RESEARCH ARTICLE

Morph- and sex-specific effects of challenging conditions on maintenance parameters in the Gouldian finch

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ABSTRACT

Intraspecific discrete polymorphism is associated with the use of alternative life-history strategies, reflected by distinct reproductive or copying strategies in individuals of different morphs. Yet, morph-specific costs and benefits related to different life-history strategies remain unclear. Here, we examined in the polymorphic Gouldian finch (*Erythrura gouldiae*) whether markers of somatic maintenance (body mass, oxidative status and telomere length) differed between red- and black-headed birds under energetically demanding conditions (during heatwaves of different intensity, and during moult or breeding following heatwaves). During heatwaves, red-headed birds showed a homogeneous response, as males and females initially tended to gain mass and had reduced plasma hydroperoxide levels (a marker of oxidative damage) irrespective of heatwave intensity. In contrast, black-headed birds showed a stronger and more heterogeneous response, as black-headed males gained mass at the beginning of the thermoneutral heatwave and showed stable oxidative status, whereas black-headed females lost mass and tended to show higher hydroperoxide levels at the end of the thermocritical heatwave. Following heatwaves, we found morph-specific oxidative costs owing to moult or reproduction, with oxidative markers varying only in black-headed birds. Again, oxidative markers varied differently in black-headed males and females, as plasma antioxidant capacity decreased in moulting or breeding females, whereas males showed higher hydroperoxide levels with larger broods. For the first time, our study highlights that within polymorphic species, some individuals appear more vulnerable than others when coping with energetically demanding conditions. In the context of climate change, such differential effects may ultimately alter the currently observed balance between morphs and sexes within natural populations.

KEY WORDS: Body mass variation, Endogenous traits, Inter-morphic variability, Moult-breeding strategy, Oxidative status, Telomere shortening

INTRODUCTION

The regulation of self-maintenance (i.e. the ability of functioning and surviving) parameters is usually assumed to be constant and generalisable across individuals within the same species. For instance, immune efficiency, moulting costs and homeostasis (e.g. redox balance) are typically defined intraspecifically (Valladares

et al., 2014). Physiological thermal tolerance is also typically defined intraspecifically although thermoneutral ranges may not necessarily be identical between individuals. Indeed, because of extrinsic (e.g. early-life experience, social environment) or intrinsic factors (e.g. genetic, maternal effects), intraspecific phenotypic variability may result in a variety of maintenance strategies within the same species (Kempenaers et al., 2008; Schwartz and Bronikowski, 2013). To reduce such interindividual physiological differences, animals may differently alter their behaviour. For example, individuals unable to adjust their physiology to extreme temperatures may still be able to keep maintenance parameters stable by reducing their overall physical activity (Beaulieu et al., 2015a; Wolf, 2000).

In polymorphic species, morphological traits typically covary with life-history traits, as reflected by distinct reproductive, copying or foraging strategies in each morph (Roulin, 2004). Such phenotypic differences between morphs may result in different maintenance costs under stressful conditions. For instance, in the polymorphic Australian painted dragon (*Ctenophorus pictus*) (Rollings et al., 2017), morph-specific reproductive strategies are associated with different maintenance costs in terms of telomere dynamics. One mechanism for the coexistence of distinct phenotypes within polymorphic species is the use of alternative life-history strategies, with the highest fitness advantages and minimal maintenance costs for each morph under different conditions (e.g. ecological conditions or morph frequency). Such intraspecific differences in maintenance investment owing to the use of different life-history strategies may be exacerbated under extreme environmental conditions if some individuals of a given morph are more affected than others by such conditions (Dingemanse et al., 2010; Schwartz and Bronikowski, 2013). Intraspecific phenotypic variability in polymorphic species therefore appears inseparable from environmental variability. However, the examination of morph differences in life-history strategies and their maintenance costs under variable environmental conditions remains mostly overlooked.

Here, we tested whether challenging conditions affected differently the somatic maintenance (body mass, oxidative status and telomere length) of Gouldian finches (*Erythrura gouldiae*) of the two main colour morphs (red- and black-headed birds). More specifically, birds were first exposed to either a thermocritical heatwave (temperature above thermoneutrality, associated with increased energetic needs for thermoregulation) or a thermoneutral heatwave (temperature within thermoneutrality, associated with minimal energetic needs for thermoregulation). Because of the higher energetic requirements expected under thermocritical conditions, we first expected maintenance parameters to be more strongly affected by thermocritical than by thermoneutral conditions (i.e. accentuated body mass loss, lower antioxidant defences, higher oxidative damage and faster telomere shortening under thermocritical than under thermoneutral conditions). We then examined whether such maintenance differences between thermal treatments led to

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subsequent differences in moult and breeding initiation between both morphs, as the exposure to challenging conditions, such as thermocritical conditions, may have postponed effects on life-history decisions (Harrison et al., 2010). Birds were therefore expected to be more likely to refrain from moulting or breeding after a thermocritical heatwave than after a thermoneutral heatwave, as these activities are themselves energetically demanding and may force animals into an allostatic overload (i.e. energy requirements exceed energy supply) that would accentuate the deterioration of maintenance parameters (Lindström et al., 1993; Monaghan and Nager, 1997; Murphy and King, 1992). Because red-headed Gouldian finches have been described as being more aggressive and dominant, and feeding more (at least males) than black-headed birds (Fragueira and Beaulieu, 2019; Pryke et al., 2012; Williams et al., 2012), the effects of thermocritical conditions on maintenance parameters were first expected to be stronger in red-headed birds, with presumably higher energy requirements than in black-headed birds. However, because red-headed birds decrease their activity more strongly during heatwaves than black-headed birds (Fragueira and Beaulieu, 2019), they may be able to minimise the effects of thermocritical conditions on maintenance parameters. In contrast, the maintenance of black-headed birds, altering their behaviour less strongly during heatwaves, may be physiologically more affected by thermocritical conditions, which in turn may reduce their capacity to moult or breed.

