On urbanisation and birds

Insights from a molecular and physiological perspective

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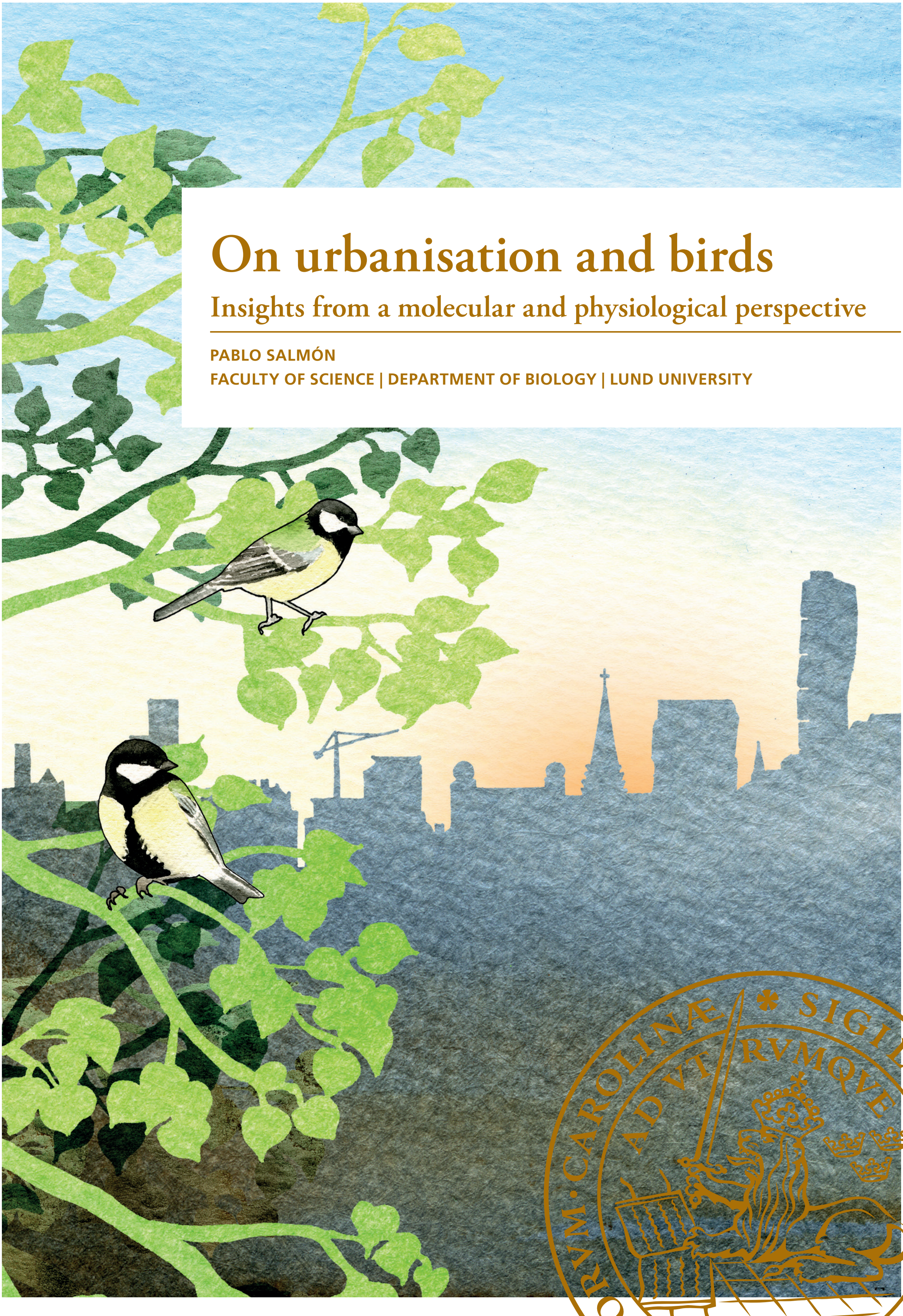

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On urbanisation and birds:

Insights from a molecular and physiological perspective

Pablo Salmón

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Abstract

Urbanisation of natural habitats is rapidly intensifying and poses a potential global threat for wildlife. Thus, it is important to explore its impact on wildlife in order to understand the present and future threats and how resilient organisms are to them. Although many species decline in abundance or even disappear once an area is urbanised, some species, populations and/or individuals are able to succeed in this new environment. Indeed, marked phenotypic differences in behaviour, morphology and physiology have been demonstrated among species inhabiting urban environments. In this thesis, using birds as model organisms, I investigate the role of urbanisation and the implications of two important life-history traits; telomere dynamics and oxidative stress physiology. In addition, I studied the genomic structure of multiple urban and natural/semi-natural populations of great tit (*Parus major*), a common urban-dwelling species, in order to explore potential common signatures of selection and local adaptation to urban habitats.

Regarding telomere dynamics, the results show that urban environments inflict an early-life detrimental effect on telomere length, probably through a combination of being exposed to multiple urban stressors and poorer diet. This early-life effect on telomere length had implications for survival, with strong selection against short telomere length individuals in the first year, especially in the urban environment. However, if the bird survives their first year, the urban environment does not seem to affect the telomere shortening more than the rural environment. Possibly, some of the urban factors such as supplementary feeding, higher temperatures or lower predation risk, may outweigh the costs of being exposed to different stressors (e.g. air and noise pollution or artificial light at night).

With respect to oxidative stress physiology, we found that in four common urban passerine species, the plasma antioxidant capacity was positively correlated with increases in urbanisation and air pollution intensity (i.e., NO_x exposure estimates). Although, their response to urbanisation/NO_x was similar, there were significant differences between the four species in absolute levels, suggesting different reaction norms to urbanisation/NO_x or different capacities in the up-regulation of the antioxidant physiology. Indeed, and despite the increase in the antioxidant capacity, one of the species, the tree sparrow (*Passer montanus*), had increased levels of protein carbonyls (oxidative damage biomarker), but only in relation to NO_x exposure. This suggests that this species is more sensitive to pollution. Furthermore, the latter result highlights the importance to study markers of oxidative stress across species and in the same environments, in order to understand how urban environmental stress affects species differently.

Finally, our genomic analysis of multiple great tit urban populations reveals that despite the overall low genetic differentiation, some loci diverged in relation to urbanisation. This implies that parallel evolution of a few key genes may play an important role for adaption to the urban environment.

In summary, the results presented in this thesis shed light and offer new perspectives in the emerging field of urban ecology, particularly for bird urban ecology, but also regarding life-history variation and the potential mechanisms involved in shaping phenotypic traits to novel environmental stressors.

Key words: air pollution, antioxidants, birds, great tit, local adaptation, nitrogen oxides, oxidative stress, parallel evolution, survival, telomere dynamics, telomeres, urbanisation.

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For Becca, Peter and all my family *sensu lato*

What is the city but the people?

Coriolanus, William Shakespeare

Content

List of papers

I. **Salmón, P.**, Nilsson, J.F., Nord, A., Bensch, S. and Isaksson, C. (2016). Urban environment shortens telomere length in nestling great tits, *Parus major*. *Biology Letters, 12(6), 20160155.*

II. **Salmón, P.**, Nilsson, J.F., Watson, H., Bensch, S. and Isaksson, C. (2017). Selective disappearance of great tits with early-life short telomeres in urban areas. *Proceedings of the Royal Society of London B: Biological Sciences, 284: 20171349.*

III. **Salmón, P.**, Stroh, E., Herrera-Dueñas, A., von Post, M. and Isaksson, C. Oxidative stress in birds along an increasing NO_x and urbanisation gradient: an interspecific approach. *Manuscript, submitted.*

IV. **Salmón, P.**, Lundberg, M., Ahrén, D., Biard, C., Dingemanse, N.J., Dominoni, D., Helm, B., Senar, J.C., Sprau, P., Visser M.E. and Isaksson, C. Low population genetic differentiation, but significant divergence in response to urbanisation in great tits, *Parus major*. *Manuscript.*

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Author contributions

I. CI, AN, JN and **PS** conceived the study. CI, AN and **PS** carried out the fieldwork and sample collection. JN and **PS** performed the laboratory work. **PS** analysed the data with the input from CI and JN. CI and **PS** drafted the initial manuscript and SB, JN and AN provided substantial comments that improved the manuscript. All authors contributed to and gave approval to the submission for publication of the final manuscript

II. **PS** conceived the study with input from CI, JN and HW. CI, **PS** and HW performed the field work and sample collection. JN and **PS** carried out the laboratory analysis. **PS** analysed the data with input from JN and HW. SB, CI, JN, **PS** and HW drafted the initial manuscript. All authors contributed to the final manuscript.

III. CI and MvP conceived the study with input from AHD and **PS**. AHD, CI, MvP and **PS** carried out the field work. Laboratory analysis on oxidative stress biomarkers were performed by AHD and **PS**. ES contributed with the modelling of the NO_x. **PS** analysed the data with input from CI. CI and **PS** drafted the initial manuscript. All authors contributed to the final manuscript.

IV. CI and **PS** conceived the study. **PS** performed most of the sample collection with additions from CB, ND, DD, BH, JC and PhS. DA, ML and **PS** carried out the analysis of the data with help from MV. CI and **PS** drafted the initial manuscript. All authors contributed to the final manuscript.

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Dag Ahrén (DA), Staffan Bensch (SB), Clotilde Biard (CB), Niels J. Dingemanse (ND), Davide Dominoni (DD), Barbara Helm (BH), Amparo Herrera-Dueñas (AHD), Caroline Isaksson (CI), Max Lundberg (ML), Johan F. Nilsson (JN), Andreas Nord (AN), Maria von Post (MvP), Pablo Salmón (PS), Joan Carlos Senar (JC), Philip Sprau (PhS), Emilie Stroh (ES), Marcel E. Visser (MV), Hannah Watson (HW).

Abstract

Urbanisation of natural habitats is rapidly intensifying and poses a potential global threat for wildlife. Thus, it is important to explore its impact on wildlife in order to understand the present and future threats and how resilient organisms are to them. Although many species decline in abundance or even disappear once an area is urbanised, some species, populations and/or individuals are able to succeed in this new environment. Indeed, marked phenotypic differences in behaviour, morphology and physiology have been demonstrated among species inhabiting urban environments. In this thesis, using birds as model organisms, I investigate the role of urbanisation and the implications of two important life-history traits; telomere dynamics and oxidative stress physiology. In addition, I studied the genomic structure of multiple urban and natural/semi-natural populations of great tit (*Parus major*), a common urban-dwelling species, in order to explore potential common signatures of selection and local adaptation to urban habitats.

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Finally, our genomic analysis of multiple great tit urban populations reveals that despite the overall low genetic differentiation, some loci diverged in relation to

urbanisation. This implies that parallel evolution of a few key genes may play an important role for adaption to the urban environment.

In summary, the results presented in this thesis shed light and offer new perspectives in the emerging field of urban ecology, particularly for bird urban ecology, but also regarding life-history variation and the potential mechanisms involved in shaping phenotypic traits to novel environmental stressors.

Popular Science Summary

The human population continues to increase and as a result, urban areas are expanding, dramatically transforming the previously natural landscape. This is an extreme challenge for many wildlife species and they will vanish from this new environment. However, some species seem to flourish in the city. Similar to humans, the urban living species are faced with challenges, in terms of being exposed to high pollution levels, but also opportunities with more food and warmer temperatures.

Many urban living species look and behave differently compared to their conspecifics living in more natural habitats. For example, many bird species sing at a different pitch and many urban animals are less shy towards humans, such as seagulls and squirrels. Differences in physical attributes and nutrition are common and have been reported in many different kinds of animal groups around the world. However, it is important to understand what the implications of these differences are for the life and survival of animals in the urban environment. Will they make the life of urban species easier and better? Or, are the differences a sign of stress and poor health? Crucially, we know that genetic changes can also occur because of human activity and this can also happen quickly, resulting in differences that may be passed on to future generations of urban living species, with consequences that as yet, we do not fully understand.

Birds are a highly visible animal group in urban areas, and we can enjoy watching them, listening to their songs and feeding them. In this thesis, we look closer at birds' physiology and the effects of the urban environment on their survival. In addition, we have screened a large part of the genome of urban and rural birds to investigate whether urban birds from a city in Sweden are more similar to urban birds in Lisbon, compared to Swedish forest birds.

One important mechanism explored in this thesis is telomeres. Telomeres are fascinating because they protect the genetic code from breaking down, by breaking down themselves i.e., shortening. This self-sacrifice makes them suitable indicators of ageing - the longer an individual's telomeres are the better chance the individual has for a longer life. In addition, it has been shown that environmental stress can accelerate the telomere shortening. Here, we used this ageing biomarker to test how the urban environment affects the telomere length in great tits, a common urban-living bird species. The results reveal that urban life is tough for young birds i.e., chicks that were reared in an urban habitat had a shorter telomere length. The environmental aspect driving this effect could possibly be poor diet or starvation, as diet plays a fundamental role in maintaining telomere length. More importantly, this shortening did not have an immediate negative effect on chick survival, but was life threatening in the long run. Namely, urban individuals that have short telomeres as chicks disappear from the population during the first year of life i.e., they die. Since the most stress-prone young individuals die, the adult population should consist of only the most stress-resistant birds. Indeed, the urban adult population consisted of individuals with long telomeres, when compared to those in a natural habitat. Thus, the negative effect of the urban habitat is less apparent later in life and the benefits of living in an urban environment outweigh the potential costs.

In addition to telomeres, there are other physiological markers of environmental stress that are important to investigate in relation to urbanisation, and specifically air pollution. Air pollution contains active oxidants, which can damage vital molecules and tissues of the body. Thus, measurements of the protective antioxidants and the potential oxidative damage will provide insights into the health status of wildlife, as well as humans, that live in urban environments. Here, we look at this in four different bird species, and all four bird species showed higher antioxidant levels with increased air pollution levels and urbanisation. Despite the observed higher antioxidant levels, one species, the tree sparrow, also had higher levels of oxidative damage in relation to air pollution. This indicates that urban birds have a boosted protective system and that this protection is sufficient enough to prevent damage in three out of four species.

