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Spatial patterns of bird-parasite interactions along an urbanisation gradient

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Spatial patterns of bird-parasite interactions along an urbanisation gradient

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Carlos Andres Delgado-Velez, BSc (Hons)

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Red-browed finch and Superb-fairy wren; passerine birds upon which this thesis is mostly based. They are affected differentially by parasites in urban areas.

CERTIFICATION

I, Carlos Andres Delgado-Velez, declare that this thesis, submitted in fulfillment of the requirements for the award of Doctor of Philosophy, in the Department of Biological Sciences, University of Wollongong, is wholly my own work unless otherwise referenced or acknowledged below. The document has not been submitted for qualifications at any other academic institution.

Carlos Andres Delgado-Velez

25 March 2015

PUBLICATIONS

This thesis includes chapters that have been written as the following journal articles:

Chapter one: Delgado-V., C.A., French, K. 2012. Parasite-bird interactions in urban areas: Current evidence and emerging questions. Landscape and Urban Planning. 105, 5-14.

Chapter three: Delgado-V., C.A., French, K. In review. Urbanisation does not increase ectoparasite prevalence in Australian passerine birds. Urban Ecosystems.

Chapter four: Delgado-V., C.A., French, K. In press. Differential influence of urbanisation on Coccidian infection in two passerine birds. Parasitology Research [DOI: 10.1007/s00436-015-4414-2].

My supervisor, as co-author on my publications, has helped with data collection, analysis, interpretation and writing but this has not been above and beyond that which is expected of a PhD supervisor. Therefore I, as primary author in all mentioned papers, have been solely responsible for submitting each manuscript for publication to the relevant journals, and have been in charge of responding to reviewers' comments.

For Chapter five, I also received contributions from Paulo Pulgarín, MSc. His contribution to this paper was focused on performing and advising on the technical aspects of the molecular screening.

STATEMENT OF CANDIDATE CONTRIBUTION

As the supervisor, I, Professor Kristine French, declare that the greater part of the work in each article listed is attributed to the candidate, Carlos Andres Delgado-Velez who led conceptual development, study design and was primarily responsible for data collection, data analysis, and data interpretation. Carlos has been solely responsible for submitting each manuscript for publication to the relevant journals, and he has been in charge of responding to reviewers' comments.

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Professor Kristine French
Supervisor
25 March 2015

_____

Carlos Andres Delgado-Velez
Candidate
25 March 2015

CONFERENCES AND OTHER PUBLICATIONS

I have also presented data from this thesis at the following national and international conferences:

- Delgado-V., C.A., French, K., 2012. Are urban birds full of parasites? Ecological Society of Australia Conference. December 3-7. Melbourne, Australia.

- Delgado-V., C.A. 2012. Bird-parasite interactions along an urbanisation gradient. Postgraduate Student Conference, University of Wollongong. November 2. Kioloa, Australia.

- Delgado-V., C.A. 2011. Bird-parasite interactions in urban areas. Postgraduate Student Conference, University of Wollongong. November 3. Kangaroo Valley, Australia.

- Delgado-V., C.A., French, K., 2011. Spatial dynamics of bird-parasite interactions along an urban gradient. International Conference on Malaria and Related Haemosporidian Parasites of Wildlife. Malaria Research Coordinator Network. Agosto 5-7. National Conservation Training Center, Shepherdstown, West Virginia, USA.

In addition to the published manuscripts listed above, during the course of my studies as PhD student at University of Wollongong I have published two journal articles which were based on observations obtained during my studies in Australia:

- Delgado-V., C.A., Correa-H., J.C. Accepted. Sugar packet opening by the Noisy Miner: a novel foraging behavior. The Wilson Journal of Ornithology.

- Delgado-V., C.A., Correa-H., J.C. 2013. An unusual foraging tactic of the Willie Wagtail. The Wilson Journal of Ornithology. 125, 846-848.

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ABSTRACT

Urbanisation is a fast growing phenomenon worldwide which has direct and indirect impacts on biodiversity. Urbanisation affects particular species but also biotic relationships and interactions. Changes in host-parasite interactions have the potential to be one of the important consequences of urbanisation, influencing the species able to exploit, establish and succeed in urban landscapes. However, how urbanisation influences the prevalence and impact of parasites and the susceptibility of different birds has not been studied in detail, especially in areas such as Australia and many Oceanic islands. The risk of parasitism is predicted to increase in cities due to changes in climatic conditions, habitat loss, habitat conversion, increased temperature, fragmentation and a greater presence of human-modified landscapes.

Integrating ecological and physiological approaches, this thesis analyses the distribution and diversity of three groups of bird parasites along an urbanisation gradient in New South Wales, Australia, and investigates how parasites and haematological and body condition may influence the structure of urban-bird communities. Given the paucity of information available in Australia, this thesis provides an inventory of a portion of Australia parasite fauna and tested whether parasite loads could influence the ability of some birds to inhabit cities. I particularly focused on two species of birds which are not particularly common in urban areas.

Contrary to expectations, my research does not support a positive association of urbanisation and bird parasites, nor does it identify detrimental effects on the immune system and body condition of the host. Instead, results suggest that urbanisation does

not necessarily result in high parasitism and lower immune system in urban-sensitive birds. This thesis found that some parasites such as louse flies, ticks and haemosporidians are less prevalent (or even disappear) while others might be either more prevalent (e.g. Coccidian parasites) or kept unchanged in urban areas (e.g. lice). My study was unable to detect a general pattern of loss of body condition associated with urban development for the species sampled suggesting that urbanisation effects are complex and might be site-, parasite- and host-specific related.

This thesis identified four new lineages of haemosporidians, indicating great potential for Australian bird parasites to be fundamentally unique. The poor knowledge available on the relationship among parasites and wildlife still requires further consideration across more species in order to determine how parasites are affected by urbanisation and whether they play a role in the establishment and success of birds in cities. At present parasite load does not appear to be a cause of the decline in urban birds.

ACKNOWLEDGMENTS

First of all, I would like to thank my supervisor Kris French for accepting me as her student and encouranging me to complete the crazy experience of conducting a PhD project overseas. As an international student new in such an immense and distant country, Kris is the kind of tutor everyone would always wish to have, not just for her insight and support to my research whenever needed, but also for her mood, generosity and unconditional kindness, aspects that were crucial to stay in and complete this process.

There were many people who helped with particular aspects of my research and time in Australia. Personally, I received generous and disinterested help from Australians: I thank Danina Scrivenor and her family for helping us to settle well and safe. Jocelyn and Ted Booth lent us their bicycles which allowed Juanis and I to transport around Wollongong with independence. Kel Magrath and, Jacqui and David Price for the warm hospitality and company they offered us. The members of the French' Lab for friendship and lab and field support. Moreover, I would like to thank Daniel Zuleta for providing advice on statistical analysis and Paulo Pulgarín for assisting with molecular screening.

This study could not have been completed without the enduring support of my family and friends in -or coming from- my natal country: Juanis offered me endless patience, love, help and company; “mi mamá” who took care of Fênix with such love during my time in Australia, ¡loquito perdón por abandonarte tanto tiempo!; thanks to Chucho for his crucial moral support while finishing and submitting the thesis; and all the “viejitos” for being always close to me.

Funding was received from The Joyce Vickery Scientific Research Fund and The Linnean Society of NSW. All urban field trips were carried out on a Yuba Mundo bicycle which was partly donated by CargoCycles (Australia) and Yuba Bicycles (USA) for conducting urban ecology studies. Finally, I received scholarships from University of Wollongong. Without them, I would have never visited Australia to study and run with kangaroos.

PROLOGUE

Urban areas are increasing in both extent and number (Evans et al. 2009) which is causing important impacts on biodiversity through a range of processes. However, while the effects of urbanisation on biodiversity structure and composition have been quite well studied, interactions and relationships amongst species are virtually unknown (Schochat et al. 2006).

Urbanisation causes significant changes to species composition, species interactions and ecological and evolutionary processes (Gaston 2010). Changes in host-parasite interactions have the potential to be one of the important consequences of urbanisation, and influence, perhaps, the species ability to exploit urban landscapes (Martin and Boruta 2014; Riley et al. 2014). However, how urbanisation influences the prevalence and impact of parasites and the susceptibility of different birds to them has not been studied in detail.

Some bird species survive and prosper in urbanised areas ("Urban Tolerant Birds"), but others are directly or indirectly affected by a wide range of anthropogenic activities and infrastructure ("Urban Sensitive Birds") (Parsons et al. 2006). An increase in some kinds of food and water, reduced presence of predators, fewer or limited competitive interactions, and a changed microclimate influence the inclusion or exclusion of birds in cities (Fokidis et al. 2008), however they do not explain (or characterise) fully the presence, establishment and success of some native species that thrive in urban environments around the world. Therefore some other significant factors that are more cryptic, and unstudied may be important in elucidating the causes

of presence (and absence) of species in urban areas.

Parasites are a dominant and diverse group which is fundamental in ecological and evolutionary aspects (Atkinson et al. 2008). For example, certain parasitic life strategies may allow the maintenance of ecological processes and will be critical to the conservation of fully functioning ecosystems in natural environments (Nichols and Gómez 2011). However, chronic alterations in parasite prevalence might cause significant changes in vertebrate hosts and change ecological processes.

Birds are host to a variety of parasites which exhibit a diverse array of form and life history, and where obligate and non-obligate taxa are recognised as vectors of pathogens and diseases (Pershikova et al. 2010). Avian blood parasites and ectoparastites, but also others protozoa, bacteria, viruses, cestodes and nematodes may play important roles such as increasing mortality (Ludwing et al. 2010); reducing fecundity (Heins and Baker 2014); altering nutritional status (Aronstein et al. 2012); reducing growth (Mouritsen and Poulin 2002); enhancing susceptibility to predation (Laferty and Morris 1996); altering mate choice (Minchella and Scott 1991); and affecting body condition (Faim et al. 2008). Therefore, parasitism could contribute greatly to the structure, diversity, conservation, function, and health of urban biodiversity (McCallum and Dobson 1995).

