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Environmental conditions shape the temporal pattern of investment in reproduction and survival

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Abstract

The relationship between environmental stress exposure and ageing is likely to vary with stressor severity, life history stage, and the time scale over which effects are measured. Such factors could influence whether stress exposure accelerates or slows the ageing process, but their interactions have not previously been experimentally investigated. We found that experimental exposure of zebra finches to mildly challenging environmental circumstances from young to old adulthood, which increased exposure to stress hormones, reduced breeding performance during early adulthood, but had positive effects when individuals were bred in old adulthood. This difference was not due to selective mortality, since the effects were evident within individuals, and no evidence of habituation in the response to the stressor was found. The more stressful environment had no effects on survival during young or old adulthood, but substantially improved survival during middle age. Changes in the effects at different ages could be due to the duration and nature of the challenging exposure, or to variation in coping capacity or strategy with age. These results show that living under challenging environmental circumstances can influence ageing trajectories in terms of both reproductive performance and longevity. Our results provide experimental support for the emerging idea that stress exposure needs to be optimised rather than minimised to obtain the best health outcomes.

Keywords: environmental stress, glucocorticoids, reproduction, survival, hormesis.

Introduction

Ageing, broadly defined as the decline in performance with advancing age, has been well documented among different animal taxa both in the wild and under laboratory conditions [1, 2]. The pattern of ageing, that is the timing of onset and the rate at which deterioration occurs, is highly variable both among and within species. One of the major focii of ageing research is

47 the endeavour to understand the causes of such heterogeneity [3-5]. This involves identifying
48 selection pressures driving the evolution of species-specific patterns of ageing [1], the
49 underlying cellular mechanisms [6], and the genetic and environmental factors that generate
50 variation among individuals of the same species [7]. Evolutionary explanations of ageing are
51 largely based on cost-benefit trade-offs. Two main theories currently predominate - a genetic
52 approach centred on the antagonistically pleiotropic effects of genes that confer beneficial
53 effects early in life but deleterious effects later in life [8, 9], and a resource allocation
54 approach, embodied in the disposable soma theory, which is concerned with the fitness effects
55 of differential investment in self-maintenance and reproduction [3, 10, 11]. These two
56 approaches are complementary, make similar predictions and have both been applied largely
57 in the context of variation in lifespan and reproductive performance among different species
58 [1, 3, 12, 13].

59 Variation among individuals of the same species in the pattern of ageing can also be
60 viewed using the same framework. Allocation of resources to self maintenance will vary due
61 to differing capacities, constraints, priorities and resource availability. It is well recognised
62 that intra-specific variation in the pattern of ageing is strongly influenced by environmental
63 conditions. Shifts in "priority rules" underlying optimal allocation of limiting resources
64 between self-maintenance and reproduction are expected to become more evident when
65 animals are exposed to challenging environments, such as when facing unpredictable, adverse
66 environmental circumstances influencing factors such as weather, food availability, disease,
67 parasite and predation risk [14, 15]. The resultant increase in energy expenditure and stress
68 exposure might directly damage the soma and result in faster age-related deterioration [16-

19]. Alternatively, harsher environmental conditions could influence the optimal balance of resource allocation between self-maintenance and reproduction with consequences for age-related reproductive effort and survival patterns [7, 20-21]. Strategic rescheduling of investment may occur, with individuals delaying reproduction if conditions are likely to improve or bringing it forward if life expectancy is likely to be reduced, with consequences for age-specific reproductive success and the pattern of senescence [22, 23].

Effects of stressful environments on ageing patterns could also vary at different life stages, for example in early life and in adulthood, or early adulthood and old age, because vulnerability to damage, and the resulting fitness consequences, may differ. An additional layer of complexity is added by the fact that the ageing process itself can alter both vulnerability and resilience to stress exposure, and stress exposure can diminish or exacerbate ageing [24]. These interactions are influenced by the severity of the stress experienced, with severe stress generally accelerating ageing, while milder stress exposure can induce resilience and extend lifespan [25]. Furthermore, the consequences of exposure to even mild stressors are likely to change with age due at least in part to impaired functioning of the stress-response systems with age [24]. Much attention has been devoted to the long lasting effects of stress exposure in early life, with much less attention being given to effects in adulthood, and less still to how these effects might change across the life course [24, 26]. In the majority of studies conducted to date, manipulations of environmental conditions have been conducted over a relatively short period, and at a single life stage. This is in part due to the time investment required, the logistics of following individuals over time, and to some extent also to the largely untested assumption that what holds at one life history stage also holds at others.

Here we report the results of an experiment in which female zebra finches (*Taeniopygia guttata*) were repeatedly exposed to a relatively mild environmental stressor, to which they did not habituate, from early in their adult lives. We have previously shown that this has no effect on survival in young adulthood, but increased survival during middle age in comparison with a control group not exposed to the environmental stressor [27]. The survival advantage could have occurred due to a re-scheduling of resource allocation to reproduction, and/or stress-induced resilience. In order to examine whether this response to the mildly stressful environment involved any differences in reproductive investment over controlled age-specific breeding events, we examined reproductive performance of these birds from young adulthood into old age. We also examined whether the previously observed survival advantage of stress-exposed birds in middle age persisted into old age, or whether there was evidence that resilience then declined. Lastly, we examined whether there were any change in baseline levels of the stress hormone corticosterone with age, and whether there was any evidence of habituation to the stressor when the birds were older.

Materials and Methods

(a) Study subjects

The study was performed in female zebra finches that were produced in two replicates from parents of the same stock population at the University of Glasgow (replicate 1 birds were produced in April-June 2011; replicate 2 birds were produced in August-September 2011). To minimise potential mate familiarity [28], the stock females were paired with

different mates in the two breeding events, and the resulting offspring used to form the two experimental replicates. For each replicate, the environmental manipulations started when the study females were young, fully grown, sexually mature adults (5 months old on average: mean ± SE: 152 ± 1 day) [27]. Females were housed in treatment-specific cages (n = 7-10 per 120 x 50 x 50 cm cage) and randomly allocated to one of two experimental groups: (1) challenging environment (replicate 1: n = 45 females; replicate 2: n = 62 females), or (2) control environment (replicate 1: n = 46 females; replicate 2: n = 61 females). When possible, females that hatched in the same nest (part of the same brood) were counterbalanced between the two treatment groups and family of origin was taken into account in all analyses. All birds were maintained throughout the experiment at a photoperiod of 14h:10h light:dark cycle and the temperature was maintained between 20-24°C. All procedures were carried out under UK Home Office Project Licence 60/4109.

(b) Environmental conditions

Upon 5 months of age, females were randomly assigned to either a challenging or control environmental condition. In the challenging environmental condition, food was made unavailable for a continuous period of 4.9 hours (~ one third of the daylight hours), 4 days per week, on a random time schedule. For the remaining two thirds of the day and on the remaining 3 days per week, they were provided with *ad libitum* food. Thus the manipulation changed the temporal availability of food, but, when available, food was abundant. Challenged females were always kept on this food regime, except when they were breeding and received *ad libitum* access to food continuously from the time they were paired with a

male or shortly afterwards until after they completed breeding (~ two months for each breeding event). Females in the control group were always provided with *ad libitum* food and experienced exactly the same breeding regime as the challenged birds (see paragraph below). During the third breeding event only, at 1.8 years old (see also paragraph below), the birds in the challenging environment were given a single, daily, exposure to the glucocorticoid stress hormone corticosterone to determine whether a more protracted environmental challenge during pre-breeding/pair formation influenced reproductive investment. Specifically, two weeks prior to this breeding event, challenged birds were given oral doses of corticosterone (Sigma-Aldrich, Poole, UK) following each period of episodic food withdrawal. The hormone was administered by providing the birds with seed soaked in corticosterone suspended in peanut oil at a concentration of 0.0825mg/ml (corticosterone dose/bird was ~ 4.075µg; 1 g of seed soaked/bird) for 10 min immediately after the end of each episodic food withdrawal. Corticosterone dosing was based on previous work in zebra finches (29). Control birds received 1g of seed soaked in peanut oil only for the same amount of time as the challenged females. The unpredictable food regime and corticosterone seed manipulation were continued until individual clutches were completed (mean ± SE: 25.8 ± 0.3 days; range: 20-33 days). A small number of females did not attempt to breed and in these birds the oral corticosterone treatment was suspended 14 days after pairing (total duration 28 days). Following this breeding event, all experimental females were placed back on the unpredictable food regime only (i.e., no exposure to corticosterone soaked seeds) until the next breeding event at 3.5 years of age (~1.5 years later). There were no effects of the duration of corticosterone supplementation on measures of reproductive performance (clutch size or number of chicks

reared) at both 1.8 years of age and 3.5 years of age (Pearson's r: $-0.02 < r < 0.2$, $p \geq 0.2$ for all), suggesting that this short-term additional corticosterone treatment did not influence breeding investment.

