

Oxidative damage and anti-oxidant capacity in two migratory bird species at a stop-over site

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Abstract

We quantified in the garden warbler (*Sylvia borin*) and the barn swallow (*Hirundo rustica*), two long-distance migratory songbirds, the early oxidative damage (ROMs) and plasma anti-oxidant capacity (OXY) variation of individuals caught at a stop-over site after a sustained flight across the sea, during spring migration. Our main goal was to quantify the oxidative damage and anti-oxidant capacity variation in these two migratory species in relation to fat and muscle stores. The birds were sampled in Ponza, a small island along the migratory route of these species. The levels of ROMs and OXY did not show any differences between the two species and in general were higher in individuals with higher fat and protein stores. Nevertheless, the balance between ROMs and OXY was better in individuals in good condition. These patterns were similar in both species. No sex differences emerged for both ROMs and OXY in the barn swallow, the only species that could be sexed. Both markers of oxidative stress did not show any significant variation across a 30-min restrained experiment. These data are the first of this kind in wild birds in a migratory context and suggest that individuals in better condition are exposed to lower oxidative stress, providing an indirect evidence of the oxidative cost caused by prolonged flights.

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1. Introduction

Migration imposes a number of physiological challenges, such as the production of energy and the maintaining of body homeostasis (McWilliams et al., 2004), to which a number of specific adaptations have evolved. To fuel long-term fasting flights, birds derive energy from fat and protein stores accumulated in the pre-migratory phase (Berthold, 2001). In particular, after an initial *phase one* of adaptation to flight, long-term fasting birds enter the so called *phase two*, during which fat stores represent the first source of energy. When lipids drop to a certain level, metabolism shifts to protein catabolism (so called *phase three*). As a consequence, birds need to consume

energy stores in the best appropriate manner to avoid stress or distress situation. Refuelling and fattening rates are actually thought to affect the timing and success of migration in birds (Alerstam and Lindstrom, 1990). For example, it has been shown that corticosterone levels are very high in birds with no visible fat stores and emaciated breast muscles (Jenni et al., 2000). These findings led to the suggestion that migrant birds with good fat and protein stores cope better with endurance flight, so not perceiving as stressful the phase of fasting and simultaneous high-level exercise (Jenni et al., 2000).

Another challenge birds might cope with is to maintain redox homeostasis, i.e. the balance between pro-oxidant production and anti-oxidant response in order to avoid oxidative stress. It is known that a high metabolic activity produces large quantities of reactive oxygen species, ROS (Leffler, 1993; Beckman and Ames, 1998). The main consequence of an overproduction of these compounds is an extensive oxidative damage to biomolecules, such as lipids, proteins, and nucleic acids. To limit

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the amount of oxidative damages, organisms have evolved sophisticated detoxifying systems based on endogenous and exogenous anti-oxidants, i.e. substances that significantly delay or inhibit oxidation of biological macromolecules (Halliwell and Gutteridge, 1989). Moreover, such self-sustained defence machinery does work in concert with specific cellular components evolved to repair oxidatively damaged molecules (Yu, 1994). It is also widely recognized that a high caloric intake increases oxidative stress (Sohal and Weindruch, 1996; Masoro, 2002). Finally, birds mainly accumulate polyunsaturated fatty acids (McWilliams et al., 2004), which are particularly susceptible to free radical damage (Bielski et al., 1983; Porter et al., 1995). As a consequence, it can be hypothesised that birds need to balance the high intake of nutrients to fuel flight (reviewed in McWilliams et al., 2004) with the risk to incur in pervasive per-oxidation. Thus, migratory birds might face with two main distinct sources of oxidative stress, i) the flight effort (i.e. high metabolic activity) and ii) the accumulation (pre-migratory hyperphagia; Bairlein, 1985) and depletion of large quantities of fats and proteins. To our knowledge, no attention has been devoted to these topics in migratory free-living birds.

The aims of this study are i) to quantify the oxidative damage and anti-oxidant capacity variation in two migratory bird species and ii) to evaluate if fat and protein stores reflect the individual oxidative status. We investigated these questions in the long-distance migrants garden warbler (*Sylvia borin*) and barn swallow (*Hirundo rustica*), two common passerine species whose migration physiology and ecology are well known. The specimens of both species were captured at stop-over site after crossing about 500 km of open Mediterranean Sea during spring migration.

2. Material and methods

2.1. Study species

The barn swallow (*H. rustica*) is a socially monogamous, sexually dimorphic trans-Saharan migratory songbird, wintering in Africa and breeding in Europe (Cramp, 1992). The arrival in the breeding grounds, which is strongly correlated with the onset of breeding, ranges between February and May, depending on the latitude of the breeding area (Møller, 1994). Males usually arrive earlier than females and within the same area males with longer tails (outermost tail feather), which arrive earlier, are the most preferred by females and have higher

reproductive success (Møller, 1994). Migratory flights are mainly diurnal, as this species feeds on aerial insects while flying (Cramp, 1992).