Parasites and pathogens may cause several detrimental effects in native species globally (Daszak et al. 2000; Cumming and Van Vuuren 2006). Since the risk of parasitism and wildlife diseases caused by ecto- and endoparasites are likely to increase due to human activity (Bradley and Altizer 2006), parasites may be an important factor affecting the

presence of wildlife species in cities. However, in contrast to the growing interest in studying factors that can influence the risk of parasitism inherent to the host, such as sex, age, reproductive status and behaviour, the habitat-specific factors that promote parasitism have been mostly ignored (Kleindorfer et al. 2006). As a result, we have very little understanding of whether parasite infestations in disturbed habitats such as cities, influence the host species that can exist in these habitats.

The prevalence and impact of wildlife diseases and parasite loads in urban areas differ amongst studies (Bradley and Altizer 2006). Some authors have found that urban centres have lower rates of parasite infection than rural or natural areas due to, for example, an increase in food availability which probably helps urban birds to create resistance through increased immunity (Gregoire et al. 2002). In contrast, others suggest a positive relationship of abundance and/or prevalence of parasites in urban areas (e.g. Cumming and Van Vuuren 2006, Morand and Poulin 1998).

Avian urban parasite studies have been carried out preferentially in Europe and North America (Delgado-V. and French 2012). Moreover, such studies have been focused on a couple of species which are abundant in cities such as European blackbirds, House sparrows and crows. As a result, although there are globally few studies to help elucidate the role of parasites in urban areas, especially in Australia, most of them are focused on Urban Tolerant Birds from temperate locations but just few have been carried out on Urban Sensitive Birds (Fokidis et al. 2008).

At present therefore, studies have not been undertaken on the groups of birds for which parasitism may be the cause of their decline in cities. Additionally, since different bird

species are indeed differentially susceptible to parasite infestation (e.g. *P. relictum* causes diseases of different severity in different passerine birds, Palinauskas et al. 2008), the consequences of parasite host dynamics due to anthropogenic environments should be explored. This means that a comparative analysis of parasite load should also be linked with measures of health in birds since species which are common in cities may be advantaged by increased immunity whereas species which are now rare in urban environments may be more susceptible. Thus different species of birds may have different susceptibility to parasite load thus influencing their ability to occupy urban centres.

This PhD project investigates the dynamics of bird-parasite interactions in the Illawarra and Sydney regions to determine the relationship between infestation loads, health and body condition in order to determine how parasites and immune status can change and potentially affect bird communities along a gradient of urbanisation.

RESEARCH AIMS

This thesis seeks to describe parasite load and the host body condition and susceptibility along urban gradients, to investigate their importance in contributing to the decline of some passerine birds in Australia (Ford et al. 2001). I hypothesized that Urban Sensitive Species may be less exposed to parasites in natural habitats where they are in better condition and have better immune systems and where parasites are less prevalent. While not all species occur in every habitat, comparisons in parasite load and health of birds in habitats where they are rare will identify if parasite load increases or decreases differentially with urbanisation.

Specifically, I will

1. Describe the diversity and direction of the bird-parasite relationships identifying prevalence of bird parasites in habitats with different degree of urbanisation development;

2. Examine the immune system and body condition of two Urban Sensitive Species; and

3. Determine if two Urban Sensitive Species are more prone to parasites in cities.

OVERVIEW OF THIS THESIS

One of the chapters (chapter one) has already been published (Delgado-V. and French 2012). Additonally, chapter four is in press (Delgado-V. and French in press). The rest of data chapters are written in the format of separate scientific papers for submission to peer-reviewed journals (for example, chapter three was submitted to *Urban Ecosystems*). Therefore there is some inevitable repetition of content between chapters. This thesis is the original work of the author, unless stated (see Publications). I provide a brief overview of each chapter

Chapter one: I compile information about bird-parasite interactions publications in urban areas around the world. This review of the literature identified a poor knowledge of this interaction. While studies identifying parasites were more common than those comparing parasite abundance or host health, studies were limited geographically and to

a few bird species. I found that urbanisation could have a positive or negative effect on the diversity and prevalence of bird parasites. Very few studies have linked parasite loads to bird health. I identify some hypotheses that need further consideration across broader regions and species to determine if parasites play a role in the establishment and success of birds in cities and how their health and body conditions are affected (Delgado-V. and French 2012).

Chapter two: I performed a brief evaluation of the dust-ruffling method using Pestene, an insecticide powder used in veterinary practices but never tested in ecological field studies. Comparison of the visual examination and dust-ruffling methods for quantification of ectoparasite infestation carried out on 13 species captured suggested that the method using Pestene is significantly more sensitive in recovering a greater number of mites and lice. Also it allowed the identification of a greater proportion of the population that is infested by mites, lice and ticks and discerned a higher number of parasites per bird. Pestene is recommended as a valuable tool to measure prevalence and intensity of lice, mites and ticks infestation and provides a sound reason for improving estimates of ectoparasite prevalence and intensity in ecological studies.

Chapter three: Urbanisation has the potential to alter the risk of parasitism on wildlife as it is associated with habitat modification, fragmentation and decreased biodiversity. As birds are commonly found in urban areas, they provide a valuable opportunity to study parasitism in urban environments. Native birds were examined along an urbanisation gradient for three groups of ectoparasites in order to detect if variation of prevalence might be directly related to urbanisation development. Prevalence of parasites declined or fluctuated with urbanisation, rather than increased. Lice were

equally prevalent regardless of the level of urban development and higher prevalence of ticks and louse flies seemed to be negatively correlated with urban intensity. Although parasite values are usually variable amongst hosts, parasite parameters exhibited similar trends within the hosts sampled which suggests that urbanisation does not necessary result in high ectoparasitism. Instead, urbanisation effects on bird parasites might be site-, parasite- and host-specific. Studies on other urban areas, seasons, parasites and a wider diversity of hosts are needed in order to understand the ecological distribution of parasitism in an urbanised world.

Chapter four: Urbanisation has the potential to increase the risk of parasitism on wildlife. Although some ectoparasite groups appear unaffected, different responses are hypothesized for parasites with simpler life histories such as gastrointestinal parasites. Two native passerine birds affected by urbanisation were examined for Coccidian parasites along an urbanisation gradient in New South Wales, Australia, in order to detect if prevalence might be directly related to the degree of urbanisation. Prevalence of *Isospora* increased in more urbanised areas on Red browed Finch but this did not significantly change with the degree of urbanisation in Superb-fairy-wren. Diet, behaviour, and habits, are suspected to be the most influential factors on the variation seen between both species with the granivorous and gregarious species being significantly infected. Since the dynamics of urban wildlife-pathogen interactions is largely unexplored, more studies are needed to corroborate if this pattern of *Isospora* infections can be extended to other passerine birds in cities from Australia and overseas.

Chapter five: Australia is one of the poorest sampled regions for parasites. Such absence of knowledge prevents an understanding of the ecology and diversity of its

Haemosporidian fauna (globally as well as regionally), and the effects of different forms due to disturbance (such as the ones due to urbanisation) on the complex host-parasite relationship. In order to better understand the prevalence and diversity of Haemosporidian parasites in eastern Australia, natural and urban sites were sampled in the Illawarra region, NSW. Of the 270 passerine birds screened using light microscopy in urban areas, a total of 0.7% were found infected, although the degree of infection varied among species. Additionally, molecular screening of 147 individuals of Superb-fairy wrens and Red-browed finches suggested that there are at least four undescribed lineages of *Leucocytozoon* and *Haemoproteus*, two respectively, and that their prevalence is higher or exclusive in natural areas. The few molecular studies of Haemosporidians in Australia do not allow to conduct a deeper analysis, however I suspect that vectors might be implicated as response since, for instance, the absence of *Haemoproteus* in urban areas might be due to the decrease of louse flies, one of its main vectors, in highly urbanised areas, as it was recorded in a concomitant study of ectoparasites which included the same sampling areas. Prevalence and diversity data here presented for first time in Eastern Australia have important insights into the epidemiology, diversity and transmission dynamics of Haemosporidian parasites in the country, even in highly disturbed areas.

Chapter six: Urban development is associated with activities and land uses changes that can cause detrimental changes in body conditions and immunology stages making wildlife more susceptible and vulnerable to different parasite pressures and diseases found in urban areas. In order to try to detect if susceptibility might be considered a factor affecting some bird species living in cities, I tested whether the degree of urbanisation affects the health and body conditions of the Red-browed finch and

Superb-fairy wren, two passerine birds considered affected by urbanisation in Australia. I monitored six blood and body condition parameters along an urban gradient and found significant differences in Haemoglobin concentration, head and bill length, H/L ratio and Total Leukocyte Count among sites. For example, Haemoglobin concentration in RBF is significant higher in natural areas while finches captured in industrial areas exhibited the highest value of H/L ratio. Contrary, the differences I detected for males and females of SFW were more subtle and restricted to H/L ratio in breeding seasons. Results suggest that while wrens might have a greater capacity to cope well with parasite infections, pathogens and diseases in urban areas, the immune system of urban finches might not be in optimal condition to respond successfully to urban pressures in cities.

Chapter seven: Here I draw together the results from previous chapters together and discuss their implications and conclude the thesis.

CHAPTER ONE: PARASITE-BIRD INTERACTIONS IN URBAN AREAS: CURRENT EVIDENCE AND EMERGING QUESTIONS

A modified version of this chapter is published in *Landscape and Urban Planning*.

Delgado-V., C.A., French, K., 2012. Parasite-bird interactions in urban areas: Current evidence and emerging questions. Landscape and Urban Planning. 105, 5-14.

