

1 **Antioxidant capacity is repeatable across years but does not consistently**
2 **correlate with a marker of peroxidation in a free-living passerine bird.**

3 Running title: Correlation of oxidative state markers

4

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21 **Authors' contributions**

22 C.R., B.D. and P.B. designed the study. C.R. carried out the fieldwork. C.R. and M.A.
23 performed the laboratory analyses and analysed the data. C.R., B.D. and P.B. drafted the
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41

42 **Abstract**

43 Oxidative stress occurs when reactive oxygen species (ROS) exceed antioxidant defences,
44 which can have deleterious effects on cell function, health and survival. Therefore, organisms
45 are expected to finely regulate pro-oxidant and antioxidant processes. ROS are mainly
46 produced through aerobic metabolism and vary in response to changes in energetic
47 requirements, whereas antioxidants may be enhanced, depleted or show no changes in
48 response to changes in ROS levels. We investigated the repeatability, within-individual
49 variation and correlation across different conditions of two plasmatic markers of the oxidative
50 balance in 1108 samples from 635 free-living adult collared flycatchers (*Ficedula albicollis*). We
51 sought to manipulate energy constraints by increasing wing load in 2012 and 2013 and by
52 providing additional food in 2014. We then tested the relative importance of within- and
53 between-individual variation on reactive oxygen metabolites (ROMs), a marker of lipid and
54 protein peroxidation, and on non-enzymatic antioxidant defences (OXY test). We also
55 investigated whether the experimental treatments modified the correlation between markers.
56 Antioxidant defences were repeatable (range of repeatability estimates = 0.128 - 0.581),
57 whereas ROMs were not (0 - 0.061). Antioxidants varied neither between incubation and
58 nestling feeding nor between sexes. ROMs increased from incubation to nestling feeding in
59 females and were higher in females than males. Antioxidant defences and ROM concentration
60 were globally positively correlated, but the correlation varied between experimental
61 conditions and between years. Hence, the management of oxidative balance in wild animals
62 appears flexible under variable environmental conditions, an observation which should be
63 confirmed over a wider range of markers.

64 Keywords: energetic constraints, food supplementation, reactive oxygen metabolites,
65 antioxidant defences, oxidative stress, *Ficedula albicollis*

66

67 **Introduction**

68 Reactive oxygen species (ROS) produced during aerobic respiration are important actors of
69 cell signalling pathways and immune responses (Valko et al. 2007; Finkel 2011; Sena and
70 Chandel 2012; Schieber and Chandel 2014), but can have deleterious effects by oxidising
71 macromolecules and thereby disrupting cell function (Avery 2014). Oxidative stress, resulting
72 from an excess of ROS relative to antioxidant defences, is thus proposed as a credible
73 mechanism underlying life-history trade-offs (Costantini 2008; Monaghan et al. 2009; Metcalfe
74 and Alonso-Alvarez 2010). To understand the potential role of oxidative stress as a constraint
75 on life-history traits, it is crucial to understand how organisms co-regulate pro- and anti-
76 oxidant molecules. There is no *a priori* expectation on how measures of antioxidant defences
77 should relate to measures of ROS levels and oxidative damages (Costantini and Verhulst 2009).
78 The antioxidant capacity could be modulated to counteract the deleterious effects of ROS, thus
79 resulting in a redox balance. Such compensation by an increase in antioxidant in response to
80 ROS production would lead to a positive, or an absence of, correlation. Conversely, a deficit
81 of energetic resources or dietary antioxidants might constrain antioxidant defences and thus
82 decrease their ability to counteract ROS effects. If circulating antioxidants are then used up to
83 protect the organism against increased reactive oxygen species, they should covary negatively
84 with ROS production or oxidative damages. However, it is not clear whether antioxidant
85 protection, especially through antioxidant enzymes, is energetically costly (Speakman and
86 Krol 2010; Isaksson et al. 2011; Gems and Partridge 2013). Even direct experimental
87 manipulations of dietary antioxidants rarely impacted antioxidant defences in the expected
88 direction (Costantini and Møller 2008; Cohen et al. 2009; Archer et al. 2015; Cecere et al. 2016;
89 Vaanholt et al. 2016; Costantini et al. 2018), despite some links in correlative studies
90 (Talegawkar et al. 2009; Wang et al. 2012; Yang et al. 2013b) and some experimental studies
91 (Beaulieu et al. 2016). Overall, results are often conflicting when different dietary antioxidants

92 are considered simultaneously (Cohen et al. 2009; Talegawkar et al. 2009), suggesting
93 compensation mechanisms between different types of antioxidant defences.

94 Variation in ROS production is also insufficiently understood. ROS are often expected to
95 increase in response to increased metabolism, but the generality of this relationship is far from
96 clear (Speakman and Selman 2011). A higher metabolic and respiratory rate might not be
97 associated with a higher ROS production (Barja 2007; Glazier 2015; Salin et al. 2015b). First, the
98 natural or experimental inhibition of mitochondrial respiration simultaneously slows down
99 the electron flow through the electron transport chain, which is then in a reduced state, and
100 increases the intra-mitochondrial concentration of oxygen, two mechanisms which promote
101 ROS production (Bonawitz et al. 2007; Salin et al. 2015a). Second, uncoupling proteins (UCPs)
102 and changes in the mitochondrial inner membrane structure modulate the inner membrane
103 conductance to protons and in turn the relationship between energy consumption and ROS
104 production (Brand 2000; Criscuolo et al. 2005; Stier et al. 2014a).

105 The interpretation of oxidative stress measures in ecological studies requires a better
106 understanding of their variability in the wild and the actual co-variation between markers, as
107 well as the link between these markers and fitness. In particular, relationships between traits
108 can differ greatly when measured at the within-individual level, where life-history trade-offs
109 can be expressed, or at the between-individual level, where differences in individual quality
110 or permanent environment are expected to play a large role (Stearns 1989; Wilson and Nussey
111 2009). To explore these questions, we measured two plasmatic markers of oxidative state,
112 namely (i) reactive oxygen metabolites, mainly driven by organic hydroperoxides acting as
113 precursors of long-term oxidative damage, through the d-ROM test and (ii) non-enzymatic
114 antioxidant capacity through the OXY test, in adults of a small passerine bird, the collared
115 flycatcher *Ficedula albicollis* (Temminck, 1815), during three consecutive breeding seasons.
116 These two markers, frequently used in wild bird populations, are sensitive to manipulations
117 of energy expenditure or mitochondrial ROS production (Stier et al. 2014b; Vaugoyeau et al.

118 2015; Récapet et al. 2016c, 2017). They have been linked to dietary antioxidant intake in some
119 studies (Beaulieu and Schaefer 2014), although the relationship was often absent (Marri and
120 Richner 2015; Leclaire et al. 2015; Cecere et al. 2016) or counter-intuitive (Costantini et al. 2007;
121 Vaugoyeau et al. 2015). These markers also depend on food and habitat quality (van de
122 Crommenacker et al. 2011; Isaksson 2013; Lendvai et al. 2014; Beaulieu et al. 2015b; Giordano
123 et al. 2015; but see Costantini 2010), as well as reproductive effort (Beaulieu et al. 2011; Markó
124 et al. 2011; Stier et al. 2012; Reichert et al. 2014; Wegmann et al. 2015b). Links to fitness
125 outcomes were found in some studies (Geiger et al. 2012; Herborn et al. 2016), but overall
126 results remain mixed (see Stier et al. 2012 for a review of the links with reproductive success;
127 Boonekamp et al. 2018). Whether variation of these markers in natural populations is driven
128 by environmental variation or permanent individual differences, in particular related to
129 differences in overall fitness or life-history strategies, thus remains unclear. Although markers
130 of oxidative state are often variable across tissues (Veskoukis et al. 2009), we focused on
131 plasma here because (i) it can be repeatedly sampled within and between years and (ii) we can
132 expect quick variation of oxidative balance in the blood in response to energetic constraints
133 (Nikolaidis et al. 2008). We first estimated the repeatability of oxidative state markers within
134 the same season, as well as between seasons. Then we assessed the correlation between these
135 markers while controlling for differences between years or between sexes that can spuriously
136 generate strong correlations between markers (Christensen et al. 2015). If non-enzymatic
137 antioxidants are constrained by availability in the diet, we expect a negative correlation
138 between antioxidant capacity and reactive oxygen metabolites and this negative correlation
139 should be stronger when feeding conditions are poorer. Conversely, if non-enzymatic
140 antioxidants are up regulated at low costs in response to increased ROS production, we expect
141 a positive correlation between these two markers, independently of the feeding conditions.