The garden warbler (*Sylvia borin*) is a well-known subject species in studies of migration ecology and physiology (e.g. Gwinner et al., 1985, 1988; Totzke et al., 1999, 2000). It is a small not sexually dimorphic trans-Saharan songbird, wintering in Africa and breeding in Central and Northern Europe (Cramp, 1988). Seasonal fattening in garden warblers is regulated by a circannual clock and is due to pre-migratory hyperphagia, to an increased food utilization efficiency and to a dietary shift (Bairlein, 1985; Bairlein and Simons, 1995). In parallel, migratory disposition includes several metabolic adjustments such as a lower glucose utilization rate (Bairlein and Totzke, 1992). The garden warbler is a typical nocturnal migrant, being normally active only during daytime, but performing migratory flights mainly at night. The physiological mechanisms underlying this dramatic transition are basically unknown, although the pineal hormone melatonin is a likely candidate for a role in this adaptation (Fusani and Gwinner, 2005).

2.2. Field work

The study was carried out in Ponza, a small (9.87 Km²) island located around 50 km off the Tyrrhenian coast (Central Mediterranean Sea) of Italy (40° 50' N, 12° 58' E). Twenty-five garden warblers (*S. borin*) and 28 barn swallows (*H. rustica*) were caught by mist nets between April 29th and May 1st 2006 (see Table 1 for descriptive statistics). Based on the capture rate at the ringing station, the bulk of migration of garden warblers and barn swallows occurred, respectively, during 6–10 May and 26–30 April.

All individuals were trapped by nets located in the upper side of the ringing station where migrant birds land. Nets were continuously monitored and all birds were bled within 30 min from the time they flew into the net.

In both species, we took a double sample of the same individual to control for potential effects of handling stress and time elapsed from the time the birds flew into the net and their bleeding. Four garden warblers and five barn swallows were bled twice. After the first bleeding, the birds were kept quiet into a bag until the second bleeding, 30 min later. This restrained protocol allows us also to control for recovery, if any, of the physiological variables considered towards resting levels. It is in theory possible that either handling stress or resting time

Table 1
Descriptive statistics of the variables considered for each species

Species	Day	Fat scores	Muscle scores	ROMs	OXY	OS	Hour	n
GW	1	1.64±0.39	1.73±0.14	3.15±0.14	151.97±12.77	22.07±1.79	14.22±0.73	11
	2	1.89±0.26	1.78±0.15	2.59±0.16	148.38±6.89	17.82±1.36	13.09±0.59	9
	3	1.40±0.68	1.60±0.24	2.90±0.22	136.29±13.65	21.94±2.34	13.79±0.91	5
BS	1	0.71±0.47	1.14±0.14	2.86±0.21	118.35±11.56	26.18±3.92	17.54±0.40	7
	2	0.38±0.18	1.15±0.10	2.75±0.20	102.84±7.45	27.76±2.08	13.11±0.68	13
	3	1.25±0.45	1.13±0.12	2.73±0.21	141.35±13.61	20.91±2.94	13.14±0.87	8

Values are shown as mean±se for each day of sampling separately (GW=garden warbler; BS=barn swallow; ROMs=reactive oxygen metabolites; OXY=plasma anti-oxidant capacity; OS=level of oxidative stress; Hour=hour of sampling).

affect metabolite or anti-oxidant levels. A 30-min time has been shown to be long enough to allow homing pigeons (*Columba livia*) and small passerines to recover some plasma metabolites to their resting levels (Jenni-Eiermann and Jenni, 1991; Schwilch et al., 1996). Only the measures of the samples of the first bleeding were added to the final analysis including all the remaining birds bled only once.

The low number of recaptures (less than 5% of trapped birds, M. Cardinale personal observation) at Ponza indicates that most birds spend less than one day or usually only few hours on the island and that birds trapped in Ponza have at maximum spent few hours after a non-stop flight. Therefore, the values should mirror the physiological status of birds that incurred a large energy loss during a previous non-stop flight. Estimates done on the neighbouring Ventotene Island suggest that birds landing in these stop-over sites had completed a 14–16 h non-stop flight (Pilastro et al., 1995; Schwilch et al., 2002).

Each specimen was bled within a few minutes (max 30 min) after flying into the net. A blood sample (100–150 μ L) was kept by Na-heparinized micro-haematocrit-tubes after puncturing the wing vein. The samples were kept cool (0–5 °C) until centrifugation, which occurred within a few hours, and the plasma was kept at –20 °C. After shipment in ice to the lab, all samples were newly stored at –20 °C.

