Early-life reproduction predicts male lifespan in turquoise killifish (*Nothobranchius furzeri*)

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Abstract

Individuals of the same species can show striking variation in lifespan. A common assumption in life-history theory is that intra-species lifespan variation can be explained by trade-offs between reproduction and survival, i.e., individuals investing more in early-life reproduction are expected to live shorter. However, the trade-off assumption can mask alternative models. For example, individual quality could affect reproduction and survival in the same direction; e.g., high-quality individuals may both reproduce more and live longer. Here, we study the naturally short-lived turquoise killifish (*Nothobranchius furzeri*), an annual teleost with a limited reproductive window, which – according to the trade-off model – should generate a selective pressure that maximizes individual investment in early-life reproduction, at the expense of survival. To test the individual co-variation in reproduction and lifespan, we focused on males and performed IVFs using ejaculates collected longitudinally throughout life. Unlike the trade-off expectation, we found that reproduction outcomes of young individuals positively correlate with male lifespan, suggesting that early-life reproduction is a *bona fide* proxy for intrinsic individual quality. Using a causal-informed simulation, we showed that a parsimonious model where *i*) intrinsic quality affects both lifespan and reproduction and *ii*) age negatively impacts reproduction, largely recapitulates the observed data, including lifespan prediction from early-life reproduction. Our findings challenge the assumption that reproductive costs are the

primary drivers of lifespan variation and instead highlight intrinsic quality as a key common cause to both lifespan and reproduction.

Introduction

Lifespan varies considerably both within and among species [1]. In humans, even though life expectancy has steadily increased over the last two centuries [2], substantial variation in lifespan persists among individuals, even within the same population, also referred to "lifespan disparity" [3], especially among males [4,5]. To explain variation in lifespan among individuals, trade-offs with reproduction are often invoked. Trade-offs emerge from the allocation of limited "resources" between reproduction and survival and are used to describe most of the variation in life-history strategies across species [6]. For instance, fast growth and early-life reproduction is predicted to be associated with an accelerated senescence and reduced longevity [7-9]. However, patterns of lifespan-reproduction co-variation can be compatible with alternative models [10-12]. For instance, variation in individual quality may explain the positive co-variation between reproduction and survival as high-quality individuals may both reproduce more and live longer [13-16].

In contrast, males are predicted to maximise reproductive success by investing in early-life reproduction, as obtaining matings entails increased risk of extrinsic mortality (e.g. conspicuous coloration, courtship behaviour, male-male competition). By investing in early-life reproduction, males are expected to incur in survival costs, which ultimately leads to the evolution of a "live fast-die young" strategy [5,17]. However, theoretical and comparative studies support the idea that sexual competition imposes strong selection on the overall individual quality [18,19], potentially favouring positive associations between reproduction and survival [17]. Yet, empirical evidence on the relationship between reproduction and survival in males remains scarce (reviewed in [20]).

Males of the short-lived *Nothobranchius furzeri* show a strong and rapid decline in reproductive functions [21,22] despite their short lifespan (from 4 to 8 months [23]). While there seems to be no clear trade-off between reproduction and survival at the species-level, trade-offs could still exist at the population level. For example, some males may reproduce at a low rate throughout life and live longer, while others could invest more in early reproduction and die sooner. Alternative life-history strategies within the same population can explain both the considerable variation in lifespan among individuals [23-25] and the

57 observed reproductive aging [21,22] because the apparent decline in reproduction at older ages may reflect
58 the selective disappearance of "live fast - die young" individuals from the population.

59 To investigate whether the variation in turquoise killifish lifespan reflects differential investment in
60 reproduction among males, in this study we employed a longitudinal experimental design in which we
61 measured male reproductive success after controlled *in vitro* fertilizations (IVF) at three different time
62 points during lifespan and recorded the age of death. Under the trade-off model, we expect to find a
63 negative association between survival and reproductive success, especially in early life. Alternatively,
64 in the absence of obvious trade-offs, individual quality may affect both individual lifespan and embryo
65 survival (through ejaculate quality), resulting in positive co-variation between reproductive success and
66 lifespan.

Results

Male reproductive success decreases with age and does not trade-off with lifespan

To investigate whether the variation in turquoise killifish lifespan reflects differential investment in reproduction among individuals, we performed IVFs at three different ages during male lifespan: at young (9 wph, 100% population alive), middle (15 wph, 85% population alive) and old (20 wph, 60% population alive) age (**Figure 1a**). To factor out the contribution of female's age on the measure of reproductive output, we used mid-age females throughout the experiment (**Materials and Methods**). As a measure of reproductive success, we counted the number of fertilized eggs (i.e., fertilization rate) and the number of embryos that survived after 72 hours after fertilization (i.e., embryo survival) (**Materials and Methods**). Here, we present the results for embryo survival. Fertilization rate largely mirrors the patterns observed in embryo survival (see **Supplementary Results, Figure S1**).

First, confirming previous work [21], we found that age significantly affected embryo survival (χ^2=26.805, p=1.512e-06). Old males produced embryos with significant lower survival compared to young males (z=4.572, p<0.0001) (**Figure 1b**). Moreover, we found that reproductive output, measured as embryo survival, significantly declines already at mid-age (z=3.764, p=0.0005) (**Figure 1b**). Second, to test for reproduction-survival trade-offs, we estimated posterior predictive distributions of the relationship between lifespan and embryo survival at the three different ages (**Figure 1c**). We fitted Bayesian binomial models [26,27] with a logit link function modelling the log-odds of embryo survival (i.e., the logit of the embryo survival probability) as a linear function of log transformed lifespan (see *Equation 1,* **Materials and Methods**). Contrary to the trade-off expectation, we found no negative correlation between individual reproductive success and lifespan (**Figure 1c**). Moreover, posterior estimates indicate that individuals with higher reproductive success at young age live longer, i.e., early-life reproductive success positively covaries with individual age at death (β_{mean}=1.07; 0.51 – 1.67, 95% credible intervals), while there is no correlation between individual survival and reproductive outcome when sampling fathers at mid (β_{mean}=-0.15; -0.95 – 0.65, 95% credible intervals) and old age (β_{mean}=-0.14; -2.98 – 2.33, 95% credible intervals) (**Figure 1d**). These results indicate a positive association between embryo survival and lifespan at young age but not later in life.