Finally, in the last part of this thesis we compared the genomes of multiple urban and rural great tit populations across Europe. The idea was to test for common signals of urbanisation. In general, we found that urban and rural great tits where very similar, which indicates that the urban environment does not exert a major barrier for movement, as it can do for many other species. We found an overall high resemblance between urban and rural birds, we did detect that some gene types where more common in urban environments across the whole of Europe. These common signatures at the genomic level may suggest that urban environments are similar in terms of the type of environmental stress and the response to it i.e., a similar selection pressure that may make genetically better suited to city life. However, further research is needed to understand if this has any positive effects for urban life.

To sum up, the results compiled in this thesis demonstrate that inhabiting urban areas poses changes and challenges to birds' physiology. These changes had different effects at different life stages and in different species. In order to better understand the current and future threats of urbanisation to wildlife, more studies of physiological health, responses and constraints are needed to be able to understand species resilience to urbanisation and its associated pollution.

Populärvetenskaplig Sammanfattning

Världens befolkning ökar och i takt med det utbredningen av städer och urbana miljöer. Urbaniseringen är en extrem utmaning för många djurarter och antalet arter har minskat i takt med urbaniseringen. Trots detta finns det en hel del arter som tillsynes verkar blomstra i staden, mycket tack vara den rika tillgången till mat. Men så finns det en också baksida, nämligen de olika miljöföreningar som stadsdjuren, liksom vi människor, exponeras för. Med andra ord har staden både för- och nackdelar för de vilda djur som bor vid sidan av oss i staden.

Många av de arter som lever i staden lever också i skogen, och stadspopulationer har visat sig vara väldigt olika de populationer som bor i mer naturliga miljöer, det kan vara allt ifrån beteende skillnader till hur de ser ut. Till exempel sjunger många fågelarter på en annan tonhöjd för att överrösta bullret i staden och de är inte lika skygga gentemot människor, detta ses tydligt hos till exempel måsar och ekorrar. De uppvisar också stora skillnader fysiologi, vilket har visats hos många olika slags djurgrupper runt om i världen. Det är emellertid viktigt att förstå vad konsekvenserna av dessa skillnader kan ha för djurens liv och överlevnad i stadsmiljön. Kommer de att göra livet för stadslevande arter lättare och bättre? Eller är skillnaderna ett tecken på stress och dålig hälsa? Viktigt att också veta är om stadspopulationer skiljer sig genetiskt ifrån de rurala populationerna. För om detta är fallet så kan fördelaktiga gentyper överföras till kommande generationer och populationen kan vara bättre anpassad för miljöstress.

Fåglar är en kanske den djurgrupp som är mest synlig i stan, vi kan njuta av att lyssna på deras sång om våren och interagera med dem när vi matar dem med våra brödrester. I denna avhandling tittar vi närmare på hur stadsmiljön påverkar just fåglar, deras fysiologi och överlevnad. Dessutom har vi undersökt genetiska skillnader mellan stads- och skogsfåglar, för att undersöka om t.e.x en fågel ifrån Malmö är mer lik en stadsfågel ifrån Lissabon, än en skogsfågel ifrån södra Sverige.

En huvudkomponent i denna avhandling är telomerer. Telomerer är fascinerande eftersom de skyddar arvsmassan från att brytas ner. Detta gör de delvis genom att brytas ner själva, d.v.s. de förkortas. Denna självuppoffring gör dem lämpliga som indikatorer för åldrande - ju längre en individs telomerer är, desto större chans har den enskilde individen till ett långt liv. Dessutom har det visat sig att miljöstressfaktorer kan påskynda telomerers förkortning. Här använder vi denna åldrande markör för att testa hur stadsmiljön påverkar telomerlängden hos talgoxar. Resultaten visar att stadslivet är tufft för unga talgoxar, d.v.s. de fågelungar som växer upp i stan har kortare telomerer. Vilken miljöfaktor det är som ligger bakom denna effekt är ännu okänd, men dålig kost och/eller svält är troliga faktorer, speciellt eftersom kost har visat sig ha en betydande roll för att upprätthålla telomerlängden. Det påföljande året följde vi dessa unga individer och kunde visa på att telomerlängden har en viktig roll för överlevnaden i stan. Alla de unga fåglar som hade korta telomerer i stan återfanns inte i populationen det kommande året, i klartext, de överlevde inte vintern. Detta innebär att de mest stresskänsliga unga individerna dör tidigt, men det innebär också att mest stresståliga individerna överlever till vuxen ålder. Detta var tydligt när vi mätte telomerlängden hos de vuxna talgoxarna, för då visade det sig att stadspopulationen bestod av individer med relativt längre telomerer i jämförelse med vuxna talgoxar ifrån skogen. Således är den negativa effekten av stadsmiljön mer prominent tidigt i livet än senare i livet, då kan t.o.m. fördelarna överväga nackdelarna.

Förutom telomerer finns det andra fysiologiska markörer för miljöstress som är viktiga att undersöka i relation till urbanisering, och speciellt i relation till luftföroreningar. Många luftföroreningar är reaktiva i kroppen som s.k. oxidanter. Dessa oxidanter kan skada vitala molekyler och vävnader i kroppen. Genom att ta blodprov och mäta nivåerna av de skyddande antioxidanterna och även de potentiella oxidativa skadorna som uppkommit så kan man få bra insikter om djurens, liksom människans, hälsotillstånd i urbana miljöer. Här tittar vi på detta i fyra olika fågelarter, och alla fyra fågelarter visade högre antioxidantnivåer med ökande luftföroreningsnivåer och urbanisering. Trots de höga antioxidantnivåerna så uppvisade pilfinken även högre nivåer av oxidativa skador på proteiner med ökad exponering för trafik-genererade luftföroreningar. Dessa resultat tyder på att stadsfåglar har ett förstärkt skyddssystem och att detta skydd är tillräckligt för att förhindra skador hos tre av fyra vanliga stadsfågelarter.

I den sista delen av den här avhandlingen studeras talgoxens genetik. Vi jämför en halv miljon små genetiska koder mellan flera stads- och landsbygdspopulationer. Dessa populationer var spridda över hela Europa - ifrån Göteborg i norr till Lissabon i söder. Syftet med detta stora projekt var att testa om det finns mer likheter mellan vitt spridda stadspopulationer än mellan en stad- och en skogspopulation som ligger nära varandra. På det stora hela så visar talgoxarna en mycket liten genetisk skillnad över hela sin utbredning, vilket tyder på att stadsmiljön inte utövar ett stort hinder för förflyttning mellan områden. Men trots att den största delen av genomet var lika, så var det en hel del gentyper som var signifikant vanligare i stadsmiljöerna över hela Europa än de var i skogen. Dessa gemensamma signaturer på gennivån tyder på att stadsmiljön är likartad m.a.p. både miljöstressfaktorerna och talgoxens respons till dessa, d.v.s. ett liknande selektionstryck som kan leda till småskalig evolution och lokalanpassning till stadsmiljön. Ytterligare forskning behövs för att förstå om detta har några positiva effekter för talgoxar i stan.

Sammanfattningsvis så visar resultaten ifrån denna avhandling att stadsmiljön utgör ett hot för en av våra vanligaste och mest älskade fågelarter. Vi har visat negativa effekter på biomarkörer kopplat till åldrande och hälsa, samt direkt negativa effekter på överlevnad. Unga talgoxar är mest känsliga, och pilfinken är mer känslig än blåmesen, talgoxen och gråsparven. För att kunna förutsäga effekterna av den fortsatt ökande urbaniseringen behövs mer studier av vilda djurs fysiologiska hälsa, och hur gentyper kopplas till detta. Detta för att förstå olika arters och livsstadiers motståndskraft och känslighet mot urbanisering och dess olika miljöföroreningar och för att kunna ha livskraftiga fågelpopulationer i stan även i framtiden.

General introduction

Urban environments represent around 2.4% of the terrestrial land surface (Potere and Schneider, 2007) and this is predicted to continue increasing (Seto et al., 2011; Seto et al., 2012). More than half of the human population live in urban areas (United Nations, 2016) and this intense occupation by humans affects the urban environment as well as its surroundings. Urbanisation is leading to a large reduction in the extent of natural habitat available and embodies irreversible transformations of natural ecosystems, e.g. changing the previously natural vegetated surface into parklands or gardens. Therefore, despite the apparently low percentage of land cover, urbanisation is regarded as one of the most pressing global issues and according to the United Nations, is considered the largest environmental challenge of our time, next to climate change, affecting the ecology and evolution of populations, species and communities across the globe (Hendry et al., 2016).

Although many wildlife species decline in abundance or even disappear once an area is urbanised, some species, populations and/or individuals are able to succeed in this new environment (McKinney, 2002; Sol et al., 2014), being able to take advantage of the new opportunities and the altered patterns e.g. of food resources that urban ecosystems provide (Catterall, 2009). Nonetheless, for many organisms, urban stressors may give rise to increased selection pressures, which can make specific life history traits more beneficial (Shochat et al., 2006; Isaksson, 2015). Indeed, marked changes in behaviour, physiology and morphology have been widely demonstrated among species inhabiting urban environments, even among those species that thrive in them (Isaksson et al., 2005; Partecke et al., 2006; Moller, 2010; Bonier, 2012). Understanding the impact of urbanisation on wildlife is key to reach a better comprehension of the present and future threats to wildlife populations and the adaptations organisms make to counter these. Furthermore, cities offer a unique scenario that can be used to test evolutionary hypotheses and explore the interface between physiology, behaviour and adaptation (Gil and Brumm, 2013), providing researchers with multiple replicates across the world.

Thesis aims

The main aim of this thesis was to investigate mechanistic underpinnings of potential costs and selection pressures associated with living in urban compared to rural environments, using wild bird populations. Two main mechanistic costs were addressed: telomere length dynamics (*Papers I-II*) and oxidative stress physiology (*Paper III*). Telomeres are of great potential interest to evolutionary biologists since they are linked to fitness costs and the biology of life span. However, research implementing this trait in an urban context is scarce. Oxidative stress physiology has been previously shown to differ between urban and rural populations (e.g. Isaksson et al., 2005) but empirical studies of different species and from different locations are needed.

Additionally, we explored patterns of parallel local adaptation to the urban environment in different urban/rural population pairs across Europe (*Paper IV*). Nowadays, it is clear that urbanisation is a widespread phenomenon driving phenotypic changes in urban populations (Alberti et al., 2017). Nonetheless, few studies have tried to address the underlying mechanisms that could generate these phenotypic differences and even more, if the patterns are the same across populations.

The individual papers included in this thesis primarily address the following questions:

Paper I: Do urban and rural rearing conditions impact nestling telomere length differently? Do maternal components (genetic or environmental) attenuate the effects of rearing conditions regarding telomere length?

Paper II: Does telomere length predict survival during both early and late life, and does the relationship differ between urban and rural habitats? Is telomere length attrition accelerated in an urban compared to a rural habitat? Does the urban environment affect the relationship between chronological age and telomere length?

Paper III: Is the oxidative stress status in four common passerine species associated with urbanisation and/or pollution levels (NO_x)? Are there species-specific responses in oxidative stress physiology to urbanisation and/or pollution levels (NO_x)?

Paper IV: Are there consistent genomic differences between urban/rural great tit population pairs across Europe? Are there potential genomic footprints of divergent selection in relation to urbanisation?

Before summarising the findings from the studies included in this thesis, I will set the scene by describing what is an urban compared to a rural environment, what are the key characteristics that distinguish them, and in what way do they affect wildlife. Here I will summarise the current literature about the known effects of urbanisation on wildlife, and more specifically, on birds.

Urban Environment

Urban societies started to developed around 5000 to 6000 years ago, which is a rather recent phenomenon from an evolutionary perspective. Historically, only a small proportion (<10 %) of the world's human population inhabited urban areas by the year 1700, distributed amongst a relatively limited number of urban centres (Berry, 1990). Nowadays, up to 54% of the world's human population live in cities and it is continuously growing (United Nations, 2016; Figure 1).

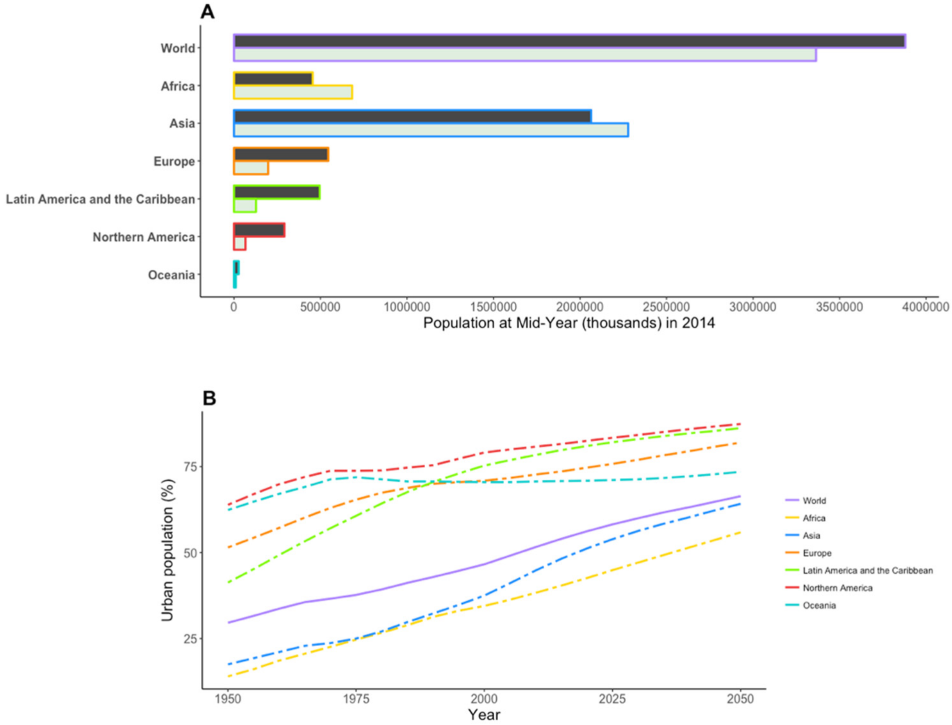

Figure 1. A) World population of humans in urban (black) and rural (grey) by region. B) Estimated and projected percentage of urban population by region between 1950 and 2050. Data from United Nations, Department of Economic and Social Affairs, Population Division (2014)

Urban environments differ markedly from natural/semi-natural habitats regarding several abiotic and biotic factors. The most pronounced effect of the urbanisation process is the permanent loss or fragmentation of the once continuous mosaic of native vegetation. This transforms the landscape into a scattered disjunction of pieces of native habitat surrounded by a matrix of cement, grass and degraded land (Marzluff and Ewing, 2001). Even where natural habitat remains in the urban landscape, its degradation is apparent, e.g. excess of non-native plant species (planted as ornaments or by invasion), which alter local habitat characteristics such as the structural complexity of the vegetation (Luck and Smallbone, 2010). While habitat loss and fragmentation are shared with other anthropogenic disturbances, e.g. agriculture, urban landscapes possess a higher disturbance from pollution related to human infrastructure and population size. This includes air pollution, light at night and noise.