After bleeding, all birds were ringed, weighted (0.1 g) with an electronic balance and their wing measured (0.5 mm) according to standard procedures, and immediately released. The sex of garden warblers is undeterminable because of the lack of morphological dimorphism. In barn swallows, sex was determined by measuring the outermost tail feathers (delta-value: ΔT males > 56 mm; ΔT females < 51 mm; ΔT undetermined 51–56 mm; Svensson, 1992). Further, fat and muscle scores were recorded. These represent two classic scores taken by bird ringers and showing high repeatability (see e.g. Jenni et al., 2000). The fat score is determined by checking the visible amount of subcutaneous fat deposits between the furcula and on the abdomen (9 level score). These scores correlate well with the amount of fat extracted from whole birds (Kaiser, 1993; Jenni et al., 2000). Instead, muscle scores quantify the thickness of the

breast muscle (4 level score: e.g. 0 = breast muscle emaciated and its cross section shaped concavely; 3 = breast muscle bulging and shaped convexly; Bairlein, 1995). Muscle scores represent an estimate of breast muscle protein mass (Jenni et al., 2000).

2.3. Measurement of reactive oxygen metabolites

Reactive oxygen metabolites (ROMs) are a marker of early oxidative damage. These include primarily hydroperoxides (ROOH). Such a marker allows also to evaluate potential future risks for the individual health, since circulating metal ions, such as iron (Fe^{2+} and Fe^{3+}) and copper (Cu^+ and Cu^{2+}), can cleave ROOH, leading to the generation of two highly reactive and histolesive pro-oxidants, namely the alkoxyl ($\text{R-O}\bullet$) and alkylperoxyl ($\text{R-OO}\bullet$) radicals. These radicals are able to propagate the oxidative cascade. The plasma concentration of ROMs was measured by the d-ROMs test (Diacron, Grosseto, Italy). The plasma (10 μ L) was first diluted with 200 μ L of a solution containing 0.01 M acetic acid/sodium acetate buffer (pH 4.8) and *N,N*-diethyl-*p*-phenylenediamine as chromogen and then incubated for 75 min at 37 °C. The acidic pH of the solution causes the release from proteins of metal ions which cleave the metabolites. After reacting with an alkyl-substituted aromatic amine solubilized in the chromogen, the metabolites produce a complex whose colour intensity is directly proportional to their concentration. After incubation, the absorbance was read with a spectrophotometer (Microplate Reader Model 550) at 490 nm. The measurements are expressed as mM of H_2O_2 equivalents in reference to a standard curve. The repeatability tested on 8 duplicates was significantly high (intraclass correlation coefficient: $r=0.95$, $p<0.001$; see Lessells and Boag, 1987). For further details see Costantini et al. (2006), Costantini and Dell’Omo (2006a,b).

2.4. Measurement of the plasma anti-oxidant capacity

The OXY-Adsorbent test (Diacron, Grosseto, Italy) allows to quantify by a colorimetric determination the ability of the total serum or plasma anti-oxidant barrier (enzymatic and non-

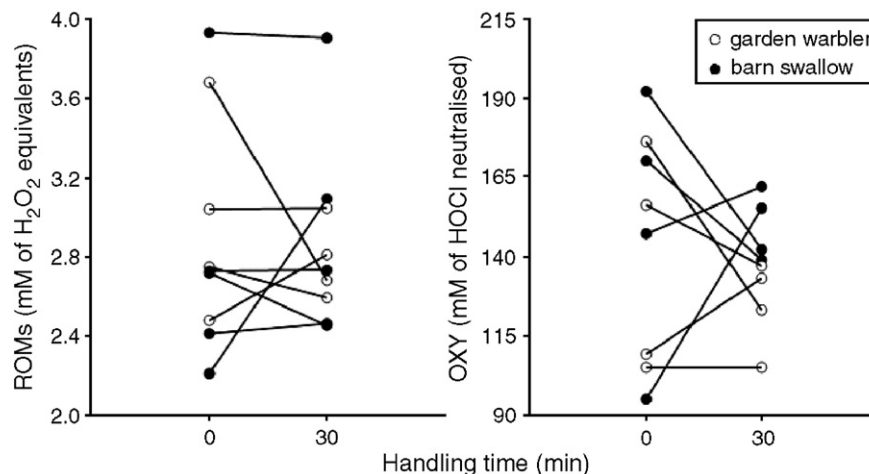


Fig. 1. The levels of reactive oxygen metabolites (ROMs; $n=9$) and plasma anti-oxidant capacity (OXY; $n=8$) did not show any significant variation across the 30-min restrained experiment.

