Supplementary Information

Extreme temperatures compromise male and female fertility in a large desert bird

Contents

1 Supplementary Tables

1.1 Supplementary Table 1: Sample sizes within years

Yearly number of females and males and observations included in the analysis. Data of egg laying, egg mass, hatchting success and number of offspring were all obtained from the same females every year.

1.2 Results from random regression models (Supplementary Tables 2-7)

1.2.1 Supplementary Table 2: Egg laying (random regression)

Testing the effect of thermal stress on egg laying rate using a random slope model in the R-package MCMCglmm.

1.2.2 Supplementary Table 3: Number of sperm (random regression)

Testing the effect of thermal stress on number of sperm using a random slope model in the R-package MCMCglmm.

1.2.3 Supplementary Table 4: Egg mass (random regression)

Testing the effect of thermal stress on egg mass using a random slope model in the R-package MCMCglmm.

1.2.4 Supplementary Table 5: Sperm viability (random regression)

Testing the effect of thermal stress on sperm viability using a random slope model in the R-package MCMCglmm.

1.2.5 Supplementary Table 6: Hatching success (random regression)

Testing the effect of thermal stress on hatching success using a random slope model in the R-package MCMCglmm.

1.2.6 Supplementary Table 7: Number of offspring (random regression)

Testing the effect of thermal stress on number of offspring using a random slope model in the R-package MCMCglmm.

1.3 Results from random regression models with year specific slopes (Supplementary Tables 8-11)

1.3.1 Supplementary Table 8: Egg laying (year specific random regression)

Testing the effect of thermal stress on egg laying rate using a (yearly) random slope model in the R-package MCMCglmm.

1.3.2 Supplementary Table 9: Number of sperm (year specific random regression)

Testing the effect of thermal stress on number of sperm using a (yearly) random slope model in the R-package MCMCglmm.

1.3.3 Supplementary Table 10: Egg mass (year specific random regression)

Testing the effect of thermal stress on egg mass using a (yearly) random slope model in the R-package MCMCglmm.

1.3.4 Supplementary Table 11: Sperm viability (year specific random regression)

Testing the effect of thermal stress on sperm viability using a (yearly) random slope model in the R-package MCMCglmm.

1.4 Results from character state models (Supplementary Tables 12-17)

1.4.1 Supplementary Table 12: Egg laying (character-state)

Testing the effect of thermal stress on egg laying rate using a character-state model in the R-package MCMCglmm.

1.4.2 Supplementary Table 13: Number of sperm (character-state)

Testing the effect of thermal stress on number of sperm using a character-state model in the R-package MCMCglmm.

1.4.3 Supplementary Table 14: Egg mass (character-state)

Testing the effect of thermal stress on egg mass using a character-state model in the R-package MCMCglmm.

1.4.4 Supplementary Table 15: Sperm viability (character-state)

Testing the effect of thermal stress on sperm viability using a character-state model in the R-package MCMCglmm.

1.4.5 Supplementary Table 16: Hatching success (character-state)

Testing the effect of thermal stress on hatching success using a character-state model in the R-package MCMCglmm.

1.4.6 Supplementary Table 17: Number of offspring (character-state)

Testing the effect of thermal stress on number of offspring using a character-state model in the R-package MCMCglmm.

1.5 Results from two-trait models (Supplementary Tables 18-22)

1.5.1 Supplementary Table 18: Phenotypic correlations from character state two-trait models

Testing for trade-offs between fertility traits in cold and hot environments. Phenotypic correlations among and within individuals were estimated using two-trait character-state models in MCMCglmm. Credible intervals not overlapping with zero are written in bold. See supplementary tables 19-20below for model details.

1.5.2 Supplementary Table 19: Character state model of number of sperm vs sperm viability

Testing for correlations between sperm viability and number of sperm in the effect of thermal stress using ^a bivariate character state model in theR-package MCMCglmm.

1.5.3 Supplementary Table 20: Character state model of egg-laing vs egg mass

Testing for correlations between egg mass and egg laying in the effect of thermal stress using ^a bivariate character state model in the R-package MCMCglmm.

