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Enzymatic antioxidants but not baseline glucocorticoids mediate the reproduction–survival trade-off in a wild bird

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The trade-off between reproductive investment and survival is central to life-history theory, but the relative importance and the complex interactions among the physiological mechanisms mediating it are still debated. Here we experimentally tested whether baseline glucocorticoid hormones, the redox system or their interaction mediate reproductive investment–survival trade-offs in wild great tits (*Parus major*). We increased the workload of parental males by clipping three feathers on each wing, and 5 days later determined effects on baseline corticosterone concentrations (Cort), redox state (reactive oxygen metabolites, protein carbonyls, glutathione peroxidase [GPx], total non-enzymatic antioxidants), body mass, body condition, reproductive success and survival. Feather-clipping did not affect fledgling numbers, chick body condition, nest provisioning rates or survival compared with controls. However, feather-clipped males lost mass and increased both Cort and GPx concentrations. Within feather-clipped individuals, GPx increases were positively associated with reproductive investment (i.e. male nest provisioning). Furthermore, within all individuals, males that increased GPx suffered reduced survival rates. Baseline Cort increases were related to mass loss but not to redox state, nest provisioning or male survival. Our findings provide experimental evidence that changes in the redox system are associated with the trade-off between reproductive investment and survival, while baseline Cort may support this trade-off indirectly through a link with body condition. These results also emphasize that plastic changes in individuals, rather than static levels of physiological signals, may mediate life-history trade-offs.

1. Introduction

Life-history theory centres on the concept that trade-offs exist between reproductive investment and survival [1–4]. Trade-offs are mediated by processes that compete with each other for resources that are potentially limited [1,4,5]. However, the physiological systems that mediate such trade-offs are still debated [6,7]. At present, two major physiological processes are being discussed as potential mediators: the endocrine and the redox systems [6,8–10]. Among endocrine signals, glucocorticoid hormones (GCs) have emerged as systemic regulators of life-history trade-offs [11–14]. GCs are often referred to as 'metabolic hormones' because one of their main functions is the mobilization of energy sources to support increased energetic needs of tissues [15–18]. These functions render GCs prime candidates for mediating trade-offs that are based on energy allocation. Indeed, high circulating concentrations of GCs, such as induced by the endocrine stress response, can directly inhibit reproductive processes through actions on the brain and the hypothalamic–pituitary–gonadal axis [13,15,16,19–21]. Hence, at high stress-induced concentrations, GCs reduce investment in current reproduction and prioritize processes that promote survival behaviours like escape and foraging to restore internal resources [19]. However, whether GCs play a role in mediating such trade-offs at low baseline concentrations is less clear, although

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their involvement has been hypothesized [22]. Baseline GCs are elevated when individuals experience increased energetic demands [11,12,17,18,22–25], for example, in the reproductive season when parents provision their offspring [18,26–31]. These findings prompted the hypothesis that baseline GCs may mediate trade-offs, but in an opposite way to stress-induced concentrations: baseline GCs would support investment in current reproduction at the expense of survival [22,32,33]. However, thus far the evidence for GCs supporting reproductive investment [22,26,34–37] or impairing survival is mixed [22].

According to the 'oxidative stress life-history theory', trade-offs can be a direct consequence of oxidative damages induced by exposure to reactive oxygen species (ROS) that have significant pro-oxidant effects [8,38–41]. If the concentrations of ROS cannot be counterbalanced by antioxidant defences, oxidative stress will ensue, which can generate cellular oxidative damage [42–44]. ROS can damage vital molecules such as DNA and proteins, impair cellular functionality and impact longevity [44–46]. Oxidative costs can arise both from an increase in oxidative damages and a change in antioxidant concentrations [47–50]. Antioxidants can be upregulated to cope with a pro-oxidant challenge or can be depleted when ROS cannot be buffered, thus exposing the organism to oxidative damage [46]. The central concept of the 'oxidative stress life-history theory' is that ROS are produced proportionally to metabolic rate. Since metabolic rate typically increases during reproduction, more resources have to be allocated to antioxidant protection [38–41,51]. While there is some evidence that reproductive investment does entail oxidative costs [52,53], the postulated positive association between metabolic rate and the production of ROS has not been widely supported [47,54,55]. At least partly, such conflicting results could arise from the correlative approaches that have been used in most investigations thus far, where individuals are allowed to decrease their reproductive effort to minimize exposure to pro-oxidants [47]. Therefore, experimental studies in which the metabolic factors that cause cellular oxidative stress are manipulated are essential to properly test this hypothesis [54]. Moreover, we still need to understand whether changes in the redox system that are within the normal physiological range, and not only acute oxidative damage, are linked to life-history trade-offs.