enzymatic activity) to cope with the oxidant action of hypochlorous acid (HOCl; oxidant of pathologic relevance in biological systems). The plasma (10 μ L) was diluted 1:100 with distilled water. A 200 μ L aliquot of a titred HOCl solution was incubated with 5 μ L of the diluted plasma for 10 min at 37 °C. Then, 5 μ L of the same chromogen solution used for the ROM determination was added. An alkyl-substituted aromatic amine solubilized in the chromogen is oxidized by the residual HOCl and transformed into a pink derivative. The intensity of the coloured complex, which is inversely related to the anti-oxidant power, was measured with the same spectrophotometer at 490 nm. Measurements are expressed as mM of HOCl

neutralised in reference to a standard curve. The repeatability tested on 8 duplicates was significantly high (intraclass correlation coefficient: $r=0.83$, $p=0.002$; see Lessells and Boag, 1987). For further details see Costantini et al. (2006), Costantini and Dell'Omo (2006a,b).

2.5. Statistical analyses

All analyses were performed by the STATISTICA package (Version 6.0, StatSoft 2001, Tulsa, USA). Generalized linear/non linear models (GLZ) were performed to evaluate which factors better explained the variation in ROMs, OXY, and level

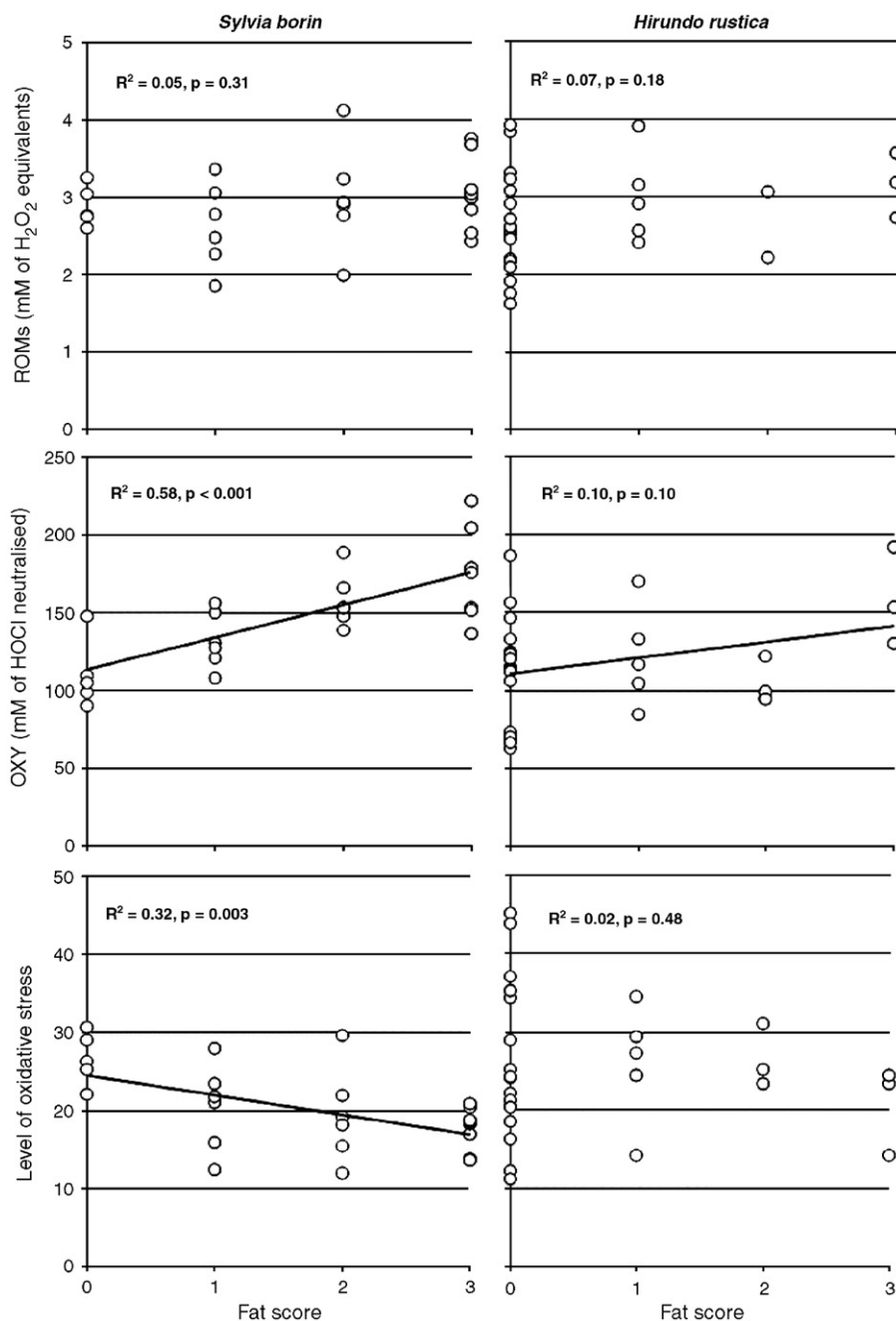


Fig. 2. Relationships between fat scores and the markers of oxidative stress shown for each species separately (ROMs=reactive oxygen metabolites; OXY=plasma anti-oxidant capacity; level of oxidative stress=ROMs/OXY \times 1000). Trend lines are shown when $p \leq 0.10$.