Many air pollutants are associated with the industrial and residential activities that characterise urban landscapes e.g. traffic-related air pollutants. The atmosphere in urban areas has higher concentrations of carbon dioxide (CO_2), nitrogen oxides (NO_x), ozone (O_3), sulphur oxides (SO_x), aerosols, metals and suspended particulates among others (Hill, 2010). These pollutants have profound implications for biogeochemical cycles, nutrient flows and primary production (Grimm et al., 2008). The levels of air pollution remain high in many areas of the globe despite the deindustrialisation process, the use of cleaner technologies and the introduction of stricter regulations in urban centres in the last decades, that will likely increase in the future (Pozzer et al., 2012; WHO, 2016).

Another common abiotic disturbance related to urbanisation is anthropogenic night light pollution, which is caused by the high number of artificial light sources commonly used in commercial signs, buildings and lit pathways. Whereas other urban disturbances can have an impact throughout the day, artificial light pollution only acts directly during the dark hours. The amount and intensity of light that organisms are exposed to during the night varies depending on e.g. the moon phase. However, light pollution can alter the natural patterns of night light exposure for example, increasing it up to six times in cities compared to suburban and rural areas (Davies et al., 2013). Despite large decreases in night light in many areas (e.g. in Europe, Bennie et al., 2014). there remains a current global trend of increasing night brightness over the last 50 years (Bennie et al., 2015)

Along with air and light pollution, noise is another important urban component. Worldwide noise levels are rising (Barber et al., 2010), and urban habitats are typically noisier than rural habitats (Butler, 2004). The urban soundscape is characterised by high levels of low-frequency noise produced by traffic and heavy machinery (Slabbekoorn and Peet, 2003). As a result, noise pollution can mask acoustic signals and alter the way animals generally perceive sounds, i.e. not only conspecific communication (Templeton et al., 2016) but also the sounds made by potential predators (Barber et al., 2010).

Another abiotic difference between urban and rural environments is temperature. In urban centres, the air temperature is often 2-10°C higher than in neighbouring areas, especially at night (Shepherd, 2005). This difference in temperature is often referred to as the "*urban heat island*" (Oke, 1997; Pickett et al., 2008) and its effects are exacerbated by a number of different factors, but it tends to increase with the number of impervious surfaces. The "urban heat island" phenomenon has direct implications for plant communities, mainly in their flowering and leafing phenology (Neil and Wu, 2006).

Among the biotic factors, the continuous presence of humans is probably the most remarkable factor and may have a profound effect on the distribution and abundance of organisms (Marzluff, 2001). Humans (we) have introduced noise and pollution to environments, and wildlife has to adapt its biorhythms in order to share the habitat with us (i.e. Gil et al., 2015). In addition, urban environments are characterised by the presence of many non-native species, of which the majority have been deliberately or accidentally introduced by people e.g. monk parakeet (*Myopsitta monachus*) in many European cities, and European starlings (*Sturnus vulgaris*) and house sparrows (*Passer domesticus*) in, for example, many American and Australian cities. These alien species may compete and displace the native wildlife (Pell and Tidemann, 1997; but see Grarock et al., 2014). However, the impact of alien biota in urban environments is more widespread and problematic regarding plants. The urban plant community, in comparison with surrounding natural habitats is more diverse, however, this is mainly due to the presence of exotic species (Luck and Smallbone, 2010), which have a negative effect on insect abundance, e.g. less suitable food resources for invertebrates such as pollinators, but also on other taxa, like birds, e.g. via less suitable nesting structures (Narango et al., 2017).

Cities are also characterised by a high abundance of food of anthropogenic origin (e.g. food waste and provisioning) that urban wildlife can take advantage of, but also of lower abundance of natural prey for species feeding on invertebrates. For example, in the United Kingdom there are approximately 12.6 million households that provide supplementary food for birds (Davies et al., 2009; Fuller et al., 2008), and a large part of this is in urban and suburban areas (Jones and James Reynolds, 2008). The potential negative effects associated with supplementary feeding are those arising from the lower nutritive quality of the food and the increase in the disease transmission at feeding stations (Jones and James Reynolds, 2008). Food resources in urban landscapes are usually patchily distributed and can markedly influence transmission of disease. Moreover, the higher densities in many urban populations relative to rural ones seems likely to increase disease transmission rates (Bradley and Altizer, 2007; Martin and Boruta, 2013). Also, the effect of predators can be different in urban areas compared with rural/natural areas in terms of new predators (e.g feral cats) and predator densities (Haskell et al., 2001).

Effects of urbanisation on wildlife

It is clear that the urban environment differs substantially from the adjacent natural ecosystems and its particular characteristics pose great challenges to many species that try, either successfully or unsuccessfully, to colonise and persist in it (Figure 2). However, it also provides opportunities for those species or individuals that thrive in this new environment and make use of the resources provided by human activities (i.e. urban exploiters). The urban landscape acts as a filter, selecting against species that are for example food specialists or require large lekking grounds or territories (i.e. urban avoiders, Clergeau et al., 2006; Sol et al., 2014). The result of this selection at the community level will change the species compositional structure (e.g. taxonomic and phylogenetic diversity: Sol et al., 2014; Sol et al., 2017; evolutionary uniqueness: Ibáñez-Álamo et al., 2016), and this effect is common to most (if not all) animal taxa such as mammals (Villaseñor et al., 2014; Gortat et al., 2014), reptiles and amphibians (Hunt et al., 2013; Hamer and McDonnell, 2010), birds (Marzluff, 2001) and invertebrates (Niemelä et al., 2002), but also for plants (McKinney, 2008). In general, the decrease in taxonomic diversity of urban animal biota, in addition to the effects of alien species and similar abiotic pressures, could drive the observed community homogenisation in cities (Morelli et al., 2016).

While patterns in biodiversity metrics provide an idea of the macro-ecological effects of urbanisation, it is the intraspecific divergence in ecological and life history traits that is more relevant to fundamental questions in evolutionary ecology, e.g. the role of phenotypic plasticity and/or genetic local adaptation to new environments (Isaksson 2015). Indeed, marked changes in behaviour, physiology and morphology have been widely demonstrated among species inhabiting both urban and rural environments, even among those species that seemingly thrive in the urban habitat.

Birds are perhaps the most visible animal group in the urban environment, being relatively diverse in terms of life-histories, ranging from birds of prey to small perching birds. In addition, they are widespread, generally relatively long-lived organisms and the urban species are well-known in terms of their ecology, behaviour and physiology (including as pollution biomonitors: Golden and Rattner, 2003). As a result, most of the research on wildlife response to urbanisation is based on birds as model species (Marzluff, 2017). From now on in this thesis, I will focus my examples on birds to exemplify the consequences on different life-history traits that urban environments can exert in wildlife populations.

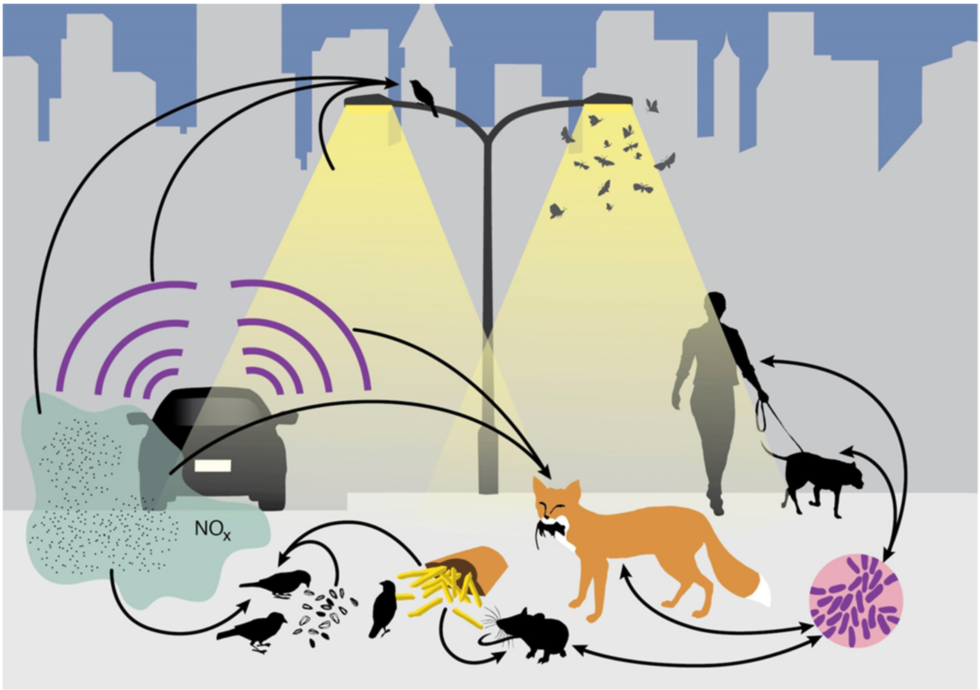

Figure 2. Schematic representation of the challenges (e.g. human presence, air, noise and light at night pollution) and opportunities (e.g. increased food opportunities) that urban environments provide for urban-dwelling wildlife.

Demographic traits

Demographic traits are those that affect population structure and growth. Timing of breeding is a crucial demographic trait that is affected, for example, by ambient temperatures, circadian rhythm (i.e. light) and female condition. Birds inhabiting urban areas experience increased temperature, light at night and food abundance, thus they are likely to have altered the breeding phenology. In fact, it has repeatedly been documented that birds breed earlier in urban centres compared to natural environments (Chamberlain et al., 2009). However, species-specific differences in life-history and local characteristics (i.e. latitude or urbanisation time) can hide a general pattern (e.g. Vaugoyeau et al., 2016). Urbanisation can also affect plant phenology, thus affecting caterpillar emergence which can have repercussions for the reproductive success of some urban bird species, through a mismatch between breeding timing and the caterpillar emergence i.e., food peak abundance (Wawrzyniak et al., 2015). Although the effect does not appear to be universal (Paridae vs Muscicapidae: Vaugoyeau et al., 2016) and similarly to the timing of reproduction, results are mixed, it has been shown that clutch size and nestling condition are reduced in urban habitats (Chamberlain et al., 2009; Sumasgutner et al., 2014, *Paper I* in this thesis).

The effects of urban stressors on survival (Rouse et al., 1999; Krebs and Davies, 2009; Hassell et al., 2017) can differ between bird life stages i.e. early-life (nestling and fledging period) or adulthood (e.g. Clobert et al., 1988). Surprisingly few studies have assessed the effect of urbanisation on birds' survival (reviewed in Chamberlain et al., 2009). While some studies lack an effect of urbanisation on post-fledging and nestling survival (Whittaker and Marzluff, 2009; Ryder et al., 2010; Ausprey and Rodewald, 2011), others have found a negative effect (e.g. Capilla-Lasheras et al., 2017). Similarly, the results on adulthood survival are mixed, with lower survival rates reported in, for example, urban great tits (*Parus major*) (Hõrak and Lebreton, 1998), while in other species they have found little differences between urban and rural populations (e.g. northern cardinal, *Cardinalis cardinalis*, Rodewald and Shustack, 2008b; Rodewald and Shustack, 2008a). Also, differences in adult survival rate have been reported between bird species in relation to urbanisation along the same urban gradient, i.e. some species exhibited higher rates of survival, others lower and some showed no response (Evans et al., 2015).

The potential change in predation in risk in, for example, small passerine birds, varies across urban and natural environments. This change will depend on the influence of human-introduced animals and natural predators present in the urban area. In certain urban habitats, there is an increased abundance of some predators such as cats and corvids (Marzluff, 2001; López-Flores et al., 2009; Rodewald and Shustack, 2008b; but see Vincze et al., 2017), thus predation pressure can be higher. Apart from corvids, other natural predators are present in urban areas (e.g. sparrow hawks, *Accipiter nisus*) but usually in lower numbers compared to natural areas, and consequently a lower predation pressure (Jokimäki et al., 2005). Nonetheless, due to the heterogeneity of the urban environment and abundance of anthropogenic food, scenarios with high predator abundance but lower predation risk, have been observed in some urban areas (predation paradox, Rivera-López and MacGregor-Fors, 2016). The differences in food availability between habitats, for example during breeding or cold winter months, both critical periods for survival in passerine birds (e.g. Nilsson et al., 2011; Gullett et al., 2014), could explain the lack of a significant effect on survival. In fact, it has been recently shown that dietary differences between blue tit (*Cyanistes caeruleus*) populations along an urbanisation gradient attained a lower reproductive success in urban and suburban individuals compared to forest populations (Pollock et al., 2017). Moreover, while it is true that urbanisation may influence demography (i.e. through predation or resource availability), the regulatory mechanisms can be species-specific (Evans et al., 2015) and potentially life stage dependent (*Paper II*), thus more studies are needed in this respect.