We have previously shown that there is no overall significant effect of the experimental treatment on body mass up to three years of age [27]. Consistent with other studies, we have also found that the experimental food manipulation resulted in increases in overall exposure to glucocorticoids [30, 31]. More specifically, at the end of the episodes of food withdrawal, the challenged birds showed higher baseline corticosterone (the predominant avian glucocorticoid hormone) levels than those birds living in the control environment and this physiological response was consistent over prolonged exposure periods (up to 6 weeks) indicating no habituation of the birds to the unpredictable food shortages (on average 1.4 fold increase; full details in [27]). These data were collected during young adulthood (< 1 year of age). We also measured corticosterone in a randomly chosen subset of study females (34 control and 32 challenging environment) when they were 3.5 years old. Average baseline corticosterone levels decreased by around 50% in both treatment groups in old adulthood compared to young adulthood, but despite this the birds in the challenging environment continued to show a similar magnitude of increase in baseline corticosterone at the end of the episodic food withdrawals also into old adulthood (on average 1.8 fold increase; Table S0; full details in Supplementary). Thus, our environmental protocol mimicked the physiological effects of an environmental stressor naturally experienced by animals living under protracted exposure to unpredictable environmental conditions [32].

(c)Breeding schedule and breeding performance

Study females from both treatment groups were allowed to produce clutches of eggs four times during the study. For breeding, females were paired with a randomly assigned, relatively young male ranging in age from 6 months to 1.8 years; experimental females were paired with the same male partner in their first and second breeding event whereas in the following two breeding events they were always paired with a different male. Control and challenged females were paired with males for the first time when they were on average 6 months old (188 ± 0.89 days of age; all females survived to this first breeding event), approximately 1 month after the start of the environmental manipulation. Each pair was housed in their own cage (60 X 50 X 50 cm) and provided with a nest box and nest material (coconut fibre and jute, Haiths Ltd). The females were paired again at the following ages: 1.1 years (408 ± 0.82 days of age), 1.8 years (653 ± 0.78 days of age), and finally when they were 3.5 years old (1270 ± 0.92 days of age) – mean ± SE for all. For the breeding event at 1.1 years the pairs were not allowed to rear any chicks since the eggs were required for assays of egg composition, and were collected shortly after laying and replaced with dummy eggs. Dummy eggs were removed once individual clutches were complete and the pairs then separated. During the breeding at 1.8 years, most of the clutches (157out of 187; logistic reasons) were cross-fostered at the end of the incubation period in order to examine egg effects on chick survival as part of a separate study to disentangle maternal from rearing environmental treatment effects; a small subset of cross-fostered clutches (43) was also subjected to brood size manipulation experiments (data to be reported in full elsewhere).

When lifetime breeding performance and survival are examined below, we only considered those birds whose clutch size was not manipulated (43 out of 214 birds excluded from these analyses; total sample size 171 birds). We quantified breeding performance of the study females by recording the following: (1) likelihood of breeding (laying a clutch), (2) latency to lay (i.e. time from pairing to the laying of the first egg); (3) clutch size; (4) fledgling success (proportional data: number of chicks fledged/clutch size), and (5) the number of chicks fledged (assessed when the offspring were ~ 30 days old, including also those females that did not lay a clutch in order to assess the overall breeding performance).

(d) Survival

We monitored the survival of the birds for 4 years (i.e. till 1456 days of age). Experimental birds were inspected daily and all the birds considered here died of intrinsic causes, not of accidental injury or aggression. Where birds showed clear signs that death was imminent and their welfare was very severely compromised (the birds were not able to fly and/or feed independently and our veterinarian confirmed that death was imminent), they were culled under the advice of our veterinarian in line with UK Home Office legislation (n = 24 out of 85 females that died – total sample size, n = 171). Generally, deaths were unpredictable with the majority of the birds being found dead on the cage floor without having shown prior symptoms.

Data analysis

220 Analyses were performed in R (version 3.2.5; R core team, 2014). Unless otherwise
221 specified, all final models included the effects of experimental design factors expected to
222 influence the response variables either as parameters of interest integral to the question being
223 investigated or for the purpose of adjustment. These relevant factors were always retained in
224 the main models rather than tested using backwards or forwards selection to avoid over-
225 fitting. We used Generalised Linear Mixed Models (GLMMs, R package "lme4" and
226 "lmerTest" - [33, 34]) to examine whether the challenging treatment influenced reproductive
227 performance and whether any potential effect of the treatment varied across the age-specific
228 breeding events at 6 months, 1.1 years, 1.8 years, and 3.5 years old as appropriate. Unless
229 otherwise specified, final models included the following factors: treatment, age, replicate, and
230 the interaction treatment x age. In initial models, we tested the potential interaction effect of
231 the treatment with replicate to check consistencies of treatment effects between the two
232 replicates. Age was modelled as categorical rather than continuous variable due to the
233 relatively reduced number of data points per individual bird (up to 2, or 4 as appropriate);
234 female individual identity was always added as random factor to control for correlations
235 between reproductive performance traits within individuals due to the presence of repeated-
236 measurements in the data. As appropriate, we also entered family of origin and male partner
237 identity as additional random factors to control for potential pseudo-replication due to the
238 presence of sisters in the experiment and because some males were used more than once
239 across the breeding events. In preliminary analyses, we also tested if previous reproductive
240 investment decision level (investment in egg laying up until the event under consideration)
241 influenced current reproductive investment (clutch size) at 1.1 years, 1.8 years, or 3.5 years in

242 an interaction with the treatment. Chick mortality is low in this captive situation, and clutch
243 size correlates well with the number of chicks reared in our population (Pearson's r = 0.6, $p <$
244 0.0001); thus investment in egg laying is a good proxy for overall reproductive investment
245 level at each breeding event. Across all breeding events, we found no interaction effect of the
246 treatment with previous reproductive decisions on clutch size ($p \geq 0.5$), excluding the
247 possibility of conditionality between previous and current reproductive decisions in relation to
248 life time environmental conditions. We first examined if there were any treatment differences
249 in whether or not the females attempted to breed (i.e. laid eggs) using GLMM with a binomial
250 error distribution and logit link function. The interactions treatment x age and treatment x
251 replicate could not be assessed in the latter model due to reduced statistical power because
252 relatively few birds did not attempt to breed during the first three breeding events. For those
253 females that bred (i.e. laid a clutch), we then analysed the latency to lay the first egg using
254 GLMMs with a Gaussian distribution error– data were log10 transformed to improve
255 normality of model residuals. Clutch size (mean: 4.3, range: 1-8 eggs) was analysed using a
256 GLMM with a Gaussian distribution error rather than with a Poisson distribution because the
257 data were strongly under-dispersed (dispersion parameter < 0.39) and model residuals were
258 normally distributed. Fledging success was analysed with GLMM using a binomial error
259 distribution and logit link function [35], and the number of chicks fledged (range 0-6 chicks)
260 was analysed using a GLMM with a Poisson distribution (dispersion parameters: 0.8-1.3). In
261 the fledging success and number of chicks fledged statistics we did not include the data at 1.8
262 years of age as these response variables could have been influenced by the cross-fostering
263 experiment conducted as part of a separate study to disentangle maternal from rearing

environmental treatment effects (data to be fully reported elsewhere). In order to assess within-female treatment effects and to exclude potential survival bias in the results caused by loss of specific phenotypes from the population (e.g. poor quality breeders dying in early adulthood), we also performed the analyses using only those females that survived to the breeding event up to 3.5 years of age (103 out of 171 birds). We used the R package "lsmeans" (36) to perform pairwise post-hoc contrasts for significant outcomes in the main models (Tukey *p* values adjustment).