MATERIALS AND METHODS
Experimental design
A total of 112 wild-type, young adult Gouldian finches [*Erythrura gouldiae* (Gould 1844); approximately 1 year old] were obtained from local breeders. Across our study, birds had access to water, shell grit, cuttlefish bones and mixed seeds (Deli Nature 40 - Exoten Basis, Beyers, Belgium) *ad libitum*, and were given a bath daily. Monomorphic breeding pairs were initially randomly assigned into experimental cages (99.6×48.0×52.0 cm) in two different climate

chambers (2.8×4.2×2.5 m; 7 red-headed and 7 black-headed pairs in each climate chamber in two sessions) set at 32±1°C (i.e. within the thermoneutral range of Gouldian finches: 31.8–38.0°C; Burton and Weathers, 2003), with 50±5% humidity and a 12 h:12 h light:dark cycle. Birds were allowed to habituate to these conditions for at least 2 weeks before measurements. Then, all birds were exposed to the same experimental event sequence (Fig. 1): (1) a pre-experimental phase at 32°C for 14 days (day −18 to day −5), followed by (2) an intermediate phase at 34°C (thermoneutral intermediate treatment) or 35.5°C (thermocritical intermediate treatment) for 4 days (day −4 to day −1), (3) a heatwave at 36°C (thermoneutral heatwave) or 39°C (thermocritical heatwave) for 14 days (day 1 to day 14), (4) an intermediate phase similar to the intermediate phase before the heatwave (34°C or 35.5°C; day 15 to day 18) and (5) a monitoring phase at 32°C for 4 months when birds could moult or breed (day 19 to day 150). During both intermediate phases (phases 2 and 4) and during the heatwaves (phase 3), birds experienced maximal temperatures only for 2 h each day from 12:00 to 14:00 h, with a progressive temperature increase before and temperature decrease after these 2 h of exposure (Fig. 1). We progressively exposed birds to temperature changes during intermediate phases so they would have time to adjust their behaviour and physiology to the new conditions. During the heatwaves, maximal temperatures were either within or beyond thermoneutrality, whereas temperature was always within this range otherwise. The 14-day-long heatwaves that we induced were designed to reflect long heatwaves that tropical birds, such as Gouldian finches, will likely experience in the future in Australia (IPCC, 2015).

After this experimental phase, each cage was supplemented with a nest box, nest material, seed sprouts and egg food (Witte Molen Eggfood, Witte Molen, Netherlands), and the light:dark cycle was changed to 13 h:11 h light:dark. Each bird had then the possibility to breed or moult. To monitor moult (i.e. start and end of moult) and breeding (i.e. clutch initiation, clutch size and brood size) status, each cage and nest was checked daily. We defined individual
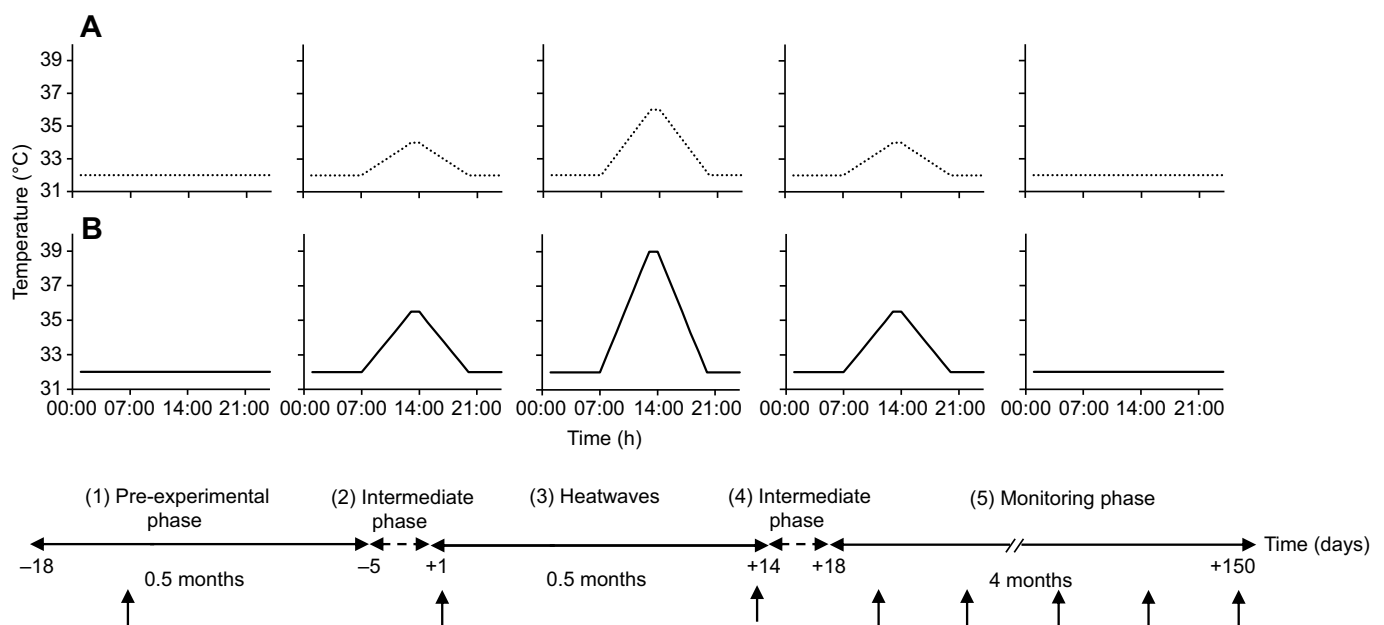

Fig. 1. Chronogram of the experiment conducted under the different thermal conditions. (A) Thermoneutral (dashed lines) and (B) thermocritical (solid lines). Arrows along the time axis represent when Gouldian finches were weighed and sampled. Each graph represents temperature variation over 24 h before the heatwaves under pre-experimental conditions (1), during the intermediate phases (2 and 4), during the heatwaves (3) and during the monitoring phase (5). This experiment was repeated twice in two different sessions conducted in two consecutive years.

moulting periods from the time birds started losing body and/or wing feathers until complete feather replacement, and breeding periods from the first egg being laid until chick fledging. For birds that both moulted and bred consecutively, we only considered the first event following heatwaves in the analyses. Moreover, three males for which moult and breeding overlapped were excluded from our analyses, as the respective effects could not be distinguished.

Maintenance measurements

Body mass, markers of oxidative status and telomere length were measured longitudinally to examine how birds regulated their maintenance across time. Temporal variation in body mass reflects how birds regulate their energy balance (Rozman et al., 2003) whereas temporal variation in oxidative status may reflect how they prioritise self-maintenance over other functions, such as reproduction (Beaulieu et al., 2015b). For instance, antioxidant defences decrease with brood size in parent zebra finches (*Taeniopygia guttata*; Alonso-Alvarez et al., 2006; Wiersma et al., 2004), thereby indicating that they sacrifice their maintenance for their current reproduction (note that this pattern is not necessarily seen in all species; Stier et al., 2012). Low antioxidant defences may lead to an imbalance between pro-oxidants [e.g. reactive oxygen species (ROS)] and antioxidant defences, and to increased levels of oxidative damage on key biological molecules, such as lipids, proteins and DNA sequences, including telomeres (i.e. non-coding sequences at the end of eukaryotic chromosomes stabilizing the genome) (Finkel and Holbrook, 2000). Accordingly, high ROS levels have been described *in vitro* as accelerating telomere shortening (Von Zglinicki, 2002), which in turn may lead to accelerated senescence and reduced survival probability (Bize et al., 2009). Variation in markers of oxidative status and telomere dynamics can therefore be used to examine how animals prioritise their maintenance (Young, 2018).