Behavioural traits

Behaviour is often the first line of response to an altered environment (Wingfield, 2003). Certain behaviours are mainly plastic (e.g. song rate and song type, but see e.g. Zollinger et al. (2017)) whereas other behaviours are partly genetically determined (such as boldness, van Oers et al., 2004), which allows selection for certain individual phenotypes. The presence of humans has a profound effect on the distribution and abundance of birds making the avian colonisation and persistence in urban areas a behavioural phenomenon as well (Sol et al., 2013). Tolerance to human presence may potentially increase boldness, an essential behavioural trait in order to thrive in urban environments. Indeed, several studies have found differences between urban and rural populations regarding distress behaviours (e.g. Moller, 2008; Møller and Ibáñez-Álamo, 2012; Torné-Noguera et al., 2014; Potvin et al., 2014). For example, urban birds generally seem to show shorter flight initiation distance (the distance at which an individual will fly away when a human approaches) than rural birds (Moller, 2008; Møller, 2012). This phenomenon has been shown to be consistent in several locations (e.g. latitudinal: Díaz et al., 2013) and sheds light on the importance of tolerance towards humans for urban-dwelling species, populations and individuals. Different urban bird species have also shown a similar distress response to human handling, showing higher frequencies of distress calling compared to rural counterparts (Møller and Ibáñez-Álamo, 2012). This effect was recently shown to be consistent within a species (the great tit) across multiple urban/rural population pairs (Senar et al. in revision).

Furthermore, birds have changed their acoustic signals to their habitat-specific sound properties in order to reduce attenuation (loss of volume), degradation (change in acoustic structure) and masking by background noise. Acoustic signalling is another well documented difference across urbanisation gradients (e.g. Slabbekoorn and Peet, 2003; Gil et al., 2014). Different species increase the minimum frequency of their acoustic signals in order to reduce the overlap with low-frequency anthropogenic noise and increase signal transmission and detectability (e.g. Wood et al., 2006; Salaberria and Gil, 2010; Hamao et al., 2011; Potvin et al., 2011). While this phenomenon is consistent across locations and species, the fitness costs and benefits of these modified signals are not fully explored (Slabbekoorn, 2013; Luther et al., 2016). However, it has been shown experimentally that intraspecific alarm calls can be masked by traffic noise (Templeton et al., 2016). This could have implications for the predation risk under urban noise and consequently affect community dynamics in noise-polluted areas.

Physiological traits

Physiological traits can influence the species response to environmental degradation (Chown and Gaston, 2008) and it can be the adjustments of these traits that enables species or populations to exploit urban environments. Stress hormones (glucocorticoid steroid hormones) play a major role to ensure survival under adverse environmental conditions (Romero, 2004). Therefore, as urban habitats potentially create new challenges, the hypothalamic-pituitary-adrenal axis system (the system responsible for glucocorticoid secretion) has garnered attention in the past years as an important mediator between behavioural and physiological responses to urban challenges (Bonier, 2012). Nonetheless, while some studies have reported lower baseline levels of corticosterone (the main avian stress hormone) in urban birds (Schoech et al., 2007; Fokidis et al., 2009), others have not found a difference, and instead found lower levels of the acute, stress-induced, corticosterone (Partecke et al., 2006; Atwell et al., 2012). While high corticosterone levels might indicate that individuals are chronically exposed to environmental stress, a reduced stress response could also be an adaptive response to frequent anthropogenic disturbances. In addition, other studies revealed no differences in the corticoid secretion in relation to urbanisation (e.g. Bókony et al., 2012; Heiss and Schoech, 2012). The lack of a common pattern in relation to stress hormone physiology could be related to the differences in life-history trajectories of urban and rural individuals/populations and the degree of tolerance to disturbance between species (Bonier, 2012).

Urban habitats, for example via light pollution at night, may alter biorhythms of urban animals, and thereby, change the secretion of certain hormones linked to reproduction and circadian rhythms (e.g., testosterone and melatonin, respectively). Indeed, the levels of these hormones have been demonstrated to differ between urban and rural bird populations (Kempenaers et al., 2010; Dominoni et al., 2013b; Dominoni et al., 2013c; Dominoni et al., 2013a; Partecke et al., 2004). These results suggest that there is a variation in the physiological timing of reproduction in relation to urbanisation, which is generally found to be earlier in urban individuals.

Moreover, the high levels of anthropogenic pollution in urban areas are expected to increase oxidative stress (see Box 2) of urban wildlife (Isaksson, 2010; *Paper III*). In birds, studies have reported differences in certain components of the oxidative stress physiology between urban and rural populations (Isaksson et al., 2005; Moller et al., 2010; Herrera-Dueñas et al., 2014; Giraudeau and McGraw, 2014; *Paper III*). For example, urban adult great tits have higher oxidative stress and antioxidant activity in their blood and lungs compared to their rural counterparts (Isaksson et al., 2005; Isaksson et al., 2007; *Paper III*). This could be a response to chemical pollution levels, such as traffic-related air pollution, as other urban stressors seem not to have a direct effect on oxidative stress levels (artificial light: Raap et al., 2016; artificial light and noise: Casasole et al., 2017). However, other studies have found an opposite pattern, i.e. lower levels of antioxidants in blood of urban birds (Herrera-Dueñas et al., 2014; Costantini et al., 2014; Giraudeau and McGraw, 2014) or no differences in oxidative damage levels (Giraudeau et al., 2015; Giraudeau and McGraw, 2014). Despite the recent interest in oxidative stress in ecological research, there are still few studies on the effects of urbanisation on oxidative stress physiology and more descriptive, empirical studies are needed (Isaksson, 2015) as the effects may potentially be species-specific (Isaksson et al., 2017; *Paper III*).

Food supplementation is a common activity in urban centres with potential repercussions for bird physiology. In general, urban animals are exposed to more anthropogenic food sources than rural individuals, such as sunflower seeds or coconut fat for birds. Some of these components, such as sunflower seeds, contain high levels of ω-6 polyunsaturated (PUFA) fatty acids (Beare-Rogers et al., 2001; Lobb et al., 2008), which can have pro-inflammatory effects and also increase the release of reactive oxygen species (ROS) (Larsson et al., 2004; Gomes et al., 2012). Thus, a higher intake of certain dietary fatty acids or an imbalance in the proportions of the different fatty acids might have implications for individuals' health (Hulbert and Abbott, 2012; Isaksson et al., 2017). Despite the potential divergent diets for urban and rural bird populations, relatively few studies have paid attention to fatty acid physiology in an urbanisation context (but see Andersson et al., 2015; Toledo et al., 2016). The fatty acid composition of an individual shows seasonal variation in birds (e.g. Pierce and McWilliams, 2005), nonetheless, it has been recently demonstrated that urbanisation also has an effect (Andersson et al., 2015). This difference in fatty acid composition across urban/rural environments can be species-specific (Isaksson et al., 2017) and have potentially, detrimental effects even in early-life (Toledo et al., 2016).

Micro-evolutionary or plastic response to urbanisation

The demographic, behavioural and physiological changes described above can be due to genetic changes (i.e., strong selection on certain traits) or to plastic, non-genetic, responses. Since the environmental changes linked to urbanisation appear to be quite homogeneous around the world, i.e. urban wildlife face similar challenges and opportunities, parallel evolution could occur for the same traits across, for example, Europe (*Paper IV*). Interestingly, some bird species show geographic differences in their response to urbanisation. For example, dunnocks (*Prunella modularis*) are absent from some urban areas, but occur in high densities in other urban areas, all within its natural range (Vogel and Toumenpuro, 1997).

Other birds, despite a wider distribution, have only recently colonised some European cities e.g., wood pigeons (*Columba palumbus*) (Fey et al., 2015). The colonisation events and the establishment of populations in novel environments altered by humans is often associated with rapid evolutionary events (Reznick and Ghalambor, 2001). Thus, it might be expected that the urbanisation process also leads to micro-evolutionary changes as a consequence of the potential novel selection regimes in urban habitats (Diamond, 1986). Recently, a study revealed the existence of global signatures of phenotypic change rates as a consequence of urban development (Alberti et al., 2017), i.e. a greater phenotypic change in urbanizing environments compared with natural and non-urban anthropogenic habitats. This study covered multiple taxa, including highly mobile organisms such as birds. Moreover, some studies have found genetic divergence at loci important for traits like immune response and metabolism in wild white-footed mice (*Peromyscus leucopus*) (Harris et al., 2013) and harm avoidance in blackbirds (*Turdus merula*) (Mueller et al., 2013) in urban populations. However, whether these divergent loci are associated with adaptive genetic change is still not clear (i.e. if there is a fitness benefit). Also, despite the similarity regarding urban abiotic factors, it is still unknown whether urbanisation can lead to parallel evolution of phenotypic change as it has been reported before in other study systems, such as for stickleback populations (e.g. Colosimo et al., 2005; Hohenlohe et al., 2010; DeFaveri et al., 2011).

Urban areas have repeatedly shown to present a barrier to gene flow for non-urban species (e.g. Ruiz-González et al., 2014), but these barriers are also within the urban habitat, such as high-ways, which could lead to disjunctive populations (Zhang et al., 2013; Beninde et al., 2016; Lourenço et al., 2017). These barriers may oppose gene flow and if there is strong selection, local differentiation can occur. In fact, a study on great tits in the city of Barcelona has shown the existence of genetic structure among different city parks embedded in the urban matrix (Björklund et al., 2010).

In addition to genetic adaptation, other mechanisms may help individuals to adjust to the urban environment. For example, most organisms exhibit phenotypic plasticity in response to environmental variation (Pigliucci, 2001), which implies changes in phenotypic traits but not necessarily at the genotypic level. It has also been recently shown, in our study populations, that the expression of genes linked to stress responses is higher in urban, compared to rural birds (Watson et al., 2017). The results of this study indicate a potential key role for another mechanism involved in phenotypic variation, which is epigenetic modifications e.g. DNA methylation. Indeed, urban great tits show higher DNA methylation in some loci with behavioural implications (Riyahi et al., 2015). However, as none of the described mechanisms are mutually exclusive, future studies exploring them simultaneously are still needed in order to understand the relative contribution of each in the phenotypic adjustments to the urban environment (Isaksson, 2015) and also whether the mechanisms are consistent across urban populations (Mueller et al., 2013).

It is clear that in the recent years, our knowledge about the effects (morphological, behavioural or physiological) of urban environments on wildlife, and particularly birds, has become much improved. However, there are still many open questions. For example, what are the implications of the divergent traits for the fitness of urban versus natural populations? Are there any suitable biomarkers that could predict survival and environmental stress in the urban environment? Could the suggested "*molecular clock*" of telomere length be a good marker? Also, under the same urbanisation intensity, is the phenotypic response similar among different species, i.e. if there is a convergent local response (e.g. in oxidative stress physiology). Another important question that still needs to be addressed is whether there are common signatures of selection between the different urban populations of a species or, alternatively, the in-principle common selective pressures related to the urbanisation process have acted independently to shape the genetic or phenotypic architecture of the different populations. This thesis tries to shed some light, and offer new perspectives on several of these unanswered questions in order to establish bases for future hypotheses and research in the relatively young field of urban ecology from a physiological and evolutionary perspective.

Material and methods

Study area

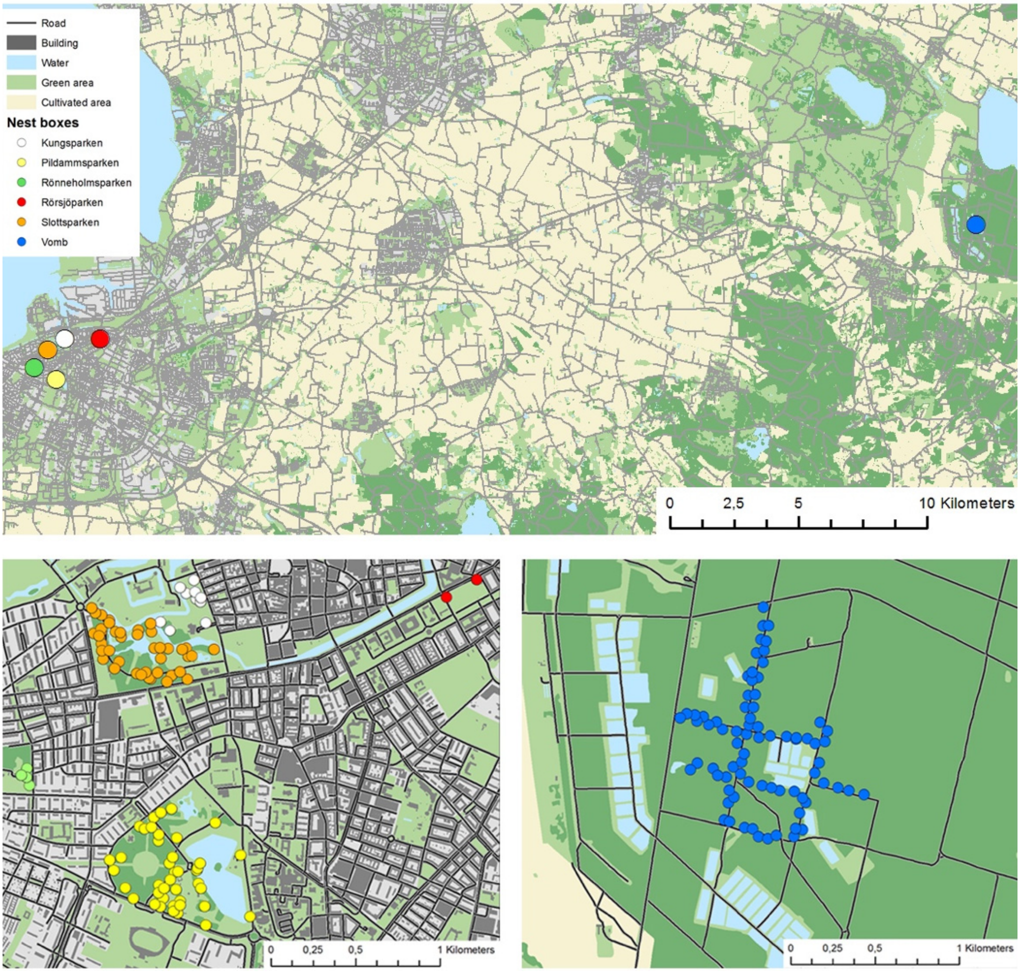

Figure 3. Map of the study area, showing in detail the two main study sites (on bottom left Malmö and on the bottom right Vombs fure) where nest-boxes have been monitored since 2013. Each point in the bottom figures represents a subset of the nest-boxes occupied by great tits (*Parus major*) and used in *Paper I-II* (for visual reasons, not all individual nest-boxes are represented in the figure).