Early life reproductive success predicts individual lifespan

Since father survival and embryo survival covary for measures taken at young father age (**Figure 1c**), we asked whether embryo survival statistically predicts variation in father lifespan across individuals. Running a Bayesian model for each age class with father lifespan as the outcome variable (**Materials and Methods, Figure 2a**), we found – as expected from the previous model – that the effect of early-life reproductive success on lifespan is positive (**Figure 2b**). Specifically, the posterior predictive distribution of the slope (young age: β_{mean}=0.33; 0.035 – 0.620, 95% credible intervals, **Figure 2c**) indicates a 95% probability that higher embryo survival is associated with increased male lifespan. For instance, an increase of 50% in embryo survival predicts a 17% proportional increase in expected lifespan (**Figure 2d**). Thus, while we do not estimate absolute lifespan from embryo survival, differences in early reproductive performance can be used as a reliable indicator of relative differences in expected male lifespan. To estimate the expected father lifespan for a given embryo survival probability (p), we extracted the posterior means of the intercept (α) and slope (β) from the model and calculated the expected lifespan at p as follows:

$$\text{Lifespan}_{(p)} = \exp(\alpha_{mean} + \beta_{mean} \times p)$$

To note, running a model using reproductive success measured at older ages, the credible intervals associated to the slope of embryo survival on lifespan include zero both at mid (β_{mean}=0.017; -0.202 – 0.229, 95% credible intervals) and old age (β_{mean}=0.052; -0.164 – 0.269, 95% credible intervals), indicating that measuring embryo survival from mid and old age fathers provides no information about their lifespan (**Figure 2c**).

Intrinsic quality explains the age-dependent change in the correlation between individual reproductive success and lifespan

Since trade-off models don't explain the positive co-variation between survival and reproduction at the species and individual level, we explored an alternative model. We hypothesized that the individual quality (*intrinsic quality*) affects both individual lifespan and embryo survival (e.g., through ejaculate quality). Age, in turn, impacts functional quality. This simple assumption leads to three important

consequences. First, variation in *intrinsic quality* among individuals leads to the positive association between survival and reproduction. Second, age negatively impacts embryo survival – but not lifespan! – hence leading to the loss of covariation between reproductive success and lifespan at older age. Furthermore, individuals with low *intrinsic quality* (and thus shorter lifespan) die at young age, reducing the remaining variation of *intrinsic quality* at the population level in middle and old age. Finally, early-life reproduction is a *bona fide* proxy for *intrinsic quality* and Darwinian fitness, and can be used to predict lifespan. To formalize our conceptual framework, we expressed our model as a direct acyclic graph (DAG) (**Figure 3a**), which helps clearly define causal relationships among variables, identifying potential confounders, mediators and colliders. To test whether the causal model specified in the DAG could reproduce the observed patterns between lifespan and embryo survival, we used simulated data. We first simulated a population of individuals differing for *intrinsic quality* (IQ). We then expressed the other variables in the DAG, i.e. lifespan, functional quality and embryo survival, as linear functions of IQ, and age classes were defined according to experimental data thresholds: "young" age class when ≥85% of the population was alive, "mid-age" age class between 85% and 60%, and "old" when ≤60% of the population is left (**Materials and Methods**). By correlating simulated embryo survival and lifespan, we found, similar to our observed data, no negative correlation between embryo survival and lifespan (**Figure 3b**). Moreover, simulated individuals with higher embryo survival at young age live longer, while embryo survival at mid and old age does not correlate with lifespan (**Figure 3b**). First, by modelling variation in *intrinsic quality* among individuals, our simulations confirmed that early-life reproductive success positively correlates with individual lifespan (**Figure 3b**), meaning that longer-lived individuals have higher early-life embryo survival compared to individuals living shorter (**Figure 3c**). Consistent with our observed data, our model confirmed that the positive correlation between reproduction and lifespan is lost at older ages (**Figure 3b**). Overall, these results indicate that *i)* inter-variation in *intrinsic quality* is sufficient to generate the positive correlation between reproduction and lifespan, and *ii)* age weakens the correlation between reproduction and lifespan at older ages.

The collinearity between age and lifespan leads to the loss of correlation across all ages

Finally, since age leads to the loss of correlation between embryos survival and lifespan, we asked whether *intrinsic quality* correlates with embryo survival in an age-dependent manner. To test the effect of age on the reproduction/ survival co-variation, we ran two separate Bayesian models on the simulated data by either excluding or including the age class variable "Age Class" as an input variable for the outcome variable, i.e. lifespan (**Materials and Methods**). In the first model, to investigate whether reproductive success can serve as a predictor of lifespan at different age classes, we ran three independent models for the young, mid and old age, respectively. We found that embryo survival in early life alone predicts individual lifespan (**Figure 3d**), in accordance with the observed data (**Figure 2b**) and the generative Bayesian model (**Figure 3b**). In the second model, we included "Age Class" as one of the input variables. Noteworthy, in this case – contrary to the observed data and our generative model – we found no correlation between reproductive success and lifespan across all age classes (**Figure 3d**). Having expressed our generative model as a DAG clarifies that since "Age Class" is collinear with lifespan through age, its inclusion as a predictor absorbs the variance explained by embryo survival. In other words, the disappearance of the association between embryo survival and lifespan in the second model is a statistical artifact of collinearity, not evidence against the genuine predictive role of embryo survival at young age.

166 **Discussion**

167 Lifespan varies both among and within-species, with important consequences for species- and
168 individual fitness. While part of lifespan variation is attributed to stochasticity, e.g., due to
169 environmental effects, yet a central question in geroscience and evolutionary biology is understanding
170 the source of lifespan variation. Theories based on the assumption of limited resources predict that
171 resources are allocated among reproduction and survival, thus explaining most of variation in lifespan
172 both among- and within-species. However, this predominant view may overlook alternative
173 explanations, especially at the individual level, such as variation in individual quality. In this study, we
174 investigated the relationship between reproductive success and lifespan in males of *N. furzeri*, a
175 vertebrate model with an exceptionally compressed life cycle. The turquoise killifish has evolved in
176 habitats where individuals can mate within a narrow time window, defined by seasonal water
177 availability. Under a trade-off model, turquoise killifish males are predicted to maximize early-life
178 reproduction at the expense of survival.

179 The use of a longitudinal experimental design allowed us to directly test for individual variation in life-
180 history strategy and to confirm previous evidence of male reproductive aging in this species [21,22]. By
181 measuring reproductive success of the same males at different ages allowed us to reveal that male highly
182 investing in reproduction in early life live longer than individuals with lower early-life reproductive
183 success. The absence of a clear trade-off between reproduction and survival at the individual level
184 allowed us also to exclude that reproductive aging in killifish is a statistical artefact resulting from the
185 age-dependent selective disappearance of short-lived males with high early-life reproduction
186 investment.

