Received: 15 September 2017 | Accepted: 10 February 2018

DOI: 10.1111/1365-2656.12822

RESEARCH ARTICLE

Journal of Animal Ecology | BRITISH ECOLOGICAL SOCIETY

Effects of an early-life paraquat exposure on adult resistance to oxidative stress, plumage colour and sperm performance in a wild bird

Sylvain Losdat[1,2] | Jonathan D. Blount[3] | Viviana Marri[2] | Lea Maronde[2] | Heinz Richner[2†] | Fabrice Helfenstein[1,2†]

[1]Institute of Biology, University of Neuchâtel, Neuchâtel, Switzerland

[2]Institute of Ecology and Evolution, University of Bern, Bern, Switzerland

[3]Centre for Ecology & Conservation, College of Life & Environmental Sciences, University of Exeter, Penryn, UK

Correspondence
Sylvain Losdat
Email: s.losdat@gmail.com

Funding information
Schweizerischer Nationalfonds zur Förderung der Wissenschaftlichen Forschung

Handling Editor: Ben Dantzer

Abstract

1. Early-life stressful conditions can shape individual phenotypes and ultimately influence fitness. Oxidative stress is a pervasive threat that affects many fitness-related traits and can modulate life-history trade-offs. Yet, the extent to which exposure to oxidative stress during early life can have long-lasting effects on key fitness-related traits remains to be elucidated, particularly in natural populations of vertebrates.
2. Using a wild population of great tits *Parus major*, we experimentally dosed 11-day-old birds with paraquat, a pro-oxidant molecule, aiming at increasing oxidative stress. One year later, we recaptured 39 of them as adult recruiting breeders and quantified effects of the paraquat exposure on their resistance to oxidative stress, carotenoid-based plumage coloration and male sperm performance.
3. Despite the absence of a short-term effect of paraquat on oxidative stress measured two days later, the pre-fledging exposure to paraquat induced a reduction in individual oxidative damage measured at adulthood. Paraquat-dosed individuals also had brighter plumage, but no effect was observed on male sperm performance.
4. For the first time in a natural population of vertebrates, we experimentally show that an early-life acute exposure to a pro-oxidant has long-lasting effects on individual resistance to oxidative stress at adulthood. Our results are in line with the environmental matching and the hormesis hypotheses but may also reflect selective disappearance of individuals with lower resistance to oxidative stress.

KEYWORDS
early environment, long-term effects, oxidative damage, plumage colour, sperm performance

1 | INTRODUCTION

Environmental conditions experienced during early stages of life can shape individual phenotypes and influence Darwinian fitness (Burton & Metcalfe, 2014; Monaghan, 2008). In fact, early-life stressful environments have been shown to be deleterious to several life-history traits, ultimately reducing individual fitness (Haussmann, Longenecker, Marchetto, Juliano, & Bowden, 2012; Marasco, Spencer, Robinson, Herzyk, & Costantini, 2013; Monaghan, 2008). Conversely, recent empirical studies have shown that early-life stressful conditions can be adaptive by increasing survival and reproduction later in life (Garratt et al., 2015; Rubenstein et al., 2016;

†Shared last authorship

Wilkin & Sheldon, 2009), potentially because individuals whose early environment matches that experienced at adulthood might achieve greater fitness (Bateson, Gluckman, & Hanson, 2014; Monaghan, 2008). However, despite major implications for individual fitness, long-lasting effects of early-life stressful environments on key physiological traits have rarely been quantified in natural populations.

Oxidative stress (OS), the imbalance between the production of reactive species and the antioxidant defences in favour of the former (Sies, 1991), arises through the deleterious effects of reactive species to lipids, proteins and DNA, ultimately impacting survival (Losdat et al., 2013; Noguera, Kim, & Velando, 2011) and reproduction (Agarwal, Aponte-Mellado, Premkumar, Shaman, & Gupta, 2012; Bize, Devevey, Monaghan, Doligez, & Christe, 2008; Stier, Reichert, Massemin, Bize, & Criscuolo, 2012). An individual's ability to resist OS is therefore considered a key physiological trait potentially mediating life-history trade-offs (Costantini, 2014; Metcalfe & Alonso-Alvarez, 2010; Monaghan, Metcalfe, & Torres, 2009). A substantial portion of the phenotypic variance in individual ability to resist OS depends on an individual's developmental environment (Costantini & Dell'Omo, 2006; Kim, Velando, Sorci, & Alonso-Alvarez, 2010; Losdat, Helfenstein, Blount, & Richner, 2014), although some of the variance may also be genetically inherited (Kahar, Debes, Vuori, Vähä, & Vasemägi, 2016; Kim, Noguera, Morales, & Velando, 2010). Therefore, exposure to early-life stressful conditions and/or early-life oxidizing environments may influence an individual's ability to keep reactive species at low levels and limit the occurrence of OS at adulthood. Recent experiments on captive zebra finches have revealed that reduced antioxidant levels during early life led to higher OS but also increased expression of secondary-sexual traits at adulthood (Romero-Haro & Alonso-Alvarez, 2015), that early-life nutritional restrictions did not increase OS at adulthood despite reducing antioxidant defences (Noguera, Monaghan, & Metcalfe, 2015), or that early-life heat stress reduced OS at adulthood when individuals were exposed to a similar heat stress (Costantini, Monaghan, & Metcalfe, 2012). Romero-Haro, Sorci, and Alonso-Alvarez (2016) recently reported the first evidence that exposure to OS for several days during early life can reduce the oxidative cost of future reproduction in captive zebra finches. However, the long-lasting effects of an early-life exposure to OS on adult resistance to OS have not yet been investigated in wild populations. Additionally, testing the long-term effect of an acute exposure to oxidative stress is crucial because acute exposures may induce hormesis, the beneficial effects of an exposure to a low dose of chemical agent that would be damaging at higher doses (Costantini, 2014).

Fitness-related traits may be particularly susceptible to OS and are hence of direct interest when investigating the long-lasting effects of an early-life exposure to OS (Dowling & Simmons, 2009; Monaghan et al., 2009; von Schantz, Bensch, Grahn, Hasselquist, & Wittzell, 1999). In particular, secondary-sexual traits such as colourful sexual signals are often under strong sexual selection and are prime predictors of individual reproductive success (e.g. Andersson & Simmons, 2006). Carotenoid-based colourful ornaments have been hypothesized to reflect individual ability to resist OS (Peters, 2007; von Schantz et al., 1999; Svensson & Wong, 2011) because animals face an allocation trade-off in the use of carotenoids either as pigments for their ornaments or as antioxidants to fight off reactive species (Andersson & Prager, 2006; Pérez-Rodríguez, 2009). Indeed, empirical studies have shown that carotenoid-based sexual coloration is increased by antioxidant supplementation (Bertrand et al., 2006) or correlated with individual resistance to OS (Losdat, Helfenstein, Gaude, & Richner, 2011; Simons, Cohen, & Verhulst, 2012). Therefore, given that OS may influence carotenoid-based sexual signals, one could expect exposure to OS during early life to affect colourful signals later in life.

Sperm performance, a major determinant of male fertilization success in species with intense sperm competition (i.e. the competition among different sperm for the fertilization of ova, Parker, 1970), might also be affected by an early-life exposure to OS. Due to the large proportion of polyunsaturated fatty acids in their membranes (Hulbert, Pamplona, Buffenstein, & Buttemer, 2007), spermatozoa are prone to oxidation by reactive species, which can impair their performance (Helfenstein, Losdat, Møller, Blount, & Richner, 2010; Losdat, Richner, Blount, & Helfenstein, 2011) and cause male infertility (Aitken & Baker, 2006; Tremellen, 2008). Therefore, if early-life exposure to OS influences individual ability to resist OS later in life, it may in turn affect sperm traits at adulthood, hence potentially affecting male (and female) reproductive fitness (Blount, Møller, & Houston, 2001; Velando, Torres, & Alonso-Alvarez, 2008).

In the present study, we experimentally exposed pre-fledging wild great tits *Parus major* to a reactive species generator and quantified the resulting effects on individual plumage coloration, resistance to OS and sperm performance measured in adults that recruited the following year into the local breeding population.

2 | MATERIALS AND METHODS

2.1 | Study system

The experiment was conducted in spring 2010 in a natural population of great tits *Parus major* breeding in nest boxes in a forest near Bern, Switzerland (46°55′N, 7°18′E). Nest boxes were regularly visited from the beginning of the breeding season to finally determine in 141 nests the start of egg laying and hatching dates (hatching date = day 0). On day 3 post-hatch, nestlings were ranked according to body mass (a correlate of hatching rank and survival, Magrath, 1990), and individually marked by removing dorsal tuft feathers.