Increasing the workload of parents during the reproductive period in free-ranging individuals has been shown to be an effective method to elevate their costs and two recent experimental studies in swallows suggest an involvement of baseline Cort in the resulting increased parental effort [56,57]. However, neither of these studies investigated the existence of costs like impaired survival or health state. Thus, the question of whether baseline Cort mediates life-history trade-offs has not been conclusively answered. Likewise, a study that feather-clipped great tits (*Parus major*) prior to the nesting phase did not reveal oxidative costs of reproduction because manipulated parents decreased their reproductive investment by laying smaller clutches [58]. A recent study raised the workload of reproducing European starling (*Sturnus vulgaris*) females by mounting a backpack radio transmitter, feather-clipping three wing feathers or combining the two handicaps [59]. Cort concentrations were higher in individuals carrying a radio transmitter but lower in individuals with both handicaps, rendering the interpretation of the findings difficult. Females subjected to both handicaps did show evidence of costs by having increased concentrations of ROS and lower return rates in the following breeding season [59].

The majority of the studies reviewed above conducted their analyses at the population level, which can obscure processes occurring at the individual level. For example, individuals can differ in trade-off strategies depending on their body condition or ecological circumstances, such that individuals in optimal condition or living in high-quality environments can maximize both survival and reproductive success [4,60–62]. Hence, analyses of individual variation are required to truly understand life-history trade-offs and to test for direct links between physiological measures and fitness.

Here, we experimentally increased energetic costs during reproduction in a free-living bird species to test for effects on fitness variables like reproductive investment and survival as well as physiological mechanisms like baseline Cort concentrations and the redox system. We designed our experiment along the lines of the 'ideal manipulation' concept [62] by avoiding any direct effects of the experimental manipulation on reproductive success (which in turn would influence the reproductive investment of the parents) or on survival (figure 1, arrows 1, 2 and 6). Instead, we aimed at inducing physiological changes in experimental individuals to test for consequences on reproductive investment and survival. Specifically, we clipped three primary feathers on both wings of male great tits (*Parus major*) during the offspring provisioning phase to increase wing load and thus energetic costs of flight and nestling provisioning (our measure of reproductive investment) [57,63–65]. With this experiment, we aimed at testing whether baseline Cort, the redox system or both may mediate the trade-off between reproductive investment and survival. In general, we expected that the increase in wing load, an unexpected worsening of conditions for parental individuals, will lead to an upregulation in baseline Cort concentrations, to an increase in oxidative damage and to a change in antioxidant concentrations (either increase or decrease) [48]. We analysed the resulting changes in male great tits both at the population and the individual level.

2. Material and methods

The study was carried out between March 2015 and November 2017, in a mixed forest located in the district of Starnberg, southern Germany (47°99′ N–11°39′ E; for further details on field work, see electronic supplementary material). Adult great tits were captured two times in their nest-box between 08.00 and 15.00 h by triggering a remote-controlled flap at the nest-box entrance (for a time line, see electronic supplementary material, figure S1). During the first trapping, when chicks were 7 days old (electronic supplementary material, figure S1), we clipped three primary feathers on both wings in experimental males (FC-males), leaving approximately 1.5 cm of the feather shaft intact. Control males (C-males) and all females were caught and handled similarly, but had no feathers cut. To assess the effect of the treatment, on chick day 12 males were re-trapped and re-measured as described above. Following each capture, a blood sample of maximally 80 µl was taken from the wing vein within less than 3 min (see electronic supplementary material) from the remote-controlled closure of the nest-box entrance. The number of 15-day-old nestlings and their body mass were recorded as proxies for fledging success.

One day before each trapping, we video-recorded the nest provisioning rate of both parents, our proxy for reproductive investment. Apparent survival was estimated by the presence/absence of an individual at the study site in the following breeding

