© 2020. Published by The Company of Biologists Ltd | Journal of Experimental Biology (2020) 223, jeb203794. doi:10.1242/jeb.203794

COMMENTARY

Urban ecophysiology: beyond costs, stress and biomarkers

Caroline Isaksson*

ABSTRACT

Natural habitats are rapidly declining due to urbanisation, with a concomitant decline in biodiversity in highly urbanised areas. Yet thousands of different species have colonised urban environments. These organisms are exposed to novel urban conditions, which are sometimes beneficial, but most often challenging, such as increased ambient temperature, chemicals, noise and light pollution, dietary alterations and disturbance by humans. Given the fundamental role of physiological responses in coping with such conditions, certain physiological systems such as the redox system, metabolism and hormones are thought to specifically influence organisms' ability to persist and cope with urbanisation. However, these physiological systems often show mixed responses to urbanisation. Does this mean that some individuals, populations or species are resilient to the urban environmental challenges? Or is something missing from our analyses, leading us to erroneous conclusions regarding the impact of urbanisation? To understand the impact of urbanisation, I argue that a more integrated mechanistic and ecological approach is needed, along with experiments, in order to fully understand the physiological responses; without knowledge of their ecological and evolutionary context, physiological measures alone can be misinterpreted. Furthermore, we need to further investigate the causes of and capacity for individual plasticity in order to understand not only the impact of urbanisation, but also species resilience. I argue that abiotic and biotic urban factors can interact (e.g. pollution with micro- and macronutrients) to either constrain or relax individual physiological responses – and, thereby, plasticity – on a temporal and/or spatial scale, which can lead to erroneous conclusions regarding the impact of urbanisation.

KEY WORDS: Anthropogenic, Endocrinology, Environmental stress, Nutrition, Oxidative stress

Introduction

As urban environments expand and rapidly encroach on natural habitats, it is increasingly important to understand the impact of urbanisation on organisms. Urban habitats are characterised by impervious surfaces and buildings, higher levels of chemicals, noise, light at night, increased ambient temperatures and availability of anthropogenic food sources that are often of poor nutritional quality (Isaksson, 2018). Although recent studies have shown that suburban habitats can host a high biodiversity (Batáry et al., 2017), highly urbanised areas show an overall decline in biodiversity (e.g. McKinney and Lockwood, 1999; Shochat et al., 2010), which is projected to decline further as urban habitats are growing (McDonald et al., 2018, 2020). Less obvious is the impact that the urban habitat has on city dwellers (e.g. pigeons, crows, rats and racoons). Thus, a key question in urban ecology is whether current urban-dwelling species will be able to cope with the expanding urban sprawl and its associated environmental changes. How challenging is the urban environment for these species?

Physiology plays a pivotal role in allowing individuals to cope and persist in the urban environment. Urbanisation and its associated anthropogenic factors are predicted to affect physiological systems such as the redox system (leading to oxidative stress; see Glossary; Box 1), inflammation, metabolism and endocrinology (Navara and Nelson, 2007; Isaksson, 2015; Isaksson and Bonier, 2020). The measurable components of these physiological systems include different anti-oxidant enzymes, organic co-factors (such as vitamins), metabolites (e.g. amino acids), proteins (such as cytokines and haptoglobin) and hormones (e.g. corticosterone). These components are regularly used as (bio)markers of effect and/or exposure (Boxes 1 and 2).

By measuring physiological markers, we can gain insight into individual- and species-level sensitivity and resilience to urbanisation, but can also begin to understand future threats of expanding urban sprawl, highlighting potential areas of concern. Conversely, we can gain insight into the potential positive outcomes of conservation efforts. Although studies of physiological markers in an urban context have the potential to be highly valuable, their interpretation is not always straightforward. Take, for example, the two most commonly investigated aspects of physiology in an urban context – oxidative stress and the hypothalamic–pituitary–adrenal (HPA) axis (Box 1) – which show mixed responses to urban life (e.g. Bonier, 2012; Isaksson and Bonier, 2020) (Table 1).

This Commentary aims to go beyond the simplistic heuristic biomarker approach (Box 2), and explore when and why results are not always as predicted. What causes the frequently observed variation in physiology across (and even within) urban environments? Does our current biomarker framework limit future advances in ecophysiology and its importance in evaluating the impact of urbanisation? I argue that this is indeed the case. I do not aim to review all challenges, responses or taxonomic groups, but instead focus on the systems with which I am most familiar (i.e. oxidative stress and vertebrates), in order to highlight general concerns that can be extrapolated to other systems and responses.

When and why is the urban environment challenging?

The field of urban ecophysiology is relatively young, going back to the early 2000s (e.g. Partecke et al., 2004; Bonier et al., 2007), and the field of urban evolutionary physiology is even younger (reviewed in Isaksson and Bonier, 2020). Most research has been on birds, with a focus on a few European and North American species, such as great tits (*Parus major*; e.g. Isaksson et al., 2009), house sparrows (*Passer domesticus*; e.g. Meillère et al., 2015), blackbirds (*Turdus merula*; e.g. Partecke et al., 2006; Dominoni et al., 2013), house finches (*Carpodacus mexicanus*; e.g. Giraudeau et al., 2015) and song sparrows (*Melospiza melodia*; Grunst et al., 2014). However, the field is rapidly growing, and knowledge about other species, taxonomic groups and their physiological responses in different geographical and climatic regions is increasing (e.g. French et al., 2008; Falfushinska, et al., 2008a, 2018b; Radwan et al., 2010; Payne et al., 2012).

Lund University, Evolutionary Ecology, SE-223 62 Lund, Sweden.

*Author for correspondence (Isaksson@biol.lu.se)

C.I., 0000-0002-6889-1386

Journal of Experimental Biology (2020) 223, jeb203794. doi:10.1242/jeb.203794

Glossary

Acclimation
The reversible process by which an organism can adjust its physiology to prevailing environmental conditions, such as by upregulating heat shock proteins or anti-oxidants to maintain cellular function during heat stress and pro-oxidant exposure, respectively.

Adaptation
Selection on a certain genetic phenotype that becomes more frequent in the population over time.

Oxidative stress
A state with a surplus of oxidants relative to anti-oxidants, which results in increased oxidative damage to lipids, proteins and/or DNA, which can result in cellular dysfunction, ultimately affecting fitness.

Physiological robustness
Integration of different physiological responses that can compensate for each other.

Plasticity
The within-individual range (reaction norm) of a physiological response under different external stimuli, such as different urban factors. Plasticity is likely to play a key role in how well individuals acclimate to the urban environment.

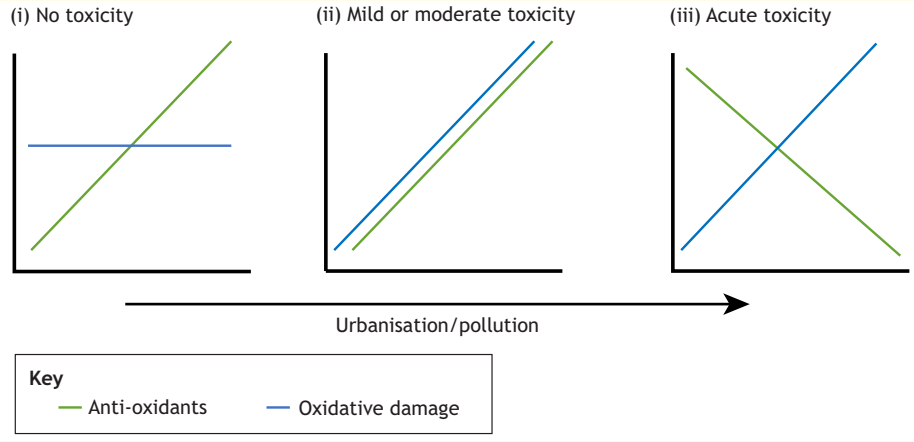
Urbanisation gradient
Refers to spatial variation in environmental factors, with urban areas having a large proportion of their surface covered in concrete and buildings and a low proportion of natural green areas (see more in Seress et al., 2014).

Although it has become clear that some aspects of the urban environment are attractive to some organisms (e.g. high abundance of food and nesting sites), the general conception is that, overall, the urban environment is challenging for its inhabitants (e.g. Austin, 1998; Samet et al., 2004; Hartz et al., 2008; Acevedo-Whitehouse and Duffus, 2009; Isaksson, 2010; Koivula and Eeva, 2010). The attractive features of the city can cause some species to preferentially settle in an urban instead of a non-urban habitat, with detrimental effects on fitness, a so-called 'ecological trap' (e.g. Demeyrier et al., 2016; Vlaschenko et al., 2019). However, the nature and magnitude of the urban challenge can vary widely, and this variation is not only a result of city size, but also of other abiotic and biotic factors that may vary within and between cities; these are discussed in more detail below.