2.2 | Experimental procedure

On day 11 post-hatch, we ringed all nestlings (n = 771) with uniquely numbered aluminium rings, weighed them (±0.1 g) and measured their tarsus length (±0.01 mm). Within each brood, nestlings were orally dosed with a solution of paraquat (Sigma-Aldrich, Switzerland) mixed in water (hereafter referred to as "paraquat") or with water only (thereafter referred to as "control"). We also

took a blood sample from the brachial vein of a subsample of offspring (n = 205) to check for a potential treatment bias with respect to initial values of oxidative damage and erythrocyte resistance to OS (sampling all nestlings was not possible for practical reasons). We chose to expose offspring to paraquat acutely in order to mimic naturally occurring transient stressors such as harsh climatic conditions or acute pathogen infections, which are known to increase offspring oxidative stress (Beaulieu, Haas, & Schaefer, 2014; Marri & Richner, 2015). Given that offspring mostly rely on maternal antioxidants for a few days after hatching (Giordano, Costantini, & Tschirren, 2015; Parolini et al., 2017; Surai & Fisinin, 2013), we exposed offspring to paraquat at the specific age of 11 days old such that the observed treatment effects primarily reflected the offspring's own physiological response. We used paraquat, the active molecule of a widely used herbicide, because it is well known to increase OS in animals through increased reactive species production (Koch & Hill, 2017). The toxicity of paraquat occurs through the production of the superoxide anion, which catalyses the formation of reactive species, in turn inducing oxidative damage (Dinis-Oliveira et al., 2008). A previous study on adult great tits showed that daily intakes of 0.24 or 0.60 mg of paraquat over a six-week period (i.e. ca. 15 and 38 mg/kg of body mass, respectively) did not affect survival but the highest dose induced symptoms of fatigue and lack of alertness (Isaksson & Andersson, 2008). With the aim of inducing an acute exposure to OS without affecting bird behaviour and survival, we dosed each individual offspring with a single dose of 0.3 mg of paraquat (0.1 ml of a solution at 3 g/L), accounting for approximately 22 mg/kg of body mass. To randomize the paraquat treatment with regard to nestling hatching rank within nest, the first hatched nestling within each nest was randomly assigned to either "paraquat" or "control" by tossing a coin and the subsequent nestlings alternately received "control" and "paraquat" doses.

On day 13 post-hatch, a few days before fledging, we blood-sampled all 771 nestlings to obtain pre-fledging estimates of individual resistance to OS. During the subsequent breeding season in spring 2011, we captured all breeding adults in the same forest aiming at recapturing as many recruits (i.e. individual breeders that survived from year 2010) as possible. Using clap traps, breeding adults were captured at their nests while feeding their offspring. A high level of sampling effort (85% of the 2011 local breeders were captured) ensured that the majority of recruits were likely captured (39 recruits). Within minutes upon capture, adults were blood-sampled for OS analyses, their plumage reflectance was recorded (see details below), and males were sperm-sampled to assess sperm performance. Post-mating competition for paternity is likely high during the entire breeding season due to the permanent opportunities for extra-pair paternity and the frequent production of second clutches. Therefore, quantifying sperm performance at this stage is biologically relevant, as shown by our previous work (Helfenstein et al., 2010; Losdat, Richner, et al., 2011). Besides measuring physiological traits in male and female recruits, we also recorded the number of offspring they produced in 2011.

2.3 | Erythrocyte resistance to OS

Erythrocyte resistance to OS was assessed in pre-fledging individuals in 2010 and in breeding adults that recruited in 2011 using the Kit Radicaux Libres® (KRL® test, Brevet Spiral, France) adapted to bird physiological parameters (Alonso-Alvarez et al., 2004). This assay provides a quantitative measurement of the erythrocyte resistance to OS as it assesses the time required to haemolyse 50% of red blood cells of the sample when exposed to a controlled free radical attack. It reflects the current availability of total antioxidant defences (enzymatic and non-enzymatic) as well as the past oxidative insults experienced by red blood cells (Brzezinska-Slebodzinska, 2001) and also reflects the rates of lipid peroxidation in the erythrocyte membrane (Zou, Agar, & Jones, 2001); 7 µl of whole blood was diluted in 255.5 µl of KRL buffer (150 mM Na^+, 120 mM Cl^-, 6 mM K^+, 24 mM $HCO3^-$, 2 mM Ca^{2+}, 340 mOsM, pH 7.4) immediately after sampling and stored at 4°C before analysis that occurred 6.2 ± 4.0 hr after blood collection. We loaded 80 µl of KRL-diluted whole blood into wells of a 96-well microplate. We subsequently added to each well 136 µl of a 150 mM solution of 2,2-azobis- (amidinopropane) hydrochloride (AAPH; a free radical generator; 646 mg of 2,2′-azobis-amidinopropane-hydrochloride) diluted in 20 ml of KRL buffer (Rojas Wahl, Liansheng, Madison, DePinto, & Shay, 1998). The rate of haemolysis was determined by the change in optical density measured at 540 nm (Bertrand et al., 2006) using a microplate reader spectrophotometer (PowerWave XS, Witec AG, Switzerland) at 40°C. Readings were made every 3.5 min for 80 min, and the microplate was shaken immediately before each reading to prevent cells from settling at the bottom of the wells. Repeatability of the KRL assay was r = .83, which was assessed by duplicating the analyses on different microplates for 76 adults captured in 2011 (not all 76 individuals are included in this study).

2.4 | Oxidative damage to lipids

MDA levels were measured in pre-fledging individuals in 2010 and in breeding adults that recruited in 2011. Plasma concentrations of MDA, formed by the β-scission of peroxidized fatty acids, were quantified using high-pressure liquid chromatography with fluorescence detection (HPLC-FLD), as described previously (Losdat et al., 2014). All chemicals were of HPLC grade, and chemical solutions were prepared using ultra pure water (Milli-Q Synthesis; Millipore, Watford, UK). Samples were first immersed in a water bath (ice cold) and sonicated for 10 min, and then microtubes were homogenized for one minute using a motorized pestle before being centrifuged at 15,338 ***g*** and 4°C for 4 min. Sample derivatization was done in 2 ml capacity screw-top microcentrifuge tubes. To a 5 µl aliquot of sample or standard (1,1,3,3-tetraethoxypropane, TEP; see below), 5 µl butylated hydroxytoluene solution (0.05% w/v in 95% ethanol), 40 µl phosphoric acid solution (0.44 M) and 10 µl thiobarbituric acid (TBA) solution (42 mM) were added. Samples were capped, vortex-mixed for 5 s, and heated at 100°C for exactly one hour in a dry bath incubator to allow formation of MDA-TBA adducts. Samples were then

cooled on ice for five minutes, before 80 µl n-butanol was added and tubes were vortex-mixed for 10 s. Tubes were then centrifuged at 13,000 rpm and 4°C for four minutes, before a 55 µl aliquot of the epiphase was collected and transferred to an HPLC vial for analysis. Samples (40 µl) were injected into a Dionex HPLC system (Dionex Corporation, California, USA) fitted with a 2-µm pre-column filter and a Hewlett-Packard Hypersil 5µODS 100 × 4.6 mm column maintained at 37°C. The mobile phase was methanol buffer (40:60, v/v), the buffer being a 50 mM anhydrous solution of potassium monobasic phosphate at pH 6.8 (adjusted using 5 M potassium hydroxide solution), running isocratically over 3.5 min at a flow rate of 1 ml/min. Data were collected using a fluorescence detector (RF2000; Dionex) set at 515 nm (excitation) and 553 nm (emission). For calibration, a standard curve was prepared using a TEP stock solution (5 µM in 40% ethanol) serially diluted using 40% ethanol. Repeatability of the assay, assessed by repeating the analysis on a different day from the same blood sample for 21 adults captured in 2011, was very high (r = .99).

2.5 | Plumage coloration

For all recaptured adults, we recorded reflectance spectra of the yellow breast plumage on both sides of the keel. For each of these two patches, we took two reflectance readings to assess repeatability of the method, removing the probe from the plumage between each measure (i.e. four measurements in total). Spectral measures were made using a USB4000 spectrophotometer, an FCR-7UV200-2-ME bifurcated reflectance probe with a 200-µm fibre core diameter, and a deuterium-halogen/tungsten light source (DH-2000-BAL, UV-VIS-NIR; Ocean Optics Inc., Netherlands). Each measurement was itself the average of four scans with a 100 ms integration time and was calculated relative to a diffuse reflectance standard (WS-1, Ocean Optics Inc., Netherlands). We used avian visual models to compile SWS ratio and double cone, which, respectively, quantify bird chromatic (spectral shape or plumage colour) and achromatic (spectral intensity or plumage achromacity) variation in plumage reflectance (Andersson & Prager, 2006; Evans, Hinks, Wilkin, & Sheldon, 2010). Recent studies have encouraged the use of models of avian vision rather than classical tristimulus scores because (1) those models describe colourful traits in the eye of a conspecific by integrating retinal cones spectral sensitivity, transmittance properties of the ocular media and ambient light irradiance spectrum rather than providing raw physical metrics, and (2) they allow to separately quantify variation in white feather structure and in carotenoid pigmentation itself, hence providing more specific information. Such models have hence been intensively and successfully used to quantify carotenoid-based coloration, particularly in great tits (e.g. Endler & Mielke, 2005; Evans & Sheldon, 2013; Evans et al., 2010; Kelber & Osorio, 2010). We quantified the quantum catches for each of the four types of single cones that are sensitive to chromatic cues in birds (very short, short, medium and long wavelengths, Hart, Partridge, Cuthill, & Bennett, 2000) and for the double cones that are sensitive to achromatic cues (Endler & Mielke, 2005). Using the "PAVO" R package (Maia, Eliason, Bitton, Doucet, & Shawkey, 2013), we computed very short, short, medium and long single cones as well as double cones using cone sensitivity data, ocular media transmittance for the blue tit *Cyanistes caeruleus* (Hart et al., 2000) and forest shade irradiance spectrum (Endler, 1993) because our population lives in forests. For each individual bird, we averaged measurement values per patch (within-patch measurement repeatability was r = .70 and r = .77 for SWS ratio and double cone, respectively) and further used the mean of both patch values per bird. Plumage chromacity and achromacity estimated from avian physiological models were highly correlated with commonly used classical measurements; SWS ratio was highly correlated (r = .89, n = 37) with carotenoid chroma (which reflects the amount of pigment deposited in the feathers, Saks, McGraw, & Hõrak, 2003), and double cone was highly correlated with total brightness (r = .97, n = 37) and with achromatic colour brightness (r = .98, n = 37), the absolute reflectance between 575 and 700 mm (Jacot, Romero-Diaz, Tschirren, Richner, & Fitze, 2010). Accordingly, our results were qualitatively unchanged when using these classical traits instead of SWS and double cone (data not shown).