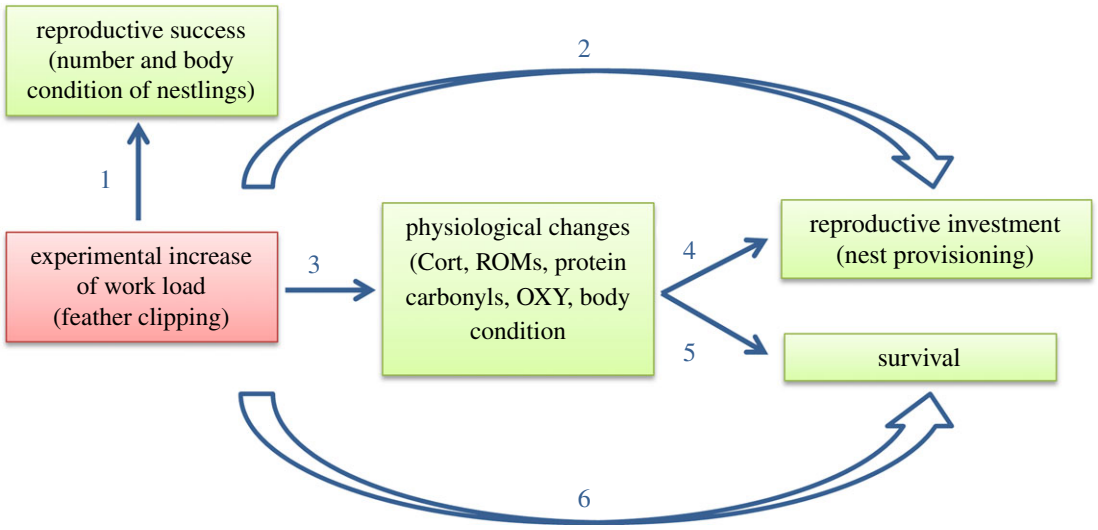

Figure 1. Conceptual representation of the statistical analyses used in the study. Our main aim was to assess the physiological changes induced by an experimentally increased workload (arrow 3), and how changes in physiology affected reproductive investment and survival (arrows 4 and 5). One major assumption for the success of this experiment was the lack of a direct effect of the manipulation on reproductive success (arrow 1), reproductive investment (arrow 2) and survival (arrow 6). This design represents the 'ideal manipulation' for studying the physiological costs of parental care [62]. For further explanations, see text.

season or during fall recaptures in the year after the experiment [26]. Previous studies have suggested that presence/absence data closely match actual survival rates of great tits [66]. Plasma corticosterone concentrations were determined using enzyme immunoassay following a double diethyl ether extraction (see electronic supplementary material). We measured changes in the redox system by quantifying its two main components: the potential oxidative damage incurred as well as the antioxidant defences (both enzymatic and non-enzymatic antioxidants). Potential oxidative damage was quantified by measuring the plasma levels of reactive oxygen metabolites (ROMs; d-ROM test kit, Diacron International). ROMs are organic hydroperoxides, end-products of the oxidation of lipids, proteins and nucleic acids that are generated by hydrogen peroxides produced primarily during mitochondrial respiration [67]. The non-enzymatic antioxidants (OXY) present in the plasma, which can be either produced by the organism or acquired through the diet were quantified with a colorimetric assay (OXY-Adsorbent test kit, Diacron International) [67]. The intra-cellular antioxidant enzymatic activity was quantified through glutathione peroxidase (GPx; kit Randox Laboratories) concentrations. GPx has the unique function to enzymatically convert hydrogen peroxides into water. We quantified protein carbonyl concentrations in red blood cells (kit Cayman Chemical Company), which indicates terminal oxidative damage to proteins [46], following a validated protocol for birds [49]. All samples were measured in duplicates (number of plates run for each assay and data on assay quality are provided in the electronic supplementary material).

We were able to sample 43 males twice (23 FC-males and 20 C-males; $n = 86$ repeated measures, but total $n = 112$ including individuals trapped only once). Before the onset of the experiment, FC- and C-males did not differ in any of the variables measured (see electronic supplementary material). To verify that the treatment had no direct effects on reproductive success (figure 1, arrow 1), we ran two separate general linear models with number of fledglings and mean body condition of fledglings as response variables. The body condition of nestlings (and that of adults, see below) was calculated as body mass scaled by wing length [68]. These models included treatment group as a fixed factor while controlling for provisioning rate recorded on day 15, year and hatching date. We next tested for direct effects of feather-clipping on reproductive investment (figure 1, arrow 2) by using a linear mixed model with nest provisioning (number of nest visits $\times$ h^{-1}) standardized for brood size as the response variable, including treatment and day of sampling (two levels: day 7 and day 12) as fixed factors in a full factorial model. Individual identity was nested into treatment and included as random factor [69]. We used similar models to determine whether the feather-clipping manipulation affected aspects of male physiology (figure 1, arrow 3), specifically using Cort concentrations (ng ml^{-1}), body mass (g) and body condition as body mass standardized for wing length (g) [68], d-ROMs (mM H_2O_2 equivalents), Oxy (mM HOCl), GPx (Units—$U\,l^{-1}$) and protein carbonyls (nmol mg^{-1} of proteins) as response variables.

To investigate whether the physiological variables that changed following the treatment could predict male reproductive investment (figure 1, arrow 4), we ran a repeated measure model with provisioning rate per chick as the response variable and Cort, body condition and GPx as predictors. Among the predictors, we also included year because in 2015 Cort was higher, but GPx lower and nestling body condition worse. The two groups were analysed separately because they showed divergent changes in physiological variables (treatment $\times$ time interactions; see Results).

Model selection was carried out using the Akaike information criterion [70] for small sample sizes (AICc). We considered the models with the lowest AICc value as well as those with an AICc difference lower than 2 (electronic supplementary material, tables S1–S3) [70]. The final model included the predictors that were present in the majority of the best models and its AICc was compared to the AICc of a null model that did not include any predictors (intercept-only model).

