportional hazards regression with contest experience, reproductive treatment, their two-way interaction, and male body size as fixed factors. We then removed the non-significant interaction between contest experience and reproduction treatment and reran the model to test the main effects (Supplementary Table S2). Being a winner (coefficient = -0.92, SE = 0.40, z = -2.44, P =

0.015), or a larger male (coefficient = -0.39, SE = 0.68, z = -0.39, P = 0.041), significantly increased the likelihood of survival. Interestingly, however, mortality did not differ between males that did or did not have the opportunity to mate (coefficient = 0.06, SE = 0.34, z = 0.06, P = 0.857). It should be noted that these are *post hoc* tests to determine if treatment type affected mortality and these tests were not listed in our OSF pre-registration.

Discussion

We used a 2 x 2 experimental design to test how a long-term history of winning or losing fights, in combination with either high or low past reproductive effort, influenced seven key life-history traits in the Eastern mosquitofish, *Gambusia holbrooki*. After 9 weeks there were significant differences between winners and losers in their mating effort; winners spent significantly more time than losers with a female and, depending on their size, also made more mating attempts. There were no differences between winners and losers in either their sperm count or sperm velocity. There was also no evidence that consistently winning or losing fights affected body growth or telomere length. In contrast, males that had previously had full access to females (i.e., greater past reproductive effort) had significantly lower current mating effort (measured as the time with female and number of successful mating attempts), slower swimming sperm (but only if the male was small bodied), and lower body growth. As with winning/losing, however, there was no detectable effect on telomere length. We discuss these findings in more detail in the following sections.

Past reproductive effort and current reproduction

We find strong evidence for a cost of past reproductive effort on current male reproduction. That is, males that had previously been able to mate (higher reproductive effort) made fewer successful copulation attempts and spent less time near females than was the case for males that were unable to mate. Males that could previously mate also had significantly lower growth (although growth during the experiment was negligible). There was therefore a detectable cost of the combined effect of incomplete access to females (hence less chasing of females) and sperm production, which is only possible for males with full access to females that could ejaculate and then had to replenish sperm, on both sexually and naturally selected traits. Intriguingly, however, there was no detectable effect of past reproductive effort on sperm quantity. The effects of past reproductive effort on sperm velocity depended on male size, but with the unexpected result that males who had previously been able to mate, hence replenish sperm, had *faster* swimming sperm, but only if they were larger males (see Figure 3C). Another experimental study of *G. holbrooki* in our lab by Chung and colleagues (2024) had a similar design but excluded a winner/loser treatment. This study reported very similar results as higher past reproductive effort caused males to spend less time chasing females, make fewer mating attempts and grow less (their 'naïve' vs 'mating + ejaculation' treatments equates to our 'with female' vs 'without female' treatments). The results differ, however, in that we did not detect a decline in ejaculate quality, while Chung and colleagues (2024) found that 'naïve' males had a significantly lower future sperm count, albeit with no decline in sperm velocity. The most substantive differences between the two studies are: (a) we had one rival male present, whereas they had two; (b) our focal males without access to a female could physically interact with their rival, while their focal males without access to a female (naïve' treatment) could only see their rivals; (c) our focal males were consistently winners or losers, while their focal males were randomly assigned rivals weekly so switched between winning and losing.

Future studies are required to determine which, if any, of these factors account for the difference in the effect of past reproductive effort on current sperm count.

Prior contest experience and current reproduction

Winners had significantly better mating performance than losers but there were no significant differences in their post-copulatory reproductive investment. That is, winners made more mating attempts and spent more time with the female than did losers but did not differ in their sperm count or sperm velocity. These results broadly replicate those of our earlier study that tested for a long-term winner-loser effect on the plasticity of male investment into pre- and post-copulatory sexually selected traits (Harrison *et al.*, 2023). Surprisingly, however, in the current study we found no differences between winners and losers in how many successful mating attempts males made. In our earlier study, males experienced only winning or only losing against a rival male in the visual presence of a female (i.e., males could perceive but not mate with a female). This experimental design is comparable to our 'contests only' treatment in the current study. It is therefore worth noting that when we directly compare winners and losers only from the 'contests only' treatment, winners did indeed have significantly more mating attempts that were successful than did losers (see Figure 2B). Additionally, our earlier study quantified current mating effort when a size-matched winner and loser directly competed for a female. In the present study, we instead quantified male mating effort in the absence of a rival. It seems plausible that direct interactions between winning and losing males influence their mating success, especially where winners monopolise access to a female. The difference between the two studies therefore helps clarify the mechanism driving our previous findings (Harrison *et al.*, 2018, 2023).

Prior contest experience and non-reproductive traits

While male-male contests can induce different stress responses in winners and losers, we found no evidence that winners have a greater change than losers in telomere length. One potential explanation is that the immune and/or endocrine system compensate when an individual is exposed to the same stressful event for a sustained period. For instance, sudden changes in social status can trigger oxidative stress (Beaulieu *et al.*, 2014), but once dominance hierarchies stabilise and fighting decreases, then the immune and endocrine systems adjust to the new group dynamics (Fialkowski *et al.*, 2021; Milewski *et al.*, 2022). In our experimental study, focal male *G. holbrooki* were unlikely to have established stable dominance hierarchies as they faced a new rival every 3-5 days. We therefore suggest that It is more plausible that the experience of always winning or losing fights imposes different types of costs, but that these induce similar levels of oxidative stress and telomere shortening (Costanzo *et al.*, 2021). For example, higher levels of testosterone (associated with winning) or of stress hormones (associated with losing) can both induce oxidative damage.