Temporal and spatial variation in urban factors

At the macro-scale, cities are rather homogeneous. Macro-level ecology is clearly valuable for understanding general features of city dwellers in comparison with those from rural areas (e.g. Møller, 2009; Møller et al., 2010). Here, however, I want to highlight the importance of the microscale for understanding the physiological variation across the urban landscape. In fact, when looking at specific urban factors, both within and across cities, cities can be highly environmentally heterogeneous (both spatially and temporally). Take, for example, air pollution. In proximity to highways and roads, air pollution can reach dangerously high levels. Even small cities (~300,000 inhabitants) can occasionally exceed the recommended threshold for acceptable levels of certain air pollutants (European Environment Agency, 2018; Salmón et al., 2018a). However, cities can improve urban air quality, by allowing

Box 1. Oxidative stress



Because of its role in detoxification, respiration and cellular functioning, oxidative stress is highly relevant to investigate in relation to urbanisation (e.g. Weissman et al., 2006; Falfushinska et al., 2008a; Salmón et al., 2018a). However, studies of oxidative stress in an urban context have produced mixed results (see Table 1). When considering oxidative stress, the general prediction is that both damage and the level of anti-oxidants should increase with increasing urbanisation, which may be valid when exposed to moderate levels of stress/urbanisation (see scenario ii). In other scenarios, this prediction is oversimplified, and has its origin in the traditional biomarker approach (see Box 2). Sometimes, the urban environment is non-toxic (scenario i), because of either a low level of urbanisation/pollution or high detoxification capacities. In this case, one could predict that there is no increase in damage due to an efficient anti-oxidant system that can cope with increasing urbanisation. However, are there long-term costs of having an upregulated intrinsic anti-oxidant system? And what role do dietary anti-oxidants play? When the urban environment becomes acutely toxic (scenario iii), oxidative damage might increase rapidly as the intrinsic anti-oxidant system collapses. These three scenarios are still somewhat simplified; however, they represent three testable categories that may elucidate the impact of urban life. Furthermore, oxidative stress is far more complex and integrated than previously acknowledged in the field of urban ecophysiology, and can not only vary across tissues, but also across life stages and life history, which can further complicate the interpretation of variable results in the wild (e.g. Costantini and Verhulst, 2009). For example, a pro-oxidative challenge can either be direct (e.g. nitrogen oxides are active pro-oxidants that directly trigger anti-oxidant responses) or indirect, via integration of different physiological systems (e.g. human presence can trigger upregulation of corticosteroids, which enhances metabolism and pro-oxidant generation, which subsequently trigger anti-oxidant responses). Thus, plasticity, acclimation and even selection can affect physiological responses that are not obviously connected to oxidative stress, although the outcome reveals changes in oxidative stress. To bring light to the variable responses to urbanisation, a better understanding of its context dependence (physiologically, ecologically and evolutionarily) is needed.

Box 2. The biomarker concept in urban environments

Biomarkers in ecology are commonly used to identify early signs of an external threat to a population, species or ecosystem. Thus, biomarkers can serve a purpose in evaluating the impact of urbanisation. There are many different kinds of biomarkers; however, here the focus is on physiological biomarkers. The traditional concept of physiological biomarkers is based on a few key criteria: (i) high specificity and consistency, (ii) context independence (i.e. a linear relationship between biomarker and the external threat across time and place), and (iii) a strong correlation with measures of health or fitness (e.g. Huggett et al., 2002; Forbes et al., 2006). Without these properties, biomarkers would be difficult to interpret and studies that relate physiological biomarkers to fitness are still scarce in an urban context (but see Lucas and French, 2012; McLay et al., 2017). Biomarkers are invaluable in toxicology and pathology. However, their value for terrestrial wildlife has been debated (e.g. Bartell, 2006; Forbes et al., 2006). The primary challenges that have been raised include difficulties in (i) developing stressor-specific biomarkers, (ii) determining dose–response functions and (iii) projecting higher-order ecological effects from cellular or subcellular biomarkers (Bartell, 2006). Commonly used physiological biomarkers include molecular damage, anti-oxidants, heat shock proteins, metabolites, antibodies and hormones. Most of the biomarkers used are not specific, being part of highly integrated physiological responses. Thus, variation in the biomarker response is to be expected, especially if the experienced stress or exposure is low to moderate and chronic rather than acute. To increase our understanding and future use of physiological biomarkers, better knowledge about the causes of variation is needed. There are multiple ways in which this can be achieved. For example, experimentally manipulating urban factors, translocating individuals across the urban–rural landscape, and performing correlative studies of microhabitat heterogeneity in urban-specific factors will allow us to identify consistent or inconsistent associations of the target biomarker across different contexts such as space, time, genetic background and previous experience of urban life.

more green areas and trees to grow (Pugh et al., 2012). Similar spatial and temporal variation can be found for other urban factors, such as noise and night light pollution (e.g. Sprau et al., 2017).

The effects of temporal and spatial variation in exposure level within a city has been shown for humans, with increased incidence of air pollution-related diseases on peak days and when living on exposed streets (e.g. Oudin et al., 2010). Recently, ecologists have started to either experimentally manipulate or quantify variation in single or multiple urban factors and relate this to physiological markers (e.g. Raap et al., 2016; Casasole et al., 2017; McLay et al., 2017; North et al., 2017; Salmón et al., 2018a). Among these studies, only McLay et al. (2017) link the physiological effects caused by artificial light to measures of fitness. By doing so, the physiological marker can more reliably be used as a biomarker of effect. Also, as physiology can respond more rapidly and directly than life-history traits to short-term environmental perturbations, the spatial and temporal variation in urban factors can be used to disentangle the effects of urban stressors on physiological responses and thereby their potential long-term effects on the population/species.

How can we detect physiological stress?

For wildlife, a ‘challenging’ environment is defined as one that causes physiological responses or outcomes to deviate from the ‘normal’, with ‘normal’ in the context of urbanisation often being nearby population(s) from a non-urban habitat. As discussed above, urbanisation presents a broad range of challenges, thereby also eliciting a broad range of physiological responses. When the challenge is extreme, such as very high pollution levels or lack of an essential micronutrient such as thiamine or calcium, there is no doubt that urbanisation negatively affects wildlife. However, in most cases, the challenges are not acutely toxic, which allows physiological responses to compensate, leading to acclimation (see Glossary) to prevailing conditions (e.g. Kight and Swaddle, 2011; Isaksson, 2015; Isaksson and Bonier, 2020). This complicates the answer to the question ‘when and why is an urban environment challenging?’.

In many cases, the interpretation of whether an urban environment is challenging, based on measured physiological responses, has been simplistic and deterministic, with the physiological responses being interpreted as either ‘good’ or ‘bad’ for the urban dwellers. This can lead to false conclusions about the impact of urbanisation. Take, for example, corticosterone, which, until recently, has been used as a measure of stress (MacDougall-Shackleton et al., 2019). Thus, when studied in the urban context, the general hypothesis has been that corticosterone levels should be higher in city dwellers compared with those of their non-urban conspecifics. According to this hypothesis, a higher corticosterone level would then trigger an appropriate behavioural response to the urban challenge (Bonier, 2012). However, the results have been highly mixed, and no general association between urbanisation and individual ‘stress’ has been established (Bonier, 2012). As discussed by MacDougall-Shackleton et al. (2019), these apparently differing responses relate to the simplistic and deterministic view of this group of hormones. Glucocorticoids are, in fact, multi-faceted – they are involved in many physiological functions (see also Jimeno et al., 2018). By no longer assuming a direct association of these hormones with ‘stress’, and realising that responses can acclimate or evolve in response to urbanisation, we will achieve a better understanding of their role in urban-dwelling organisms. This also applies to a wider range of physiological responses, such as oxidative stress and metabolism.

Apart from the physiological complexity, there is also variation in degree of urbanisation across studies. Thus, a lack of an effect in one study may simply be a result of lower degree of urbanisation compared with another study with stronger physiological effects (for oxidative stress, see Table 1). Although it is becoming more common to include standardised urbanisation indexes in the description of study sites (Seress et al., 2014), this should be common practice so that the physiological responses can be compared across studies and evaluated based on degree of urbanisation.

Moving beyond biomarkers and physiological determinism

The biomarker approach (Box 2) in ecophysiology originates from the fields of medicine and ecotoxicology. This approach has, to some extent, also been valuable in urbanisation research, allowing researchers to identify specific challenges, and determine the physiological cost and health status of the population (Isaksson, 2010; Murray et al., 2019). However, as discussed above, the physiological responses in the wild show high variability (e.g. Falfushinska et al., 2008a, 2008b; Koivula and Eeva, 2010; Bonier, 2012; Abdel-Halim et al., 2013; Gillis et al., 2014; Herrera-Dueñas et al., 2014; Salmón et al., 2018a, 2018b; see also Table 1), which is why we need to broaden our view and to explore the underlying causes for this variability.

