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Neither artificial light at night, anthropogenic noise nor distance from roads are associated with oxidative status of nestlings in an urban population of songbirds

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Abstract

Increasing urbanization is responsible for road-related pollutants and causes an unprecedented increase in light and noise pollution, with potential detrimental effects for individual animals, communities and ecosystems. These stressors rarely act in isolation but studies dissecting the effects of these multiple stressors are lacking. Moreover, studies on urban stressors have mainly focused on adults, while exposure in early-life may be detrimental but is largely ignored. To fill this important knowledge gap, we studied if artificial light at night, anthropogenic noise and road-related pollution (using distance from roads as a proxy) explain variation in oxidative status in great tit nestlings (*Parus major*) in an urban population. Artificial light at night, anthropogenic noise and distance from roads were not associated with variation of the nine studied metrics of oxidative status (superoxide dismutase-SOD-, glutathione peroxidase-GPX, catalase-CAT-, non-enzymatic total antioxidant capacity-TAC-, reduced glutathione-GSH-, oxidized glutathione-GSSG-, ratio GSH/GSSG, protein carbonyls and thiobarbituric acid reactive substances-TBARS). Interestingly, for all oxidative status metrics, we found that there was more variation in oxidative status among individuals of the same nest compared to between different nests. We also showed an increase in protein carbonyls and a decrease of the ratio GSH/GSSG as the day advanced, and an increase of GPX when weather conditions deteriorated. Our study suggests that anthropogenic noise, artificial light at night and traffic-related pollution are not the most important sources of variation in oxidative status in great tit nestlings. It also highlights the importance of considering bleeding time and weather conditions in studies with free-living animals.

Keywords

Distance from road, great tit, light pollution, nestlings, noise pollution, oxidative stress.

1. Introduction

Our world is urbanizing rapidly, with more than half of the global population already living in cities, and this proportion is still growing (UN-Habitat, 2012). The creation and growth of cities and towns impact heavily on ecological processes and have been identified as major threats to global biodiversity (McDonald et al., 2008; McDonnell & Hahs, 2015). Urbanization is responsible for modification, fragmentation and loss of habitat, with a consequent decline of many animal species (Seto et al., 2012; McDonnell & Hahs, 2015), and furthermore for chemical pollution (Grimm et al., 2008). Recently, there has been a growing interest in two major threats that are closely linked to urbanization, artificial light at night (ALAN), also known as light pollution (Hölker et al., 2010), and noise pollution (Barber et al., 2010). Light pollution is mainly caused by street lights and noise pollution is mainly caused by traffic noise (Seress & Liker, 2015).

Recent research has shown that road-related pollutants such as air pollution and both light and noise pollution may influence a large variety of physiological and behavioural aspects (Pauley, 2004; Isaksson et al., 2005; Blask, 2009; Isaksson, 2015; Swaddle et al., 2015). Chemical pollution has been suggested to affect physiology in wild animals (reviewed in Isaksson, 2015). For example, oxidative status appeared to be affected in urban wild great tits, *Parus major* (Isaksson et al., 2005; but see also Isaksson et al., 2009). ALAN may have direct effects on animal physiology, e.g. it suppresses melatonin (Bedrosian et al., 2016) and increases stress hormones (corticosterone) in songbirds (e.g. Russ et al., 2015, Ouyang et al., 2016). Indirect effects on physiology may also occur through altered animal behaviour, e.g. ALAN affects daily rhythms (e.g. Dominoni et al., 2014), disrupts sleep (e.g. Raap et al., 2015), affects foraging (e.g. Russ et al., 2015) and nestling provisioning in songbirds (Tracey et al., 2014). Likewise, noise pollution may have direct effects on bird physiology as it increased stress hormones (Crino et al., 2013) and decreased telomere length (Meillère et al.,

2015). Noise may also indirectly affect animal physiology, e.g. through impairment of parent-offspring communication which is likely to affect offspring health (Lucass et al., 2016).

An important aspect of the physiological system that might be affected by urbanisation through road-related pollutants, such as chemical, light and noise pollution, is the oxidative balance (Isaksson, 2015). Recent work has shown that oxidative stress may be a mediator of some life-history trade-offs (Costantini, 2014). This is because increased molecular damage and depletion of antioxidants may influence growth, reproductive strategies and survival. In the urban environment, stressors such as light, noise and chemical pollution may influence the oxidative balance through their effect on hormones like melatonin and corticosterone (Isaksson, 2015). In fact previous studies have shown that melatonin, which is also an antioxidant, and corticosterone, which has pro-oxidant effects, can affect the oxidative status (Yadav & Haldar, 2014; Costantini et al., 2011) together with chemical pollutants (Isaksson, 2010). Furthermore, in an urban environment, stressors rarely act in isolation. Studies that dissect the effects of these multiple stressors (e.g. light and noise pollution) are lacking and therefore they are urgently needed to comprehend the impact of urbanization on wild animals and for effective management of protected areas (Barber et al., 2010; Isaksson, 2015; Swaddle et al., 2015).

To fill this important knowledge gap, we examined if road-related pollutants can explain variation in oxidative status in an urban population of the great tit, an important model species. Because conditions experienced during early-life may produce effects that can persist throughout life, we used developing nestlings (Lindstrom, 1999; Metcalfe & Monaghan, 2001; Fonken & Nelson, 2016). We tested whether the variation in oxidative status was better explained by distance of the nest box from the road/highway (which is used as a proxy that integrates information on environmental stress at different levels), noise or light pollution in isolation or by a combination of noise and light pollution. Given the complexity of the

oxidative balance (Dotan et al., 2004; Cohen & McGraw, 2009; Costantini et al., 2013), we examined multiple metrics of antioxidant protection and oxidative damage.

2. Material and methods

2.1 Study area

The study was carried out in the surroundings of Wilrijk (Antwerp), Belgium (51°9'44''N, 4°24'15''E) in a free-living urban population of great tits breeding in nest boxes. Great tits are cavity breeders that readily accept nest boxes for breeding in spring and roosting during winter. Nest boxes are made out of plywood (120 mm wide × 155 mm deep × 250 mm high) with a metal ceiling and an opening of 30 mm ϕ . They were installed in an urbanized area in patches of deciduous woodlands crossed by small roads illuminated during the night by street lights and close to a highway which represents the main source of background noise (Lefebvre et al., 2011). Therefore, nest boxes are exposed to different levels of artificial light and noise depending on their location (in proximity of roads or far away). Since their installation in 1997, the nest boxes have been monitored all year round (see e.g. Rivera-Gutierrez et al., 2010; 2012; Van Duyse et al., 2005; Vermeulen et al., 2016). To allow individual identification, all individuals are metal-ringed as nestlings or when first caught.