of oxidative stress (OS). A normal error function (all variables are normally distributed) and an identity-link function were applied. A backward removal, with a critical $p < 0.05$ was used to build the minimum model significantly explaining the observed variance.

Species was included as categorical variable, whilst time of sampling, fat and muscle scores as covariates. Two-, three- and four-way interactions were included in all the models. Body mass was not included in the models because redundant with fat ($r = 0.53$, $p < 0.001$) and muscle ($r = 0.43$, $p = 0.001$) scores. As far as OS is concerned, two different analyses have been performed. In a first model, OS was quantified as the ratio

between ROMs and OXY ($\times 1000$) according to Costantini et al. (2006) with higher values meaning higher OS. In general, the use of ratios is suggested when both variables are significantly correlated (Hayes and Shonkwiler, 1996). However, pro-oxidant and anti-oxidant compounds may or may not be significantly correlated. In fact, their relationship can be affected by the ability of the organism to cope with pro-oxidant production or by the time needed to mount an effective anti-oxidant response (e.g. Sohal et al., 1994). Moreover, a recent meta-analysis revealed that most anti-oxidants exhibit a non-monotonic relationship with other markers of OS, with all markers correlating only under severe pathological conditions

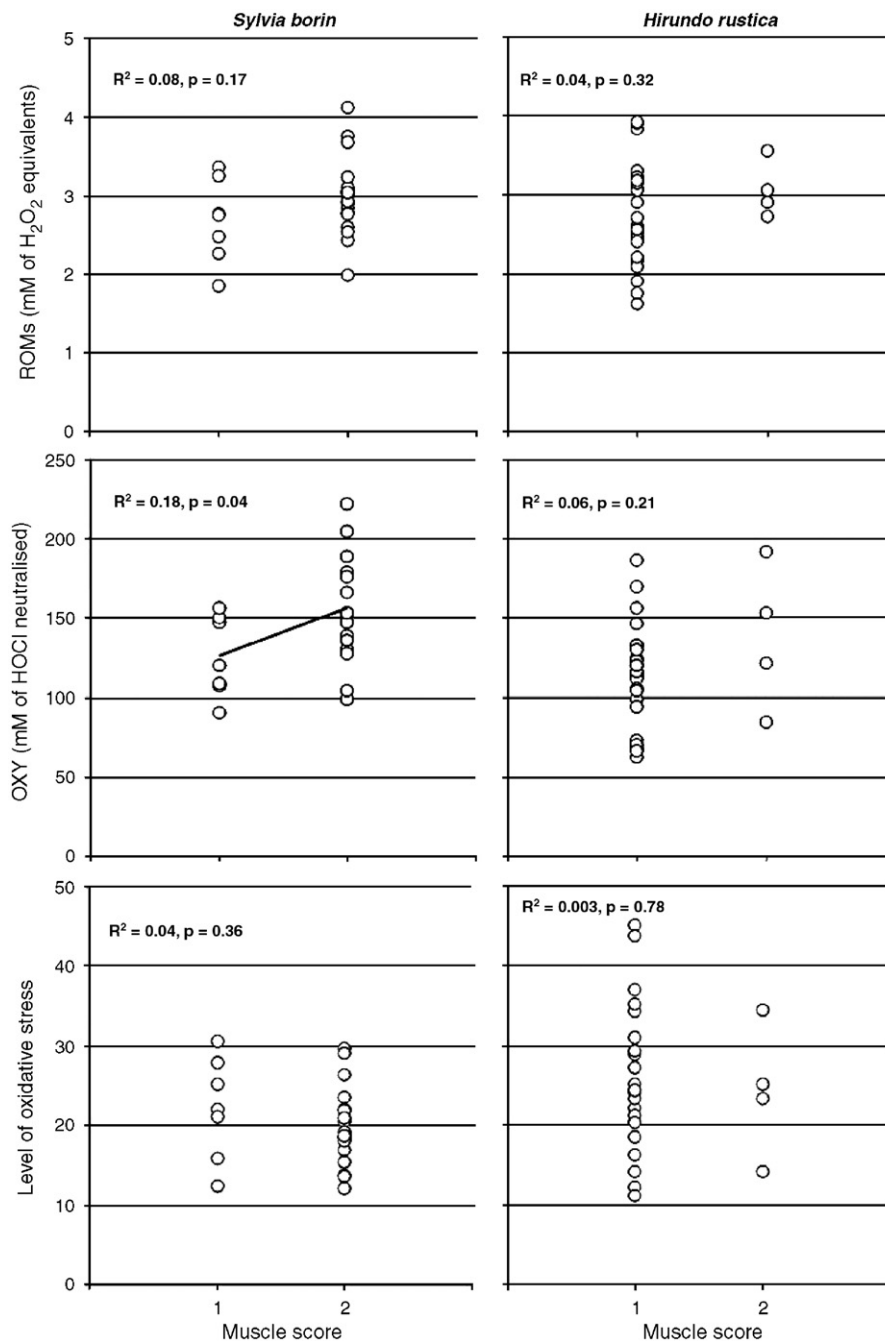


Fig. 3. Relationships between muscle scores and the markers of oxidative stress shown for each species separately (ROMs=reactive oxygen metabolites; OXY=plasma anti-oxidant capacity; level of oxidative stress=ROMs/OXY $\times 1000$). Trend lines are shown when $p \leq 0.10$.

(Dotan et al., 2004). For example, nestling kestrels show increased levels of ROMs and decreased anti-oxidant barrier during an immune challenge (Costantini and Dell’Omo, 2006a). In the present case, ROMs and OXY were not significantly correlated ($r=0.14$, $n=53$, $p=0.33$). Therefore, in a second model ROMs was included as response variable and OXY was included as covariate according to a similar approach for body condition analysis (see García-Berthou, 2001). The other covariates included in both the above described models were hour of sampling, fat and muscle scores. Since we performed two distinct models for OS to test the same hypothesis, Bonferroni correction was considered ($p=0.025$).

Because of the small sample size, the effects of handling time and sex were assessed by the Wilcoxon test and by the Mann–Whitney U test, respectively. Pearson correlation coefficient was used to test the association between the tail delta-value of barn swallows (a trait used as a proxy of fitness in this species, see discussion) with ROMs, OXY, OS, fat score, or muscle score.