We have previously shown in the birds from the same study population used here that the challenging environmental conditions improved life expectancy up to three years of age (Mixed Effects Cox Models, $p = 0.02$; full details in [27]). We have also shown that there was no link between body mass at 1 year of age and subsequent survival up to three years of age [27]. Importantly, the positive effect of the challenging treatment on survival was evident prior to the start of the additional short-term corticosterone manipulation at ~1.8 years of age (data right-censored at 600 days of age: Mixed Effects Cox Models, $p = 0.04$) excluding the possibility that the short-term change in the severity of the stress treatment at 1.8 years of age per se was the main factor triggering the change in the survival trajectories of our study birds. Here, we further examined survival in old age (between three and four years) and tested the extent to which survival probability was dependent on individuals' lifetime reproductive effort. We excluded the females that were subjected to the brood size manipulation experiments at 1.8 years of age (43 birds) from all breeding performance and survival analyses performed here to exclude any possibility that those manipulations altered subsequent survival independently of the environmental conditions. However, the results do

not differ qualitatively when these birds are included (data not shown). Data were right censored to allow inclusions of birds still alive at the end of the survival monitoring period (49.7% out of 171 birds). We first checked if our annual measurements of body mass (i.e. 1 year, 2 years, and 3 years) predicted survival up to 4 years of life using time-dependent covariate Cox model analyses (R package "survival" [37]) and found no effect of this covariate on survival (body mass, body mass x treatment, body mass x replicate, $p \geq 0.2$). Therefore body mass was dropped from the following analyses. In the following Cox Model analyses (R packages: "survival" and "coxme" [37, 38] we entered treatment, replicate, and their interaction as fixed factors, and family identity as a random factor as appropriate. Model diagnostics using Schoenfeld's residuals plotting suggested that the proportional hazards hypothesis was not met due to a non-linear effect of the treatment with time emerging after 3 years of age, whereas it was met in our previous analysis up to 3 years. As mortality rates were clearly very low from 5 months to 1 year (4 control and 2 challenged birds dead out of 171 females), and because of the change in the effect of the treatment over time after 3 years of age, we consequentially introduced in the analyses treatment time-dependent coefficients by breaking the data into three time intervals: (1) young adulthood, from the start of the experiment (5 months old) up to 1 year of age; (2) middle adulthood, from 1 to 3 years of age, and (3) old adulthood, from 3 to 4 years of age. In the model we also checked the potential interaction effect of treatment with replicate. The proportional hazard assumption was met in these models. To test if survival was influenced by the individual's lifetime reproductive effort, we performed separate GLMs (binomial family distribution error with logit link function) entering replicate, along with lifetime egg laying effort (calculated as lifetime

number of eggs laid divided by total number of breeding events, ranging from 1 to 4 events depending on the individual's lifespan), or chick rearing effort (calculated as lifetime number of chicks reared by each female divided by total number of breeding events in which chicks were reared, including the event at 1.8 years of age, ranging from 1 to 3 events depending on the individual's lifespan) as continuous covariate – this standardisation allowed us to overcome collinearity between longevity and lifetime number of eggs laid/chicks reared (Pearson's r = 0.1, $0.07 < p < 0.2$) as females that survived longer ended up with larger number of eggs and chicks reared over the lifespan (Pearson's r = 0.5-0.7, $p < 0.0001$ for both covariates). We performed the latter GLMs separately by treatment in order to simplify model interpretation and avoid issues of collinearity between the treatment and the lifetime reproductive effort. Unless otherwise specified, values are presented as means ± SE.

Results

Breeding failure

Irrespective of environmental conditions, the probability of breeding failure was influenced by female age. More females failed to produce a clutch in the later breeding events at 1.8 years and 3.5 years of age than the earlier events (1.8 years *vs* 6 months and 1.8 years *vs* 1.1 years, $p \leq 0.048$; 3.5 years months *vs* 6 months and 3.5 years *vs* 1.1 years, $p \leq 0.0007$; full results in Table S1a, Supplementary; descriptive statistics in Table S2, Supplementary). There was no difference in the probability of breeding failure between the breeding events at 6 months and 1.1 years old ($p = 1.0$), or between 1.8 years and 3.5 years of age ($p = 0.2$; Table

S1a, S2). We found no significant effect of the treatment or replicate on the likelihood of breeding failure (Table S1a). Similar results were obtained when we carried out this analysis using only those females that survived to breed in old age at 3.5 years old (Table S3a and S4, Supplementary).

Latency to lay the first egg within the clutch

Irrespective of their environmental conditions, females at 1.1 years and 1.8 years laid their first egg sooner following pairing than they did at at 6 months of age (1.1 years *vs* 6 months, and 1.8 years *vs* 6 months, $p < 0.0001$ for both; full results in Table S1b, Supplementary; Figure 1a); there were no differences in latency between 1.8 and 1.1 years ($p = 0.4$; Figure 1a). Latency to lay increased again when the birds were old at 3.5 years to a level similar to that at 6 months of age ($p = 0.8$; Table S1b; Figure 1a). Replicate 2 birds laid their first clutches slightly sooner compared to replicate 1 birds (replicate 1: 8.5 ± 0.4 days; replicate 2: 7.2 ± 0.3 days), and there were no treatment effects on latency to lay either as a main factor or in its interaction with age (Table S1b; Figure 1a). Again, similar estimate parameters were obtained when carrying out the analysis only on those females that opted to breed and survived to the breeding event at 3.5 years of age (Table S3b and Figure S1a, Supplementary).

Clutch size

Irrespective of environmental conditions, clutch size (range 1-8 eggs) was influenced by female age: it increased at 1.1 years relative to 6 months of age ($p = 0.007$), did not differ

between 1.1 years and 1.8 years of age (p = 0.15), and then decreased in old adulthood relative to the earlier life breeding events ($p \leq 0.0001$ for all contrasts, full results in Table S1c, Supplementary; Figure 1b). We found no effect of treatment on clutch size (Table S1c; Figure 1b). Replicate 2 females produced overall slightly larger clutches than replicate 1females (replicate 1: 4.0 ± 0.1 eggs; replicate 2: 4.5 ± 0.1 eggs - Table S1c). Similar parameter estimates were obtained when carrying out the analyses only using those females that opted to breed and survived up the breeding event at 3.5 years of age (Table S3c and Figure S1b, Supplementary).

Fledging success

We examined fledging success at the first and last breeding event (no chicks were reared at the breeding event at 1.1 years, and at 1.8 years of age a separate egg cross-fostering experiment was performed so these data have not been included). Fledging success was reduced when the birds were 3.5 years relative to 6 months ($p < 0.0001$, full results in Table S1d, Supplementary; Figure 1c). There was no effect of replicate either as a main factor or in its interaction with the treatment (Table S1d). The effect of the treatment on fledging success was age-dependent (Table S1d, Figure 1c). At 6 months of age there was no detectable reduction in fledging success in the challenged females relative to controls (p = 0.2; Figure 1c), while at 3.5 years, challenged females had higher fledging success than the age-matched controls (p = 0.01; Figure 1c). The same results were observed when the analysis was carried out using only those females that opted to breed and survived to the breeding event at 3.5 years of age (Table S3d and Figure S1c, Supplementary).