2.2 Data sampling

During the breeding season of 2015, the nest boxes were regularly checked as part of our long-term study to obtain information about the start of nest-building, egg-laying, clutch size, hatching date and fledging success. All nestlings were ringed with a numbered metal ring when they were between 10 and 13 days old (hatch day = 1). On day 15 post-hatch, between 7:45 and 15:45 (between 4th of May and 29th of June), a blood sample ($\leq 150 \mu\text{l}$) was taken from the brachial vein of the nestlings with a Microvette CB 300 lithium-heparin tube

(Sarstedt, Numbrecht, Germany). The time of blood collection of each nest was recorded. Directly after blood collection, the body mass of the nestlings (± 0.1 g) was measured using a digital balance (Kern TCB 200-1). The blood samples were kept cool and centrifuged (10,000 rpm for 5 minutes; within an average of two hours after sampling) to separate plasma from red blood cells. After centrifugation, the resulting plasma and red blood cells of each sample were divided into different tubes to avoid repeated thawing and freezing of the same aliquots and then stored at -80°C . In total, a sample of blood from 561 nestlings (85 nests) was collected. Due to blood volume limitations, sample sizes vary between oxidative status metrics ($N = 543-561$).

2.3 Light and noise measurements

Ambient light intensity and noise were measured at each nest box after sunset (May 26-28 2015, between 22:00 and 01:00). Additional noise measurements were taken during the day between 09:30 and 12:30 (June 3-5 2015). Maximum light intensity (lux) was measured with an ILM 1335 light meter (ISO-TECH, Northamptonshire, UK) by placing the photo detector vertically at the nest box opening. Noise amplitude (dB SPL re 20 μPa) was measured with a DVM 401 environmental meter (Velleman Inc., Fort Worth, TX, USA) by placing the microphone at the level of the nest box opening and registering the highest value of background noise amplitude. Both light intensity and noise amplitude were measured at the nest box opening in order to minimize disturbing the nestlings and/or parents. These measurements were considered as a proxy of the levels of light and noise pollution the nestlings were exposed to inside the nest box. The reliability of our noise measurements was confirmed by the high correlation of noise measurements over years (see 2.8 Statistical analyses).

2.4. Distance to the road and the highway

We determined the distance between each nest box and the road and highway in metres using Google Earth. For 13 nest boxes the closest road was the highway and the same value is used for distance to either.

2.5 Collection of data on weather condition

Weather may affect oxidative status, especially low temperatures (Costantini, 2014). Therefore, when the nestlings were sampled, we collected data on weather conditions (rain in mm, average wind speed in km/h and temperature in °C) from a local meteorological station for the area of the city of Antwerp.

2.6 Sex determination

DNA was extracted from 1 µl of red blood cells (RBC) using Chelex 100 resin (Bio-Rad Laboratories; Walsh et al. 1991). CHD-W and CHD-Z genes were amplified from sex chromosomes using polymerase chain reaction with primers P2 and P8 (Griffiths et al., 1998). PCR products were separated by electrophoresis on agarose gel stained with ethidium bromide and visualized by UV transillumination. Birds were sexed according to the presence of a single Z-band for males and Z- and W-bands for females.

2.7 Oxidative status analyses

We analysed in detail different aspects of oxidative status because, given its complexity, it is very difficult to have a good understanding from only one or two measures (Cohen & McGraw, 2009; Costantini et al., 2013; Dotan et al., 2004). We assessed both markers of

oxidative protection and oxidative damage because either low or high antioxidant levels do not necessarily indicate damage is or is not occurring (Costantini & Verhulst, 2009).

Specifically, in RBCs we measured: 1) activity of enzymatic antioxidants, superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX), 2) non-enzymatic total antioxidant capacity (TAC) and 3) molecular antioxidants (reduced glutathione-GSH, oxidized glutathione-GSSG- and the ratio between reduced glutathione and oxidized glutathione-GSH/GSSG). Finally, two markers of oxidative damage were also measured: protein carbonyls (in RBC) and thiobarbituric acid reactive substances (TBARS; in plasma).

Activity of GPX, CAT and SOD was determined from 5 μ l of RBC homogenized in 250 μ l of extracting buffer (pH 7.4; 1.15% KCl and 0.02 M EDTA in 0.01 M PBS) using a MagNALyser (Roche, Vilvoorde, Belgium). All activity measurements were made using a micro-plate reader (Synergy Mx, Biotek Instruments Inc., Vermont, USA). GPX activity was assessed by measuring the decrease in NADPH absorbance measured at 340 nm and calculated from the $6.22 \text{ mM}^{-1} \text{ cm}^{-1}$ extinction coefficient (Drotar et al., 1985). CAT activity was estimated by monitoring the rate of decomposition of H_2O_2 at 240 nm according to the procedure of Aebi (1984). SOD activity was determined by measuring the inhibition of nitroblue tetrazolium (NBT) reduction at 560 nm according to the protocol in Dhindsa et al. (1981). SOD activity was quantified by using a standard curve using known units of purified SOD enzyme under identical conditions against the % of NBT reduction. TAC was assessed using a ferric ion reducing antioxidant power (FRAP) assay following Benzie & Strain (1996). 10 μ l of the RBC (homogenized in the extracting buffer) were mixed with 100 μ l of FRAP reagent (pH 3.6; 0.3 M acetate buffer, 0.01 mM TPTZ in 0.04 mM HCl and 0.02 M $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$) and the absorption was measured at 600 nm after 30 min. In the assay 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) was used as the standard.

GSH and GSSG were measured in RBC using reversed-phase high-performance liquid chromatography (HPLC) with electrochemical detection (Shimadzu, Hai Zhonglu, Shanghai), following the protocol of Sinha et al. (2014). The concentrations of GSH and GSSG was expressed as micromole per gram fresh weight of RBC. GSH/GSSG ratio was calculated as an index of redox state with higher values indicating lower oxidative stress (Jones, 2006).

The concentration of TBARS in plasma was estimated as a measure of lipid peroxidation according to the protocol in El-Shafey & Abdelgawad (2012). Five μl of plasma was mixed with 0.5% (w/v) thiobarbituric acid (TBA) in 20% trichloroacetic acid (TCA). The samples were incubated at 90°C for 45 minutes, then centrifuged at 10.000 g after letting them cool. Absorbance was measured at 532, 600 and 450 nm and amount of malondialdehyde (MDA) equivalents was calculated using the following formula: $6.45 \times (A_{532} - A_{600}) - 0.56 \times A_{450}$ and expressed as nmol MDA equivalents g^{-1} plasma. Protein carbonyls were also measured in RBC as measure of oxidative damage to proteins following the protocol in Levine et al. (1990). RBC were diluted with extracting buffer in order to have a concentration of 2 mg proteins ml^{-1} , as measured using the Bradford protein assay (Bradford, 1976). The concentration of protein carbonyls was expressed as nanomoles per milligram proteins.

2.8 Statistical analyses

We analysed all data using R (R Core Team 2015). When doing correlation analyses we either used Spearman Rank or Pearson correlation depending on the distribution of the data (Zuur et al., 2009). In our statistical analyses we used average values of the noise measured (in 2015) during the day and the night because they were highly correlated (Pearsons $r = 0.63$, $P < 0.001$). Using the average may thus give an indication of the level of noise exposure to which the nestlings are exposed to on a 24 hour basis. Noise levels during the night are highly

correlated between the previous years (2012, 2013, 2014, 2015; Pearson's $r \geq 0.52$, $P < 0.001$). Furthermore it was shown that noise levels are similar during the day and between working days and weekends (Departement L.N.E., 2016). Data on rain, average wind speed and temperature were used in a Principal Component Analysis (PCA) to obtain an overall variable for weather conditions. We used the First Principal Component Analysis (PC1) in the subsequent analyses as a variable that summarizes the weather conditions. PC1 explains 54.5% of the variance for temperature, rain and wind. The loadings are -0.538 for temperature, 0.596 for rain and 0.595 for wind. When the variable increases, this indicates a deterioration of the weather conditions: colder, windier and rainier.