2.6 | Sperm performance

For males captured in 2011, a sperm sample was collected by gently massaging males' cloacal protuberance (Wolfson, 1952). Collected sperm (ca. 1 µl) were mixed immediately with pre-warmed (40°C) Dulbecco's modified Eagle's medium (4,500 mg glucose/L, 110 mg sodium pyruvate/L, 4 mM L-glutamine, Sigma-Aldrich, Switzerland); 9 µl of sperm/Dulbecco solution was immediately (within ca. one minute) transferred to a dark-field phase-contrast microscope, where sperm motion was video-recorded, following standard protocols (e.g. Losdat, Richner, et al., 2011). Temporal dynamics of sperm motion were later analysed after 0, 30, 60, 90 and 120 s of video recording using a computer-assisted sperm analysis plug-in implemented in IMAGEJ software (Wilson-Leedy & Ingermann, 2007). We quantified sperm motility (the proportion of motile vs. immotile sperm) at time 0 and across the 0–120 s of video recording (i.e. sperm longevity) and straight-line velocity (VSL) of motile sperm at time 0 and across the 0–120 s of video recording (i.e. sperm stamina). These sperm traits were chosen because they predict male fertilization success in several species (e.g. Hunter & Birkhead, 2002; Pizzari & Parker, 2009). VSL, our measure of sperm velocity, was highly correlated with two variables that are commonly used to quantify sperm velocity; VCL (curvilinear velocity) and the PC1 scores of a principal component analysis including VSL, VCL, average path velocity VAP and the number of motile sperm. Since our results remained similar when using VCL and PC1 scores, we only present the output of the models based on VSL.

2.7 | Statistical analyses

To quantify effects of the pre-fledging paraquat treatment on adult resistance to OS and plumage colour in males and females, we fitted four generalized linear mixed-effect models. Dependent variables

were oxidative damage, erythrocyte resistance to OS, SWS ratio and double cone, all of which have been measured in one-year-old adults captured in 2011. Fixed effects were treatment (two-level factor, control or paraquat), sex (two-level factor, male or female) as well as pre-fledging oxidative damage and pre-fledging erythrocyte resistance to OS that had been measured prior to fledging in 2010. Models included interactions of treatment by sex, treatment by pre-fledging erythrocyte resistance to OS and treatment by pre-fledging oxidative damage to test whether the paraquat treatment effect was sex dependent and/or depended on individual pre-fledging resistance to OS. We also initially tested for effects of brood size and laying date, but since those were never significant (α set at 0.05) we chose not to include them in the final models. We fitted random nest identity effects to control for the non-independence among offspring that grew in the same nests in 2010.

To quantify the paraquat effect on male sperm performance, we fitted two repeated-measures mixed-effect models. Dependent variables were sperm motility and velocity at all time points (five values per ejaculate, allowing estimation of sperm longevity and sperm stamina). Both models included random male identity effects (nested within nest identity) to account for the five repeated measures per male. Models included a fixed regression on time since start of video recording and interactions of time by treatment, time by pre-fledging oxidative damage and time by pre-fledging erythrocyte resistance to OS to test for treatment effects and pre-fledging OS values on the rate of decrease in motility and velocity.

Further, we tested for an effect of the paraquat treatment on the reproductive output of the recruiting adults captured one year later in 2011. We ran a linear model with the (log-transformed) number of offspring produced in 2011 as dependent variable and paraquat treatment as explanatory variable.

Last, we tested for potential selective disappearance by investigating whether exposure to paraquat and pre-fledging resistance to oxidative stress predicted recruitment probability. We ran a generalized linear mixed-effect model with binomial distribution including the paraquat exposure, pre-fledging oxidative damage, pre-fledging erythrocyte resistance to OS and the interactions between the paraquat exposure and the two markers of OS as fixed factors. Pre-fledging body mass was also included as a fixed effect due to its known effect on future survival. Nest identity was fitted as random factor.

Model reduction was limited to removing interactions with $p > .10$ following Whittingham, Stephens, Bradbury, and Freckleton (2006); final models hence contained all fixed effects and interactions with $p < .10$. However, our treatment effects were unchanged when removing non-significant interactions and fixed effects (data not shown). Analyses were run in R 3.3.2 (R Core Team 2016) using "LME4" package (Bates & Maechler, 2009).

3 | RESULTS

Of the 771 offspring that received the treatment on day 11 post-hatch in 2010, 39 individuals (25 males, 14 females) were recaptured as adult breeders the following year (rate: 5.0%, a low but rather typical figure in great tit populations, Losdat et al., 2013; van Oers, Drent, Dingemanse, & Kempenaers, 2008; Norte, Sheldon, Sousa, & Ramos, 2009; Wilkin, King, & Sheldon, 2009). Of those 39 recruits, 21 had received paraquat (seven females, 14 males) and 18 received the control treatment (seven females, 11 males) at the pre-fledging stage. The 39 recruits originated from 33 different broods that contributed one recruit (28 broods), two recruits (four broods) or three recruits (one brood).

3.1 | Short-term effect of the exposure to paraquat

On day 11 post-hatch, when offspring were exposed to paraquat, hatching date, brood size, oxidative damage and erythrocyte resistance to OS measured in a subsample of 205 offspring did not differ between paraquat and control groups (all $F_{1,160–205} < 1.44$, $p > .23$) and nor did offspring hatching rank ($\chi^2_7 = 1.89$, $p = .96$), therefore supporting the absence of any initial bias. Within the subsample of 205 offspring sampled before and after the paraquat exposure (on days 11 and 13 post-hatch, respectively), none of the markers of OS were affected by the paraquat exposure (KRL: $\beta = -0.06 \pm 0.08$, $F_{1,361} = 0.48$, $p = .49$; MDA: $\beta = -0.06 \pm 0.04$, $F_{1,310} = 1.83$, $p = .18$). Across all 771 offspring measured on day 13 post-hatch, none of the markers of OS differed between paraquat-exposed and control offspring (KRL: $\beta = -0.05 \pm 0.05$, $F_{1,702} = 1.06$, $p = .30$ MDA: $\beta = 0.01 \pm 0.02$, $F_{1,654} = 0.28$, $p = .60$).

3.2 | Effect of pre-fledging exposure to paraquat on adult resistance to OS and coloration

Oxidative damage (i.e. MDA levels) at adulthood was significantly influenced by the paraquat exposure; individuals that were paraquat-exposed at the pre-fledging stage had lower oxidative damage at adulthood (Table 1, Figure 1a). Oxidative damage was not significantly influenced by any other main effect or interaction (Table 1). Erythrocyte resistance to OS (i.e. KRL values) could only be estimated for 22 of the 39 recruits (but the results are presented for completeness) and was significantly influenced only by pre-fledging oxidative damage, such that higher pre-fledging oxidative damage led to higher erythrocyte resistance to OS at adulthood (Table 1). There was also a non-significant tendency for paraquat-exposed individuals to have higher erythrocyte resistance to OS at adulthood (Table 1, Figure 1b) but since only a subsample of individuals was included in this analysis it should be cautiously interpreted due to the high risk of type I error. Interestingly however, the direction of the effect is similar to the one observed on oxidative damage. None of the markers of OS differed between the sexes.

There was a non-significant tendency for plumage chromacity (i.e. SWS ratio) to be affected by the sex by paraquat treatment interaction ($p = .07$, Table 1). Post hoc tests comparing the four corresponding groups between each other showed that paraquat-dosed females displayed higher chromacity compared to control females (Tukey-adjusted post hoc test: $z = 3.55$, $p = .002$, Figure 1c), an

TABLE 1 Linear mixed models testing for effects of the paraquat treatment, sex, pre-fledging oxidative damage and pre-fledging erythrocyte resistance to oxidative stress and their interactions on malondialdehyde levels, erythrocyte resistance to oxidative stress (KRL) plumge chromacity (SWS ratio) and plumage achromacity (double cone) all measured at adulthood. Estimates (Est.) are shown ± 1 standard error (*SE*). Parameters retained in the final models are highlighted in bold