1.1. Introduction

Urbanisation is a fast-growing phenomenon which is recognised as the major cause of biotic homogenisation, a process characterised by a loss of diversity and a concomitant progressive increase in similarity where the abundance of some species can increase while others generally decline (McKinney 2006). Urban growth has direct and indirect impacts on biodiversity with an increasing number of studies detecting detrimental effects on diversity of plants, arthropods, amphibians, birds, and mammals in temperate (Chace and Walsh 2006; McKinney 2008) and tropical countries (Lim and Sodhi 2004; Biamonte et al. 2011; Ortega-Álvarez and MacGregor-Fors 2011). For birds, highly urbanised places generally exhibit a high abundance of some groups, but an overall poor diversity, compared with adjacent natural areas (Blair 1996; Biamonte et al. 2011; Faeth et al. 2011), although some studies have identified a higher abundance in sites of intermediate development (Tratalos et al. 2007).

Urban ecology studies have been numerous in recent years (Schochat et al. 2006) but little emphasis has been given to the effects of urbanisation on ecological and

evolutionary processes (Alvey 2006; Chace and Walsh 2006; Christie and Hochuli 2009; Sumoski et al. 2009). However, urbanisation influences ecological interactions (Faeth et al. 2005, 2011; Parson et al. 2006) such as pollination (Neil and Wu 2006) and seed dispersal (Cheptou et al. 2008), as well as competition and predation among vertebrates (Faeth et al. 2011). It might also significantly affect the interaction between parasites and their hosts causing changes in host composition.

Although parasites contribute to biodiversity (Hudson et al. 2006), they are causative factors of distress and vectors of disease for wild communities (Atkinson et al. 2008). Previous reviews have summarised parasite-bird research for natural areas (e.g. Boyd, 1951; Mackerras and Mackerras 1960; Forrester and Spalding 2003), but not identified how such interactions are influenced by anthropogenic land change. Changes in parasite loads in response to urbanisation can influence the prevalence (proportion of infected hosts) and impact of wildlife diseases in urban areas (Bradley and Altizer 2006). Changes might be influenced by a range of features in the urban environment including the increased presence of exotic species (Boal et al. 1998), poorer quality of habitat (Patz et al. 2004), potential transference from domestic and exotic animals (Lehrer et al. 2010) and climate change, for example through warmer conditions (Cumming and Van Vuuren 2006) associated with urban heat islands (Alekseev 2006). Thus, identifying parasites which are potentially affected by urbanisation is important in understanding urban biodiversity.

Changes in host-parasite interactions have the potential to influence which species are capable of inhabiting urban areas and the susceptibility of different species to parasites is likely to influence survival and presence in cities. Despite this, our understanding of

these interactions as a process structuring urban wildlife communities appears limited. In this review, I gather the current knowledge about the relationship between ecto-, blood and gastro-intestinal parasites and one particular host taxon; birds. I base my review on birds as hosts since they are the vertebrate group best studied in cities around the world, are ubiquitous, have been used to explore effects and responses to urban development, and exhibit a complex community system in cities. They are used as bio-indicators of environmental quality in cities (Chace and Walsh 2006; Ortega-Álvarez and MacGregor-Fors 2011) and as sentinels for diseases and pathogens (Ludwig et al. 2010).

Birds are host to a variety of parasites which exhibit a diverse array of form and life history, and where obligate and non-obligate taxa are recognised as vectors of pathogens and diseases (Atkinson et al. 2008) which can be transmitted to humans (Epstein et al. 2007). Avian blood parasites and ectoparasites, but also others protozoa, bacteria, viruses, cestodes and nematodes may play important roles in sexual selection and reproductive success in birds (McCallum and Dobson 1995; Atkinson et al. 2008). Therefore, parasitism could contribute greatly to the structure, diversity, conservation, function, and health of urban biodiversity.

In this review I seek to answer three specific questions about the relationship between parasites and urban birds:

1. What parasite fauna has been recorded on urban birds?

2. Are parasites, in terms of their intensity, diversity and prevalence, a relevant factor

influencing the establishment and success of birds in cities?

3. How is health and body condition of urban birds related to their parasite infestation?

Throughout the review I identify future areas of research and emerging questions based on responses obtained for these specific questions in order to better understand how urbanisation as a form of extreme disturbance, biodiversity homogenisation and deforestation (although the level of urbanisation and associated characteristics of a particular region or country might differ in intensity and extension, Gaston 2010), affects bird-parasite interactions.

Since parasites are also associated with transmission of infectious pathogens to humans and other fauna (Haag-Wackernagle 2005; Keesing et al. 2010; Reye et al. 2010; Sehgal 2010), my review could also have implications for wildlife management and public health in urban areas where, for example, exotic species, such as rock doves, come into closer contact with humans (Bart et al. 2008) or where wildlife may be a source of disease for domestic animals (Lehrer et al. 2010). In this sense, comparisons of bird/host interactions in natural and urbanised areas can detect human risks (Daszak et al. 2000) as well as detecting changes in this fundamental ecological interaction.

1.2. Literature Review

1.2.1. Methods

An intensive literature search was performed in the databases PubMed, Web of Science,

Biological Abstracts, Summon and Google Scholar from February to October 2011. A multi-keywords field search (in English and correspondent Spanish translations) included the following terms: "parasite*", "pathogen*", "disease*", "bird*" and "urban*, always using AND as a connector, and * as a proper truncation sign on each database. After this general search, a more specific one was performed adding specific parasites known to be associated with birds in wild areas such as "haemoparasite*" or "ectoparasite*". I also screened articles referenced by the initial identified publications in order to expand our literature. Research carried out on pets, poultry, seabirds (which have a recent review of their parasite fauna, Quillfeldt et al. 2011), or any captive manipulative study or laboratory trial in zoos or animal facilities were excluded from analysis although they were carried out within urban centres. I did not include brood parasitism by vertebrates in our review. Although this is a bird-related parasitic interaction which is widely recognised to be altered by anthropogenic factors, including urbanisation (Schmidt and Whelan, 1999), I omitted this particular mode of vertebrate-vertebrate interaction.

1.3. Results

My search gathered 73 papers dealing with issues related with one or more search topics in urban areas.

According to my questions, I categorised papers into three groups: 1. *Descriptive Studies* (Table 1.1), which include research that recognises and cites the occurrence of bird parasites in urban areas; 2. *Spatial patterns* (Table 1.2), which includes research that compares parasite parameters such as prevalence and diversity of parasites in urban

with adjacent rural or natural areas; and 3. *Parasite Effects* (Table 1.3), which summarises multi-approach studies that seek to determine correlations between parasite load and the condition or health status of their urban bird host.

Descriptive Studies were the most common (Figure 1.1). There were no Spatial Pattern studies from Oceania, no Parasite Effect studies from either Asia or Oceania and no urban bird-parasite research published for Africa (Figure 1.1).

1.3.1. Urban areas, parasites and bird hosts

Information was recovered for 24 countries, and about 60 cities (Table 1.1). The parasite fauna is better known for American and European urban birds (Table 1.1), however, data are limited and restricted to few bird hosts from cities located in a limited geographic region. For example, Brazilian (9 studies) and US cities (17 studies) are centres for research within the Americas. The tropics were less well known (12 studies) than the temperate regions (42 studies) (Table 1.1).

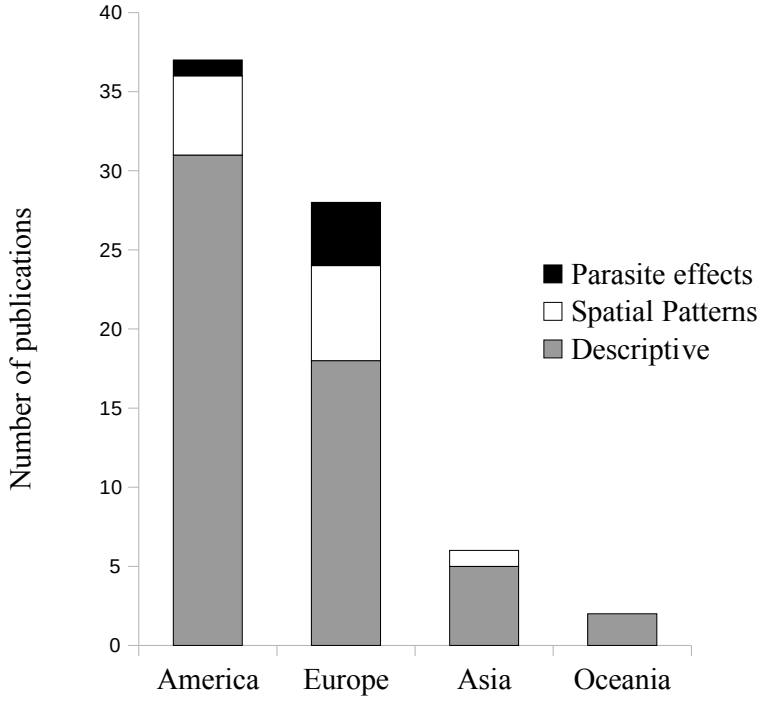

Figure 1.1 Number of publications and main type of research carried out in four continents on parasite-bird interactions in urban areas. No urban-parasite research was published for Africa.

Efforts to identify and develop inventories for haemoparasites and ectoparasites have received more attention than other types of parasites, but there is a growing interest in avian bacteria and viruses (Table 1.1) due to the potential of human transmissions (Bradley et al. 2008). Despite this, knowledge is still rudimentary for all groups. For example, *Ixodes* ticks have been studied more than other arthropod groups (Bardach 1981; Gregoire et al. 2002; Arzúa et al. 2003) probably because they can be detected easily by visual examination (Gregoire et al. 2002), but new descriptions and biogeographic extensions of bird parasites still take place (e.g. Barros-Battesi et al. 2003), suggesting the field is in its infancy.

