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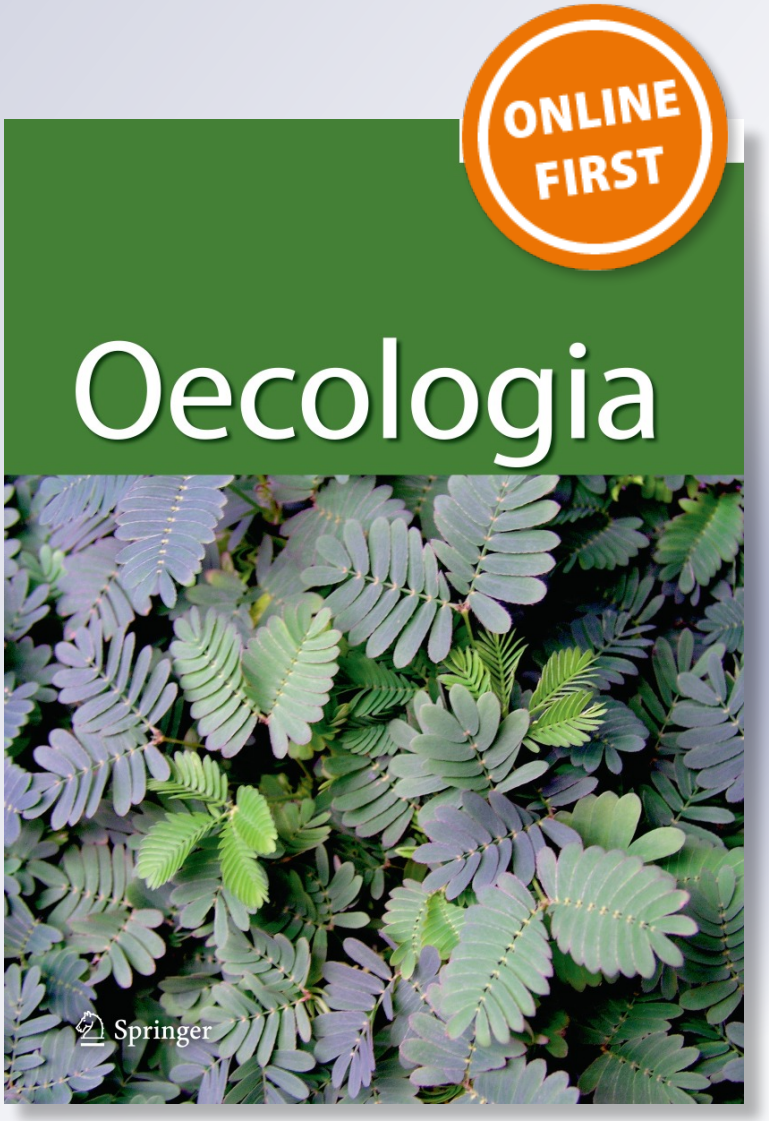

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PHYSIOLOGICAL ECOLOGY - ORIGINAL RESEARCH

Elevation impacts the balance between growth and oxidative stress in coal tits

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Abstract The short favorable period of time available for the growth in seasonal environments could constrain the resources allocation between growth and other life-history traits, and the short-term fitness benefits of increased growth rate may prevail over other functions. Accelerated growth rates have been associated with long-term deleterious consequences (e.g., decreased lifespan), and recently oxidative stress (the imbalance between pro-oxidants generation and antioxidant defenses) has been suggested as a mediator of these effects. Here, we examined the impact of elevation on growth rate and self-maintenance parameters (resting metabolism, oxidative damage, and antioxidant defenses) of coal tit chicks (*Periparus ater*). We predicted that the shorter favorable season at the higher-elevation site could lead to a reallocation of resources towards growth at the expense of self-maintenance processes. We found that chicks at high elevation grew significantly faster in terms of body mass and body size. Chicks from the high-elevation site presented higher resting metabolism, higher oxidative damage level, but similar antioxidant defenses, compared to low-elevation chicks. Interestingly, the chicks exhibiting the better antioxidant defenses at 7 days were also those with the highest resting metabolic rate, and the chicks that grew at the faster rate within the high-elevation site were those with the highest levels of oxidative damage on DNA. Our study supports the idea that increasing elevation leads to a higher growth rate in coal tit chicks, possibly in response to a shorter favorable season. In accordance with life-history theory, a bigger investment in growth was done at the expense of body maintenance, at least in terms of oxidative stress.