The redox system and its response to oxidative stress (Box 1) is the primary aspect of physiology that is used as a source of biomarkers for environmental stress (see Table 1). In the urban environment, oxidative stress can change in response to, for example, increased pollution and ambient temperatures, and poorer food quality. The intriguing aspect of oxidative stress is its association with cellular ageing and dysfunction; it thus provides a link to a physiological cost, which can be manifested as reduced fitness (e.g. Metcalf and Alonso-

Table 1. A representative summary of selected key studies of oxidative stress in relation to urbanisation

Reference	Taxon	Species	Urban site/country	Tissue	AOX/ damage	Marker(s)	Effect
Amri et al. (2017)	Aves	Hybrid sparrows, *Passer domesticus*×*Passer hispaniolensis*	Gabes/Tunisia	Liver	AOX	SOD, CAT	(–), (–)
		Hybrid sparrows, *P. domesticus*×*P. hispaniolensis*	Gabes/Tunisia	Liver	Damage	TBARS	(+)
de la Casa-Resino et al. (2015)	Aves	White stork, *Ciconia ciconia*	Cáceres/Spain	Blood	AOX	GSH, GST	(+), (+)
		White stork, *C. ciconia*	Cáceres/Spain	Blood	Damage	TBARS	(0)
Giraudeau and McGraw (2014)	Aves	House finch, *Haemorhous mexicanus*	Phoenix/USA	Blood	AOX	Vitamin E, vitamin A, carotenoids	(–), (–), (–)
		House finch, *H. mexicanus*	Phoenix/USA	Blood	Damage	TBARS	(0)
Herrera-Dueñas et al. (2014)	Aves	House sparrow, *P. domesticus*	Madrid/Spain	Blood	AOX	TotAOX, SOD, CAT, GSH/GSSG	(–), (0), (0), (0)
		House sparrow, *P. domesticus*	Madrid/Spain	Blood	Damage	PC, TBARS	(0), (0)
Herrera-Dueñas et al. (2017)	Aves	House sparrow, *P. domesticus*	Madrid/Spain	Blood	AOX	TotAOX, SOD, GPx	(–), (0), (+)
		House sparrow, *P. domesticus*	Madrid/Spain	Blood	Damage	PC, TBARS	(+), (+)
Isaksson et al. (2009)	Aves	Great tit, *Parus major*	Göteborg/Sweden	Lung	AOX	GR, GST, CAT	(0), (0), (0)
		Great tit, *P. major*	Göteborg/Sweden	Lung	Damage	TBARS	(0)
		Great tit, *P. major*	Göteborg/Sweden	Liver	AOX	GR, GST, CAT, carotenoids	(0), (0), (0), (+)
		Great tit, *P. major*	Göteborg/Sweden	Liver	Damage	TBARS	(0)
Isaksson et al. (2007)	Aves	Great tit, *P. major*	Göteborg/Sweden	Blood	AOX	TotAOX, carotenoids	(+ old males), (0)
Kurhalyuk et al. (2009)	Aves	Feral pigeons, *Columba livia domestica*	Słupsk/Poland	Blood	AOX	TotAOX, SOD, CAT, GR, GPx	(–), (+), (–), (+), (–)
		Feral pigeons, *C. livia domestica*	Słupsk/Poland	Blood	Damage	PC, TBARS	(–), (–)
Salmón et al. (2018a,b)	Aves	Great tit, *P. major*	Malmö/Sweden	Blood	AOX	TotAOX, SOD, GSH,	(+), (0), (0)
		Great tit, *P. major*	Malmö/Sweden	Blood	Damage	PC, TBARS	(0), (0)
		House sparrow, *P. domesticus*	Malmö/Sweden	Blood	AOX	TotAOX, SOD, GSH,	(+), (0), (0)
		House sparrow, *P. domesticus*	Malmö/Sweden	Blood	Damage	PC, TBARS	(0), (0)
		Tree sparrow, *Passer montanus*	Malmö/Sweden	Blood	AOX	TotAOX, SOD, GSH	(+), (0), (0)
		Tree sparrow, *P. montanus*	Malmö/Sweden	Blood	Damage	PC, TBARS	(+), (0)
		Blue tit, *Cyanistes caeruleus*	Malmö/Sweden	Blood	AOX	TotAOX, SOD, GSH	(+), (0), (0)
		Blue tit, *C. caeruleus*	Malmö/Sweden	Blood	Damage	PC, TBARS	(0), (0)
Falfushinska et al. (2008a)	Amphibia	Marsh frog, *Rana ridibunda*	Ternopil/Ukraine	Liver	AOX	SOD, CAT, GSH	(–), (0), (+)
		Marsh frog, *R. ridibunda*	Ternopil/Ukraine	Liver	Damage	PC, TBARS	(–), (–)
Regoli et al. (2006)	Gastropoda	Garden snail, *Helix aspersa*	Ancona/Italy	Digestive gland	AOX	TotAOX, CAT, GR, GST, GPx	(+), (+), (+), (–), (0)
		Garden snail, *H. aspersa*	Ancona/Italy	Digestive gland	Damage	DNA TD	(+)

All studies included compare natural variation in anti-oxidants and oxidative damage in animals living in urban versus non-urban habitats. (+) and (–) indicate that the marker shows a higher and lower value in the urban compared with the non-urban population, respectively. (0) indicates no difference between the urban and non-urban populations. AOX, anti-oxidants; CAT, catalase; DNA TD, DNA total damage; GSH, glutathione; GSH/GSSG, glutathione/glutathione disulphide; GPx, glutathione peroxidase; GR, glutathione reductase; GST, glutathione-*S*-transferase; PC, protein carbonyls; SOD, superoxide dismutase; TBARS, thiobarbituric acid reactive substances; TotAOX, total anti-oxidant capacity (note that there are different assays included in this marker).

Alvarez, 2010; Isaksson et al., 2011). Yet the data that support these links in an urban context are still scarce (but see Lucas and French, 2012).

In recent years, it has been emphasised that one must measure many components (biomarkers) of oxidative stress in order to fully understand the oxidative stress status of an individual (e.g. Beaulieu and Costantini, 2014; Speakman et al., 2015). However, even when doing so, the results can be challenging to interpret (e.g. if individual markers show opposite responses; Table 1). Hence, the use of the strict biomarker definition when using components of the redox system to evaluate whether an urban environment is challenging may only be reliable and valuable when the urban challenge is very high and toxic (Box 1). This may also apply to other physiological responses. Moving beyond physiological determinism and instead focusing on context-dependent constraints in physiology and the processes leading to a specific response will be much more rewarding. I will expand on this below.

Who finds the urban environment challenging?

The way in which species, populations and individuals physiologically respond to urbanisation can be influenced by, for example, phylogenetic constraints, time since colonisation, life history, life stage, and current and previous diseases, along with nutrition and other environmental factors. Thus, extrapolations about the significance of a certain marker from one context to another can be misleading. To answer the question of who finds the urban environment challenging, at the physiological level, will rely on detailed knowledge about the species/population and the urban

environment, but also on the capacity for physiological plasticity and robustness (see Glossary).

Physiological plasticity: chronic and acute responses

Within an individual, physiology is highly plastic and responsive to environmental perturbations. Some physiological responses occur immediately (within seconds to hours), whereas others require days to weeks to adjust. Likewise, some responses rapidly subside, whereas others are maintained for a longer time. Maintaining a strong response over an extended period is often associated with a cost, such as chronic inflammation and/or alternative resource allocation (i.e. resources are withdrawn from other functions).

Usually, urban factors are not acutely detrimental (but see Calderón-Garcidueñas et al., 2014, 2015), and thus are manageable in the short term (Isaksson and Bonier, 2020). On longer time scales, they can, however, be detrimental. Acclimation and other kinds of physiological plasticity are likely to play an important role in reducing the short- and long-term effects, thereby increasing species resilience towards urbanisation. Despite the high interest in plasticity, studies of urban physiology (like most urban ecology studies) mainly use effect size analyses and summary statistics between urban and non-urban sites, which obscure the potential to explore the underlying variance across different contexts (see Terblanche and Hoffmann, 2020). Cross-fostering, common garden and translocation studies would also be useful for understanding both the capacities for plasticity across naïve and pre-exposed individuals and populations, and the underlying mechanism(s) behind the variation (i.e. genetic or 'non-genetic') (Costantini et al., 2014; Romero-Haro and Alonso-Alvarez, 2015; Isaksson and Bonier, 2020). Indeed, a direct plastic response of the anti-oxidant enzyme superoxide dismutase was detected using a cross-fostering design, where urban-raised chicks had an increased activity of the enzyme regardless of nest or habitat of origin (Salmón et al., 2018b). This plastic response was, however, not sufficient to prevent generation of lipid peroxides in late-hatched urban broods.

Furthermore, the distinction between baseline and acute level responses is common practice in some ecophysiological fields, such as endocrine ecology and immuno-ecology (Quaye, 2008; Bonier et al., 2009; Matson et al., 2012; Hegemann et al., 2013; Vermeulen et al., 2016; Ouyang et al., 2017). This distinction is unfortunately rarely seen in fields such as oxidative stress (but see Costantini et al., 2014; Raap et al., 2016). Instead, the levels of both anti-oxidants and oxidative damage are usually measured at a single time point and without an experimental challenge to elicit a plastic response. Within-individual changes can reveal important information about the range and capacity of responses across time and contexts. Although oxidative damage is not directly a physiological response, but an outcome, it was recently shown that even oxidative damage to lipids can vary rapidly within an individual (Eikenaar et al., 2020). Thus, it would be highly valuable to also view oxidative damage as a variable trait, and to explore patterns of clearance and regeneration of oxidative damage over time to establish when it actually becomes a threat to fitness.

Physiological robustness

In addition to displaying physiological plasticity, physiological markers and systems are also highly integrated. For example, hormones can affect metabolism, which in turn affects the production of reactive oxygen species (ROS), thereby affecting components in the anti-oxidant system. This integration is a crucial part of physiological function and robustness (see also Cohen et al., 2012; Martin and Cohen, 2015). However, ecophysiological studies – and not just in urban contexts – rarely consider this.