Number of chicks fledged

As with the fledging success, we examined overall breeding performance at the first and last breeding event. As expected from the clutch size results, the number of chicks fledged (0-6) was much reduced in old adulthood compared to 6 months of age in both control and challenged females ($p < 0.0001$, full results in Table S1e, Supplementary; Figure 1d). Replicate 2 birds reared more fledglings than replicate 1 birds (replicate 1: 1.6 ± 0.1 chicks; replicate 2: 2.1 ± 0.1 chicks, Table S1e), however this effect was consistent between control and challenged females (Table S1e). The effect of the treatment on the number of chicks fledged was influenced by female age. Challenged females fledged fewer chicks (on average 20%) compared to controls at 6 months of age ($p = 0.04$; Figure 1c), whereas at 3.5 years, challenged females reared more offspring compared to age-matched controls ($p = 0.008$, Figure 1d). Similar parameter estimates were obtained when performing analyses only using those females that survived to 3.5 years of age ($p = 0.1$, Table S3e and Figure S1d, Supplementary).

Survival

Mortality was very low between 5 months and 1 year of age and there were no differences in survival between the two treatment groups ($p = 0.5$, full results in Table S5, Supplementary; Figure 2a). Survival curves started diverging after 1 year of age (Figure 2b), and from 1 to 3 years old, the challenged females had on average a 48% reduction in relative risk of death

393 compared to controls (p = 0.03, Table S5), as previously shown [27]. However, when we
394 examined survival during old age, between ages 3 and 4, this effect disappeared; survival of
395 challenged birds was no longer better than controls (p = 0.8, Table S5; Figure 2c). There was
396 no effect of replicate as main factor or in its interaction with the treatment (Table S5). When
397 examining survival up to 4 years of age in relation to lifetime breeding effort, we found no
398 relationships between either laying effort, nor chicks rearing effort within both treatment
399 groups (Table S6, Supplementary).

400

401 **Discussion**

402 This is the first experimental longitudinal study in a vertebrate species to directly compare the
403 effects of living in a challenging environment at different adult life stages, from early to old
404 adulthood. Our key findings are that (i) regardless of environmental conditions, female
405 reproductive performance changed across adult life (6 months, 1.1 years, 1.8 years and 3.5
406 years) with peak performance generally occurring during middle adulthood (1.1 and 1.8
407 years) followed by a marked decline in old adulthood (3.5 years) – importantly this later life
408 decline occurred within individuals consistent with previous literature on ageing across
409 diverse vertebrate taxa [2], (ii) females exposed to the challenging environmental
410 circumstances produced relatively fewer chicks than those living in the control environmental
411 conditions when they were young (6 months of age), but, in contrast, were able to rear more
412 chicks when they were old (3.5 years of age), again this effect occurred within individuals,
413 (iii) females living in the more challenging conditions showed no difference relative to
414 controls in more benign conditions in the probability of survival when they were young adults

(5 months to 1 year of age), had a higher probability of survival in middle age (1 to 3 years of age), with this benefit then disappearing at older ages (from 3 to 4 years).

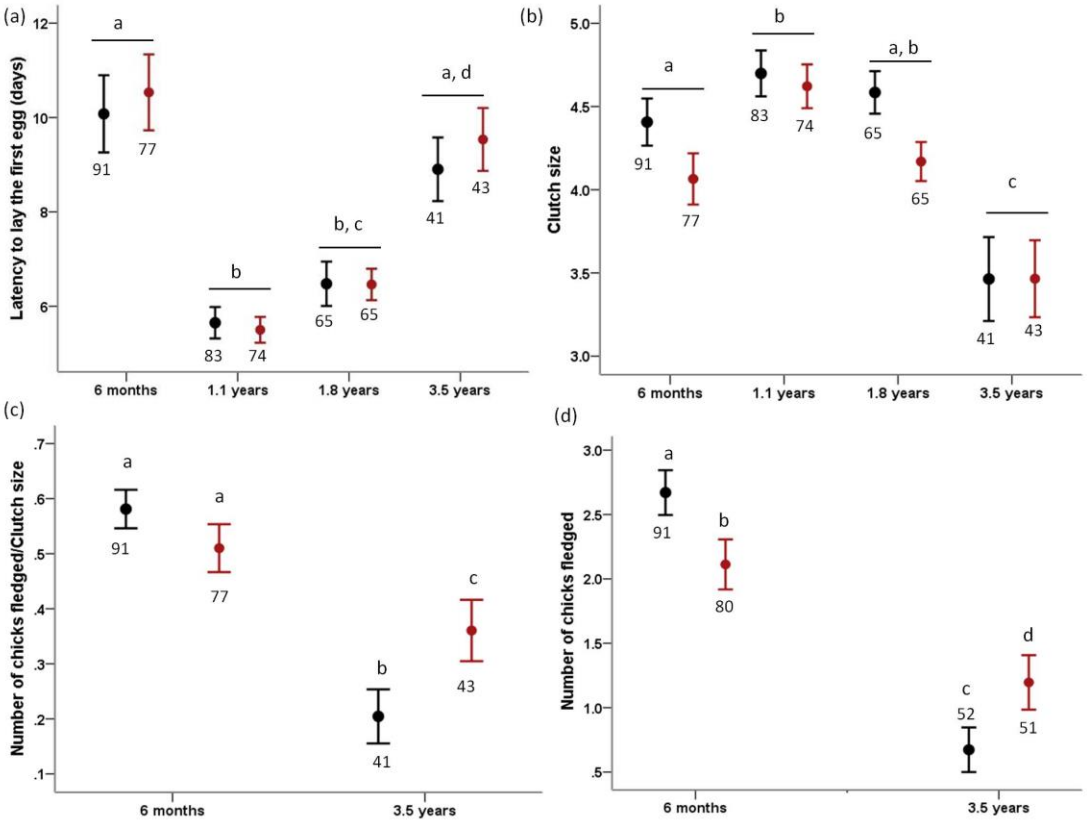
Our stressful environmental protocol did not influence either the likelihood of breeding or the latency to lay the first egg when the females were given the opportunity to breed across the four breeding events, from early to old adulthood. During young adulthood, the challenged females showed an overall reduction in the number of fledglings produced compared to the controls. This effect on overall breeding performance was due to additive treatment-dependent reduction in performance observed at the clutch (primarily) and fledging success level. Interestingly, in old adulthood (3.5 years of age), challenged females, despite laying similar clutch sizes to the controls, fledged proportionally more of their chicks than females living in the more predictable environment, possibly due to treatment differences in parental behaviour and/or in egg quality. Altogether, our results thus show that the mild stress exposure induced by the challenging environmental conditions resulted in females showing a relatively reduced breeding performance when they were young, but increased performance in old age. This effect occurred within individuals, and thus was not due to any differential survival effects. It could be due to challenged females having either an impaired breeding capacity in young adulthood as a result of their exposure to increased levels of glucocorticoid hormones, or to their showing a strategic restraint in breeding effort during early adulthood. We have shown that our challenging environmental protocol did increase overall exposure to stress hormones without causing habituation (measured to old adulthood, 3.5 years). A reduction in reproductive performance in response to stress exposure has been reported in other studies that examined responses to stressful environments, including food shortages or