Past reproductive effort and non-reproductive traits

Greater male reproductive effort is generally associated with decreased somatic maintenance and a shorter lifespan (e.g., Papadopoulos *et al.*, 2010). There is general evidence in many taxa that the energetic costs of male courtship effort reduce somatic maintenance and lifespan (e.g., Cordts & Partridge, 1996; Martin & Hosken, 2004). It is therefore surprising that we found no significant difference in the telomere lengths of males with full or no access to females.

While reproduction is assumed to be energetically costly, there is, however, limited direct evidence that it increases oxidative damage (Speakman & Garratt, 2013), which tends to shorten telomeres. More research is needed to test the generality of our results.

A final, unexpected finding from our study was that smaller males had both higher mortality and significantly shorter telomeres than large males. It is particularly surprising that smaller males had shorter telomeres because body size is often negatively correlated with telomere length (Ringsby *et al.*, 2015). In mammals, a negative relationship between body size and telomere length is thought to be an adaptive response to a higher cancer risk associated with greater cellular replication (Pepke & Eisenberg, 2022) such that larger individuals better suppress telomerase activity, or have relatively longer telomeres at birth to account for expected higher telomeric attrition associated with body growth (Monaghan & Ozanne, 2018; Risques & Promislow, 2018). However, the relationship between telomere length and body size is not universally negative (Monaghan & Ozanne, 2018), and telomerase activity is far more variable in ectotherms than endotherms (Olsson *et al.*, 2018). It is possible that smaller male *G. holbrooki* in our study lacked the energetic resources necessary to sustain prolonged male-male competition, regardless of whether they consistently won or lost, thereby elevating mortality. Our findings suggest that large males can better manage the costs of competition and reproduction, although the mechanisms involved remain unclear. Males that could fully invest into reproduction by mating had slower growth than males that did not, suggesting that reproduction reduces somatic maintenance, but not lifespan (as inferred from telomere length). The combination of fewer resources to invest and a shift in allocation could produce the pattern we observed in *G. holbrooki*; lower mating effort but no change in ejaculates (sperm count or velocity), when males had a greater past reproductive effort.

Conclusions

Collectively, our findings suggest that: 1) both losing contests and full access to females (hence greater past reproductive effort) tend to lower current mating effort, 2) neither winning/losing contests nor full access to females (hence greater past reproductive effort) affect telomere length, and 3) there are size-dependent costs of access to females (hence past reproductive effort) and winning/losing fights that affect current reproductive effort, but do not affect growth or telomere length.

Data accessibility

All data and code are freely available in a Dryad Digital Repository (https://doi.org/10.5061/dryad.rjdfn2zp3) and through our pre-registration on the OSF: https://osf.io/saj46/.

Author contributions

L.M.H.: conceptualization, investigation, methodology, project administration, formal analysis, data curation, visualisation, and writing – original draft. O.P.S.: methodology, investigation, and writing – review and editing. M.D.J.: conceptualization, funding acquisition, project administration, resources, and writing – review and editing.

Conflict of interest statement

The authors declare no conflict of interest.

Funding

This research was supported by an Australasian Society for the Study of Animal Behaviour (ASSAB) small student research grant awarded to L.M.H., an Australian Government Research Training Program scholarship to L.M.H. and O.P.S. and an Australian Research Council (ARC) Discovery Grant awarded to M.D.J. (grant no. DP190100279).

Acknowledgements

We thank the ANU Animal Services team for their assistance with fish maintenance, and N. Aitken and L. Broady for their invaluable advice regarding molecular lab work.

References

Anderson, J.A., Johnston, R.A., Lea, A.J., Campos, F.A., Voyles, T.N., Akinyi, M.Y., *et al.* 2021. High social status males experience accelerated epigenetic aging in wild baboons. *Elife* **10**: 1–22.

Angelier, F., Costantini, D., Blévin, P. & Chastel, O. 2018. Do glucocorticoids mediate the link between environmental conditions and telomere dynamics in wild vertebrates? A review. *General and Comparative Endocrinology* **256**: 99–111.

Bauch, C., Riechert, J., Verhulst, S. & Becker, P. 2016. Telomere length reflects reproductive effort indicated by corticosterone levels in a long-lived seabird. *Molecular Ecology* **25**: 5785–5794.

Beaulieu, M., Mboumba, S., Willaume, E., Kappeler, P.M. & Charpentier, M.J.E. 2014. The oxidative cost of unstable social dominance. *Journal of Experimental Biology* **217**: 2629–2632.

Blackburn, E.H. 1984. Telomeres: Do the ends justify the means? *Cell* **37**: 7–8.

Bonduriansky, R., Maklakov, A., Zajitschek, F. & Brooks, R. 2008. Sexual selection, sexual conflict and the evolution of ageing and life span. *Functional Ecology* **22**: 443–453.

Brooks, M.E., Kristensen, K., van Benthem, K.J., Magnusson, A., Berg, C.W., Nielsen, A., *et al.* 2017. glmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. *The R Journal* **9**: 378–400.