Physiological integration (and thereby robustness) might explain the lack of a linear relationship between a specific marker and environmental stress. It has been suggested that this might account for difficulties in revealing physiological trade-offs in the wild (Metcalf and Alonso-Alvarez, 2010; Williams, 2018). Physiological integration means that the 'value' of a physiological marker is not necessarily constant across contexts and species. To exemplify this, I will use a bank analogy used by Cohen et al. (2017) in a theoretical paper about 'currencies' in life-history trade-offs, but will expand it to the present context. Before presenting the analogy, I will summarise three main points about physiological trade-offs (i.e. 'currencies') in an ecological context. First, we know that there are multiple possible 'currencies', such as dietary anti-oxidants and calories, that can be allocated to different functions. Second, the value of these currencies is context dependent and non-linear (i.e. they display diminishing marginal returns and are sometimes non-monotonic: it is possible to have too much; e.g. Isaksson and Bonier, 2020). This means that the 'values' of a marker vary across species, environments, populations and territories, for example. Third, the value of the currencies is not necessarily equal for different functions, such as reproduction versus somatic maintenance, and some level of each currency is necessary (as can be seen from nutrient shortages causing health problems even when there are sufficient calories).

Now to the analogy (see Fig. 1). Imagine that a bank offers reward points (RPs) as one form of currency. In this context the RPs can be carotenoids (AOX1) – a group of dietary substances that has multiple biological functions (Britton, 1995; Svensson and Wong, 2011). AOX1 can be invested in either 'savings' (i.e. stored in tissues for later use) or in 'spending' (i.e. directly used). In addition to RPs, there is also a second currency: 'money' (i.e. resources such as calories). If the value of the money and RPs are equal (or at least similar) to different functions such as survival and reproduction, there are almost an unlimited number of ways that you could allocate the RPs and money to still arrive at your optimum allocation. However, if the money has different values in savings versus spending, the presence of two currencies will allow more optimised solutions. Now, if the relative values in savings and spending are different in different populations and the different currencies do not have equal market values (i.e. there are different resource availabilities, which can also vary over time), then your allocation strategy will become more important for the final outcome. Additionally, different banks (territories) provide different RPs (AOX1 is instead AOX2, -3, -4 or a combination of two or more). In other words, the dietary availability of certain nutrients (RPs) differs in different territories (banks), which means that the optimum strategy (phenotype) is reached in different ways in different territories either within or across urban environments (Fig. 1). This analogy is in line with current evolutionary theories that promote the importance of the ecological context for understanding life-history strategies (e.g. Williams, 2018).

Physiological compartmentalisation is also part of physiological robustness. Different tissues are differentially affected by urban stressors. For example, inhaled air pollutants most strongly affect the lungs and respiratory tract, whereas ingested toxins may most affect the liver. This will influence the level of a physiological response in certain tissues, but also means that different stressors will affect different markers. Not surprisingly, blood is the most commonly used tissue in ecophysiology, as its sampling is non-lethal. However, when assessing markers of oxidative stress and inflammation, blood is probably the tissue that is most integrated and potentially the most variable in its response (Speakman et al., 2015; Isaksson, 2015; Ouyang et al., 2018; Costantini, 2019; Isaksson and Bonier, 2020).

Journal of Experimental Biology (2020) 223, jeb203794. doi:10.1242/jeb.203794

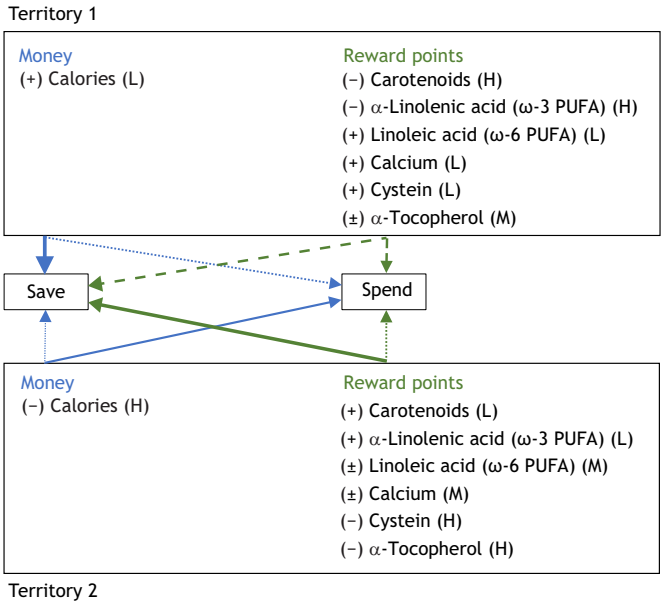


Fig. 1. Simplified illustration of the 'bank analogy' – habitat heterogeneity within or across urban and rural habitats influences the value of currencies, affecting the physiological processes that underlie fitness. The two territories (banks) represent either an urban and a rural territory, or two different territories within an urban or rural habitat. The signs (−, ±, +) indicate the abundance of a resource, where (−) is low, (±) is intermediate and (+) is high abundance; greater abundance means a lower value (L), low abundance means a high value (H) and intermediate abundance means a medium value (M). The two territories differ in the availability of calories (money), with more calories available in Territory 1 (T1) than in Territory 2 (T2). Organisms in T1 can afford to 'save' a large proportion of their money (solid blue arrow versus dotted arrow) because it is easily available, whereas individuals in T2 choose to 'spend' a larger proportion. Money is therefore more valuable for individuals in T2. Additionally, the bank offers reward points (RPs), which can be different micronutrients (some examples are listed). Similar to money, rare RPs are of higher value than abundant RPs. T1 and T2 have different values (availabilities) of the different micronutrients: in T1, carotenoids and ω-3 polyunsaturated fatty acids (PUFAs) are highly desired, whereas in T2 cystein and α-tocopherol are more valuable, which means that the optimum (phenotype, fitness) is reached in different ways. The green arrows indicate how individuals in the two territories 'spend' versus 'save' RPs. For example, individuals in T1 have intermediate abundance of α-tocopherol and choose to 'spend' some and 'save' some, whereas individuals in T2 choose to 'save' a larger proportion (solid green arrow versus dashed arrow). However, if a function linked to current survival or reproduction is constrained, T2 individuals might also choose to 'spend' their rare RPs to maximise current fitness at the expense of future functions. For a more detailed description and theoretical model of this analogy, see Cohen et al. (2017).

For example, different cytokines have different half-lives, which means that any point measurement indicates both current and previous responses, which could either obscure, under- or over-estimate an association with fitness. Hence, when using blood compared with other tissues, it is even more important to understand and investigate the causes for individual and population level variation, to be able to assess its suitability as a biomarker (Box 2).

Nutritional factors can constrain, mitigate and enhance physiological responses

Not only do environmental conditions trigger physiological responses: they can also be part of the response. For example, many different nutrients such as trace metals, essential fatty acids and amino acids are required for multiple cellular functions and structures, and their environmental availability can influence an individual's response to urban factors, leading to city-specific responses.

The high availability of anthropogenic food is a key factor allowing city fauna to thrive (e.g. Murray et al., 2016; Kumar et al., 2018). Luring wild animals to feed on these anthropogenic sources can, however, lead to a less diverse and nutritious diet (Birnie-Gauvin et al., 2017; Shulte-Hostedde et al., 2018), affecting their physiology. For example, city dwellers tend to have increased cholesterol, decreased dietary anti-oxidants and altered fatty acid composition compared with rural individuals (Andersson et al., 2015; Isaksson et al., 2017; Townsend et al., 2019). These are conditions that, in humans, are associated with increased risk of developing cancer, metabolic and cardiovascular diseases, and hence reduced lifespan. Although cancer has recently been highlighted as a concern also for wild animals (Madsen et al., 2017), urban great tits show no difference in the expression of anti-cancer genes compared with their rural conspecifics, which the authors argue could represent a lag in the evolutionary response to a highly oncogenic environment (Giraudeau et al., 2020). However, it could also be that anti-cancer genes are expressed in other tissues than blood. Moreover, it is not always the absolute concentration of a certain macro- or micronutrient that is important, but the balance between them (Simpson and Raubenheimer, 2014). For example, in humans, the modern Western diet increases the relative intake of omega-6 to omega-3 polyunsaturated fatty acids (PUFAs), which triggers a downstream pro-inflammatory response, leading to a state of chronic inflammation (Calder, 2011). Similar dietary changes have been shown for urban birds (Isaksson et al., 2017); this could further enhance inflammatory responses in urban wildlife. However, it could also stimulate evolutionary changes that reduce the physiological value of nutrients such as omega-3 PUFAs in urban habitats over time, such that these become less essential in urban-dwelling individuals. This is not unrealistic; for example, hummingbirds have evolved to survive on an extreme sugar diet, with very few other macro- or micronutrients.

Ecological studies rarely detect or screen for diseases linked to malnutrition. Instead, several nutritional markers such as carotenoids, cholesterol, Mead acid and omega-6/omega-3 ratio have been associated with 'body condition' (Andersson et al., 2015; Townsend et al., 2019), a state used to infer survival prospects and 'health' in a broad sense, which – under natural conditions – is highly reliable (Peig and Green, 2009). The high availability of anthropogenic food can reduce the immediate risk of starvation by increasing the body reserves of urban-dwelling animals; thus, a positive association between anthropogenic food resources and body condition could be predicted (reviewed in Jones and Reynolds, 2008). However, in the future, we also need to consider whether high intake of anthropogenic food, and thereby high body condition, might make wildlife more susceptible to inflammatory diseases and environmental stress. Indeed, in urban black sparrowhawks (*Accipiter melanoleucus*) relatively low body condition is associated with increased survival (Sumasgutner et al., 2016; Tate and Amar, 2017).