To avoid possible pseudoreplication, we examined whether our data exhibited spatial autocorrelation (Zuur et al., 2009) but this was not detected. Following Anderson & Burnham (2002), we then used an information-theoretic approach to model selection in order to evaluate which factors had an influence on nestling oxidative status.

In order to examine which anthropogenic variable (light and noise, distance to nearest road or distance to highway) best explained variation in oxidative status, three different “full models” were constructed of each oxidative status metric (Table S1; lme4 package, Bates et al., 2014). Three separate full models (A, B and C; see below) were used because of the risk of over fitting the model and because of significant correlations between anthropogenic variables (Table 1) and possible problems with a high degree of collinearity (e.g. the interaction light and distance to the highway: VIF (Variance Inflation Factor)=49.68). Models always included “nest” as random factor because of pseudo replication of sampling nestlings from the same nest. Following the recommendations in Anderson & Burnham (2002), we reduced the number of possible models. We did this by using minimal models that always included the fixed factors sex, laying date, brood size and body mass. These variables have been shown to contribute to explain variation in the oxidative balance in birds (Cohen et al.,

2009; Costantini et al., 2010; De Coster et al., 2012; Giordano et al., 2015; Norte et al., 2009; Tschirren et al., 2003). Furthermore, to all ‘minimal’ models we also added the fixed factors bleeding time and weather condition to evaluate if also these factors could explain variation in nestling oxidative status.

Full model A included light and noise and the three way interaction with sex and all two-way interactions; for full model B we included distance from nearest road and its interaction with sex; for full model C we included distance from highway and its interaction with sex (see Table S1). We compared all models, meaning all possible models from the full model down to the “minimal” model, per oxidative status metric using a model selection approach based on Akaike’s information criterion (AICc was used, Anderson & Burnham, 2002; MuMIn package Barton, 2016). Only models within $\Delta AICc < 4$ from the top ranked model were considered (cf. Halfwerk et al., 2011). In order to meet model assumptions all OS metrics except TBARS were square root transformed, TBARS itself was log transformed. Using “nest” as a random factor allowed us to assess within and among nest variation in oxidative status metrics. Following Xu (2003) we quantified the variation explained by the entire mixed model (R^2).

2.9 Ethical note

The nestling manipulations (blood sampling, weighing) were approved by the ethical committee for animal testing (ECD) of the University of Antwerp (ID number 2014-88) and they were performed in accordance with Belgian and Flemish law. The Belgian Royal Institute for Natural Sciences (Koninklijk Belgisch Instituut voor Natuurwetenschappen) provided ringing licenses for authors and technical personnel.

3. Results

3.1 Artificial light, anthropogenic noise and distance from road

Nest boxes in our study population were exposed to artificial light at night with a light intensity ranging between 0.01 and 6.4 lux. Noise amplitude ranged from 44.5 to 86.5 dB during the day and from 33 to 76.5 dB during the night. Distance of nest boxes from the nearest road ranged from 2 to 294 m and distance of the nest box from the highway from 5 to 1687 m.

Several of the anthropogenic factors (light, noise and distance from road/highway) were correlated with each other (Table 1). Light and noise levels were, however, not correlated in our urban population and therefore used as two independent factors in our statistical analyses. Noise amplitudes were significantly negatively correlated with both distance from the highway and from the nearest road, while light levels were not correlated with both variables. Distance from the highway and from the road were weakly positively correlated, given that for 13 nests they coincide.

3.2 Artificial light, anthropogenic noise, distance from road and markers of oxidative status

Overall, none of the anthropogenic factors (artificial light at night, noise and distance from road/highway) explained variation in any of the oxidative status markers (descriptive statistics are shown in Table 2): none of the models with these factors received any support in the models selection procedure (Table 3). Only for CAT, the model including light received support, but very weakly ($\Delta AICc = 3.2$). In fact, overall, the best supported model to explain variation in CAT was the “minimal model” which is the model including only sex, laying date, brood size and body mass ($\Delta AICc = 0$).

3.3 Within and among nest variation in oxidative status

For all oxidative status metrics the within nest variation was larger than the between nest variation (Table 3). For all OS metrics (except GPX), the proportion of variation explained by between nest variation ranged between 0.16 and 0.37, while the proportion of variation explained by within nest variation was between 0.63 and 0.84. For GPX, the proportion of variation explained by within and between nest variation was rather similar.

3.4 Bleeding time, weather conditions and oxidative status metrics

The best-supported model for the ratio GSH/GSSG and for protein carbonyls included the variable “bleeding time”. Protein carbonyls increased and the ratio GSH/GSSG decreased as the day progressed (Figure 1). Weather conditions explained variation of GPX, as the model including weather conditions received substantial support with a Δ AICc of 0.9 (Table 3). When the weather conditions deteriorated nestlings’ levels of GPX activity increased (Figure 2). Therefore nestlings showed higher levels of GPX when it was colder, windier and rainier.

4. Discussion

4.1 Artificial light, anthropogenic noise and distance to road

We investigated whether the variation in oxidative status in an urban population of great tit nestlings was better explained by distance of the nest box from the road and from the highway (which is used as a proxy that integrates information on environmental stress at different levels), noise or light pollution alone or by a combination of noise and light pollution. While light and noise pollution often coincide (Swaddle et al., 2015), in our study area light at night and noise were not correlated. Neither noise, light pollution nor a combination of both explained variation in oxidative status. Nestling oxidative status was also unrelated to distance from the road/highway.

4.2 Light pollution and oxidative status

Artificial light at night did not explain variation in any of the markers of oxidative status measured. The model selection procedure showed that there was weak support for a model that included light as a factor to explain variation in CAT. This result was however not convincing enough to infer that light acted as a stressor which affected the oxidative status of the offspring.

In our study area, light intensity at the nest box opening ranged between 0.01 and 6.4 lux. Although this intensity was used as a proxy of light pollution and nestlings in the nest box may have been exposed to lower levels of light intensity, it has repeatedly been shown that very low light intensities can already significantly affect bird physiology and behaviour (Dominoni et al., 2013; de Jong et al., 2016). Urban-like night illumination (0.3 lux) advanced the timing of reproductive physiology in blackbirds, *Turdus merula* (Dominoni et al., 2013) and a light intensity as low as 0.05 lux was found to advance the onset of daily activity and increase nocturnal activity in great tits (de Jong et al., 2016). Therefore, we had expected that the oxidative status of the nestlings would have been affected in our urban population. Previous experiments in the field showed that one night of light exposure (1.6 lux) affected nightly activity/sleep of nestling great tits (Raap et al., 2016a) which may increase metabolic demand leading to oxidative stress (Noguera et al., 2010; Boncoraglio et al., 2012; Costantini, 2014). Also, two nights of experimental light exposure (3 lux) in the nest box caused an increase in haptoglobin and decrease in nitric oxide (Raap et al., 2016b), which are two important indicators of immunity and health state (Sild & Horak, 2009; Matson et al., 2012), and reduced body mass gain of nestlings (Raap et al., 2016c), which is a good proxy for condition (Both et al., 1999; Maness & Anderson, 2013). Although the same light treatment (two nights, 3 lux) did not affect the oxidative status of the nestlings (Raap et al., 2016c),

nestlings in the present study were exposed to light at night during the entire development and this might have affected their oxidative status. It would have been also in accordance with laboratory studies which showed that long term exposure to ALAN can affect the oxidative status of an organism (Baydas et al., 2001; Cruz et al., 2003; Hardeland et al., 2003; Ashkenazi & Haim, 2013). Moreover, nestling oxidative status might have been affected through indirect effects such as altered adult behaviour (e.g. nestling provisioning; Stracey et al., 2014). However, our study suggests that wild organisms do not suffer from the same health consequences of ALAN than laboratory organisms. Furthermore, effects on oxidative stress may differ between nestlings and adults as for example adult great tits exposed to air pollution showed increased oxidative stress but nestlings did not (Isaksson et al., 2005).