Keywords Elevation · Altitude · Oxidative stress · Metabolism · Life history trade-off · Ageing

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Introduction

Early development is a key period conditioning an organism's life history for at least two types of reasons: (i) adult size is often related to fitness (Reeve et al. 2000; Richner 1989) and (ii) organisms are more vulnerable during growth (Arendt 1997; Calow 1982), developmental time being inversely related to future fitness (Roff 1980). Consequently, growing as fast as possible should be a general rule, when in fact most organisms are capable of growth

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rates superior to those observed under "normal" conditions (Calow 1982). Ecological and physiological costs of rapid growth rates (reviewed in Metcalfe and Monaghan 2001) effectively suggest that resource allocation towards growth could be done at the expense of other life-history components. Then, an individual's growth rate is usually optimal rather than maximal (even with ad libitum access to a high-quality food), and growth rates are flexible and regulated (Dmitriew 2011; Metcalfe and Monaghan 2001). In particular, the resource part invested in growth seems to be balanced against allocation toward longevity (Lee et al. 2013), probably through a decrease investment in body self-maintenance processes.

In recent years, more attention has been given to the specific nature of the mechanisms that underlie the global idea of trade-offs (Zera and Harshman 2001), and oxidative stress frequently appears to be involved (Dowling and Simmons 2009; Metcalfe and Alonso Alvarez 2010; Monaghan et al. 2009). Oxidative stress is defined as an unbalanced situation between reactive oxygen species (ROS) production—which are mainly produced by the mitochondria during normal energy processing—and the capacity of the various anti-oxidant systems to deal with them (Halliwell and Gutteridge 2007). Such an unbalanced situation will cause oxidative damage to various biomolecules, and the accumulation of damage with time is thought to be one potential cause of ageing (Finkel and Holbrook 2000). In this context, accelerated growth was proposed to conduct to oxidative stress (as predicted by Mangel and Munch 2005), either by increasing ROS production (Rosa et al. 2008) or decreasing resistance to an oxidative challenge (Alonso-Alvarez et al. 2007; Kim et al. 2011), both leading ultimately to elevated levels of oxidative damage (Geiger et al. 2012b; Nussey et al. 2009). Interestingly, a higher growth rate was also associated with increased metabolism (Careau et al. 2013), either measured by resting metabolic rate (RMR) or daily energy expenditure. The energy cost of fast growth was often used as a putative proximate factor to explain its oxidative stress consequences (i.e., higher growth rate leading to higher O_2 consumption, which could lead to higher ROS production), but relationships between ROS production/oxidative stress and metabolism are still under debate (e.g., see Barja 2007; Fletcher et al. 2013; Stier et al. 2014a).

Environmental conditions are important constraints on life-history trait evolution (Naef-Daenzer et al. 2012; Stearns 1992). Accordingly, it was suggested that in seasonal environments, the duration of the favorable period of time available for the growth might affect its fitness consequences (Abrams et al. 1996; Gotthard 2008). Elevation (see McVicar and Körner 2012 for distinction between altitude and elevation) is known to impact phenology, with shorter favorable season at higher elevation (Dittmar and Elling 2005; Pellerin et al. 2012). Elevation has been suggested to be a major ecological constraint for birds, partly due to the restrained time-window of food abundance that coincides with breeding time at high-elevation areas (Lack 1968). Therefore, elevation could also be a constraint for the growth period, with potential consequences in terms of resource allocation in other life-history components. However, there has been no data examining the impact of such environmental conditions on the trade-off between growth and self-maintenance processes (i.e., processes allowing an organism's survival and preventing premature ageing).

The timing of reproduction for tits (*Parus* sp.) is crucial since breeders have to match the quick seasonal changes in food availability (known as the "caterpillar peak"; Betts 1955; Naef Daenzer and Keller 1999). Indeed, they have to supply enough food to their chicks to ensure proper growth and body condition, which are important factors determining post-fledging survival (Naef-Daenzer et al. 2001). Coal tit chicks have a limited period of growth within the nest before fledging (around 17–20 days, pers. obs.) and their survival seems markedly affected by fledging date (Naef-Daenzer et al. 2001). Therefore, one hypothesis is that coal tit chicks could grow faster at higher elevation, in order to take full advantage of the shorter favorable season, and fledge as soon as possible since an earlier fledging date is associated with higher survival (Naef-Daenzer et al. 2001). However, such an accelerated growth could have a negative impact on body self-maintenance (Geiger et al. 2012b).

To test this hypothesis, we followed environmental conditions (i.e., air temperature) and the breeding activity of coal tits along an elevational gradient (1,300 and 1,900 m a.s.l.) in 2011. Body size and body mass growth of chicks were recorded and self-maintenance parameters (resting metabolism, oxidative damage, and antioxidant defenses) were evaluated during the growth period. We aimed at both testing the impact of elevation on our different parameters and also at discriminating potential predictors of oxidative stress (i.e., growth rate vs. metabolic rate) in a free-living species.