142 To experimentally test the effect of energetic and nutritional constraints on oxidative state,
143 we manipulated wing load in breeding females (i.e. handicapped females) to increase their

144 energy expenditure in 2012 and 2013. Measures of field metabolic rate on a subset of females
145 showed that this manipulation was effective at increasing energy expenditure, although we
146 cannot determine the exact contribution of behavioural and metabolic changes (Récapet et al.
147 2016c). In another year (2014), we food supplemented breeding pairs during the nestling
148 feeding stage to alleviate nutritional constraints and foraging energy expenditure. Less than
149 40% of the data analysed in this study (264 observations out of 663 for ROMs and 316 out of
150 828 for antioxidant capacity) have already been used as separate variables to investigate
151 differences in the management of reproductive trade-offs between dispersing and philopatric
152 individuals (Récapet et al. 2016c, 2017). Here, we are focusing on the impact of energetic
153 constraints on the correlation between the two markers and to this end we pooled together
154 measures on birds of known and unknown dispersal status.

155

156 **Material and methods**

157 *Study population*

158 The study was conducted during spring 2012 to 2014 in a natural population of collared
159 flycatchers breeding on the island of Gotland, Sweden (57°07'N, 18°20'E). This hole-nesting
160 bird readily breeds in the artificial nest boxes erected in the nine forest plots used for the study
161 (between 13 and 78 nest boxes per plot distributed homogeneously in space). Nests were
162 visited regularly to estimate laying date, clutch size and hatching date. Females were first
163 caught during incubation (on average \pm S.D. 7.2 ± 1.2 days after the start of incubation), before
164 the start of any experimental treatment, then males and females were both caught when
165 feeding nestlings (on average when nestlings were 9.0 ± 2.1 days old). For females, the interval
166 between both captures was thus 13.6 ± 2.5 days on average (\pm S.D.). Despite the potential
167 interest of measuring short-term repeatability in blood parameters, blood samples for this
168 study were only taken once at each stage to avoid the risk of taking a larger amount of blood

169 than advised by ethical guidelines and to reduce the negative impact of such disturbance on
170 reproductive success. Upon capture, birds were ringed if previously unringed and blood
171 sampled (see below).

172

173 *Experimental manipulations of energetic constraints*

174 In 2012 and 2013, we increased energetic constraints on females from the second half of the
175 incubation period by cutting the two innermost primaries of each wing at their base, to mimic
176 feather loss at the onset of moult (Moreno et al. 1999; Sanz et al. 2000; Ardia and Clotfelter
177 2007; Récapet et al. 2016c). Upon capture during incubation, females were assigned to the
178 manipulated (handicapped) or the control group (same handling conditions but no feathers
179 cut) alternatively by blocks of two females to avoid the two experimental groups to differ in
180 treatment date. The manipulation was successful at increasing female energy expenditure,
181 measured through the doubly-labelled water method (Récapet et al. 2016c). We did not
182 manipulate the wing load of males in this experiment and found no effect of our manipulation
183 on males, for example through compensatory behaviour (Récapet et al. 2016c).

184 Conversely, in 2014, we relieved energetic constraints on both parents during the
185 nestling feeding period by providing additional food (Récapet et al. 2016a, 2017). When
186 nestlings were two days old, transparent plastic containers were attached to the front side of
187 the nest box. For supplemented pairs, 30g live maggots (larvae of *Calliphora erythrocephala*, Fibe
188 AB, Kungsängen, Sweden) were placed in the containers once a day until nestlings were 12
189 days old (i.e. over a total of 11 days). This corresponded to approx. 150 individual larvae of
190 200mg per day, i.e. 25 larvae per nestling for a brood of 6 nestlings. Thus, the food
191 supplementation spared about half of the approx. 360 daily parental visits estimated in control
192 nests in the same year (Récapet et al. 2017). Control pairs received no food, but were visited
193 daily to control for human disturbance. Pairs were assigned either to the control or

194 supplemented group alternatively in space for a given hatching date, so as to distribute
195 treatments homogenously in space both within and between study plots and in time within
196 the breeding season.

197

198 *Measures of markers of the oxidative balance*

199 To measure blood markers of oxidative state, a 40 μ L blood sample was taken from the brachial
200 vein into heparin-coated Microvettes (Sarstedt, Nümbrecht, Germany). Blood samples were
201 maintained at 5°C in the field before being centrifuged in the evening to separate plasma from
202 red blood cells. Plasma and red blood cells were then stored at -80°C until being analysed in
203 the laboratory. A total of 860 blood samples was collected on nestling feeding males and
204 females, and 256 on incubating females. Because of the low amount of plasma available for
205 each sample, we restricted our laboratory analyses to two oxidative state markers: reactive
206 oxygen metabolites (ROMs) concentration and plasma antioxidant capacity, following
207 protocols adapted to small samples (Récapet et al. 2016c, 2017). Each sample was analysed on
208 the same day in the laboratory for both ROM concentration and antioxidant capacity to avoid
209 freeze-thaw cycles. Samples were distributed randomly on 96-well plates relative to breeding
210 pair, breeding area or laying date. The samples collected in different years were analysed in
211 different years, so potential differences between years in ROM and OXY levels might partly
212 reflect experimental variation and are thus not interpreted as possible biological effects later
213 on. When studying correlations, these variables were however standardized within years, so
214 that differences in their correlation coefficient could not stem from between-year differences.

215 Plasma concentration of ROMs was measured using the d-ROMs test (MC0001 kit,
216 Diacron International, Grosseto, Italy). Haemolysed samples with a light orange or pink to
217 bright red colouration were excluded visually, as well as opaque hyperlipemic samples with
218 $OD_{800nm} > 0.100$ (N = 229, out of a total of 1116 samples). ROMs were measured on 5 to 7
219 different 96-well plates each year. The intra-plate repeatability [95% CI] was 0.797 [0.67; 0.897]

220 on 24 duplicates and the inter-plate repeatability was 0.694 [0.579; 0.787] on 28 duplicates
221 (calculated using the rptR package version 0.9.2; Nakagawa and Schielzeth 2010). Dietary
222 hydroperoxides are degraded in the stomach (Kanazawa and Ashida 1998) and have a low
223 uptake by intestinal cells (Maestre et al. 2013). Their contribution to plasma ROM
224 concentration is most likely low. Plasmatic ROM concentration might however be influenced
225 by the concentration of triglycerides in the plasma (Pérez-Rodríguez et al. 2015). Preliminary
226 analyses on a subset of our samples provide no support for such association in our study
227 species, and thus we did not control for the concentration of triglycerides in our statistical
228 analyses (Appendix 1).

229 Plasma antioxidant capacity was measured by the capacity of plasma to oppose the
230 oxidative action of the hypochlorous acid HClO (OXY adsorbent test, MC434 kit, Diacron
231 International, Grosseto, Italy). This measure reflects the concentrations of ascorbate (vitamin
232 C), flavonoids, carotenoids, glutathione and albumin, which are efficient scavengers of HClO
233 (Folkes et al. 1995; de Groot and Rauen 1998; Pennathur et al. 2010), and to a lesser extent
234 tocopherols (vitamin E) and ubiquinol which are less reactive toward hypochlorous acid (Carr
235 et al. 2001; Pattison et al. 2003; Nguyen and Southwell-Keely 2007; Niki 2014; Chen et al. 2016).
236 We chose this assay because it is less sensitive to variations in uric acid concentration in the
237 plasma than other methods (Costantini 2011). Antioxidant capacity was measured on 5
238 different 96-well plates each year. The intra-plate repeatability [bootstrap 95% CI] was 0.914
239 [0.876, 0.946] on 30 duplicates and the inter-plate repeatability was 0.858 [0.802, 0.901] on 24
240 duplicates.