Blood was collected from each bird across the experimental period (once before the heatwaves, twice during the heatwaves, then monthly across the 4 months of monitoring; Fig. 1) at a fixed time (13:00–16:00 h). Immediately after capture, blood (ca. 80 µl) was taken from the brachial vein, and then the birds were weighed ($\pm$0.1 g). Blood was centrifuged for 10 min at 4000 g, and plasma and erythrocytes were separated and stored at $-80°C$ until analysis.

To examine plasma oxidative status, we measured one marker of antioxidant defence (total plasma antioxidant capacity using the OXY-adsorbent test; Diacron International, Grosseto, Italy) and one marker of oxidative damage (plasma hydroperoxide levels using the d-ROM test; Diacron International). Both markers of oxidative status have previously been measured in the plasma of Gouldian finches and other bird species (Beaulieu et al., 2014; Beaulieu and Schaefer, 2014; for measurement principles, see Costantini, 2016). In the assays, samples were balanced across plates for morph, sex and treatment. Intra- and inter-plate coefficients of variation for the OXY and ROM were 15% (n=48 replicates) and 7% (n=42 replicates), and 16% (n=42 replicates) and 9% (n=42 replicates), respectively, which fall within the range reported in Costantini (2011).

Telomere length was measured in erythrocytes before the heatwaves and at the end of the monitoring period using the terminal restriction fragment (TRF) procedure as previously described in birds (Salomons et al., 2009) but adjusted for Gouldian finches. This procedure consists in extracting DNA from ca. 5 µl of red blood cells using the CHEF Mammalian Genomic DNA Plug kit (Bio-Rad Laboratories). The extracted DNA in agarose plugs was then digested overnight at 37°C with a mixture of the restriction enzymes HinfI (15 U), HindIII (30 U) and MspI (30 U) in NEB2 buffer. The restricted DNA and the ^{32}P

end-labelled size standards [(a) 1 kb DNA ladder, New England Biolabs; Molecular Weight Marker XV, Roche Diagnostics, Basel, Switzerland, range 2.4–48.5 kb; (b) Ultra-long DNA ladder, PFG Marker I, NEB Midrange] were separated through a 0.8% agarose gel by pulsed-field gel electrophoresis at 14°C for 22 h with 4.8 V cm^{-1}. In a second step, gels were dried (gel dryer model 538, Bio-Rad Laboratories) and hybridised overnight at 37°C with a ^{32}P-end-labelled oligonucleotide [(5′-CCCTAA-3′)] bound to the single-stranded overhang of telomeres. Finally, gels were exposed overnight to a phosphor screen (PerkinElmer, Waltham, MA, USA), and the radioactive signal, obtained from the phosphor image (CycloneTM Storage Phosphor System, PerkinElmer), could be further analysed through densitometry using ImageJ 1.50i. The lower limit of the measurement was set at 2.4 kb, corresponding to the smallest observable band of the 1 kb DNA ladder and the upper limit at 195 kb, corresponding to the highest observable band of the Ultra-long DNA ladder. The background, set between 200 and 250 kb, was subtracted from the optical density measurements. The inter-gel coefficient of variation was 10.5%. All raw data generated in the study are available as Dataset 1.

Statistics

First, we analysed variation in body mass and oxidative status during the heatwaves using general (plasma antioxidant capacity) and generalised (body mass, plasma hydroperoxide levels; gamma-distributed) linear mixed models with thermal treatment (thermoneutral, thermocritical), sex (male, female), morph (black- and red-headed) and time (day -10, day $+1$, day $+14$) as independent factors, and individual birds nested in their cage as a random factor.

For birds that started moult or breeding, we analysed whether the thermal treatment affected moult/breeding strategy using a generalised linear mixed model with a binary function with moult–breeding status as a dependent factor (moult: 0, breeding: 1), thermal treatment (thermoneutral, thermocritical), sex (male, female) and morph (black-, red-headed) as independent factors, and cage as a random factor. Birds were considered breeding when at least one egg was laid.

To examine whether maintenance parameters were differently affected by moult and breeding in both morphs, we used general (antioxidant capacity, log telomere length) and generalised (body mass, plasma hydroperoxide levels; gamma-distributed) linear mixed models with morph, sex, thermal treatment, time (before, during moulting or breeding) and moult/breeding strategy (not moulting/not breeding; moult; breeding) as independent factors, and birds nested in their cage as a random factor. In these analyses, we only considered birds with fledglings (i.e. having completed reproduction) as breeding birds, in order for all breeding individuals to be comparable between each other in terms of breeding advancement and to cover the whole reproduction period. We also examined whether brood size had an impact on the temporal dynamics of the measured physiological traits [i.e. difference (Δ) between before and during breeding] using general (Δantioxidant capacity) and generalised (Δbody mass, Δhydroperoxide levels and Δtelomere length corrected for regression to the mean) mixed models with sex, morph and brood size as independent factors, and cage as a random factor.

Final models were selected using the corrected Akaike's information criterion (AICc) in a backwards-deletion procedure. When the factor 'morph' was involved in a significant three- or four-way interaction, we repeated our models for each morph separately to clarify how each morph responded across conditions. For all other interactions, *post hoc* comparisons were performed using Benjamini–Hochberg corrections. All analyses were performed

using SPSS (v. 22). For clarity, results (given as means±s.e.m.) are presented depending on the statistical outcomes (i.e. depending on significant factors or interactions between factors).

Ethics
The animal care and experimentation complied with the EU animal experimentation regulations approved by the ethical committee of Mecklenburg-Western Pomerania (LALLF: permit no. 7221.3-2-041/15; ZooBea3936/3/15).

RESULTS
Maintenance status during heatwaves (day −10 to day +14)
During the heatwaves, body mass varied depending on morph, sex and heatwave intensity (Table 1). After repeating our model within each morph, we found that red-headed birds tended to gain mass at the beginning of the heatwaves and irrespective of their intensity (time: $F_{2,155}=13.84$, $P<0.001$; post hoc test: $P_{-10-1d}=0.07$; Table S1, Fig. 2A), but they lost this gain afterwards ($P_{1-14d}<0.001$). Similarly, black-headed males gained mass at the beginning of the heatwave but only during the thermoneutral heatwave (thermal treatment×time×sex: $P=0.034$; post hoc test: $P_{-10-1d}=0.049$; Table S1). In contrast, the body mass of black-headed males during the thermocritical heatwave and the body mass of females during the thermoneutral heatwave remained constant across time (all $P>0.17$). Black-headed females during the thermocritical heatwave also maintained their body mass constant at the beginning of the thermocritical heatwave (post hoc test: $P_{-10-1d}=0.29$), but their body mass significantly decreased afterwards (post hoc test: $P_{1-14d}=0.03$, $P_{-10-14d}=0.045$; Table S1, Fig. 2B).

