

MF magnitude does not affect body condition, pro-oxidants and anti-oxidants in Eurasian kestrel (*Falco tinnunculus*) nestlings

David Costantini^{a,*}, Stefania Casagrande^{b,c}, Giacomo Dell’Omo^d

^aDipartimento di Biologia Animale e dell’Uomo, Università La Sapienza, Viale dell’Università 32, 00185 Roma, Italy

^bDipartimento di Biologia Evolutiva e Funzionale, Università di Parma, Parco Area delle Scienze 11, 43100 Parma, Italy

^cZoological Laboratory, Research Group Behavioural Biology, University of Groningen, Kerklaan 30, 9751 NN Haren, Groningen, The Netherlands

^dOrnis italica, Piazza Crati 15, 00199 Roma, Italy

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Abstract

Pylons of utility lines are commonly used by breeding birds as structures for supporting their nests. Nesting near power lines, however, exposes adult birds and their offspring to the electric and magnetic fields (EMFs) produced by the current. Therefore, we searched for possible relationships between the magnetic field (MF) magnitude experienced by wild kestrel (*Falco tinnunculus*) nestlings grown on pylons and different health-related variables: body condition, serum concentration of carotenoids, reactive oxygen metabolites (ROMs; marker of early oxidative damage), serum anti-oxidant capacity (OXY), and the ratio between ROMs and OXY (index of oxidative stress). No significant relationships were found between the MF magnitude or squared MFs and any of the variables considered. Comparisons with values recorded in nestlings from non-exposed nests seem to confirm the absence of any effect of exposure to MFs produced by power lines on the variables considered.

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1. Introduction

Overhead power lines represent a relevant threat for wild birds because of injuries and death they cause by collision or electrocution (Bevanger, 1998; Ferrer and Janss, 1999). Another potential threat not adequately investigated so far in free-living birds is the exposure to low-frequency (50/60 Hz) electric and magnetic fields (EMFs) generated by power lines.

Nowadays, many bird species nest regularly on the electric pylons, either in nest-boxes or directly on the structure, or use the pylons for perching or as observational points for hunting. Therefore, they can be exposed for extended periods to EMFs. It has been argued that the exposure of birds to EMFs may represent a source of

potential noxious effects to the health status (Ferne and Reynolds, 2005).

Exposure to low-frequency EMFs is actually of relevant interest because of a supposed causal relationship between exposure and the appearance of neurodegenerative diseases or cancer in humans mediated by oxidative stress (Preece et al., 2000). For example, it has been suggested that the formation of DNA–DNA cross-links or DNA strand breaks in MF-exposed brain cells of rats could result from pervasive free radical damage (Singh and Lai, 1998; Lai and Singh, 2004). However, a previous study reported no significant effect of a 60-Hz magnetic field (MF) on DNA single-strand breaks in Chinese hamster cells (Reese et al., 1988).

As far as birds are concerned, the potential effects of exposure to EMFs similar to those produced by power lines on a number of morphological, behavioural, or physiological variables have been recently reviewed for several captive and wild birds (Ferne and Reynolds, 2005). However, on the basis of such a review, it is difficult to

*Corresponding author. Dipartimento di Biologia Animale e dell’Uomo, Università La Sapienza, Viale dell’Università 32, 00185 Roma, Italy.

Fax: +39 06 4958 259.

E-mail address: david.costantini@uniroma1.it (D. Costantini).

draw a clear picture about the effects and consequences of them on the health of birds. The picture is further complicated by the different sensitivities to EMF exposure bird species appear to have. For example, tree swallows (*Tachycineta bicolor*) show reduced reproductive success under environmentally relevant EMF conditions (Doherty and Grubb, 1996). In contrast, free-living eastern bluebirds (*Sialia sialis*), house wrens (*Troglodytes aedon*), ravens (*Corvus corax*), golden eagles (*Aquila chrysaetos*), and red-tailed hawks (*Buteo jamaicensis*) do not appear to be reproductively sensitive to EMFs from power lines (Steenhof et al., 1993; Doherty and Grubb, 1996).

Fernie et al. (2000) reported that laboratory exposure of captive American kestrels (*Falco sparverius*) to 60-Hz EMFs (30 μ T, 10 kV/m) caused reduced eggshell thickness and hatching success, increased fertility, egg size, and fledging success. In another paper on the same set of experiments, EMF exposed kestrel nestlings were heavier and had longer tarsi while the growth of ninth primaries and central rectrices were unaffected (Fernie and Bird, 2000).