4.3 Noise pollution and oxidative status

Nestlings from noisier environments did not show higher levels of oxidative stress than nestlings exposed to lower noise amplitudes. The level of ambient noise exposure ranged between 39.5 to 78.5 dB in our population. Previous studies showed that noise levels of ≥ 63 dB may already reduce telomere length in nestlings of the house sparrow, *Passer domesticus* (Meillère et al., 2015). Also, laboratory studies have shown that exposure to anthropogenic noise may affect the oxidative status (e.g. Demirel et al., 2009; Cheng et al., 2011). Surprisingly, we found no association between noise pollution and any of the oxidative status metrics. Meillère et al. (2015) also found no effect of noise pollution on nestling body size, body condition or baseline corticosterone in nestling house sparrows. In song sparrows (*Melospiza melodia*) noise also appeared not to affect stress physiology or condition (Grunst et al., 2014). Furthermore, although a long term population study showed a negative correlation between great tit reproduction and noise (Halfwerk et al., 2011), an experimental

study found no effect of noise on great tit reproduction (clutch size, chick growth, fledging mass and number of fledglings; Halfwerk et al., 2016).

4.4 Distance from the road /highway and oxidative status

Nest box distance from the closest road or from the highway did not contribute to explain the variation in oxidative status in our population. Distance from the road is a complex variable including habitat fragmentation, sensory disturbance (light and noise pollution) and chemical pollution (McClure et al, 2013; Fahrig & Rytwinski, 2009). Oxidative stress measured in great tit lungs and livers appeared not to be affected by urbanisation (Isaksson et al., 2009). However, while urban adult great tits had increased oxidative stress (GSH/GSSG ratio) nestlings did not (Isaksson et al., 2005). Our results are in line with these findings. Isaksson et al. (2005) offered the possible explanation that adults may be exposed to more/other stressors (e.g. predation, poor nutrition from previous winter) compared to nestlings that are in a relatively safe and warm environment inside the nest box.

4.5 Within and among nest variation in oxidative status

To our knowledge, our study is the first to show that there is a high heterogeneity in all measured oxidative status parameters within nests both in antioxidant protection and oxidative damage. We found that there was more variation in oxidative status parameters between individuals of the same nest compared to between different nests. This was true for all metrics of oxidative status, apart from GPX in which the proportion of variation explained between and within nests was rather similar. Likewise, Vermeulen et al. (2015) found that there was a higher heterogeneity within nests than among nests for several aspects of the innate immune function (haptoglobin, nitric oxide, circulating natural antibodies and the complement system) and for metal pollutants (arsenic, lead and cadmium). The high heterogeneity found in our

study indicates that an individual-based approach is required to investigate oxidative status in wild populations.

4.6 Bleeding time, weather conditions and oxidative status

Both bleeding time and weather conditions appeared to be important variables to take into account when studying oxidative status in wild nestlings. Later during the day there was an increase in oxidative damage. Protein carbonyls (oxidative protein damage) increased and the ratio GSH/GSSG (index of redox state with lower values indicating higher oxidative stress; Jones, 2006) decreased. Our results are in line with laboratory studies and confirm rhythmicity of oxidative status markers in free-living animals. Diurnal time rhythms of oxidative damage compounds such as protein carbonyls (Coto-Montes et al., 2001; Burkhardt et al., 1999; Coto-Montes & Hardeland, 1997) and of antioxidant protection as glutathione activity (Beaver et al., 2012; Hardeland et al., 2003) have already been shown in other taxa in laboratory studies. It is still unclear if the rhythms of antioxidants and oxidation production depend on differential expression of genes, on daily fluctuations in secretion of stress hormones or melatonin, on daily fluctuation of mitochondrial activity, on variation in food intake and/ or on a response to environmental stimuli (Costantini, 2014). Future studies may clarify the mechanism behind daily rhythms in oxidative status metrics.

We also found that weather conditions (summarized in a vector explaining temperature, wind and rain) could explain variation in one of the markers of antioxidant protection. GPX increased when the weather conditions deteriorated (i.e. when it was colder, windier and rainier). GPX is an antioxidant enzyme which could have been up-regulated to counterbalance the production of oxidative damage compounds (Rodriguez et al., 2004). Bad weather conditions, such as lower temperatures, could have acted as an environmental stressor and the up-regulation of GPX might explain why we did not show higher levels of oxidative

damage. Cold stress can affect the oxidative balance through multiple mechanisms (Costantini, 2014). For example, metabolic rate may be increased to maintain a constant body temperature which in its turn causes an up-regulation of the antioxidants and/or increase of oxidative damage compounds. Cold stress can also decrease the mitochondrial membrane fluidity which may lead to an increase in reactive species production. In our study the variable “weather condition” was composed out of several weather related variables: temperature, wind speed and rain. When it was colder there was also more precipitation and higher wind speeds. However, variation in oxidative status is most likely explained by temperature variation because the nestlings were sheltered from wind and rain inside their nest box.

4.7 Conclusions

Our study suggests that anthropogenic noise, artificial light at night and traffic-related pollution are not the most important sources of variation in oxidative status in great tit nestlings in the urban population under study. The high heterogeneity in oxidative status found within nests may have masked possible effects of pollution on the oxidative balance of the nestlings.

Our study highlights the importance of considering the timing of bleeding as well as the weather conditions at the time of sampling when using free-living animals. Finally, given the correlative nature of our study, it would be important to use an experimental approach to confirm our findings.

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References

Aebi, H., 1984, Catalase in vitro, *Methods in Enzymology* 105, 121–126.

Anderson, D., R., & Burnham, K., P., 2002, Avoiding pitfalls when using information-theoretic methods, *Journal of Wildlife Management* 66, 912-918.

Ashkenazi, L., & Haim, A., 2013, Effect of light at night on oxidative stress markers in Golden spiny mice (*Acomys russatus*) liver, *Comparative Biochemistry and Physiology A Molecular and Integrative Physiology* 165, 353-7.

Barber, J., R., Crooks, K., R., Fristrup, K., M., 2010, The cost of chronic noise exposure for terrestrial organisms, *Trends in Ecology and Evolution* 25, 180-189.

Barton, K., 2016, MuMIn Multi-model inference. Available at <https://CRAN.R-project.org/package=MuMIn>.

Bates, D., Maechler, M., Bolker, B., Walker, S., 2014, lme4: Linear mixed-effects models using Eigen and S4. R package version 1.0-6. Available at <http://CRAN.R-project.org/package=lme4>.