Antioxidant capacity remained unchanged across heatwaves, irrespective of morph and sex (Table 1, Fig. 2C,D). However, similar to body mass, plasma hydroperoxide levels tended to vary differently across time depending on morph, sex and heatwave intensity (Table 1). After repeating our model within each morph, we found that plasma hydroperoxide levels varied across time in red-headed birds (time: $F_{2,155}=3.40$, $P=0.036$; Table S1), as they slightly decreased at the beginning of the heatwaves ($P_{-10-1d}=0.048$) and returned to initial levels afterwards ($P_{1-14d}=0.57$, $P_{-10-14d}=0.09$;

Fig. 2E). Plasma hydroperoxide levels also appeared to vary across time in black-headed birds, but depending on sex and heatwave intensity (thermal treatment×sex×time: $F_{2,156}=3.17$, $P=0.045$; Table S1). Indeed, hydroperoxide levels tended to increase in black-headed females at the end of the thermocritical heatwave (post hoc test: $P_{-10-14d}=0.07$, $P_{1-14d}=0.10$) whereas they did not vary in other black-headed birds (all other post hoc tests: $P>0.10$; Fig. 2F).

Effects of moult and breeding on maintenance status (day +21 to day +150)
In the monitoring period, 95 birds out of the 112 birds included in our study (85%) moulted or bred (i.e. produced at least one egg). Among these birds, the proportion of birds moulting (53%) or reproducing first (47%) did not differ between black- and red-headed birds (Table 1). Irrespective of morph and thermal treatment, maintenance parameters did not significantly differ between pre-moulting and pre-breeding birds (body mass: $F_{1,80}=1.91$, $P=0.17$; antioxidant capacity: $F_{1,46}=0.38$, $P=0.54$; hydroperoxide levels: $F_{1,78}=0.88$, $P=0.35$), even though telomeres tended to be longer in pre-moulting than in pre-breeding birds ($F_{1,48}=3.47$, $P=0.07$; Table S2).

Body mass varied differently through time in moulting and breeding birds (Table 2). Indeed, moulting birds kept their body mass stable ($P_{before-during\ moult}=0.55$) and comparable to that of non-moulting/non-breeding birds ($P=0.63$) whereas breeding birds exhibited a significant mass loss ($P_{before-during\ breeding}=0.015$; Fig. 3A). This body mass loss (i.e. difference between before and during breeding) was independent of colour morph and brood size (all $P>0.09$; Table S3).

Even though antioxidant capacity did not differ between moulting and breeding individuals (Fig. 3C), a significant interaction between sex, morph and time suggested different antioxidant dynamics in birds depending on their sex and morph (Table 2). Although no sex differences were observed in red-headed birds across time after repeating our model within each morph (sex×time: $F_{1,39}=1.35$, $P=0.25$; Table S4, Fig. 4A), antioxidant capacity varied differently in black-headed females and males (sex×time: $F_{1,38}=6.14$, $P=0.018$; Table S4). Multiple comparison analyses revealed that antioxidant capacity was higher in black-

Table 1. Summary table of statistical tests examining the effects of thermal treatment (TT), time (−10, +1, +14 days before/after the start of the heatwaves), sex and colour morph on Gouldian finch body mass, plasma antioxidant capacity and plasma hydroperoxide levels during the heatwaves

Test used	GLMM – gamma log link with repeated measures			LMM – repeated measures			GLMM – gamma log link with repeated measures			GLMM – binary logit link		
Response variable	Body mass			Antioxidant capacity			Hydroperoxide level			Proportion of moulting and breeding birds		
Explanatory variables	d.f.	F	P	d.f.	F	P	d.f.	F	P	d.f.	F	P
TT	1, 311	0.11	0.74	1, 104	1.98	0.16	1, 310	0.88	0.35	1, 87	0.25	0.62
Time	2, 311	9.85	<0.001	2, 207	1.94	0.15	2, 310	2.62	0.08			
Morph	1, 311	0.65	0.42	1, 104	2.25	0.14	1, 310	0.07	0.80	1, 87	0.08	0.78
Sex	1, 311	0.07	0.79	1, 104	1.53	0.22	1, 310	0.002	0.96	1, 87	0.08	0.78
TT×Time	2, 311	2.11	0.12	2, 207	1.04	0.36	2, 310	0.25	0.78			
TT×Morph	1, 311	0.86	0.35	1, 104	0.06	0.80	1, 310	1.62	0.20	1, 87	0.25	0.62
TT×Sex	1, 311	0.07	0.79	1, 104	0.09	0.77	1, 310	0.14	0.71	1, 87	0.005	0.94
Time×Morph	2, 311	0.13	0.88	2, 207	0.58	0.56	2, 310	0.45	0.64			
Time×Sex	2, 311	0.54	0.59	2, 207	0.01	0.99	2, 310	1.17	0.31			
Sex×Morph	1, 311	2.03	0.16	1, 104	0.16	0.69	1, 310	3.15	0.08	1, 87	0.08	0.78
TT×Time×Morph	2, 311	0.50	0.61	2, 207	1.10	0.34	2, 310	3.04	0.049			
TT×Sex×Morph	1, 311	0.57	0.45	1, 104	1.53	0.22	1, 310	0.28	0.60			
TT×Time×Sex	2, 311	0.71	0.50	2, 207	2.28	0.11	2, 310	2.39	0.09	1, 87	0.02	0.89
Sex×Morph×Time	2, 311	3.30	0.038	2, 207	2.31	0.10	2, 310	0.25	0.78			
TT×Time×Morph×Sex	2, 311	5.87	0.003	2, 207	1.01	0.37	2, 310	2.67	0.07			