Furthermore, other micronutrients such as minerals do not show a consistently lower or higher availability when comparing urban versus rural habitats, but instead show a high overall variation in environmental availability. Thus, independently of urbanisation, environmental differences in nutrient availability can contribute to inconsistencies in physiological responses in urban habitats. For example, the availability of calcium is known to vary across environments, and it limits uptake and accumulation of heavy metals such as arsenic, lead and cadmium (Dauwe et al., 2006; Scheuhammer, 1996). Thus, a deficiency in calcium can have toxic effects, whereas calcium supplementation can mitigate the potential negative effects of living in an otherwise highly polluted environment. In addition, calcium intake seems to mitigate the inhibitory effect that heavy metals

have on the anti-oxidant system; thus, variation in calcium intake across urban sites and across urbanisation gradients (see Glossary) can influence the anti-oxidant response (Sánchez-Virosta et al., 2019).

Thus, macro- and micronutrients have potentially constraining, mitigating and enhancing effects on physiological responses, thereby influencing resilience/sensitivity towards urbanisation. These effects have been overlooked in ecophysiology, and require more attention.

Future directions

The first and most urgent future direction in urban ecophysiology is to link physiological components to fitness under different urban conditions (e.g. temporal or spatial differences, nutrient availability or pollution) using experiments or long-term data sets. This will allow a better understanding of the underlying causes for physiological variability or consistency under different conditions, and thereby its potential use as a 'biomarker of effect'. Well-designed experiments will also be able to disentangle the underlying mechanisms of acclimation (or other types of plasticity), heritability or genetic adaptation (see Glossary), or will determine whether the responses are coping strategies or even maladaptive, allowing us to understand the impact of urbanisation. These kinds of experimental studies are still rare (but see Lucas and French, 2012; McLay et al., 2017; Salmón et al., 2018b; Tuzun and Stoks, 2020).

Another future direction to explore physiological integration and robustness can be to use 'omics' (i.e. proteomics, metabolomics, transcriptomics; see Box 3) approaches, allowing over-represented pathways, functions and metabolites to be identified (e.g. Suarez and Moyes, 2012; Watson et al., 2017; Gouveia et al., 2019). This knowledge can then allow us to target key physiological markers or systems that are affected by urbanisation, which can potentially lead to faster progress relative to measuring single physiological markers without prior knowledge of which physiological components are affected by urbanisation.

Furthermore, in order to increase our understanding of urban ecophysiology at multiple levels and scales (from species-level to cellular responses, from macro- to micro-ecological processes, and from current to future responses) the field would benefit greatly from multi-disciplinary projects and open access data-sharing. This would allow us to disentangle the effects of different pollutants (either alone or in combination), abiotic factors (e.g. ambient temperature, soil acidity and water stress) and biotic factors (e.g. food availability and pathogen transmission), while maintaining the ecological relevance.

Conclusions

Urban environments are home to a range of different species. Although some of these species thrive, they face a number of physiological challenges, such as increased pollution and ambient temperatures. The traditional biomarker approach to detect physiological costs of urban life has gained mixed results. In this Commentary, I argue that unless the urban challenge is severe, the biomarker approach could lead to inaccurate conclusions about the physiological responses to urban challenges due to heterogeneity in the abiotic and biotic factors that are integrated into the physiological response. Furthermore, the fundamental properties of a biomarker – namely that their levels should be consistent for a certain state, as well as across time and contexts, e.g. season and life-stage – are often violated or ignored, resulting in the misuse and misinterpretation of physiological markers in response to urbanisation.

Physiological complexity and variability may seem daunting; however, rather than dismissing physiology as an ecological tool to estimate the impact of a given stressor, a conceptual paradigm shift is needed, where physiological, ecological and evolutionary processes are integrated in much greater detail. For this to happen, we need to move beyond the biomarker concept and physiological determinism, and instead focus on context-specific causes for variation, physiological plasticity and robustness and evolutionary processes within, as well as across, urban environments. Only then can ecophysiology claim to increase our understanding of the underlying causes of environmental stress, and accurately estimate the resilience of species, populations and individuals to future anthropogenic developments. Finally, I wish to finish this Commentary by rephrasing Dobzhansky's old saying from 1973: Nothing in urban ecophysiology makes sense except in its ecological and evolutionary context (Dobzhansky, 1973). I hope that this Commentary will inspire (especially young) researchers to take a more holistic and integrated view of physiology and provide a framework for the design of future studies to gain a better understanding of the physiological responses and thereby the impact of urbanisation. Overall, I think that urban ecophysiology has an important role to play for understanding species resilience to urbanisation and that it has an exciting future ahead.

Box 3. The potential use of 'omics' for increasing our understanding of species resilience to urbanisation

'Omics' is a broad collective term for a simultaneous quantification of multiple biological molecules that relate to function and biochemical dynamics of an organism. Its application is gaining interest across many disciplines from ageing research to risk assessment (e.g. van der Oost et al., 2003; Suarez and Moyes, 2012; Martyniuk, 2018; Solovev et al., 2020). Transcriptomics is probably the best known 'omics' approach in ecology. Transcriptomics provides a complete picture of which genes are expressed (and at what level) across the whole genome. One important piece of knowledge to be gained from using transcriptomics is that an integrated mechanistic response to urban stressors is automatically obtained, where pathway analyses can provide a central step in our understanding of how different genes and their expression are interconnected or decoupled (e.g. Watson et al., 2017; Bertucci et al., 2018). These changes could then be followed up with targeted physiological measurements to estimate the actual effect, the physiological robustness, plasticity and its potential effect on fitness and species resilience. It should be noted that gene expression does not necessarily translate into changes in enzymatic activity or protein expression (e.g. due to post-translation control), thus transcriptomics data should not be used as an indicator of 'effect' but rather 'exposure' (see e.g. Regoli and Giuliani, 2014). Other examples of 'omics' approaches relevant for urban ecophysiologists are epigenomics, metabolomics and proteomics, of which the latter two are more directly linked to function (e.g. Suarez and Moyes, 2012; Gouveia et al., 2019). The overall challenge and potential drawback with all kinds of 'omics' data compared with physiological biomarkers is that they are rather laborious, expensive and often require extensive bioinformatics. Other challenges are similar to those affecting the use of physiological markers, such as selection of reference site, standardised sampling, the effect of spatial and temporal variation and the need to link the results to fitness. Yet, I emphasise that there is value in understanding molecular and biochemical networks, in conjunction with traditional physiological biomarkers, in order to further our understanding of physiological variability and integration in response to urbanisation.

Acknowledgements

I thank Alan Cohen and three anonymous reviewers for highly valuable and insightful feedback that greatly improved this paper.

Competing interests

The author declares no competing or financial interests.

References

Abdel-Halim, K. Y., Abo El-Saad, A. M., Talha, M. M., Hussein, A. A. and Bakry, N. M. (2013). Oxidative stress on land snail *Helix aspersa* as a sentinel organism for ecotoxicological effects of urban pollution with heavy metals. *Chemosphere* **93**, 1131-1138. doi:10.1016/j.chemosphere.2013.06.042

Acevedo-Whitehouse, K. and Duffus, A. L. J. (2009). Effects of environmental change on wildlife health. *Phil. Trans. R. Soc. B* **364**, 3429-3438. doi:10.1098/rstb.2009.0128

Amri, N., Rahmouni, F., Chokri, M. A., Rebai, T. and Badraoui, R. (2017). Histological and biochemical biomarkers analysis reveal strong toxicological impacts of pollution in hybrid sparrow (*Passer domesticus*×*Passer hispaniolensis*) in southern Tunisia. *Environ. Sci. Pollut. Res.* **24**, 17845-17852. doi:10.1007/s11356-017-9352-3

Andersson, M. N., Wang, H.-L., Nord, A., Salmón, P. and Isaksson, C. (2015). Composition of physiologically important fatty acids in great tits differs between urban and rural populations on a seasonal basis. *Front. Ecol. Evol.* **3**, 93.

Austin, B. (1998). The effects of pollution on fish health. *J. Appl. Microbiol.* **85**, 234S-2342. doi:10.1111/j.1365-2672.1998.tb05303.x

Bartell, S. M. (2006). Biomarkers, bioindicators, and ecological risk assessment – a brief review and evaluation, environmental bioindicators. *Environ. Bioind.* **1**, 60-73. doi:10.1080/15555270591004920

Batáry, P., Kurucz, K., Suarez-Rubio, M. and Chamberlain, D. E. (2017). Non-linearities in bird responses across urbanization gradients: a meta-analysis. *Glob. Change Biol.* **24**, 1046-1054. doi:10.1111/gcb.13964

Beaulieu, M. and Costantini, D. (2014). Biomarkers of oxidative status: missing tools in conservation physiology. *Conserv. Physiol.* **2**, cou014. doi:10.1093/conphys/cou014

Bertucci, A., Pierron, F., Gourves, P. Y., Klopp, C., Lagarde, G., Pereto, C., Dufour, V., Gonzalez, P., Coynel, A., Budzinski, H. et al. (2018). Whole-transcriptome response to wastewater treatment plant and stormwater effluents in the Asian clam, *Corbicula fluminea*. *Ecotox. Environ. Safety* **165**, 96-106. doi:10.1016/j.ecoenv.2018.08.090

Birnie-Gauvin, K., Peiman, K. S., Raubenheimer, D. Cooke, S. J. (2017). Nutritional physiology and ecology of wildlife in a changing world. *Conserv. Physiol.* **5**, cox030.