Bustin, S.A., Benes, V., Garson, J.A., Hellemans, J., Huggett, J., Kubista, M., *et al.* 2009. The MIQE guidelines: Minimum information for publication of quantitative real-time PCR experiments. *Clinical Chemistry* **55**: 611–622.

Caldwell, M.C. & Caldwell, D.K. 1962. Monarchistic dominance in small groups of captive male mosquitofish, *Gambusia affinis patruelis*. *Bulletin of the Southern California Academy of Sciences* **61**: 37–43.

Carré, J.M., Campbell, J.A., Lozoya, E., Goetz, S.M.M. & Welker, K.M. 2013. Changes in testosterone mediate the effect of winning on subsequent aggressive behaviour. *Psychoneuroendocrinology* **38**: 2034–2041.

Casagrande, S. & Hau, M. 2019. Telomere attrition: Metabolic regulation and signalling function? *Biology Letters* **15**: 20180885.

Cawthon, R.M. 2002. Telomere measurement by quantitative PCR. *Nucleic Acids Research* **30**: e47.

Chatelain, M., Drobniak, S.M. & Szulkin, M. 2020. The association between stressors and telomeres in non-human vertebrates: a meta-analysis. *Ecology Letters* **23**: 381–398.

Chung, M.-H.J., Fox, R.J. & Jennions, M.D. 2024. Male allocation to ejaculation and mating effort imposes different life history trade-offs. *PLoS Bioogyl* **22**: e3002519.

Chung, M.-H.J., Jennions, M.D. & Fox, R.J. 2021. Quantifying the costs of pre- and postcopulatory traits for males: Evidence that costs of ejaculation are minor relative to mating effort. *Evolution Letters* **5**: 315–327.

Cordts, R. & Partridge, L. 1996. Courtship reduces longevity of male *Drosophila melanogaster*. *Animal Behaviour* **52**: 269–278.

Costanzo, A., Ambrosini, R., Parolini, M., Caprioli, M., Secomandi, S., Rubolini, D., *et al.* 2021. Telomere shortening is associated with corticosterone stress response in adult barn swallows. *Current Zoology* **68**: 93–101.

Criscuolo, F., Bize, P., Nasir, L., Metcalfe, N.B., Foote, C.G., Griffiths, K., *et al.* 2009. Real-time quantitative PCR assay for measurement of avian telomeres. *Journal of Avian Biology* **40**: 342–347.

Dantzer, B. & Fletcher, Q.E. 2015. Telomeres shorten more slowly in slow-aging wild animals than in fast-aging ones. *Experimental Gerontology* **71**: 38–47.

Earley, R.L., Lu, C.K., Lee, I.H., Wong, S.C. & Hsu, Y. 2013. Winner and loser effects are modulated by hormonal states. *Frontiers in Zoology* **10**: 6–19.

Eastwood, J.R., Dupoué, A., Delhey, K., Verhulst, S., Cockburn, A. & Peters, A. 2023. When does early-life telomere length predict survival? A case study and meta-analysis. *Molecular Ecology* **32**: 3000–3013.

Fialkowski, R., Aufdemberge, P., Wright, V. & Dijkstra, P. 2021. Radical change: temporal patterns of oxidative stress during social ascent in a dominance hierarchy. *Behavioral Ecology and Sociobiology* **75**: 43.

Filice, D.C.S. & Dukas, R. 2019. Winners have higher pre-copulatory mating success but losers have better post-copulatory outcomes. *Proceedings of the Royal Society B* **286**: 20182838.

Gao, J. & Munch, S.B. 2015. Does reproductive investment decrease telomere length in *Menidia menidia*? *PLoS One* **10**: 1–13.

Goymann, W. & Wingfield, J.C. 2004. Allostatic load, social status and stress hormones: the costs of social status matter. *Animal Behaviour* **67**: 591–602.

Hardy, I.C.W. & Briffa, M. 2013. *Animal contests*. Cambridge University Press, Cambridge.

Harrison, L.M., Jennions, M.D. & Head, M.L. 2018. Does the winner-loser effect determine male mating success? *Biology Letters* **14**: 20180195.

Harrison, L.M., Vega-Trejo, R. & Jennions, M.D. 2023. The effect of brief or prolonged bouts of winning or losing male-male contests on plasticity in sexually selected traits. *The American Naturalist* **201**: 442–459.

Harshman, L.G. & Zera, A.J. 2007. The cost of reproduction: the devil in the details. *Trends in Ecology and Evolution* **22**: 80–86.

Hartig, F. 2020. DHARMa: Residual diagnostics for hierarchical (multi-level/mixed) regression models.

Haussmann, M.F. & Marchetto, N.M. 2010. Telomeres: Linking stress and survival, ecology and evolution. *Current Zoology* **56**: 714–727.

Heidinger, B.J., Blount, J.D., Boner, W., Griffiths, K., Metcalfe, N.B. & Monaghan P. 2012. Telomere length in early life predicts lifespan. *Proceedings of the National Academy of Sciences* **109**: 1743–1748.

Houben, J.M.J., Moonen, H.J.J., van Schooten, F.J. & Hageman, G.J. 2008. Telomere length assessment: Biomarker of chronic oxidative stress? *Free Radical Biology and Medicine* **44**: 235–246.