Baydas, G., Ercel, E., Canatan, H., Donder, E., Akyol, A., 2001, Effect of melatonin on oxidative status of rat brain, liver and kidney tissues under constant light exposure, *Cell Biochemistry and Function* 19, 37-41.

Beaver, L., M., Klichko V., I., Chow, E.S., Kotwica-Rolinska, J., Williamson, M., Orr, W., C., Radyuk, S., N., Giebultowicz, J., M., 2012, Circadian regulation of glutathione levels and biosynthesis in *Drosophila melanogaster*, PLoS ONE 7, e50454.

Bedrosian, T., A., Fonken, L., K., Nelson, R., J., 2016, Endocrine Effects of Circadian Disruption, Annual Review of Physiology 78, 109-131.

Benzie, I., F., & Strain, J., J., 1996, The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay, Analytical Biochemistry 239, 70-76.

Blask, D., E., 2009, Melatonin, sleep disturbance and cancer risk, Sleep Medicine Reviews 13, 257-264.

Boncoraglio, G., Caprioli, M., Saino, N., 2012, Solicitation displays reliably reflect oxidative damage in barn swallow nestlings, Behavioural Ecology and Sociobiology 66, 539-546.

Both, C., Visser, M., E., Verboven, N., 1999, Density-dependent recruitment rates in great tits: the importance of being heavier, Proceedings of the Royal Society B 266, 465-469.

Bradford, M., 1976, A Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein-Dye Binding, Analytical Biochemistry 72, 248-254.

Burkhardt, S., Coto-Montes, A., Hardeland, R., 1999, Diurnal rhythm of protein carbonyl in *Gonyaulax polyedra*. In: Hardeland R (ed) Studies on antioxidants and their metabolites. Cuvillier, Göttingen.

Cheng, L., Wang, S.-H., Chen, Q.-C., Liao, X.-M., 2011, Moderate noise induced cognition impairment of mice and its underlying mechanisms, *Physiology & Behavior* 104, 981-988.

Cohen, A., A., & McGraw, K., J., 2009, No simple measures for antioxidant status in birds: complexity in inter- and intraspecific correlations among circulating antioxidant types, *Functional Ecology* 23, 310-320.

Cohen, A., A., McGraw, K., J., Robinson, W., D., 2009, Serum antioxidant levels in wild birds in relation to diet, season, life history strategy, and species, *Oecologia* 161, 673–683.

Costantini, D., 2014, *Oxidative Stress and Hormesis in Evolutionary Ecology and Physiology—A Marriage between Mechanistic and Evolutionary Approaches*. Heidelberg: Springer-Verlag, Berlin.

Costantini, D., Carello, L., Fanfani, A., 2010, Relationships among oxidative status, breeding conditions and life-history traits in free-living Great Tits *Parus major* and Common Starlings *Sturnus vulgaris*, *Ibis* 152, 793-802.

Costantini, D., Marasco, V., Møller, A., P., 2011, A meta-analysis of glucocorticoids as modulators of oxidative stress in vertebrates, *Journal of Comparative Physiology B* 181, 447-456.

Costantini, D., Monaghan, P., Metcalfe, N., B., 2013, Loss of integration is associated with reduced resistance to oxidative stress, *Journal of Experimental Biology* 216, 2213-20.

Costantini, D., & Verhulst, S., 2009, Does high antioxidant capacity indicate low oxidative stress? *Functional Ecology* 23, 506-509.

Coto-Montes, A., & Hardeland, R., 1997, Diurnal time patterns of protein carbonyl in *Drosophila melanogaster*: comparison of wild-type flies and clock mutants. In: Hardeland R (ed) *Biological rhythms and antioxidative protection*, Cuvillier, Göttingen.

Coto-Montes, A., Boga, J., A., Tomás-Zapico, C., Rodríguez-Colunga, M., J., Martínez-Fraga, J., ToliviaCadrecha, J., Menéndez, G., Hardeland, R., Tolivia, D., 2001, Porphyrin enzymes in hamster Harderian gland, a model of damage by porphyrins and their precursors. A chronobiological study on the role of sex differences, *Chemico-Biological Interactions* 134, 135–149.

Crino, O., L., Johnson, E., E., Blickley, J., L., Patricelli, G., L., Breuner, C., W., 2013, Effects of experimentally elevated traffic noise on nestling white-crowned sparrow stress physiology, immune function and life history, *Journal of Experimental Biology* 216, 2055-2062.

Cruz, A., Padillo, F., J., Granados, J., Túnez, I., Muñoz, M., C., Briceño, J., Pera-Madrado, C., Montilla, P., 2003, Effect of melatonin on cholestatic oxidative stress under constant light exposure, *Cell Biochemistry and Function* 21, 377-80.

De Coster, G., De Neve, L., Verhulst, S., Lens, L., 2012, Maternal effects reduce oxidative stress in female nestlings under high parasite load, *Journal of Avian Biology* 43, 177–185.

De Jong, M., Jeninga, L., Ouyang, J., Q., Oers, K., V., Spoelstra, K., Visser, M., E., 2016, Dose-dependent responses of avian daily rhythms to artificial light at night, *Physiology and Behavior* 155, 172-179.

Demirel, R., Mollaoğlu, H., Yeşilyurt, H., Üçok, K., Ayçiçek, A., Akkaya, M., Genç, A., Uygur, R., Doğan, M., 2009, Noise Induces Oxidative Stress in Rat, *European Journal of General Medicine* 6, 20-24.

Departement Leefmilieu Natuur en Energie, 2016, Geluidsmetnet: cijfers en rapporten, from <https://www.lne.be/geluidsmetnet-cijfers-en-rapporten>.

Dhindsa, R., S., Plumb-Dhindsa, P., Thorpe, T., A., 1981, Leaf Senescence: Correlated with Increased Levels of Membrane Permeability and Lipid Peroxidation, and Decreased Levels of Superoxide Dismutase and Catalase, *Journal of Experimental Botany* 32, 93–101.

Dominoni, D., M., Quetting, M., Partecke, J., 2013, Artificial light at night advances avian reproductive physiology, *Proceedings of the Royal Society B* 280, 20123017.

Dominoni, D., M., Carmona-Wagner, E., O., Hofmann, M., Kraunstauber, B., Partecke, J., 2014, Individual-based measurements of light intensity provide new insights into the effects of artificial light at night on daily rhythms of urban-dwelling songbirds, *Journal of Animal Ecology* 83, 681-692.

Dotan, Y., Lichtenberg, D., Pinchuk, I., 2004, Lipid peroxidation cannot be used as a universal criterion of oxidative stress, *Progress in Lipid Research* 43, 200-27.

Drotar, A., Phelps, P., Fall, R., 1985, Evidence for glutathione peroxidase activities in cultured plant cells, *Plant Science* 42, 35–40.

El-Shafey, N., M., & AbdElgawad, H., 2012, Luteolin, a bioactive flavone compound extracted from *Cichorium endivia* L. subsp *divaricatum* alleviates the harmful effect of salinity on maize, *Acta Physiologiae Plantarum* 34, 2165-2177.

Fahrig, L., & Rytwinski., T., 2009, Effects of roads on animal abundance: an empirical review and synthesis, *Ecology and Society* 14, 21.