Inadequate baseline information on parasites in urban areas will hamper our understanding of patterns of host infestation and the role of parasites in cities. Few bird species have been studied and only in a few urban areas (Arzúa and Barros-Battesti 1999). Most studies have focused on well-adapted or introduced species (categorised as urban adapters and exploiters, Kark et al. 2007), such as rock doves (Marques et al. 2007) probably because they are ubiquitous, abundant and generally associated with zoonotic diseases (Zarrin et al. 2010). Very little data have been obtained for whole communities or for species that are more sensitive to urbanisation. The study by Arzúa and Barros-Battesi (1999) is one of the exceptions. They studied parasitism of ticks on birds in a Brazilian city providing new host records. However, for parasites with multiple hosts, few studies have identified secondary hosts and parasite prevalence in urban areas (Jansen et al. 2009).

1.3.2. Spatial patterns along an urbanisation gradient

Comparative data on the prevalence and intensity of infection by ectoparasites, blood parasites and other parasites in urban and natural populations of birds are mostly available for Europe and America (Figure 1.1). These provide insights about patterns of parasitism in cities, and whether they are possible factors influencing urban colonisation by birds. Some studies on other vertebrates in urban centres have been undertaken (Skoracki et al. 2003).

The studies identify two different trends of parasite abundance along gradients from natural and/or rural to more urban developed areas. Some studies detect lower

parasitism in urbanised areas (compared with adjacent rural and natural areas) while others found a greater prevalence and intensity of infection by bird parasites in the more urbanised places (Table 1.2). Results apparently vary for city, host studied and type of parasite considered. For example, while Geue and Partecke (2008) found lower blood parasite prevalence for urban blackbirds in Munich, Germany, Boal et al. (1998) found a higher prevalence of other protozoa (Trichomonas) in urban hawks in Tucson, USA. Differences in parasite prevalence might be explained by the type of parasite studied and their way of transmission. While malaria is a vector-borne disease, Trichomoniasis is transmitted by direct (for example through predation on infected birds which is case for raptor birds) or indirect (through a common use of water resources or feeding stations) contact (Atkinson et al. 2008). Therefore, a general pattern about how parasites are affected by urbanisation was not detectable in my review and identifies a significant research gap.

A further explanation for differences in observed urban patterns is the possibility that some studies may not detect microscopic parasites using blood smears (Braga et al. 2011). For example, while Evans et al. (2009) found a reduced prevalence of blood parasites in 8 out of 11 European cities studied using microscopy as the method of detection, Belo et al. (2011) found higher prevalence in Brazil using microscopy and molecular methods. While microscopy has certainly been used for urban studies and is considered a powerful tool for the study of haemosporidians (Braga et al. 2011), future research should include molecular techniques, to get more comprehensive prevalence and diversity measures for blood parasite in urban areas.

Table 1.1. Summary of the descriptive research about the diversity and occurrence of parasites on urban birds.

Country	City	Parasite Type	Host	Reference
Argentina	Córdoba	Virus	*Furnarius rufus*	Tauro et al. 2009
Argentina	San Juan	Ectoparasites	*Columba livia*	Vallve et al. 1995
Argentina	Corrientes	Ectoparasites	*Columba livia*	Bar et al. 1993
Australia	Gold Coast	Bacteria and virus	*Threskiornis molucca*	Epstein et al. 2007
Australia	Cairns, Brisbane, Newcastle and Sydney	Blood parasites	Various spp	Jansen et al. 2009
Austria	Vienna	Ectoparasites	*Columba livia*	Bardach 1981
Brazil	Lages	Ectoparasites, Blood parasites, and Gastro-intestinal parasites	*Columba livia*	Marques et al. 2007
Brazil	Saõ Paulo	Fungus	*Columba livia*	Montenegro and Paula 2000
Brazil	Curitiba	Ectoparasites	Various spp	Arzúa and Barros-Battesti 1999
Brazil	Serrinha, Mata de São João and Coceição	Toxoplasma	*Passer domesticus*	Gondim et al. 2010
Brazil	Jaboticabal	Bacteria, Virus and Toxoplasma	*Columba livia*	de Sousa et al. 2010
Brazil	Saõ Paulo, Bauru, Soracata, and Botacatu.	Bacteria and Toxoplasma	*Columba livia*	de Lima et al. 2011
Brazil	Pelotas	Gastro-intestinal	*Columbina picui*	Coimbra et al. 2009
Brazil	Porto Alegre	Gastro-intestinal	*Passer domesticus*	Calegaro and Amato 2010
Brazil	Brasília, Jataí and Uberlândia	Blood parasites	*Passer domesticus*	Lima et al. 2010

Canada	Quebec	Virus	*Corvus brachyrhynchos*	Ludwig et al. 2010
Chile	Santiago	Bacteria, Virus, Ectoparasites, Blood parasites, and Gastro-intestinal parasites.	*Columba livia*	Toro et al. 1999
Croatia	Zagreb	Bacteria	*Columba livia*	Prukner-Radovcic et al. 2005
Croatia	Zagreb	Bacteria and Fungi	*Corvus frigilegus*	Gomercic et al. 2010
Czech Republic	Certak	Ectoparasites	Various spp	Dubska et al. 2009
England	Central England	Toxoplasma	*Sturnus vulgaris*	Peach et al. 1989
Spain	Madrid	Bacteria	*Columba livia*	Muñoz et al. 2010
Spain	Murcia	Fungus	*Columba livia*	Haro et al. 2005
Spain	Santa Cruz	Ectoparasites, Blood parasites, and Gastro-intestinal parasites	*Columba livia*	Foronda et al. 2004
France	Besançon	Bacteria	*Corvus frugilegus*	Boutterfroy et al. 1997
Germany	Berlin	Ectoparasites	*Columba livia*	Dautel et al. 1991
Iran	Ahwaz	Fungus	*Columba livia*	Zarrin et al. 2010
Italy	Napoli	Bacteria	*Columba livia*	Santaniello et al. 2007
Italy	Bari	Bacteria	*Columba livia*	Tarsitano et al. 2010
Japan	Hokkaido	Ectoparasites	Various spp	Iwasa et al. 1995
Malayasia	Bangsar	Bacteria and Gastro-intestinal	*Corvus* spp	Yong et al. 2008
Poland	Cracow	Ectoparasites	*Columba livia*	Rosciszewska et al. 2000
Portugal	Lisboa	Toxoplasma	*Columba livia*	Waap et al. 2008
Slovak Republic	Košice	Virus	*Columba livia*	Gronesová et al. 2009

Slovak Republic	Bratislava	Bacteria	*Columba livia*	Rehacek et al. 1984
The Netherlands	Amsterdam	Bacteria	*Columba livia*	Heddema et al. 2006
The Netherlands	Amsterdam	Fungus	*Columba livia*	Bart et al. 2008
USA	Houston	Bacteria and Gastro-intestinal	*Molothrus ater*	Johnson et al. 2004
USA	Illinois	Ectoparasites	*Cyanocitta cristata*	Mannelli et al. 1993
USA	Detroit	Gastro-intestinal	*Quiscalus quiscula*	Seneviratna et al. 1975
USA	Urbana	Bacteria	Various spp	Marrow et al. 2009
USA	Boston	Bacteria	*Branta canadensis*	Fox et al. 2006
USA	Chicago	Virus	Various spp	Loss et al. 2009
USA	Various, especially New York, New Jersey, Connecticut	Virus	Various spp., particularly *Corvus brachyrhynchos*	McLean 2006
USA	Memphis	Virus	*Passer domesticus*	McLean et al. 1983
USA	New Jersey	Toxoplasma	*Tyto alba* and *Columba livia*	Kirkpatrick et al. 1990
USA	New Jersey	Blood parasites	Various spp	Kirkpatrick and Sluthers 1988
USA	New Jersey	Ectoparasites	*Tyto alba*	Kirkpatrick and Colvin 1989
USA	Fort Collins	Bacteria	*Branta canadensis*	Kullas et al. 2002
USA	New Jersey	Bacteria	*Carpodacus mexicanus*	Hartup et al. 2001
USA	Baltimore	Bacteria	*Falco peregrinus*	Dement et al. 1986
USA	Honolulu	Blood parasites	Various spp	Shehata et al. 2001
USA	Milwaukee, Grand Forks and East Grand Forks	Gastro-intestinal	*Accipiter cooperi*	Rosenfield et al. 2009
USA	Fort Collins	Bacteria	*Columba livia*	Pedersen et al. 2006

Table 1.2. Summary of studies investigating spatial patterns of parasite-bird interactions.