	Malondialdehyde levels			KRL			SWS ratio			Double cone		
Effect	**Est. ± *SE***	F_{df}	***p***	**Est. ± *SE***	F_{df}	***p***	**Est. ± *SE***	F_{df}	***P***	**Est. ± *SE***	F_{df}	***p***
Intercept	1.95 ± 0.33	–	–	0.03 ± 0.68	–	–	1.48 ± 0.10	–	–	0.57 ± 0.06	–	–
Paraquat treatment[a]	**−0.29 ± 0.11**	$\mathbf{6.07}_{1,24.4}$	**.02**	**0.50 ± 0.25**	$\mathbf{3.30}_{1,15.0}$	**.09**	**0.13 ± 0.04**	$\mathbf{8.32}_{1,11.4}$	**.01**	**0.23 ± 0.07**	$\mathbf{3.30}_{1,28.7}$	**.005**
Individual sex[a]	**0.06 ± 0.12**	$\mathbf{0.23}_{1,25.6}$	**.64**	**−0.42 ± 0.32**	$\mathbf{1.28}_{1,12.0}$	**.28**	**0.09 ± 0.04**	$\mathbf{3.41}_{1,24.4}$	**.08**	**0.03 ± 0.02**	$\mathbf{2.61}_{1,32.6}$	**.12**
Pre-fledge MDA	**0.04 ± 0.10**	$\mathbf{0.13}_{1,30.0}$	**.72**	**0.57 ± 0.24**	$\mathbf{5.53}_{1,15.9}$	**.03**	**0.02 ± 0.03**	$\mathbf{0.37}_{1,33.0}$	**.55**	**−0.07 ± 0.02**	$\mathbf{5.53}_{1,15.9}$	**.03**
Pre-fledge KRL	**−0.01 ± 0.08**	$\mathbf{0.01}_{1,24.9}$	**.94**	**0.10 ± 0.16**	$\mathbf{0.32}_{1,15.8}$	**.58**	**−0.04 ± 0.02**	$\mathbf{3.72}_{1,17.3}$	**.07**	**0.01 ± 0.10**	$\mathbf{0.79}_{1,31.5}$	**.38**
Treatment[a] × Sex[b]	−0.32 ± 0.23	$1.65_{1,17.6}$	.22	−0.18 ± 0.71	$0.04_{1,8.1}$	.86	**−0.12 ± 0.06**	$\mathbf{3.88}_{1,15.0}$	**.07**	−0.04 ± 0.03	$1.74_{1,30.1}$	.20
Treatment[a] × Pre. MDA	−0.18 ± 0.19	$0.74_{1,20.7}$	.40	−0.01 ± 0.54	$0.01_{1,12.9}$	.40	0.05 ± 0.05	$0.59_{1,11.4}$	.46	**0.07 ± 0.03**	$\mathbf{7.44}_{1,30.0}$	**.01**
Treatment[a] × Pre. KRL	0.26 ± 0.16	$2.02_{1,26.8}$	.17	−0.26 ± 0.41	$0.23_{1,12.7}$	.64	0.02 ± 0.05	$0.21_{1,24.7}$	.65	−0.02 ± 0.02	$1.03_{1,27.1}$	.32
Random nest identity	0.03 ± 0.18			0.00 ± 0.00			0.008 ± 0.09			0.00 ± 0.00		

[a]Paraquat-dosed relative to control.
[b]Males relative to females.

effect not observed in males (z = .22, p = .98, Figure 1c). Plumage achromacity (i.e. double cones) was significantly influenced by the treatment by pre-fledging oxidative damage interaction such that across paraquat-dosed individuals there was a significant negative relationship between oxidative damage prior to fledging and plumage achromacity at adulthood, a relationship that was not significant across controls (Table 1, Figure 1d). Paraquat-dosed individuals with lower oxidative damage prior to fledging hence expressed a brighter plumage at adulthood (Figure 1d).

3.3 | Effect of pre-fledging exposure to paraquat on sperm performance and reproductive output

Sperm velocity, stamina (i.e. rate of decrease in sperm velocity), motility and sperm longevity (i.e. rate of decrease in sperm motility) were not significantly influenced by the paraquat treatment, as shown by the absence of significant paraquat treatment by time post-ejaculation interaction (Table 2, Figure 2). Pre-fledging OS also did not significantly predict male sperm performance at adulthood (Table 2). The total number of offspring produced by the recruiting males and females in 2011 (i.e. one year post-treatment) did not significantly vary between paraquat (mean ± *SD*: 8.0 ± 1.7) and control birds (mean ± *SD*: 7.5 ± 1.6, $F_{1,28}$ = 0.56, p = .46).

3.4 | Effect of pre-fledging resistance to oxidative stress and paraquat exposure on recruitment probability

Recruitment probability was strongly predicted by offspring body mass prior to fledging (Table 3), as commonly observed in passerines. The paraquat exposure and interactions of paraquat by pre-fledging MDA and paraquat by pre-fledging KRL did not significantly influence recruitment probability (Table 3), therefore showing that paraquat-dosed offspring that had relatively low pre-fledging resistance to oxidative stress were not more likely to disappear and hence to elude sampling as adults. In contrast, pre-fledging MDA levels significantly predicted recruitment probability such that offspring with higher pre-fledging MDA levels were more likely to recruit (Table 3).

4 | DISCUSSION

In a natural population of vertebrates, we experimentally show that an early-life acute exposure to paraquat, despite the absence of a short-term effect, had long-lasting effects leading to lower levels of oxidative damage in recruiting adults. Females (but not males) exposed to paraquat as nestlings also exhibited a more colourful adult plumage. The acute paraquat exposure also had a positive effect on the adult plumage brightness of males and females that had low pre-fledging oxidative damage. However, early stress did not affect first year reproductive success or male sperm performance.

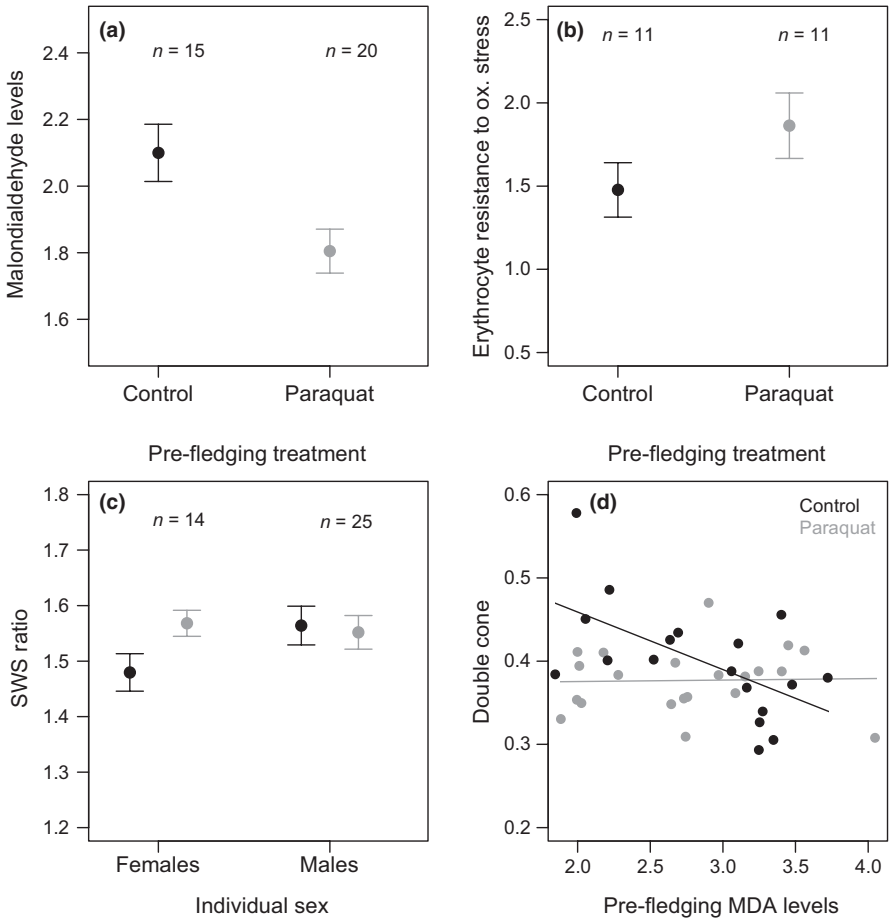

FIGURE 1 Mean values *M* (±standard error *SE*) of (a) oxidative damage (log-transformed) at adulthood, (b) erythrocyte resistance to oxidative stress (log-transformed) at adulthood, and (c) adult plumage chromacity measured as SWS ratio in relation to the pre-fledging paraquat treatment (black dots: control, grey dots: paraquat). (c) also shows the sex-specific treatment effect on SWS ratio. (d) shows the relationship between adult plumage achromacity measured as double cone and pre-fledging oxidative damage in the control (black dots) and paraquat (grey dots) experimental groups. Linear regression lines are shown for control (black) and paraquat (grey) groups

TABLE 2 Linear mixed models testing for effects of the paraquat treatment and of pre-fledging oxidative damage and erythrocyte resistance to OS, on the decrease in sperm velocity (i.e. sperm longevity) and sperm motility (i.e. sperm stamina) with time (both variables were square-root-transformed). Interactions of time post-ejaculation by paraquat treatment were kept in the final models because they test the treatment effect on the rates of change in velocity and motility. Estimates (Est.) are shown ± 1 standard error (*SE*). Parameters retained in the final models are highlighted in bold

	Sperm velocity			Sperm motility		
Effect	**Est. ± *SE***	**F_{df}**	***p***	**Est. ± *SD***	**F_{df}**	***p***
Intercept	**9.7 ± 32.1**	**–**	**–**	**0.75 ± 0.19**	**–**	**–**
Paraquat treatment[a]	**−0.29 ± 0.89**	**$0.10_{1, 34.4}$**	**.75**	**−0.016 ± 0.08**	**$0.04_{1, 22.9}$**	**.84**
Time	**−0.02 ± 0.006**	**$8.90_{1, 82.0}$**	**.004**	**−0.001 ± 0.0004**	**$12.5_{1, 82.0}$**	**.0007**
Pre-fledging MDA	**−0.40 ± 0.63**	**$0.37_{1, 16.6}$**	**.55**	**−0.05 ± 0.06**	**$0.71_{1, 16.7}$**	**.41**
Pre-fledging KRL	**−0.29 ± 0.44**	**$0.20_{1, 9.5}$**	**.66**	**−0.02 ± 0.04**	**$0.10_{1, 4.6}$**	**.84**
Treatment[a] × Time	**0.003 ± 0.009**	**$0.16_{1, 82.0}$**	**.69**	**−0.0002 ± 0.0005**	**$0.14_{1, 82.0}$**	**.71**
Treatment[a] × Pre. MDA MDA	0.005 ± 0.008	$0.42_{1, 81.0}$	.52	0.0005 ± 0.0005	$1.29_{1, 81.0}$	.26
Treatment[a] × Pre. KRL	−0.003 ± 0.006	$0.26_{1, 80.0}$	.61	−0.0001 ± 0.0003	$0.15_{1, 80.0}$	.70

[a]Paraquat-dosed relative to control.