Most of the fieldwork in this thesis was performed in the province of Scania in Southern Sweden (*Papers I-III*). In Swedish terms, the province is densely populated (approx. 1.2 million people in 11,000 km2) and Sweden's third largest city, Malmö (more than 350,000 inhabitants), is located in the area. Moreover, the air pollution levels throughout the county differ considerably due to its geographically varying population density, the proximity to Copenhagen (approx. 2,000,000 inhabitants in the metropolitan area), and the vehicle emissions on motorways and other major roads, as well as transportation to and from harbours (*Paper III*). These characteristics make the study area a suitable scenario for the thesis hypothesis. Since 2013, we have intensively monitored nest-boxes installed in two contrasting areas, one within the city of Malmö (four different urban parks) and another in a natural/semi-natural habitat, Vombs fure, separated by approximately 35 km (*Papers I-II*; Figure 3). In addition, in *Paper IV* we used 9 urban/rural population pairs of great tits across Europe (including our urban rural pair in Scania, Malmö/Vombs fure) in order to better understand the effects of urbanisation at the genome level (Figure 4).

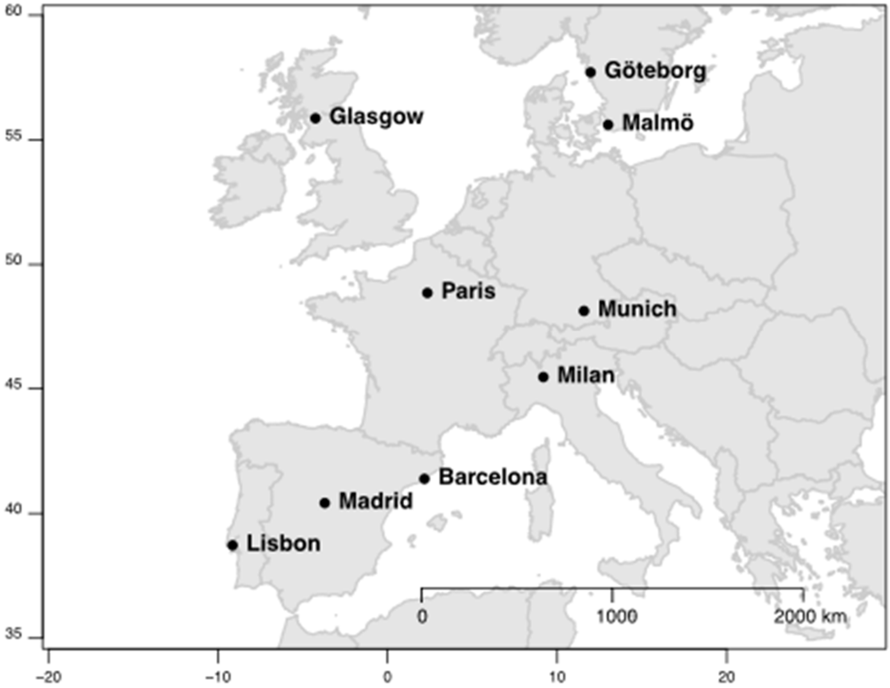

Figure 4. Map of the major areas where urban/rural paired sampling of great tits (*Parus major*) was carried out for *Paper IV*.

Study species

The biology and life-history of birds is well known owing to a large historical interest in this taxon. This is perhaps because birds are easily observed and captured, as well as considered to be aesthetically appealing. Whilst urban avifaunae have been the subject of investigation for over a century (Cramp, 1980), the study of the impact of urban dwelling on their life history variation is a relatively young field and the number of studies continue to increase (Marzluff, 2017). Because of their importance as model organisms, the results in this thesis are based on studies of four common bird species present in urban and rural environments, with emphasis on one of them; the great tit (*Parus major*, L.1758).

The great tit (Figure 5 A, *Papers I-IV*) is a common passerine bird (ca 20 g) with a distribution from Europe and North of Africa to Asia. In its wide distribution range, this species occupies a broad diversity of habitats such as gardens and city parks to complex deciduous and coniferous forests (Gosler and Clement, 2007). The species is mainly insectivorous during spring and summer (nestling rearing period), shifting to a more generalised omnivorous diet, including seeds and fruits in winter. This plasticity, in addition to its affinity for man-provided artificial nesting boxes (nest-boxes) has made great tits an excellent model to understand basic ecological and evolutionary processes - from life history evolution (e.g. Boyce and Perrins, 1987; Van Noordwijk and Scharloo, 1981), responses to climate change (e.g. Charmantier et al., 2008), variation in rates of ageing (Bouwhuis et al., 2009) to physiology, including oxidative stress (Isaksson and Andersson, 2008). In addition, the species is also widely used in studies of urban avian ecology (e.g. Senar et al., 2014; Bailly et al., 2016; Watson et al., 2017). For example, previous studies have reported phenotypic differences between urban and rural great tit populations regarding important life-history traits such as oxidative stress physiology (Isaksson et al., 2007; Isaksson et al., 2017), behavioural ecotypes (Charmantier et al., 2017) and breeding performance (Sprau et al., 2017). Further, great tit populations are globally stable and do not need any precautions in terms of conservation (BirdLife-International, 2016b)

The Eurasian blue tit (Figure 5 B, *Cyanistes caeruleus*, L. 1758; *Paper III*) is a small passerine bird (ca 11 g), phylogenetically related to the great tit with which it co-occurs for much of its range, sharing many ecological similarities (Cramp and Perrins, 1993; Gosler and Clement, 2007). Similar to the great tit, blue tit populations are globally stable (BirdLife-International, 2016a) and in southern Sweden, a substantial part of the population is year-round resident on the breeding territories. Nonetheless, part of the population migrates for short distances, in particular during years of high density and low winter food availability (Smith et al., 1987).

The house sparrow (Figure 5 C, *Passer domesticus*, L. 1758; *Paper III*) is a small (ca 30 g) non-migratory and highly sedentary bird (rarely forages more than 1 km from the nest) specialised on foraging on seeds, particularly grains, except during breeding when they feed their nestlings with insects (Anderson, 2006; Summers-Smith, 1963). It is commensal to humans and depends on food and nest-sites available in and around human dwellings, making its worldwide distribution a result of this relationship (Saetre et al., 2012). However, since the mid 1970's the NW European house sparrow populations have drastically decreased in both urban and rural habitats (e.g. Robinson et al., 2005), partly as a result of farming intensification in rural habitats (Hole et al., 2002), but the causes of the decline in many urban centres are still unclear (Summers-Smith, 2003).

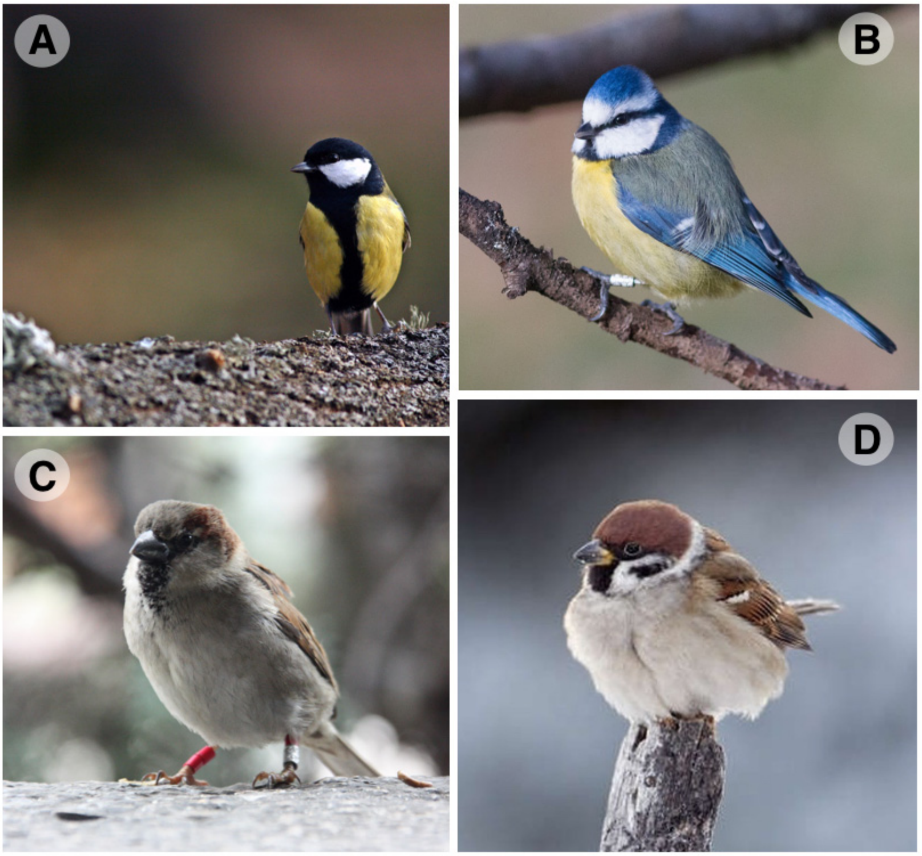

Figure 5. The study species used in this thesis: A) The great tit (*Parus major*), *Papers I-IV*; B) The blue tit (*Cyanistes caeruleus*), *Paper III*; C) The house sparrow (*Passer domesticus*), *Paper III*; D) The tree sparrow (*Passer montanus*), *Paper III.* Photos A-C: © Elena Tena. Photo D: Markus Varesvuo /© Nature Picture Library / Universal Images Group.

The Eurasian tree sparrow (Figure 5 D, *Passer montanus*, L.1758; *Paper III*) is another small granivorous bird (ca 21 g) connected with human settlements, but contrary to the closely related house sparrow, it is less strongly associated to humans in some aspects of its ecology e.g. it often uses natural cavities (Summers-Smith, 1995). Tree sparrow populations in Europe are largely sedentary (Hagemeijer and Blair, 1997). In a similar way to the house sparrow, the tree sparrow's conservation status is of least concern according to the IUCN but the current population trend is decreasing (BirdLife-International, 2016c). However, the population trend decline in this species shows large geographic differences (Eaton et al., 2009).

Laboratory analysis

Telomere length measurement *(Paper I-II)*

Relative telomere length (RTL) was measured in red blood cells (RBC) by quantitative real-time amplification method (qPCR) (Cawthon, 2002), following a procedure previously validated for birds (Criscuolo et al., 2009). This method provides a relative measure of the telomere length suitable for within-species comparisons (Nussey et al., 2014). Briefly, RTL was measured as the ratio (T/S) of telomere repeat copy number (T) to control gene copy number (S), relative to a reference sample using a Mx3005P (Stratagene). The control, single-copy gene used was glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Previous studies have confirmed the suitability of this gene as an invariant control gene in great tits (Atema et al., 2013). To account for variation in amplification efficiencies between telomere and GAPDH, we used the Pfaffl method to calculate T/S ratios (Pfaffl, 2001).

Oxidative stress biomarkers (*Paper III*)

Several oxidative stress biomarkers were measured in RBCs or in plasma in order to obtain a general picture of the oxidative stress physiology (antioxidants and oxidative damage) in four common urban-dwelling passerine species. Superoxide dismutase (SOD) and total glutathione (tGSH), both in RBCs and total antioxidant capacity (AOX) in plasma, were used as antioxidant biomarkers. Thiobarbituric acid reactive substances (TBARS) and protein carbonyls (PC) in RBCs were used as oxidative damage biomarkers. We followed standard protocols previously used in different bird species (Herrera-Dueñas et al., 2014; Isaksson, 2013; de la Casa-Resino et al., 2015; e.g. Kamiński et al., 2009). Briefly, SOD was measured by pyrogallol oxidation method (Marklund and Marklund, 1974), quantifying the amount of enzyme that inhibited the autoxidation of pyrogallol by 50%. The tGSH content of RBC was determined following Baker et al. (1990); and used before in passerine birds by e.g. Isaksson et al. (2013). The total AOX of plasma was measured using the ferric reducing antioxidant power (FRAP) assay (Benzie and Strain, 1996), which gives the overall reducing potential i.e. non-enzymatic antioxidant potential. The TBARS were used as a lipid peroxidation estimate according to the method of Ohkawa et al. (1979). PC were measured in RBC according to Reznick and Packer (1994) as an indicator of oxidative damage to proteins (Dalle-Donne et al., 2003).

Several studies have found that up to 90 % of the variation in avian plasma AOX can be due to the effect of uric acid (Cohen et al., 2007; Costantini, 2011). In order to correct our AOX values, uric acid levels were measured in plasma using a commercial kit (SPINREACT, Sant Esteve d'en Bas, Spain) based on the uricase/peroxidase method. In addition, total protein concentration in RBC was measured for correcting the SOD activity, following Bradford (1976).

All the samples were run in duplicates (intra-assay coefficient of variability < 10%) using 96-well microplates and the absorbance was measured in a FLUOstar OMEGA (BMG LABTECH) microplate reader. The chemicals used for all the analysis were purchased from Sigma-Aldrich (Stockholm, Sweden).

Modelled air NO_x exposure levels (*Paper III*)

A high-resolution emission database (Stroh et al., 2005) was used to model the hourly and weekly levels of NO_x ($\mu g/m^3$) for each sampling locality during the study period (11th of January to the 25th of February 2015). Briefly, the emission database consists of a broad list of potential emission sources in the county of Scania, such as road traffic, industrial emissions, ship and aviation traffic, heat and energy emissions as well as long range transport. In addition, the emission model takes source location, emission levels, time pattern and intensity as well as meteorological factors into account when calculating the NO_x exposure levels. The model has been previously validated and displays a high correlation with in situ measured values ($r^2 = 0.85$, $p < 0.001$) (see for more details Stroh et al., 2012). Each individual bird was assigned with the GPS coordinates and the time of capture and thereafter the model computed the current NO_x exposure levels for these coordinates during the assigned period. All the modelling analysis was done with the software *EnviMan* (OPSIS).

