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Oxidative stress in birds along a NO_x and urbanisation gradient: An interspecific approach

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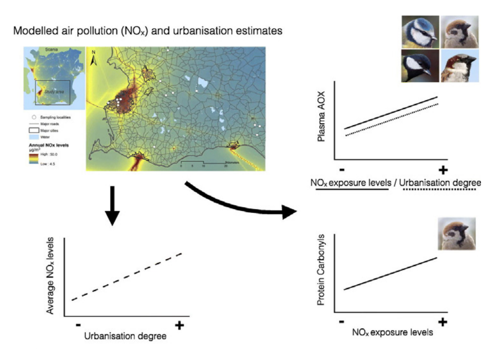
HIGHLIGHTS

- Estimated NO_x and urbanisation levels were positively correlated with AOX in all four species.
- Tree sparrows showed higher levels of protein damage in relation to NO_x level exposure.
- None of the other oxidative biomarkers correlated with NO_x or urbanisation levels.
- Future wildlife urbanisation studies should pay attention to species-specific variation in oxidative stress physiology.

GRAPHICAL ABSTRACT



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ABSTRACT

Urbanisation is regarded as one of the most threatening global issues for wildlife, however, measuring its impact is not always straight forward. Oxidative stress physiology has been suggested to be a useful biomarker of health and therefore, a potentially important indicator of the impact that urban environmental stressors, especially air pollution, can have on wildlife. For example, nitrogen oxides (NO_x), released during incomplete combustion of fossil fuels, are highly potent pro-oxidants, thus predicted to affect either the protective antioxidants and/or cause oxidative damage to bio-molecules. To date, epidemiological modelling of the predicted association between oxidative stress and NO_x exposure has not been performed in wild animals. Here, we address this short-coming, by investigating multiple oxidative stress markers in four common passerine bird species, the blue tit (*Cyanistes caeruleus*), great tit (*Parus major*), house sparrow (*Passer domesticus*) and tree sparrow (*Passer montanus*), living along a gradient of NO_x and urbanisation levels in southern Sweden. First of all, the results revealed that long- and medium-term (one month and one week, respectively) NO_x levels were highly correlated with the level of urbanisation. This confirms that the commonly used urbanisation index is a reliable proxy for urban air pollution. Furthermore, in accordance to our prediction, individuals exposed to higher long- and medium-term NO_x levels/urbanisation had higher plasma antioxidant capacity. However, only tree sparrows showed higher oxidative damage (protein carbonyls) in relation to NO_x levels and this association was absent with urbanisation. Lipid peroxidation, glutathione and superoxide dismutase levels did not co-vary with NO_x/urbanisation. Given that most oxidative stress biomarkers showed strong species-specificity, independent of variation in NO_x/urbanisation, the present study highlights the need to study variation in oxidative stress across contexts, seasons and life-stages in order to understand how the ecology and phylogeny of species interact to affect species resilience to urban environmental stress.

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1. Introduction

In recent times, urban development has increased exponentially and nowadays up to 54% of the world's population live in cities. Urbanisation is expected to expand further as populations continue to rise (United Nations, 2016). In comparison to natural/semi-natural habitats, urban landscapes show pronounced differences regarding abiotic factors such as noise, artificial light at night and air pollution levels (Butler, 2004; Künzli et al., 2006; Grimm et al., 2008; Pickett et al., 2008). In addition, urban wildlife also has to cope with other biotic factors such as new food resources or increased pathogen prevalence (Davies et al., 2009; Evans et al., 2009). Despite the substantial differences from the adjacent ecosystems, some species, populations and/or individuals are able to succeed in this new and urbanised environment (McKinney, 2002; Sol et al., 2014), whereas others struggle (McIntyre, 2000; Ibáñez-Álamo et al., 2017). Nonetheless, for many urban-dwelling organisms, urban stressors may give rise to increased selection pressures, which can make certain life-history traits more or less beneficial (Shochat et al., 2006). Indeed, marked changes in behaviour, physiology and morphology have been widely demonstrated among species inhabiting urban environments, even among those that thrive in them (Isaksson et al., 2005; Partecke et al., 2006; Møller, 2010; Bonier, 2012).

Oxidative stress physiology is an important life-history trait (Monaghan et al., 2009), and has been highlighted as the unifying feature underlying the toxic action of most pollutants, including atmospheric ones (Halliwell and Gutteridge, 2015). Most air pollutants e.g. particulate matter (PM) and gaseous pollutants such as nitrogen oxides (NO_x) or tropospheric ozone (O_3) have the ability to directly or indirectly act as pro-oxidant molecules, thereby causing damage to lipids, DNA and/or proteins, unless the antioxidant system is up-regulated. Nonetheless, a surplus of pro-oxidants can occur and as a consequence, the rate in which damaged molecules accumulate may increase. This pathology is commonly referred to as oxidative stress (Menzel, 1994; Halliwell and Gutteridge, 2015). NO_x are among the most abundant urban chemical pollutants and they are considered to be reliable indicators of general urban air pollution (WHO, 2006). Emissions occur almost anywhere where combustion takes place (e.g., car engines and fire), with motor vehicles accounting for >50% of overall emissions (Hill, 2010). Anthropogenic pollution (including traffic-related air pollution) is known to have detrimental effects on human health, e.g., via increased susceptibility to oxidative stress-related diseases such as pulmonary and cardiovascular disorders (Halonen et al., 2008; Brown et al., 2009). Thus, it is important to understand how, in wildlife populations, the different components of the oxidative stress system vary in response to NO_x, as they could provide a mechanistic link between environmental stressors and fitness (Isaksson, 2015).