The positive effect of the early-life acute exposure to paraquat on oxidative damage measured at adulthood is somewhat surprising, particularly in light of the well-known negative long-term effects of adverse conditions experienced in early life (Haussmann et al., 2012; Herborn et al., 2014; Monaghan, 2008). In addition, negative long-lasting effects have been shown specifically on resistance to OS; early-life experimental reduction in offspring antioxidant levels and experimental increase in corticosterone levels in eggs both reduced resistance to OS at adulthood in captive birds (Haussmann et al., 2012; Noguera et al., 2015; Romero-Haro & Alonso-Alvarez, 2015; Zimmer & Spencer, 2015). On the other hand, early-life stressful conditions have been shown to induce positive long-term effects by

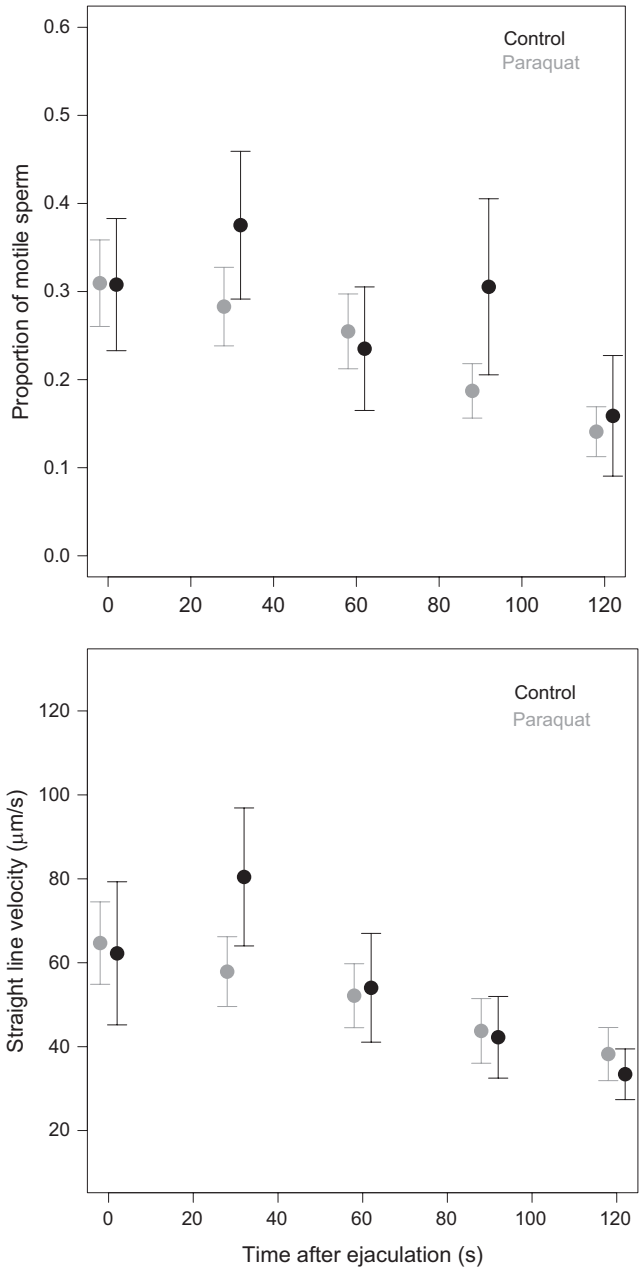

FIGURE 2 Decrease over time in the proportion of motile sperm (i.e. sperm longevity) and in straight-line velocity (i.e. sperm stamina) measured at adulthood (age one year) in relation to the pre-fledging paraquat treatment. Black and grey dots represent means *M* (±standard error *SE*) for the control and paraquat experimental groups, respectively

TABLE 3 Generalized linear mixed-effect model with binomial distribution testing effects of offspring body mass prior to fledging, paraquat exposure, pre-fledging oxidative damage (MDA) and erythrocyte resistance to OS (KRL) and interactions of paraquat by pre-fledging MDA and paraquat by pre-fledging KRL on recruitment probability. Estimates (Est.) are shown ± 1 standard error (*SE*). Parameters retained in the final models are highlighted in bold

	Recruitment probability		
Effect	**Est. ± *SE***	**χ^2**	***p***
Intercept	−11.4 ± 2.4	–	–
Paraquat exposure[a]	**0.28 ± 0.35**	**0.64**	**.42**
Pre-fledging body mass	**0.36 ± 0.11**	**10.43**	**.001**
Pre-fledging MDA	**0.76 ± 0.34**	**5.02**	**.025**
Pre-fledging KRL	**−0.05 ± 0.22**	**0.06**	**.81**
Treatment[a] × Pre-fledging MDA	−0.15 ± 0.44	0.12	.73
Treatment[a] × Pre-fledging KRL	−0.25 ± 0.61	0.17	.68

[a]Paraquat-dosed relative to control.

positively affecting secondary-sexual traits at adulthood (Romero-Haro & Alonso-Alvarez, 2015), by boosting adulthood resistance to OS (Costantini et al., 2012; Galván et al., 2014) or by reducing the oxidative cost of future reproduction (Romero-Haro et al., 2016). Together with these latter studies, our results are in line with and, our results are in line with "environmental matching hypothesis" of developmental plasticity according to which early-life adverse conditions can be adaptive (Bateson et al., 2014; Monaghan, 2008) and/or with the "hormesis" hypothesis stating that exposure to a low dose of a chemical agent, which is damaging at higher doses, can induce an adaptive beneficial effect (Calabrese, 2008; Costantini, 2014). One aspect of our experiment, which may have promoted long-term hormetic effects rather inducing long-term costs, is its acute (not chronic) nature, because acute stressors generally induce more limited costs compared to chronic stress (e.g. Boonstra, 2013). However, the positive effect observed here may simply reflect selective disappearance through higher mortality and/or emigration of individual adults with lower resistance to oxidative stress and darker plumage. Here, we were able to exclude the possibility that paraquat-dosed offspring and/or paraquat-dosed offspring with relatively lower pre-fledging resistance to oxidative stress were less likely to recruit in the population the following year, because neither the paraquat exposure itself nor its interaction with pre-fledging resistance to oxidative stress significantly predicted recruitment probability. On the contrary, offspring that originally had higher oxidative damage were more likely to recruit, which renders our long-term positive effect rather conservative. However, despite the absence of selective disappearance at early age, it remains possible that adults with lower resistance to oxidative stress were more likely to emigrate or die before we could trap and measure them, particularly given the low (5%) recruitment rate.

Given the absence of negative short-term (after two days) effect of the paraquat exposure on offspring OS markers in blood, the proximate mechanisms underlying our long-term positive effect remain unknown. Studies using paraquat to manipulate OS levels in birds showed that deleterious effects on OS were only reported at high paraquat doses (reviewed in Koch & Hill, 2017). Here, the paraquat dose administered was higher than those used in all previous studies on non-poultry birds (see Table 2 in Koch & Hill, 2017). In addition, house sparrows dosed with paraquat showed limited effects

on markers of OS shortly after the exposure but strong downstream effects (see Figure S1 in Mora, Firth, Blareau, Vallat, & Helfenstein, 2017). Consequently, one might still expect our paraquat exposure to have increased OS some days post-exposure. However, we cannot rule out the possibility that systemic OS was not increased after the paraquat exposure. Non-mutually exclusive explanations for the long-term effect observed despite the absence of a short-term effect could be that (1) the paraquat exposure increased oxidative stress several days post-exposure (i.e. more than two days later) and was hence not detected, (2) the paraquat exposure increased oxidative stress shortly after the exposure but this effect only manifested in other tissues than blood and was hence not detected, and/or (3) because we administered paraquat orally, one might expect the paraquat to have induced oxidative damage in the digestive system while not being absorbed in the blood. Should the paraquat have induced oxidative stress in the digestive system, one could expect an hormetic response in resistance to oxidative stress at the whole organism level. Irrespective of the short-term effect, our long-term positive effect supports the "environmental matching" and the "hormesis" hypotheses, which posit that increased exposure to reactive species at an early stage of life may shape individuals' antioxidant phenotype for relatively more oxidative future environments, in the same way pre-birth maternal effects adaptively shape offspring development trajectories to match their future environment (Marshall & Uller, 2007). Accordingly, Noguera et al. (2015) showed in zebra finches that individuals growing on a low-micronutrient diet had higher antioxidant defences at adulthood when their diet at adulthood was also low in micronutrients. Here, since we did not manipulate the environmental conditions at adulthood, the question of the costs and benefits of upregulating the antioxidant machinery to reduce oxidative damage arises. Notably, the number of offspring produced by recruiting males and females was not affected by their early exposure to paraquat, hence providing no evidence for a reproductive benefit nor for a reproductive cost to compensating for a bad start, at least in terms of oxidative damage to lipids. One obvious potential benefit of reduced oxidative damage could be greater lifespan (Agarwal et al., 2012; Costantini, 2014; Metcalfe & Alonso-Alvarez, 2010) and/or increased future reproductive output (Agarwal et al., 2012; Romero-Haro et al., 2016; Stier et al., 2012; Zimmer & Spencer, 2015) but we did not monitor the population in subsequent years. Alternatively, should our findings reflect selective disappearance, the proximate mechanisms involved could be differential mortality or increased natal dispersal distance for offspring that were more affected by the paraquat exposure (i.e. lower "quality" individuals). Notably, offspring are generally more likely to disperse further from their birth or hatching location when environmental conditions experienced during early life are harsher (e.g. Pasinelli & Walters, 2002; Studds, Kyser, & Marra, 2008). Whatever the fate of those individuals, dispersion or death, selective disappearance would imply condition-dependence in the expression of resistance to OS and plumage coloration, because only the higher "quality" individuals from the paraquat group would have been able to recruit in the local population.

