Supplementary Material

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

Valeria Marasco[1, 2], Winnie Boner[1], Kate Griffiths[1], Britt Heidinger[1, 3], Pat Monaghan[1*]

[1]Institute of Biodiversity, Animal Health and Comparative Medicine, Graham Kerr Building, University of Glasgow, Glasgow, G12 8QQ, UK

Current Addresses:

[2] Department of Integrative Biology and Evolution, Konrad Lorenz Institute of Ethology, Vetmeduni Vienna, Savoyenstraβe 1a, A-1160, Vienna, Austria.

[3] Biological Sciences Department, Stevens Hall, North Dakota State University, Fargo, ND 58108, USA

*Corresponding author: Pat.Monaghan@glasgow.ac.uk

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 ~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 13.00 and 16.00 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%. Seven 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: 3.78 ± 0.48; 3.5 years, control: 1.11 ± 0.18 ng/ml; challenged: 2.03 ± 0.23 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).

References

1. Wingfield, J. C., Smith, J. P. & Farner, D. S. 1982 Endocrine Responses of White-Crowned Sparrows to Environmental-Stress. *Condor* 84, 399-409.
2. Marasco, V., Boner, W., Heidinger, B., Griffiths, K. & Monaghan, P. 2015 Repeated exposure to stressful conditions can have beneficial effects on survival. *Experimental Gerontology* 69, 170-175.
3. Bates D., Mächler M., Bolker B., Walker S. 2015 Fitting Linear Mixed-Effects Models Using lme4. *Journal of Statistical Software*; **1**(1).

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 factors (likelihood ratio test, $p > 0.05$) were removed from the final model.

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			
Residual	0.043			
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)	0.297			

Partner identity (r)	0.037			
Family identity (r)	0.015			
Residual	1.192			
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

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}	3.8%, n = 80^{1}
1.1 years	3.5%, n = 86^{1}	1.3%, n = 75^{1}
1.8 years	12.2%, n = 74^{2}	7.1%, n = 70^{2}
3.5 years	21.2%, n = 52^{2}	15.7%, n = 51^{2}

Table S3. 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. These analyses are performed only using the females that were alive up to the final breeding event at 3.5 years of age. Fixed factors estimates are indicated in parenthesis, r indicates random factor and its associated variance. Significant factors are highlighted in bold and post-hoc pairwise comparisons for significant outcomes are shown in Table S4 and Figure S1. 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)	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)	-0.710	1.239	-0.573	0.566
Age (1.8 years)	-2.050	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			
Residual	0.041			
Intercept	0.975	0.040	24.367	<0.0001
Treatment (challenging environment)	0.012	0.050	0.233	0.816
Replicate (2)	**-0.095**	**0.033**	**-2.866**	**0.005**
Age (1.1 years)	**-0.237**	**0.040**	**-5.948**	**<0.0001**
Age (1.8 years)	**-0.205**	**0.047**	**-4.332**	**<0.0001**
Age (3.5 years)	-0.028	0.050	-0.548	0.584
Treatment x Age (1.1 years)	0.030	0.057	0.53	0.597
Treatment x Age (1.8 years)	0.020	0.067	0.301	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)	0.140			
Family identity (r)	0.062			
Residual	1.067			
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**	**2.300**	**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

Table S4. 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 within the pool of females that survived up to the final breeding event at 3.5 years of age; sample sizes refers to the total number of birds within each treatment group. Different letters indicate significant differences ($p < 0.05$ after Tukey multiple comparison adjustment).

Age at breeding	Control	Challenging
6 months	0%, n = 52^{1}	2.0%, n = 51^{1}
1.1 years	1.9%, n = 52^{1}	2.0%, n = 51^{1}
1.8 years	5.8%, n = 52$^{1, 2}$	7.8%, n = 51$^{1, 2}$
3.5 years	21.2%, n = 52^{2}	15.7%, n = 51^{2}