To assess whether the physiological changes observed during the experiment explained annual survival (figure 1, arrow 5; 0 = not survived, 1 = survived), we included change in GPx, Cort and standardized body mass (i.e. all variables that were affected by the treatment; changes were calculated as the difference between days 12 and 7) into a logistic model, together with year and treatment. This model also allowed us to test for a direct effect of feather-clipping on survival (figure 1, arrow 6). For reasons of sample size, the two treatment groups were pooled for this analysis. Also, *changes* in physiological variables were calculated because repeated measurements of individuals were not available due to the nature of the survival data. To provide a similarly structured analysis for reproductive investment, we used changes in GPx, Cort and standardized body mass as predictors, together with year and treatment in a model run for both treatment groups combined.

In addition to the analyses depicted in figure 1, we assessed whether the Cort concentrations explained variations in the redox system (i.e. whether Cort could potentially be a mediator of

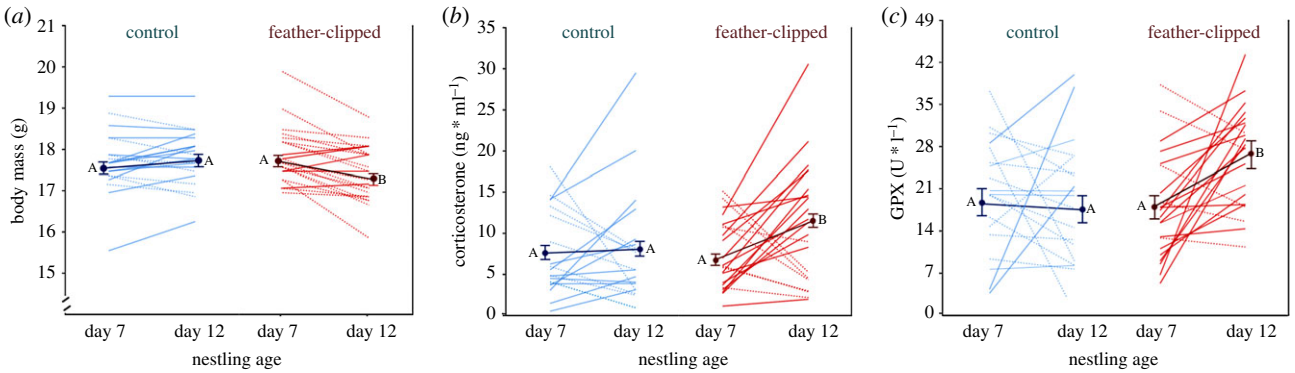

Figure 2. (*a*) Body mass (C-males $n = 20$, FC-males $n = 23$), (*b*) corticosterone concentrations (C-males $n = 19$, FC-males $n = 22$) and (*c*) glutathione peroxidase (GPx; C-males $n = 19$, FC-males $n = 22$) concentrations of males on chick days 7 and 12. Different capital letters indicate a significant difference between days 7 and 12 (Tukey's HSD *post hoc* tests). Thin solid lines: positive individual changes, thin dotted lines: negative individual changes, thick lines: group least squared means (±1 s.e.m.).

trade-offs). For this, we ran separate models for FC- and C-males with the response variables being GPx, OXY, ROM and protein carbonyl, respectively, and Cort being the predictor. A similar model was used to assess the relationship between body condition and Cort.

All analyses were performed using JMP v. 12.2.0 (SAS Institute Inc. Cary, NC, USA), which provides a *p*-value for each fixed effect in the model based on *F*- or χ^2-statistics by testing the null hypothesis that the parameters associated with that effect are zero. In line with this approach, we analysed *post hoc* within-individual differences with the Tukey's HSD test. For all models, we used *z*-score normalized variables. All data are given as means ± s.e.m. Further statistical details are provided in the electronic supplementary material.

3. Results

(a) Treatment effects on reproductive success and parental provisioning rate

Feather-clipping male great tits did not affect their reproductive success (figure 1, arrow 1). Our final model for the number of fledglings as a function of the feather-clipping included hatching date and year as predictors and showed that FC- and C-males did not differ in number of fledglings produced (electronic supplementary material, table S1). Supporting this finding, this model had a lower fit than the intercept-only model. Similarly, the final model for the body condition of fledglings as a function of feather-clipping showed that fledgling condition did not differ for treated versus control males (electronic supplementary material, table S1). Only year explained the body condition of nestlings ($F_{(1,43)}$=6.91, $p = 0.01$), with condition being worse in 2015. Again, the null model had a better AICc value than the final model.