Since it is recognised that adverse conditions experienced during growth and development can affect negatively the health at adulthood in birds (Lindström, 1999; Metcalfe and Monaghan, 2001), it is pivotal to evaluate if EMF exposure in the wild can affect health-related variables in nestlings. Therefore, during the breeding seasons 2002 and 2003, we carried out a study on wild nestling Eurasian kestrels (*Falco tinnunculus*) hatched in nest boxes mounted on high-voltage 50-Hz power lines. The birds were exposed to EMFs from their development *in ovo* until fledging. In the light of the potential shielding of the electric fields (EFs) by the pylon structure, MFs have the potential for greater contribution in causing biological effects. Indeed, MFs are uniformly distributed around the conductors and are not influenced by the structure of the pylon (Garrido et al., 2003).

The study was designed to evaluate the relationships between the MF magnitude experienced by the nestlings and a series of variables marking their health status in order to assess the ecological relevance of such an exposure. The variables considered were body condition (body mass corrected for age differences), which is a variable commonly used to investigate offspring condition in birds (Conway et al., 1994; Ardia, 2006), serum carotenoid concentration, serum reactive oxygen metabolites (ROMs; marker of early oxidative damage), serum anti-oxidant capacity (OXY), and the ratio between ROMs and OXY. This latter variable can be considered as an index of oxidative stress, i.e., the balance between pro-oxidants and anti-oxidants, with higher values indicating higher oxidative stress (see Costantini et al., 2006). If exposure to MFs produced by power lines is detrimental to the chicks' health, we expect the MF magnitude to correlate negatively with body condition, carotenoid concentration, and OXY, and positively with ROMs and oxidative stress.

2. Materials and methods

2.1. Model species

The Eurasian kestrel (*F. tinnunculus*) is a small open-country raptor widespread throughout the Palaearctic, Afrotropical, and Oriental regions. This species readily uses nest boxes mounted on electric pylons in suitable habitats. Since nestlings raised in such nest boxes are exposed to MFs from their development *in ovo* until fledging, this species can be conveniently used as a model to study exposure to MFs in the wild.

2.2. Study area and measurement of MFs

The field study was carried out during the 2002–2003 breeding seasons in a 1200 km² area around Rome. We collected data from nestlings (100 in 2002, 151 in 2003) raised in nest boxes (27 in 2002, 35 in 2003) mounted on the utility lines of two local electric power companies. About 2–6 nestlings were sampled from each nest-box. The power lines included single or double conductors with 60, 150, 220, or 380 kV. Nest boxes were attached on the pylons between 7 and 20 m of height from the ground depending on the structure of the pylons and the morphology of the landscape. Therefore, for a given current, the level of exposure of each nest varied not only as a function of the current intensity carried through the conductor, but also as a consequence of the distance of exposure from the source. Of each nest box used in this study, the MF was measured inside the nest with a polarised magnetometer (UGN.3503/U, Sprague) along each of the three main orthogonal axes (x – z) centred on the internal base of the nest and parallel to the borders. The place of measurements corresponded roughly with the area where eggs were laid and chicks raised. The resulting vectorial component was calculated as the square root of the sum of the square values measured along the three axes ($\sqrt{x^2 + y^2 + z^2}$) (Bowman et al., 1998). The intensity of the resulting vector was used as a measure of the MF level and was expressed in μ T (T = Tesla). In addition, data were also collected from nestlings in non-exposed nest boxes (controls). These included nest boxes mounted on abandoned electric pylons, non-electric pylons (structures used as windmill pumps or observation points), and abandoned buildings.

2.3. Blood collection

A sample of blood (~400 μ L) was taken from the brachial vein (range of age: 5–31-day old in 2002 and 9–31-day old in 2003). Samples were kept cool (0–5 °C) and centrifuged (2 min, 10,000 rpm) within 4–8 h. Serum was stored to –20 °C until laboratory analyses. Serum was used to measure carotenoid concentration (breeding season 2002 and 2003), ROMs and OXY (breeding season 2003). At the time of bleeding, we measured the wing length (maximum chord, mm) and the body mass (g) as well.