241 Due to various technical problems during sample preparation, conservation and
242 during laboratory assays, ROM concentration could not be measured for 11 out of 887 samples
243 of adequate quality and plasma antioxidant capacity could not be measured for 27 out of 1116
244 samples. The total sample size was thus $N = 876$ for ROM concentration and $N = 1089$ for
245 plasma antioxidant capacity. For correlation analyses, the sample size with data for both

246 markers was $N = 857$. More detailed sample sizes by years, breeding stages, sexes and
247 experimental treatments can be found in tables 1 to 4.

248

249 *Statistical analyses*

250 First, to assess the inter-annual repeatability of ROM and antioxidant capacity markers,
251 we used samples collected on different years but at the same breeding stage (incubation or
252 nestling feeding) for a given individual. We ran a linear mixed-effect model for each breeding
253 stage because only females were sampled at the incubation stage whereas both males and
254 females were sampled at the nestling feeding stage. We included individual identity (nested
255 within sex) and plate (nested within year) as random factors, and year as a fixed factor.
256 Individuals for which only one measure was available were included in the models to improve
257 the estimates for residual variance (Martin et al. 2011). Previous studies have not shown any
258 strong effect of wing load manipulation on antioxidant capacity or reactive oxygen metabolites
259 (Wegmann et al. 2015a; Récapet et al. 2016c, 2017), so data from manipulated individuals were
260 included in the repeatability analyses while still including an effect of the experimental
261 manipulation to account for the fact that previous analyses were based on smaller samples.
262 The effects of sex (two-level factor) and experimental manipulations (three-level factor:
263 “control”, “wing load manipulation”, “food supplementation”), as well as their interaction,
264 were included in the models describing the markers during nestling feeding. Second, to assess
265 the repeatability of the oxidative state markers (ROM concentration and antioxidant capacity)
266 measured at different breeding stages (incubation and nestling feeding) within the same year
267 in a given female, we used linear mixed-effect models with individual identity within a year,
268 individual identity within a breeding stage across years (to account for the potential
269 correlation between measures of an individual at a given breeding stage between years), and
270 plate (nested within year) as random factors, and year, breeding stage, manipulation and the
271 interaction of breeding stage and manipulation as fixed factors, on females sampled during

272 incubation and/or nestling feeding. Repeatability was calculated as the ratio of individual
273 (resp. individual within year) random variance on the sum of individual (resp. individual
274 within year), between-plate and residual variances. This allowed us to calculate repeatability
275 estimates adjusted for measurement errors, by multiplying the between-plates and residual
276 variances by the technical repeatability, to better reflect the actual biological variance.
277 Confidence interval for these estimates were calculated through 1000 parametric bootstraps
278 and we tested whether the repeatability differed from zero through a likelihood-ratio test with
279 a mixture distribution of Chi-square distributions with zero and one degree of freedom as
280 reference, using the *rptR* package version 0.9.2 (Nakagawa and Schielzeth 2010).

281 Including body mass as a covariate in any of the models above did not change the
282 variance estimates, and there was no significant effect of body mass, thus the models reported
283 here do not include body mass. The parameters of the univariate models for the repeatability
284 analyses were estimated by restricted maximum likelihood (REML) using the *lmer* function in
285 R (Bates et al. 2014). The significance of the fixed effects was tested using F-tests with
286 Satterthwaite estimation for the denominator degree of freedom, using the function *anova* from
287 the *lmerTest* library (Kuznetsova et al. 2016).

288 To investigate the correlation between ROM concentration and antioxidant capacity,
289 the two variables were modelled as response variables in bivariate mixed-effect models with
290 plate as distinct random factors for antioxidant capacity and ROM concentration, as well
291 individual identity as a common random factor when pooling multiple years. This allowed us
292 to estimate the covariance, and thus correlation coefficients, at the between-individual and
293 within-individual (residual) levels, while correcting for the random structure of both
294 variables. The response variables were standardized to mean zero and variance one within
295 each year, treatment and sex to account for potential differences in mean and variance between
296 these groups. The covariance was estimated at the between- and within-individual levels
297 when studying multiple years together. The total phenotypic variance-covariance matrix was

298 computed as the sum of the between- and within-individual variance-covariance matrices.
299 Only the phenotypic covariance is reported when there were less than 10 individuals with
300 multiple measures.

301 The parameters of the bivariate models were estimated in a Bayesian framework that
302 allowed us to fit different random effects for each response variable and to estimate their
303 covariance at the between- and within-individual levels. The priors for the fixed effects
304 estimates were set to a multinomial distribution with expected values of 0 and a diagonal
305 variance-covariance matrix with a low strength of belief (10^{10}). The priors were set to inverse-
306 Wishart distributions with the variances set to $1/n_i$ where n_i was the number of variance
307 components estimated for the parameter i , null covariances, and a degree of belief equal to the
308 dimension of the variance-covariance matrix for the parameter. Preliminary analyses showed
309 that the priors used for the covariances were quite informative on the posterior distribution of
310 the between- and within-individual correlations, but not on the total phenotypic correlation.
311 The analyses were performed with Markov chain Monte Carlo sampling using the
312 *MCMCglmm* function in R (Hadfield 2010), with 1020000 iterations, a burn-in period of 20000,
313 and a thinning interval of 500, to obtain autocorrelation values lower than 0.06 and an effective
314 sample size higher than 2000 for all correlation estimates. We reported the mode of the
315 posterior distribution as point estimate for the correlations and the Highest Posterior Density
316 as 95% credibility interval. All estimates passed convergence diagnostic tests using the
317 Cramer-von-Mises statistic with $P > 0.05$ (package CODA version 0.18-1; Plummer et al. 2006).

318 To test the effect of the wing load manipulation on the correlation between the two
319 markers, we compared two bivariate models describing the standardized markers in 2012 and
320 2013, one with homogeneous within-individual (residual) covariances according to the
321 experimental treatment and a second allowing for heterogeneous within-individual
322 covariances according to the experimental treatment. Similarly, we tested the effect of the food
323 supplementation on the correlation by comparing bivariate models for the standardized

324 markers in 2014 with or without heterogeneous covariances according to the experimental
325 treatment. Finally, the effect of temporal variation in the environment was tested by
326 comparing two models for the control groups in all years, with or without heterogeneous
327 covariances according to year. The Deviance Information Criterion was used as an indication
328 to compare models with different random variance structures, with a DIC difference larger
329 than five interpreted as a significantly better model (DIC; Spiegelhalter et al. 2002).

330

331 **Results**

332 *Inter-annual repeatability of OXY and ROM*

333 In females sampled during incubation in different years, the repeatability of antioxidant
334 capacity was significant (r [95% confidence interval] = 0.581 [0.327; 0.825]; Table 1, Fig. 1).
335 Considering that approximately 14.2% of the within-individual variance could be considered
336 measurement error in the context of this study, the biological repeatability was approximately
337 0.617. In contrast, the repeatability of ROM concentration was very low and did not
338 significantly differ from zero (0.032 [0; 0.660]; Table 1, Fig. 1). As measurement error accounted
339 for 30.6% of the within-individual variance, the biological repeatability corrected for
340 measurement error was approximately 0.046.

341 In males and females sampled during feeding in different years, the repeatability of
342 antioxidant capacity was low but significant (0.124 [0.018; 0.254], adjusted for measurement
343 error: 0.142; Table 1, Fig. 1), whereas the repeatability of ROM concentration did not
344 significantly differ from zero (0.061 [0; 0.257], adjusted for measurement error: 0.085; Table 1,
345 Fig. 1). There was no effect of the manipulations, even in interaction with sex, on antioxidant
346 capacity (manipulations x sex = $F_{2,796} = 0.08$, $P = 0.92$; manipulations: $F_{2,812} = 1.55$, $P = 0.21$;

347 Fig. 2) or ROM concentration (manipulations \times sex = $F_{2,640} = 0.17$, $P = 0.84$;
348 manipulations: $F_{2,650} = 0.41$, $P = 0.67$;

349 Fig. 3). During feeding, antioxidant capacity was independent of sex ($F_{1,646} = 0.39$, $P =$
350 0.54;

351 Fig. 2) but ROM concentration was lower in males than females (-0.095 ± 0.045 , $F_{1,512} =$
352 12.34, $P = 0.0005$;