187 Our results align with emerging evidence from other species in which positive associations between
188 early-life reproduction and survival – rather than trade-off – define the primary axis of variation among
189 individuals [12]. For example, in ungulate populations of bighorn sheep females producing more lambs
190 tend to live longer [16]. Similarly, the number of eggs produced in early-life by female killifish positively
191 covaries with lifespan [22]. While most of the reported positive associations between reproduction and

192 survival are found in females, in general males are expected to evolve a "live fast-die young" strategy
193 due to the increased extrinsic mortality associated with obtaining mates [17]. Our results show that males
194 as well can show a positive co-variation between lifespan and reproduction.

195 Growing evidence from humans indicates male infertility as an indicator of poor health and/or lifestyle
196 [28], which is associated with both an increased risk of certain diseases, such as cancers, metabolic and
197 cardiovascular diseases [29], and early mortality [30]. Moreover, a recent meta-analysis found that adult
198 men with high motile sperm count live significantly longer than men with low motile count [31],
199 suggesting that ejaculate parameters can serve as a potential biomarker for long-term survival.
200 Accordingly, we found that higher early-life embryo survival predicts increased male lifespan,
201 indicating that reproductive success early in life can be used to predict lifespan.

202 To explain our observed data, we propose that the positive co-variation between reproduction and
203 lifespan lies on the individual variation in *intrinsic quality*. *Intrinsic quality* here refers to stable,
204 individual-specific differences that affect both reproductive and somatic performance, potentially due
205 to genetic, developmental, or physiological factors [32]. High-quality individuals are able to
206 simultaneously achieve greater reproductive success and extended survival. The variation in *intrinsic*
207 *quality* among individuals can also explain the loss of correlation between reproduction and lifespan at
208 older ages since, as low-quality individuals die earlier, the remaining population becomes increasingly
209 homogeneous in quality. We formalized these hypotheses using a causal framework, represented as a
210 directed acyclic graph approach (DAG). The DAG was the basis for our simulation, which recapitulates
211 the main findings from our observed data, while also revealing novel insights. For instance, the relation
212 between "Lifespan" and "Age", which emerges as a propriety of the DAG structure, bears an important
213 statistical consideration. When we included "Age Class" as input variable in the model, the correlation
214 between embryo survival and lifespan disappeared at all ages, despite being present in the original
215 model. This loss of correlation can be explained by the collinearity between lifespan and age, and as a
216 consequence, with the "Age Class" parameter. Longer-lived individuals, by definition, reach later age
217 classes compared to short-lived individuals, and including data from different age classes in the same

model introduces redundancy that can obscure real associations. Our results warn from the practice of using all available variables in a model without a causal framework, as this practice may mask true associations or even generate spurious ones (Table 2 fallacy) [33]. This result underscores the importance of carefully considering model structure and potential collinearity when interpreting complex biological data.

Beyond the immediate findings, our results have broader implications for understanding variation in lifespan. The turquoise killifish has been widely adopted as a model for aging due to its short life and rapid senescence, yet surprisingly little is known about the factors affecting lifespan variation within populations (despite the effort to understand variation among populations and species [23,34,35]). Our data suggest that a substantial portion of the individual variation in lifespan reflects differences in individual quality rather than strategic trade-offs. This result raises the possibility that biomarkers of reproductive performance in early life, such as embryo survival or ejaculate traits, may serve as useful predictors of healthspan and lifespan in killifish, and potentially in other species.

More broadly, our results highlight how life-history trade-offs, while useful and intuitive [36], may not always explain life-history relationships within and between populations. Our findings do not invalidate trade-off theories, but rather provide evidence to support a broader framework of life-history variation (both among and within-species) that incorporates variation in individual quality, ecological, demographic factors, and diversity in life stages duration [10,12]. By integrating reproductive performance and survival within a unified quality-based model, our results offer a revised perspective about the interplay between reproduction and aging, with implications for life-history evolution and the biology of aging.

Materials and Methods

Fish husbandry

The turquoise killifish used in this study were laboratory-reared fish from GRZ-D strain maintained at the Fish Facility of the Leibniz Institute on Aging (Jena, Germany) under standard laboratory conditions (12:12 light–dark cycle; water temperature 26 ± 1 °C; pH 6.5-7.5). Fish were maintained in 3.2L tanks (density1.6L/fish, Aqua Schwarz recirculating system) provided with enrichments. Fish were fed *ad libitum* twice per day with live brine shrimps (*Artemia salina*) until week 10. From week 4 fish are fed also with bloodworm larvae (*Chironomus spp.*) once per day throughout their life. Extending details on housing and breeding conditions are described in a previous publication [37]. The experiment was carried out under permission of the following animal experimental license: FLI-22-107.

In vitro fertilizations

To investigate how investment in reproduction affects lifespan, we performed in vitro fertilizations (IVFs) at three different time points throughout male's lifespan. The average age of males was 62.8±6.2 days (mean±sd) at young age, 103.5±6.8 days at mid-age, and 140±4.7 at old age. Between young and mid-age n=8 (~16%) males died, while between mid and old age n=20 (~50% of the males alive until that point) males died. The males that survived until old age were n=20 (~41% of the initial males). The median lifespan of the males was 154 days (141-176 95% confidence intervals). Before IVF, we standardized recent social/ sexual history by placing each experimental male with two females (age ranging between 80 and 110 days) in a 10L-tank (Acqua Schwarz) and we let them mate for 5 days. To ensure the replenishment of sperm reserves after the mating, each male was isolated from females for 5 days. The male had visual and olfactory access to a female without the possibility to mate with it. At day 10, we performed IVFs by following the protocol that we published previously [38]. To collect sperm from the males, each male was anesthetized in a water bath containing Tricaine (200mg/L) and then placed on a moist sponge under a stereomicroscope (Leica S9D). After drying the urogenital area with a tissue, sperm were collected using a 5-µl microcapillary by gently pressing the male's abdomen. The ejaculate was then placed in 35µl Hank's buffer solution and maintained in ice and used within 20 minutes from collection. To collect eggs from the females, each female was anesthetized as above, then

267 dried with a tissue and placed on standard microscope slide. Eggs were collected by pressing the
268 female's abdomen with an angle chisel sharper. Eggs were then placed in a petri dish and immerged in
269 a 4% BSA solution at room temperature until use. For each IVF trial, we pooled eggs from at least 3-4
270 females to limit the effect of female quality on fertilization rate and embryo survival (mean±sd number
271 of eggs/IVF trial: 33.1±9.16). Immediately before the IVF, we removed the BSA with a disposable
272 Pasteur pipette and 30µl of the sperm solution was mixed with 120µl of tank water and then added to
273 the eggs. The remaining 5µL of the sperm solution was used to measure sperm concentration using a
274 cell counter (TC20, BioRad). After 5 minutes from the IVF, eggs were immerged in a diluted solution
275 of methylene blue (1:1000) and incubated at 28°C. We performed a total of 108 IVF trials (n=48 at
276 young age, n=40 at mid-age, n=20 at old age) using 72 females in total. The age of the females was
277 maintained constant across the experiment (mean±sd female age: 98.2±7.60 days). The same female
278 was used for a maximum of two IVFs with 2-week break between the trials.