We also report a positive effect of the early-life acute paraquat exposure on the achromatic component (i.e. brightness, Andersson & Prager, 2006; Pérez-Rodríguez, 2009) of both male and female yellow plumage and on the chromatic component of female yellow plumage. Plumage achromacity is a highly dynamic trait that shows marked seasonal variations (Evans & Sheldon, 2012) and has been shown to depend on early-life conditions in great tits (Jacot et al., 2010). However, the social and sexual cues conveyed by plumage achromacity (i.e. brightness) itself remain unclear (Evans & Sheldon, 2013; Peters, Kurvers, Roberts, & Delhey, 2011). In contrast, chromatic yellow coloration has proved to reflect both male and female reproductive performance in other passerines (Doutrelant et al., 2008; Henderson, Heidinger, Evans, & Arnold, 2013; Whittingham & Dunn, 2016) and may convey cues for mutual mate choice (e.g. Kraaijeveld, Kraaijeveld-Smit, & Komdeur, 2007). Therefore, the increased plumage chromacity of paraquat-exposed females and the increased plumage brightness of both paraquat-exposed males and females that had lower pre-fledging MDA levels (Figure 1), may have influenced males' ability to increase their fitness through extra-pair fertilizations, and females' ability to mate with higher "quality" males. Information about total and extra-pair reproductive success as well as survival of the next generation would, however, be required to address this question. In contrast, male sperm performance was not affected by our early-life acute exposure to paraquat, potentially stemming from the strong selective pressures acting on male sperm performance and fertility (Pizzari & Parker, 2009), particularly in species with frequent extra-pair reproduction. Overall, our study shows a long-term positive effect of an early-life exposure to a pro-oxidant molecule on future resistance to oxidative stress and on coloration traits, with potential beneficial effects for individual fitness.

ACKNOWLEDGEMENTS

We thank Alessandra Basso for assistance in field data collection. This study was approved by the Ethical Committee of the Agricultural Office of the Canton Bern, Switzerland (experimentation permit 24/10 to SL) and the Federal Office for the Environment, which provided a ringing permit to SL (ID 2739).

AUTHORS' CONTRIBUTIONS

S.L. and F.H. designed the study and wrote the manuscript; S.L. carried out field/laboratory data collection and conducted the statistical analyses; J.D.B. contributed to the oxidative damage analyses; L.M., V.M. and F.H. contributed to field data collection; H.R. and F.H. supervised the study.

DATA ACCESSIBILITY

The datasets supporting this article can be accessed in the following Dryad Digital Repository: https://doi.org/10.5061/dryad.8n5r3v1 (Losdat et al., 2018).

ORCID

Sylvain Losdat http://orcid.org/0000-0001-7673-4877
Jonathan D. Blount http://orcid.org/0000-0002-0016-0130

REFERENCES

Agarwal, A., Aponte-Mellado, A., Premkumar, B. J., Shaman, A., & Gupta, S. (2012). The effects of oxidative stress on female reproduction: A review. *Reproductive Biology and Endocrinology*, *10*, 1.

Aitken, R. J., & Baker, M. A. (2006). Oxidative stress, sperm survival and fertility control. *Molecular and Cellular Endocrinology*, *250*, 66–69. https://doi.org/10.1016/j.mce.2005.12.026

Alonso-Alvarez, C., Bertrand, S., Devevey, G., Prost, J., Faivre, B., & Sorci, G. (2004). Increased susceptibility to oxidative stress as a proximate cost of reproduction. *Ecology Letters*, *7*, 363–368. https://doi.org/10.1111/j.1461-0248.2004.00594.x

Andersson, S., & Prager, M. (2006). Quantification of avian coloration. In G. E. Hill & K. J. McGraw (Eds.), *Bird coloration, part 1: Mechanisms and measurements* (pp. 41–89). Cambridge, UK: Harvard University Press.

Andersson, M., & Simmons, L. W. (2006). Sexual selection and mate choice. *Trends in Ecology and Evolution*, *21*, 296–302. https://doi.org/10.1016/j.tree.2006.03.015

Bates, D., & Maechler, M. (2009). *lme4: Linear mixed-effects models using S4 classes*. R package.

Bateson, P., Gluckman, P., & Hanson, M. (2014). The biology of developmental plasticity and the predictive adaptive response hypothesis. *The Journal of Physiology*, *592*, 2357–2368. https://doi.org/10.1113/jphysiol.2014.271460

Beaulieu, M., Haas, A., & Schaefer, H. M. (2014). Self-supplementation and effects of dietary antioxidants during acute thermal stress. *Journal of Experimental Biology*, *217*, 370–375. https://doi.org/10.1242/jeb.092825

Bertrand, S., Alonso-Alvarez, C., Devevey, G., Faivre, B., Prost, J., & Sorci, G. (2006). Carotenoids modulate the trade-off between egg production and resistance to oxidative stress in zebra finches. *Oecologia*, *147*, 576–584. https://doi.org/10.1007/s00442-005-0317-8

Bize, P., Devevey, G., Monaghan, P., Doligez, B., & Christe, P. (2008). Fecundity and survival in relation to resistance to oxidative stress in a free-living bird. *Ecology*, *89*, 2584–2593. https://doi.org/10.1890/07-1135.1

Blount, J. D., Møller, A. P., & Houston, D. C. (2001). Antioxidants, showy males and sperm quality. *Ecology Letters*, *4*, 393–396. https://doi.org/10.1046/j.1461-0248.2001.00255.x

Boonstra, R. (2013). Reality as the leading cause of stress: Rethinking the impact of chronic stress in nature. *Functional Ecology*, *27*, 11–23. https://doi.org/10.1111/1365-2435.12008

Brzezinska-Slebodzinska, E. (2001). Erythrocyte osmotic fragility test as the measure of defence against free radicals in rabbits of different age. *Acta Veterinaria Hungarica*, *49*, 413–441. https://doi.org/10.1556/004.49.2001.4.5

Burton, T., & Metcalfe, N. B. (2014). Can environmental conditions experienced in early life influence future generations? *Proceedings of the Royal Society B: Biological Sciences*, *281*, 20140311. https://doi.org/10.1098/rspb.2014.0311

Calabrese, E. J. (2008). Converging concepts: Adaptive response, preconditioning, and the Yerkes–Dodson Law are manifestations of hormesis. *Ageing Research Reviews*, *7*, 8–20. https://doi.org/10.1016/j.arr.2007.07.001

Costantini, D. (2014). *Oxidative stress and hormesis in evolutionary ecology and physiology*. Berlin, Germany: Springer-Verlag. https://doi.org/10.1007/978-3-642-54663-1

Costantini, D., & Dell'Omo, G. (2006). Environmental and genetic components of oxidative stress in wild kestrel nestlings (*Falco tinnunculus*). *Journal of Comparative Physiology B Biochemical Systemic and Environmental Physiology*, *176*, 575–579. https://doi.org/10.1007/s00360-006-0080-0

Costantini, D., Monaghan, P., & Metcalfe, N. B. (2012). Early life experience primes resistance to oxidative stress. *The Journal of Experimental Biology*, *215*, 2820–2826. https://doi.org/10.1242/jeb.072231

Dinis-Oliveira, R., Duarte, J., Sanchez-Navarro, A., Remiao, F., Bastos, M., & Carvalho, F. (2008). Paraquat poisonings: Mechanisms of lung toxicity, clinical features, and treatment. *Critical Reviews in Toxicology*, *38*, 13–71. https://doi.org/10.1080/10408440701669959

Doutrelant, C., Grégoire, A., Grnac, N., Gomez, D., Lambrechts, M., & Perret, P. (2008). Female coloration indicates female reproductive capacity in blue tits. *Journal of Evolutionary Biology*, *21*, 226–233. https://doi.org/10.1111/j.1420-9101.2007.01451.x

Dowling, D. K., & Simmons, L. W. (2009). Reactive oxygen species as universal constraints in life-history evolution. *Proceedings of the Royal Society of London B Biological Sciences*, *276*, 1737–1745. https://doi.org/10.1098/rspb.2008.1791

Endler, J. A. (1993). The color of light in forests and its implications. *Ecological Monographs*, *63*, 2–27.