437 increased predation pressure [32, 39-41]. However, because the birds in our study then
438 showed increased breeding performance in old adulthood, despite still being exposed to
439 higher levels of stress hormones, suggests that their breeding capacity was not impaired and
440 supports a strategic restraint interpretation. It has been suggested that stress exposure induces
441 shifts in energy allocation in order to promote self-maintenance strategies at the expense of
442 reproductive behaviours and parenting [42]. It has also been suggested that environmental
443 stressors could trigger protective and compensatory effects on reproductive physiology (see
444 [15] for a review on the potential mechanisms). Therefore, increases in stress exposure levels
445 experienced by the challenged birds might have activated adaptive changes that allowed
446 individuals to better cope with the protracted exposure to the somewhat harsher
447 environmental conditions, at the expense of earlier reproductive investment perhaps in favour
448 of long-term maintenance processes, including survival [43]. We found no relationships
449 between lifetime breeding effort (egg laying/chicks rearing) and survival within both
450 treatment groups. The slight treatment-dependent reduction in clutch size during the early-
451 middle adulthood breeding events within the pool of birds that survived up to the final
452 breeding event in old adulthood provides only very limited support to this possibility. It
453 would be interesting in future studies to see whether similar treatment effects would be
454 observed in animals free to reproduce. Such a design was not possible in our experiment since
455 we were interested in determining the varying effects of the treatment with maternal age on
456 breeding performance, while controlling for the age of the male partner. Our experimental
457 design does not allow us to separate the effects of age and duration of the challenging
458 exposure, since the two are interlinked as would be the case in nature. Our comparison

between exposure to repeated stress or not simulates responses of animals living in environments in which the occurrence of key stressors such as low food availability, high population density or high predation risk differ, as has been recorded in the wild in diverse species, such as black-legged kittiwakes *Rissa tridactyla* [44], Belding's ground squirrels *Spermophilus beldingi* [45], snowshoe hares *Lepus americanus* (see 46 for further discussion of this). The facts that breeding performance increased at old age in the birds living in the more stressful environment, and that the stress response of the birds to the random food withdrawals was not diminished with age, suggests that the observed effects on reproductive performance are not due to any accumulated negative effects of stress exposure. Our data on survival show that exposure to the challenging environmental conditions had little effect on survival probability when the birds were young, as mortality was very low during this period in our study population as in previous work in captive zebra finches [17,47]. Survival of the birds in the challenging environments was better that the controls during middle age, with this effect disappearing into old adulthood. Our environmental exposure protocol only affected the temporal availability of food, which was otherwise abundant and thus the effects on survival that we found are not likely to be attributable to caloric restriction. Indeed, body mass was not predictive of survival in our study. The challenge induced by our environmental manipulation was mild, giving rise to repeated and prolonged increases in baseline glucocorticoid secretion (this study, 27). The effect of the treatment on survival was substantial, with the challenged birds having on average 48% decrease in the relative risk of mortality compared to control females during middle age. It is possible that the challenging environment may have induced effects that reduced the rate of ageing through hormetic processes [48, 49]. This possibility

fits also with our reproductive data in old adulthood as the challenged females showed less pronounced age-specific declines in reproductive performance relative to those females exposed to the more benign environmental conditions. These long-term beneficial effects of mild challenging exposure resemble those induced by various low-levels/mild repeated stressors that have been shown to delay or slow the onset of senescence across a large variety of animals, including humans [49-53]. Our data are therefore compatible with the treatment exposure having induced stimulatory hormetic responses that slowed at least in part the rate of ageing. The majority of the work focussing on hormetic effects have used single or repeated exposure to mild stressors over relatively brief periods [54, 55]. There is good experimental evidence that exposure to mild stressors can 'prime' responses such that individuals are better able to cope with challenges experienced in later life [52, 56, 57]. However, the survival benefits seem to be contingent on the environmental conditions to which the physiology of the animal has been conditioned being encountered again later in life [47]. In our study, the birds exposed to the challenging environment were continuously exposed to it from when they first experienced it at five months old, which may have enabled them to reap the best survival benefit from the resilience induced by the challenging exposure. We do not know the mechanism underlying the disappearance of the positive effect of the challenging environmental conditions on survival in old age. Overall our data highlights the need of more longitudinal/long-term studies to further our understanding of interacting effects among duration of exposure to stress, stressor severity, and aging patterns – disentangling such factors would require exposing animals of different ages to different stressors duration and severity.

In conclusion, the results of this study suggest that the apparent organismal effects of living in a mildly challenging environment might vary at different life stages, something which has previously received very little consideration. We found evidence of negative effects of living under challenging environmental conditions on breeding performance across young adulthood, but positive effects in old age. Survival was not affected in young adulthood, improved in middle age, but then not affected in old age. These results, in addition to showing that exposure to challenging environments can modulate life histories with consequences for patterns of senescence, also emphasise that the duration of studies, the life history stage at which they take place, and the point at which the effects are examined can influence the interpretation. That repeated exposure to stress might slow the ageing process is an extremely interesting prospect and fits with the emerging idea that, rather than being minimised, exposure to stress levels across the life course needs to be optimised in order to obtain the best health benefits [25, 48].



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518 **Figure 1**

519

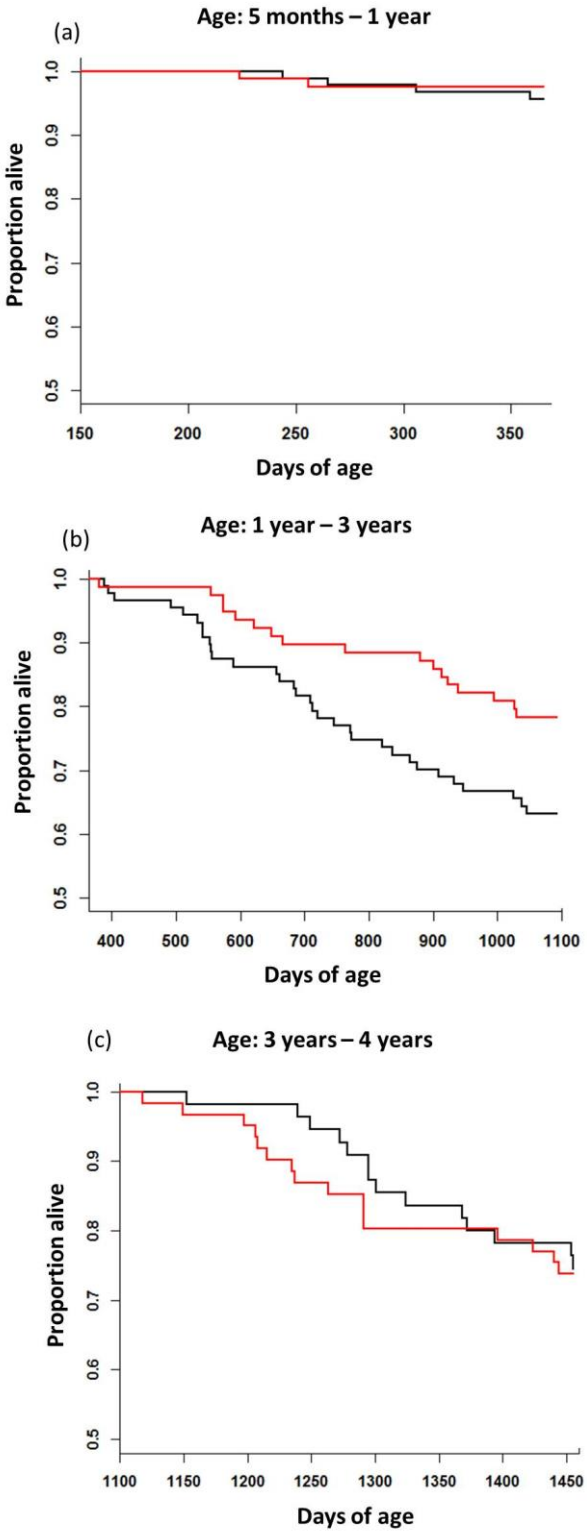

Figure 2

Figure legends

Figure 1. (a) Latency to lay the first egg, (b) clutch size, (c) fledging success (number of chicks fledged/clutch size; proportional data) and (d) number of chicks fledged by the females exposed to the challenging environmental conditions (in red) and control environmental conditions (in black) across the age-specific breeding events. Note that eggs were allowed to hatch only during the breeding event at six months, 1.8 years and 3.5 years of age; at 1.8 years, cross-fostering was used and these data were omitted from these analyses (full details in 'Data Analysis'). Different letters indicate significant post hoc pairwise contrasts ($p < 0.05$ after Tukey's multiple comparison adjustment—full statistics in electronic supplementary material, table S1); numbers indicate sample sizes separately by treatment and age.