Hsu, Y., Earley, R.L. & Wolf, L.L. 2006. Modulation of aggressive behaviour by fighting experience: Mechanisms and contest outcomes. *Biological Reviews of the Cambridge Philosophical Society* **81**: 33–74.

Hunt, J., Brooks, R., Jennions, M.D., Smith, M.J., Bentsen, C.L. & Bussière, L.F. 2004. High-quality male field crickets invest heavily in sexual display but die young. *Nature* **432**: 1024–1027.

Kahn, A.T., Kokko, H. & Jennions, M.D. 2013. Adaptive sex allocation in anticipation of changes in offspring mating opportunities. *Nature Communications* **4**: 1603.

Kasumovic, M.M., Elias, D.O., Sivalinghem, S., Mason, A.C. & Andrade, M.C.B. 2010. Examination of prior contest experience and the retention of winner and loser effects. *Behavioral Ecology* **21**: 404–409.

Lemaître, J.-F., Gaillard, J.-M. & Ramm, S.A. 2020a. The hidden ageing costs of sperm competition. *Ecology Letters* **23**: 1573–1588.

Lemaître, J.-F., Moorad, J., Gaillard, J.-M., Maklakov, A.A. & Nussey, D.H. 2024. A unified framework for evolutionary genetic and physiological theories of aging. *PLoS Biology* **22**: e3002513.

Lemaître, J.F., Ronget, V., Tidière, M., Allainé, D., Berger, V., Cohas, A., *et al.* 2020b. Sex differences in adult lifespan and aging rates of mortality across wild mammals. *Proceedings of the National Academy of Sciences* **117**: 8546–8553.

Lewin, N., Treidel, L.A., Holekamp, K.E., Place, N.J. & Haussmann, M.F. 2015. Socioecological variables predict telomere length in wild spotted hyenas. *Biology Letters* **11**: 20140991.

Lukas, D. & Clutton-Brock, T. 2014. Costs of mating competition limit male lifetime breeding success in polygynous mammals. *Proceedings of the Royal Society B: Biological Sciences* **281**: 20140418.

Martin, O.Y. & Hosken, D.J. 2004. Copulation reduces male but not female longevity in *Saltella sphondylli* (Diptera: Sepsidae). *Journal of Evolutionary Biology* **17**: 357–362.

McPeek, M.A. 1992. Mechanisms of sexual selection operating on body size in the mosquitofish (*Gambusia holbrooki*). *Behavioral Ecology* **3**: 1–12.

Milewski, T.M., Lee, W., Champagne, F.A. & Curley, J.P. 2022. Behavioural and physiological plasticity in social hierarchies. *Philosophical Transactions of the Royal Society B: Biological Sciences* **377**: 20200443.

Monaghan, P., Metcalfe, N.B. & Torres, R. 2009. Oxidative stress as a mediator of life history trade-offs: Mechanisms, measurements and interpretation. *Ecology Letters* **12**: 75–92.

Monaghan, P. & Ozanne, S.E. 2018. Somatic growth and telomere dynamics in vertebrates: relationships, mechanisms and consequences. *Philosophical Transactions of the Royal Society B: Biological Sciences* **373**: 20160446.

Monteforte, S., Cattelan, S. & Grapputo, A. 2020. Maternal predator-exposure affects offspring size at birth but not telomere length in a live-bearing fish. *Ecology and Evolution* **10**: 2030–2039.

Morbiato, E., Cattelan, S., Pilastro, A. & Grapputo, A. 2023. Sperm production is negatively associated with muscle and sperm telomere length in a species subjected to strong sperm competition. *Molecular Ecology* **32**: 5812–5822.

O'Dea, R.E., Jennions, M.D. & Head, M.L. 2014. Male body size and condition affects sperm number and production rates in mosquitofish, *Gambusia holbrooki*. *Journal of Evolutionary Biology* **27**: 2739–2744.

Olsson, M., Wapstra, E. & Friesen, C. 2018. Ectothermic telomeres: It's time they came in from the cold. *Philosophical Transactions of the Royal Society B: Biological Sciences* **373**: 20160449.

Papadopoulos, N.T., Liedo, P., Muller, H.G., Wang, J.L., Molleman, F. & Carey, J.R. 2010. Cost of reproduction in male medflies: the primacy of sexual courting in extreme longevity reduction. *Journal of Insect Physiology* **56**: 283– 287.

Parolini, M., Romano, A., Costanzo, A., Khoriauli, L., Santagostino, M., Nergadze, S.G., *et al.* 2017. Telomere length is reflected by plumage coloration and predicts seasonal reproductive success in the barn swallow. *Molecular Ecology* **26**: 6100–6109.

Pepke, M. Le & Eisenberg, D.T.A. 2022. On the comparative biology of mammalian telomeres: Telomere length co-evolves with body mass, lifespan and cancer risk. *Molecular Ecology* **31**: 6286–6296.

Pfaffl, M.W. 2001. A new mathematical model for relative quantification in real-time RT–PCR. *Nucleic Acids Research* **29**: E45.

R Development Core Team. 2020. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Ringsby, T.H., Jensen, H., Pärn, H., Kvalnes, T., Boner, W., Gillespie, R., *et al.* 2015. On being the right size: Increased body size is associated with reduced telomere length under natural conditions. *Proceedings of the Royal Society B: Biological Sciences* **282**: 20152331.