The treatment did not affect the rate at which nestlings were fed by their parents (figure 1, arrow 2). The final model selected for nest provisioning as a function of the feather-clipping included year, mate nest provisioning and hatching date, but treatment did not significantly explain nest provisioning and this model had a similar fit than an intercept-only model (electronic supplementary material, table S2). Overall, the provisioning rate of males was positively associated with that of their female partners ($\beta = 0.48 \pm 0.14$, $F_{1,85} = 12.45$, $p < 0.001$; electronic supplementary material, table S2).

(b) Treatment effects on body mass, corticosterone and redox system

Feather-clipping induced changes in three physiological parameters of male great tits (figure 1, arrow 3). First, body mass was significantly affected by the treatment, as FC-males decreased body mass (change: -0.36 ± 0.10 g, $p = 0.004$) while C-males did not (0.13 ± 0.11 g, $p = 0.59$; figure 2*a*; table 1; electronic supplementary material, table S3A). The final model also included number of fledglings, with heavier males producing more fledglings (table 1; electronic supplementary material, table S3). Male body condition was similarly affected by the treatment, as was body mass (table 1; electronic supplementary material, table S3B). However, in the final model for body condition, the number of fledglings was not retained (electronic supplementary material, table S1B).

Second, variation in baseline Cort concentrations was significantly explained by the treatment (figure 2*b*; table 1; electronic supplementary material, table S3C), as FC-males increased Cort over time after feather-clipping (change: 4.53 ± 1.44 ng ml^{-1}, $p = 0.015$), while C-males did not (0.31 ± 1.56 ng ml^{-1}, $p = 0.99$; figure 2; electronic supplementary material, table S1C). The final model for Cort also included bleeding duration, which increased with longer blood sampling times (table 1; electronic supplementary material, table S3C).

Third, red blood cell GPx concentrations were affected by the treatment and increased from day 7 to day 12 in FC-males (change: 8.14 ± 2.74 U l^{-1}, $p = 0.02$) but not in C-males (0.69 ± 2.89 U l^{-1}, $p = 0.99$; figure 2*c*; table 1; electronic supplementary material, table S3D). Year was also included in the final model (table 1, electronic supplementary material, table S3D).

By contrast, feather-clipping did not affect ROM concentrations (electronic supplementary material, table S3E) or protein carbonyl concentrations (electronic supplementary material, table S3G), and the null model had the best fit for ROMs and equal fit for protein carbonyl model. We then included GPx in the models for ROMs, because hydrogen peroxides are produced by this enzyme, and obtained a better fit of the final model compared to the null model (electronic supplementary material, table S3F). Treatment still had no effect on ROM concentrations, but ROMs were positively related to GPx concentrations (table 1; electronic supplementary material, table S3F). Finally, feather-clipping did not change plasma OXY concentrations (electronic supplementary material, table

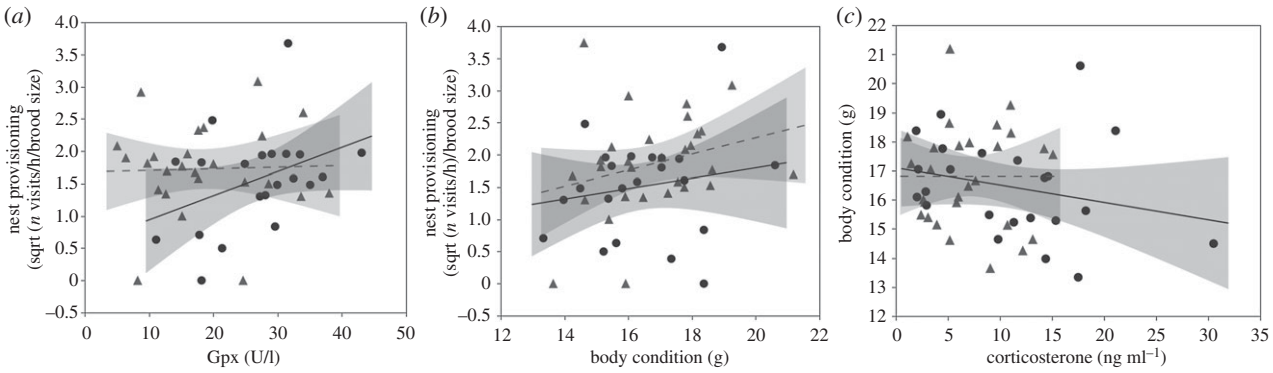

Figure 3. Relationships in FC-males between (*a*) nest provisioning rate and glutathione peroxidase (GPx) concentrations (day 7: $n = 25$, day 12: $n = 21$), (*b*) nest provisioning rate and corticosterone concentrations (day 7: $n = 28$, day 12: $n = 22$), and (*c*) body condition and corticosterone concentrations (day 7: $n = 28$, day 12: $n = 23$). Grey triangles and dotted lines for chick day 7; black circles and solid lines for chick day 12.