Background, results and discussion

Telomeres and its dynamics in an urban context

The physiological differences that have been documented between urban and rural bird populations (reviewed in the section *Effects of urbanisation on wildlife* in this thesis), can be a potential sign of a cost or alternatively, an adaptation. There are suggested links between these physiological traits and fitness but such studies are by and large lacking. Nonetheless, if the urban and rural environments exert differential selection pressures on life-history traits, such as on physiology, these should subsequently have implications for individual adaptation and fitness (Shochat et al., 2006).

Telomere research has recently gained attention in the fields of evolutionary biology and ecology, probably as a result of the increasing evidence for the role of telomere length in survival and life span in laboratory and field populations (Bize et al., 2009; Heidinger et al., 2012; Barrett et al., 2013, *Paper II*). From a molecular perspective, telomeres are non-coding DNA repeats at the extreme ends of eukaryotic chromosomes with a capital role in cell senescence and genome protection (see Box 1 for details). Studies in a range of organisms have shown that telomeres shorten with age in different somatic tissues (reviewed in Dantzer and Fletcher, 2015), thus telomere attrition has been suggested to be one of the possible mechanisms associated with age-related declines in physiological function (i.e. senescence) and therefore fitness (Monaghan, 2010; but see Simons, 2015). In addition to the general pattern between telomere length and age across taxonomic groups, individual telomere attrition rate can be accelerated by factors related to physiological stress e.g. increased glucocorticoid levels (Herborn et al., 2014) and oxidative stress (von Zglinicki, 2002; but see Boonekamp et al., 2017).

Recent research provides compelling evidence about the role of environmental factors as relevant drivers in telomere dynamics; factors such as habitat (Angelier et al., 2013; McLennan et al., 2016; Stier et al., 2016), breeding colony (Young et al., 2013), latitude (Quirici et al., 2016; Kirby et al., 2017) or foraging performance (Young et al., 2015). Many of the urbanisation-related environmental stressors can increase oxidative stress and inflammatory responses (reviewed in Isaksson, 2015), and both have previously been linked to telomere length shortening (O'Donovan et al., 2011; von Zglinicki, 2002). Indeed, a recent study in house sparrows has experimentally shown a negative impact of anthropogenic noise exposure on early-life telomere length (Meillère et al., 2015). Therefore, implementing research on telomere dynamics in an urban context could develop our understanding of how anthropogenic stressors may shape patterns of ageing and survival in wildlife. We explored this possibility in *Papers I* and *II* in this thesis, in order to understand the effects of the urban environment on individual telomere length.

Box 1. What are telomeres?

Telomeres are highly conserved tandem repeats of non-coding DNA (5'-TTAGGG-3')*n* at the ends of eukaryotic chromosomes (Blackburn, 1991). Together with protein complexes they play an important role in genome stability and replication, protecting the coding sequence of the genome (O'Sullivan and Karlseder, 2010; De Lange et al., 1990). Telomeres typically end in a single-stranded DNA that curls around in a long circle (T-loop) where the telomeric DNA invade the double-stranded DNA generating a displacement loop (D-loop) (de Lange, 2004). This structure prevents chromosomes ends to be recognised as double stranded breaks. Telomeres shorten each time a cell divides as DNA polymerases are not able to replicate chromosomes completely, this is known as the "end-replication problem" (Hug and Lingner, 2006). However, telomere length can be restored by the telomerase enzyme activity but in most somatic cells this enzyme is inactive and as a consequence telomeric repeats shorten in each division until they reach a critical length, in which the cell stops dividing and undergo apoptosis (Bodnar et al., 1998). In addition, because of its guanine (G) content telomeric sequences are highly sensitive to damage by oxidative stress (Henle et al., 1999), alkylation (Petersen et al., 1998) or UV irradiation (Oikawa et al., 2001) increasing telomere shortening rate.

Paper I:

In *Paper I*, we assessed the effect of the urban habitat on early-life telomere length (TL) in great tit nestlings, using a partial cross-fostering experiment (i.e. Merilä, 1996; Nilsson et al., 2009) between our urban (Malmö) and rural (Vombs fure) populations. Our overall aim was to determine if urban rearing conditions had an impact on individual TL. In addition, our experimental setup allowed us to disentangle the influences from the genetic background and the environment i.e. rearing habitat.

Our results showed that early-life TL (15 days old) differed by 10.7% between habitats (shorter TL in the urban habitat than in the rural habitat, Figure 6) and that differences were independent of nestling genetic origin (urban or rural). The result suggests that urban and rural rearing conditions have an impact on telomere maintenance even during a relatively brief exposure (less than two weeks) to the new environments. TL, as other life-history traits, has the potential to be genetically determined and for example, in human populations, the heritability is on average around 0.7 % (Broer et al., 2013). In non-human animals, the results are less consistent ranging from 0.09 to 0.99 %, but birds early-life TL seems to be highly heritable (Asghar et al., 2015; Atema et al., 2015; but see Becker et al., 2015). Our results also showed that early-life TL is at some degree genetically inherited as the nest of origin was significant in our analysis (Table 1 in *Paper I*). However, the differences between habitats (urban versus rural) seem to have a larger impact than the inherited effects, which shows the importance of both genetics and habitat quality as determinants of nestling TL.

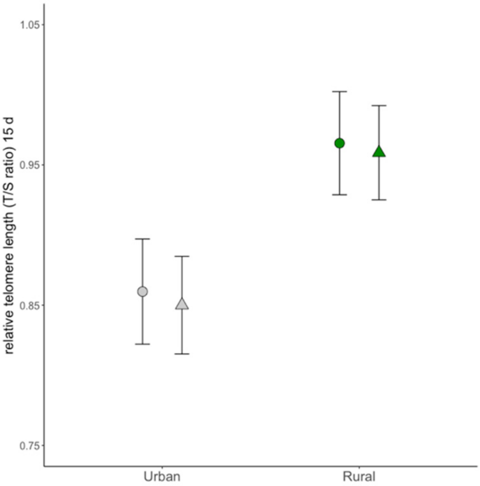

Figure 6. Relative telomere length (RTL) in great tit nestlings. Mean (± SE) RTL (T/S ratio) at 15d in great tit nestlings, reared in an urban (grey) or a rural (green) habitat (n = 16 nest pairs; 156 nestlings). Triangles represent cross-fostered nestlings while circles non-fostered nestlings. [Adapted from Figure 1 in *Paper I*]

In this study we did not measure any urban stressor simultaneously so we can only speculate about the underlying mechanisms driving the observed habitat differences. A recent study in urban great tit nestlings found a lower constitutive immune response in urban reared individuals compared to rural individuals (Bailly et al., 2016). This result, in combination with ours on TL, suggests that urban nestlings might be constrained by resource limitation during development as nutritional status plays an important role in immune response (Houston et al., 2007) and TL maintenance (Paul, 2011). Moreover, similar to TL, the body condition of the nestlings reared in the urban habitat was lower than the body condition of rural nestlings, supporting the idea of dietary restrictions during development (supplementary information *Paper I*). This, in addition to the other urban associated stressors (e.g. air pollution, traffic noise), could drive an accelerated early life telomere loss (Watson et al., 2016).

To summarise, the results from *Paper I* indicate that an urban environment can have detrimental effects on TL during post-hatching development. It is well understood that exposure to stressors in early-life can have profound effects on longevity and performance (Metcalfe and Monaghan, 2001). Understanding the subsequent effects of short early-life TL in an urban habitat and its implications on longevity and performance are crucial in order to interpret the consequences of urbanisation on wildlife.

Paper II

Our results in *Paper I* show that being reared in an urban environment has implications on early-life TL, where nestlings in the urban population have shorter TL at 15 days than nestlings in the rural environment. In *Paper II*, the aim was to investigate the missing link between telomere dynamics and survival in relation to urbanisation. During three consecutive breeding seasons, we sampled nestling and adult great tits in the urban and rural populations for measurements of RBC's TL and survival. We found that indeed, early-life TL was a strong predictor of post-fledging survival and recruitment in both urban and rural populations, but the results showed that selective disappearance was more pronounced in the urban habitat (results section in *Paper II*; Figure 7 A). Despite fledging with shorter telomeres in the urban habitat (*Paper I*), the present study demonstrates that urban recruits have significantly longer TL than rural recruits and that this is driven, presumably, by strong selective disappearance of individuals with short telomeres (Figure 7 B). Previous research on certain life stages, e.g. nestling survival (Ryder et al., 2010) or post-fledging survival (Whittaker and Marzluff, 2009), have failed to report a negative effect of urbanisation (but see Capilla-Lasheras et al., 2017). However, the effect of early-life conditions may not be revealed until later in life and could be mediated by early-life telomere dynamics, as is supported by our results. Urban rearing conditions have detrimental effects on TL (*Paper I*) and the relation between this biomarker and survival suggest that individuals experiencing accelerated early telomere attrition have disappeared from the populations during their first year (Boonekamp et al., 2014). In contrast, in adulthood, there were no differences between the urban and rural environments with respect to TL and how it links to survival. Nonetheless, TL was once again positively related to survival (Figure 8). Despite the expectation that the urban environment poses less favourable conditions, we did not find any difference in the rate of telomere attrition, neither during the first year of life – from fledging to 2nd calendar year – nor during later life between the urban and rural habitats. This could, in part, be explained by the elimination of poor-quality individuals' due to selective disappearance of those with short telomeres (Haussmann and Mauck, 2008b; Barrett et al., 2013). It is also possible that the urban environment may not present the same level of stress to adult birds, as it does to young inexperienced and naive birds, and as a result of early life conditions, urban adult birds have longer TL than rural birds in our study (see Figure 4 in *Paper II*). However, the two mechanisms are not necessarily mutually exclusive. In cities, if individuals survive the critical early life-stage, the benefits of the urban environment may outweigh the costs. For example, feeding birds in urban areas is a common practice (e.g. Jones and James Reynolds, 2008; Davies et al., 2009) which may increase adult survival (reviewed in Robb et al., 2008).

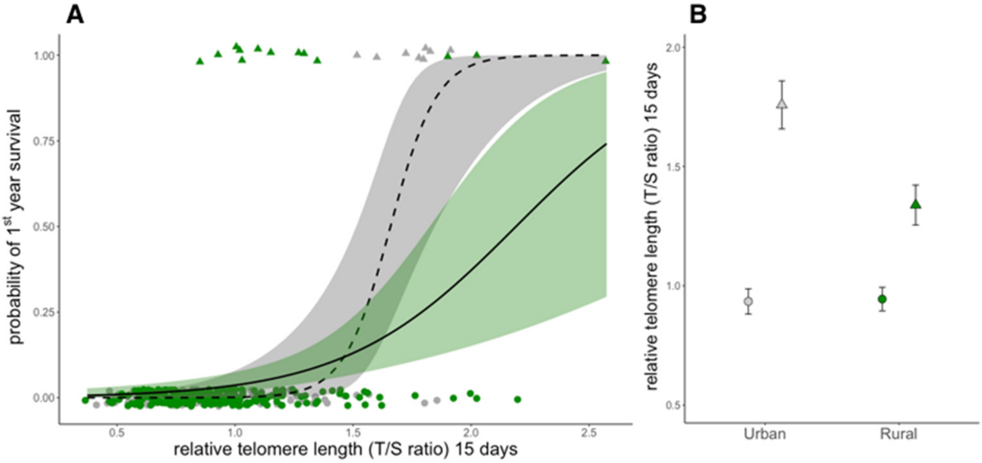

Figure 7. Relationship between relative telomere length (RTL, T/S ratio) at 15d and survival and recruitment to the subsequent breeding season in urban (grey) and rural (green) great tits. Data points represent individuals present (triangles) or absent (circles) in the populations (a) Predicted probability of 1st year survival and recruitment in relation to habitat and RTL at 15d. Fitted lines represent model predictions for urban (dotted) and rural (solid) populations with 95% confidence intervals (shaded area) (b) Mean ± SE RTL (T/S) at 15d of the individuals that are present and absent in their 1st breeding season. [Adapted from Figure 1 in *Paper II*]

Finally, we did not find an effect of age on TL in our populations. Early work on TL in an ecological context suggested that TL could be a suitable biomarker of chronological age (Nakagawa et al., 2004; Haussmann and Mauck, 2008a). However, several studies, mostly based on cross-sectional data, have failed to find a clear link between TL and chronological age (e.g. Ujvari and Madsen, 2009; Angelier et al., 2013; Kirby et al., 2017) but also in some longitudinal analysis (Pauliny et al., 2012; Ujvari et al., 2016). However, these longitudinal studies were carried out in long-lived organisms in which telomerase activity has been shown to be maintained in adult somatic cells (e.g. Haussmann et al., 2007; Ujvari et al., 2016). The role of telomerase in maintaining TLs is still very poorly studied in non-human organisms and future studies are needed in order to get a better understanding of telomere dynamics.

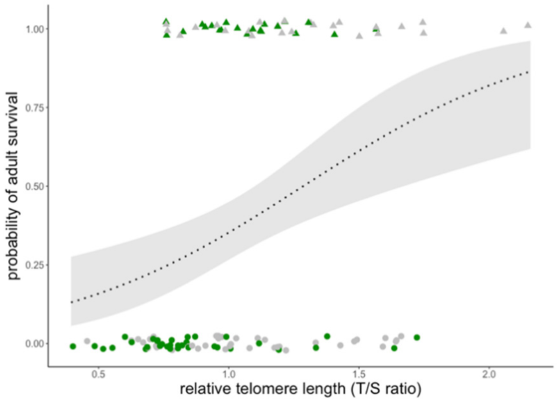

Figure 8. Relationship between relative telomere length (RTL, T/S ratio) and survival to the following breeding season in adult great tits from urban (grey) and rural (green) populations. The adult great tits are 2nd calendar year or older. Triangles denote presence in the population while circles denote absence. Predicted probability of survival in relation to RTL is plotted as a dotted line with 95% confidence intervals shown by shaded area. [Adapted from Figure 2 in *Paper II*]

In *Paper II*, we demonstrated a clear effect of the urban environment on telomere dynamics and survival in early life, but the urban effect was lacking for adults (Figure 8). This indicates adverse consequences of exposure to urban-related stressors during early life, while suggesting that individuals are more resilient in later life and/or the benefits of the urban environment may outweigh the costs of being exposed to urban environmental stressors. Our results provide, to the best of our knowledge, the first evidence of differential selective disappearance, in relation to TL, between two contrasting environments. Moreover, this study highlights the importance of research on general demographic patterns and how life-history traits link to individual fitness in order to better understand how anthropogenic stressors may shape urban wildlife populations.