Endler, J. A., & Mielke, P. W. (2005). Comparing entire colour patterns as birds see them. *Biological Journal of the Linnean Society*, *86*, 405–431. https://doi.org/10.1111/j.1095-8312.2005.00540.x

Evans, S. R., Hinks, A. E., Wilkin, T. A., & Sheldon, B. C. (2010). Age, sex and beauty: Methodological dependence of age- and sex-dichromatism in the great tit *Parus major*. *Biological Journal of the Linnean Society*, *101*, 777–796. https://doi.org/10.1111/j.1095-8312.2010.01548.x

Evans, S. R., & Sheldon, B. C. (2012). Quantitative genetics of a carotenoid-based color: Heritability and persistent natal environmental effects in the great tit. *The American Naturalist*, *179*, 79–94. https://doi.org/10.1086/663198

Evans, S. R., & Sheldon, B. C. (2013). Pigments versus structure: Examining the mechanism of age-dependent change in a carotenoid-based colour. *Journal of Animal Ecology*, *82*, 418–428. https://doi.org/10.1111/1365-2656.12008

Galván, I., Bonisoli-Alquati, A., Jenkinson, S., Ghanem, G., Wakamatsu, K., Mousseau, T. A., & Møller, A. P. (2014). Chronic exposure to low-dose radiation at Chernobyl favours adaptation to oxidative stress in birds. *Functional Ecology*, *28*, 1387–1403. https://doi.org/10.1111/1365-2435.12283

Garratt, M., Lemaître, J.-F., Douhard, M., Bonenfant, C., Capron, G., Warnant, C., … Gaillard, J.-M. (2015). High juvenile mortality is associated with sex-specific adult survival and lifespan in wild roe deer. *Current Biology*, *25*, 759–763. https://doi.org/10.1016/j.cub.2014.11.071

Giordano, M., Costantini, D., & Tschirren, B. (2015). Sex-specific effects of prenatal and postnatal nutritional conditions on the oxidative status of great tit nestlings. *Oecologia*, *177*, 123–131. https://doi.org/10.1007/s00442-014-3100-x

Hart, N. S., Partridge, J. C., Cuthill, I. C., & Bennett, A. T. D. (2000). Visual pigments, oil droplets, ocular media and cone photoreceptor distribution in two species of passerine bird: The blue tit (*Parus caeruleus*) and the blackbird (*Turdus merula*). *Comparative Biochemistry and Physiology B Comparative Biochemistry*, *186*, 375.

Haussmann, M. F., Longenecker, A. S., Marchetto, N. M., Juliano, S. A., & Bowden, R. M. (2012). Embryonic exposure to corticosterone modifies the juvenile stress response, oxidative stress and telomere length. *Proceedings of the Royal Society B: Biological Sciences*, *279*, 1447–1456. https://doi.org/10.1098/rspb.2011.1913

Helfenstein, F., Losdat, S., Møller, A. P., Blount, J. D., & Richner, H. (2010). Sperm of colourful males are better protected against oxidative stress. *Ecology Letters*, *13*, 213–222. https://doi.org/10.1111/j.1461-0248.2009.01419.x

Henderson, L. J., Heidinger, B. J., Evans, N. P., & Arnold, K. E. (2013). Ultraviolet crown coloration in female blue tits predicts reproductive success and baseline corticosterone. *Behavioral Ecology*, *24*, 1299–1305. https://doi.org/10.1093/beheco/art066

Herborn, K. A., Heidinger, B. J., Boner, W., Noguera, J. C., Adam, A., Daunt, F., & Monaghan, P. (2014). Stress exposure in early post-natal life reduces telomere length: An experimental demonstration in a long-lived seabird. *Proceedings of the Royal Society B: Biological Sciences*, *281*, 20133151.

Hulbert, A. J., Pamplona, R., Buffenstein, R., & Buttemer, W. A. (2007). Life and death: Metabolic rate, membrane composition, and life span of animals. *Physiological Reviews*, *87*, 1175–1213. https://doi.org/10.1152/physrev.00047.2006

Hunter, F. M., & Birkhead, T. R. (2002). Sperm viability and sperm competition in insects. *Current Biology*, *12*, 121–123. https://doi.org/10.1016/S0960-9822(01)00647-9

Isaksson, C., & Andersson, S. (2008). Oxidative stress does not influence carotenoid mobilization and plumage pigmentation. *Proceedings of the Royal Society of London B Biological Sciences*, *275*, 309–314. https://doi.org/10.1098/rspb.2007.1474

Jacot, A., Romero-Diaz, C., Tschirren, B., Richner, H., & Fitze, P. (2010). Dissecting carotenoid from structural components of carotenoid-based coloration: A field experiment with great tits (*Parus major*). *The American Naturalist*, *176*, 55–62. https://doi.org/10.1086/653000

Kahar, S., Debes, P. V., Vuori, K. A., Vähä, J.-P., & Vasemägi, A. (2016). Heritability, environmental effects, and genetic and phenotypic correlations of oxidative stress resistance-related enzyme activities during early life stages in Atlantic salmon. *Evolutionary Biology*, *43*, 215–226. https://doi.org/10.1007/s11692-016-9368-5

Kelber, A., & Osorio, D. (2010). From spectral information to animal colour vision: Experiments and concepts. *Proceedings of the Royal Society of London B: Biological Sciences*, *277*, 1617–1625. https://doi.org/10.1098/rspb.2009.2118

Kim, S. Y., Noguera, J. C., Morales, J., & Velando, A. (2010). Heritability of resistance to oxidative stress in early life. *Journal of Evolutionary Biology*, *23*, 769–775. https://doi.org/10.1111/j.1420-9101.2010.01942.x

Kim, S. Y., Velando, A., Sorci, G., & Alonso-Alvarez, C. (2010). Genetic correlation between resistance to oxidative stress and reproductive lifespan in a bird species. *Evolution*, *64*, 852–857. https://doi.org/10.1111/j.1558-5646.2009.00862.x

Koch, R. E., & Hill, G. E. (2017). An assessment of techniques to manipulate oxidative stress in animals. *Functional Ecology*, *31*, 9–21. https://doi.org/10.1111/1365-2435.12664

Kraaijeveld, K., Kraaijeveld-Smit, F. J., & Komdeur, J. (2007). The evolution of mutual ornamentation. *Animal Behaviour*, *74*, 657–677. https://doi.org/10.1016/j.anbehav.2006.12.027

Losdat, S., Blount, J. D., Marri, V., Maronde, L., Richner, H., & Helfenstein, F. (2018). Data from: Effects of an early-life paraquat exposure on adult resistance to oxidative stress, plumage colour and sperm performance in a wild bird. *Dryad Digital Repository*, https://doi.org/10.5061/dryad.8n5r3v1

Losdat, S., Helfenstein, F., Blount, J. D., Marri, V., Maronde, L., & Richner, H. (2013). Nestling erythrocyte resistance to oxidative stress predicts fledging success but not local recruitment in a wild bird. *Biology Letters*, *9*, 20120888.

Losdat, S., Helfenstein, F., Blount, J., & Richner, H. (2014). Resistance to oxidative stress shows low heritability and high common environmental variance in a wild bird. *Journal of Evolutionary Biology*, *27*, 1990–2000. https://doi.org/10.1111/jeb.12454

Losdat, S., Helfenstein, H., Gaude, B., & Richner, H. (2011). Reproductive effort transiently reduces antioxidant capacity in a wild bird. *Behavioral Ecology*, *22*, 1218–1226. https://doi.org/10.1093/beheco/arr116

Losdat, S., Richner, H., Blount, J. D., & Helfenstein, F. (2011). Immune activation reduces sperm quality in the great tit. *PLoS ONE*, *6*, e22221. https://doi.org/10.1371/journal.pone.0022221

Magrath, R. D. (1990). Hatching asynchrony in altricial birds. *Biological Reviews*, *65*, 587–622. https://doi.org/10.1111/j.1469-185X.1990.tb01239.x

Maia, R., Eliason, C. M., Bitton, P. P., Doucet, S. M., & Shawkey, M. D. (2013). pavo: An R package for the analysis, visualization and organization of spectral data. *Methods in Ecology and Evolution*, *4*, 906–913.

Marasco, V., Spencer, K. A., Robinson, J., Herzyk, P., & Costantini, D. (2013). Developmental post-natal stress can alter the effects of pre-natal stress on the adult redox balance. *General and Comparative Endocrinology*, *191*, 239–246. https://doi.org/10.1016/j.ygcen.2013.07.003

Marri, V., & Richner, H. (2015). Immune response, oxidative stress and dietary antioxidants in great tit nestlings. *Comparative Biochemistry and Physiology Part A: Molecular and Integrative Physiology*, *179*, 192–196. https://doi.org/10.1016/j.cbpa.2014.10.013

Marshall, J. D., & Uller, T. (2007). When is a maternal effect adaptive? *Oikos*, *116*, 1957–1963. https://doi.org/10.1111/j.2007.0030-1299.16203.x

Metcalfe, N. B., & Alonso-Alvarez, C. (2010). Oxidative stress as a life-history constraint: The role of reactive oxygen species in shaping phenotypes from conception to death. *Functional Ecology*, *24*, 984–996. https://doi.org/10.1111/j.1365-2435.2010.01750.x

Monaghan, P. (2008). Early growth conditions, phenotypic development and environmental change. *Philosophical Transactions of the Royal Society of London B Biological Sciences*, *363*, 1635–1645. https://doi.org/10.1098/rstb.2007.0011

Monaghan, P., Metcalfe, N. B., & Torres, R. (2009). Oxidative stress as a mediator of life history trade-offs: Mechanisms, measurements and interpretation. *Ecology Letters*, *12*, 75–92. https://doi.org/10.1111/j.1461-0248.2008.01258.x

Mora, A. R., Firth, A., Blareau, S., Vallat, A., & Helfenstein, F. (2017). Oxidative stress affects sperm performance and ejaculate redox status in subordinate house sparrows. *The Journal of Experimental Biology*, *220*, 2577–2588. https://doi.org/10.1242/jeb.154799

Noguera, J. C., Kim, S.-Y., & Velando, A. (2011). Pre-fledgling oxidative damage predicts recruitment in a long-lived bird. *Biology Letters*, *8*, 61–63.