GLMM, generalized linear mixed model; LMM, linear mixed model. Italicized values indicate $P<0.05$.
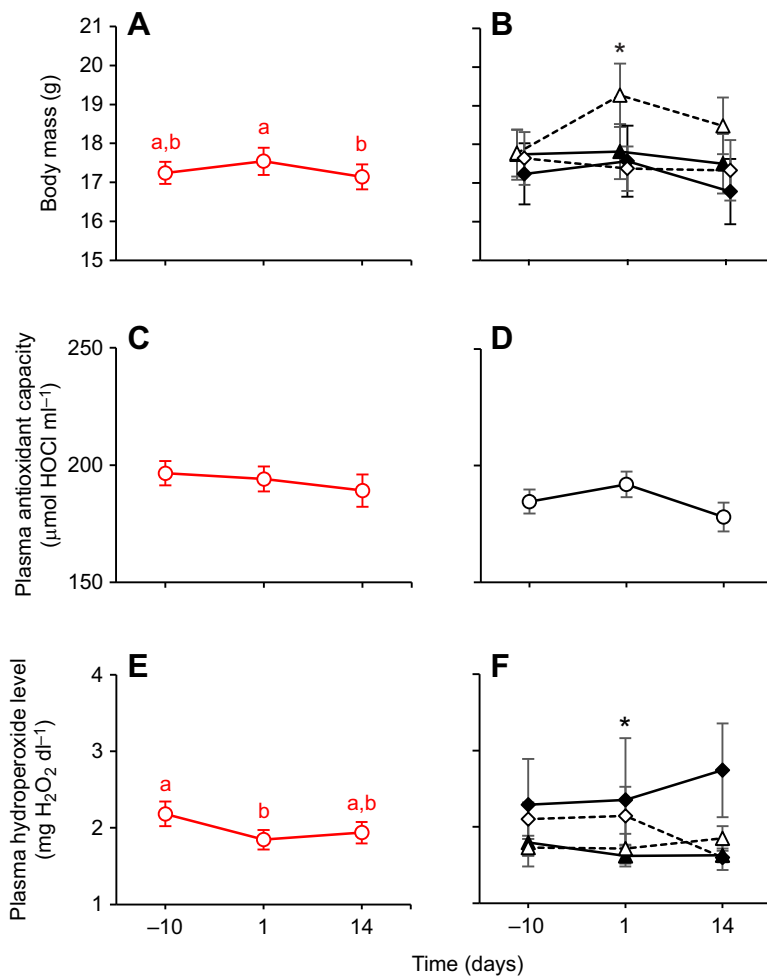
Fig. 2. Maintenance status during the heatwaves. Body mass, plasma antioxidant capacity and plasma hydroperoxide levels across time in red-headed Gouldian finches (A,C,E), irrespective of sex and thermal treatment (○; N=56), and in black-headed Gouldian finches (B,D,F) for each sex (females, ◇, N=28; males, △, N=28) and thermal treatment (thermoneutral, dashed black lines; thermocritical, solid black lines). Asterisks indicate significant interactions between time, sex and thermal treatment in black-headed birds. Different letters indicate significant differences between time points in red-headed birds.

headed females than in black-headed males (*post hoc* test: $P=0.019$; Fig. 4B) before moulting or breeding. Moreover, antioxidant values decreased with time only in moulting or breeding black-headed females (*post hoc* tests: $P_{\text{black-females}}=0.019$, $P_{\text{red-females}}=0.23$, $P_{\text{black-males}}=0.73$, $P_{\text{red-males}}=0.38$), resulting in no remaining sexual differences in black-headed birds during moult or breeding (*post hoc* test: $P=0.76$; Fig. 4B). Even though plasma hydroperoxide levels showed the same temporal pattern as body mass, multiple comparison analyses revealed no differences between groups (all $P>0.24$; Fig. 3D).

Morph differences in the regulation of oxidative status were also visible when examining temporal variation in oxidative markers (i.e. difference between before and during breeding) in relation to brood size. Indeed, Δantioxidant capacity and Δhydroperoxide levels tended to differ between sexes, morphs and brood sizes (sex×morph×brood size: $P=0.06$; Table S3), and Spearman correlations conducted for each sex within each morph revealed that Δantioxidant capacity positively correlated with brood size only in red-headed females ($r_S=0.593$, $P=0.05$, $n=10$), whereas Δoxidative damage positively correlated with brood size only in black-headed males ($r_S=0.702$, $P=0.035$, $n=9$; Fig. S1). All other correlations between temporal variation in oxidative markers and brood size were not significant (all $P>0.27$).

Telomere length showed a significant morph×sex interaction (Table 2), with red-headed males exhibiting shorter telomeres than red-headed females (39.3 ± 1.07 and 43.8 ± 1.13 kbp, respectively; *post hoc* test: $P=0.012$) and black-headed males (42.4 ± 0.91 kbp;

post hoc test: $P=0.021$). In contrast to males, telomere length tended to be shorter in black-headed females (39.9 ± 0.65 kbp) than in red-headed females (*post hoc* test: $P=0.07$). However, telomere length did not vary across time, nor between moulting or breeding birds, irrespective of the thermal conditions they previously experienced (Table 2, Fig. 3B).

DISCUSSION
Whether individuals of different morphs are differently affected by challenging conditions and differently engage in costly activities is largely unknown. In this study, we monitored longitudinally markers of somatic maintenance in red- and black-headed Gouldian finches across a sequence of different events: first, a thermally challenging experimental period, and second, a subsequent monitoring period when birds could freely moult or breed. Overall, we found that Gouldian finches of different morphs responded differently to energetically demanding conditions (heatwaves, moult and reproduction), with red-headed males and females showing a homogeneous response, whereas the response of black-headed males and females diverged.

Maintenance status during heatwaves
We found that red-headed males and females tended to gain body mass at the beginning of the thermoneutral and thermocritical heatwaves. The food provided to birds, their low physical activity in breeding cages, and the absence of competition with other birds probably explain this mass gain across time. However, red-headed

Table 2. Summary table of statistical tests examining the effects of thermal treatment, time (before and during moult or breeding), moult/breeding status (i.e. moult/breeding strategy: moult or reproduction), sex and morph on body mass, plasma antioxidant capacity, plasma hydroperoxide levels and telomere length