Materials and methods

Field work and bird sampling

We monitored the breeding activity and chicks' growth rates of coal tits (*Periparus ater*) in the French Alps (Vallorcine, France) during the 2011 season (April–June). Artificial nest boxes (Schwegler 1B, Germany, $n = 34$ per site) were placed following two transects [see Electronic Supplementary Material (ESM) fig. S1], the first around 1,300 m (mean ± SE 1,329.7 ± 2.1 m a.s.l.) of elevation and the second around 1,900 m (1,907.3 ± 2.7 m a.s.l.). During the early season, nests were checked regularly

before hatching to determine hatching date, clutch size, clutch mass, and estimated incubation time (assuming that incubation started just after clutch completion). In 2011, eight nest boxes were occupied at the lower site, and six at the higher site.

We selected five chicks per nest to be sure of including only chicks being born the first day of hatching, since we have data on the great tit suggesting that hatching asynchrony affects growth and self-maintenance parameters (Stier et al., unpublished work). We measured body mass (BM) and body size (BS: wing length, tarsometatarsus length, head size) every 2 days following hatching (hatching = day 1) using an electronic balance (0.1 g precision) and a digital caliper rule (0.1 mm precision). We evaluated the repeatability of our BS measurements by measuring a subsample ($n = 8$) of chicks four times at three ages (1–2 days, 7–8 days, and 17–18 days). The mean ± SE coefficients of variation (CV) for the head size were 2.09 ± 0.26, 1.38 ± 0.21, and 0.75 ± 0.07 %, for the three classes of age respectively. Similarly, the CVs for the tarsometatarsus length were of 3.85 ± 0.42, 1.78 ± 0.29, and 1.12 ± 0.14 %; and of 3.89 ± 0.60, 2.63 ± 0.53 and 1.18 ± 0.11 % for the wing length. Chicks were followed until approximately 16 days, which is close to fledging (≈17–20 days, pers. obs.). We avoided returning to the nest after day 17–18, in order to avoid compromising fledging success. One small blood sample (≈30 µl) was taken from the brachial vein with a heparinized glass capillary at day 7 (mean ± SE 6.94 ± 0.12 days) and day 16 (15.75 ± 0.08 days). Blood was kept on ice until the plasma was separated from blood cells by centrifugation (10 min, 1,500×g) and samples were subsequently stored at −80 °C before analysis. We paid attention to have a similar time duration before centrifugation for both sites. Only one 1,300-m "monitored" chick died during the study, which gave a final sample size of 69 chicks (39 at 1,300 m and 30 at 1,900 m). Sex determination was also done on DNA extracted from red blood cells following an adapted method from Griffiths et al. (1998)

Temperature monitoring

To ensure that our two elevation sites actually differed in terms of "favorable period", we choose to evaluate the impact of elevation on a key environmental variable, namely the air temperature. One meteorological station (developed for the "Phénoclim project" of the CREA) was therefore implanted at each site (1,300 and 1,900 m). Those stations recorded air temperature at standard height (2 m) every 15 min during the entire year of 2011, and transferred data via a GSM network (for full description, see Pellerin et al. 2012). We used daily mean temperature for further statistical analysis.

Growth analysis

As an estimate of the overall body size (BS), we used the first principal axis (PC1) resulting from a principal component analysis (PCA) of the measurements of wing, tarsometatarsus and head, which explained 96.3 % of the total variance of BS (Freeman and Jackson 1990). Therefore body size is expressed in arbitrary units (AU).

BM and BS growth curves were fitted with the following logistic equation: $Y(x) = \frac{A}{[1+\exp(-K\times x-B)]}$, which was the best-fitting model based on R^2. $Y(x)$ represents the size or mass of a chick at age x (in AU or g), A is the asymptotic mass or size (i.e., mass or size at fledging), K is the growth rate constant (an increase in K value implies an increase in the rate at which mass or size increases from initial value to asymptotic value) and B is a constant determining the initial mass. Growth fitting was performed with the nonlinear regression procedure in SPSS (SPSS 20.0, 1989–2011, SPSS Inc., USA) for each chick. The equation obtained for each chick allows reconstructing the entire growth curve, estimating mass and size at each time point. We checked that growth fitting was not significantly different between our two sites for BM (mean R^2 ± SE: 1,300 m = 0.995 ± 0.005, 1,900 m = 0.992 ± 0.005; linear mixed model: $df = 1$, $F = 1.02$, $p = 0.33$) and BS (mean R^2 ± SE: 1,300 m = 0.996 ± 0.001, 1,900 m = 0.995 ± 0.001; linear mixed model: $df = 1$, $F = 2.53$, $p = 0.12$).