Noguera, J. C., Monaghan, P., & Metcalfe, N. B. (2015). Interactive effects of early and later nutritional conditions on the adult antioxidant defence system in zebra finches. *Journal of Experimental Biology*, *218*, 2211–2217. https://doi.org/10.1242/jeb.120956

Norte, A. C., Sheldon, B. C., Sousa, J. P., & Ramos, J. A. (2009). Environmental and genetic variation in body condition and blood profile of great tit *Parus major* nestlings. *Journal of Avian Biology*, *40*, 157–165. https://doi.org/10.1111/j.1600-048X.2008.04461.x

van Oers, K., Drent, P. J., Dingemanse, N. J., & Kempenaers, B. (2008). Personality is associated with extrapair paternity in great tits, *Parus major*. *Animal Behaviour*, *76*, 555. https://doi.org/10.1016/j.anbehav.2008.03.011

Parker, G. A. (1970). Sperm competition and its evolutionary consequences in the insects. *Biological Reviews*, *45*, 525–567. https://doi.org/10.1111/j.1469-185X.1970.tb01176.x

Parolini, M., Khoriauli, L., Possenti, C. D., Colombo, G., Caprioli, M., Santagostino, M., … Saino, N. (2017). Yolk vitamin E prevents oxidative damage in gull hatchlings. *Royal Society Open Science*, *4*, 170098.

Pasinelli, G., & Walters, J. R. (2002). Social and environmental factors affect natal dispersal and philopatry of male red-cockaded woodpeckers. *Ecology*, *83*, 2229–2239. https://doi.org/10.1890/0012-9658(2002)083[2229:SAEFAN]2.0.CO;2

Pérez-Rodríguez, L. (2009). Carotenoids in evolutionary ecology: Re-evaluating the antioxidant role. *BioEssays*, *31*, 1116–1126. https://doi.org/10.1002/bies.200900070

Peters, A. (2007). Testosterone and carotenoids: An integrated view of trade-offs between immunity and sexual signalling. *BioEssays*, *29*, 427–430. https://doi.org/10.1002/(ISSN)1521-1878

Peters, A., Kurvers, R. H. J. M., Roberts, M. L., & Delhey, K. (2011). No evidence for general condition-dependence of structural plumage colour in blue tits: An experiment. *Journal of Evolutionary Biology*, *24*, 976–987. https://doi.org/10.1111/j.1420-9101.2011.02229.x

Pizzari, T., & Parker, G. A. (2009). Sperm competition and sperm phenotype. In T. R. Birkhead, D. J. Hosken & S. Pitnick (Eds.), *Sperm biology: An evolutionary perspective* (pp. 207–245). San Diego, California: Academic Press. https://doi.org/10.1016/B978-0-12-372568-4.00006-9

R Core Team. (2016). *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing.

Rojas Wahl, R. U., Liansheng, Z., Madison, S. A., DePinto, R. L., & Shay, B. J. (1998). Mechanistic studies on the decomposition of water soluble azo-radical-initiators. *Journal of the Chemical Society, Perkin Transactions*, *2*, 2009–2018. https://doi.org/10.1039/a801624k

Romero-Haro, A. A., & Alonso-Alvarez, C. (2015). The level of an intracellular antioxidant during development determines the adult phenotype in a bird species: A potential organizer role for glutathione. *The American Naturalist*, *185*, 390–405. https://doi.org/10.1086/679613

Romero-Haro, A. A., Sorci, G., & Alonso-Alvarez, C. (2016). The oxidative cost of reproduction depends on early development oxidative stress and sex in a bird species. *Proceedings of the Royal Society B: Biological Sciences*, *283*, 20160842.

Rubenstein, D. R., Skolnik, H., Berrio, A., Champagne, F. A., Phelps, S., & Solomon, J. (2016). Sex-specific fitness effects of unpredictable early life conditions are associated with DNA methylation in the avian glucocorticoid receptor. *Molecular Ecology*, *25*, 1714–1728. https://doi.org/10.1111/mec.13483

Saks, L., McGraw, K., & Hõrak, P. (2003). How feather colour reflects its carotenoid content. *Functional Ecology*, *17*, 555–561. https://doi.org/10.1046/j.1365-2435.2003.00765.x

von Schantz, T., Bensch, S., Grahn, M., Hasselquist, D., & Wittzell, H. (1999). Good genes, oxidative stress and condition-dependent sexual signals. *Proceedings of the Royal Society of London B Biological Sciences*, *266*, 1–12. https://doi.org/10.1098/rspb.1999.0597

Sies, H. (1991). *Oxidative stress: Oxidants and antioxidants*. London, UK: London Academic Press.

Simons, M. J. P., Cohen, A. A., & Verhulst, S. (2012). What does carotenoid-dependent coloration tell? Plasma carotenoid level signals immunocompetence and oxidative stress state in birds–a meta-analysis. *PLoS ONE*, *7*, e43088. https://doi.org/10.1371/journal.pone.0043088

Stier, A., Reichert, S., Massemin, S., Bize, P., & Criscuolo, F. (2012). Constraint and cost of oxidative stress on reproduction: Correlative evidence in laboratory mice and review of the literature. *Frontiers in Zoology*, *9*, 1.

Studds, C. E., Kyser, T. K., & Marra, P. P. (2008). Natal dispersal driven by environmental conditions interacting across the annual cycle of a migratory songbird. *Proceedings of the National Academy of Sciences*, *105*, 2929–2933. https://doi.org/10.1073/pnas.0710732105

Surai, P., & Fisinin, V. (2013). Natural antioxidants in hens' embryogenesis and antistress defence in postnatal development. *Sel'skokhozyaistven Biologiya*, *2*, 3–18.

Svensson, P. A., & Wong, B. B. M. (2011). Carotenoid-based signals in behavioural ecology: A review. *Behaviour*, *148*, 131–189. https://doi.org/10.1163/000579510X548673

Tremellen, K. (2008). Oxidative stress and male infertility - a clinical perspective. *Human Reproduction Update*, *14*, 243–258. https://doi.org/10.1093/humupd/dmn004

Velando, A., Torres, R., & Alonso-Alvarez, C. (2008). Avoiding bad genes: Oxidatively damaged DNA in germ line and mate choice. *BioEssays*, *30*, 1212–1219. https://doi.org/10.1002/bies.20838

Whittingham, L. A., & Dunn, P. O. (2016). Experimental evidence that brighter males sire more extra-pair young in tree swallows. *Molecular Ecology*, *25*, 3706–3715. https://doi.org/10.1111/mec.13665

Whittingham, M. J., Stephens, P. A., Bradbury, R. B., & Freckleton, R. P. (2006). Why do we still use stepwise modelling in ecology and behaviour? *Journal of Animal Ecology*, *75*, 1182–1189. https://doi.org/10.1111/j.1365-2656.2006.01141.x

Wilkin, T. A., King, L. E., & Sheldon, B. C. (2009). Habitat quality, nestling diet, and provisioning behaviour in great tits *Parus major*. *Journal of Avian Biology*, *40*, 135–145. https://doi.org/10.1111/j.1600-048X.2009.04362.x

Wilkin, T. A., & Sheldon, B. C. (2009). Sex differences in the persistence of natal environmental effects on life histories. *Current Biology*, *19*, 1998–2002. https://doi.org/10.1016/j.cub.2009.09.065

Wilson-Leedy, J. G., & Ingermann, R. L. (2007). Development of a novel CASA system based on open source software for characterization of zebrafish sperm motility parameters. *Theriogenology*, *67*, 661–672. https://doi.org/10.1016/j.theriogenology.2006.10.003

Wolfson, A. (1952). The cloacal protuberance: A means for determining breeding condition in live male passerines. *Bird-Banding*, *23*, 159–165. https://doi.org/10.2307/4510381

Zimmer, C., & Spencer, K. A. (2015). Reduced resistance to oxidative stress during reproduction as a cost of early-life stress. *Comparative Biochemistry and Physiology A Comparative Physiology*, *183*, 9–13. https://doi.org/10.1016/j.cbpa.2014.12.032

Zou, C.-G., Agar, N. S., & Jones, G. L. (2001). Oxidative insult to human red blood cells induced by free radical initiator AAPH and its inhibition by a commercial antioxidant mixture. *Life Sciences*, *69*, 75–86. https://doi.org/10.1016/S0024-3205(01)01112-2

How to cite this article: Losdat S, Blount JD, Marri V, Maronde L, Richner H, Helfenstein F. Effects of an early-life paraquat exposure on adult resistance to oxidative stress, plumage colour and sperm performance in a wild bird. *J Anim Ecol.* 2018;87:1137–1148. https://doi.org/10.1111/1365-2656.12822