Fonken, L., K., & Nelson, R., J., 2016, Effects of light exposure at night during development, *Current Opinion in Behavioral Sciences* 7, 33-39.

Giordano, M., Costantini, D., Tschirren, B., 2015, Sex-specific effects of prenatal and postnatal nutritional conditions on the oxidative status of great tit nestlings, *Oecologia* 177, 123-131.

Griffiths, R., Double, M. C., Orr, K., Dawson, R., J., G., 1998, A DNA test to sex most birds, *Molecular Ecology* 7, 1071–1075.

Grimm, N., B., Faeth, S., H., Golubiewski, N., E., Redman, C., L., Janguo, W., Wu, J., Bai, X., Briggs, J., M., 2008, Global change and the ecology of cities, *Science* 319, 756-760.

Grunst, M., L., Rotenberry, J., T., Grunst, A., S., 2014, Variation in adrenocortical stress physiology and condition metrics within a heterogeneous urban environment in the song sparrow *Melospiza melodia*, *Journal of Avian Biology* 45, 574-583.

Halfwerk, W., Both, C., Slabbekoorn, H., 2016, Noise affects nest-box choice of 2 competing songbird species, but not their reproduction, *Behavioral Ecology*, 1-9.

Halfwerk, W., Holleman, L., J., M., Lessells, C., M., Slabbekoorn, H., 2011, Negative impact of traffic noise on avian reproductive success, *Journal of Applied Ecology* 48, 210-219.

Hardeland, R., Coto-Montes, A., Poeggeler, B., 2003, Circadian rhythms, oxidative stress, and antioxidative defense mechanisms, *Chronobiology International* 20, 921-962.

Hölker, F., Wolter, C., Perkin, E., K., Tockner, K., 2010, Light pollution as a biodiversity threat, *Trends in Ecology and Evolution* 25, 681-682.

Isaksson, C., 2010, Pollution and its impact on wild animals: a meta-analysis on oxidative stress, *EcoHealth* 7, 342-350.

Isaksson, C., 2015, Ecology of organisms in urban environments-Urbanization, oxidative stress and inflammation: a question of evolving, acclimatizing or coping with urban environmental stress, *Functional Ecology* 29, 913-923.

Isaksson, C., Ornborg, J., Stephensen, E., Andersson, S., 2005, Plasma glutathione and carotenoid coloration as potential biomarkers of environmental stress in great tits, *EcoHealth* 2, 138–146.

Isaksson, C., Sturve, J., Almrot, B., C., Andersson, S., 2009, The impact of urban environment on oxidative damage (TBARS) and enzymatic and non-enzymatic defence system in lungs and liver of great tits, *Parus major*, *Environmental Research* 109, 46–50.

Jones, D., P., 2006, Redefining oxidative stress, *Antioxidant & Redox Signaling* 8, 1865–1879.

Lefebvre, W., Schillemans, L., Op 't Eyndt, T., Vandersickel, M., Poncelet, P., Neuteleers, C., Dumez, J., Janssen, S., Vankerkom, J., Maiheu, B., Janssen, L., Buekers, J., Mayeres, I., 2011, Voorstel van maatregelen om de luchtkwaliteit te verbeteren en de geluidshinder te beheersen in de stad Antwerpen, VITO.

Levine, R., L., Garland, D., Oliver, C., N., Amici, A., Climent, I., Lenz, A., G., Ahn, B., W., Shaltiel, S., Stadtman, E., R., 1990, Determination of carbonyl content in oxidatively modified proteins. *Methods in Enzymology* 186, 464–478.

Lindstrom, J., 1999, Early development and fitness in birds and mammals, *Trends in Ecology and Evolution* 14, 343-348.

Lucass, C., Eens, M., Muller, W., 2016, When ambient noise impairs parent-offspring communication, *Environmental Pollution* 212, 592-597.

Maness, T., J., & Anderson, D., J., 2013, Predictors of juvenile survival in birds, *Ornithological Monographs* 78, 1-55.

Matson, K., D., Horrocks, N., P., Versteegh, M., A., Tieleman, B., I., 2012, Baseline haptoglobin concentrations are repeatable and predictive of certain aspects of a subsequent experimentally-induced inflammatory response, *Comparative Biochemistry and Physiology - Part A: Molecular & Integrative Physiology* 162, 7–15.

McClure, C., J., W., Ware, H., E., Carlisle, J., Kaltenecker, G., Barber, J., R., 2013, An experimental investigation into the effects of traffic noise on distributions of birds: avoiding the phantom road, *Proceedings of the Royal Society B* 280, 20132290.

McDonald, R., I., Kareiva, P., Forman, R., T., T., 2008, The implications of current and future urbanization for global protected areas and biodiversity conservation., *Biological Conservation* 141, 1695-1703.

McDonnell, M., J., & Hahs, A., K., 2015, Adaptation and Adaptedness of Organisms to Urban Environments, *Annual Review of Ecology, Evolution and Systematics* 46: 261-280.

Meillère, A., Brischoux, F., Ribout, C., Angelier, F., 2015, Traffic noise exposure affects telomere length in nestling house sparrows, *Biology Letters* 11, 20150559.

Metcalf, N., B., & Monaghan, P., 2001, Compensation for a bad start: grow now, pay later?, *Trends in Ecology and Evolution* 16, 254–260.

Noguera, J., C., Morales, J., Pérez, C., Velando, A., 2010, On the oxidative cost of begging: antioxidants enhance vocalizations in gull chicks, *Behavioural Ecology* 21, 479–484.

Norte, A., C., Sheldon, B., N., Sousa, J., P., Ramos, J., A., 2009, Environmental and genetic variation in body condition and blood profile of Great Tit *Parus major* nestlings, *Journal of Avian Biology* 40, 157–165.

Ouyang, J., Q., Lendvai, A., Z., Moore, I., T., Bonier, F., Haussmann, M., F., 2016, Do Hormones, Telomere Lengths, and Oxidative Stress form an Integrated Phenotype? A Case Study in Free-Living Tree Swallows, *Integrative and Comparative Biology* 56, 138-145.

Pauley, S., M., 2004, Lightening for the human circadian clock: recent research indicates that lightening has become a public health issue, *Medical Hypotheses* 63, 588-596.

R Core Team., 2015, R: A language and environment for statistical computing. Available at <https://www.R-project.org/>

Raap, T., Pinxten, R., Eens, M., 2015, Light pollution disrupts sleep in free-living animals, *Scientific Reports* 5, 13557.

Raap, T., Pinxten, R., Eens, M., 2016a, Artificial light at night disrupts sleep in female great tits (*Parus major*) during the nestling period, and is followed by a sleep rebound, *Environmental Pollution* 215, 125-134.

Raap, T., Casasole, G., Pinxten, R., Eens, M., 2016b, Early life exposure to artificial light at night affects the physiological condition: An experimental study on the ecophysiology of free-living nestling songbirds, *Environmental Pollution* 218, 909-914.

Raap, T., Casasole, G., Costantini, D., AbdElgawad, H., Asard, H., Pinxten, R., Eens, M., 2016c, Artificial light at night affects body mass but not oxidative status in free-living nestling songbirds: an experimental study, *Scientific Reports* 6, 35626.

Rivera-Gutierrez, H., F., Pinxten, R., Eens, M., 2010, Multiple signals for multiple messages: great tit, *Parus major*, song signals age and survival, *Animal Behaviour* 80, 451-459.