Test used	GLMM with repeated measures – gamma log link			LMM – repeated measures			GLMM with repeated measures – gamma log link			LMM with repeated measures – log transformed		
Response variable	Body mass			Plasma antioxidant capacity			Plasma hydroperoxide level			Telomere length		
Explanatory variable	d.f.	F	P	d.f.	F	P	d.f.	F	P	d.f.	F	P
TT	1, 147	0.16	0.69	1, 85	0.03	0.86	1, 149	2.43	0.12	1, 98	0.001	0.97
Time	*1, 147*	*14.38*	*<0.001*	1, 83	1.36	0.25	1, 149	0.62	0.43	1, 77	0.05	0.82
Strategy	2, 147	0.38	0.68	2, 84	0.03	0.97	2, 149	1.73	0.18	2, 92	0.86	0.43
Morph	1, 147	0.77	0.38	1, 85	0.03	0.86	*1, 149*	*5.09*	*0.025*	1, 99	0.04	0.85
Sex	1, 147	0.03	0.87	1, 86	0.57	0.45	1, 149	3.71	0.06	1, 92	1.04	0.31
TT×Time	1, 147	0.66	0.43	1, 80	2.19	0.14	1, 149	1.72	0.19	1, 80	1.02	0.32
TT×Strategy	2, 147	0.32	0.73	2, 85	0.15	0.86	2, 149	2.35	0.10	2, 96	0.18	0.84
TT×Morph	1, 147	2.84	0.09	1 83	0.09	0.76	1, 149	0.01	0.94	1, 92	1.49	0.23
Time×Strategy	*2, 147*	*7.25*	*0.001*	2, 82	0.01	0.99	*2, 149*	*3.21*	*0.043*	2, 77	0.11	0.90
Time×Morph	1, 147	0.05	0.83	1, 80	2.72	0.10	1, 149	0.02	0.88	1, 80	0.001	0.98
Strategy×Morph	2, 147	0.15	0.86	2, 85	1.00	0.37	*2, 149*	*3.18*	*0.045*	2, 96	1.62	0.20
TT×Sex	1, 147	0.20	0.66	1, 82	0.87	0.36	1, 149	0.15	0.70	1, 92	1.17	0.28
Sex×Strategy	2, 147	0.04	0.96	2, 86	0.04	0.96	2, 149	0.28	0.76	2, 92	0.15	0.86
Sex×Morph	1, 147	3.85	0.05	1, 83	3.51	0.07	1, 149	0.13	0.72	*1, 91*	*9.04*	*0.003*
TT×Time×Strategy				2, 81	0.17	0.85	2, 149	1.09	0.34	2, 79	0.31	0.74
TT×Time×Morph				1, 81	3.24	0.08	1, 149	0.12	0.73	1, 77	1.64	0.20
TT×Strategy×Morph				2, 84	0.29	0.75	2, 149	0.30	0.74			
Time×Strategy×Morph				2, 80	0.004	>0.99	2, 149	2.16	0.12	2, 79	1.08	0.35
TT×Sex×Strategy				2, 83	0.07	0.93	2, 149	0.64	0.53			
TT×Sex×Morph				1, 84	0.23	0.63	1, 149	0.14	0.71			
TT×Sex×Time				1, 80	1.61	0.21	1, 149	1.62	0.21	1, 78	0.35	0.55
Sex×Strategy×Morph	2, 147	0.10	0.91	2, 83	0.60	0.55	2, 149	0.43	0.65			
Sex×Morph×Time	1, 147	0.20	0.66	*1, 80*	*9.70*	*0.003*	1, 149	0.43	0.52	1, 77	0.37	0.54
Sex×Time×Strategy				2, 79	1.47	0.24	2, 149	0.53	0.59	2, 75	0.19	0.83

Italicized values indicate P<0.05.

birds still lost mass at the end of the heatwaves, suggesting that initial adjustments were only temporary and not sufficient to compensate for the effects of high temperature on their maintenance in the long term. Similarly, black-headed males under thermoneutral conditions also gained mass at the beginning of the heatwave. The fact that black-headed males experiencing thermocritical conditions were unable to do so suggests that either their energy requirements were higher because of the activation of thermoregulatory processes

(Wolf, 2000), or they maintain fat reserves at a low level to reduce insulation (Clark, 1979). Similarly, black-headed females losing body mass at the end of the thermocritical heatwave, may have used these strategies. Irrespective of the mechanisms underlying mass changes in Gouldian finches exposed to variable thermal conditions, the fact that these changes differ between red- and black-headed males and females suggests that birds of both morphs and sexes may show different thermal tolerances. This may be due
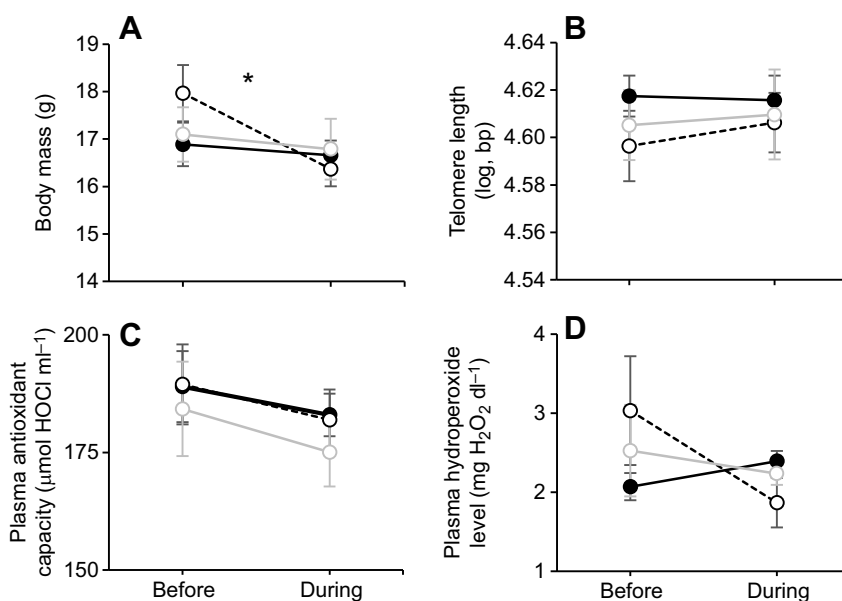

Fig. 3. Maintenance status during moult/reproduction. (A) Body mass, (B) telomere length, (C) plasma antioxidant capacity and (D) plasma hydroperoxide levels before and during moult (solid line; N=51), breeding (dashed line; N=33) and in non-moulting/non-breeding individuals (grey line; N=17) irrespective of sex, thermal treatment and colour morph. The asterisk indicates a significant interaction between time and strategy.
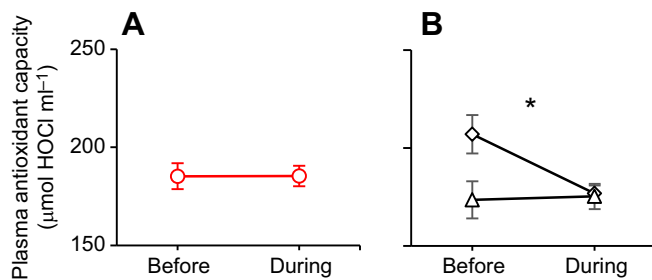
Fig. 4. Plasma antioxidant capacity before and during moult or breeding. (A) Red-headed males and females together (○; N=56), and (B) black-headed females (◇; N=28) and males (△; N=28) separately. The asterisk indicates a significant interaction between time and sex.

to the fact that red-headed birds reduce their activity during heatwaves (Fragueira and Beaulieu, 2019), which may allow them to save on costly thermoregulatory mechanisms at least at the beginning of heatwaves, thus keeping their total energy expenditure constant. In contrast, black-headed birds negligibly reducing their activity (Fragueira and Beaulieu, 2019) may have to use such costly physiological strategies immediately at the beginning of thermocritical heatwaves (males) or during heatwaves irrespective of their intensity (females).