279 *Fertilization rate and embryo survival*

280 After 3.5 hours post IVF we checked the fertilization rate by counting the fertilized and unfertilized
281 eggs under a stereomicroscope. Fertilized eggs are scored by the presence of diving cells (two- to four-
282 cell stage). Unfertilized eggs were removed, and the methylene blue solution was changed. The survival
283 of the embryos was then scored 72 hours post fertilization (hpf) by counting the number of embryos
284 still alive. We scored embryo survival at 72hpf, which corresponds to the end of the epiboly stage [39],
285 because our previous study revealed that 85% of total embryo mortality occurs within this time frame
286 [21]. In an independent pilot experiment, we found a strong positive correlation (r=0.50, p<0.001) between
287 embryo survival following IVFs and natural matings, suggesting that IVF-derived embryo mortality is
288 not an artefact of the procedure but reflects a biological effect.

289 *Effect of male age on reproductive success and correlation with lifespan*

290 We ran the following analyses using R v 4.2.0 [40]. To test the effect of male age on embryo survival, we
291 fitted a generalized mixed effect model using the "glmer" function in the "lme4" package [41] and
292 assumed a binomial error distribution and a logit link function. We fitted the model with Age class
293 (levels: young, mid-age, old) as a categorical fixed effect, sperm concentration as a continuous fixed

effect (i.e. covariate) and male ID as random effect to account for the non-independence of the data. The model was checked for overdispersion by using the "dispersion_glmer" function within the "lme4" package and corrected by including an observation-level random effect. We calculated *p* values of the fixed effects by Type II Wald chi-square tests using the "Anova" function in the "car" package [42]. To calculate pairwise effects among the different age classes (young *vs* mid-age; mid-age *vs* old; young *vs* old) we performed post-hoc analyses of contrasts with the "lsmeans" function in the "emmeans" package [43] using the Tukey method adjusted for multiple comparisons. To investigate the correlation between reproductive success and lifespan we ran Pearson's correlations between the proportion of embryos alive (or the proportion of fertilized eggs) and lifespan (in days) for each age class and for the all dataset, using the "cor.test" function in the "psych" package [44]. The same statistical tests were also applied to fertilization rate (**Supplementary Results**).

Bayesian models on observed data

To estimate the parameters that fit the observed relationship between lifespan and reproductive success, we employed a Bayesian approach in Stan [27] via the R interface "rstanarm" [26]. For each age class (levels: young, mid-age, old), we ran a Bayesian model to assess whether embryo survival at a specific age was associated with male lifespan. We modelled the number of fertilized eggs that survived 72hpf as a binomial outcome with a logit link function. The log-odds of embryo survival were modelled as a linear function of the log-transformed lifespan of the father. The model included an intercept (α) and a slope (β) with weakly informative priors (see *Equation 1*). Each model was run with 4 chains, each consisting of 2000 iterations, of which 1000 for warmup.

(Equation 1)

For each individual *i*:

$$n_embsurv_i \sim Binomial_Logit(n_fertilized_i, \alpha + \beta \times \log(lifespan_i))$$

$$\text{Priors: } \alpha \sim Normal(0, 10); \beta \sim Normal(0, 2.5)$$

Where: $n_embsurv_i$= number of embryos survived for individual *i;* $n_fertilized_i$= number of fertilized eggs for individual *i*; α= intercept; β= slope (effect of log-lifespan on embryo survival); and $\log(lifespan_i)$= log-transformed lifespan of individual *i.* We performed similar models for fertilization rate by modelling the number of eggs that were fertilized as the binomial outcome. We assessed model

convergence using visual inspection of trace plots ("traceplot" function) and checked whether all parameters had R-hat values<1.01, indicating good convergence. Posterior probabilities of the intercept and slope were extracted ("extract" function) for each age class, and the posterior predictions of embryo survival were computed across a regular sequence of 100 father log-lifespan values. We plotted individual posterior predictions and the mean posterior prediction for each age class using "ggplot2" package [45] (see associated code).

To estimate the predictive power of embryo survival for father lifespan we ran a Bayesian model for each age class with lifespan as the outcome variable. In these models the log-transformed lifespan was modelled as a linear function of the proportion of embryos that survived 72hpf. The model assumed normally distributed residuals and included an intercept (α), a slope (β), and a residual standard deviation (σ) and weakly informative priors (see *Equation 2*). As before, each model was run with 4 chains, each consisting of 2000 iterations, of which 1000 for warmup.

(Equation 2) For each individual *i*:

$$\log(\text{lifespan}_i) \sim \text{Normal}(\alpha + \beta \times \text{embsurv}_i, \sigma)$$

$$\text{Priors: } \alpha \sim \text{Normal}(0, 10);\ \beta \sim \text{Normal}(0, 2.5);\ \sigma \sim \text{Exponential}(1)$$

Where: $\log(\text{lifespan}_i)$ = log-transformed lifespan of individual *i;* embsurv = proportion of embryos survived for individual *i;* α= intercept; β= slope (effect of embryo survival on log-lifespan); σ= residual standard deviation. We performed similar models for fertilization rate by using the proportion of fertilized eggs. We assessed model convergence as before (see associated code). As before posterior probabilities of the intercept and slope were extracted ("extract" function) for each age class, and the posterior predictions of lifespan were computed across a regular sequence of 100 embryo survival values. As before, we plotted individual posterior predictions and the mean posterior prediction for each age class using "ggplot2" (see associated code). The same statistical procedures were also applied to fertilization rate (**Supplementary Results**).

Simulation and model comparison

To test whether the hypothesized causal model (DAG, **Figure 3a**) could reproduce the observed patterns between lifespan and embryo survival, we used simulated data. We generated a population of 1000 individuals, each assigned an *intrinsic quality* (IQ) produced from a normal distribution (mean = 0, sd

= 1). Lifespan (LS) was then simulated for each individual as a log-normal transformation of QI, while functional quality (FQ) was modelled to decline linearly as age increases. At each age (i.e. from 1 until the end of the individual's lifespan), we generated embryo survival (ES) from a normal distribution with mean determined by FQ and age. Age classes were defined according to population survival thresholds, consistent with the experimental data: "young" when ≥85% of the population was alive, "mid-age" between 85% and 60%, and "old" when ≤60% of the population is left.