All procedures performed in this study were consistent with the European directive for the protection of vertebrate animals used for experimental and other scientific purposes (86/609/EEC).

2.4. Measurement of carotenoids

The serum (100 μ L) was diluted with absolute methanol (1:8) and the flocculent proteins were precipitated by centrifugation at 12,000g for 5 min. Carotenoids were quantified by means of a Beckman DU 7400 spectrophotometer at 476 nm. The carotenoid concentration is estimated as μ g/mL of serum using the standard absorbance curve of lutein (alpha-carotene-3,3'-diol; Sigma–Aldrich), which represents about 90% of the total xanthophyll content in both blood and skin of the Eurasian kestrel (zeaxanthin is the only other carotenoid present; Casagrande et al., 2006).

2.5. Measurement of 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 alkoxy (R-O^\bullet) and alkylperoxy (R-OO^\bullet) radicals. These radicals are able to propagate the oxidative cascade. The serum concentration of ROMs was measured by the d-ROMs test (Diacron, Grosseto, Italy). The serum (10 μL) was first diluted with 200 μL 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 favours the release of iron and copper from serum proteins, which catalyse the cleavage of ROOH in the alkoxy and alkylperoxy radicals. When such compounds react with an alkyl-substituted aromatic amine (A-NH₂) solubilized in the chromogen, they produce a complex whose colour intensity (pink) is directly proportional to their concentration. After incubation, the absorbance was read with a spectrophotometer (Microplate Reader Model 550) at 490 nm and the concentration of ROMs was calculated by comparison with a standard curve obtained by measuring the absorbance of a standard solution. The results of the d-ROMs test are expressed in arbitrary units called “Carratelli units” (CARR U), where 1 CARR U is equivalent to 0.08 mg of H₂O₂/100 mL. Values are expressed as mM of H₂O₂ equivalents (for further details, see Costantini et al., 2006; Costantini and Dell’Omo, 2006a, b).

2.6. Measurement of the serum 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-enzymatic activity) to cope with the oxidant action of hypochlorous acid (HOCl; oxidant of pathologic relevance in biological systems). The serum (10 μL) was diluted 1:100 with distilled water. A 200 μL aliquot of a titred HOCl solution was incubated with 5 μL of the diluted serum for 10 min at 37 °C. Then, 5 μL of the same chromogen solution used for the ROMs determination was added. An alkyl-substituted aromatic amine solubilized in the chromogen is oxidised 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. Values are expressed as mM of HOCl neutralised in reference to a standard curve (for further details see Costantini et al., 2006; Costantini and Dell’Omo, 2006a, b).

2.7. Statistical analyses

The response variables are body condition, serum carotenoid concentration, ROMs, OXY, and oxidative stress (i.e. ROMs/OXY \times 1000). The body mass was used as an index of body condition (corrected for age in the partial correlation) following the approach in Garcia-Berthou (2001). In the graphics, we scored body condition as residuals of body mass on the wing length (linear measure of body size). The wing length was used as an index of age (Costantini et al., 2006). Within-nest mean values were used to avoid pseudoreplication (Hurlbert, 1984). The values of ROMs and oxidative stress were square-root transformed to meet the normality assumption of parametric tests. The ratio between ROMs and OXY can prove useful as a general index of the individual oxidative stress (higher values indicating higher oxidative stress; Costantini et al., 2006; Costantini and Dell’Omo, 2006a). The relationships between MF magnitude and all the response variables were checked by partial correlations to correct for the effect of age because a large span was present (range: 5–31-day old in 2002 and 9–31-day old in 2003). Firstly, we performed the analyses excluding control nests. Subsequently, the analyses were repeated after inclusions of those. We did so because control nests were assigned a nominal value of 0, which could have biased the partial correlation analyses. All the analyses were repeated including the squared MFs in spite of MFs to assess the possible nonlinear relationship between the variables.

The electric current on the lines is not constant during the day, but varies with the electricity demand, and hence the intensity of the MFs may

be expected to vary. The measurements of MFs made throughout the embryonic and nestling development were, however, relatively stable (intra-class correlation coefficient: $r = 0.73$, $P < 0.001$; see Lessells and Boag, 1987), so, an average nest value obtained from 3 to 5 measurements for each nest was included in the analyses.