Resting metabolism

Resting oxygen consumption (VO_2 expressed in ml O_2 consumed per minute) was determined in the dark to reduce the chicks' movements and stress. We recorded O_2 consumption during 30 min with a field open-circuit respirometry system (FOXBOX, Sable Systems, USA), at day 11 or 12 for 46 chicks (1,300 m: $n = 23$ and 1,900 m: $n = 23$). We choose to define the resting VO_2 as the lowest consecutive 2 min within the 30-min recording, and we included mean value of body mass (measured both before and after the respirometry measurement) as a covariate in the statistical model to control for body mass effect on metabolism. Ambient air temperature was also simultaneously recorded to control for a potential temperature effect.

DNA oxidative damage

Oxidative damage was determined on DNA extracted (Nucleospin Blood QuickPure, Macherey–Nagel, Düren, Germany) from red blood cells (at 7 days and 16 days) using the 8-hydroxy-2-deoxy Guanosine (8-OHdG) EIA kit (StressMarq Biosciences Inc., Victoria, BC, Canada), after

enzymatic digestion as described by Quinlivan and Gregory (2008). 8-OHdG is produced by the oxidative damage of DNA by reactive oxygen species (ROS) and increased levels of this marker have been associated with the ageing process. Blood DNA damage is expressed as pg/ml and intra-individual variation based on duplicates was low (CV = 1.95 ± 0.26 %) as well as inter-plate variation based on a standard sample repeated over plates (CV = 5.13 %).

Antioxidant defenses

Antioxidant capacity was measured using the OXY-Adsorbent test (5 µl of 1:100 diluted plasma; Diacron International, s.r.l, Italy) following the manufacturer's protocol (for detailed description of this test and its use in evolutionary ecology studies, see Stier et al. 2012). The OXY adsorbent test allows quantifying the ability of the plasma antioxidant components to buffer a massive oxidation through hypochlorous acid. Antioxidant capacity is expressed as mM of HClO neutralized and intra-individual variation based on duplicates was low (respectively CV = 6.17 ± 0.54 %) as well as inter-plate variation based on a standard sample repeated over plates (CV = 10.03 %).

Statistics

We tested temperature differences among sites by the use of a general additive model (GAM) because it allows capturing the shape of a relationship between y and x without prejudging the issue by choosing a particular parametric form (Wood 2006). Daily mean temperature was the response variable against day of year as smooth term and site (1,300 vs. 1,900 m) as fixed factor. The GAM model was fitted using the software R (version 2.15; R Development Core Team 2010).

The elevation effect on parameters measured only one time [asymptotic BM and BS (A), BM and BS growth rate (K), and resting O_2 consumption] was done using LMMs, with the nest as random effect (since the five chicks within one nest are not independent statistical units) and elevation as the fixed effect. Sex and other covariates (e.g., mass or size at hatching) were also included in starting statistical models.

We tested the elevation effect on parameters measured two times (i.e., DNA damage and antioxidant defenses) by the use of repeated linear mixed models, with chick age (7 or 16 days) as the repeated effect, the nest as a random effect and the elevation as a fixed effect. Growth rate and RMR (i.e., the residuals from the regression between VO_2 and body mass) were included as covariates, and all interactions were included in initial models.

We choose to present the most parsimonious final models, where parameters presenting $p > 0.05$ were sequentially removed (starting by the interactions between parameters). Linear mixed models were fitted with a normal error distribution (SPSS 20.0), and data were tested for normality and homoscedasticity. All tests were two-tailed tests and p values ≤ 0.05 were considered significant. Means are quoted ± SE.

Results

Annual pattern of air temperature

Both sites followed the same temperature pattern all along the year (GAM: $\beta = 7.82 \pm 0.18$, $df = 8.66$, $p < 0.001$, adjusted $R^2 = 0.71$, see ESM fig. S2). Nevertheless, the 1,300-m site was on average 2.33 ± 0.26 °C warmer all along the year than the 1,900-m site (GAM: $p < 0.001$, ESM fig. S2).

Nests parameters

As illustrated in ESM table S3, few nest parameters were significantly different among sites. Indeed, clutch size, clutch mass, mean egg mass, brood size at hatching and fledging, hatching success and fledging success were not statistically different between the 1,300- and the 1,900-m sites. However, hatching date was significantly earlier for the lowest elevation site (Mann–Whitney test: $Z = -2.52$, $p = 0.012$), with a mean hatching date being approximately 1 week earlier than for the 1,900-m site. Moreover, estimated incubation time appeared to be significantly longer for the 1,900-m site (≈+ 1.6 days, Mann–Whitney test: $Z = -2.33$, $p = 0.020$).