353 Fig. 3).

354

355 *Intra-annual repeatability of OXY and ROM*

356 In females sampled at different breeding stages within the same year, the repeatability of
357 antioxidant capacity was low but significant (0.131 [0.030; 0.253], adjusted for measurement
358 error: 0.149; Table 1, Fig. 1), whereas the repeatability of ROM concentration between stages
359 was null and non-significant (<0.001 [0; 0.174], adjusted for measurement error: <0.001; Table
360 1, Fig. 1). Food supplementation had a positive effect on the increase in antioxidant capacity
361 between the incubation and breeding stage, although this effect was not very strong relative
362 to the overall variation in antioxidant capacity (interaction breeding stage x manipulations:
363 $F_{2,293} = 3.63, P = 0.028$;

364 Fig. 2). There was no effect on ROM concentration of the manipulations (

365 Fig. 3), either alone ($F_{2,519} = 1.08$, $P = 0.34$) or in interaction with the breeding stage ($F_{2,429} = 0.14$,
366 $P = 0.87$). ROM concentration was higher during the nestling feeding stage compared to the
367 incubation stage ($+0.165 \pm 0.058$, $F_{1,100} = 13.14$, $P = 0.0005$;

368 Fig. 3).

369

370 *Correlations between physiological markers*

371 Overall, there was a positive correlation at the phenotypic level between ROM concentration
372 and antioxidant capacity during nestling feeding (N = 527 individuals; n = 646 observations;
373 posterior mode [95% credibility interval]: $V_{\text{phenotypic}} = 0.102$ [0.028; 0.181]). Considering that
374 measurement error accounted for 14.2% (resp. 30.6%) of the within-individual variance in
375 antioxidant capacity (resp. ROM concentration) and that measurement errors were
376 uncorrelated between the two markers, the adjusted correlation would be approximately
377 0.125. The between-individual and within-individual correlations, when estimated separately,
378 were however not significantly different from zero ($V_{\text{between-individual}} = 0.242$ [-0.271; 0.618],
379 $V_{\text{within-individual}} = 0.099$ [-0.043; 0.193]). The strength of this phenotypic correlation differed
380 according to the experimental group: the correlation was stronger in wing load manipulated
381 (handicapped) females compared to control ones (model with heterogeneous covariances
382 according to manipulation compared to homogeneous covariances: $\Delta\text{DIC} = -12.6$; Table 2).
383 This effect seemed to be driven by a difference between experimental groups in 2013 ($V_{\text{phenotypic}}$
384 = 0.417 [0.126; 0.590] in handicapped females vs. -0.038 [-0.325; 0.208] in control females),
385 whereas there was no difference in 2012 ($V_{\text{phenotypic}} = -0.110$ [-0.421; 0.200] in handicapped
386 females vs. 0.081 [-0.291; 0.341] in control females), although measuring differences in within-
387 individual correlation due to the experimental treatment was impossible within a single year.
388 There was no difference in correlation between males whose females were handicapped and
389 controls ($\Delta\text{DIC} = -4.0$; Table 2). Conversely, there was no effect of the food supplementation in
390 2014 on the correlation between antioxidant capacity and ROM concentration, since the
391 positive correlation found in supplemented pairs was similar to that in control ones (model
392 with heterogeneous covariances according to food supplementation compared to
393 homogeneous covariances: $\Delta\text{DIC} = +4.6$; Table 3). Finally, in control pairs, the strength of the

394 correlation was also higher in 2014 compared to 2012 and 2013 (model with heterogeneous
395 covariances according to year compared to homogeneous covariances: $\Delta\text{DIC} = -26.3$; Table 4).
396 Evidence for a positive correlation between markers was weaker in incubating females
397 compared to nestling-feeding adults (Table 4).

398

399 **Discussion**

400 In this study, we aimed at describing the correlation structure between two plasmatic markers
401 of the oxidative balance and its variation in different natural and experimental conditions.
402 Individual identity and food availability were important determinants of antioxidant capacity
403 but did not influence ROM concentration. These two physiological markers covaried
404 positively. Importantly, the correlation between markers was stronger in experimentally
405 handicapped (wing-load manipulated) females and varied between years, while relieving
406 energetic constraints through food supplementation did not change the correlation. Overall,
407 our findings refute the existence of a stable correlation structure between these two widely
408 used markers. Context dependence should thus be better taken into account when interpreting
409 correlations between markers of oxidative stress.

410

411 *Stability and variation of individual markers*

412 Antioxidant capacity was repeatable between years at a given breeding stage, as well as within
413 year between incubation and nestling feeding stages (for females). The repeatability was
414 higher during incubation compared to nestling feeding (between years) or between stages
415 (within the same year), suggesting that uncontrolled environmental factors had a stronger
416 effect during nestling feeding. It thus seems that the between-year variation in antioxidant
417 capacity is partly determined by individual characteristics, perhaps including the ability to
418 find higher quality food rich in antioxidants, to acquire a better territory, or to produce more

419 enzymatic antioxidants and spare the dietary antioxidant pools. This could be due to genetic
420 differences or to permanent individual differences due to early-life effects. Similarly,
421 significant within-individual repeatability in antioxidant capacity among breeding seasons
422 was found in barn swallows *Hirundo rustica* ($r = 0.49$, $P < 0.001$, Saino *et al.* 2011), Seychelles
423 warblers *Acrocephalus sechellensis* ($r = 0.122$, $P = 0.043$, van de Crommenacker *et al.* 2011),
424 laboratory mice *Mus musculus* ($r = 0.65$, Stier *et al.* 2012) and European shags *Phalacrocorax*
425 *aristotelis*, although repeatability varied with age (2-9 years old: $r = 0.20$, $P = 0.36$; 10 to 22 years
426 old: $r = 0.33$, $P = 0.020$; Herborn *et al.* 2015). The lower repeatability at the nestling feeding
427 stage, or between breeding stages within the same year, might be partly explained by
428 individual variation in the response to environmental conditions, such as the increase in
429 antioxidant capacity in food-supplemented females between incubation and nestling feeding.
430 Previous experimental studies that manipulated energy expenditure or oxidative balance in
431 birds showed that measures of antioxidant capacity were only repeatable in control birds
432 (Meitern *et al.* 2013) or were not correlated across different treatments (Beamonte-Barrientos
433 and Verhulst 2013).

434 ROM concentration was not repeatable either between- or within-year; variations in ROM
435 concentration were thus mainly determined by external factors or physiological changes, apart
436 from a difference between males and females. The lower methodological reliability of ROM
437 measurements compared to antioxidant capacity, mainly due to low values of ROMs levels,
438 could also have reduced our ability to detect weak but biologically significant repeatability.
439 This result here contrasts with studies of ROMs that found individual consistency even under
440 different experimental treatments ($r = 0.38$ in laboratory mice, Stier *et al.* 2012; $r = 0.42$ in zebra
441 finches *Taeniopygia guttata*, Beamonte-Barrientos and Verhulst 2013; $r = 0.35$ for individuals
442 younger than 10 years but 0.13 for older ones in European shags, Herborn *et al.* 2016) although
443 some studies found similarly low repeatabilities ($r = 0.00$ in Seychelles warblers, van de
444 Crommenacker *et al.* 2011). Individual consistency in ROM measurements can however vary

445 with age (Herborn et al. 2016). ROM concentration was not influenced by the experimental
446 manipulations of energetic constraints (wing load manipulation or food supplementation).
447 These results contrast with the finding that food supplementation decreased ROM
448 concentration but had no effect on antioxidant capacity in breeding great tit females (Giordano
449 et al. 2015). In our study, ROM concentration strongly increased between incubation and the
450 nestling feeding period in females. The increase between breeding stages in females might
451 reflect the costs of reproduction: ROM concentration increased in breeding females, but not in
452 non-breeding ones, in mice (Stier et al. 2012) and in breeding female Seychelles warblers
453 naturally infected with malaria although the relationship was reversed in non-infected females
454 (van de Crommenacker et al. 2012). In Eurasian kestrel, such increase in ROMs between
455 mating and nestling rearing was only measured in males, not in females (Casagrande et al.
456 2011). Alternatively oxidative damage might be adaptively reduced in females during egg-
457 laying to avoid negative effects on offspring, a process called oxidative shielding (Giordano et
458 al. 2015; Blount et al. 2016; Vitikainen et al. 2016).