Effects of air pollution and urbanisation on oxidative stress physiology

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 (Halliwell and Gutteridge, 2015), including the atmospheric pollutants. Most air pollutants (e.g. particulate matter, gaseous pollutants) have the ability to act directly as pro-oxidants attacking lipids and proteins or increase the production of reactive oxygen/nitrogen species (ROS/RNS), unbalancing the cellular redox status and inducing oxidative stress (Menzel, 1994; Halliwell and Gutteridge, 2015, see Box 2). Urbanisation, via its high levels of anthropogenic pollution can increase oxidative stress in wildlife populations (Isaksson, 2010). However, despite the recent interest and attention in oxidative stress in ecological research, there are still few studies that have measured the different components of the oxidative stress physiology machinery (it is either damage or antioxidant levels) in an urbanisation context (but see Isaksson et al., 2009; Giraudeau et al., 2015; Casasole et al., 2017).

Box 2. Oxidative stress: a molecular balancing act

Cells live in a dynamic redox environment, whose status is determined by a complex balance between pro-oxidant (reactive oxygen/nitrogen species -ROS/RNS) and antioxidant molecules both crucial for cellular signalling and function (Costantini, 2014). However, if a surplus of pro-oxidants is present, a condition named oxidative stress occurs (Halliwell and Gutteridge, 2015), which may be defined as the rate at which oxidative damage is generated to the different biomolecules such as lipids, proteins and nucleic acids (Costantini and Verhulst, 2009). Consequently, organisms have evolved a diversity of mechanisms to avoid or minimise the production of reactive species as well as to limit their pro-oxidant activity (Halliwell, 1999). The different compounds involved in the redox homeostasis maintenance can be grouped as endogenous (e.g. enzymes and low molecular weight compounds) or exogenous (e.g. carotenoids and vitamins) according to their synthesis origin. In general, antioxidant function comprises the delay, inhibition or prevention of oxidation via i.e. quenching, scavenging or binding to reactive species. In addition, antioxidants are usually connected to each other, coping synergistically with pro-oxidants and creating a complex defence network (Figure 10).

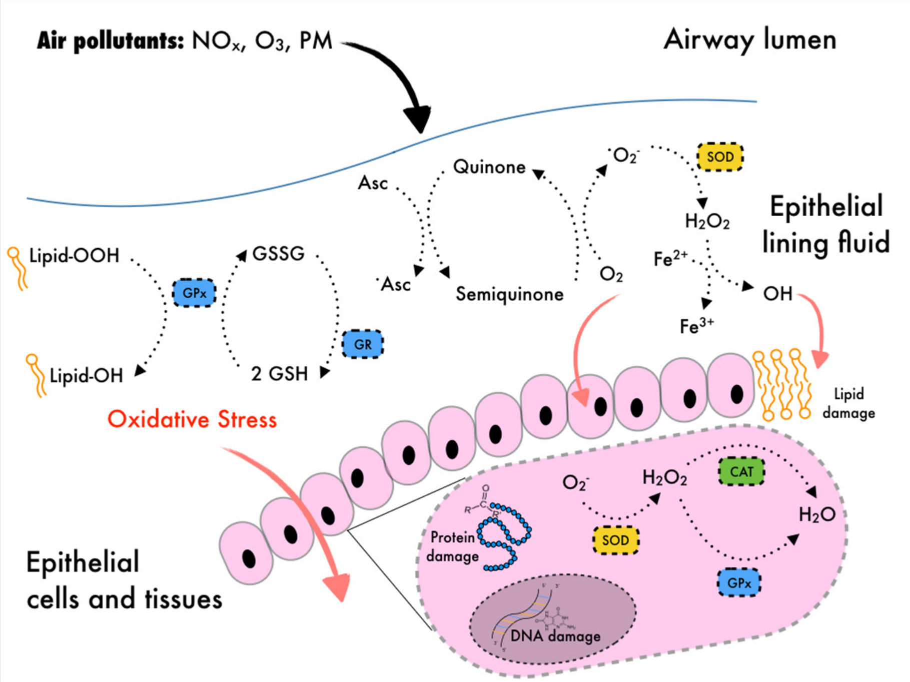

Figure 10. Schematic interaction between atmospheric pollutants, pro-oxidants and the antioxidant machinery in the respiratory tract. Air pollutants and oxygen radicals react with the different antioxidant molecules: low molecular compounds e.g. glutathione (GSSG: Oxidised glutathione; GSH: Reduced glutathione), enzymes (SOD: Superoxide dismutase; CAT: Catalase; GPx: Glutathione peroxidase; GR: Glutathione reductase), vitamins e.g. Asc: ascorbate (vitamin C). Redox active components of PM (Particulate Matter) such as quinones or iron ions can generate reactive substances and oxidative stress. Based on Lakey et al. (2016).

In nature, stressors rarely act in isolation. For instance, the three common urban stressors artificial light at night, air pollution and noise often coincide (Swaddle et al., 2015). Thus, it may be prudent to use a general index of urbanisation to capture all stress that the urban wildlife is exposed to, and indeed this is the most common approach in urban ecology (e.g. Liker et al., 2008; Bókony et al., 2012; Vincze et al., 2016; *Paper III*). Studies that try to dissect the effects of a single urban stressor (e.g. light at night or nitrogen oxides) are still rare, but are needed in order to understand the direct and specific impact of individual stressors on, for example, oxidative stress physiology in wildlife (Casasole et al., 2017). Anthropogenic pollution (including traffic-related air pollution) is known to have detrimental effects on human health, e.g., increase susceptibility to respiratory diseases (Brown et al., 2009; Halonen et al., 2008). Less is known about wildlife, but some studies have found e.g. higher oxidative stress levels in birds reared in metal contaminated environments (Berglund et al., 2007; Koivula et al., 2011). Nitrogen oxides (NO_x) are among the most common urban chemical pollutants and they are considered to be good indicators of general air pollution (WHO, 2006). Its emissions occur almost anywhere where combustion takes place (e.g. car engines and fire). The emissions are particularly high at elevated temperatures and motor vehicles account for more than 50% of overall emissions (Hill, 2010). NO_x is a potent pro-oxidant, thus likely to play a large role in oxidative stress physiology of urban animals. Indeed, previous studies reporting differences in oxidative stress physiology between urban and rural environments highlight air pollution as a likely explanation (Herrera-Dueñas et al., 2014; Isaksson et al., 2009) but no studies to date have simultaneously measured oxidative stress levels and air pollution exposure (e.g. NO_x) in wildlife.

Paper III

In *Paper III*, the aim was to understand how multiple components of the oxidative stress physiology (damage and antioxidants) were associated with urbanisation and/or long (one month)-, medium (one week)- and short-term (one day) estimates of NO_x exposure. Moreover, we simultaneously compared four passerine species that commonly inhabit urban and semi-natural/rural habitats, to investigate whether the physiological response to urbanisation/pollution is general or species-specific.

The results showed that the urbanisation index, commonly used in ecological research (e.g. Seress et al., 2014; *Paper IV*), was highly correlated with the NO_x air pollution levels, both the one month and the one week averages, and showed a trend with 24h NOx average. This supports the use of the urbanisation index as a proxy for urban-associated long- and medium-term pollution levels and specifically, traffic-related air pollution. The short-term levels (24 h) showed much more variation, and are likely to be more affected by local weather conditions, thus, having a less reliable link to urbanisation. Among the different analysed oxidative stress biomarkers, we found that plasma AOX was associated with the long- and medium-term NO_x exposure estimates but not with the short-term average (Figure 10 A). In addition, AOX was positively correlated with urbanisation index, lower AOX in less urbanised localities (Figure 10 B). Here, our study cannot disentangle the causal effect of NO_x level and urbanisation on AOX. This result was general across all four species, suggesting that the response of this biomarker is similar to urbanisation/NOx pollution. However, the level of the response is species-specific. Furthermore, the lack of relationship between the AOX and the (daily) changes in NO_x exposure suggests that variation in this short-term single stressor is not the major player in the differences in AOX, but is one out of several stressors that together shape the urban environmental AOX response.

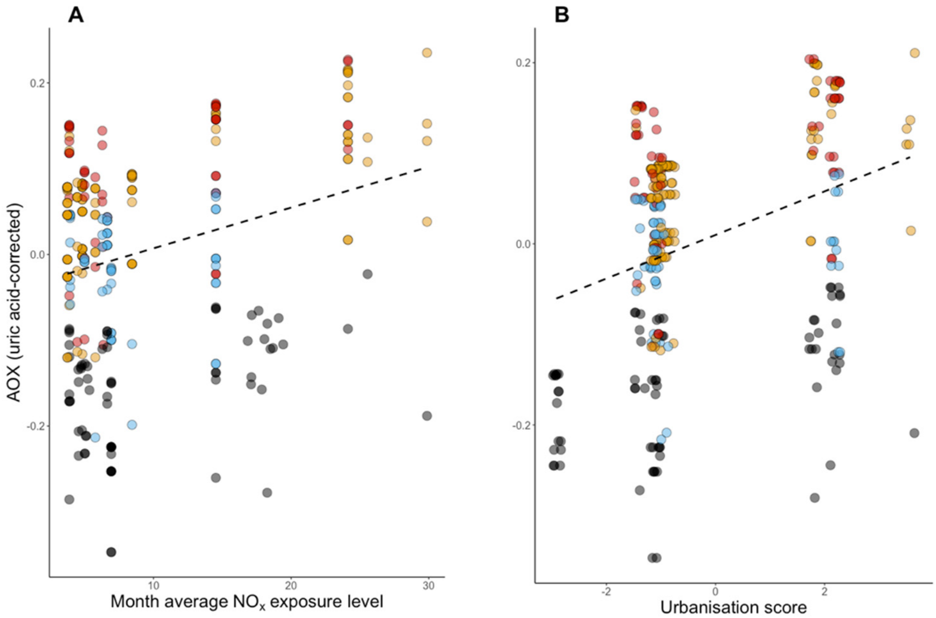

Figure 10. Effects of NOx level and urbanisation on plasma antioxidant capacity (AOX) in four passerine species. A) Relationship between the month NOx estimated exposure levels before sampling and AOX (FRAP assay corrected for uric acid levels); B) Relationship between the estimated urbanisation indices (positive values indicate higher degree of urbanisation) and AOX (FRAP assay corrected for uric acid levels). The relationship between week estimated exposure levels and AOX is not represented, as month and week estimates of NO_x levels are highly correlated and show the same results. The four passerine species are blue tit (BT, blue), great tit (GT, black), house sparrow (HS, yellow) and tree sparrow (HS, red). [Adapted from Figure 3 in *Paper III*]

In contrast, none of the other antioxidant biomarkers measured in the study (tGSH and SOD) showed a correlation with the NO_x exposure estimates or the degree of urbanisation. Previously, both of these antioxidant biomarkers have been reported to be sensitive to heavy metal and air pollution levels (Mateo et al., 2003; Delfino et al., 2009), however, here this effect is absent. Possibly, these antioxidants are more responsive to heavy metal pollution rather than NO_x levels and other urban associated stressors, and these are pollutants that are not necessarily correlated with urbanisation. Alternatively, the study site has too low pollution levels to trigger these enzymes, and an up-regulated AOX is sufficient. Generally, birds have a superior antioxidant defence to, for example, mammals, which has co-evolved with the higher metabolism needed for flight. Hence, birds may already have a high enough tGSH and SOD activities in urban areas, at least to combat the current air pollution levels. Moreover, both tGSH and SOD revealed strong species-specificity, which would be interesting to explore further in relation to life-history and ecology of the four species.

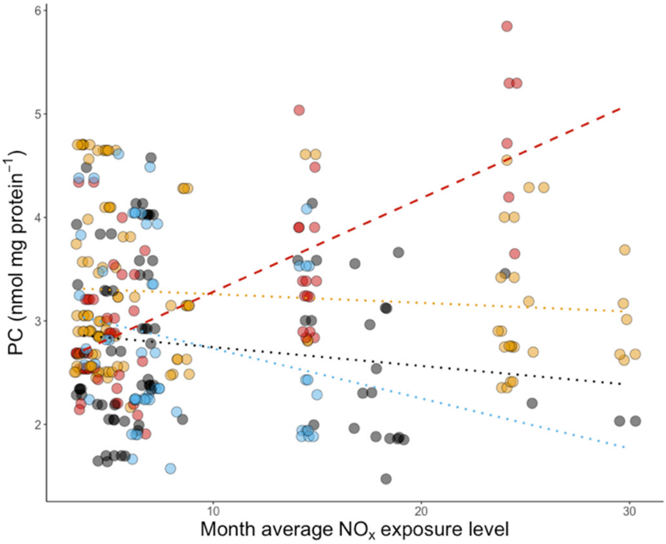

Figure 11. Relationship between long-term (month average) NOx exposure levels and protein carbonyls levels (PC) in four passerine species. The dashed line indicates a significant relationship, while dotted non-significant. The relationship with medium- (one week) and short-term (24h) NO_x estimated exposure levels is not presented here, but reveal a similar result. The four passerines species are: blue tit (BT, blue), great tit (GT, black), house sparrow (HS, yellow), and tree sparrow (TS, red) [Adapted from Figure 5 in *Paper III*]

Regarding oxidative damage biomarkers, protein carbonyl levels were related to the estimated NO_x exposure levels, but interestingly, not to the degree of urbanisation in tree sparrows (Figure 11). This result indicates that among the species included in the study, tree sparrows are more sensitive to NO_x (pro-oxidant) exposure (Grune et al., 2003), despite the high AOX levels observed in plasma. On the other hand, lipid peroxidation levels (the other damage biomarker analysed in the study) were not related to either air pollution exposure or urbanisation degree, but similar to tGSH and SOD they presented strong species-specific differences. This result enhances the idea that lipid damage could be more species-specific and possibly more nutrition dependent than previously appreciated (Isaksson et al., 2017).