Although there is a recent increase in the interest and attention for linking mechanistic approaches to ecological research, there are still few studies that have measured multiple components of the oxidative stress machinery (i.e., different types of oxidative damage and antioxidants) in species inhabiting urban environments (but see Isaksson et al., 2009; Giraudeau et al., 2015; Raap et al., 2016; Casasole et al., 2017). Moreover, studies comparing the physiological responses in multiple species in response to urbanisation and NO_x are currently lacking. Among the existing literature from wildlife, the results are mixed and this is not surprising given the differences in pollution type and levels along with the species-specific variation in life-history strategies (e.g. Almroth et al., 2005; Berglund et al., 2007; Koivula et al., 2011; Herrera-Dueñas et al., 2014; Giraudeau et al., 2015; Espín et al., 2017). In general, un-specific pollution seems to, first of all, affect the antioxidant machinery through an increase in the antioxidant enzymatic activity (Isaksson, 2010, 2015). Previous studies reporting differences in oxidative stress physiology between urban and rural environments, specifically, highlight air pollution as a likely cause (Isaksson et al., 2009; Herrera-Dueñas et al., 2014; Herrera-Dueñas et al., 2017), but no studies have, to date, performed detailed NO_x exposure models at urban/rural sampling sites, taking weather, topography, and traffic load into account prior sampling, and linked it to oxidative stress physiology of wildlife.

The present study has two main aims; *firstly*, to test if a commonly estimated urbanisation index (Seress et al., 2014) is associated with modelled estimates of NO_x levels. This test is essential in order to validate the use of urbanisation index as a proxy for anthropogenic NO_x pollution, i.e. traffic-related air pollution levels. *Secondly*, we investigate how multiple components of the oxidative stress physiology in four common passerine species are associated with long (one month)-, medium (one week)- and short-term (one day) estimates of NO_x exposure and/or urbanisation. The overall prediction is that the level of antioxidants and oxidative damages will be higher with increased NO_x levels and/or urbanisation. To our knowledge this is the first study to perform a multiple-species comparison of oxidative stress status along an urbanisation gradient and in addition, to test for chronic and acute physiological responses in relation to NO_x levels exposure in natural conditions.

2. Material and methods

2.1. Study area and sampling

Fieldwork was performed in the province of Scania in Southern Sweden from the 11th of January to the 25th of February 2015. In Swedish terms, the province is densely populated (approx. 1.2 million people in 11,000 km^2) and Sweden's third largest city, Malmö (>350,000 inhabitants), is located in the area. In addition, the air pollution levels throughout the county differ considerably due to its geographically varying human population density, the proximity to Copenhagen (approx. 2 million inhabitants in the metropolitan area), and the vehicle emissions on motorways and other major roads, as well as transportations to and from harbours (Fig. 1). Birds were caught along an approximated urbanisation gradient, with parks in Malmö city being the most urbanised sites and farmsteads or forests being rural sites. Urbanisation gradient was later quantified specifically for each site, for calculations/methods see "*2.4. Urbanisation gradient*". Four common bird species were captured along the study area: the blue tit (*Cyanistes caeruleus*), great tit (*Parus major*), house sparrow (*Passer domesticus*) and tree sparrow (*Passer montanus*). From an ecological perspective, the four species differ in their sensitivity to urbanisation and they can be grouped into two main categories: "urban adapters", blue tit and great tit, and "urban exploiters", house and tree sparrow, (Mckinney, 2002). The first category comprises species that facultatively use urbanised areas, with the greatest benefits during the cold winter months. During spring and summer urban adapters also make wide use of natural resources, such as caterpillars for raising their chicks (Johnston, 2001). On contrast, "urban exploiters", called synanthropes, often depend on human resources year around and are presumably well adapted to the intensely modified urban environments (Johnston, 2001; Marzluff, 2001).

Adult birds were caught using mist-nets ($n_{tot} = 364$). The sample size for each locality is summarised in Table 1. Each bird was ringed with a numbered metal ring and sexed when plumage characteristics permitted (blue tit, great tit and house sparrow) according to Svensson (1992). Tree sparrows are sexually monomorphic; hence these birds were sexed using molecular techniques (Griffiths et al., 1998). Morphometric measures were taken (tarsus and body mass) and a blood sample was collected from the jugular vein using a syringe and immediately placed on ice. The blood samples were centrifuged within 0–1 h for 10 min at 1800 rpm to separate the plasma from the red blood cells (RBC) and stored at −80 °C until analysis.

2.2. Scaled mass index (SMI) calculation

To assess the body condition in each species, we calculated the scaled body mass index (SMI) following Peig and Green (2010) based