To test whether "Age Class" should be explicitly included as a predictor of lifespan, we compared two Bayesian models in Stan. Similarly to *Equation 2*, in Model 1, we modelled lifespan independently for each age class as a linear function of Z-transformed embryo survival. As before, each model was run with 4 chains, each consisting of 2000 iterations, of which 1000 for warmup. In Model 2, we modelled lifespan as a linear function of Z-transformed embryo survival by including an additional categorical predictor for age class (*Equation 3*). We ran Model 2 with 4 chains, each consisting of 4000 iterations, of which 2000 for warmup.

(*Equation 3*) For each individual i:

$$LS_i \sim \text{Normal}(\alpha + \beta \times \text{Z-ES}_i + \gamma(AC_i), \sigma)$$

$$\text{Priors: } \alpha \sim \text{Normal}(0, 10);\ \beta \sim \text{Normal}(0, 10);\ \gamma \sim \text{Normal}(0, 10);\ \sigma \sim \text{Cauchy}(0, 5)$$

We assessed model convergence as above (see associated code). As before, posterior probabilities of the intercept and slope were extracted ("extract" function) for each age class, and the posterior predictions of lifespan were computed across a regular sequence of 100 embryo survival values. We plotted mean posterior predictions for Model 1 and Model 2 for each Age Class using "ggplot2" (see associated code).

Data and Code Availability

The datasets and code underlying this study will be made publicly available on GitHub. A permanent DOI will be assigned via Zenodo upon acceptance of the manuscript.

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384 ## Figure Captions

385 **Figure 1. Male reproductive success decreases with age and does not trade-off with lifespan. a)**
386 Survival curves of the GRZ-D male cohort used in the study (median lifespan: 154 days). At three age
387 classes (young, mid-age and old) across males' lifespan, we collected the ejaculate and performed IVFs.
388 We measured embryo survival, i.e. proportion of survived embryos at 72 hours after IVF. Lifespan of
389 each male was also recorded. **b)** Boxplot and Kernel density plot depicting embryo survival at young
390 (mean±sd: 63.2±24.3%), mid (mean±sd: 40.4±29.6%) and old (mean±sd: 26.6±31.0%) age. Pairwise
391 comparisons are significant (p<0.001) for all groups except between mid and old age. **c)** Scatterplot
392 showing the relationship between lifespan and embryo survival across the three age classes. Dots
393 represent observed data from each individual male. Mean posterior predictions (black lines) and
394 posterior predictive distributions (coloured lines) from Bayesian models are fitted. Embryo survival
395 was modelled as a binomial outcome with a logit link and log-transformed lifespan was added as a
396 predictor in the model. For visualization purposes, observed data for embryo survival and lifespan is
397 transformed using the logit function and the log scale, respectively. **d)** Forest plot of β estimates for
398 each age class. Points indicate posterior means and horizontal bars represent 95% credible intervals,
399 relative to zero as the null effect.
400
401 **Figure 2. Early life reproductive success predicts individual lifespan. a)** Equation used to run
402 Bayesian models predicting father lifespan from embryo survival. Log-transformed lifespan was
403 modelled as a linear function of embryo survival, where α represents the intercept, β is the slope (i.e.
404 effect of embryo survival on lifespan), and σ is the residual standard deviation. Each model (one per
405 age class) was run with 4 chains, each consisting of 2000 iterations, of which 1000 for warmup. **b)**
406 Mean posterior predictions (black lines) and posterior predictive distributions (coloured lines) from
407 Bayesian models run for each age class. Lifespan was modelled as the outcome variable. For
408 visualization purposes, lifespan was back-transformed from the log scale by exponentiation. **c)** Forest
409 plot of β estimates for each age class. Points indicate posterior means and horizontal bars represent 95%
410 credible intervals, relative to zero as the null effect. At young age the posterior distribution of the slope

411 indicates a 95% probability that higher embryo survival is associated with increased male lifespan, as
412 the 95% credible intervals does not include 0. **d)** Example of posterior predictions for father lifespan at
413 young age. To estimate the expected father lifespan for a given embryo survival probability (p), we
414 extracted the posterior means of the intercept (α) and slope (β) from the model and calculated the
415 expected lifespan at p as follows: $\text{Lifespan}_{(p)} = \exp(\alpha_{mean} + \beta_{mean} \times p)$. For instance, an increase of 50%
416 in embryo survival (e.g. from 25% to 75%) translates into a 17% increase in lifespan. Lifespan
417 differences are indicated on the survival curves.
418
419 **Figure 3. Intrinsic quality explains the age-dependent change in the correlation between**
420 **individual reproductive success and lifespan. a)** Direct acyclic graph showing the causal relationships
421 among variables. *Intrinsic quality* influences both lifespan and functional quality, which in turn affects
422 reproductive success (e.g. embryo survival). Age impacts reproductive success both directly and
423 indirectly through functional quality. Longer-lived individuals reach later ages compared to short-lived
424 individuals, making lifespan collinear with age class (e.g. young, mid-age, old). The collinearity
425 between variables can introduce statistical artifacts and thus must be carefully considered when
426 structuring the models. **b)** Scatterplot showing the correlation between embryo survival and lifespan in
427 in a simulated population in which individuals differ for their *intrinsic quality*. Lifespan and embryo
428 survival were expressed as linear functions of *intrinsic quality* and age classes were defined according
429 to experimental data thresholds. Each dot represents an individual. Similarly to observed data, simulated
430 individuals with higher embryo survival at young age live longer, while embryo survival at mid and old
431 age does not correlate with lifespan. **c)** Individual trajectories of the relationship between embryo
432 survival and lifespan across ages from simulated data. Each line represents an individual. Individuals
433 with high embryo survival at young age tend to live longer (orange lines) compared to individual with
434 low embryo survival at young age (black lines). **d)** Mean posterior predictions from two separate
435 Bayesian models on the simulated data by either excluding (Model 1) or including (Model 2) the "Age
436 Class" variable as an input variable lifespan. In Model 1 (purple lines), embryo survival at young age
437 but not at mid and old age, predicts individual lifespan, in accordance with the observed data. In Model
438 2, there is no correlation between embryo survival and lifespan across all age classes. When included

Supplementary Results

Early-life reproduction predicts male lifespan in turquoise killifish (*Nothobranchius furzeri*)

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Supplementary Results

To investigate how investment in reproduction affects lifespan, we performed in vitro fertilizations (IVFs) at three different time points throughout male's lifespan. As a measure of reproductive success we recorded fertilization rate and embryo survival. Specifically, after 3.5 hours post IVF we checked the fertilization rate by counting the fertilized and unfertilized eggs under a stereomicroscope. The survival of the embryos was then scored 72 hours post fertilization (hpf) by counting the number of embryos still alive. Here we present the results for fertilization rate (i.e. the number of fertilized eggs on the total number of eggs).