459

460 *A highly variable correlation structure*

461 Antioxidant capacity was positively correlated with ROM concentration when considering all
462 individuals irrespective of their experimental treatment. This positive correlation could not be
463 fully explained by permanent differences between individuals (van Noordwijk and de Jong
464 1986; Wilson and Nussey 2009) as it was also found at the within-individual level. It is
465 inconsistent with the hypothesis that the dynamics of protection against increased reactive
466 oxygen species are dominated by the depletion of a limited pool of available circulating
467 antioxidants. Indeed, in such a case, antioxidant defences and oxidative damages should
468 correlate negatively or be modified in opposite ways depending on individual and
469 environmental factors, such as was observed in several mammalian and bird species (Fletcher
470 et al. 2013; Yang et al. 2013a; Hanssen et al. 2013; López-Arrabé et al. 2014, 2015; Marri and

471 Richner 2015). Such relationships were however highly dependent of the tissues and markers
472 studied (Xu et al. 2014; Marasco et al. 2017). Regarding the two markers studied here, most
473 studies directly testing their correlation showed a positive or non-significant relationship
474 (Table 5), including when correlation was studied at the within-individual level (van de
475 Crommenacker et al. 2011, 2012). Differences in the same direction were also observed in
476 relation to individual characteristics, such as sex and breeding status (Stier et al. 2012; van de
477 Crommenacker et al. 2012; Isaksson 2013; Wegmann et al. 2015a; Silva et al. 2018). Changes
478 within individuals or in response to environmental or experimental conditions were mostly
479 unrelated (Costantini et al. 2007, 2010; Bonisoli-Alquati et al. 2010; Costantini 2010b; Beaulieu
480 et al. 2011; van de Crommenacker et al. 2011, 2012; Isaksson 2013; Wegmann et al. 2015b; Marri
481 and Richner 2015; Vaugoyeau et al. 2015; Récapet et al. 2016c, 2017; Herborn et al. 2016) or in
482 opposite directions (Costantini et al. 2008, 2010; Beaulieu et al. 2010; Marri and Richner 2015;
483 Viblanc et al. 2018; Kulaszewicz et al. 2018), despite some exceptions (Piccione et al. 2013;
484 Cornell and Williams 2017; Silva et al. 2018). In collared flycatchers, antioxidant protection as
485 reflected by the OXY test thus seems to adaptively build up to face increased exposure to ROS
486 in periods of higher energy demands, especially in females during the early breeding stages,
487 when oxidative stress can be particularly harmful for developing offspring, but changes in
488 habitat quality and food resources tend to impact these two markers independently, similar to
489 what is observed in the present study.

490 The correlation between ROM concentration and antioxidant capacity was sensitive to
491 variation in the conditions experienced by individuals. It was stronger in handicapped
492 females, which experienced stronger energetic constraints compared to control ones.
493 Conversely, experimentally increased food availability did not appear to influence the
494 correlation between the markers, as there was no noticeable difference in this correlation
495 between control and food-supplemented pairs. The strength of the correlation also increased
496 in control birds from 2012 to 2014. This hints at a role of environmental conditions in

497 modulating the correlation between antioxidant defences and oxidative damages. Indeed,
498 particularly sunny and dry meteorological conditions in 2012 and particularly rainy and cold
499 conditions in 2014 resulted in strong differences in mortality rate among nestlings and thus in
500 reproductive performances, with 2013 in between (mean fledging success in control nests \pm
501 S.D. (N) = 4.7 ± 2.6 (89) in 2012, 3.0 ± 2.4 (99) in 2013, and 1.5 ± 2.0 (82) in 2014). The correlation
502 between oxidative damage and antioxidant capacity might thus be stronger when
503 environmental conditions are harsher, although this cannot be formally tested here with only
504 three study years. These differences in correlation coefficients are not an artefact of a larger
505 range of values for the markers (heterogeneity in individual responses) in more constrained
506 conditions, as variances for ROM concentration and plasma antioxidant capacity actually
507 tended to be larger in control females than in handicapped females, and in 2012 and 2013 than
508 in 2014 (data not shown). Although the exact sources of the observed differences in the
509 correlation remain speculative, our results clearly show that the relationships between
510 different components of the oxidative balance are not fixed but may be modulated by
511 individual or environmental factors.

512 Despite our limited understanding of oxidative stress and redox signalling across animal
513 species (Halliwell and Gutteridge 2015; Jones and Sies 2015), a stable relationship between
514 oxidative damage and antioxidant protection is often assumed: it underpins some proposed
515 measures of oxidative stress, such as the ratio between a marker of ROS production/oxidative
516 damages and a marker of antioxidant protection (e.g. the ratio between ROMs and antioxidant
517 capacity initially proposed by Costantini et al. 2006, which use has declined in recent years;
518 but see Cornell and Williams 2017; Injaian et al. 2018; Kulaszewicz et al. 2018), or the extraction
519 of principal components from PCAs on a set of these markers (Hörak and Cohen 2010).
520 Previous studies however failed to find consistent correlations between multiple markers of
521 oxidative balance, despite large longitudinal samples (Romero-Haro and Alonso-Alvarez
522 2014; Christensen et al. 2015). This could partly be due to the correlation structure being much

523 more labile than previously thought. Correlations between antioxidants alone were found to
524 vary strikingly among bird species (Cohen and McGraw 2009), and correlation coefficients
525 between ROMs and antioxidant capacity varied from 0.623 ($P = 0.002$) to -0.533 (P unknown)
526 among domestic pig breeds (Brambilla et al. 2002). Our results show that such correlations
527 could also vary at the within-individual level, according to environmental conditions.
528 Redundancy of antioxidant systems is classically invoked to explain the low correlation
529 between any individual antioxidant and markers of oxidative damages (Jacob 1995). Another
530 non-exclusive explanation is that, when challenged, organisms would shift trade-offs in favour
531 of those traits that are particularly important for fitness, so that these traits remain invariant
532 in response to environmental challenges, a phenomenon originally termed canalisation in the
533 context of development (Waddington 1942). This concept was recently extended to plastic
534 traits such as redox markers: in young jackdaws, antioxidant protection is associated with
535 survival but not influenced by experimental challenges, whereas markers of oxidative damage
536 respond well to experimental challenges but do not predict survival (Boonekamp et al. 2018).
537 As a result, variation in canalised traits would be uncoupled from variation in traits less crucial
538 to fitness.

539 Beyond defining reference values for different environmental conditions and life-stages
540 (Beaulieu and Costantini 2014), the stability of the correlation structure under varying
541 conditions should thus be tested in any study species before using ratios or principal
542 components as response variables. In particular, multivariate analyses and comparison of
543 covariance matrices (Steppan et al. 2002; Garcia 2012) can be used to detect potential
544 differences in the correlation structure. However, more mechanistic models of oxidative
545 homeostasis may be required to properly measure oxidative stress. This type of mechanistic
546 models is now relatively well implemented and widely used for studying energetic trade-offs,
547 building upon thermodynamics and chemistry principles (Jusup et al. 2017). These models
548 already include nutritional trade-offs due to dietary restrictions in some nutrients, especially

549 in plants. Other potential sources of trade-offs, such as oxidative stress, but also immune
550 function or glucose homeostasis (Récapet et al. 2016b; Montoya et al. 2018), are however not
551 modelled explicitly, probably due to the complexity of their interactions (Cohen et al. 2012).
552 Theoretical models have however shown that multiple, partially independent physiological
553 mechanisms underlying life-history trade-offs could relax the overall trade-off observed at the
554 individual level and thus change the evolutionary outcome (Cohen et al. 2017). The
555 physiological “machinery” evolved to respond to environmental challenges can be seen as an
556 evolutionary constraint that does matter and should thus be taken into account when
557 projecting the future evolution of organisms.

558

559 **Conclusions**

560 In our study, individual antioxidant capacity was repeatable within and between years, but
561 ROM concentration was not, which suggest that an individual’s ability to acquire antioxidant-
562 rich food plays an important role in its ability to respond to oxidative stress. The two markers
563 were positively correlated, but this relationship was conditional on the energetic constraints
564 experienced by each individual: correlations between markers were stronger when wing load
565 was increased experimentally or when environmental conditions were naturally poorer. This
566 probably comes from a tighter adjustment of antioxidant defences to ROS production when
567 conditions were constrained, which could be particularly important to maintain cell redox
568 homeostasis. Given the diversity of physiological and ecological factors that seem to affect the
569 correlation structure between markers of oxidative state in a single population, the
570 discrepancies in these correlations among studies, species and populations come as no
571 surprise. Our results thus question our ability to interpret and make ecological inferences from
572 markers of oxidative state without a more flexible, mechanistic understanding of their
573 interactions.