Bonier, F. (2012). Hormones in the city: endocrine ecology of urban birds. *Horm. Behav.* **61**, 763-772. doi:10.1016/j.yhbeh.2012.03.016

Bonier, F., Martin, P. R. and Wingfield, J. C. (2007). Urban birds have a broader environmental tolerance. *Biol. Lett.* **3**, 670-673. doi:10.1098/rsbl.2007.0349

Bonier, F., Martin, P. R., Moore, I. T. and Wingfield, J. C. (2009). Do baseline glucocorticoids predict fitness? *Trends Ecol. Evol.* **24**, 634-642. doi:10.1016/j.tree.2009.04.013

Britton, G. (1995). Structure and properties of carotenoids in relation to function. *FASEB J.* **9**, 1551-1558. doi:10.1096/fasebj.9.15.8529834

Calder, P. C. (2011). Fatty acids and inflammation: the cutting edge between food and pharma. *Eur. J. Pharmacol.* **668**, S50-S58. doi:10.1016/j.ejphar.2011.05.085

Calderón-Garcidueñas, L., Calderón-Garcidueñas, A., Torres-Jardón, R., Avila-Ramírez, J., Kulesza, R. J. and Angiulli, A. D. (2014). Air pollution and your brain: what do you need to know right now. *Prim. Health Care Res. & Dev.* **26**, 1-17.

Calderón-Garcidueñas, L., Kulesza, R. J., Doty, R. L., D'Angiulli, A. and Torres-Jardóne, R. (2015). Megacities air pollution problems: Mexico City metropolitan area critical issues on the central nervous system pediatric impact. *Environ. Res.* **137**, 157-169. doi:10.1016/j.envres.2014.12.012

Casasole, G., Raap, T., Costantini, D., AbdElgawas, H., Asard, H., Pinxten, R. and Eens, M. (2017). Neither artificial light at night, anthropogenic noise nor distance from roads are associated with oxidative status of nestlings in an urban population of songbirds. *Comp. Biochem. Physiol. A* **210**, 14-21. doi:10.1016/j.cbpa.2017.05.003

Cohen, A. A., Martin, L. B., Wingfield, J. C., McWilliams, S. R. and Dunne, J. A. (2012). Physiological regulatory networks: ecological roles and evolutionary constraints. *Trends Ecol. Evol.* **27**, 428-435. doi:10.1016/j.tree.2012.04.008

Cohen, A. A., Isaksson, C. and Salguero-Gómez, R. (2017). Co-existence of multiple trade-off currencies shapes evolutionary outcomes. *PLoS One* **12**, e0189124. doi:10.1371/journal.pone.0189124

Costantini, D. (2019). Understanding diversity in oxidative status and oxidative stress: 714 the opportunities and challenges ahead. *J. Exp. Biol.* **222**, jeb194688. doi:10.1242/jeb.194688

Costantini, D. and Verhulst, S. (2009). Does high antioxidant capacity indicate low oxidative stress? *Funct. Ecol.* **23**, 506-509. doi:10.1111/j.1365-2435.2009.01546.x

Costantini, D., Greives, T. J., Hau, M. and Partecke, J. (2014). Does urban life change blood oxidative status in birds? *J. Exp. Biol.* **217**, 2994-2997. doi:10.1242/jeb.106450

Dauwe, T., Snoeijs, T., Bervoets, L., Blust, R. and Eens, M. (2006). Calcium availability influences lead accumulation in a passerine bird. *Anim. Biol.* **56**, 289-298. doi:10.1163/157075606778441822

de la Casa-Resino, I., Hernández-Moreno, D., Castellano, A., Soler Rodríguez, F. and Pérez-López, M. (2015). Biomarkers of oxidative status associated with metal pollution in the blood of the white stork (*Ciconia ciconia*) in Spain. *Toxicol. Environ. Chem.* **97**, 588-598. doi:10.1080/02772248.2015.1051484

Demeyrier, V., Lambrechts, M. M., Perret, P. and Grégoire, A. (2016). Experimental demonstration of an ecological trap for a wild bird in a human-transformed environment. *Anim. Behav.* **118**, 181-190. doi:10.1016/j.anbehav.2016.06.007

Dobzhansky, T. (1973). Nothing in biology makes sense except in the light of evolution. *Am. Biol. Teacher* **35**, 125-129. doi:10.2307/4444260

Dominoni, D., Quetting, M. and Partecke, J. (2013). Artificial light at night advances avian reproductive physiology. *Proc. R. Soc. B: Biol. Sci.* **280**, 20123017. doi:10.1098/rspb.2012.3017

Eikenaar, C., Winslott, E., Hessler, S. and Isaksson, C. (2020). Oxidative damage to lipids is rapidly reduced during migratory stopovers. *Funct. Ecol.* **34**, 1215-1222. doi:10.1111/1365-2435.13540

European Environment Agency (2018). *Europe's urban air quality – re-assessing implementation challenges in cities*. EEA Technical Report No. 24.

Falfushinska, H. I., Loumbourdis, N., Romanchuk, L. D. and Stolyar, O. B. (2008a). Validation of oxidative stress responses in two populations of frogs from Western Ukraine. *Chemosphere* **73**, 1096-1101. doi:10.1016/j.chemosphere.2008.07.060

Falfushinska, H. I., Romanchuk, L. D. and Stolyar, O. B. (2008b). Different responses of biochemical markers in frogs (*Rana ridibunda*) from urban and rural wetlands to the effect of carbamate fungicide. *Comp. Biochem. Physiol. C* **148**, 223-229.

Forbes, V. E., Palmqvist, A. and Bach, L. (2006). The use and misuse of biomarkers in ecotoxicology. *Environ. Toxicol. Chem.* **25**, 272-280. doi:10.1897/05-257R.1

French, S. S., Fokidis, H. B. and Moore, M. C. (2008). Variation in stress and innate immunity in the tree lizard (*Urosaurus ornatus*) across an urban–rural gradient. *J. Comp. Physiol. B* **178**, 997-1005. doi:10.1007/s00360-008-0290-8

Gillis, P. L., Higgins, S. K. and Jorge, M. B. (2014). Evidence of oxidative stress in wild freshwater mussels (*Lasmigona costata*) exposed to urban-derived contaminants. *Ecotoxicol. Environ. Saf.* **102**, 62-69. doi:10.1016/j.ecoenv.2013.12.026

Giraudeau, M. and McGraw, K. J. (2014). Physiological correlates of urbanization in a desert songbird. *Integr. Comp. Biol.* **54**, 622-632. doi:10.1093/icb/icu024

Giraudeau, M., Chavez, A., Toomey, M. B. and McGraw, K. J. (2015). Effects of carotenoid supplementation and oxidative challenges on physiological parameters and carotenoid-based coloration in an urbanization context. *Behav. Ecol. Sociobiol.* **69**, 957-970. https://doi.org/10.1007/s00265-015-1908-y

Giraudeau, M., Watson, H., Vincze, O., Thomas, F., Sepp, T., Ujvari, B., Le Loc'h, G. and Isaksson, C. (2020). Will urbanisation affect the expression level of genes related to cancer of wild great tits?. *Sci. Total Environ.* **20**, 13579.

Gouveia, D., Almunia, C., Cogne, Y., Pible, O., Degli-Esposti, D., Salvador, A., Cristobal, S., Sheehan, D., Chaumot, A., Geffard, O. et al. (2019). Ecotoxicoproteomics: a decade of progress in our understanding of anthropogenic impact on the environment. *J. Proteomics* **198**, 66-77. doi:10.1016/j.jprot.2018.12.001

Grunst, M. L., Rotenberry, J. T. and Grunst, A. S. (2014). Variation in adrenocortical stress physiology and condition metrics within a heterogeneous urban environment in the song sparrow *Melospiza melodia*. *J. Avian Biol.* **45**, 574-583. doi:10.1111/jav.00459

Hartz, A. M. S., Bauer, B., Block, M. L., Hong, J.-S. and Miller, D.-S. (2008). Diesel exhaust particles induce oxidative stress, proinflammatory signaling, and P-glycoprotein up-regulation at the blood–brain barrier. *FASEB J.* **22**, 2723-2733. doi:10.1096/fj.08-106997

Hegemann, A., Matson, K. D., Versteegh, M. A., Villegas, A. and Tieleman, B. I. (2013). Immune response to an endotoxin challenge involves multiple immune parameters and is consistent among the annual-cycle stages of a free-living temperate zone bird. *J. Exp. Biol.* **216**, 2573-2580. doi:10.1242/jeb.083147

Herrera-Dueñas, A., Pineda, J., Antonio, M. T. and Aguirre, J. I. (2014). Oxidative stress of house sparrow as bioindicator of urban pollution. *Ecol. Indic.* **42**, 6-9. doi:10.1016/j.ecolind.2013.08.014

Herrera-Dueñas, A., Pineda-Pamliega, J., Antonio-Garcia, M. T. and Aguirre, J. I. (2017). The influence of urban environments on oxidative stress balance: a case study on the house sparrow in the Iberian Peninsula. *Front. Ecol. Evol.* **5**, 106. doi:10.3389/fevo.2017.00106

Huggett, R. J., Kimerle, R. A., Mehrle , Jr., P. M. and Bergman, H. L. (2002). *Biomarkers: Biochemical, physiological, and histological markers of anthropogenic stress*. Lewis, Boca Raton, FL, USA.