Growth parameters

Estimated mean growth curves for both elevations are presented in Fig. 1a for BM and in Fig. 1b for BS. Chicks from the two sites did not significantly differ at hatching (day 1) in terms of BM (mean ± SE: 1,300 m = 0.89 ± 0.22 vs. 1,900 m = 1.04 ± 0.22 g; LMM for elevation effect: $df = 1$, $F = 1.68$, $p = 0.22$) and BS (1,300 m = 0.25 ± 0.07 vs. 1,900 m = 0.28 ± 0.07 AU; LMM for elevation effect: $df = 1$, $F = 0.64$, $p = 0.44$). Similarly, we were unable to find significant differences at fledging (asymptotic value: A), neither for BM (1,300 m = 10.11 ± 0.47 g, 1,900 m = 9.85 ± 0.48 g; LMM for elevation effect: $df = 1$, $F = 0.97$, $p = 0.34$) nor for BS (1,300 m = 3.10 ± 0.11 AU, 1,900 m = 3.10 ± 0.11 AU; LMM for elevation effect: $df = 1$, $F = 0.007$, $p = 0.93$).

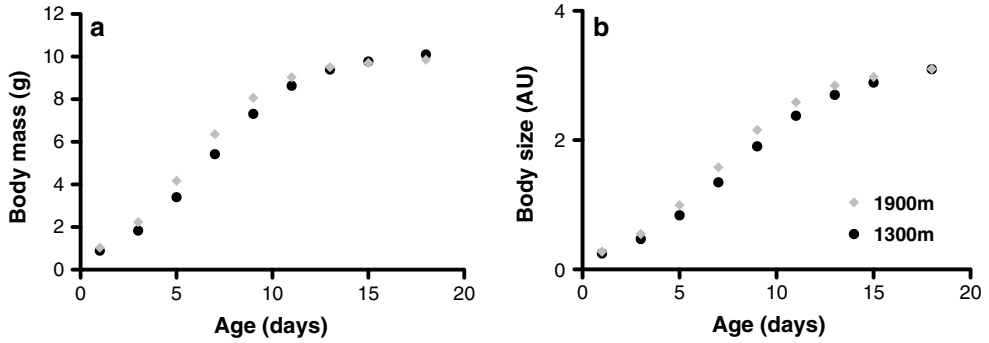

Fig. 1 Mean growth curves ± SE for **a** body mass (BM) and **b** body size (BS) of coal tit (*Periparus ater*) chicks. *Black dots* and *grey diamonds* represent, respectively, the lower (1,300 m) and the higher elevation (1,900 m). Note here that mass and size are estimated following a logistic equation (see Methods for details). BS is defined as the first axis of a PCA performed on three morphologic measurements (head, tarsometatarsus, and wing), and is therefore expressed in Arbitrary Units (AU))

Table 1 Results of mixed models testing differences between 1,300 and 1,900 m in terms of growth rate *K* for (a) body mass (BM) and (b) body size (BS)

See Methods for detailed description of growth constant determination and for body size estimation. Estimates for fixed factors are given for the following levels: elevation = 1,300 m

Significant effects ($p \leq 0.05$) are highlighted in bold

	Estimate	SE	*F*	*p* value
(a) BM growth rate (K)				
Random effect				
Nest	0.0013	0.0006		
Fixed effects and covariates				
Constant	0.46	0.04	135.86	**<0.001**
Elevation	−0.05	0.02	4.72	**0.050**
Mass at hatching				NS
Sex				NS
(b) BS growth rate (K)				
Random effect				
Nest	0.0002	<0.0001		
Fixed effects and covariates				
Constant	0.49	0.02	582.78	**<0.001**
Elevation	−0.05	0.01	25.18	**<0.001**
Size at hatching	−0.30	0.04	63.67	**<0.001**
Sex				NS

Table 2 Results of a linear mixed model testing differences between 1,300 and 1,900 m in terms of resting O_2 consumption

Estimates for fixed factors are given for the following levels: elevation = 1,300 m and age = 11 days

Significant effects ($p \leq 0.05$) are highlighted in bold

VO_2 (ml/min)	Estimate	SE	*F*	*p* value
Random effect				
Nest	0.005	0.004		
Fixed effects and covariates				
Constant	−0.72	0.48	20.21	**<0.001**
Elevation	−1.36	0.45	8.96	**0.005**
Sex				NS
Age	−0.24	0.06	17.29	**0.004**
Mass	0.10	0.04	44.20	**<0.001**
Temperature	−0.02	0.01	6.88	**0.017**
BM growth rate	1.19	0.56	4.57	**0.042**
Elevation × mass	0.14	0.05	7.57	**0.009**

Table 3 Results of mixed models testing differences between 1,300 and 1,900 m in terms of (a) DNA damage in the blood and (b) plasma antioxidant defenses, at 7 and 16 days