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

Parameter	**Coef**	**Exp (Coef)**	**SE (Coef)**	**Z**	**p**
Treatment:Age interval 150-365 days	-0.553	0.575	0.866	-0.640	0.523
Treatment:Age interval 365-1096 days	**-0.656**	**0.519**	**0.300**	**-2.180**	**0.029**
Treatment:Age interval 1096-1456 days	0.111	1.118	0.367	0.300	0.762
Replicate	0.272	1.313	0.221	1.230	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

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 removed from the final models (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

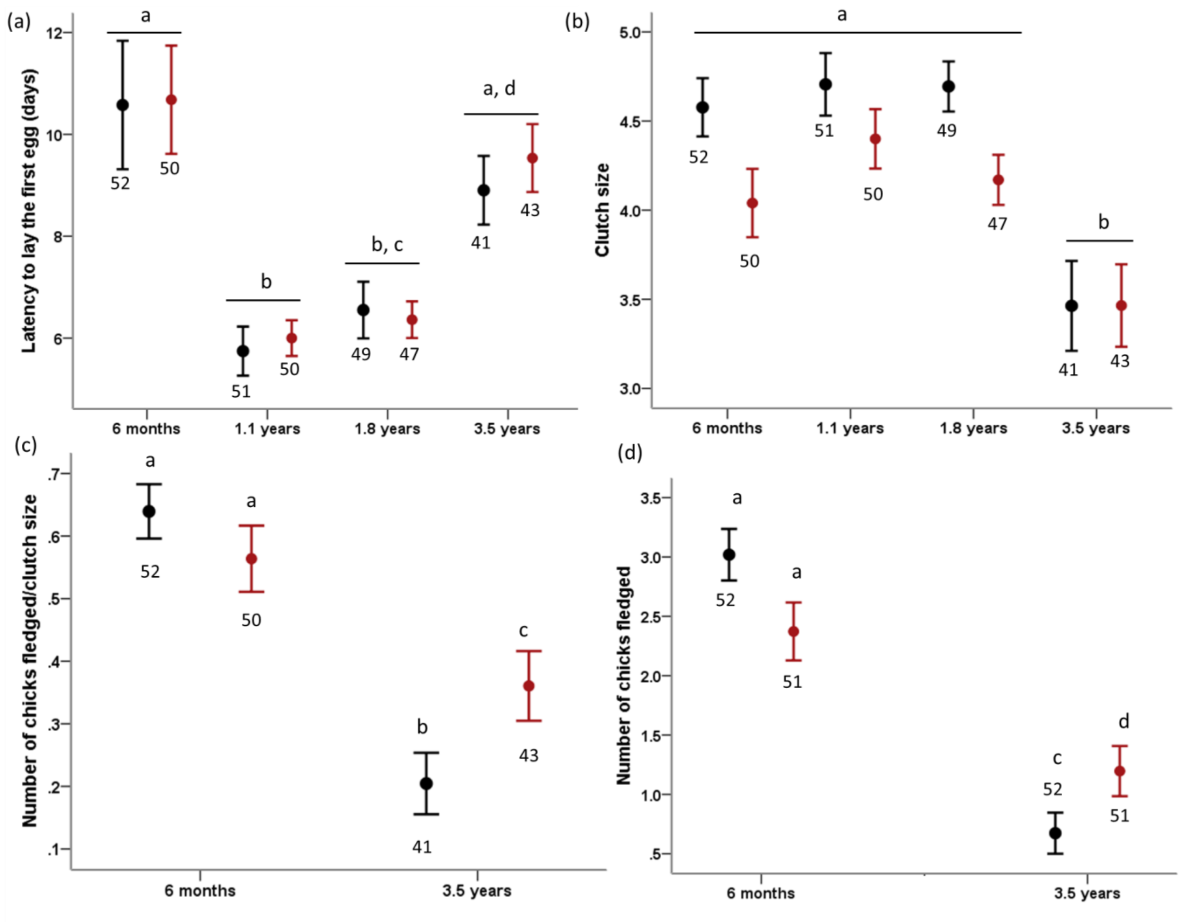

Figure S1. (a) Latency to lay the first egg, (b) clutch size, (c) fledging success (number of chicks fledged/clutch size; proportional data), and (d) number of chicks fledged in the females exposed to the challenging environmental conditions (in red) and control environmental conditions (in black) across 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.