Risques, R.A. & Promislow, D.E.L. 2018. All's well that ends well: Why large species have short telomeres. *Philosophical Transactions of the Royal Society B: Biological Sciences* **373**: 20160448.

Rollings, N., Friesen, C.R., Sudyka, J., Whittington, C., Giraudeau, M., Wilson, M., *et al.* 2017. Telomere dynamics in a lizard with morph-specific reproductive investment and self-maintenance. *Ecology and Evolution* **7**: 5163–5169.

Snyder-Mackler, N., Burger, J.R., Gaydosh, L., Belsky, D.W., Noppert, G.A., Campos, F.A., Bartolomucci, A., Yang, Y.C., Aiello, A.E., O'Rand, A., Harris, K.M., Shively, C.A., Alberts, A.C. & Tung, J. 2020. Social determinants of health and survival in humans and other animals. *Science* **368**: eaax9553 (2020).

Speakman, J.R. & Garratt, M. 2013. Oxidative stress as a cost of reproduction: beyond the simplistic trade-off model. *BioEssays* **36**: 93–106.

Stearns, S.C. 1989. Trade-offs in life-history evolution. *Functional Ecology* **3**: 259–268.

Stoffel, M.A., Nakagawa, S. & Schielzeth, H. 2017. rptR: repeatability estimation and variance decomposition by generalized linear mixed-effects models. *Methods in Ecology and Evolution* **8**: 1639–1644.

Sudyka, J. 2019. Does reproduction shorten telomeres? Towards integrating individual quality with life-history strategies in telomere biology. *BioEssays* **41**: 1900095.

Taff, C.C. & Freeman-Gallant, C.R. 2017. Sexual signals reflect telomere dynamics in a wild bird. *Ecology and Evolution* **7**: 3436–3442.

Vega-Trejo, R., Jennions, M.D. & Head, M.L. 2016. Are sexually selected traits affected by a poor environment early in life? *BMC Evolutionary Biology* **16**: 1–12.

Vega-Trejo, R., O'Dea, R.E., Jennions, M.D. & Head, M.L. 2014. The effects of familiarity and mating experience on mate choice in mosquitofish, *Gambusia holbrooki*. *Behavioral Ecology* **25**: 1205–1211.

Wood, E.M., Capilla-Lasheras, P., Cram, D.L., Walker, L.A., York, J.E., Lange, A., Hamilton, P.B., Tyler, C.R., & Young, A.J. 2021. Social dominance and rainfall predict telomere dynamics in a cooperative arid-zone bird. *Molecular Ecology* **31**: 6141–6154.

Zane, L., Nelson, W.S., Jones, A.G. & Avise, J.C. 1999. Microsatellite assessment of multiple paternity in natural populations of a live-bearing fish, *Gambusia holbrooki*. *Journal of Evolutionary Biology* **12**: 61–69.

Figures and Tables

Table 1. Descriptive statistics for each of the life history traits measured.

	Winners						**Losers**					
	Contests Only			Contests + Reproduction			Contests Only			Contests + Reproduction		
Trait	*Mean*	*SD*	*n*	*Mean*	*SD*	*n*	*Mean*	*SD*	*n*	*Mean*	*SD*	*n*
Mating attempts	26.27	19.17	41	18.11	17.20	37	19.55	20.88	31	14.55	12.60	33
Successes	2.49	2.40	41	1.11	1.58	37	1.35	1.85	31	1.24	1.71	33
Time with female (s)	438.31	218.15	41	241.54	148.23	37	248.12	242.52	31	125.73	78.00	33
Sperm count (x1000)	2087	1927	32	1820	1675	29	2429	2701	25	1975	1502	25
Sperm velocity ($\mu m/s^{-1}$)	165.13	22.10	29	166.28	23.54	25	155.36	22.23	21	167.60	24.69	21
Male size (SL) at week 0 (mm)	20.34	1.59	41	20.57	1.55	37	20.58	1.63	31	20.27	1.42	33
Growth* (mm)	0.64	0.82	41	0.46	0.48	37	0.56	0.41	31	0.33	0.33	33
Relative telomere length (average)	0.87	0.45	39	0.82	0.43	36	0.90	0.45	31	0.95	0.36	31

*growth calculated as (male size (SL) at week 9 – male size (SL) at week 0)

Table 2. Model estimates from generalised linear mixed models for each of the reproduction and life-history traits measured. Significant effects are highlighted in bold. The estimate is for the level of the factor shown in parentheses.