Rivera-Gutierrez, H., F., Pinxten, R., Eens, M., 2012, Tuning and fading voices in songbirds: age-dependent changes in two acoustic traits across the life span, *Animal Behaviour* 83, 1279-1283.

Rodriguez, C., Mayo, J., C., Sainz, R., M., Antolín, I., Herrera, F., Martín, V., Reiter, R., J., 2004, Regulation of antioxidant enzymes: a significant role for melatonin, *Journal of Pineal Research* 36, 1-9.

Russ, A., Rüger, A., Klenke, R., 2015, Seize the night: European Blackbirds (*Turdus merula*) extend their foraging activity under artificial illumination, *Journal of Ornithology* 156, 123-131.

Seress, G., & Liker, A., 2015, Habitat urbanization and its effects on birds, *Acta Zoologica Academiae Hungaricae* 61, 373-408.

Seto, K., C., Güneralp, B., Hutyra, L., R., 2012, Global forecasts of urban expansion to 2030 and direct impacts on biodiversity and carbon pools, *Proceedings of the National Academy of Sciences of the United States of America* 109, 16083-16088.

Sild, E., & Horak, P., 2009, Nitric oxide production: an easily measurable condition index for vertebrates, *Behavioral Ecology and Sociobiology* 63, 959–966.

Sinha, A., K., AbdElgawad, H., Giblen, T., Zinta, G., De Rop, M., Asard, H., Blust, R., De Boeck, G., 2014, Anti-oxidative defences are modulated differentially in three freshwater teleosts in response to ammonia induced oxidative stress, *PLoS ONE* 9, e95319.

Stracey, C., M., Wynn, B., Robinson, S., K., 2014, Light Pollution Allows the Northern Mockingbird (*Mimus polyglottos*) to Feed Nestlings After Dark, *The Wilson Journal of Ornithology* 126, 366-369.

Swaddle, J., P., Francis, C., D., Barber, J., R., Cooper, C., B., Kyba, C., C., Dominoni, D., M., Shannon, G., Aschehoug, E., Goodwin, S., E., Kawahara, A., Y., Luther, D., Spoelstra, K., Voss, M., Longcore, T., 2015, A framework to assess evolutionary responses to anthropogenic light and sound, *Trends in Ecology and Evolution* 30, 550-560.

Tschirren, B., Fitze, P., S., Richner, H., 2003, Sexual dimorphism in susceptibility to parasites and cell-mediated immunity in great tit nestlings, *Journal of Avian Ecology* 72, 839–845.

UN-Habitat, 2012, State of the World's Cities 2010/2011. *Cities for All: Bridging the Urban Divide*, London, Sterling, VA.

Van Duyse, E., Pinxten, R., Snoeijs, T., Eens, M., 2005, Simultaneous treatment with an aromatase inhibitor and an anti-androgen decreases the likelihood of dawn song in free-living male great tits, *Parus major*, *Hormones and Behavior* 48, 243-251.

Vermeulen, A., Müller, W., Matson, K., D., Tieleman, B., I., Bervoets, L., Eens, M., 2015, Sources of variation in innate immunity in great tit nestlings living along a metal pollution gradient: an individual-based approach, *Science of the Total Environment* 508, 297-306.

Vermeulen, A., Zaid, E., Eens, M., Müller, W., 2016, Baseline innate immunity does not affect the response to an immune challenge in female great tits (*Parus major*), *Behavioral Ecology and Sociobiology* 70, 585-592.

Walsh, P., S., Metzger, D., A., Higuchi, R., 1991, Chelex 100 as a medium for simple extraction of DNA for PCR/based typing from forensic material, *Biotechniques* 10, 506-513.

Xu, R., 2003, Measuring explained variation in linear mixed effects models, *Statistics in Medicine* 22, 3527-3541.

Yadav, S., K., & Haldar, C., 2014, Experimentally induced stress, oxidative load and changes in immunity in a tropical wild bird, *Perdicula asiatica*: involvement of melatonin and glucocorticoid receptors, *Zoology* 117, 261-268.

Zuur, A., F., Ieno, E., N., Walker, N., J., Saveliev, A., A., Smith, G., M., 2009, Mixed effects models and extensions in ecology with R. Springer, New York.

Tables and figures

Table 1 Correlation matrix for the four investigated anthropogenic factors potentially affecting oxidative status (road distance, highway distance, light and noise). Values presented are obtained by Spearman Rank Correlations adjusted for multiple testing (Holm correction). Values indicated in bold are significant correlations (N of nests = 85, $P < 0.05$).

	Highway Distance	Light	Noise
Road distance	0.30	-0.13	-0.44
Highway Distance		-0.13	-0.57
Light			0.20

Table 2 Descriptive values of the non-transformed oxidative status metrics (OS metrics) with sample size (N), Mean and standard deviation (SD).

OS metrics	N	Mean	SD
GSH ($\mu\text{mol/g}$ of fresh weight of red blood cells)	543	1.620	0.746
GSSG ($\mu\text{mol/g}$ of fresh weight of red blood cells)	543	0.930	0.826
GSH/GSSG ($\mu\text{mol/g}$ of fresh weight of red blood cells)	543	3.709	4.465
GPX ($\mu\text{mol NADPH/mg}$ protein per minute)	556	0.009	0.006
CAT ($\mu\text{mol H}_2\text{O}_2/\text{mg}$ protein per minute)	556	0.329	0.258
SOD (units/mg protein per minute)	557	0.463	0.214
TAC ($\mu\text{M trolox/g}$ red blood cells)	556	0.096	0.049
Protein carbonyls (nmol/mg protein)	552	3.715	2.225
TBARS (nmol MDA equivalents/g of plasma)	561	61.280	17.177

Table 3 Models that explained variation in oxidative status metrics after model selection procedure. Model selection was based on an information-theoretic approach following Anderson & Burnham (2002), see 2.8 Statistical analyses. Full models included the anthropogenic factors: light, noise, distance to road/ highway as well as bleeding time and weather (see Table S1). All models included the random factor “nest”. The “minimal” model always includes the factors body mass, sex, laying date and brood size. For each oxidative status metric and model, the “Between” and “Within” nest variation is shown as a proportion of the total variation. Results are based on nestlings (N) from 85 nests. Only models with $\Delta AICc < 4$ are shown for each oxidative status metric.