3. Results

The levels of ROMs ($Z=0.30$, $p=0.77$) and OXY ($Z=0.51$, $p=0.61$) did not show any significant variation across the 30-min restrained experiment (Fig. 1). The same outcome emerged considering the two species separately (ROMs: garden warbler, $Z=0.37$, $p=0.72$; barn swallow, $Z=0.94$, $p=0.35$; OXY: garden warbler, $Z=0.53$, $p=0.59$; barn swallow, $Z=0.00$, $p=0.99$). One barn swallow was not included in the OXY analysis because the amount of plasma was not sufficient for the analyses.

ROMs, OXY, OS, fat or muscle scores did not correlate with the time of sampling considering both species altogether ($n=53$, all r coefficients = -0.07 to 0.07 , all p -values ≥ 0.60) or separately (garden warbler, $n=25$: all r coefficients = -0.08 to 0.22 , all p -values ≥ 0.29 ; barn swallow, $n=28$: all r coefficients = -0.16 to 0.24 , all p -values ≥ 0.22).

The variance of ROMs was significantly explained by the interaction between time of sampling, fat and muscle scores (Wald statistics = 4.93, $p=0.026$, Figs. 2 and 3): i) ROMs were positively correlated with fat scores; ii) expect for the fat

category 4, within all the other, individuals with muscle score of 2 showed higher levels than individuals with 1; iii) this pattern was not time-dependent. The interaction between species, hour of sampling, fat and muscle scores was not significant ($p=0.20$). The variance of OXY was significantly explained by the interaction between fat and muscle scores (Wald statistics = 38.88, $p<0.001$; Figs. 2, 3 and 4): i) OXY was positively correlated with fat scores; ii) within the fat categories 0 and 1, individuals with a muscle score of 2 showed lower values of OXY than individuals with 1; iii) within the fat categories 2 and 3, the opposite pattern was observed. The variance in OS was significantly explained by the interaction between fat and muscle scores (Wald statistics = 9.74, $p=0.002$; Figs. 2, 3 and 4): i) OS was negatively correlated with fat scores; ii) within the fat categories 0 and 1, individuals with a muscle score of 2 showed higher values of OS than individuals with 1; iii) within the fat categories 2 and 3, the opposite pattern was observed. The same result was obtained including in the model ROMs as dependent variable and OXY among the covariates (Wald statistics = 5.68, $p=0.017$). These patterns were similar in both species as shown by the nonsignificant interaction between species, fat and muscle scores found in all the full models tested (ROMs: $p=0.18$; OXY: $p=0.34$; OS: $p=0.42$). The main effect of species was always not significant (ROMs: $p=0.32$; OXY: $p=0.31$; OS: $p=0.53$).