	Estimate	SE	*F*	*p* value
(a) DNA damage (8-OHdG)				
Random effect				
Nest	3,193.03	1,712.35		
Repeated effect				
Age	6,317.55	1,166.85		
Fixed effects and covariates				
Constant	370.17	205.84	2.97	0.093
Elevation	316.40	255.33	5.12	**0.028**
Age				NS
Sex				NS
BS growth rate	595.36	419.44	4.25	**0.044**
RMR				NS
Elevation × BS growth rate	−914.89	567.91	6.43	**0.014**
(b) Antioxidant capacity (OXY)				
Random effect				
Nest	186.76	123.47		
Repeated effect				
Age	339.61	92.63		
Fixed effects and covariates				
Constant	181.54	15.04	139.01	**<0.001**
Elevation				NS
Age	−21.26	6.94	6.06	**0.017**
Sex	−11.36	6.16	0.051	0.822
BS growth rate				NS
RMR	−29.43	21.00	0.005	0.942
Age × RMR	56.16	27.09	4.30	**0.042**
Age × sex	20.49	8.96	5.23	0.025

Estimates for fixed factors are given for the following levels: elevation = 1,300 m, age = 7 days and sex = female

Significant effects ($p \leq 0.05$) are highlighted in bold

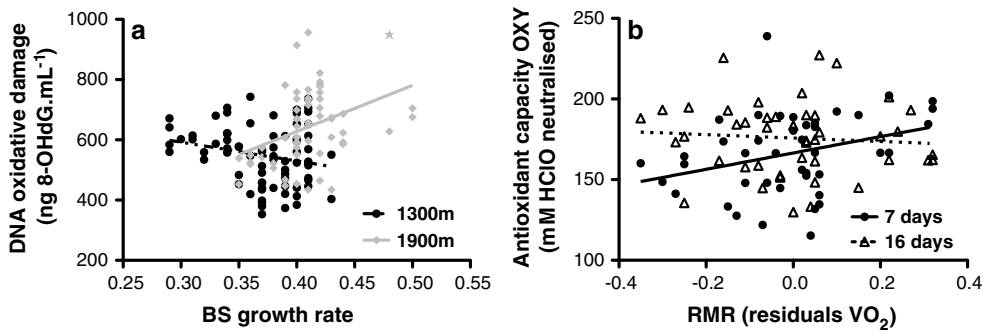

Fig. 2 Relationships between **a** DNA oxidative damage and body size (BS) growth rate according to elevation, and **b** plasma antioxidant capacity and RMR according to chick age. In both cases, the statistical interaction between either BS growth rate and elevation (**a**), or RMR and chick age (**b**) was significant (see text for details). The individual in the right top of Fig. 2a (*grey star*) might appear as an outlier since the removal of this point induces the loss of significance of the statistical interaction ($p = 0.091$)

The growth rates (K) in terms of BM (1,300 m = 0.419 ± 0.006 vs. 1,900 m = 0.465 ± 0.009; Table 1a) and BS (1,300 m = 0.370 ± 0.006 vs. 1,900 m = 0.409 ± 0.009; Table 1b) were significantly higher for chicks at 1,900 m than at 1,300 m (Fig. 1). In addition, BS growth rate was negatively affected by the size at hatching (Table 1b).

Resting metabolism

Elevation significantly affected the resting O_2 consumption, with chicks coming from the higher elevation being those with the higher resting VO_2 (mean ± SE 1,300 m = 0.83 ± 0.08 vs. 1,900 m = 0.97 ± 0.09 ml

O_2/min; Table 2). Resting O_2 consumption also increased according to body mass and body mass growth rate, and decreased with increasing ambient temperature (Table 2). Finally, resting metabolism was also affected by chick age (older chicks consuming more O_2), and by the interaction between body mass and elevation.

Oxidative stress parameters

DNA damage measured on blood cells DNA was significantly higher for chicks growing at the higher elevation (mean ± SE 1,300 m = 549.8 ± 68.0 vs. 1,900 m = 643.0 ± 69.3 pg/ml, Table 3a), but irrespectively of chick age or sex. DNA damage levels were not affected by chick RMR, but were significantly influenced by the BS growth rate and the interaction between elevation and BS growth rate (Table 3a). The latter interaction was explained by the positive correlation existing between DNA damage and BS growth rate for the higher elevation only (Fig. 2a).

Plasma antioxidant capacity was not significantly affected by the elevation (mean ± SE 1,300 m = 168.9 ± 15.3 vs. 1,900 m = 177.8 ± 15.6 mM HClO neutralized, Table 3b), or the sex, but was significantly affected by chick age and by the interaction between sex and age. Indeed, as revealed by a separate analysis for each sex, antioxidant defenses increased significantly with age but only for males (males: 7 days = 163.2 ± 13.6 vs. 16 days = 189.0 ± 13.6 mM HClO neutralized, LMM for age effect: $df = 1$, $F = 25.46$, $p < 0.001$; females: 7 days = 166.2 ± 14.3 vs. 16 days = 172.5 ± 14.3 mM HClO neutralized, LMM for age effect: $df = 1$, $F = 1.76$, $p = 0.19$). Plasma antioxidant capacity was not significantly affected by the BS growth rate or the RMR of individuals, but was significantly influenced by the interaction between RMR and chick age (Table 3b). Accordingly, the latter interaction was explained by the positive correlation existing at 7 days old only between plasma antioxidant capacity and RMR (Fig. 2b).