Model Parameters	**Estimate**	**SE**	***z***	***P*-value**
1) Number of mating attempts[‡]				
Intercept	**2.58**	**0.16**	**15.86**	**<0.0001**
Male size (Centred and standardised)	-0.32	0.17	-1.87	0.062
Experience (Winning)	0.23	0.22	1.02	0.308
Treatment (Contests only)	0.34	0.23	1.47	0.141
Female size (Centred and standardised)	0.11	0.09	1.28	0.200
Male size x Experience (Winning)	**0.59**	**0.22**	**2.60**	**0.009**
Male size x Treatment (Contests only)	**0.60**	**0.23**	**2.63**	**0.009**
Experience (Winning) x Treatment (Contests only)	0.12	0.31	0.40	0.691
Male size x Experience x Treatment	**-0.97**	**0.30**	**-3.23**	**0.001**
2) Number of successful mating attempts[§]				
Intercept	-0.04	0.20	-0.19	0.849
Male size (Centred and standardised)	-0.18	0.12	-1.54	0.124
Experience (Winning)	0.29	0.21	1.40	0.161
Treatment (Contests only)	**0.50**	**0.21**	**2.45**	**0.014**
Female size (Centred and standardised)	0.14	0.11	1.30	0.194
3) Time with female (seconds[§]				
Intercept	**4.80**	**0.13**	**36.56**	**<0.0001**
Male size (Centred and standardised)	0.05	0.08	0.67	0.501
Experience (Winning)	**0.66**	**0.12**	**5.33**	**<0.0001**
Treatment (Contests only)	**0.64**	**0.12**	**5.21**	**<0.0001**
Female size (Centred and standardised)	0.07	0.07	0.96	0.337
4) Total sperm count (log transformed)[§]				
Intercept	**13.95**	**0.25**	**56.92**	**<0.0001**
Male size (Centred and standardised)	**0.31**	**0.16**	**1.99**	**0.047**
Experience (Winning)	-0.05	0.22	-0.25	0.804
Treatment (Contests only)	0.12	0.22	0.55	0.582
5) Sperm velocity (weighted average VCL)[§]				
Intercept	**164.30**	**4.76**	**34.51**	**<0.0001**
Male size (Centred and standardised)	6.16	4.49	1.37	0.170
Experience (Winning)	4.05	4.18	0.97	0.333
Treatment (Contests only)	-5.63	4.21	-1.34	0.181
Male size x Treatment (Contests only)	**-12.49**	**4.43**	**-2.82**	**0.005**
6) Growth (male size (SL) at week 9, mm)[§]				
Intercept	**2.91**	**0.59**	**4.96**	**<0.0001**
Male size (SL) at week 0 (Centred and standardised)	**0.87**	**0.03**	**30.64**	**<0.0001**
Experience (Winning)	0.11	0.09	1.23	0.219
Treatment (Contests only)	**0.21**	**0.09**	**2.37**	**0.018**
7) Relative telomere length (rTL)[§]				
Intercept	**0.94**	**0.06**	**14.68**	**<0.0001**
Male size (Centred and standardised)	**0.09**	**0.04**	**2.43**	**0.015**
Experience (Winning)	-0.09	0.07	-1.28	0.201
Treatment (Contests only)	0.00	0.07	0.01	0.995

[‡] full model is shown for mating attempts where there is a significant three-way interaction.

§ reduced models presented. See Supplementary Material for full models.

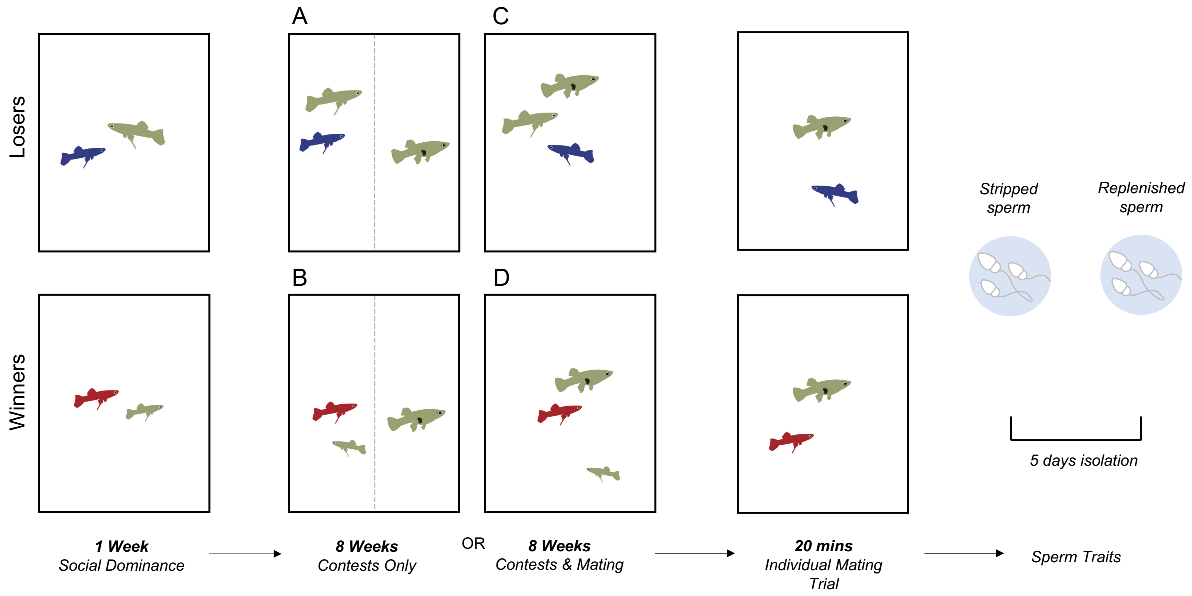

Figure 1. Experimental design to create winners and losers. Following 1 week of isolation, focal males (winners in red; losers in blue) were randomly assigned to become either a loser or winner by being paired with a larger or smaller rival, respectively. Males had 1 week of contests facing new rivals daily to establish social dominance. Focal males were then randomly assigned to experience either only male-male contests (A and B) or male-male contests with the opportunity to mate (C and D) for another 8 weeks. Rival males and females were rotated every few days. After 9 weeks, males were placed with a novel adult female to quantify their mating behaviour during individual mating trials. Males were then immediately stripped of their sperm. After 5 days in isolation, focal males were again stripped of their sperm to measure their replenished ejaculates, then euthanized to measure relative telomere length.