Intriguingly, we found that red-headed birds showed reduced levels of plasma hydroperoxides at the beginning of the heatwaves. This is in sharp contrast with previous studies showing higher oxidative damage in birds exposed to hot conditions (Altan et al., 2003; Lin et al., 2008), and highlights the difficulty in predicting the effects of thermal conditions on oxidative markers. Low levels of hydroperoxides in red-headed birds may be explained by the fact that birds were chronically exposed to high temperatures (as opposed to an acute exposure), which allowed them to activate uncoupling proteins. However, this hypothesis seems unlikely, as activating uncoupling proteins generating heat would increase even more the risk of hyperthermia for birds. Low levels of hydroperoxides in red-headed birds may rather be due to the overall reduction of their physical activity during heatwaves (Fragueira and Beaulieu, 2019) to minimize the effects of heat exposure on their overall energy expenditure and ROS production (Beaulieu et al., 2015a). Conversely, the absence of behavioural adjustments in black-headed birds may explain why black-headed females tended to show higher levels of hydroperoxides in the long term under thermocritical conditions.

Overall, our results suggest that the maintenance of Gouldian finches was more affected by thermal conditions in black-headed birds than red-headed birds, presumably because of a lack of behavioural adjustments in black-headed birds. These results are in contrast to the hypothesis that potentially higher energy requirements in dominant red-headed birds should result in higher maintenance costs under thermally challenging conditions. For instance, red-headed males feeding more than black-headed males (thereby suggesting higher energy requirements; Fragueira and Beaulieu, 2019) were not more affected by thermal conditions. However, the maintenance of black-headed females feeding more than black-headed males (Fragueira and Beaulieu, 2019) appears more affected than that of males. Higher energetic requirements in black-headed females may therefore explain differential maintenance costs between black-headed males and females. Consequently, the hypothesis that higher intrinsic requirements should result in higher maintenance costs under thermocritical conditions appears to hold true between black-headed males and females, but not between red- and black-headed birds.

Effects of moult and breeding on maintenance status

Although we exposed birds to conditions promoting reproduction by providing nest material and supplementary food, only less than half of them started breeding over the 4-month period following heatwave exposure. This low proportion was not due to the severity of the thermal conditions that birds previously experienced, as it was similar in birds previously experiencing the thermoneutral or the thermocritical heatwave. The low proportion of breeders may rather be due to the fact that the monitoring period was too short or that partners were not fully compatible despite belonging to the same colour morphs (morphs usually mate assortatively in this species; Pryke and Griffith, 2007). In contrast to our prediction, the intensity of the heatwave that birds previously experienced did not affect their propensity to preferentially start moulting or breeding. Moreover, this result was independent of their colour morph, thereby suggesting that black- and red-headed birds follow similar rules to initiate either moult or breeding. The fact that birds moulting first tended to show longer telomeres supports the hypothesis that telomere dynamics and feather regeneration are inter-related, possibly because of the common effects of telomerase on cell proliferation (Reichert et al., 2014). Overall, our results suggest that birds of both morphs recovered from the heatwaves before moulting or breeding, and that the initiation of moult and breeding is an individually based rather than a morph-specific strategy.

Body mass and plasma hydroperoxide levels showed the same temporal pattern, with pre-breeding birds showing higher values than pre-moulting and non-moulting/non-breeding birds, but converging towards them while breeding. This suggests that reproduction per se affected more strongly the maintenance of birds than moulting irrespective of their morph. However, the antioxidant capacity of birds still showed morph-specific temporal variation, with initially elevated antioxidant defences decreasing in moulting or breeding black-headed females, while remaining stable in red-headed females and in males of both morphs. This suggests that black-headed females may anticipate antioxidant requirements by increasing antioxidant defences before moulting or breeding, as they may be limited to do so during these energetically demanding events. Accordingly, black-headed females did not increase antioxidant capacity with brood size whereas red-headed females did so. In contrast to red-headed females, black-headed males showed higher levels of hydroperoxides with increasing brood size. Overall, these results suggest that the oxidative balance of moulting or breeding black-headed birds is more variable than that of red-headed birds, and that black-headed males and females alter different components of this balance while breeding.

Telomeres did not shorten over the course of our 5-month study irrespective of the morph or thermal treatment of birds. This is surprising given that telomeres typically shorten rapidly in short-lived species (Tricola et al., 2018), such as Gouldian finches (maximal longevity: 6 years, AnAge; Tacutu et al., 2012). The absence of telomere shortening in our study may be due to the fact that telomeres were measured in adults (as opposed to growing individuals; Salomons et al., 2009) and that Gouldian finches exhibit very long telomeres (mean telomere length of 40 kb in our study versus 25 kb in zebra finches or 47 kb in great tits, which also exhibit ultralong telomere length; Atema et al., 2019), which may mask the attrition of shorter telomeres. Despite this limitation, we still found that red-headed males exhibited the shortest telomeres among all birds, suggesting that they hatch with shorter telomeres, that their growth has stronger effects on telomere attrition, and/or that their telomeres shorten more rapidly over periods of time longer than that considered here. The same hypotheses might apply to black-headed females tending to show shorter telomeres than red-headed females.

Conclusions

In polymorphic species, colour polymorphism has been found to be related to distinct life-history strategies. Accordingly, our results show that somatic maintenance in Gouldian finches of different morphs was differently affected by energetically demanding conditions, with red-headed males and females showing a slight but homogeneous physiological response, whereas black-headed males and females showed a stronger but heterogeneous response. How differences in maintenance regulation between morphs and sexes contribute to distinct fitness differences in this species remains to be examined. Notably, it would be interesting to examine the ultimate consequences of a homogeneous physiological response between breeding partners relative to a heterogeneous response. In the context of climate change, such an approach would allow us to determine whether differences in maintenance regulation between morphs and sexes make some individuals within polymorphic species more vulnerable than others to novel conditions, which in turn may strongly affect population dynamics under natural conditions.

Supplementary information

Supplementary information available online at
http://jeb.biologists.org/lookup/doi/10.1242/jeb.196030.supplemental

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Supplementary material

Table S1 Summary table of generalised linear mixed model (GLMM) examining the effects of thermal treatment (TT), time (-10, +1, +14 before/after the start of the heatwaves) and sex, separated by morphs, on body mass and plasma hydroperoxide levels. Italicized values indicate $p < 0.05$.