574

575 **Compliance with Ethical Standards**

576 The authors declare that they have no conflict of interest.

577 All applicable international, national, and/or institutional guidelines for the care and use of
578 animals were followed. Permission for catching and ringing adult and young birds was
579 granted by the Ringing Centre from the Museum of Natural History in Stockholm (license
580 number 471:M009 to C.R.). Permission for blood taking and experimental procedures was
581 granted by the Ethical Committee for Experiments on Animals in Sweden (license number C
582 108/7).

583

584 **Data availability**

585 The datasets and as well as the results of the MCMC sampling generated during the current
586 study are available in the figshare repository, [PERSISTENT WEB LINK TO DATASETS].

587

588

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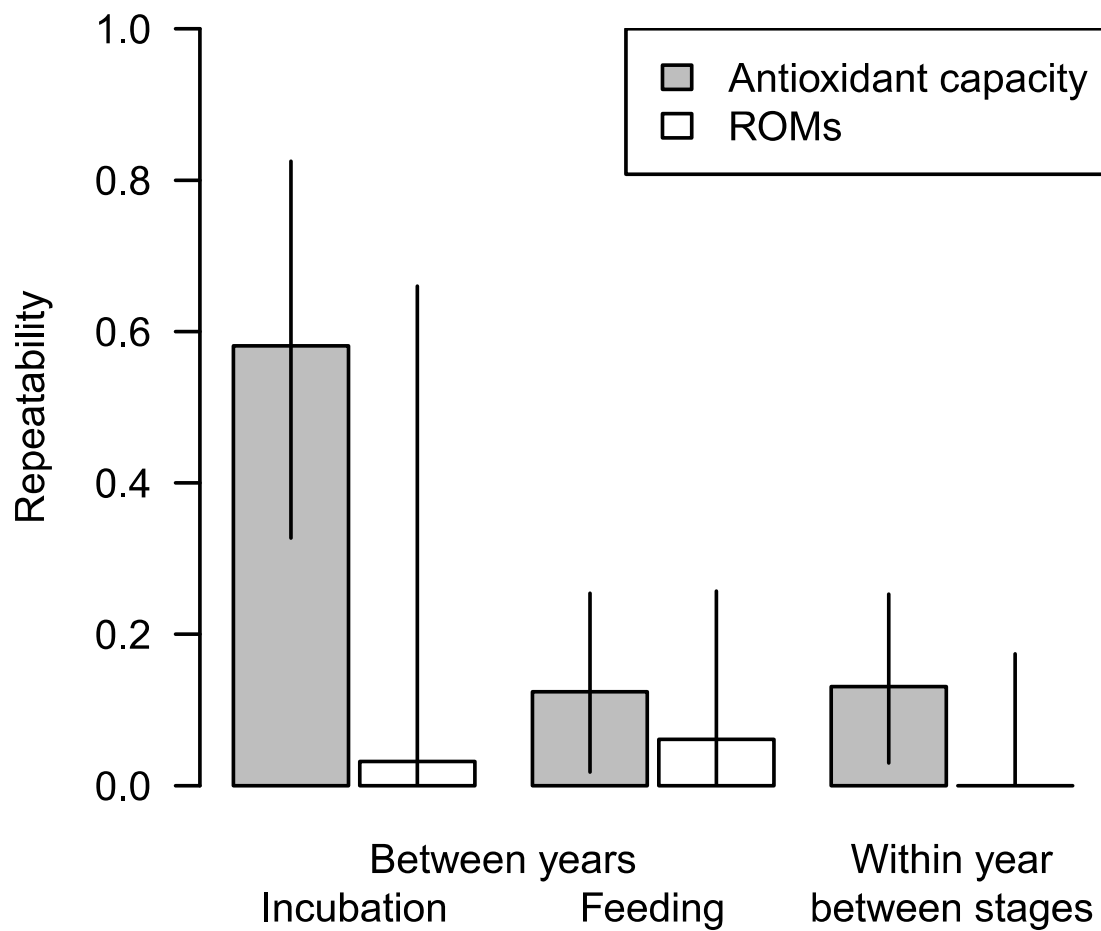
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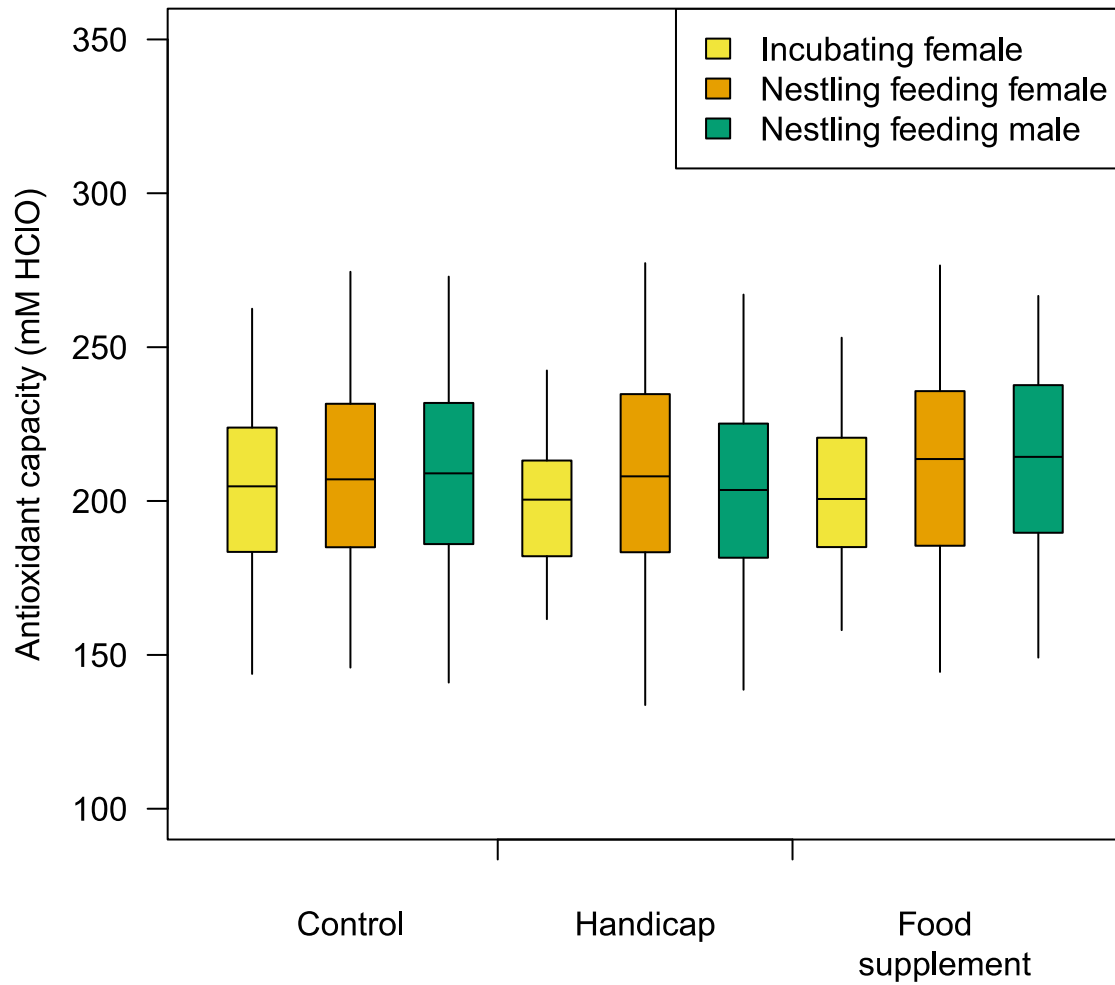
945 **Fig. 1 Repeatability estimates and 95% confidence intervals for antioxidant capacity (in**
946 **grey) and ROMs concentration (in white) over different time scales. Males were only**
947 **sampled during the nestling feeding stage, so repeatability in incubation between years and**
948 **between breeding stages within years was only estimated for females.**