Overall, our study cannot disentangle the direct effects of NO_x levels from those of urbanisation per se, however, the results reveal a convergent species response in plasma antioxidant capacity (AOX). Moreover, the study reports a strong species-specific difference in the measured oxidative stress biomarkers, which suggest that tree sparrows are the most vulnerable species. Future studies are needed to understand the variation in oxidative stress across contexts, seasons and life-stages and how the ecology and phylogeny of species interacts in order to interpret the effects of urbanisation on wildlife species resilience.

Local adaptation to the urban environment

There is now evidence that micro-evolutionary adaptations can occur within much shorter timescales than previously thought, particularly in response to human activities (Palumbi, 2001; Alberti, 2015). However, to date, it is not clear whether the observed phenotypic differences between urban and rural populations are a result of divergence at particular loci underlying adaptive evolution (i.e. local adaptation to urban environments) or acclimatisation without any genetic change (e.g. via epigenetic mechanisms), although both strategies are not mutually exclusive (Isaksson, 2015). In addition, phenotypic and genetic differentiation between a particular pair of populations might also occur without involving adaptations, e.g. genetic drift or random selection. Moreover, it may be that neither adaptation nor acclimatisation occur and instead organisms in urban habitats might have to cope with particular stressors, in which case a reduction in fitness (directly or indirectly) should be apparent.

Despite the recent interest in the role of urbanisation as a driver of evolution in wildlife populations, few studies have investigated the potential genomic signatures of divergent selection between urban and rural populations (Harris et al., 2013; Mueller et al., 2013; Munshi-South et al., 2016). These footprints in the genome could help to deepen the understanding of underlying mechanistic pathways driving the adaptation to urban environments (i.e. which genes are under selection) and the observed phenotypic differences.

Paper IV

In this paper, we tried to identify signatures of local adaptation to the urban environment. To that end, we performed a genome-wide analysis to evaluate the presence of consistent genetic divergence across nine paired urban and rural great tit populations. We used genotype-by-environment (GEA) analysis to identify the signatures of local adaptation, while correcting for population structure. Our paired sampling design across the species European distribution range might increase the power to detect genes with weak selection (Lotterhos and Whitlock, 2015), in contrast with transect designs across urbanisation gradients. Overall, our results showed low genetic differentiation between the studied urban/rural populations, suggesting a weak effect of urbanisation on great tit gene flow between the urban matrix and the adjacent natural/semi-natural habitat (Figure 12 A). This result is in accordance with previous research done in the species across its European distribution range, where low F_{ST} values have been reported between multiple distant populations (Laine et al., 2016; Lemoine et al., 2016). Birds are highly mobile organisms and in contrast to other taxa (e.g. reptiles: Beninde et al., 2016; mammals: Munshi-South et al., 2016; amphibians: Lourenço et al., 2017), human infrastructure such as buildings or motorways do not prevent gene flow between their populations (e.g. Zhang et al., 2013; Björklund et al., 2010). However, for a large number of loci across the genome (1,766 SNPs), we found slight but highly significant allele frequency differences between the urban and rural environment (Figure 12 B). This result needs to be followed up in depth, e.g. by investigating the consistency of divergence across the pairs of populations and by detailed analyses of the genes linked to the significant SNPs. Nonetheless, the result suggests that although urban and rural populations are genetically very homogeneous, a substantial fraction of the genome shows evidence of differentiation. This result agrees with previously described differences for the species in multiple phenotypic traits between urban and rural population pairs across Europe (e.g. Isaksson et al., 2005; Charmantier et al., 2017; Isaksson et al., 2017; Sprau et al., 2017; *Paper I-II* in this thesis). The existence of divergence at the SNP level, despite the gene flow, imply that natural selection imposed by urbanisation is present and potentially strong (Kawecki and Ebert, 2004). This potential evidence for parallel evolution will contribute to a better understanding of the mechanistic underpinnings involved in adaptation to the urban environment.

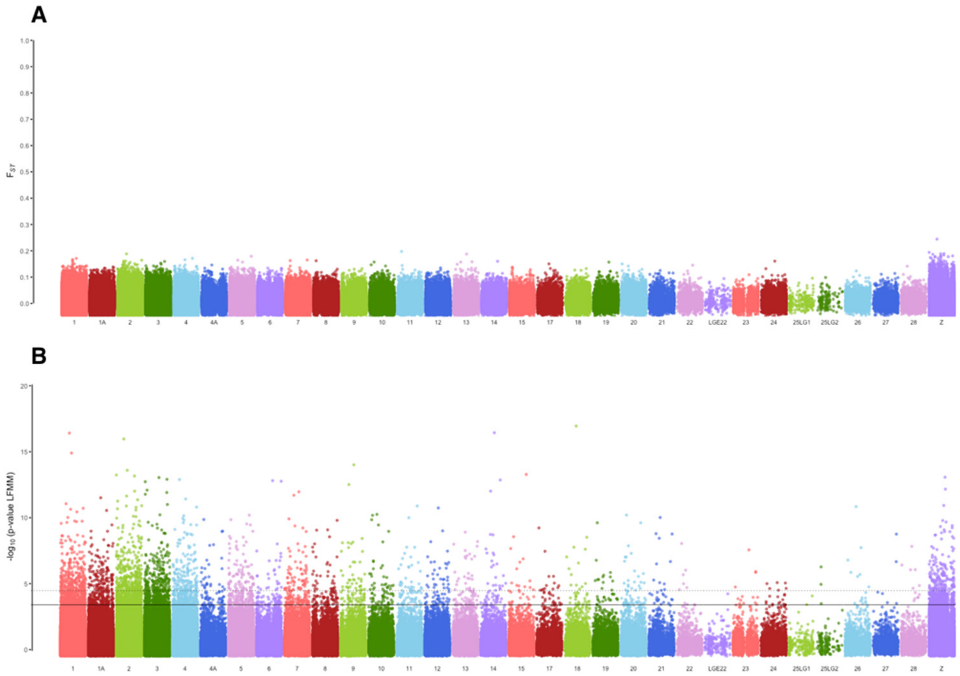

Figure 12. Manhattan plots showing genomic differences between 9 urban and rural great tit population pairs. A) Average pairwise F_{ST} values between all the sampled urban and rural great tit populations B) GEA (genotype-by-environment association) analysis for the association with the degree of urbanisation based on median log10 p-values adjusted for FDR from 10 independent runs of LFMM. The solid line corresponds to the top 5% while the dotted line corresponds to the top 1% of the distribution range. [Adapted from Figure 4 in Paper IV]

Conclusions

It is generally accepted that urban areas present both challenges and opportunities for wildlife, and that the consequences of these opposing effects can be species and context-specific. In this thesis, I have shown, using a multidisciplinary approach, negative effects of urbanisation at the molecular, physiological and ecological level, particularly in nestlings. However, there was also a sign of an opportunity, as adult birds seemed not to be affected by urbanisation at the molecular and ecological level. In addition, there is also indication of parallel evolution, suggesting a strong selection. This confirms the general point about urbanisation, that it is challenging (i.e., strong selection against certain loci and individuals), but that it could create a great opportunity for those phenotypes that selection has acted for.

To summarise, we have found, for first time, a detrimental effect of urban rearing conditions on telomere length, a commonly used biomarker, of cellular ageing (*Paper I*). This result supports the idea that urban habitats might present constraints during early development, at least in some passerine species. Moreover, the observed pattern seems to have implications later in life (*Paper II*) as our results demonstrate a stronger selective disappearance in the urban population of individuals with short telomere length in early-life compared to the rural population. However, this negative effect of urbanisation only persisted in the first year of life, while in adulthood, the benefits of living in an urban area could outweigh the physiological costs for birds. Hence, as mentioned above, these results reinforce the idea of urban areas as an ecological paradox and also, they link a phenotypic trait with differential selection pressures between urban and rural environments.

This work also aimed to link one common urban-related stressor (air pollution) to oxidative stress physiology (*Paper III*), a crucial mechanism that acts as a currency for life-history traits. Though our results are not able to disentangle the effect of the air pollution levels from those of urbanisation, we still found a consistent response in the antioxidant capacity in four different bird species. This result suggests that urbanisation and its related stressors trigger similar mechanisms on birds' physiology. In addition, one of the analysed species showed higher levels of protein damage despite the increased antioxidant capacities with urbanisation, but here it was only in relation to air pollution levels. Overall, our results on oxidative stress physiology, reinforce 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.

Urbanisation is a worldwide phenomenon, but apart from differences in urbanisation intensity, there are other local differences that can have implications for wildlife. This includes, for example latitude, size of the metropolitan area and/or time since urbanisation. Taking these differences into account, the genomic signatures of urbanisation in multiple urban great tit populations indicates that despite the low genetic differentiation, there is still a potential for selection on several genes (*Paper IV*). These genes may indeed be related to pathways or traits that provide a fitness advantage to urban individuals, and thus be involved in local adaptation processes. However, thriving in a new environment does not always attain a genetic change and further research is still needed, mostly to assess the relative role of plasticity and genetic change in generating trait divergence in an urbanisation context.

To conclude, the work presented in this thesis confirms the existence of phenotypic divergence between urban and rural bird populations. Furthermore, we have demonstrated that some of these traits can exert fitness consequences in an urban context, which reinforces the idea of specific selective pressures acting in these habitats. Further research is needed now in order to understand the specific mechanisms involved in adaptation, coping strategies and/or acclimatisation to the relatively novel scenario that urban environments pose for wildlife, as well as the relative contribution from each of urban-related stressors.

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Here I am, writing the last lines of this "*journey*", which of course, I have not accomplished alone. Nevertheless, it would be an endless task to list all names and contributions without missing any. Instead, I will mention some and leave the rest to the individual dedicatory.

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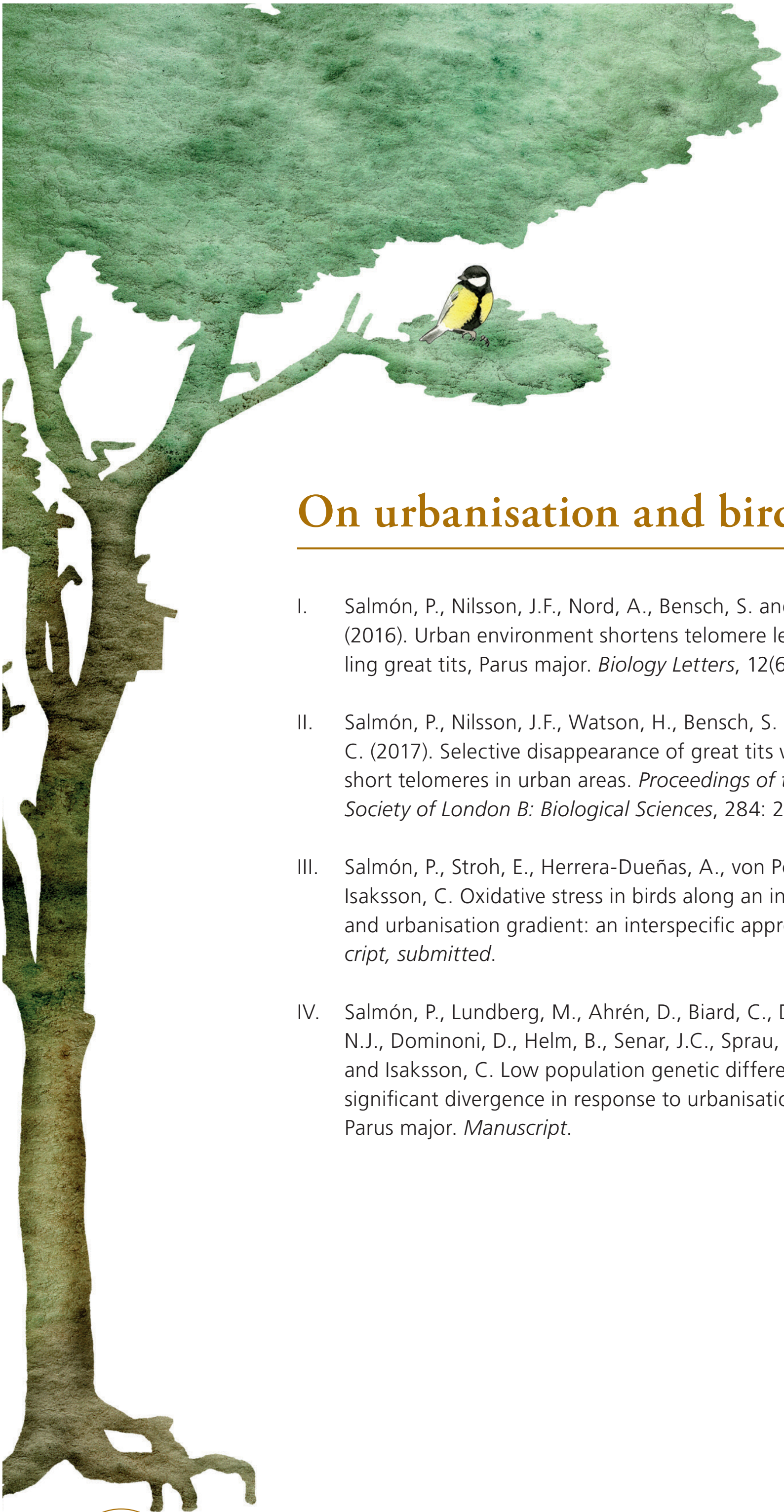

On urbanisation and birds

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