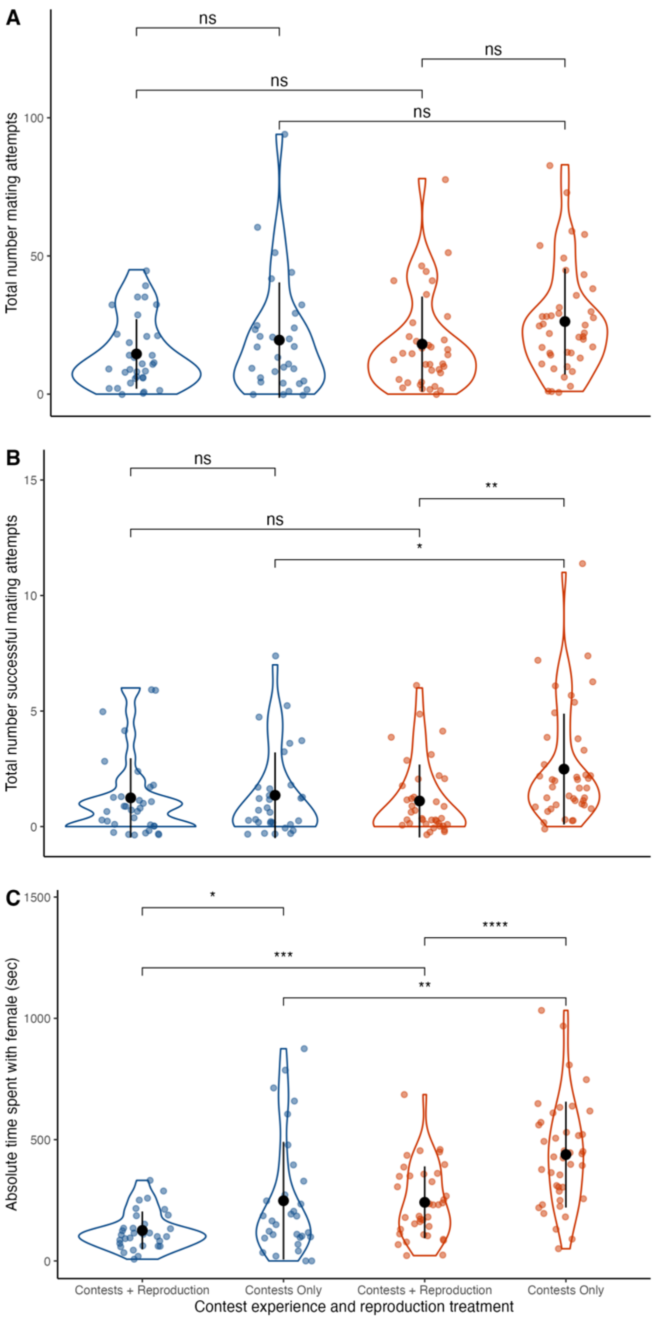

Figure 2. Violin plots depicting the mating behaviour of winners (red) and losers (blue) when males had either no access (Contests Only) or full access to females (Contests + Reproduction) for nine weeks. Asterisks (*) indicate significant pairwise differences between winners and losers within each reproduction treatment or between reproduction treatments for either winners or losers (ns = no significant difference). Mean and standard deviation shown in black. Raw data are shown but the statistical analyses (in text) corrected for random effects.