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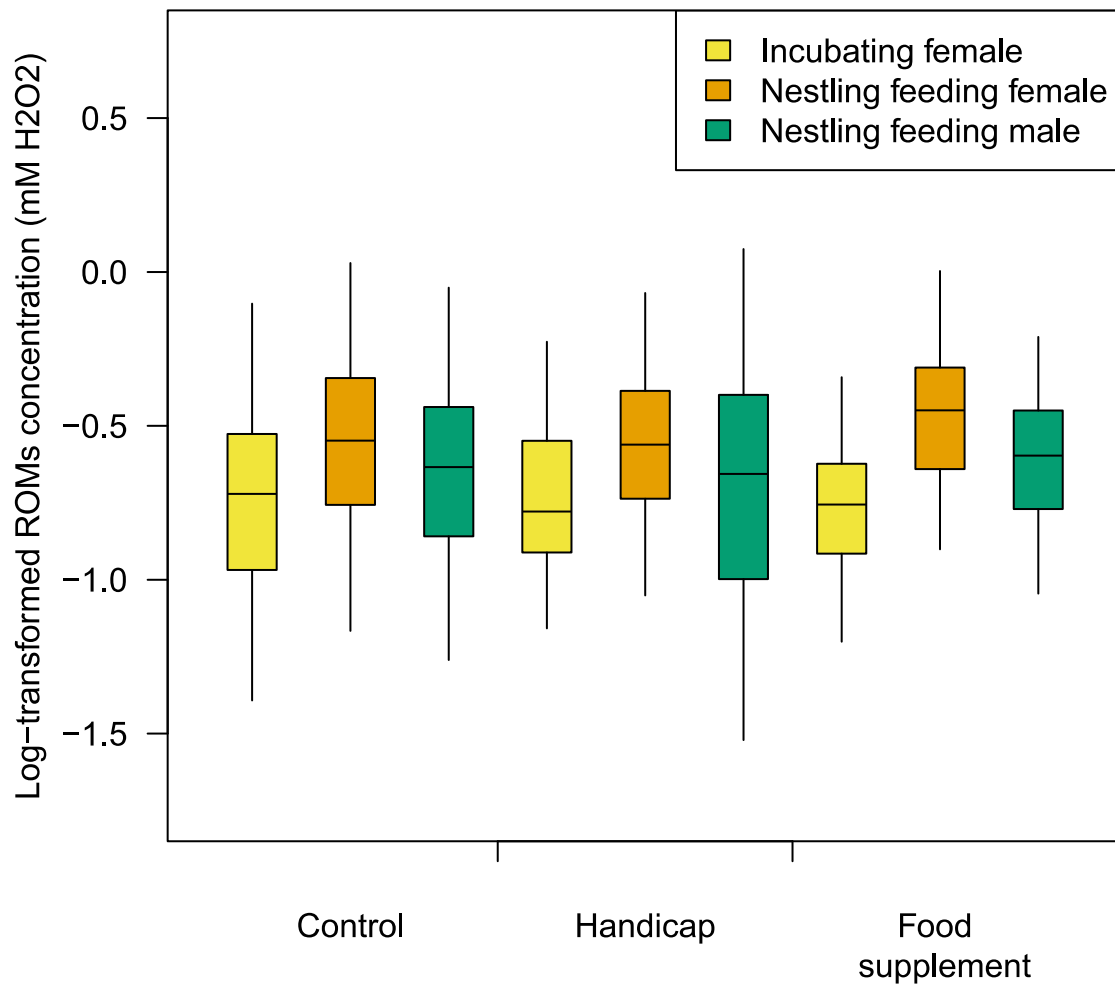
951 **Fig. 2 Antioxidant capacity in collared flycatchers according to experimental treatment, sex**
952 **and breeding stage (incubation = pre-treatment, feeding = post-treatment).** Males were only
953 sampled during the nestling feeding stage. Values were corrected for the year effect.



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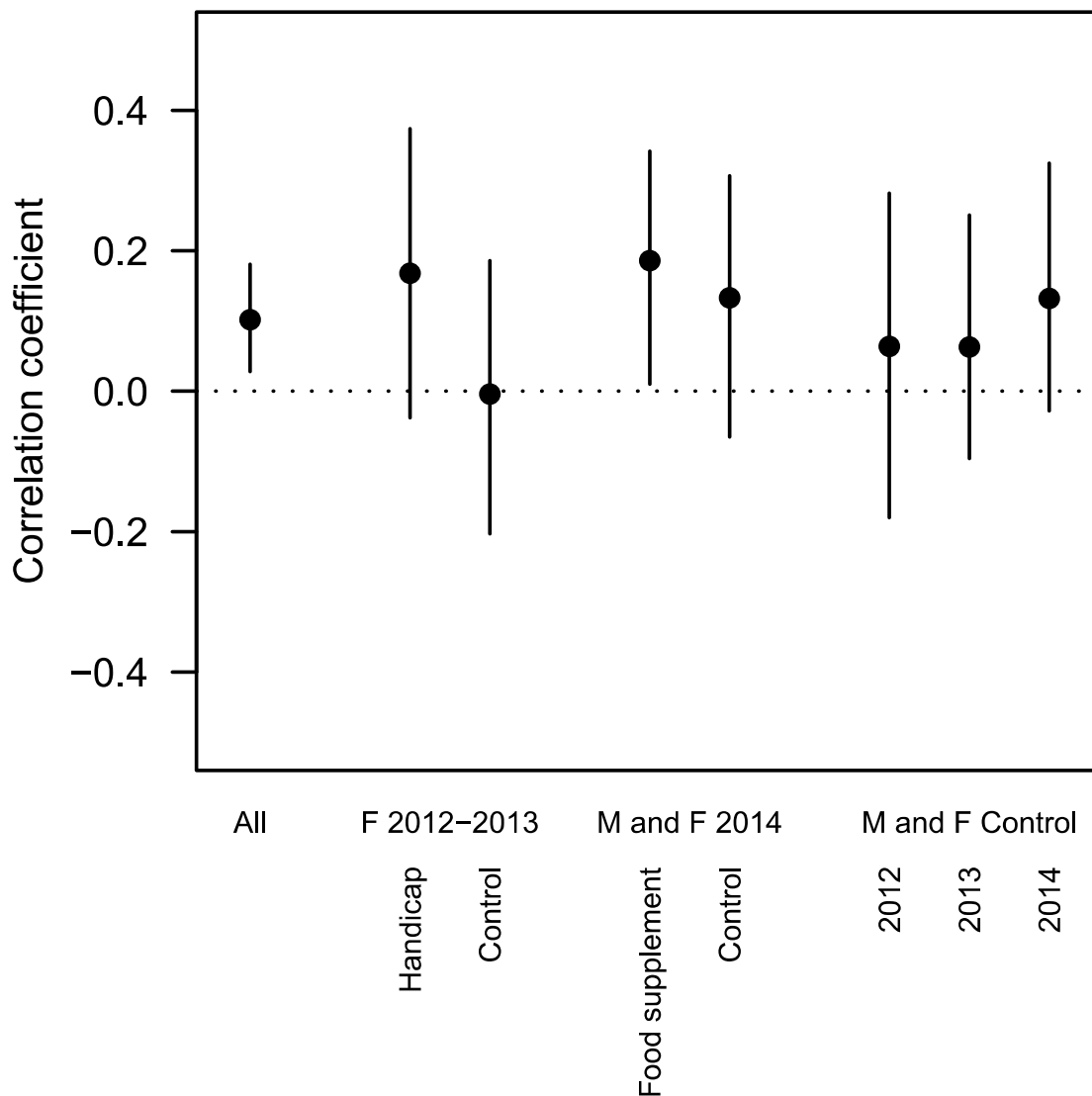
956 **Fig. 3 Log-transformed ROMs concentration in collared flycatchers according to**
957 **experimental treatment, sex and breeding stage (incubation = pre-treatment, feeding = post-**
958 **treatment).** Males were only sampled during the nestling feeding stage. Values were corrected
959 for the year effect.