Isaksson, C. (2010). Pollution and its impact on wild animals: a meta-analysis on oxidative stress physiology. *EcoHealth* **7**, 342-350. doi:10.1007/s10393-010-0345-7

Isaksson, C. (2015). Urbanisation, oxidative stress and inflammation: a question of evolving, acclimatizing or coping with urban environmental stress. *Funct. Ecol.* **29**, 913-923. doi:10.1111/1365-2435.12477

Isaksson, C. (2018). Impact of urbanization on birds. In *Bird Species: How they Arise, Modify and Vanish* (ed. D.T. Tietze), pp. 235-257. Springer, Cham. https://doi.org/10.1007/978-3-319-91689-7_13

Isaksson, C. and Bonier, F. (2020). Urban evolutionary physiology. In *Urban Evolutionary Biology* (ed. M. Szulkin, J. Munshi-South and A. Charmantier), pp. 215-231. Oxford University Press, Oxford.

Isaksson, C., Andersson, M. N., Nord, A., von Post, M. and Wang, H.-L. (2017). Species-dependent effects of the urban environment on fatty acid composition and oxidative stress in birds. *Front. Ecol. Evol.* **5**, 44. doi:10.3389/fevo.2017.00044

Isaksson, C., McLaughin, P., Monaghan, P. and Andersson, S. (2007). Carotenoid pigmentation does not reflect total non-enzymatic antioxidant activity in plasma of adult and nestling great tits, *Parus major. Funct. Ecol.* **21**, 1123-1129. doi:10.1111/j.1365-2435.2007.01317.x

Isaksson, C., Sheldon, B. C. and Uller, T. (2011). The challenges of integrating oxidative stress into life history biology. *Bioscience* **60**, 194-202. doi:10.1525/bio.2011.61.3.5

Isaksson, C., Sturve, J., Almrot, B. C. and 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*. *Environ. Res*. **109**, 46-50. doi:10.1016/j.envres.2008.10.006

Jimeno, B., Hau, M. and Verhulst, S. (2018). Corticosterone levels reflect variation in metabolic rate, independent of 'stress'. *Sci. Rep.* **8**, 13020. doi:10.1038/s41598-018-31258-z

Jones, D. N. and Reynolds, J. S. (2008). Feeding birds in our towns and cities: a global research opportunity. *J. Avian Biol.* **39**, 265-271. doi:10.1111/j.0908-8857.2008.04271.x

Kight, C. R. and Swaddle, J. P. (2011). How and why environmental noise impacts animals: an integrative, mechanistic review. *Ecol. Lett.* **14**, 1052-1061. doi:10.1111/j.1461-0248.2011.01664.x

Koivula, M. J. and Eeva, T. (2010). Metal-related oxidative stress in birds. *Environ. Pollut.* **158**, 2359-2370. doi:10.1016/j.envpol.2010.03.013

Kumar, N., Gupta, U., Jhala, Y. V., Qureshi, Q., Gosler, A. G. and Sergio, F. (2018). Habitat selection by an avian top predator in the tropical megacity of Delhi: human activities and socio-religious practices as prey-facilitating tools. *Urban Ecosyst.* **21**, 339-349.

Kurhalyuk, N., Hetmański, T., Antonowicz, J. and Tkachenko, H. (2009). Oxidative stress and protein oxidation affected by toxic metals in feral pigeons (*Columba livia*) from northern Poland. *Baltic Coastal Zone* **13**, 187-197.

Lucas, L. D. and French, S. S. (2012). Stress-induced tradeoffs in a free-living lizard across a variable landscape: consequences for individuals and populations. *PLoS One* **7**, e49895. doi:10.1371/journal.pone.0049895

MacDougall-Shackleton, S. A., Bonier, F., Romero, L. M. and Moore, I. T. (2019). Glucocorticoids and 'stress' are not synonymous. *Integr. Organ. Biol.* **1**, obz017. doi:10.1093/iob/obz017

Madsen, T., Arnal, A., Vittecoq, M., Bernex, F., Abadie, J., Labrut, S., Garcia, D., Faugère, D., Lemberger, K., Beckmann, C. et al. (2017). Cancer prevalence and etiology in wild and captive animals. In *Ecology and Evolution of Cancer* (ed. B. Ujvari, B. Roche and F. Thomas), pp 11-46. Academic Press, Elsevier, London. https://doi.org/10.1016/B978-0-12-804310-3.00002-8

Martin, L. B. and Cohen, A. A. (2015). Physiological regulatory networks: the orchestra of life? In *Integrative Organismal Biology* (ed. L.B. Martin, C.K. Ghalambor and H.A. Woods), pp. 137-152. Hoboken, NJ: John Wiley & Sons.

Martyniuk, C. J. (2018). Are we closer to the vision? A proposed framework for incorporating omics into environmental assessments. *Environ. Toxicol. Pharmacol.* **59**, 97-93. doi:10.1016/j.etap.2018.03.005

Matson, K. D., Horrocks, N. P. C., Versteegh, M. A. and Tieleman, I. B. (2012). Baseline haptoglobin concentrations are repeatable and predictive of certain aspects of a subsequent experimentally-induced inflammatory response. *Comp. Biochem. Physiol. A. Mol. Integr Physiol.* **162**, 7-15. doi:10.1016/j.cbpa.2012.01.010

McDonald, R. I., Güneralp, B., Huang, C.-W., Seto, K. and You, M. (2018). Conservation priorities to protect vertebrate endemics from global urban expansion. *Biol. Conserv* **224**, 290-299. doi:10.1016/j.biocon.2018.06.010

McDonald, R. I., Mansur, A. V., Ascensão, F., Colbert, M., Crossman, K., Elmqvist, T., Gonzalez, A., Güneralp, B., Haase, D., Hamann, M. et al. (2020). Research gaps in knowledge of the impact of urban growth on biodiversity. *Nat. Sustain.* **3**, 16-24. https://doi.org/10.1038/s41893-019-0436-6

McKinney, M. L. and Lockwood, J. L. (1999). Biotic homogenization: a few winners replacing many losers in the next mass extinction. *Trends Ecol. Evol.* **14**, 450-453. doi:10.1016/S0169-5347(99)01679-1

McLay, L. K., Nagarajan-Radha, V., Green, M. P. and Jones, T. M. (2017). Dim artificial light at night affects mating, reproductive output, and reactive oxygen species in *Drosophila melanogaster*. *J. Exp. Zool. Part A* **329**, 419-428.

Meillère, A., Brischoux, F., Parenteau, C. and Angelier, F. (2015). Influence of urbanization on body size, condition, and physiology in an urban exploiter: a multi-component approach. *PLoS ONE* **10**, e0135685. doi:10.1371/journal.pone.0135685

Metcalf, N. B. and Alonso-Alvarez, C. (2010). Oxidative stress as a life-history constraint: the role of reactive oxygen species in shaping phenotypes from conception to death. *Funct. Ecol.* **24**, 984-996. doi:10.1111/j.1365-2435.2010.01750.x

Møller, A. P. (2009). Successful city dwellers: a comparative study of the ecological characteristics of urban birds in the Western Palearctic. *Oecologia* **159**, 849-858. doi:10.1007/s00442-008-1259-8

Møller, A. P., Erritzøe, J. and Karadas, F. (2010). Levels of antioxidants in rural and urban birds and their consequences. *Oecologia* **163**, 35-45. doi:10.1007/s00442-009-1525-4

Murray, M. H., Hill, J., Whyte, P. and St. Clair, C. C. (2016). Urban compost attracts coyotes, contains toxins, and may promote disease in urban-adapted wildlife. *EcoHealth* **13**, 285-292. doi:10.1007/s10393-016-1105-0

Murray, M. H., Sánchez, C. A., Becker, D. J., Byers, K. A., Worsley-Tonks, K. E. L. and Craft, M. E. (2019). City sicker? A meta-analysis of wildlife health and urbanization. *Front. Ecol. Evol.* **17**, 575-583. doi:10.1002/fee.2126

Navara, K. J. and Nelson, R. J. (2007). The dark side of light at night: physiological, epidemiological, and ecological consequences. *J. Pineal. Res.* **43**, 215-224. doi:10.1111/j.1600-079X.2007.00473.x

North, M. A., Kinniburgh, D. W. and Smits, J. E. G. (2017). European starlings (*Sturnus vulgaris*) as sentinels of urban air pollution: a comprehensive approach from noninvasive to post mortem investigation. *Environ. Sci. Technol.* **51**, 8746-8756. doi:10.1021/acs.est.7b01861

Oudin, A., Strömberg, U., Jakobsson, K., Stroh, E. and Björk, J. (2010). Estimation of short-term effects of air pollution on stroke hospital admissions in southern Sweden. *Neuroepidemiology* **34**, 131-142. doi:10.1159/000274807

Ouyang, J. Q., de Jong, M., van Grunsven, R. H. A., Matson, K. D., Haussmann, M. F., Meerlo, P., Visser, M. E. and Spoelstra, K. (2017). Restless roosts: light pollution affects behavior, sleep, and physiology in a free-living songbird. *Glob. Change Biol.* **23**, 4987-4994. doi:10.1111/gcb.13756

Ouyang, J. Q., Isaksson, C., Schmidt, C., Hutton, C., Bonier, F. and Dominoni, D. (2018). A new framework for urban ecology: an integration of proximate and ultimate responses to anthropogenic change. *Integrat. Comp. Biol.* **58**, 915-928.