3. Results

Since some pairs could have contributed to the data set in both the study years, the analyses were carried out for each year separately in order to avoid pseudoreplication.

In 2002, we included in the analyses data collected from 27 nests (100 nestlings). Both body condition ($r = 0.30$, $P = 0.14$) and serum carotenoids ($r = 0.001$, $P = 0.99$) did not correlate significantly with the MFs (mean \pm se: $5.27 \pm 1.01 \mu\text{T}$; min/max: 0.60/20.44 μT ; Fig. 1) or the squared MFs.

In 2003, we included in the analyses data collected from 35 nests (151 nestlings). Again, the MF magnitude (mean \pm se: $4.72 \pm 0.67 \mu\text{T}$; min/max: 0.20/15.46 μT) did not correlate with any of the variables considered: body condition, $r = 0.16$, $P = 0.36$; carotenoid concentration, $r = 0.21$, $P = 0.23$; ROMs, $r = 0.03$, $P = 0.85$; OXY, $r = 0.09$, $P = 0.60$; oxidative stress, $r = -0.01$, $P = 0.94$. Similar results emerged including in the analyses the squared MFs.

The analyses regarding the data of 2003 were repeated after including the measures collected from non-exposed nests (exposure = 0). In total, 5 nests (13 nestlings) were included in the model of body condition and serum carotenoids and 4 nests (12 nestlings) in the models of the remaining variables. Again, all the variables did not correlate with MFs (body condition, $r = 0.22$, $P = 0.17$; carotenoid concentration, $r = 0.18$, $P = 0.28$; ROMs, $r = 0.01$, $P = 0.93$; OXY, $r = 0.12$, $P = 0.46$; oxidative stress, $r = -0.05$, $P = 0.75$; Fig. 2) or squared MFs.

4. Discussion

The present study failed to detect any significant relationship between MF magnitude and body condition, carotenoid concentration, pro-oxidants, anti-oxidants, or general oxidative status in wild kestrel nestlings. The results show that the capability to store nutrients and to maintain a stable redox homeostasis is not related to the prolonged exposure to the MFs generated by the power lines. Further, the values recorded in non-exposed nests are in the range of those measured in exposed ones.

In contrast to our results, captive nestling American kestrels (*F. sparverius*) exposed to EMFs were found to be heavier and have longer tarsi than controls. Yet in the same study other growth parameters were unaffected such as the antebrachial bone, feather lengths, and growth rate (Ferne and Bird, 2000). It should be noted that nestling American kestrels were exposed to a constant and uniform higher exposure intensity than were nestling in our study. In addition, nestling Eurasian kestrels ranged in age from 5 to 31 days while American kestrel chicks were 21-day old.

As far as the physiological status is concerned, short-term (i.e., one breeding season) exposed male American kestrels had suppressed melatonin levels at 42 days and elevated at 70 days of EMF exposure, whilst no effects were found in females (Ferne et al., 1999). Short-term exposed male American kestrels also had suppressed levels of total proteins, haematocrits, erythrocytes, lymphocytes, and carotenoids (Ferne and Bird, 2001). It has been suggested that the suppression of these physiological variables, and previously of melatonin (Ferne et al., 1999), should reflect a higher oxidative stress level in exposed individuals (Ferne and Bird, 2001). None of such variables, however, are reliable markers of oxidative stress (see e.g. de Zwart et al., 1999; Prior and Cao, 1999; Floyd et al., 2001; Dotan et al., 2004). The degree of oxidative stress is currently defined by the balance between pro-oxidant production and anti-oxidant defences (e.g. Finkel and Holbrook, 2000), both of which were not quantified in the study of Ferne and Bird (2001). The suggestion that the exposure to MFs may increase the oxidative stress in birds is therefore not deeply supported by the Ferne et al. series of experiments. Our results suggest that exposure to MFs produced by utility lines does not affect organism capability to maintain redox homeostasis. The increase of