Discussion

To sum up, our study shows that coal tit chicks grew faster at higher elevation. This higher allocation in growth was done at the expense of body maintenance, as stressed by the elevated levels of oxidative damage on DNA of chicks growing at the higher elevation site. Interestingly, growth rate appeared as a better predictor of oxidative damage than metabolic rate, potentially because of a compensatory response as suggested by the positive link existing between antioxidant defenses and RMR in our study.

Elevation, environmental conditions, and growth rates

As expected, even a moderate increase in elevation (≈600 m between our two sites) significantly modified air temperature in terms of absolute value (−2.33 ± 0.26 °C for the 1,900-m site compared to the lower site), but not in terms of annual pattern. Therefore, if we theoretically consider that a favorable period is defined by a number of days above a certain temperature threshold, the favorable season will automatically be shorter at our higher elevation. Concomitantly to this change in environmental conditions, chicks growing at the higher elevation site grew approximately 11 % faster, despite similar mass and size at hatching and just before fledging, and similar brood size. This suggests that chicks might have been able to fledge earlier, even if we cannot check this assumption because the exact fledging date was not recorded. Still, the optimal window for growth (and the optimal fledging date) is determined according to diverse factors depending on the species (food availability, predation, social interaction), and our data confirmed that under natural conditions, as the window shortens, growth rate might be increased (Bize et al. 2006; Geiger et al. 2012b). Then, at the higher elevation, chicks might be more prone to show a higher growth rate when conditions are good to reach the critical size required to ensure immediate survival within the nest (e.g., due to thermal constraint), growth rate being subsequently slowed down, maybe because of an earlier tissue maturation process (Ricklefs 1979). Alternatively, since chicks from the higher elevation are expected to face overall harsher environmental conditions, they might be able to exhibit higher growth rates under benign conditions. So, a multiannual monitoring is required to fully understand the relationships between elevation, environmental conditions, and growth.

As illustrated for body size by Tsuchiya et al. (2012), the effect of elevation on growth rate could be either genetically driven, or reflect phenotypic plasticity. Considering the proximity between the two sites in our study (see ESM fig. S1), a genetic isolation/difference between the two subpopulations seems unlikely to occur. Therefore, we rather suggest that the higher growth rate observed for the high-elevation site is due to resources re-allocation (i.e., developmental flexibility), especially from self-maintenance processes toward growth (Dmitriew 2011). However, an increased growth rate may also be linked to increased parental feeding (i.e., parental phenotypic flexibility). For instance, it was suggested by a large comparative study that birds breeding in high-elevation habitat demonstrated increased parental effort (Badyaev and Ghalambor 2001). Following this idea, parents breeding in high elevation areas may pay an additional reproductive cost that will be informative to follow in the future.

In contrast to the higher post-natal growth rate measured in this study, estimated incubation time seems to be longer for the higher elevation site (as suggested by Badyaev and Ghalambor 2001), which could reflect a slower pre-natal development. This effect could be linked to ambient temperature differences, even if relationships between ambient temperature, incubation temperature, and incubation duration are far from being simple (Conway and Martin 2000). The accelerated post-natal growth at higher elevation could then also be viewed as a compensatory response to a slower embryonic growth rate, as it was shown for young rats which faced an intrauterine protein restriction (Tarry-Adkins et al. 2009). However, we have to keep in mind that the estimated incubation time might be longer at higher elevation only due to a delayed incubation, a phenomenon that has already been described in tits especially under cold conditions (Monrós et al. 1998; Naef-Daenzer et al. 2004).

Elevation and self-maintenance processes

Interestingly, chicks growing at the higher-elevation site presented a higher resting O_2 consumption. This higher metabolism could be explained by a higher metabolic activity to sustain fast growth (Careau et al. 2013) and we found partial support for this hypothesis, since we demonstrate a significant correlation between growth rate and resting O_2 consumption. However, since elevation effect remains significant even after accounting for differences in growth rate (Table 2), an additive effect of elevation per se seems conceivable (see Hayes 1989 for a similar effect on deer mice, but see also Weathers et al. 2002 for a contradictory result on adult bird). This elevation effect could be linked to higher thermogenic capacities developed by high-altitude chicks, since they have to face overall colder temperatures during the growth period (data not shown) than low-elevation chicks.