Partecke, J., Schwabl, I. and Gwinner, E. (2006). Stress and the city: urbanization and its effects on the stress physiology in European blackbirds. *Ecology* **87**, 1945-1952. doi:10.1890/0012-9658(2006)87[1945:SATCUA]2.0.CO;2

Partecke, J., Van't Hof, T. and Gwinner, E. (2004). Differences in the timing of reproduction between urban and forest European blackbirds (*Turdus merula*): result of phenotypic flexibility or genetic differences? *Proc. Roy. Soc. B* **271**, 1995-2001. doi:10.1098/rspb.2004.2821

Payne, C. J., Jessop, T. S., Guay, P.-J., Johnstone, M., Feore, M. and Mulder, R. A. (2012). Population, behavioural and physiological responses of an urban population of black swans to an intense annual noise event. *PLoS One* **7**, e45014. doi:10.1371/journal.pone.0045014

Peig, J. and Green, A. J. (2009). New perspectives for estimating body condition from mass/length data: the scaled mass index as an alternative method. *Oikos* **118**, 1883-1891. doi:10.1111/j.1600-0706.2009.17643.x

Pugh, T. A. M., MacKenzie, A. R., Whyatt, J. D. and Hewitt, S. N. (2012). Effectiveness of green infrastructure for improvement of air quality in urban street canyons. *Environ. Sci. Technol.* **46**, 7692-7699. doi:10.1021/es300826w

Quaye, I. K. (2008). Haptoglobin, inflammation and disease. *Trans. R. Soc. Trop. Med. Hyg.* **102**, 735-742. doi:10.1016/j.trstmh.2008.04.010

Raap, T., Casasole, G., Costantini, D., AbdElgawad, H., Asard, H., Pinxten, R. and Eens, M. (2016). Artificial light at night affects body mass but not oxidative status in free-living nestling songbirds: an experimental study. *Sci. Rep.* **6**, 35626. doi:10.1038/srep35626

Radwan, M. A., El-Gendy, K. S. and Gad, A. F. (2010). Biomarkers of oxidative stress in the land snail, *Theba pisana* for assessing ecotoxicological effects of urban metal pollution. *Chemosphere* **79**, 40-46. doi:10.1016/j.chemosphere.2010.01.056

Regoli, F. and Giuliani, M. E. (2014). Oxidative pathways of chemical toxicity and oxidative stress biomarkers in marine organisms. *Marine Environ. Res.* **93**, 106-117. doi:10.1016/j.marenvres.2013.07.006

Regoli, F., Gorbi, S., Fattorini, D., Tedesco, S., Notti, A., Machella, N., Bocchetti, R., Benedetti, M. and Piva, F. (2006). Use of the land snail *Helix aspersa* as sentinel organism for monitoring ecotoxicologic effects of urban pollution: an integrated approach. *Environ. Health Persp.* **114**, 63-69. doi:10.1289/ehp.8397

Romero-Haro, A. A. and Alonso-Alvarez, C. (2015). The level of an intracellular antioxidant during development determines the adult phenotype in a bird species: a potential organizer role for glutathione. *Am. Nat.* **185**, 390-405. doi:10.1086/679613

Salmón, P., Stroh, E., Herrera-Dueñas, A., von Post, M. and Isaksson, C. (2018a). Oxidative stress in birds along a NO_x and urbanization gradient: an interspecific approach. *Sci. Tot. Environ.* **622–623**, 635-643. doi: 10.1016/j.scitotenv.2017.11.354

Salmón, P., Watson, H., Nord, A. and Isaksson, C. (2018b). Effects of the urban environment on oxidative stress in early life: insights from a cross-fostering experiment. *Integr. Comp. Biol.* **58**, 986-994.

Samet, J. M., DeMarini, D. M. and Malling, H. V. (2004). Do airborne particles induce heritable mutations?. *Science* **304**, 971-972. doi:10.1126/science.1097441

Sánchez-Virosta, P., Espín, S., Ruiz, S., Stauffer, J., Kanerva, M., García-Fernández, A. J. and Eeva, T. (2019). Effects of calcium supplementation on oxidative status and oxidative damage in great tit nestlings inhabiting a metal-polluted area. *Environ. Res.* **171**, 484-492. doi:10.1016/j.envres.2019.01.047

Scheuhammer, A. M. (1996). Influence of reduced dietary calcium on the accumulation and effects of lead, cadmium, and aluminium in birds. *Environ. Pollut.* **94**, 337-343. doi:10.1016/S0269-7491(96)00084-X
Seress, G., Lipovits, Á., Bókony, V. and Czúni, L. (2014). Quantifying the urban gradient: a practical method for broad measurements. *Landscape Urban Plan.* **131**, 42-50. doi:10.1016/j.landurbplan.2014.07.010
Shochat, E. Lerman, S. B., Anderies, J. M., Warren, P. S., Faeth, S. H. and Nilson, C. H. (2010). Invasion, competition, and biodiversity loss in urban ecosystems. *Bioscience* **60**, 199-208. doi:10.1525/bio.2010.60.3.6
Shulte-Hostedde, A. I., Mazal, Z., Jardine, C. M. and Gagnon, J. (2018). Enhanced access to anthropogenic food waste is related to hyperglycemia in raccoons (*Procyon lotor*). *Conserv. Physiol.* **6**, coy026.
Simpson, S. J. and Raubenheimer, D. (2014). Perspective: tricks of the trade. *Nature* **58**, S66. doi:10.1038/508S66a
Solovev, I., Shaposhnikov, M. and Moskalev, A. (2020). Multi-omics approaches to human biological age estimation. *Mech. Ageing Dev.* **185**, 111192. doi:10.1016/j.mad.2019.111192
Speakman, J. R., Blount, J. D., Bronikowski, A. M., Buffenstein, R., Isaksson, C., Kirkwood, T. B. L., Monaghan, P., Ozanne, S. E., Beaulieu, M., Briga, M. et al. (2015). Oxidative stress and life histories: unresolved issues and current needs. *Ecol. Evol.* **5**, 5745-5757. doi:10.1002/ece3.1790
Sprau, P., Mouchet, A. and Dingemanse, N. J. (2017). Multidimensional environmental predictors of variation in avian forest and city life histories. *Behav. Ecol.* **28**, 59-68. doi:10.1093/beheco/arw130
Suarez, R. K. and Moyes, C. D. (2012). Metabolism in the age of 'omes'. *J. Exp. Biol.* **215**, 2351-2357. doi:10.1242/jeb.059725
Sumasgutner, P., Tate, G. J., Koeslag, A. and Amar, A. (2016). Family morph matters: factors determining survival and recruitment in a long-lived polymorphic raptor. *J. Anim. Ecol.* **85**, 1043-1055. https://doi.org/10.1111/1365-2656.12518
Svensson, P. A. and Wong, B. B. M. (2011). Carotenoid-based signals in behavioural ecology: a review. *Behaviour* **148**, 131-189. doi:10.1163/000579510X548673
Tate, G. J. and Amar, A. (2017). Morph specific foraging behavior by a polymorphic raptor under variable light conditions. *Sci. Rep.* **7**, 9161. doi:10.1038/s41598-017-07829-x
Terblanche, J. S. and Hoffmann, A. A. (2020). Validating measurements of acclimation for climate change adaptation. *Curr. Opin. Insec. Sci.* **41**, 7-16. doi:10.1016/j.cois.2020.04.005
Townsend, A. K., Staab, H. A. and Barker, C. M. (2019). Urbanization and elevated cholesterol in American crows. *Condor* **121**, duz040. doi:10.1093/condor/duz040
Tuzun, N. and Stoks, R. (2020). Lower bioenergetics costs but similar immune responsiveness under a heatwave in urban compared to rural damselflies. *Evol. Appl.*, https://doi-org.ludwig.lub.lu.se/10.1111/eva.13041
van der Oost, R., Beyer, J. and Vermeulen, N. P. E. (2003). Fish bioaccumulation and biomarkers in environmental risk assessment: a review. *Environ. Toxicol. Pharmacol.* **13**, 57-149. doi:10.1016/S1382-6689(02)00126-6
Vermeulen, A., Eens, M., Zaid, E. and Muller, W. (2016). Baseline innate immunity does not affect the response to an immune challenge in female great tits (*Parus major*). *Behav. Ecol. Sociobiol.* **70**, 585-592. doi:10.1007/s00265-016-2077-3
Vlaschenko, A., Kovalow, V., Hukov, V., Kravchenko, K. and Rodenko, O. (2019). An example of ecological traps for bats in the urban environment. *Eur. J. Wildl. Res.* **65**, 20. doi:10.1007/s10344-019-1252-z
Watson, H., Videvall, E., Andersson, M. N. and Isaksson, C. (2017). Transcriptome analysis of a wild bird reveals physiological responses to the urban environment. *Sci. Rep.* **7**, 44180. doi:10.1038/srep44180
Weissman, L., Fraiberg, M., Shine, L., Garty, J. and Hochman, A. (2006). Responses of antioxidants in the lichen *Ramalina lacera* may serve as an early-warning bioindicator system for the detection of air pollution stress. *FEMS Microbiol. Ecol.* **58**, 41-53. doi:10.1111/j.1574-6941.2006.00138.x
Williams, T. D. (2018). Physiology, activity and cost of parental care in birds. *J. Exp. Biol.* **221**, 1-8.