Male fertilization rate decreases with age and does not trade-off with lifespan

In line with previous work [21], we found that age significantly affected fertilization rate (χ^2=56.5612, p=5.223e-13). Moreover, sperm concentration significantly affected fertilization rate: males producing more sperm fertilize more eggs (χ^2=7.0762, p=0.0078). Old males fertilized significantly less eggs than young males (z=7.167, p<0.0001) (**Figure S1a**). Similarly to embryo survival, fertilization rate, significantly declines already at mid-age (z=4.919, p<0.0001) (**Figure S1a**).

To test for reproduction-survival trade-offs, we estimated posterior predictive distributions of the relationship between lifespan and fertilization rate at the three different ages (**Materials and Methods**). We found no negative correlation between fertilization rate and individual lifespan (**Figure S1b**). Posterior estimates indicate that males achieving higher fertilization rate at young age live longer (β_{mean}=0.906; 0.48 – 1.31, 95% credible intervals), while there is no correlation between individual

survival and fertilization rate when sampling males at mid (β_{mean}=0.14; -0.38 – 0.31, 95% credible intervals) and old age (β_{mean}=-0.61; -1.98 – 0.84, 95% credible intervals) (**Figure S1c**).

Early fertilization rate predicts individual lifespan

Since father survival and fertilization rate covary, despite weakly, for data collected at young father age (**Figure S1b**), we asked whether fertilization rate statistically predicts variation in father lifespan across individuals. Running a Bayesian model for each age class with father lifespan as the outcome variable (**Materials and Methods, Figure S1d**), we found – as expected from the previous model – that the effect of early-life fertilization rate on lifespan is positive (**Figure S1d**). Specifically, the posterior predictive distribution of the slope at young age (β_{mean}=0.34; 0.006 – 0.69, 95% credible intervals) indicates a 95% probability that higher fertilization rate is associated with increased male lifespan (**Figure S1e**). On the contrary, running a model using fertilization rate measured at older ages, the credible intervals associated to the slope of fertilization rate on lifespan include zero both at mid (β_{mean}=0.037; -0.49 – 0.52, 95% credible intervals) and old age (β_{mean}=-0.13; -0.60 – 0.35, 95% credible intervals), indicating that measuring fertilization rate from mid and old age fathers provides no information about their lifespan (**Figure S1e).**

Figure S1. Fertilization rate largely mirrors the patterns observed in embryo survival. a) Boxplot and Kernel density plot depicting fertilization rate at young, mid and old age. All pairwise comparisons are significant. **b)** Scatterplot showing the relationship between lifespan and fertilization rate across the three age classes. Dots represent observed data from each individual male. Mean posterior predictions (black lines) and posterior predictive distributions (coloured lines) from Bayesian models are fitted. For visualization purposes, observed data for fertilization rate and lifespan is transformed using the logit function and the log scale, respectively. **c)** Forest plot of β estimates for each age class. Points indicate posterior means and horizontal bars represent 95% credible intervals, relative to zero as the null effect. **d)** Mean posterior predictions (black lines) and posterior predictive distributions (coloured lines) from Bayesian models run for each age class. Lifespan was modelled as the outcome variable. For visualization purposes, lifespan was back-transformed from the log scale by exponentiation. Black lines represent posterior means. **e)** Forest plot of β estimates for each age class. Points indicate posterior means and horizontal bars represent 95% credible intervals, relative to zero as the null effect.

in the model, Age Class absorbs the variance explained by embryo survival due to its collinearity with lifespan.