No sex differences in barn swallows (10 males and 12 females) were detected for both variables (ROMs: $Z=-0.82$, $p=0.42$; OXY: $Z=-1.19$, $p=0.25$). The tail delta-value of barn swallows did not correlate with ROMs ($r=-0.13$, $p=0.53$), OXY ($r=-0.25$, $p=0.20$), or OS ($r=0.11$, $p=0.57$). Further, tail did not correlate with fat scores ($r=-0.01$, $p=0.97$), whilst a marginally significant negative correlation emerged with muscle scores ($r=-0.36$, $p=0.06$). Similar results emerged considering the sexes separately.

4. Discussion

Migration is a highly energy demanding activity which exposes birds with different physiological costs. For example, metabolic rate during flapping flight can be increased up to 30 times that of basal activity (Berthold, 2001). A further cost

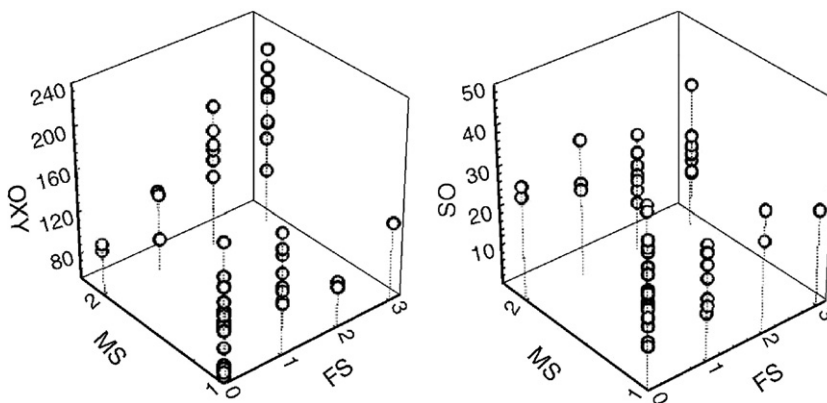


Fig. 4. Trivariate plots showing the interaction among fat scores (FS), muscle scores (MS) and plasma anti-oxidant capacity (OXY; multiple $R=0.62$, $p<0.001$) or level of oxidative stress (OS; multiple $R=0.40$, $p=0.02$).

migratory birds might cope with is the increase in free radical production due to the intense flight effort.

In our study, the levels of ROMs and OXY measured in migratory birds at a stop-over site were higher in individuals with higher fat and protein stores but the balance between ROMs and OXY was better in individuals in good condition. No sex differences emerged for both ROMs and OXY in the barn swallow.

It has been shown that birds arriving in phase two and phase three of fasting show reduced levels of flight muscles and digestive organs (Schwiltz et al., 2002). Such a status of poor condition could be assimilated to that of nutritional inadequacy, which is known to alter various parameters of oxidative stress. For example, it has been recently shown that protein malnutrition can decrease the total anti-oxidant capacity in the rat brain (Feoli et al., 2006). Our data show that fat and muscle scores taken separately are in general not good predictors of the individual oxidative status, particularly in barn swallows. Instead, taken together, they seem to be good predictors of the serum anti-oxidant capacity and of the level of oxidative stress (Fig. 4). Specifically, individuals in better condition, i.e. with higher levels of fats and proteins, show a higher anti-oxidant capacity, but also slightly higher levels of early oxidative damage. The latter result may be explained by a high lipid remobilization for energy production, which is still possible in individuals with good nutrient stores, and consequent increase of lipid per-oxidation. However, the balance between ROMs and OXY suggests that individuals in good condition are able to maintain better redox homeostasis, so incurring in lower level of oxidative stress. The higher anti-oxidant capacity may actually show that these individuals are mounting an effective anti-oxidant response. This suggests that birds in good condition still have good reserves of dietary or non-enzymatic endogenous anti-oxidants (main components of the avian anti-oxidant barrier in the blood; Lin et al., 2004; Costantini and Dell'Omo, 2006b) by which they can control the level of oxidative damage. For example, the higher levels of circulating carotenoids (dietary anti-oxidants) and of carotenoid depletion shortly after settling in the breeding area, both observed in late arriving barn swallows, led to the suggestion that these birds may have needed carotenoids to cope with a higher pro-oxidant production (Ninni et al., 2004). In this respect, nevertheless, it should be noted that recent evidences suggest that the anti-oxidant role of carotenoids in nestling and adult birds may not be as important as previously thought (*Falco tinnunculus* in Costantini et al., 2006, Costantini and Dell'Omo, 2006a; *Carduelis chloris* in Hórak et al., 2006; *Parus major* in Tummeleht et al., 2006).