Country	City	Parasite type	Host	Factors studied	Summary of Results	References
Brazil	Curitiva	Ectoparasites	Various spp	Prevalence in urban and natural places	A lesser prevalence of infested birds was found in the urban peripheral area than the natural sites in 17 host birds	Arzúa et al. 2003
Brazil	Palmas	Blood parasites	Various spp	Prevalence and diversity in urban and natural areas	Diversity and prevalence higher in the urban area	Belo et al. 2011
France	Dijon	Ectoparasites	*Turdus merula*	Prevalence and intensity urban and rural	Tick infestation significantly lower in urban areas	Gregoire et al. 2002
Germany	Munich	Blood parasites	*Turdus merula*	Prevalence urban and rural	Lower parasite prevalence in urban areas	Geue and Partecke 2008
Germany	Berlin	Trichomonas	*Accipiter gentilis*	Trichomonas infectation	High infestation in urban areas	Krone et al. 2005
Germany	Bonn	Bacteria and Ectoparasites	No name provided	Prevalence along an urbanisation gradient	Generally, suburban and urban sites with dense tree populations exhibited higher prevalence	Maetzel et al. 2005
Japan	Dijon	Bacteria	*Turdus merula*	Prevalence urban and rural	Weaker parasitism in urban area	Bentz et al. 2006

Luxembourg	Luxembourg	Ectoparasites	No name provided	Prevalence along an urbanisation gradient	Positive correlation between the grade of urbanisation and infection of ticks,	Reye et al. 2010
Paleartic region	33 countries	Blood parasites	Various spp.	Richness	Heterogeneously bird species had higher diversity	Møller et al. 2010
USA	Tucson	Trichomonas	*Accipiter cooperi*	Prevalence urban and exurban	Prevalence of *T. gallinae* was significantly greater in urban than natural nestling	Boal et al. 1998
USA	Atlanta	Virus	Various spp., especially *Cardinalis cardinalis*	Prevalence along an urbanisation gradient	Prevalence increased from rural to urban sites	Bradley et al. 2008
USA	Tucson and Weslaco	Ectoparasites	*Zenaida macrura* and *Columbina inca*	Abundance comparation in two urban areas with arid and humid environments	Birds in arid regions have fewer lice than birds in humid region	Moyer et al. 2002
Western paleartic region (Tunisia, Spain, The Netherlands, UK, Germany, Poland, Latvia, Czech Republic and Estonia)	Tunis, Valencia, Madrid, Groningen, Sheffield, Berlin, Szczecin, Prague, Krakow, Riga, Tallinn	Ectoparasites and Blood parasites	*Turdus merula*	Prevalence and intensity in 11 paired urban and rural populations	Reductions in tick prevalence and intensity in urban areas. There are also large reductions in the prevalence of avian malaria in many, but not all, urban areas	Evans et al. 2009

Table 1.3. Summary of studies on parasite effects on birds in urban centres.

Country	City	Parasite type	Host	Factors studied	Summary of Results	References
Estonia	Tartu	Blood parasites	*Parus major*	Health impact	Infected individuals were heavier than uninfected ones in the urban area	Ots and Hõrak 1998
Estonia	Tartu	Blood parasites	*Parus major*	Coloration and parasite prevalence	Plumage coloration generally higher in rural birds. Reduction of coloration of urban birds could be the results of a greater exposure to infectious diseases	Hõrak 2001
France	Paris	Blood parasites	*Columba livia*	Immunity, parasite intensity and melanin-based coloration	Darker individuals had lower parasite intensity and greater immune response	Jacquin et al. 2011
Japan	Gifu	See Summary of Results	*Corvus macrorhynchos* and *Corvus corone*	Evaluation of pathological conditions	It is not clear what parasite caused pathological issues	Hirata et al. 2010
Spain	Barcelona	Blood parasites	*Columba livia*	Age-related differences in parasitism in urban areas	Higher intensity in young birds	Sol et al. 2003
USA	Phoenix	Blood parasites	*Pipilio aberti, Mimus polyglottos*, *Pipilo fuscus*, *Toxostoma curvirostre*, and *Passer domesticus*	Comparison between rural and urban populations for blood parasite infection, body condition (stored energy reserves), and immunity	In general, urban birds had less blood parasitism. However, some birds exhibited higher immune value associated with probable stress or infection in urban areas	Fokidis et al. 2008

Improved rigour in methodology would facilitate measurements of other parasite groups as well. For example, visual examination is the predominant method for determining arthropod parasites, however other sensitive techniques, such as dusting, for collection and quantification are likely to improve the accuracy of prevalence estimates (Clayton and Walther 1997; see Chapter 2).

In contrast to the growing interest in studying factors that can influence the risk of parasitism inherent to the host, such as sex, age, and reproductive status, the number of publications about the habitat-specific factors that promote parasitism is comparatively small (Merilä et al. 1995; Kleindorfer et al. 2006), particularly for urban areas. One exception is the research carried out by Evans et al. (2009) who studied 11 paired urban and rural European populations of one of the most urbanised birds, *Turdus merula*. Although they found a notable reduction of ticks in urban areas (with no ticks detected for 5 cities), the consistent and evident reduction was less conspicuous for blood parasites (Table 1.2). We need new studies carried out in other regions, for example in the Neotropics where urbanisation is chaotic (Biamonte et al. 2011) or in Australia where cities are more recent than in Europe.

Theoretical hypotheses about epidemiology suggest a positive relationship between abundance and/or prevalence of parasites and host density in some areas (e.g. Morand and Poulin 1998). Moreover, recent predictions suggest an increase of certain parasites (e.g ticks, Kleindorfer et al. 2006) and pathogens in new environments as a consequence of climate change (Cumming and Van Vuuren 2006), thus bringing together the possibility of emerging diseases (Reye et al. 2010). Australia, Latin America and many Oceanic islands are bioregions of major concern in this regard (Cumming and Van

Vuuren 2006). Bradley et al. (2008) found that antibody prevalence of the West Nile Virus on birds increased from rural to urban sites. Similarly, the prevalence of *Trichomonas* has expanded which has been associated with decline of some birds (Lehikoinen et al. 2011). Supporting evidence has been published for some other vertebrates (Conner and Miller 2005).

Urbanisation can cause loss of host species diversity and an increase in the relative abundance of particular host species (McKinney 2008; Biamonte et al. 2011; Faeth et al. 2011). In cities, denser populations of some host species associated with overall low host diversity might mean that some host species are more susceptible to declines as a result of parasite infestation (Reisen et al. 2006; Koenig et al. 2010). Additionally, a number of studies documenting the effects of biodiversity in exposure, risk and transmission of diseases in ecological communities has found that a loss of host species diversity could change the infection rate between different hosts (Keesing et al. 2006). However, since the patterns of diversity and abundance of hosts could vary between cities, generalisation about theories of disease risk and parasite exposure in urban areas and the presence of urban bird species might be difficult. For example parasite loads on a particular host are likely to be different in cities where they are abundant but associated with a poor alternative host diversity, compared to loads in cities with a lower population density of the particular host or in a city with a high diversity of other host species (Pongsiri et al. 2009; Keesing et al. 2010). This suggests that it might be difficult to discriminate between the effects of density and diversity. Therefore, to identify mechanisms involved in bird-parasite dynamics, studies should consider not just community structure and diversity of hosts, but also parasite dependent factors such as frequency of vectors, parasite identity, parasite parameters (e.g. prevalence,

intensity), parasite stages, potential interactions among different parasites, and specific traits (e.g. vector-borne or non vector-borne transmission).

Since bird hosts occurring in urban areas are interacting directly or indirectly with other individuals or species, and potential vectors, which can potentially affect parasite transmission and disease risk, cities with low diversity but a high abundance of urban species could experience an increase in diseases and pathogen prevalence due to the Dilution Effect (Bradley and Altizer 2006), a phenomenon which explains why disease risk varies inversely with host diversity (Keesing et al. 2006). In brief, the Dilution Effect describes a mechanism of increased disease risk and parasite incidence when host diversity is reduced. Thus when urban diversity is lower, transmission of diseases might be mediated by more frequent contact between hosts and infected vectors as a result of high density, followed by transmission to other susceptible hosts (as a result of increased contact due to high density), and a slow recovery from infection (Swaddle and Calos 2008). Similarly, avian communal roost sites and perches (Harkinezhad et al. 2009), as well as human food sources (Jones and Reynolds 2008) could be implicated in the transmission of parasites and diseases (Ward et al. 2006; Diuk-Wasser et al. 2010). Other hypotheses, such as the Enemy Release Hypothesis (success of exotic species due to elimination of parasites from their original habitat), the Novel Weapon Hypothesis (susceptibility of native species due to exotic urban colonisers that bring new pathogens) (Marzal et al. 2011) and Amplification Effects (where biodiversity and disease risk are directly related) (Keesing et al. 2010) are valid frameworks which should be considered but remain to be studied in order to interpret how urban environments can affect parasite dynamics. As a result, we have very little understanding of whether parasites in cities could be an important constraint influencing

the species that can exist in these habitats.

While some bird species survive and prosper in urbanised areas, others are directly or indirectly affected by a wide range of anthropogenic activities and infrastructure (see Evans et al. 2011). Birds in urban areas are exposed to an increase of some kinds of food and water, predation, competitive interactions, warmer temperatures and different types of pollution such as noise, artificial lighting and toxins (Chace and Walsh 2006). Although some of these factors are potentially contributing to the inclusion or exclusion of birds in cities, they do not explain fully the presence, establishment and success of some native species that thrive in urban environments. It implies that other factors that are more cryptic might also be important.

Globally, I have found few studies to help elucidate the role of parasites in urban areas and the mechanisms that could influence the bird-parasite relationship. Although a reduction of parasite infestation was found as a potential factor favouring the presence of urban birds (Geue and Partecke 2008), species which are rare in urban environments might be more susceptible. Thus tolerant and sensitive urban birds might exhibit different susceptibility to parasite load thus influencing their ability to occupy urban centres. However, few studies have tested the relative importance of parasites and susceptibility in determining a species presence in urban areas (Geue and Partecke 2008). More comparative surveys (Evans et al. 2009; Evans 2010) using different parasite parameters between tolerant and urban sensitive birds and their conspecifics in adjacent natural areas will help to understand the dynamics of parasites and disease risk in urban centres.

The higher prevalence of diseases and parasites of birds recorded in some studies might be partially a result of a spillover from domestic and exotic animals (Lehrer et al. 2010). Moreover, high population density of urban-adapted hosts and urban colonisation by exotic species could influence contact rates with, and among, host species and vectors and might favour the transmission of parasites (Keesing et al. 2006). Some are recognised as sources of potential diseases in humans (Polley 2005; Tsiodras et al. 2008), such as the West Nile Virus (Bradley et al. 2008) and Lyme disease (Maetzel et al. 2005). Additionally, pollutants deposited in urban areas could cause depressed immune systems increasing the impact of parasites (Combes 2001).