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961

962 **Fig. 4** Estimates and 95% credibility intervals for the phenotypic correlation between
 963 **antioxidant capacity and ROMs concentration depending on experimental treatments and**
 964 **years.** Variables were standardized between years, sexes and treatments, so that the
 965 correlation coefficient obtained when pooling multiple groups would not be influenced by
 966 differences between years, sexes and treatments in antioxidant capacity or ROMs
 967 concentration. "F" refers to females and "M" to males. The effect of feather handicap is only
 968 represented for females because males were not directly manipulated.



969
 970

971 **Table 1: Repeatability of the measures of reactive oxygen metabolites (ROMs) and antioxidant capacity (OXY test) at different time scales**
 972 **and different breeding stages.** Estimates are obtained from linear-mixed models, 95% confidence interval from parametric bootstraps and P
 973 values from likelihood-ratio tests. The number of individuals is given in parentheses below the number of observations.

Repeatability	Sex	Variable	N	<i>r</i>	CI _{95%}	P
Between years during incubation	F	OXY	251 (234)	0.581	[0.327; 0.825]	0.02
Between years during incubation	F	ROMs	214 (202)	0.032	[0; 0.660]	0.44
Between years during nestling feeding	F & M	OXY	838 (652)	0.124	[0.018; 0.254]	0.02
Between years during nestling feeding	F & M	ROMs	662 (535)	0.061	[0; 0.257]	0.24
Between breeding stages within a year	F	OXY	681 (359)	0.131	[0.030; 0.253]	0.02
Between breeding stages within a year	F	ROMs	535 (310)	0.000	[0; 0.174]	0.50

974

975

976 **Table 2: Effect of the wing load manipulation in 2012 and 2013 on the correlation between reactive oxygen metabolites (ROMs) and**
977 **antioxidant capacity (OXY test).** Mode and 95% credibility interval of the posterior distribution of the coefficients of correlation between ROM
978 concentration and antioxidant capacity, for different experimental groups, sexes and years. The number of individuals is given in parentheses
979 below the sample sizes when the samples covered multiple years, and thus some individuals were sampled more than once. When 10 or more
980 individual birds were measured repeatedly, the within-individual correlation (residual correlation) is reported separately from the between-
981 individual correlation.

Treatment	Sex	N	DIC	Between-individual correlation	Within-individual correlation	Total phenotypic correlation
Both groups, with the same correlation coefficients	F	187 (176)	982.4	0.143 [-0.477; 0.729]	0.088 [-0.177; 0.376]	0.099 [-0.045; 0.245]
Both groups, with different correlation coefficients	F	187 (176)	969.9	0.279 [-0.364; 0.731]	0.151 [-0.273; 0.475]	in manipulated females
					-0.083 [-0.368; 0.275]	in control females
Wing load manipulation	F	91 (87)		-	-	0.168 [-0.038; 0.374]
Control	F	96 (93)		-	-	-0.004 [-0.203; 0.186]
Both groups, with the same correlation coefficients	M	220 (191)	1195.6	0.337 [-0.343; 0.714]	0.028 [-0.172; 0.217]	0.046 [-0.075; 0.189]

Both groups, with different correlation coefficients	M	220 (191)	1191.6	0.320 [-0.342; 0.726]	-0.065 [-0.347; 0.171]	in males paired with manipulated females
					0.104 [-0.163; 0.360]	in control males
Wing load manipulation	M	120 (112)		-	-	0.028 [-0.165; 0.202]
Control	M	100 (94)		-	-	0.138 [-0.062; 0.331]

982

983

984 **Table 3: Effect of the food supplementation in 2014 on the correlation between reactive oxygen metabolites (ROMs) and antioxidant capacity**
 985 **(OXY test) with both sexes pooled.** Mode and 95% credibility interval of the posterior distribution of the coefficients of correlation between ROM
 986 concentration and antioxidant capacity for males and females in 2014.

Treatment	N	DIC	Phenotypic correlation
Both groups, with the same correlation coefficients	239	1283.5	0.169 [0.050; 0.290]
Both groups, with different correlation coefficients	239	1288.0	0.181 [0.006; 0.339] in the food supplemented group 0.148 [-0.055; 0.313] in the control group
Food supplementation	128		0.186 [0.010; 0.342]
Control	111		0.133 [-0.065; 0.307]

987

988

989 **Table 4: Temporal and seasonal variation in the correlation between reactive oxygen metabolites (ROMs) and antioxidant capacity (OXY test).**
 990 Mode and 95% credibility interval of the posterior distribution of the coefficients of correlation between ROM concentration and antioxidant
 991 capacity, in nestling-feeding males and females from control groups and in incubating females before the start of the treatments. The number of
 992 individuals is given in parentheses below the sample sizes when the samples covered multiple years, and thus some individuals were sampled
 993 more than once. When 10 or more individual birds were measured repeatedly, the within-individual correlation (residual correlation) is reported
 994 separately from the between-individual correlation.

Sex and stage	Years	N	DIC	Between-individual	Within-individual	Total phenotypic
F (incubating)	2012-2014	211 (199)		-0.101 [-0.822; 0.691]	0.050 [-0.175; 0.298]	0.049 [-0.090; 0.190]
F + M (feeding)	2012-2014	307 (274)	1646.3	0.042 [-0.637; 0.482]	0.137 [-0.066; 0.320]	0.104 [-0.019; 0.200]
F + M (feeding)	2012-2014	307 (274)	1620.1	-0.114 [-0.585; 0.549]	0.087 [-0.277; 0.445] in 2012	
					0.114 [-0.171; 0.373] in 2013	
					0.262 [-0.083; 0.545] in 2014	
F + M (feeding)	2012	72		-	-	0.064 [-0.180; 0.282]
F + M (feeding)	2013	124		-	-	0.063 [-0.096; 0.251]
F + M (feeding)	2014	111		-	-	0.132 [-0.028; 0.325]

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996

997 **Table 5: Previous studies on the correlation between antioxidant capacity measured through**

998 **the OXY test and reactive oxygen metabolites.** The correlation coefficient was not available

999 in some studies. Bold values are significantly different from zero.

Species	<i>r</i>	<i>P</i>	References
Birds			
Adelie penguins <i>Pygoscelis adeliae</i>	-0.463	0.005	(Beaulieu et al. 2010)
greenfinches <i>Carduelis chloris</i>	-	0.22	(Herborn et al. 2011)
Seychelles warblers <i>Acrocephalus sechellensis</i>	0.25	< 0.001	(van de Crommenacker et al. 2011)
	-	0.40	(van de Crommenacker et al. 2012)
blue tits <i>Cyanistes caeruleus</i>	-	0.34	(Arnold et al. 2015)
great tits <i>Parus major</i>	0.16	0.08	(Wegmann et al. 2015b)
	0.50	< 0.01	(Vaugoyeau et al. 2015)
European starlings <i>Sturnus vulgaris</i>	0.68	< 0.05	(Fowler et al. 2018)
Mammals			
domestic goats <i>Capra aegagrus</i>	-	n.s.	(Celi et al. 2010)

1000

1001 **Appendix 1: Correlation between markers of oxidative balance and nutritional state in the**
1002 **plasma.**

1003 In 2014, the concentrations of triglycerides, glucose and lactate were measured in whole blood
1004 immediately after blood taking using a portable test-strips reader designed for point-of-care
1005 measures in humans (Accutrend, Roche Diagnostics), whereas antioxidant capacity and ROM
1006 concentrations were measured through the OXY and d-ROMs tests, following the protocol
1007 described in the main text. Individuals were only measured once. The quantity of blood
1008 deposited on the test-strip was 10 μ L for triglycerides and glucose and 15 μ L for lactate. ROM
1009 concentration were not significantly correlated with triglycerides (Spearman's rank correlation
1010 test: $\rho = -0.01$, $N = 76$, $S = 74136$, $P = 0.91$), lactate ($\rho = 0.18$, $N = 19$, $S = 937$, $P = 0.47$), or glucose
1011 concentration ($\rho = -0.37$, $N = 20$, $S = 1817$, $P = 0.11$). There was no correlation between the total
1012 antioxidant capacity of the plasma and triglycerides ($\rho = -0.16$, $N = 93$, $S = 155677$, $P = 0.12$),
1013 lactate ($\rho = 0.30$, $N = 21$, $S = 1072$, $P = 0.18$) or glucose concentrations ($\rho = -0.09$, $N = 24$, $S =$
1014 2510 , $P = 0.67$) either.

1015