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Figure 1

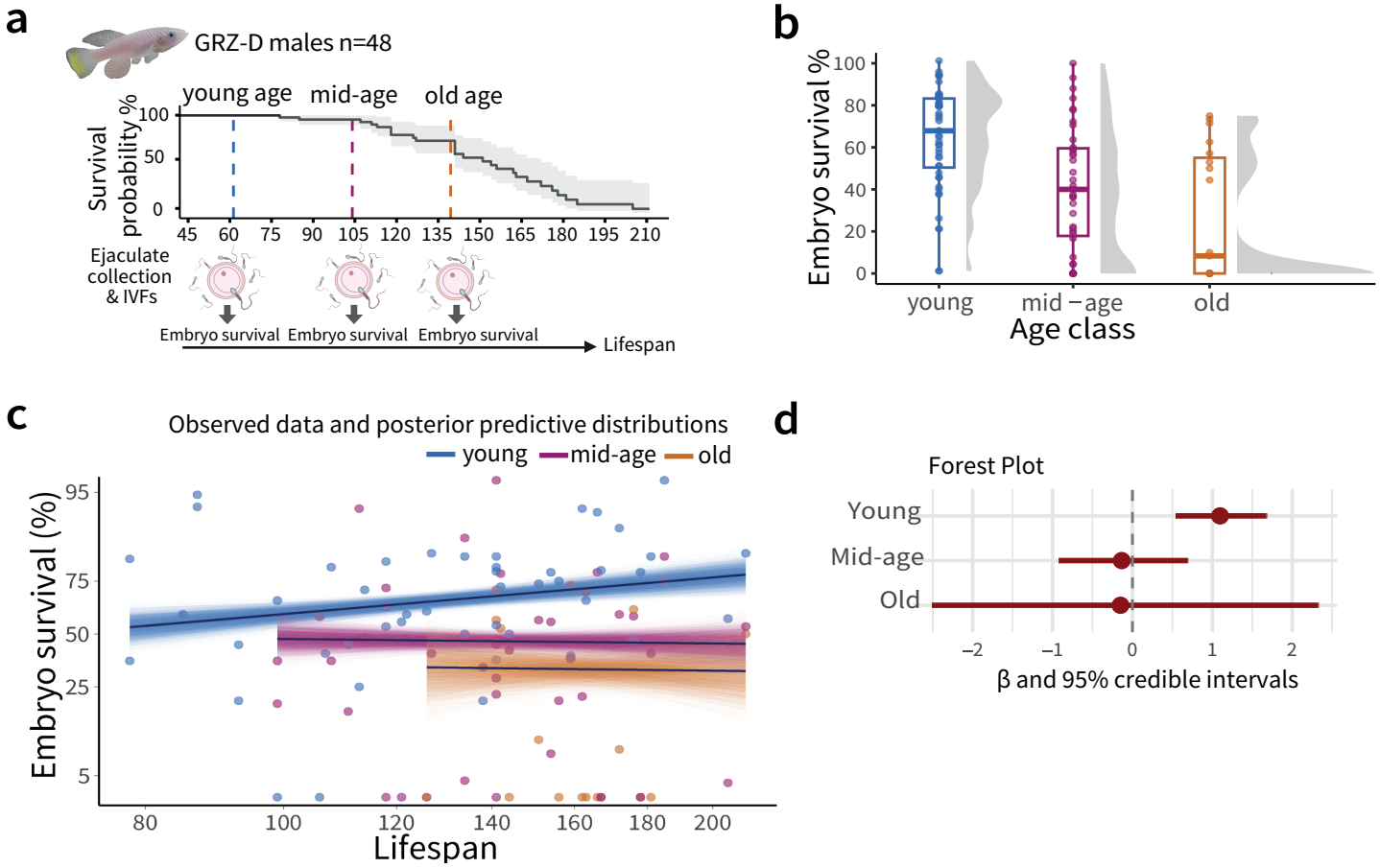

a
GRZ-D males n=48
young age
mid-age
old age
Survival probability %
100
50
0
45 60 75 90 105 120 135 150 165 180 195 210
Ejaculate collection & IVFs
Embryo survival
Embryo survival
Embryo survival
Lifespan
b
Embryo survival %
100
80
60
40
20
0
young
mid−age
old
Age class
c
Observed data and posterior predictive distributions
young
mid-age
old
Embryo survival (%)
95
75
50
25
5
80
100
120
140
160
180
200
Lifespan
d
Forest Plot
Young
Mid-age
Old
−2
−1
0
1
2
β and 95% credible intervals

Figure 2

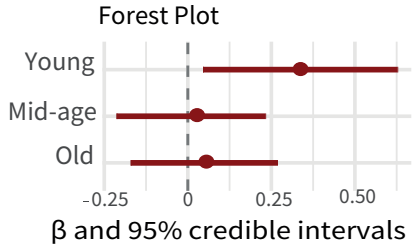
a

Bayesian model

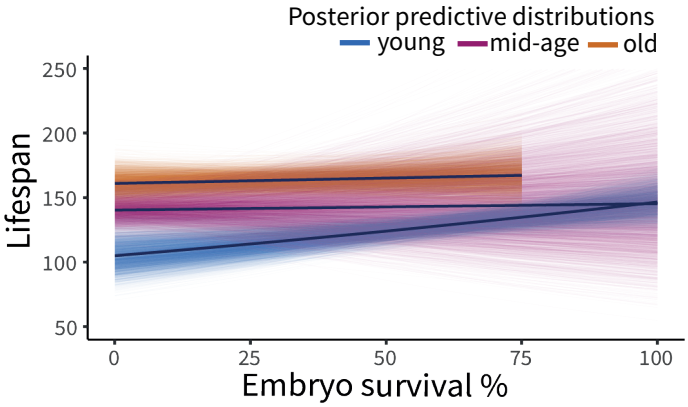
For each individual i:

$\log(\text{lifespan}_i) \sim \text{Normal}(\alpha + \beta \times \text{embsurv}_i, \sigma)$