Figure 2. (a) Survival trajectories from the start of the experiment up to 1 year of age (i.e. 150-365 days); (b) from 1 to 3 years of age (i.e. 365-1096 days) and (c) from 3 to 4 years of age (i.e. 1096-1456 days) of zebra finch females exposed to challenging (in red) or control (in black) environmental conditions. Birds exposed to the challenging environment showed improved survival from 1 to 3 years of age ($p = 0.03$), whereas no treatment effects were found either from five months to 1 year of age, or from 3 to 4 years of age (full statistics in electronic supplementary material, table S5).

Ethics statement. All procedures were carried out under Home Office Project Licence (60/4109).

Data accessibility. Data are available from Dryad Digital Repository: https://doi.org/10.5061/dryad.6r273.

Authors' contributions. VM, WB, BH, and PM designed the study; all authors conducted the experiment, VM and PM analysed the data and wrote the manuscript, all authors contributed to manuscript revisions of earlier drafts of the manuscript.

Competing interests. We declare we have no competing interests.

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Supplementary Material

Environmental conditions shape the temporal pattern of investment in reproduction and survival

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Baseline corticosterone monitoring

Sampling and laboratory analyses

To monitor the effects of the unpredictable food withdrawals on baseline corticosterone levels we sampled a subset of randomly selected birds from both replicates when the birds were ca 3.5 years old (1266.5 ± 1.5 days of age, mean ± SE; control: 34 females; challenged: 32 females), and after approximately 1.5 years of non-interrupted exposure to the unpredictable food withdrawals (since the termination of the breeding round at ca 1.8 years of age). At the end of a period of food withdrawal in the challenged birds, birds from both treatment groups were blood sampled (~ 75 ul) within 3 min of entering the room to obtain a baseline blood sample (1). We recorded bleed time from each individual bird. Blood samples were stored on ice, centrifuged to separate plasma from red blood cells, and frozen at -80 °C until laboratory analyses. Blood samples were always collected between 13.15 and 15.50 h. Corticosterone levels were measured using an enzyme-immunoassay (EIA - Assay Designs Corticosterone Kit 901-097, Enzo Life Sciences, Exeter UK) following the same method as described previously (2). Briefly, corticosterone was extracted two times in 1 ml of diethyl ether (Rathburn Chemicals, Walkerburn, UK) from plasma aliquots (~17 µl). Tracer amounts (~1500 v.p.) of corticosterone label ([1, 2, 6, 7-3M] NET 399, PerkinElmer, Waltham, MA, USA) were added to each sample to estimate extraction efficiencies. After extraction, corticosterone concentrations (ng/ml) were measured following the manufacturer's instructions. Samples from both treatment groups were standardised across assay plates and the average extraction efficiency was 85%, the average intra-assay coefficient of variation (CV) was 10%, and the inter-assay CV calculated using the same quality control sample run in each plate was 11%. Eight samples fell below the detection limit of the assay and were assigned the minimum detectable value (0.37 ng/ml). The same quality control sample used in the current batch of assays was also used when we measured baseline corticosterone levels from samples collected in early adulthood (~ 6 months of age) from randomly selected birds from the same study population (26 controls and 29 challenged birds - full data published elsewhere, REF 2), and corticosterone concentrations in the quality control were also comparable with the earlier assays (inter-assay CV was 12%).

Data analysis

By including our previous corticosterone data collected from birds in the same study population when the birds were ~ 6 months of age (full results published elsewhere, 2), we used Generalised Linear Mixed Models (GLMMs) with Gaussian error distribution to monitor the effects of age and/or the unpredictable food withdrawals on baseline corticosterone levels

("lme4" package in R, [3]). In the final model fixed factors were treatment, age (6 months *vs* 3.5 years), replicate, and the interaction treatment and age; family identity and individual identity were entered as random factors as there were sisters in the experiment and a few individuals (n = 15) were sampled at both ages. We checked the potential co-variation between the response variable and bleed time, as well as the interaction of the treatment with replicate to assess consistency of treatment effects on baseline corticosterone between the two replicates. CORT levels were ln-transformed to improve normality of model residuals.

Results

There was a main effect of age due a decrease in baseline corticosterone in the birds sampled at 3.5 years of age relative to those sampled at 6 months in both treatment groups (age: $p < 0.0001$, interaction: $p = 0.3$, full model output in Table S0). However, at both age periods the challenged birds responded with similar baseline corticosterone increases to the random episodes of food withdrawals relative to the age-matched controls sampled at the same time of the day (6 months, control: 2.32 ± 0.21, challenged: 3.93 ± 0.52; 3.5 years, control: 1.11 ± 0.19 ng/ml; challenged: 2.03 ± 0.36 ng/ml, un-transformed mean ± SE for all; treatment: $p = 0.02$, Table S0). There was no effect of replicate on baseline corticosterone levels (Table S0).

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Table S0. GLMM modelling (Gaussian error distribution) to assess the effects of the random episodes of food withdrawals on baseline corticosterone levels. Fixed factors estimates are indicated in parenthesis, r indicates random factor and its associated variance. Significant factors are highlighted in bold. The non-significant interaction treatment x replicate (likelihood ratio test, $p > 0.05$) was removed from the final models.

Factor	**Estimate**	**SE**	**t-value**	***p*-value**
Family identity (r)	0			
Individual identity (r)	0			
Residual	0.396			
Intercept	0.789	0.138	5.718	<0.0001
Treatment (challenging environment)	**0.405**	**0.170**	**2.381**	**0.019**
Age (3.5 years)	-0.936	0.164	-5.705	<0.0001
Replicate (2)	-0.098	0.115	-0.856	0. 394
Treatment x Age	0.321	0.230	1.394	0.166
Bleed time				0.4
Treatment x Replicate				0.6

Table S1. GLMM modelling to test the effects of treatment, age at breeding, and selected fixed parameters (see "Data Analysis", Material and Methods) on (a) whether or not the females attempted to breed (i.e. laid eggs); (b) latency to lay the first egg; (c) clutch size, (d) fledging success, and (e) number of chicks fledged. Fixed factors estimates are indicated in parenthesis, r indicates random factor with its estimated variance. Significant factors are highlighted in bold and post-hoc pairwise comparisons for significant outcomes are shown in Table S2 and Figure 1. The non-significant interaction treatment x replicate was removed from the final models (likelihood ratio test, $p > 0.05$). In (a) the additional random factors family identity and male partner identity were dropped from final analysis because the models did not converge.

(a) Breeding failure

Parameter	**Estimate**	**SE**	**Z**	***p***
Female ring identity (r)	1.429			
Intercept	4.254	0.798	5.328	<0.0001
Treatment (challenging environment)	0.383	0.430	0.891	0.373
Replicate (2)	0.465	0.439	1.059	0.289
Age (1.1 years)	-0.376	0.782	-0.481	0.630
Age (1.8 years)	**-1.943**	**0.677**	**-2.872**	**0.004**
Age (3.5 years)	**-2.817**	**0.704**	**-4.001**	**<0.0001**
Treatment x Age	–	–	–	–
Treatment x Replicate	–	–	–	–

(b) Latency to lay the first egg

Parameter	**Estimate**	**SE**	**t**	***p***
Female ring identity (r)	0.008			
Partner identity (r)	0.009			
Family identity (r)	0.005			
Intercept	0.947	0.032	30.044	<0.0001
Treatment (challenging environment)	0.026	0.039	0.674	0.500
Replicate (2)	**-0.058**	**0.029**	**-1.991**	**0.049**
Age (1.1 years)	**-0.221**	**0.032**	**-6.994**	**<0.0001**
Age (1.8 years)	**-0.198**	**0.038**	**-5.190**	**<0.0001**
Age (3.5 years)	-0.023	0.045	-0.514	0.608
Treatment x Age (1.1 years)	-0.026	0.046	0.553	0.581
Treatment x Age (1.8 years)	0.014	0.055	0.257	0.798
Treatment x Age (3.5 years)	-0.012	0.064	-0.189	0.850
Treatment x Replicate				0.2