Table 1. Significant predictors of the best-supported repeated-measure models for physiological variables (for chick days 7 and 12). Model selection, whole model and variance analysis of random effects are reported in electronic supplementary material, table S3A–F.

	estimate	s.e	d.f.	*F*	*p*-value
body mass ($R^2 = 0.89$)					
time × treatment	−0.03	0.009	142.26	11.45	<0.01
number of fledglings	0.27	0.12	147.31	5.03	0.03
body condition ($R^2 = 0.98$)					
time × treatment	−0.12	0.03	142.64	13.80	<0.001
corticosterone ($R^2 = 0.51$)					
time [day 7]	−0.04	0.02	147.41	5.21	0.03
time × treatment	0.04	0.02	147.48	3.93	0.05
year [2015]	0.06	0.02	156.23	9.23	<0.01
bleeding duration	0.24	0.11	181.4	4.72	0.03
GPx ($R^2 = 0.16$)					
treatment [C]	−0.05	0.02	136.84	4.47	0.04
time × treatment	0.05	0.02	141.23	4.92	0.03
year [2015]	−0.07	0.02	141.93	41.92	<0.01
ROMs ($R^2 = 0.65$)					
GPx	0.18	0.07	1.74.69	5.80	0.02
OXY ($R^2 = 0.55$)					
body mass	0.32	0.17	167.11	4.24	0.04

S1H), but males with higher body mass had higher OXY levels (table 1; electronic supplementary material, table S3H).

(c) Effects of physiological variables on reproductive investment and survival rate

Reproductive investment (i.e. provisioning rate) of FC-males was positively associated with variation in GPx between days 7 and 12 (figure 3*a*; table 2; electronic supplementary material, table S4), but was not explained by Cort variation (arrow 4 in figure 1; electronic supplementary material, table S4). FC-males in better body condition provided more parental care (figure 3*b*; table 2; electronic supplementary material, table S4). In a separate analysis, we found that in FC-males, body condition was negatively related to circulating levels of Cort ($ß = -0.14$, $F_{(1,22.91)} = 6.74$, $p = 0.016$; figure 3*c*), but not to GPx (FC-males: $F_{1,21} = 1.81$, $p = 0.19$). The provisioning rate of C-males was not explained by changes in Cort, GPx or body condition (electronic supplementary material table S4). Body condition of C-males was not explained by Cort levels ($F_{(1,20.48)} = 0.55$, $p = 0.55$) or GPx ($F_{(1,20.79)} = 0.0005$, $p = 0.98$). Since the model for survival (see below) was run by pooling the two treatment groups and including the *changes* in physiological variables from chick days 7 to 12 as predictors, we ran the same model for reproductive investment as well. None of Cort, GPx or body condition changes explained male provisioning rates (electronic supplementary material, table S5).

In the year after each experimental manipulation, we recorded the presence of 9 FC-males (39% survived) and 6 C-males (30% survived) out of the 43 birds for which we have repeated measures (figure 1, arrows 5 and 6). Irrespective of the treatment (which did not affect survival), individuals that

Table 2. Only significant predictors of the best supported repeated measure model on provisioning rate as response variable for the two groups separately. Model selection and variance analysis of random effects are reported in the electronic supplementary material, table S4.

standardized provisioning rate	estimate	s.e	d.f.	*F*	*p*-value
feather-clipped ($R^2 = 0.40$)					
year [2015]	0.08	0.034	124.77	5.91	0.022
body condition	0.30	0.13	119.54	5.50	0.030
GPx	0.34	0.15	139.89	5.17	0.028
controls ($R^2 = 0.55$)					
none					

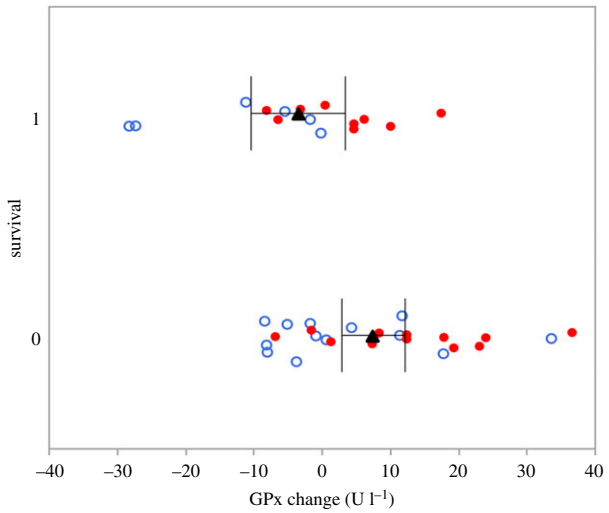

Figure 4. Apparent survival (1 = yes, 0 = no) versus change in glutathione peroxidase concentrations (GPx; chick day 12—chick day 7). Red filled circles indicate FC-males, blue open circles indicate C-males, black triangles show mean values (±95% confidence intervals).

increased GPx more were less likely to survive (electronic supplementary material, table S6; $\beta = 4.84 \pm 2.28$, $F_{1,37} = 6.18$, $p < 0.01$; figure 4). Neither changes in Cort or in body condition were related to survival (electronic supplementary material, table S5). Change in Cort concentrations also did not explain any redox variable in either group (electronic supplementary material, table S7).