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1.5.4 Supplementary Table 21: Random regression model of number of sperm vs sperm viability

Testing for correlations between sperm viability and number of sperm in the effect of thermal stress using ^a bivariate random slope model in theR-package MCMCglmm.

1.5.5 Supplementary Table 22: Random regression model of egg laying vs egg mass

Testing for correlations between egg mass and egg laying in the effect of thermal stress using ^a bivariate random slope model in the R-package MCMCglmm.

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2 Supplementary Figures

2.1 Supplementary Fig. 1: Identification of critical thermal window for egg traits

The sensitivity of female and male fertility traits to local temperature fluctuations may not only be a product of the immediate environment, but also time-lag effects. When comparing population-level GLMs using different windows to predict the sensitivity of egg production to fluctuating temperatures, we found improved predictive power of the days preceding egg-laying compared to the days around egg-laying (A) . AVG-T_{MAX} of the window spanning 4 to 2 days before egg-laying was the best predictor of egg-laying (i.e. the critical thermal window). This is in accordance with the duration of egg formation in the ostrich being approximately 2 days. For egg mass **(B)** the ranking of AIC was highly sensitive to outliers and we therefore removed eggs laid at the 0.5% hottest and 0.5% coldest days. The critical thermal windows of 2 to 0 days before egg-laying was choosen the best predictor, but several windows show similar performance.

2.2 Supplementary Fig. 2: Identification of critical thermal window for sperm traits

For the number of sperm **(A)** produced by males, we also found improved model fit in the days before ejaculation, with AVG-T $_{MAX}$ of the window spanning 4 to 2 days before delivery shown to be the critical thermal window. Sperm viability (B) showed a similar pattern but with the best model fit using AVG-T_{MAX} being the window spanning 6 to 4 days before ejaculation. The vertical lines indicate the time of egg-laying or time of ejaculation.

2.3 Supplementary Fig. 3: The relationship between ambient temperature and egg-laying.

Points show observed probabilities of egg laying $(n = 652 \text{ females})$ for the binned temperature variable. Data are presented as mean values $+/-$ SEM.

2.4 Supplementary Fig. 4: The relationship between ambient temperature and number of sperm.

Points show observed number of sperm $(n = 22 \text{ males})$ for the binned temperature variable. Data are presented as mean values $+/-$ SEM.

2.5 Supplementary Fig. 5: Acclimation duration in the start of a new breeding season.

When ostrich pairs are established in the enclosures in May/June (normal start: 746 females) there is a steep increase in rate of egg-laying for the first 75 days. This increase likely reflects two effects, one being the increase in temperature at this time of year and the other being the acclimation to the enclosure and assigned partner. To determine the duration of acclimation to enclosure and partner, we investigated the reaction norm of ostrich pairs that, for various reasons, started their breeding season between July and October (Late start: 93 females) (note that these pairs are not included in the analyses presented in the main document). These late starters also show an increase in laying probability at the start and seem to be fully acclimated around 45 days after they were assigned to an enclosure. Estimated probability and 95% confidence band were obtained from a cubic spline model. Days after 100 days since start of breeding season are not shown.

2.6 Supplementary Fig. 6: Female age influences egg-laying.

Data are presented as mean values +/- SEM across females (n = 756 females). Only 7 females had an age $>$ 11 and are not shown.

2.7 Supplementary Fig. 7: Inspecting ranking of one-day and two-day thermal windows in egg-laying

The temperature at several days before are important for egg-laying, showing that a window of three days is an acceptable way to capture the most important immediate thermal fluctuations. Best thermal windows (lowest QAIC values) are consistent with the critical three-day thermal window identified in **Supplementary Fig. 2**.

2.8 Supplementary Fig. 8: Change in egg mass with number of days since previous egg.

When more than three days pass since the previous egg was laid, the egg mass of the next egg starts to decline. A decrease of 70 g is substantial, since the average standard deviation of egg mass for an ostrich pair amounts to 73 g. If more than eight days passed since the previous egg, we assigned eight days since previous egg for illustration purposes. Data are presented as mean values $+/-$ SEM across females (n = 652).