b



c



d

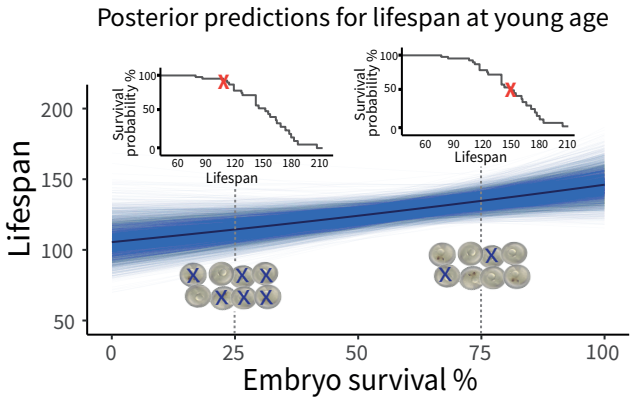


Figure 3

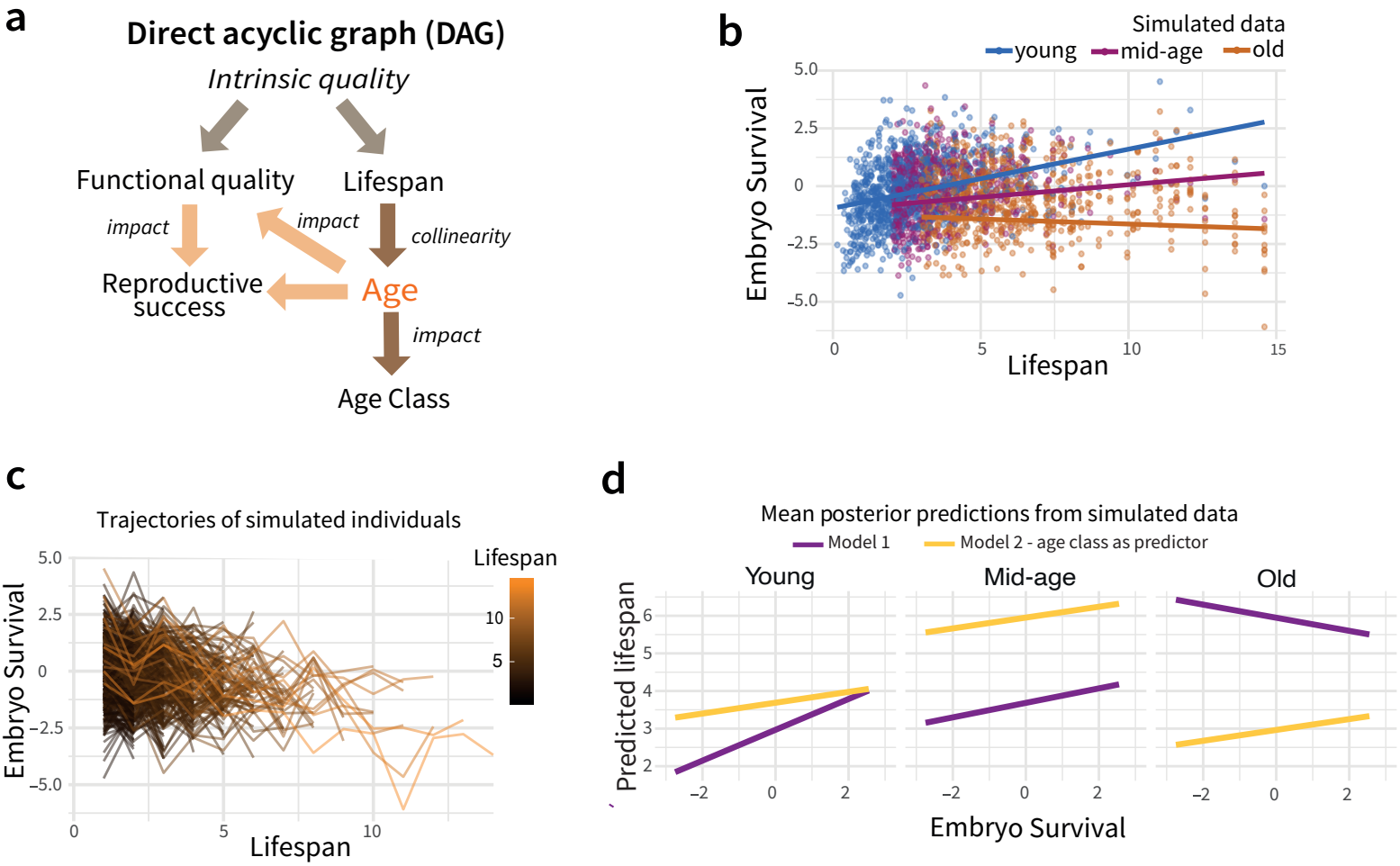
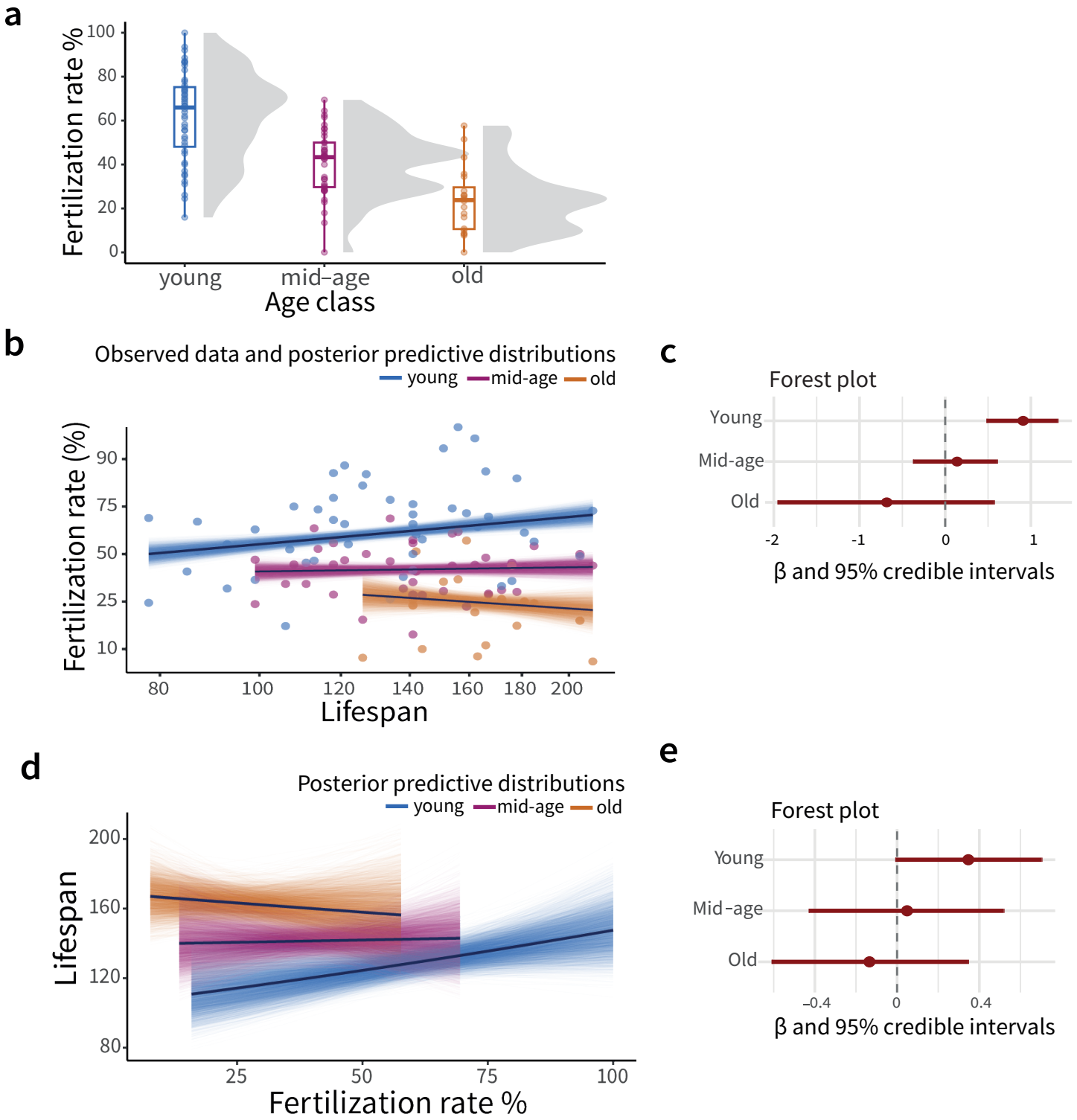

a
Direct acyclic graph (DAG)
Intrinsic quality
Functional quality
Lifespan
impact
impact
collinearity
Reproductive success
Age
impact
Age Class
b
Simulated data
young
mid-age
old
Embryo Survival
Lifespan
c
Trajectories of simulated individuals
Lifespan
Embryo Survival
Lifespan
d
Mean posterior predictions from simulated data
Model 1
Model 2 - age class as predictor
Young
Mid-age
Old
Predicted lifespan
Embryo Survival

Figure S1


a
Fertilization rate %
100
80
60
40
20
0
young
mid–age
old
Age class
b
Observed data and posterior predictive distributions
young
mid-age
old
Fertilization rate (%)
90
75
50
25
10
80
100
120
140
160
180
200
Lifespan
c
Forest plot
Young
Mid-age
Old
-2
-1
0
1
β and 95% credible intervals
d
Posterior predictive distributions
young
mid-age
old
Lifespan
200
160
120
80
25
50
75
100
Fertilization rate %
e
Forest plot
Young
Mid-age
Old
–0.4
0
0.4
β and 95% credible intervals