In any case, assigned high resting metabolism as a "good or bad thing" in terms of self-maintenance is difficult per se, since a high metabolic rate should theoretically induce high ROS production (Beckman and Ames 1998), but high metabolism could also decrease ROS production/oxidative stress if achieved through mitochondrial uncoupling (Salin et al. 2012; Stier et al. 2014a). In our particular study, we did not find a significant relationship between metabolic rate and oxidative damage (Table 3a), which could potentially be linked to a large implication of mitochondrial uncoupling in the resting metabolism of the chicks or to an adequate antioxidant response. We found partial support for the latter hypothesis, since we have shown that the chicks exhibiting the higher metabolic rate were also those exhibiting the higher antioxidant defenses during the peak of growth (i.e., 7 days, Fig. 2b). It has been previously proposed that an increase in antioxidant defenses is done in response to an increase in oxidative stress (Geiger et al. 2012a). We could then hypothesize that antioxidant defenses follow metabolic intensity, and therefore, that maximal antioxidant capacities may constrain individual metabolic rates, in our case during growth.

Nevertheless, we found that levels of oxidative damage on DNA were higher for chicks growing at the higher elevation at both 7 and 16 days, while antioxidant levels were not significantly affected by the elevation. This result suggests that higher oxidative stress occurs more probably because of higher ROS production rather than defect in antioxidant protection, which may be explained by a positive association between high energy expenditure and ROS production as suggested by (Fletcher et al. 2013). Elevated levels of oxidative damage might be linked to the accelerated growth observed at higher elevation, as suggested by experimental studies demonstrating a causal relationship between accelerated somatic growth and susceptibility to oxidative stress (Alonso-Alvarez et al. 2007; Tarry-Adkins et al. 2008). Accordingly, we demonstrated in this study a significant correlation between growth rate and oxidative damage, although it was only the case for the high-elevation site (Table 3a; Fig. 2a). This result could suggest that chicks growing at the lower elevation might stay above a deleterious threshold of growth rate (in terms of oxidative stress), whereas chicks growing at the higher elevation might exceed such a threshold. Such a mechanism could be consistent with a possible constraining role of ROS production for life-history traits evolution (Dowling and Simmons 2009), and could contribute to explain the weakness of the (nevertheless significant) relationships observed between growth rate and oxidative stress markers by some studies in natural conditions (Kim et al. 2011; Nussey et al. 2009). Still, it is important to note that factors other than growth rate per se are likely to affect oxidative stress levels early in life (e.g., environmental conditions, see Stier et al. 2014b). Still, the oxidative cost of growth appeared more linked to the rate of growth itself than to the metabolic activity of the chicks in the present study, but our limited sample size provide a really low statistical power to really exclude the impact of metabolic rate. In addition, we cannot completely exclude that oxidative stress occurs for other reasons related to elevation, such as O_2 availability (Jefferson et al. 2004), differences in food quality (Costantini 2008), or in thermogenic capacities.

Our sample size is relatively limited (2011, $n = 69$ chicks from 14 nests) despite the number of nestboxes available at each site ($n = 34$/site), which limit considerably the statistical power of our analyses (as mentioned above). However, the nestboxes were implanted in 2009 and the occupancy rate did not increase in 2012, thereby suggesting that the novelty of nestboxes was not necessarily the primary factor explaining the low occupancy. In

addition, one important point to keep in mind is that our study is restricted to short-term costs, and delayed costs of accelerated growth on fitness related-traits could also occur (see Criscuolo et al. 2008, 2011, for delayed costs of rapid growth rate in terms of resting metabolism and flight performances). Since oxidative stress could also accelerate telomere erosion (von Zglinicki 2002), chicks growing at higher elevation could also be more susceptible to a higher telomere erosion rate (see Zhong et al. 2011 for a link between elevation and telomere length in humans) with ultimate deleterious impacts on survival/lifespan (Bize et al. 2009; Heidinger et al. 2012).

Conclusions

The present study highlights the idea that environmental conditions are primary shapers of life-history trade-offs. Indeed, elevation appears as a key factor modulating on one side the growth pattern of coal tit chicks, and on the other side the variations of parameters related to the ageing process (i.e., metabolism and oxidative damage). Since oxidative stress has recently been shown to impair fitness-related traits, such as recruitment (Noguera et al. 2012) or reproductive performances (Bize et al. 2008; Stier et al. 2012), our results highlight potential fitness costs of accelerated growth pattern in the wild. If experimental studies where elevation will be manipulated (or with cross-fostering between low- and high-elevation sites) are now required to determine causal relationships between elevation, growth rate, and oxidative stress/ageing rate, our study encourages future research to integrate environmental conditions to better understand the physiological mechanisms underlying life-history trade-offs.

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