4. Discussion

Increasing the workload of male great tits during nestling provisioning by feather-clipping did not directly alter their nestling provisioning rates, reproductive success or survival. These were three important requirements for our study (figure 1, arrows 1, 2 and 6) that enabled us to assess how increased workload affects the physiological mechanisms that mediate the life-history trade-off between reproductive investment and survival. In response to the experimental treatment, feather-clipped males upregulated both Cort and GPx, and lost 3% of their body mass (similar results were obtained for body condition). Furthermore, the increase in GPx in feather-clipped males was positively related to their nest provisioning rates, while individuals that upregulated GPx suffered lower survival rates across treatment groups. We could not detect similar physiological changes in control males and, in both groups, individual changes in Cort concentrations did not explain male provisioning rate or overwinter survival. These results indicate a role of the redox system, in particular of GPx concentrations, in the life-history trade-off between reproductive investment and survival. While they cannot discount an involvement of baseline Cort, the relationships observed in feather-clipped males between body condition, parental care and Cort suggest that its effect may be indirect, perhaps through a link with body condition.

As predicted, baseline Cort concentrations increased in feather-clipped male great tits. Our experimental manipulation was successful, as Cort concentrations of FC-males at the end of our experiment were within the range of baseline levels observed for this species [26]. However, we found no association between changes in Cort and nest provisioning rate of males, our measure for post-hatching reproductive investment. This finding suggests that baseline Cort does not mediate investment in current reproduction. We cannot exclude an indirect relationship between Cort and nest provisioning rates via Cort's link with body condition, because FC-males that lost more body mass (standardized for body size) provisioned their offspring less often, but showed higher levels of Cort (figure 2).

An indirect involvement of Cort in regulating reproductive investment via body condition may in fact explain some inconsistencies in the current literature. While some studies show that absolute levels, as well as plastic changes, of baseline Cort are positively associated with the degree of parental care [26,27,36,71,72], other investigations disagree. For example, two recent correlative studies showed a negative association between chick feeding rates and baseline Cort concentrations [30,73]. Thus, the relationship between Cort and reproductive investment (or success) appears to vary across species and/or environmental context. This is not surprising, because if baseline Cort is intrinsically related to the energetic state of an individual [16], variation in body condition or environmental settings during the breeding season are expected to shape the link between this hormone and fitness traits [74]. Indeed, a recent three-year study in blue tits (*Cyanistes caeruleus*) demonstrated that the relationship between circulating levels of Cort during the parental phase and number of fledglings varied among years with contrasting environmental conditions [75]. Likewise, a multi-year study in parental tree swallow females showed that Cort increased after feather-clipping in females that also decreased body mass [76]. Together with our present data, these studies highlight that only by incorporating measures of individual state (such as body condition) and environmental context into our analyses can we achieve a full understanding of the role of Cort in the regulation of life-history

trade-offs (see also [74]). The present study focused on baseline Cort, but other Cort traits like stress-induced levels (see Introduction), the strength of the negative feedback to normalize stress-induced concentrations production and the maximal capacity to secrete Cort may also all play a role in regulating life-history trade-offs [23].

Our experimental treatment did not reduce male nest visits, demonstrating that we provided a metabolic challenge during the reproductive season that allowed males to maintain their initial degree of parental care [62]. This finding also demonstrates that male great tits can cope with a deterioration in conditions during parental care rather well, probably aided by the observed changes in physiology. It is plausible that the mass loss in FC-males, which has been observed in other species subjected to a similar treatment [57,59], helped the birds to decrease wing load and save energy [59]. Strikingly, the mass loss recorded in our study was similar to that shown by female starlings (3%), irrespective of the severity of their experimental handicap [59]. However, mass loss did not attenuate oxidative costs associated with feather-clipping as we did not find any relationship between changes in mass and GPx concentrations.

We did not identify a link between Cort and survival in the present study. Even though our sample size was limited, the finding that survival rate was predicted by GPx changes demonstrates that we had sufficient power to detect links between physiological variables and this fitness-relevant trait. The lack of an association between Cort and survival casts further doubt on the idea that baseline Cort mediates reproductive investment–survival trade-offs, at least in this species.