OS metric	Model	AICc	$\Delta AICc$	Weight	R ²	Between	Within
GSH ($N=543$)	minimal	106.0	0.0	1	0.44	0.33	0.67
GSSG ($N=543$)	minimal	448.8	0.0	1	0.44	0.36	0.64
GSH/GSSG ($N=543$)	minimal + bleeding time	1386.3	0.0	0.79	0.32	0.18	0.82
	minimal	1388.9	2.6	0.21	0.33	0.24	0.76
GPX ($N=556$)	minimal	-2381.9	0.0	0.62	0.54	0.47	0.53
	minimal + weather condition	-2381.0	0.9	0.38	0.54	0.43	0.57
CAT ($N=556$)	minimal	-203.9	0.0	0.83	0.29	0.22	0.78
	minimal + light	-200.7	3.2	0.17	0.29	0.21	0.79
SOD ($N=557$)	minimal	-454.8	0.0	1	0.27	0.20	0.80
TAC ($N=556$)	minimal	-1273.7	0.0	1	0.22	0.16	0.84
Protein Carbonyls ($N=552$)	minimal + bleeding time	888.8	0.0	1	0.45	0.37	0.63
TBARS ($N=561$)	minimal	44.5	0.0	1	0.36	0.28	0.72

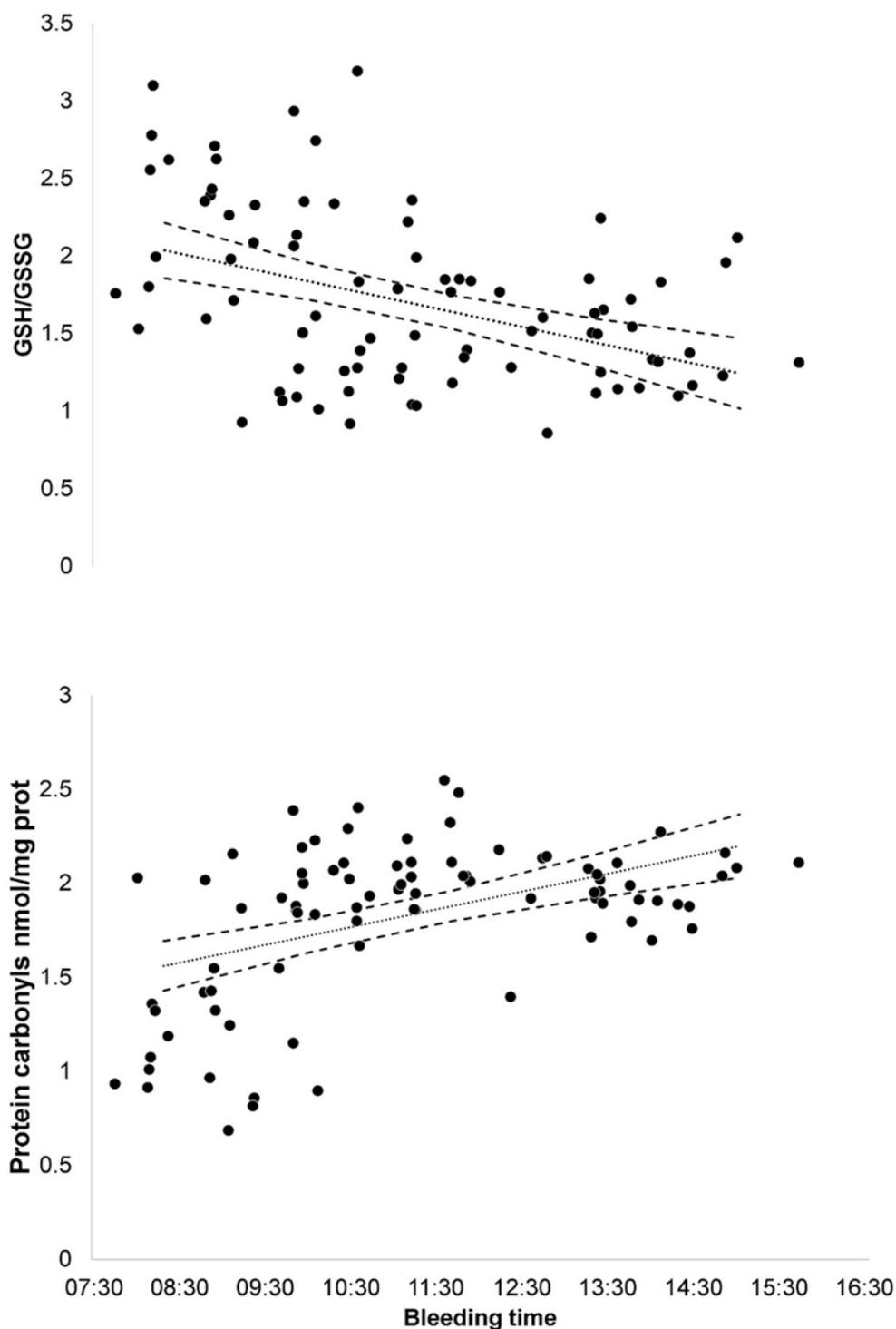


Figure 1 As the day progresses the ratio GSH/ GSSG decreases (top panel) and protein carbonyls increase (lower panel), both indicating higher oxidative damage. Averaged raw data per nest are given together with the estimated effect of bleeding time and the 95% confidence interval from the mixed models (see Table 2). Both estimates indicate higher damage later during the day. Note that both metrics have been square root transformed.

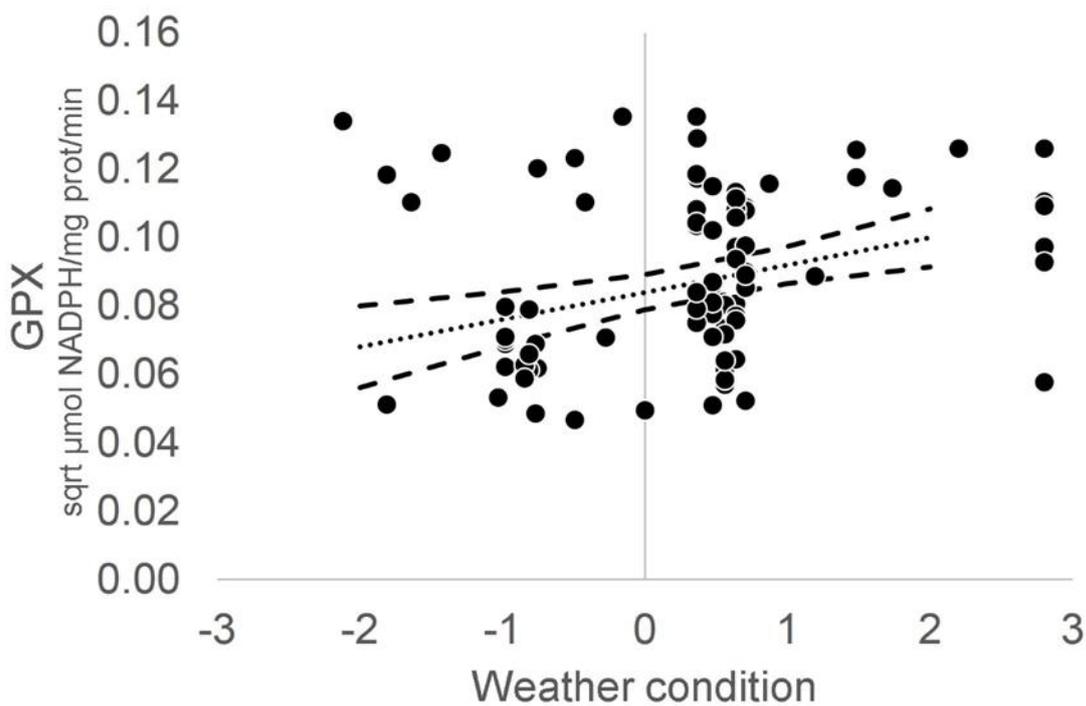


Figure 2 GPX activity increases with harsher weather conditions. Averaged raw data per nest are given together with the estimated effect of weather condition (summarized in a vector explaining temperature, wind and rain) and the 95% confidence interval from the mixed model (see Table 2). Note that all values have been square root transformed.