Passerine migrants show differences in various traits which could play some role in shaping the individual oxidative status. For example, metabolism during flight in hirundines (barn swallow in our case) and swifts is 49.3%–72.6% lower than other birds of similar size (Hails, 1979). Moreover, unlike most other passerines, hirundines rarely show regular flapping flight (Bruderer et al., 2001) which has the highest metabolic rate of any form of vertebrate endurance locomotion (Pennycuik, 1990). Finally, unlike passerines such as the garden warbler, hirundines are diurnal long-distance migrants and hunt in free flight even during migration. The lack of differences in ROMs,

OXY, or OS between garden warblers and barn swallows we found does not support any role of such traits as determinants of the oxidative cost related to the long-term flight. This suggests that the two species evolved different strategies of migration resulting, however, in a similar oxidative cost.

The length of outermost tail feathers in barn swallows has been shown to reflect condition of males. For example, it has been shown that males with longer tails live longer than males with shorter tails and have higher reproductive success (Møller, 1994). In our case, tail length did not prove as good predictor of the general oxidative status of birds. The marginally significant negative correlation between tail and muscle score could reflect a higher cost in bearing a long tail, though, it was not reflected at the physiological level. This point, however, deserves further investigation because of our small sample size. Moreover, future studies should take into account also a large arrival time span since it is suggested to be a condition-dependent trait in barn swallows (Ninni et al., 2004).

The levels of anti-oxidant capacity of garden warblers and barn swallows are lower than those measured in wild nestling Eurasian kestrels, *F. tinnunculus* (Costantini et al., 2006; Costantini and Dell'Omo, 2006a,b), slightly lower than those in wild nestling yellow-legged gulls *Larus michahellis* (Rubolini et al., 2006), or comparable to those in captive adult Eurasian kestrels, and pigeons, *C. livia* (see Table 2). In contrast, the levels of ROMs of garden warblers and barn swallows are higher than baseline or stress levels measured in wild nestling (Costantini et al., 2006; Costantini and Dell'Omo, 2006a,b) or captive adult Eurasian kestrels, and adult homing pigeons (see Table 2).

The free radical theory of ageing (or oxidative damage theory of ageing *sensu* Martin et al., 1996) states that pro-oxidant production more than anti-oxidant capacity is the most proximate explanation of ageing in vertebrates (Harman, 1956, 1972; Leffler, 1993; Beckman and Ames, 1998). All evidences so far suggest that the longer the life-span, the lower the rate of mitochondrial oxygen radical production (Perez-Campo et al., 1998). In our case, the different levels of ROMs of garden warblers, barn swallows, Eurasian kestrels, and pigeons seem to

Table 2

Oxidative profile of the avian species investigated so far for ROMs (Reactive Oxygen Metabolites) and OXY (anti-oxidant capacity)

	<i>n</i>	ROMs	<i>n</i>	OXY
Nestling kestrels ^a	261	0.21±0.17	261	286.8±83.7
Nestling kestrels ^b	31	0.42±0.20	31	365.9±85.6
Nestling kestrels ^c	68	0.31±0.16	76	347.1±71.0
Captive adult kestrels ^d	18	0.68±0.23	18	168.2±26.2
Homing pigeons ^e	20	0.45±0.24	20	144.1±15.4
Nestling gulls ^f	–	–	181	144.0±27.8
Nestling gulls ^g	–	–	119	215.4±48.5

Mean ± SD are shown.

^a Costantini et al. (2006).

^b Costantini and Dell'Omo (2006a).

^c Costantini and Dell'Omo (2006b).

^d Unpublished results of the first author.

^e Unpublished results of the first author.

^f 4-day-old nestlings, Rubolini et al. (2006).

^g 8-day-old nestlings, Rubolini et al. (2006).

be in agreement with both the theoretical and experimental evidences of the free radical theory of ageing since pigeons actually live longer than kestrels which in turn live longer than both garden warblers and barn swallows. However, because of the highly different situations under which the species have been studied general conclusions and speculations have to be toned down.

In conclusion, the covariation between the markers of oxidative stress and the fat and muscle scores provides an indirect evidence that long-term fasting flights may jeopardize the redox homeostasis of birds increasing cytotoxic effects mediated by oxidative stress. However, our results do not allow to conclude to which extent the levels of both markers of oxidative stress mirror or do not mirror the oxidative cost of migration because of the lack of data on birds that are not in the migratory disposition. Finally, our data provide correlative support for the free radical theory of aging in free-living birds.

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