As migratory birds serve as reservoirs and/or mechanical vectors for numerous parasites, migratory species may play a significant role in the dispersion of pathogens (Hubálek 2004; Alekseev 2006; Atkinson 2008; Tsiodras et al. 2008; Sehgal 2010) as has been recently confirmed for *Trichomonas* (Lawson et al. 2011). MacGregor-Fors et al. (2010) found that urban areas could offer winter habitat for some Neotropical migrant birds. If increasing urbanisation results in an increase in usage of cities by migratory birds, it could potentially cause a spread of parasites in cities and between natural and urban areas. Monitoring will tell more about the emergence of this potential issue.

While factors such as increased presence of exotic species or poorer quality of habitat (Reye et al. 2010) might suggest an increase in parasitism in urban areas (Table 1.2), the situation is not conclusive with a range of studies showing lesser prevalence; for example, in Palaearctic (Evans et al. 2009) and Asian (Bentz et al. 2006) cities. Urban environmental conditions which alter diversity and abundance of vectors, some of

which could be essential for the transmission of parasites, could cause lower parasite abundance in urban areas (Bradley and Altizer 2006). The lack of vegetation in urban areas, as well as pesticide applications might inhibit the development of some ticks as suggested (but not tested) by Evans et al. (2009) (see also Evans 2010). Further, other authors have suggested that an increase in food availability probably helps urban birds through increased immunity (Fokidis et al. 2008).

The role of other factors, such as warmer microclimates apparently has differential effects on the parasite prevalence of bird parasites. For example, the urban heat island effect could increase the desiccation of ticks in urban areas (Evans et al. 2009), however, Alekseev (2006) suggested this could promote parasitism in cities. Warmer microclimates could make mosquitoes feed and reproduce faster (Githeko et al. 2000).

Parasites that persist in urban areas are apparently less known than those present in rural or natural areas (Mackerras and Mackerras 1960; McFarland 1986; Rojas et al. 1999; Scheuerlein and Ricklefs 2004; Movila et al. 2008; Averis et al. 2009; Ogrzewalska et al. 2010). While, Chasar et al. (2009) explored the effect of degraded rainforest habitats on blood parasite diversity in African passerines, I found no study for passerines in urban areas in the African continent and very little information is available around the world. Additionally, studies on other parasites are few. Bacteria-bird interaction studies in urban areas are scarce and mainly focused in detecting negative effect groups (de Sousa et al. 2010; de Lima et al 2011). Bacteria, however, can also be beneficial for the host (Moreno et al. 2003).

1.3.3. Parasite Effects on Host health

Even fewer studies investigated health and body condition in urban birds relative to parasite load. Papers were mostly published in Europe (Figure 1.1). Immunological and pathological conditions were used as health measures to detect responses potentially caused by blood parasite infestation (Table 1.3). For example, Fokidis et al. (2008) focused on blood parasites in Phoenix (USA), and showed how haemosporidians, body condition, and immunity covary in an urban versus rural setting in five passerines (Table 3). I found no paper focused on the effects of any other parasite types on health in urban areas.

If bird-parasite interactions are important in structuring avian communities in cities, then evidence is needed for urban sensitive species in conjunction with data for urban tolerant species. Future research should test if tolerant birds have less parasite infestation and better (or similar) health and body condition in cities than in natural areas, and if sensitive birds exhibit higher infestation by parasites in urban areas than natural areas which would also impact their health and body condition. Differences in parasite susceptibility may be related to different life history strategies (Atkinson et al. 2008) and might explain why birds with particular traits are more common in cities. For example, Jacquin et al. (2011) found that darker urban pigeons had lower levels of a blood parasite and their immune systems responded faster to infection. Møller et al. (2010) showed that heterogeneously distributed bird species rarely colonised habitats as well as homogeneously distributed birds and that they were host to a greater diversity of blood parasites. Thus comparisons in parasite load between common and uncommon urban species should also be combined with comparisons amongst species with different

life history traits. Parasite load should also be linked with measures of health and stress in birds since parasites that do not influence fitness are unlikely to be factors in determining whether birds live in urban areas. However, an integrative approach among parasitism, immunology and physiology remains to be developed in urban areas.

Further, in Australia and the Neotropics, the bird fauna are derived from different evolutionary histories with many, in Australia, showing unusual cooperative breeding life histories which may well influence infestation (Oorebeek and Kleindorfer 2009). Geue and Partecke (2008) suggested that the lower parasite prevalence could be one of the factors favouring the invasion of certain birds into urban ecosystems but studies are needed to help elucidate the role of parasites as a potential factor to maintain and regulate urban diversity (Hamer and McDonnell 2008). Finally, it will also be useful to understand the emergence and ecology of human and wildlife diseases as urban areas are where domestic animals and humans interact closely with wildlife (Bradley and Altizer 2006).

Factors of stress such as noise and pollution, impact on the health of some vertebrates, including birds (e.g. Eens et al. 1999; Burger et al. 2004; Partecke et al. 2006; French et al. 2008). Such stresses may increase the likelihood of parasite infestations, affecting the capacity of hosts to occupy cities. In contrast, animals exhibit a variety of documented mechanisms to combat ectoparasites, including morphological, behavioural and immune responses (Boughton et al. 2011). Immune responses of animals in cities might explain the low prevalence of parasites in cities (Geue and Partecke 2008) for example in adult birds (Sol et al. 2003), and although self grooming is considered the first line of defence (Hart 2000), grooming and immunological competence has not been addressed in the

urban context. Additionally, although there are a wide number of possible techniques available for immuno-ecology (Boughton et al. 2011), most studies have only used leukocyte profiles from microscopic slides (Davis et al. 2008). A range of other assays could complement our understanding of the link between stress, health and parasite loads.

Host immunity is also dependent on feeding habits (Sorci and Cornet 2010). In cities, for example, it is thought that higher immunity may be caused by a high abundance of food (Gregoire et al. 2002). Native parasites might have greater impact on introduced host species due to absence of appropriate immune response and/or resistance mechanisms (Dobson and May 1986). Similarly foreign parasites associated with exotic host species might spread to native hosts for the same reason (Freed et al. 2008). Therefore, an integrative approach should explore food resources and parasite availability in cities and the effects on immune responses.

1.4. Conclusions

Parasite interactions have the potential to determine the distribution of their hosts, particularly where hosts are stressed on in poor quality environments. With cities growing globally, and urban diversity becoming important in the conservation of biodiversity, it is important to understand the role that parasites might have in limiting urban biodiversity. My review revealed a very large research gap in this area. In order to improve our understanding of host/parasite interactions in anthropogenic landscapes, a better understanding of the types of parasites, the mechanisms that influence their prevalence and their impact is needed.

CHAPTER TWO: EVALUATION OF A COMMERCIAL INSECTICIDE POWDER FOR QUANTIFICATION OF BIRD ECTOPARASITES IN ECOLOGICAL STUDIES

2.1. Introduction

Parasites are a dominant and diverse group which is fundamental in influencing ecological and evolutionary processes. Certain parasitic life strategies may allow the maintenance of ecological processes and will be critical to the conservation of fully functioning ecosystems in natural environments. For example, parasites can maintain species coexistence across space and time as they can have a detrimental effect on host fitness (Arostein et al. 2012). However, chronic alterations in parasite prevalence and introduction of parasites and pathogens to new environments might cause significant changes in vertebrate host populations and change ecological processes as has been recorded in remote islands (Samuel et al. 2011). Therefore, parasitism could contribute greatly to the structure, diversity, conservation, function and health of biodiversity.

In new environments, it is predicted that there will be an increase of certain parasites and pathogens which can increase the possibility of emerging diseases not experienced in natural undisturbed areas (Reye et al. 2010). Australia, Latin America and many Oceanic islands are regions of major concern (Cumming and Van Vuuren 2006) due to changes in climatic conditions, habitat loss, fragmentation and a greater presence of human-modified landscapes. As a result, identifying and monitoring parasites is important to understand if the risk of parasitism and wildlife diseases caused by

ectoparasites increases due to human activity (Bradley and Altizer 2006).

A large diversity of arthropod ectoparasites has been recorded on birds (Clayton et al. 2010). They have detrimental effects on growth, fitness and survival of their bird hosts (Moyer and Clayton 2004) and they could be vectors of a large variety of pathogens, which include viruses, bacteria and protozoa (Atkinson et al. 2008).

Ectoparasites such as lice (Phthiraptera), fleas (Siphonaptera), bugs (Hemiptera), flies (Diptera), and mites and ticks (Acari) are known to occur on birds but data about their prevalence and their specific detrimental effects on hosts are still scarce. However, information of this type is especially important in tropical regions where parasite pressure is predicted to be greater (Poiani 1993; Cumming and Vuuren 2006) due to drastic environmental changes associated with urbanisation, habitat conversion, increased temperatures and fragmentation (McCallum and Dobson 1995; Daszak et al. 2000; Sutherst 2001; Bradley and Altizer 2006; Cumming and Vuuren 2006).

The scarcity of information on bird-ectoparasite interactions is partially compounded by the lack of powerful and comparable detection methods. Four ectoparasite collection methods on live birds are available in the literature (Clayton and Walther 1997): visual examination, anesthesia, trapping and dust-ruffling. For trapping, birds are restrained in cages and ectoparasites are sampled by placing the host in a cage over a pan of water into which the parasites can be collected, anesthesia using chloroform vapours (Poiani 1992) in fumigation chambers allow the bird to be examined once unconscious, although a safer design is to anaesthetise parasites of conscious birds using modified jars that do not expose the bird heads to the fumes. However, these chemicals are still

dangerous for researchers and birds (Clayton and Drown 2001). As a result, many bird-ectoparasite assessments have used visual examination on caught birds without restraint or anesthetic (McFarland 1986; Gregoire et al. 2002; Kleindorfer et al. 2006; Carrillo et al. 2007; Dubska et al. 2009; Evans et al. 2009; Oorebeek and Kleindorfer 2009). This technique usually samples a particular region of the body and there is an increased inaccuracy as observers use no magnification tool. While there is good comparative strength within each study, the ability to compare across studies will be hampered if different areas of the body are searched. Furthermore, overall assessments of prevalence may be badly underestimated, preventing broader generalizations being made. Therefore, it has been suggested that other methods for quantification might offer a higher degree of accuracy in some parameters (Delgado-V. and French 2012) such as richness (number of taxa present), prevalence (proportion of infested birds in a population), and mean intensity (number of individuals of a particular parasite group in a sample of a host species divided by the number of infected individuals of the host species in the sample) (Clayton and Moore 1997).