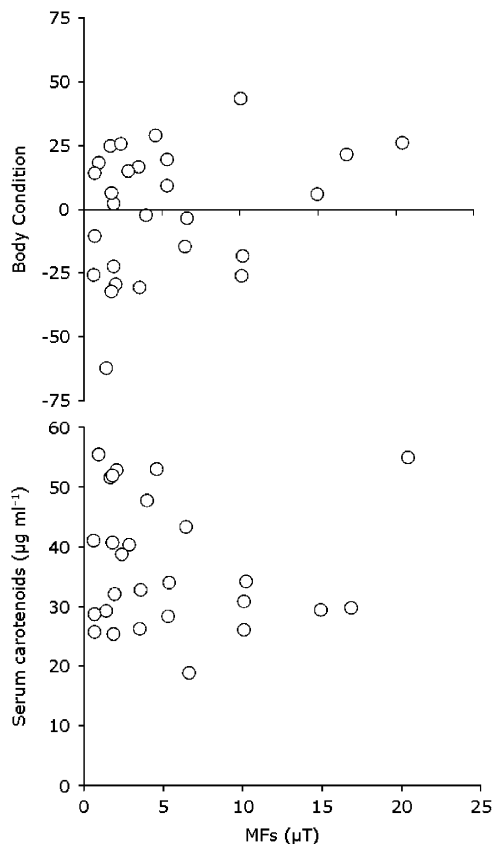


Fig. 1. The scatter plots show the absence of a relationship between the magnitude of magnetic fields (MFs) and (i) body condition (residuals of body mass on the wing length; $F_{1,25} = 30.91$, $R^2 = 0.55$, $P < 0.001$) and (ii) serum carotenoids measured in 2002. Within-nest mean values are shown (27 nests, 100 nestlings).

ROM concentration in the circulating system is indeed a consequence of the imbalance of the redox system consequently to a stressor (Costantini and Dell’Omo, 2006a). In particular, it is known that the dysregulation of the regulatory systems of the redox state leads to a chronic oxidative stress, which, in turn, may give rise to pathological conditions (e.g., Dröge, 2002). Although nestlings were exposed to relevant environmental levels of MFs for a crucial period of their life, i.e., prehatching and early posthatching phase, our study could not detect any effect of “real world” exposure to MFs on carotenoids (i.e., a class of anti-oxidants), ROMs, OXY, or oxidative status in the circulating system. In fact, the levels of carotenoids, ROMs, and OXY measured in the exposed chicks were well in the range of those measured in non-exposed wild (Casagrande et al., 2006) and captive (Costantini, 2006) adult kestrels.

There are a number of differences between the present work and that of the Ferne et al. series of experiments which may explain the lack of significant physiological differences between captive American and wild Eurasian kestrels.

The different species used in the two experiments may indicate differential sensitivity to MF exposure. In toxicological experiments whereby birds are exposed to different chemicals (e.g., polychlorinated biphenyls, flame retardants), it is well documented that chickens are much more sensitive to these chemicals than quails, who in turn are more sensitive than American kestrels (e.g., Scanes and McNabb, 2003).

The different ages of the birds, nestlings (present study) versus adults (Ferne et al. series of experiments), could also account for some of such differences. It is known that nestlings have an immature physiological system, e.g., in terms of anti-oxidant or immune capacity. For example, nestlings have high levels of oxidative stress due to the high metabolic activity caused by the growth and development processes (Surai, 2002; Costantini et al., 2006, 2007). Given this, one would expect nestlings to be less capable than adults to cope with stressors. Consequently, a higher impact of MFs is expected on chicks than on adults, which was not the case in our study.

Male American kestrels, but not females, were found to demonstrate a potential for increased oxidative stress in response to EMF exposure (Ferne et al., 1999). We did not account for sex in the analyses since we previously showed that there are no differences in the oxidative status between male and female nestling kestrels (Costantini et al., 2006).

The length of MF exposure was different between the two experiments. Our field study involved nestling birds which experienced approximately two months of MF exposure as embryos and nestlings. In contrast, the captive adult American kestrels were exposed for a period of three months. This difference in the length of exposure could be indicative of cumulative effects occurring over a longer time period than was measured in our model species.