(c) Clutch size

Parameter	Estimate	SE	t	*p*
Female ring identity (r)	0.545			
Partner identity (r)	0.037			
Family identity (r)	0.015			
Intercept	4.124	0.149	27.768	<0.0001
Treatment (challenging environment)	-0.340	0.192	-1.775	0.077
Replicate (2)	**0.525**	**0.132**	**3.975**	**0.0002**
Age (1.1 years)	0.253	0.167	1.520	0.130
Age (1.8 years)	0.132	0.183	0.720	0.472
Age (3.5 years)	**-0.992**	**0.215**	**-4.606**	**<0.0001**
Treatment x Age (1.1 years)	0.295	0.244	1.209	0.228
Treatment x Age (1.8 years)	-0.030	0.263	-0.115	0.910
Treatment x Age (3.5 years)	0.462	0.306	1.511	0.132
Treatment x Replicate				0.9

(d) Fledging success

Parameter	Estimate	SE	Z	*p*
Female ring identity (r)	1.044			
Family identity (r)	<0.0001			
Intercept	0.316	0.197	1.599	0.110
Treatment (challenging environment)	-0.305	0.235	-1.296	0.195
Replicate (2)	0.298	0.219	1.357	0.175
Age (3.5 years)	**-2.068**	**0.274**	**-7.561**	**<0.0001**
Treatment x Age (3.5 years)	**1.196**	**0.358**	**3.336**	**0.0009**
Treatment x Replicate				0.7

(e) Number of chicks fledged

Parameter	Estimate	SE	Z	*p*
Female ring identity (r)	0.128			
Family identity (r)	<0.0001			
Intercept	0.797	0.100	8.005	<0.0001
Treatment (challenging environment)	**-0.235**	**0.115**	**-2.043**	**0.041**
Replicate (2)	**0.221**	**0.106**	**2.076**	**0.038**

Age (3.5 years)	**-1.415**	**0.183**	**-7.728**	**<0.0001**
Treatment x Age (3.5 years)	**0.831**	**0.238**	**3.495**	**0.0005**
Treatment x Replicate				0.6

820

821

Table S2. Percentage values of zebra finch females subjected to the control or challenging environmental conditions that did not opt to breed (i.e. did not attempt to lay a clutch) during the four age-specific breeding events; sample sizes refers to the total number of birds within each treatment group, the gradual decrease in sample size with age was due to mortality of experimental females across the experiment. Different letters indicate significant differences ($p < 0.05$ after Tukey multiple comparison adjustment).

Age at breeding	Control	Challenging
6 months	0%, n = 91[1]	3.9%, n = 80[1]
1.1 years	3.6%, n = 86[1]	1.4%, n = 75[1]
1.8 years	13.8%, n = 74[2]	7.7%, n = 70[2]
3.5 years	26.8%, n = 41[3]	18.6%, n = 51[3]

831 **Table S3.** GLMM modelling to test the effects of treatment, age at breeding, and selected
832 fixed parameters (see "Data Analysis", Material and Methods) on (a) whether or not the
833 females attempted to breed (i.e. laid eggs); (b) latency to lay the first egg; (c) clutch size, (d)
834 fledging success, and (e) number of chicks fledged. These analyses are performed only using
835 the females that were alive up to the final breeding event at 3.5 years of age. Fixed factors
836 estimates are indicated in parenthesis, r indicates random factor and its associated variance.
837 Significant factors are highlighted in bold and post-hoc pairwise comparisons for significant
838 outcomes are shown in Table S4 and Figure S1. The non-significant interaction treatment x
839 replicate was removed from the final models (likelihood ratio test, $p > 0.05$). In (a) the
840 additional random factors family identity and male partner identity were dropped from final
841 analysis because the models did not converge.

842

(a) Breeding failure

Parameter	**Estimate**	**SE**	**Z**	***p***
Female ring identity (r)	0.979			
Intercept	4.949	1.153	4.292	<0.0001
Treatment (challenging environment)	0.088	0.473	0.185	0.853
Replicate (2)	0.146	0.475	0.308	0.758
Age (1.1 years)	-0.71	1.239	-0.573	0.566
Age (1.8 years)	-2.05	1.086	-1.887	0.059
Age (3.5 years)	**-3.275**	**1.055**	**-3.105**	**0.002**
Treatment x Age	_	_	_	_
Treatment x Replicate	_	_	_	_

(b) Latency to lay the first egg

Parameter	**Estimate**	**SE**	**t**	***p***
Female ring identity (r)	0.006			
Partner identity (r)	0.014			
Family identity (r)	0.004			
Intercept	0.975	0.04	24.367	<0.0001
Treatment (challenging environment)	0.012	0.05	0.233	0.816
Replicate (2)	**-0.095**	**0.033**	**-2.866**	**0.005**
Age (1.1 years)	**-0.237**	**0.04**	**-5.948**	**<0.0001**
Age (1.8 years)	**-0.205**	**0.047**	**-4.332**	**<0.0001**
Age (3.5 years)	-0.028	0.05	-0.548	0.587
Treatment x Age (1.1 years)	0.03	0.057	0.53	0.597
Treatment x Age (1.8 years)	0.02	0.067	0.0301	0.763
Treatment x Age (3.5 years)	0.004	0.071	0.05	0.957

Treatment x Replicate				0.1

(c) Clutch size

Parameter	**Estimate**	**SE**	**t**	***p***
Female ring identity (r)	0.249			
Partner identity (r)	0.14			
Family identity (r)	0.062			
Intercept	4.263	0.195	21.899	<0.0001
Treatment (challenging environment)	-0.468	0.244	-1.920	0.056
Replicate (2)	**0.568**	**0.164**	**3.464**	**0.001**
Age (1.1 years)	0.103	0.204	0.505	0.615
Age (1.8 years)	0.119	0.220	0.540	0.590
Age (3.5 years)	**-1.110**	**0.234**	**-4.747**	**<0.0001**
Treatment x Age (1.1 years)	0.256	0.291	0.878	0.382
Treatment x Age (1.8 years)	0.006	0.315	0.020	0.984
Treatment x Age (3.5 years)	0.535	0.330	1.618	0.107
Treatment x Replicate				0.3

(d) Fledging success

Parameter	**Estimate**	**SE**	**Z**	***p***
Female ring identity (r)	0.691			
Family identity (r)	<0.0001			
Intercept	0.510	0.233	2.185	0.029
Treatment (challenging environment)	-0.234	0.274	-0.851	0.395
Replicate (2)	0.395	0.241	1.638	0.101
Age (3.5 years)	**-2.125**	**0.277**	**-7.668**	**<0.0001**
Treatment x Age (3.5 years)	**1.128**	**0.365**	**3.095**	**0.002**
Treatment x Replicate				1.0

(e) Number of chicks fledged

Parameter	**Estimate**	**SE**	**Z**	***p***
Female ring identity (r)	0.077			
Family identity (r)	0			
Intercept	0.907	0.117	7.729	<0.0001
Treatment (challenging environment)	-0.214	0.134	-1.601	0.109
Replicate (2)	**0.273**	**0.119**	**2.3**	**0.021**

Age (3.5 years)	**-1.501**	**0.186**	**-8.056**	**<0.0001**
Treatment x Age (3.5 years)	**0.816**	**0.243**	**3.353**	**0.0008**
Treatment x Replicate				0.7

843

844 **Table S4.** Percentage values of zebra finch females subjected to the control or challenging
845 environmental conditions that did not opt to breed (i.e. did not attempt to lay a clutch) during
846 the four age-specific breeding events within the pool of females that survived up to the final
847 breeding event at 3.5 years of age; sample sizes refers to the total number of birds within each
848 treatment group. Different letters indicate significant differences ($p < 0.05$ after Tukey
849 multiple comparison adjustment).