Dust-ruffling with an insecticide represents a simple method that may improve estimates of parasite loads. Although, there are few field empirical data that quantitatively compare dust ruffling to visual inspection, studies have mostly been undertaken on a reduced number of species (e.g. the rock dove and the house sparrow) in temperate zones (Walther and Clayton 1997a, b) and/or for the screening of a particular group of arthropods (i.e. lice) on captive individuals (Clayton and Drown 2001).

Dust-ruffling methods have normally used products such as Pyrethrum (pyrethrin) and Dri-Die (silicon dioxide) (Southwood and Henderson 2000) but there is no

documentation about the efficiency of other commercial products. Since Pyrethrum is not 100% effective and Dri-die has lethal side-effects on birds (Southwood & Henderson 2000), an improved rigour in methodology using new commercial resources might facilitate measurements of ectoparasite quantification.

Pestene is an insect powder (Composition: Sulphur: 50 g/kg, Rotenone: 10 g/kg; Inca Co. Pty Ltd, St Mary's, Sydney, NSW, Australia) that is considered harmless for birds. It is commonly used in veterinary treatment and is becoming popular for control of lice, mites and fleas of domestic and laboratory animals (see Courtney Jones et al. 2012) but it has never been tested in ecological field studies. I compared dust-ruffing using Pestene with the visual examination method for detection and quantification of ectoparasites on some Australian small passerines in order to determine if Pestene might be chosen as a quick, accurate and suitable methodological approach for ecological parasite studies.

2.2. Methods

Bird capture was undertaken at University of Wollongong, Wollongong, New South Wales (34˚ 24' 17" S 150˚52' 15" E). Birds were mistnetted from February until May 2012. Once removed from the mist net, birds were individually transported in a clean cotton bag to the processing station where the ectoparasite methods were performed. Each bird was released at the capture site immediately after dust-ruffling processing (see below) and not resampled if caught again.

I conducted a visual inspection for any kind of ectoparasite arthropod by searching

head, throat, neck, under wings, around eyes, legs and belly for 3 minutes per bird by gently blowing feathers away. Additionally, I examined the right wing against a bright background in order to count parasites which are known to be between the barbs of the flight feathers of the wings and tail (e.g. feather mites). Parasites identified through visual examination were counted but not removed.

Immediately after the visual test, birds were dust-ruffled following Walther and Clayton (1997) and Poiani et al. (2000) with some variations. I dusted each bird with ca. 2 grams of Pestene powder on rump, tail, wings, back and belly. The bird was held over a pan with a clear piece of paper as the collecting surface. Following dusting, the powder was distributed homogeneously all over the body for about 1 min through gentle rubbing with fingertips. The procedure was never done near face in order to avoid any potential irritating effect in the bird's eyes, however the use of Pestene is considered harmless for birds.

Birds were placed back in the cotton bag or in a paper bag and held for 10 min to allow the powder to kill parasites. Bags were big enough to allow preening and stretching out.

Afterwards, feathers all over the body were ruffled and ectoparasites were collected on the paper underneath. Ruffling was carried out for 1 min per bird. The collecting paper and holding bag were deposited in a clean ziplock plastic bag and assessed on the same day in the laboratory. Using a stereoscopic microscope (under 10x and 40x), ectoparasites were counted and collected from both the bag and the paper. Parasites were identified as ticks (Acari: Ixodida), lice (Insecta: Phthiraptera), feather mites (Acari: Astigmata) or louse flies (Diptera: Hippoboscidae). Numbers of parasites found

by each method were compared using a paired t test. Prevalence was calculated as the number of birds caught with parasites while intensity was the number of individuals of a particular parasite group in a sample of a host divided by the number of infected individuals in the sample. Work was undertaken under UOW Ethics Number: AE11/20.

2.3. Results

Seventy-two individuals of 13 bird species were captured and sampled for ectoparasites using both methods: Red-browed finch [n = 32, *Neochima temporalis*], Superb-fairy-wren [n = 17 *Malurus cyaneus*], Silvereye [n = 6, *Zosterops lateralis*], Eastern yellow robin [n = 4, *Eopsaltria australis*], White-browed scrubwren [n = 4, *Sericornis frontalis*], Grey fantail [n = 3, *Rhipidura fuliginosa*], Golden whistler [n = 3, *Pachycephala pectoralis*], Eastern spinebill [n = 1, *Acanthorhynchus tenuirostris*], Lewin's honeyeater [n =1, *Meliphaga lewinii*], Large-billed scrubwren [n = 1, *Sericornis magnirostra*], Yellow-throated scrubwren [n = 1, *Sericornis citreogularis*], Brown thornbill [n = 1, *Acanthiza pusilla*], and Bassian thrush [n = 1, *Zoothera lunulata*]). A total of 704 ectoparasites were counted by visual examination and 1718 by dust-ruffling (Table 2.1).

Ectoparasites were mostly feather mites on all species (691 [Mean: 9.47 ± SD: 11.81, Range: 0-47] by visual and 1521 [20.84 ± 20.91, 0-99] by dust-ruffling) (Table 2.1). Lice were recorded on seven species but not on Eastern yellow robin, Eastern spinebill, Lewin's honeyeater, Yellow-throated scrubwren, and Brown thornbill. Only 8.6% of lice were visually detected in advance (12 [0.4 ± 1.52, 0-8]) compared to 140 [4.67 ± 5.55, 0-27] recovered by the dust-ruffling method. Ticks (54 [3 ± 4.96, 0-23]) were only recovered using dust-ruffling on 6 species and were not found on Silvereye, Grey

fantail, Golden whistler, Large-billed scrubwren, Lewin's honeyeater, Brown thornbill, and Yellow-throated scrubwren. Four louse flies detected during the study were counted by visual examination on Superb-fairy wren, Red-browed finch and Eastern yellow robin, but one more was recovered by the use of Pestene dust-ruffling on the only individual of Bassian thrush.

Results show that the dust-ruffling method with Pestene is significantly more sensitive than the visual examination in recovering a greater number of feather mites and lice (paired t-test mites; $t_{60} = -5.49$, $p < 0.001$, lice; $t_{29} = -5.30$, $p < 0.001$) (Table 2.1).

The proportion of infected birds (prevalence) varied between the methods. Furthermore, the estimates of infestation levels (intensity) also differed. Dust-ruffling was notably more sensitive for counting ectoparasites, identifying a greater proportion of the population that are infested by mites, lice and ticks and discerning a higher number of parasites per bird (Table 2.2). Contrary, louse fly counting was better through visual examination.

Table 2.1. Mean ± SD, range and total numbers of feather mites, lice, ticks and louse flies recovered from 72 passerines using visual examination and Pestene dust-ruffling methods.

	Visual examination	**Pestene dust-ruffling**
Mites	9.59 ± 11.81, 0-47, 691	21.12 ± 20.91, 0-99, 1521
Lice	0.16 ± 1.52, 0-8, 12	1.94 ± 5.55, 0-27, 140
Ticks	0	0.75 ± 4.96, 0-23, 54
Flies	0.05 ± 0.71, 0-2, 4	0.01 ± 0.43, 0-1, 1

Table 2.2. Differences in prevalence and mean intensity of mites, lice, flies and ticks from a sample of 72 birds using visual examination and the Pestene dust-ruffling.

	Prevalence	Mean intensity
Visual examination		
Mites	72.2%	13.3
Lice	4.2%	4
Flies	4.2%	1.3
Ticks	0%	0
Pestene dust-ruffling		
Mites	90%	25.8
Lice	41.6%	4.7
Flies	1.4%	1
Ticks	25%	3

2.4. Discussion

I recorded a clear disparity between the methods with dust-ruffling recovering more parasites. My results suggest that some previous research using the visual examination method (e.g. Gregoire et al. 2002; Evans et al. 2009) might have underestimated parasite loads. Accurate estimates of parasite loads are needed especially when relating parasites to the health and condition of birds and to understand declines (Ford et al. 2001; Valera et al. 2006; Delgado-V. and French 2012).

While visual examination is thought to be sensitive for the detection of ticks and lice, in our case it failed in detecting all ticks and only recorded a reduced fraction of lice that were recorded using Pestene. Visual examination has been employed for detection and counting of lice (Carrillo et al. 2007), however this technique has some limitation in

birds with dense plumage and small bodied lice (Clayton and Walther 1997), a problem I encountered. Visual examination works well with big and permanent ectoparasites such as Ixodid hard ticks which are easy to see and count since they are normally imbedded on the host skin (Clayton and Walther 1997), particularly in naked areas. However, tick quantification is difficult when they are small and immersed in dense plumage, especially on small passerines.

Contrary to more permanent ectoparasites, visual examination should be employed for the collection of louse flies because they leave the host quickly after capture, suggesting that examination for these should occur as soon as the bird is mistnetted (Poiani 1992; Clayton and Moore 1997). However, caution should be exhibited when processing bigger birds by visual examination since small flies could be hidden on the plumage. In our case, one fly was undetected visually in a Bassian thrush remaining hidden until the dust-ruffling was conducted.