In the Ferne et al. works, birds were exposed to uniform and constant $30\mu\text{T}$ MFs whereas in our case

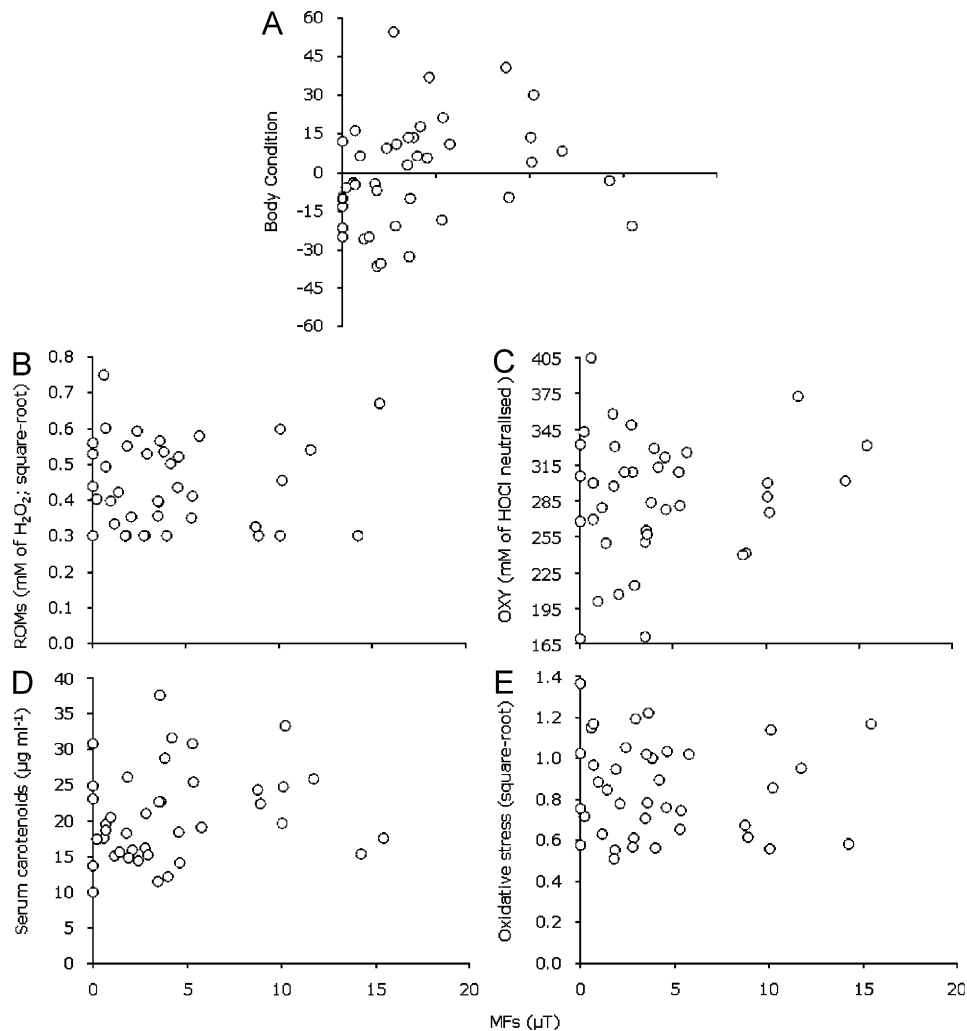


Fig. 2. The scatter plots show the absence of a relationship between the magnitude of magnetic fields (MFs) and (A) body condition (residuals of body mass on the wing length; $F_{1,38} = 25.82$, $R^2 = 0.40$, $P < 0.001$), (B) reactive oxygen metabolites (ROMs), (C) serum anti-oxidant barrier (OXY), (D) serum carotenoids, and (E) oxidative stress (ROMs/OXY $\times 1000$) measured in 2003. Within-nest mean values are shown (40 nests, 164 nestlings: body condition, serum carotenoids; 39 nests, 163 nestlings: ROMs, OXY, oxidative stress). The values of ROMs and oxidative stress are square-root transformed.

exposure was lower and fluctuated during the day. This difference in exposure levels would be more indicative of threshold effects whereby effects occur only at higher MF levels and not at lower MF levels. Our results show that the correlation between MF magnitude and a series of variables marking the health status of the nestlings cannot be adjusted to a quadratic relation. However, further work is needed to evaluate if MFs could be deleterious for the health of nestlings over a certain threshold.

In conclusion, our work failed to find any detrimental effects of MF exposure on body condition, serum carotenoids, or oxidative stress in wild kestrel nestlings. It may not be excluded a priori that potential detrimental effects of ecological relevance on the birds raised in exposed nest boxes may emerge at adulthood. However, in light of the high reproductive success that our population is experiencing since 1998, it is reasonable to assume that it should not be the case.

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