However, as suggested by the oxidative stress life-history hypothesis [8,38,40], our study did identify an involvement of the redox system in the reproductive investment–survival trade-off. Feather-clipped males that invested more in parental provisioning also upregulated GPx more strongly, and an increase in GPx was associated with a lower survival rate across treatment groups (figure 3). This pattern, however, did not emerge in a statistical model in which we pooled the two groups and included the calculated changes in the physiological variables between the nestling days 7 and 12. This discrepancy in results could be due to the fact that only FC-males, but not C-males, showed changes in GPx and body condition, which probably masked any association with a labile trait such as nest provisioning. For this reason, we drew our conclusions from the repeated measure model, which better represents the real-time variation in the physiological and behavioural traits of individuals in our experiment. The enzyme GPx is directly involved in detoxifying intra-cellular hydrogen peroxides by oxidizing glutathione [46], and its increase is generally associated with an increase in metabolic rate [77]. For example, in model species ranging from rats to humans GPx can be upregulated by 20–177% during low–medium endurance exercise [78]. Likewise, GPx was also elevated in female great tits caring for experimentally enlarged broods [79], in migratory European robins (*Erithacus rubecula*) during nocturnal endurance flight [80], and in short-tailed field voles (*Microtus agrestius*) thermoregulating at low ambient temperatures [77]. However, high workload can also result in decreased levels of GPx, as observed in zebra finches raising experimentally increased brood sizes [50]. Indeed, GPx synthesis is expected to be inhibited when the oxidative challenge is too high to be buffered, because oxidative stress can damage the genes responsible for encoding GPx expression [81].

The link between metabolic rate and GPx reviewed above could be the cause of the observed GPx upregulation in our feather-clipped male great tits (figure 3). Feather-clipping increases the costs of flight [33,57,63–65], and because feather-clipped males maintained provisioning rates at levels similar to those of control males (table 1), they probably had to elevate metabolic rate. The GPx increase apparently buffered any workload-induced production of ROMs, as plasma ROM concentrations did not change following feather-clipping. However, we observed a positive association between GPx and ROMs, suggesting that individuals that increased GPx more also experienced a greater oxidative challenge. Moreover, individual males that upregulated GPx had decreased chances to survive to the subsequent year. Mounting an antioxidant defence can be a costly process in terms of allocated resources, especially when it involves the re-establishment of original levels of reduced gluthatione (which are oxidized by GPx to buffer the production of hydrogen peroxides [46]). While this is the first study to report a survival cost of increases in GPx concentrations, breeding Seychelles warblers (*Acrocephalus sechellensis*) that upregulated total non-enzymatic capacity (OXY) during reproduction also had a lower probability to survive to the next year [82].

The negative association between GPx concentrations and survival rate may represent a delayed cost, induced in two potential ways: via cumulative damage caused by the initiation of a costly process involving this enzyme [46,54] or, alternatively, by a cell-signalling role of ROS [41,54,83,84]. Studies on intra-cellular signalling of ROS, especially of peroxide molecules, show that they are able to regulate several processes related to cell growth [84]. We did not find an increase in plasma ROMs (i.e. extracellular organic hydrogen peroxides), but in our study red blood cell GPx levels were positively related to plasma ROM concentrations. We cannot exclude the possibility that ROS concentrations increased inside the cells of diverse tissues. Trade-offs could be mediated by just small alterations of the redox system, and low ROS levels can activate signalling pathways that regulate complex biological processes [85]. Furthermore, not only ROS but also antioxidants can have signalling effects [86]. For example, besides its role as antioxidant, GPx can block the biosynthesis of prostaglandins, lipids with extensive vital roles including the regulation of the vascular system, cell growth and inflammatory processes, or affect apoptosis [86]. Thus, similar to hormones, specific components of the redox system could function as active mediators of life-history trade-offs.

5. Conclusion

Our results are in line with general predictions from life-history theory but are the first experimental data that indicate a role of the redox system in the reproduction–survival trade-off. The current study also highlights the importance of experimental approaches and individual-level analyses in investigations of trade-offs. Furthermore, our findings emphasize the need for further theoretical work. Thus far, the vast majority of hypotheses that have considered the physiological processes underlying life-history trade-offs propose associations between absolute values of physiological traits measured at a single point in time with those of life-history traits also measured once in an individual. However, the physiological system is crucial for responding to an array of external and internal

challenges, and therefore analysing individual changes (or reaction norms) are perhaps as important as absolute values, or even more important [23,31,87–91]. The field is now ripe for the development of quantitative predictions for the links between changes in physiology and life-history decisions of individuals.

Ethics. All experimental procedures were conducted according to the legal requirements of Germany and were approved by the governmental authorities of Oberbayern, Germany.

Data accessibility. This article has no additional data.

Authors' contributions. M.H. and S.C. conceived and designed the study; S.C. carried out the fieldwork, laboratory work and statistical analysis; M.H. participated in statistical analysis; S.C. wrote a first version of the manuscript; M.H. contributed in drafting its final version. Authors gave final approval for publication.

Competing interests. We declare we have no competing interests.

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