Response variable	Body mass						Plasma hydroperoxide level					
	Red-headed birds			Black-headed birds			Red-headed birds			Black-headed birds		
Explanatory variables	df	F	P	df	F	P	df	F	P	df	F	P
TT	1, 155	0.18	0.67	1, 156	0.75	0.39	1, 155	2.48	0.12	1, 155	0.07	0.80
Time	*2, 155*	*13.84*	*< 0.001*	*2, 156*	*3.67*	*0.028*	*2, 155*	*3.40*	*0.036*	2, 155	0.37	0.69
Sex	1, 155	0.70	0.41	1, 156	1.35	0.25	1, 155	1.70	0.19	1, 155	1.52	0.22
TT*Time	2, 155	2.11	0.13	2, 156	1.71	0.19	2, 155	1.57	0.21	2, 155	1.58	0.21
TT*Sex	1, 155	0.54	0.46	1, 156	0.11	0.74	1, 155	0.02	0.89	1, 155	0.42	0.52
Time*Sex	2, 155	1.52	0.22	2, 156	2.70	0.07	2, 155	1.51	0.22	2, 155	0.16	0.85
TT*Time*Sex	2, 155	2.64	0.08	*2, 156*	*3.44*	*0.034*	2, 155	1.61	0.20	*2, 155*	*3.17*	*0.045*

Table S2 Summary table of the linear mixed model (LMM) examining the effects of moult-breeding initiation (Strategy), sex and morph initial telomere length (at time -10). The italicized value indicate $p < 0.05$.

Response variable	Initial telomere length		
Explanatory variables	df	F	P
Strategy	1, 48	3.47	0.07
Sex	1, 39	0.18	0.68
Morph	1, 42	0.05	0.83
Strategy*Sex	1, 42	0.52	0.48
Strategy*Morph	1, 48	0.24	0.63
Sex*Morph	*1, 39*	*5.72*	*0.022*
Strategy*Sex*Morph	1, 42	0.68	0.42
Test used	LMM – log transformed		

Table S3 Summary table of linear mixed models (LMM) examining the effects of sex, morph and brood size on temporal variation (Δ) in body mass, plasma antioxidant capacity, plasma hydroperoxide levels and telomere length between before and during moult or breeding.

Response variable / Explanatory variables	Δ Body mass			Δ Antioxidant capacity			Δ Hydroperoxide level			Δ Telomere length		
	df	F	P	df	F	P	df	F	P	df	F	P
Sex	1, 32	1.15	0.29	1, 16	0.05	0.83	1, 14	0.01	0.92	1, 25	0.57	0.46
Morph	1, 32	0.63	0.43	1, 16	0.41	0.53	1, 16	0.50	0.49	1, 25	0.16	0.70
Brood size	1, 32	0.25	0.62	1, 16	1.30	0.27	1, 15	0.02	0.88	1, 25	0.75	0.40
Sex*Morph	1, 32	0.001	0.97	1, 16	2.35	0.15	1, 14	2.07	0.17	1, 25	0.020	0.88
Sex*Brood size	1, 32	2.98	0.09	1, 16	0.18	0.68	1, 13	1.38	0.26	1, 25	0.52	0.48
Morph*Brood size	1, 32	0.24	0.63	1, 16	0.07	0.79	1, 16	0.010	0.92	1, 25	0.03	0.87
Sex*Morph*Brood size	1, 32	0.29	0.60	1, 16	4.02	0.06	1, 13	4.51	0.05	1, 25	0.30	0.59

Table S4 Summary table of linear mixed models (LMM) examining the effects of thermal treatment (TT), time (before and during moult or breeding), moult-breeding status and sex, separated by morphs, on plasma antioxidant capacity. Italicized values indicate $p < 0.05$.

| Response variable / Explanatory variables	Plasma antioxidant capacity					
	Red-headed birds			Black-headed birds		
	df	F	P	df	F	P
TT	1, 42	0.07	0.79	1, 40	0.06	0.82
Time	1, 39	0.23	0.63	1, 37	3.87	0.06
Strategy	2, 41	0.55	0.58	2, 41	0.49	0.62
Sex	1, 42	0.51	0.48	*1, 40*	*4.68*	*0.037*
TT*Time	1, 39	2.40	0.13	1, 37	0.002	0.97
TT*Strategy	2, 41	0.02	0.98	2, 41	0.40	0.67
TT*Sex	1, 42	0.001	0.97	1, 40	1.51	0.23
Time*Strategy	2, 39	0.003	> 0.99	2, 38	< 0.001	> 0.99
Time*Sex	1, 39	1.35	0.25	*1, 37*	*6.14*	*0.018*
Strategy*Sex	2, 41	0.20	0.82	2, 41	0.43	0.65
TT*Time*Strategy	2, 39	0.36	0.70	2, 38	0.50	0.61
TT*Time*Sex	1, 39	0.04	0.84	1, 37	1.67	0.20
TT*Strategy*Sex	2, 41	0.06	0.94	2, 41	0.25	0.78
Time*Strategy*Sex	2, 39	1.26	0.30	2, 38	0.45	0.64
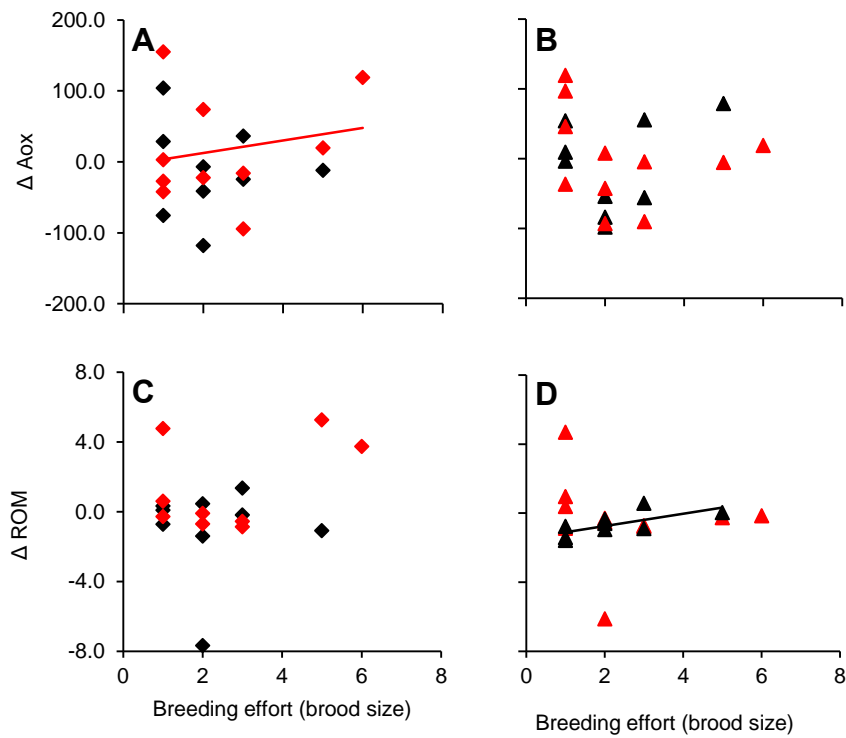
Figure S1 Temporal variation in plasma antioxidant capacity (Aox) and hydroperoxide levels (ROM) difference (Δ) between before and during breeding in relation to brood size in red- (red symbols) and black-headed (black symbols) females (◊) and males (Δ). Only significant regression lines are represented.

Dataset 1. Raw data from the study

Click here to Download Dataset 1