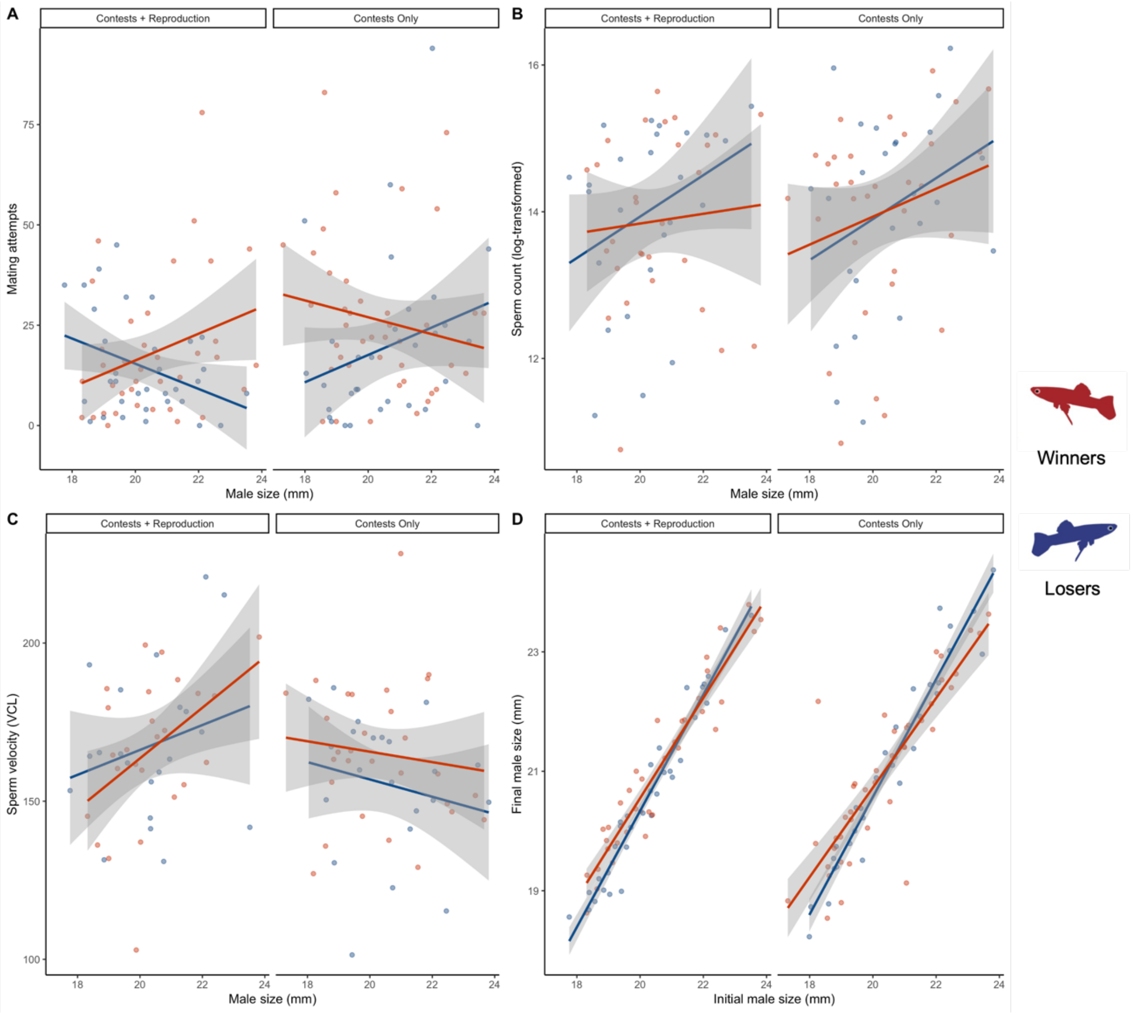

Figure 3. Simple linear regressions with 95% confidence intervals (grey ribbons) highlight interactions between male body size and several key reproductive traits: the number of mating attempts (A), total sperm counts (B), and sperm velocity (C). After nine weeks, winners (red) and losers (blue) differed in the number of mating attempts made (A); large winners that had access to mates (Contests + Reproduction) made more mating attempts than large winners without access (Contests Only). Larger males produced more sperm than did smaller males regardless of treatment (B), while larger males produced faster sperm than smaller males, but only for males that had full access to mates (C). Males without full access to females (Contests Only) grew significantly faster than males that could mate (Contests + Reproduction) (D). Raw data are shown but statistical analyses (in text) corrected for random effects.

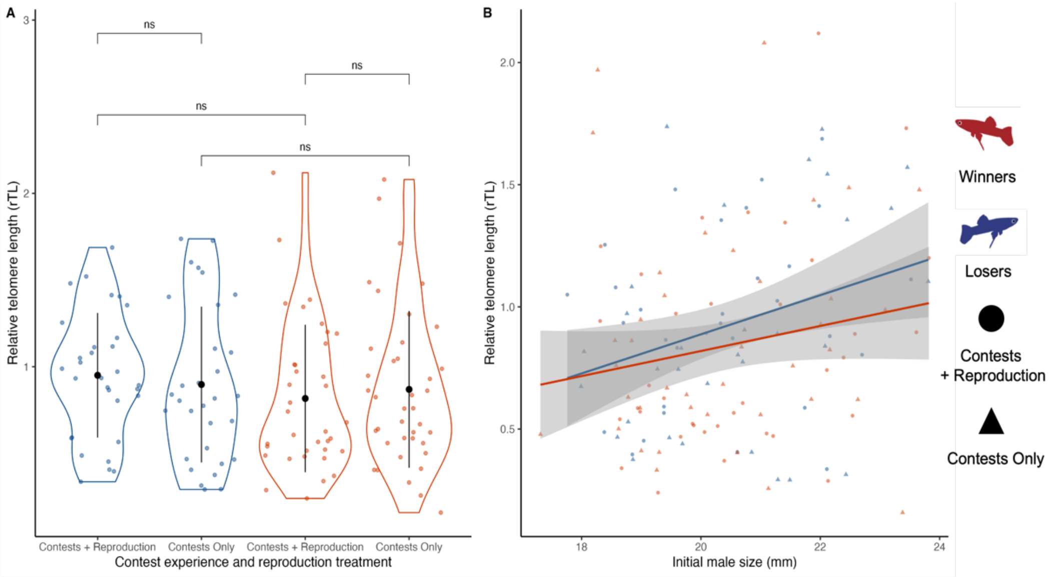

Figure 4. Violin plots depicting the relative telomere length (rTL) of winners (red) and losers (blue) that had either access to females (Contests + Reproduction) or no access to females (Contests Only) for 9 weeks (A). Simple linear regressions with 95% confidence intervals (grey ribbons) for the relationships between male body size and mean rTL (B). Larger males had significantly longer telomeres than smaller males, but there was no effect of winning (red) or losing (blue) on rTL. Raw data are shown but statistical analyses (in text) corrected for random effects.