850

Age at breeding	Control	Challenging
6 months	0%, n = 52[1]	3.9%, n = 51[1]
1.1 years	3.6%, n = 52[1]	1.4%, n = 51[1]
1.8 years	13.8%, n = 52[1, 2]	7.7%, n = 51[1, 2]
3.5 years	26.8%, n = 52[2]	18.6%, n = 51[2]

851

852 **Table S5.** Time-dependent Cox Regression modelling to test the effects of the treatment on
853 survival. Coefficient estimates are referred to treatment = challenging environment, replicate
854 = 2; Coef indicates the hazard rate; Exp (Coef) indicates the hazard ratios, and SE (Coef)
855 indicates the standard error of the hazard rate. The non-significant interaction term of
856 replicate with treatment was consequentially removed from the final model.

857

Parameter	Coef	Exp (Coef)	SE (Coef)	Z	p
Treatment:Age interval 150-365 days	-0.553	0.575	0.866	-0.64	0.523
Treatment:Age interval 365-1096 days	**-0.656**	**0.519**	**0.300**	**-2.18**	**0.029**
Treatment:Age interval 1096-1456 days	0.111	1.118	0.367	0.30	0.762
Replicate	0.272	1.313	0.221	1.23	0.218
Treatment:age interval 150-365 days:Replicate					0.8
Treatment:Age interval 365-1096 days:Replicate					0.4
Treatment:Age interval 1096-1456:Replicate					0.2

858

Table S6. GLMs modelling to assess whether the probability of survival up to 4 years of age was influenced by lifetime breeding effort (a) lifetime egg laying effort, or (b) lifetime chick rearing effort; see "Statistical analysis" paragraph for full details) within the females exposed to the control environmental conditions or challenging environmental conditions. Fixed factor estimates are indicated in parenthesis. Significant effects are highlighted in bold. The non-significant factor replicate in interaction with the treatment was subsequentially removed from the final model (likelihood ratio test, $p > 0.05$).

(a) Lifetime egg laying effort

Control environment

Parameter	**Estimate**	**SE**	**Z**	**p**
Intercept	-0.069	0.801	-0.086	0.932
Lifetime egg laying effort	0.044	0.200	0.222	0.824
Replicate (2)	0.166	0.439	0.379	0.704
Lifetime egg laying effort x Replicate				0.7

Challenging environment

Parameter	**Estimate**	**SE**	**Z**	**p**
Intercept	-1.549	0.897	-1.727	0.084
Lifetime egg laying effort	0.239	0.228	1.047	0.295
Replicate (2)	0.696	0.482	1.444	0.149
Lifetime egg laying effort x Replicate				0.3

(b) Lifetime chick rearing effort

Control environment

Parameter	**Estimate**	**SE**	**Z**	**p**
Intercept	-0.083	0.486	-0.170	0.865
Lifetime chick rearing effort	0.094	0.198	0.475	0.635
Replicate (2)	0.165	0.427	0.388	0.698
Lifetime chick rearing effort x Replicate				0.5

Challenging environment

Parameter	**Estimate**	**SE**	**Z**	**p**
Intercept	-0.339	0.474	-0.715	0.474
Lifetime chick rearing effort	-0.222	0.211	-1.051	0.293

	Replicate (2)	**0.967**	**0.484**	**2.000**	**0.046**
	Lifetime chick rearing effort x Replicate				0.1

867

868

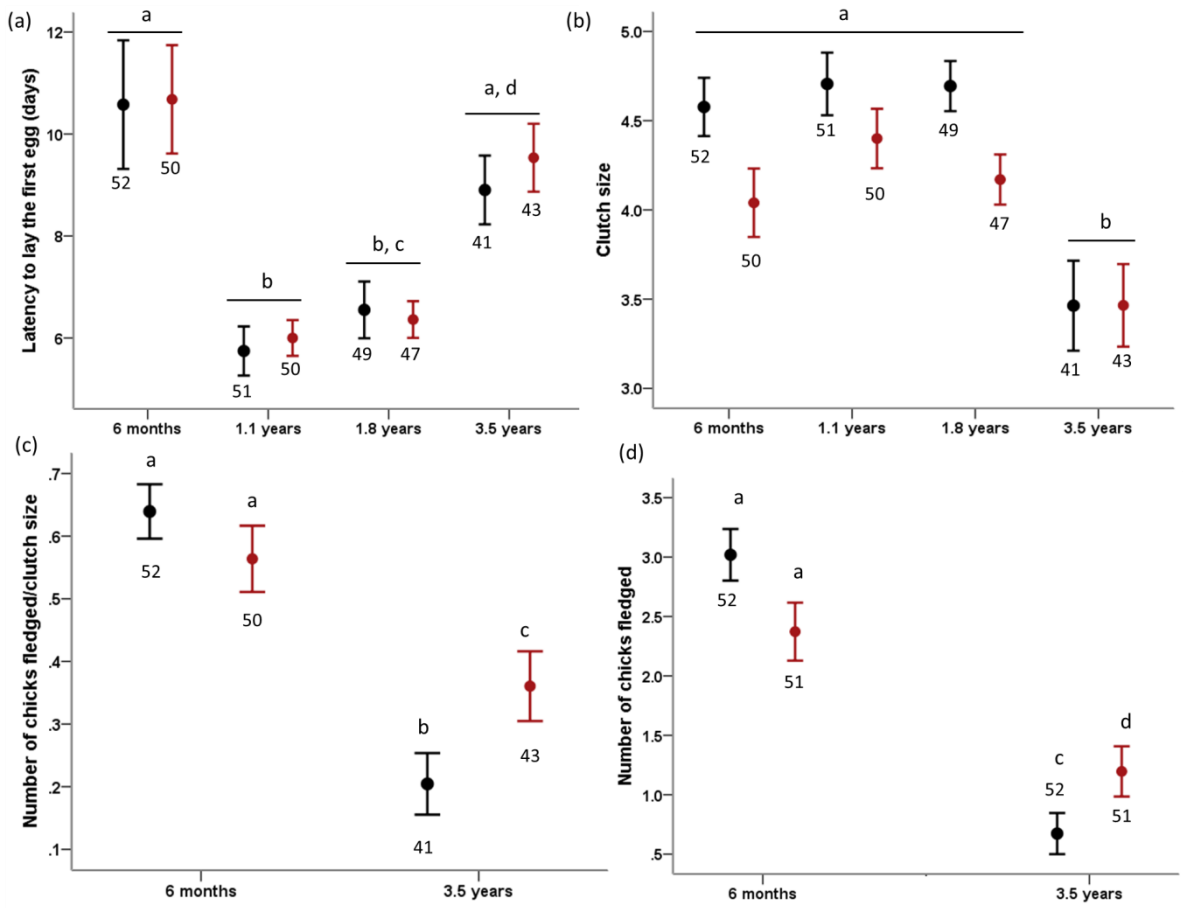

Figure S1. (a) Latency to lay the first egg, (b) clutch size, (c) fledging success (number of chicks fledged/clutch size; proportional data), and (d) number of chicks fledged in the females exposed to the challenging environmental conditions (in red) and control environmental conditions (in black) across the age-specific breeding events in the experimental birds that were alive up to 3.5 years of age. Data are shown as means ± SE. Note that eggs were allowed to hatch only during the breeding event at 6 months, 1.8 years and 3.5 years of age; cross-fostering experiments were conducted at 1.8 years of age an these data were dropped from analyses of fledging success and number of chicks fledged (full details in "Data Analysis"). Different letters indicate significant differences (post-hoc tests, $p < 0.05$ after Tukey multiple comparison adjustment – full statistics in Table S3); numbers indicate sample sizes separately by treatment and age.