Based on my trial, I would suggest that Pestene could be used for ectoparasite studies to estimate levels of infestation. Additionally, intense visual searches for ectoparasites can cause eyestrain and headaches (Clayton and Moore 1997), and might be prone to error in detection when bird sampling is numerous and taken from small-sized birds. Despite the fact the visual examination could take less time compared on the dust-ruffling, to rely exclusively on the former method might undersample an important number and diversity of ectoparasites. However, since processing a bird that is caught often includes a visual exercise of measuring and evaluating condition for each bird, visual searches of ectoparasites can be partially done at the same time. This could help detect other ectoparasites that quickly abandon the captured hosts (such as fleas and louse flies) or

other static parasites which are deeply attached to the skin of the host (Clayton and Walther 1997).

Estimates of feather mites might have been more similar between methods in the event that the visual screening had been carried out on both wings and tail (Clayton and Moore 1997; Clayton and Drown 2001). However, the time spent in detecting and counting mites along all these parts could be similar to the time spent dust-ruffling with Pestene, which might recover other ectoparasites.

2.5. Conclusions

Based in this brief trial, Pestene has a number of advantages that merits its use. It is a sensitive, practical, easy to perform, low cost technique for sampling a wide variety of ectoparasites. It is a quick method to quantify and provides a realistic estimate of prevalence and intensity. Its use is a valuable tool to explore the occurrence of ectoparasites on birds as well as the potential role of parasites in determining the structure, conservation and maintenance of bird communities, especially in small passerine birds.

CHAPTER THREE: URBANISATION DOES NOT INCREASE ECTOPARASITE PREVALENCE IN AUSTRALIAN PASSERINE BIRDS

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3.1. Introduction

Parasites are a dominant and diverse group which plays a fundamental role in ecological and evolutionary processes. Certain parasitic life strategies may allow ecological processes to be maintained and will be critical to the conservation of fully functioning ecosystems in natural environments (Samuel et al. 2011; Zylberberg et al. 2013). Parasites can maintain species coexistence across space and time as they affect host fitness, reduce dominance of host species and can additionally play important roles in determining species distributions (Nichols and Gómez 2011). However, chronic alterations in parasite prevalence and introduction of novel parasites and pathogens to environments might cause significant changes in host populations. High levels of protozoan parasites on bumblebees, for instance, can be fatal to both individuals and colonies (Goulson and Brown 2010).

In contrast to the growing interest in studying factors that can influence the risk of parasitism inherent to the host, such as sex, age, reproductive status and behaviour, the habitat-specific factors that promote parasitism have been mostly ignored (Kleindorfer

et al. 2006). However, human modifications to the environment, such as deforestation, can disturb the ecology of host-parasite relationships although particular consequences of growing global phenomena like urbanisation are largely unknown (Bradley and Altizer 2006; Delgado-V. and French 2012). This is despite the fact that the risk of parasitism and wildlife diseases caused by ecto- and endoparasites are likely to increase with human activity (Bradley and Altizer 2006). Parasites and pathogens in conjunction with habitat degradation (caused for example by urbanisation) have been recently identified as major concerns in conservation biology as they may cause several detrimental effects in native species (Daszak et al. 2000; Cumming and Van Vuuren 2006).

Urbanisation causes a loss of biodiversity in Australia and overseas (Gaston 2010), and might increase transmission of animal and plant infectious diseases (Keesing et al. 2010). Cities are also places of noise and pollution causing physiological stress which may increase the likelihood of parasitism (Pedersen et al. 2011). Urbanisation confronts animals with changes in habitat conversion and fragmentation. The reaction of animals to those disturbed and fragmented landscapes may affect the host parasite relationships in a complex fashion (Pedersen et al. 2011).

Theoretical arguments about epidemiology suggest a positive relationship between abundance and/or prevalence of parasites in urban areas (e.g. Morand and Poulin 1998; Cumming and Van Vuuren 2006). However, the prevalence and impact of wildlife diseases and parasite loads in urban areas globally varies (Bradley and Altizer 2006; Delgado-V. and French 2012; Brearley et al. 2013). Some authors have found that urban centres have lower rates of parasite infection than rural or natural areas in some groups

such as blowflies (Dykstra et al. 2012), ticks (Gregoire et al. 2002) and blood parasites (Fokidis et al. 2008; Geue and Partecke 2008; Evans et al. 2009) not just in birds but also in other hosts (Page et al. 2008). In contrast, others have found that abundance and/or prevalence of parasites is higher in urban areas (Hammer et al. 2012; Kellner et al. 2012).

I studied natural and human-altered locations to test whether these habitats caused different levels of parasite infestation in small birds. I predicted that urbanisation caused a reduction in ectoparasite prevalence. This prediction has been corroborated in urban tolerant birds in Europe and South America (Gregoire et al. 2002; Arzúa et al. 2003; Evans et al. 2009) but there is still no information about ectoparasite infestation dynamics in urban sensitive birds or other less urbanised birds.

Birds are the most prevalent vertebrate in cities and the best-studied taxonomic group. As they are ubiquitous, they provide an excellent opportunity to study parasitism and its relation to urban development. However, most urban studies of bird parasites around the world have been conducted on a few urban-adapted species such as blackbirds, pigeons and house sparrows (Delgado-V. and French 2012). Here I investigate the spatial dynamics of ectoparasites in areas along an urbanisation gradient in two small passerines cataloged as urban sensitive birds in Australia (see Parsons et al. 2006).

3.2. Methods

I caught birds at five sites each along a gradient of urbanisation within and around Wollongong, NSW, Australia (34° 26' S 150° 53'E). Using satellite images via Google

Earth (2014) analyzed using ArcGIS (10.2.1 version) ESRI (Redlands, CA, USA), we quantitatively determine the degree of urbanisation. Particularly, I calculated an approximate percentage cover of urban areas (buildings, infrastructure and sealed roads) surrounding study sites and used it to develop an Urbanisation Index (UI) as described in Gómez et al. 2008 and Hamer et al. 2012: UI = (100% – % green space + % impervious surface)/2 for the area within 1000 m of the sampling point. Surfaces considered as impervious included artificial structures such as asphalt, concrete, brick, and rooftops. Green space was defined as land having tree, shrub or grass cover.

The sites ranged from natural woodland to heavily urbanised areas. Sampling sites were: 1. Murramarang National Park (hereafter MNP, 35˚ 32’ 46” S 150˚ 21’ 49” E), a natural site situated ca. 300 km far from Wollongong to ensure the birds were well removed from urbanisation effects, UI = 1.05%; 2. Mount Keira (hereafter MK, 34˚ 24’ 48” S 150˚ 51’ 19” E) was a sub-natural area located relatively close to urban development (i.e. 10k from Wollongong city), UI = 12.23%; 3. Ecological Research Centre (hereafter ERC, 34˚ 24’ 17” S 150˚ 52’ 15” E) was a woodland edge surrounded by human altered habitats (e.g. urban gardens, paddocks and edge habitats), where there is a dense, shrubby understorey, UI = 28.12%; 4. Dalton Park (hereafter DP, 34˚ 23’ 47” S 150˚ 53’ 55” E) was a city park surrounded by a matrix of house blocks with variable amounts of mowed grass and ornamental shrubs with occasional large trees, UI = 41.83%; and 5. Green House Park (hereafter GHP, 34˚ 26’ 31” S 150˚ 53’ 31” E), a small park situated between the industrial and commercial centre of the city which exhibits high traffic streets and scarce trees and bushes, UI = 48.41%. Sites were a minimum of 3 km apart located near the coast (within 0.5 – 4.9 km of the coast) where high infection has been found in Australia due to the wet environment (see Oorebeek

and Keindorfer 2009) (Figure 3.1).

Birds were caught using mist-nets erected from sunrise until noon during the breeding season 2012-2013 (from August to February). Nets were checked for captured birds every 10 to 15 min. Birds were examined for any signs of unusual stress upon capture in mist nets (e.g. panting, collapse, extreme shock molt), and any individuals judged to be in extreme distress was immediately released without processing.

Birds were individually transported in a clean cotton bag to the nearby processing station where they were sampled for ectoparasites. Birds were fitted with an aluminum leg band (supplied by the Australian Bird and Bat Banding Scheme) and released at the capture site immediately after processing. They were not included in the analysis if caught again. Birds were dust-ruffled following Walther and Clayton (1997) and Poiani et al. (2000) with some variations (Delgado-V. and French unpublished data, Chapter 2). I dusted each bird with ca. 2 grams of Pestene powder on rump, tail, wings, back and belly. Pestene is an insect powder (Composition: Sulphur: 50 g/kg, Rotenone: 10 g/kg; Inca Co. Pty Ltd, St Mary's, Sydney, NSW, Australia) that is considered harmless for birds. It is commonly used in veterinary treatment and is becoming popular for control of lice, mites and fleas of domestic and laboratory animals (see Courtney Jones et al. 2012). The bird was held over a pan with a clear piece of paper as the collecting surface. Following dusting, the powder was distributed homogeneously all over the body for about 1 min through gentle rubbing with fingertips. The procedure was never done near face in order to avoid any potential irritating effect in the bird's eyes, however the use of Pestene is considered harmless for birds and is commonly used in veterinary treatment. Birds were placed back in the cotton bag or in a paper bag and held for 10 min to allow

the powder to kill parasites. Bags were big enough to allow preening and stretching. After this period, the feathers all over the body were ruffled for 1 min and ectoparasites were collected on the paper underneath. The collecting paper and holding bag were placed in a clean ziplock plastic bag and analyzed in the laboratory.

Using a stereoscopic microscope (under 10x and 40x), ectoparasites were counted from both the bag and the paper. Ectoparasites were determined as lice (Phthiraptera), ticks (Ixoxida), and louse flies (Diptera: Hippoboscidae) which are ectoparasite groups which may cause direct (i.e. consumption of blood, skin, muscles, and other host's tissue) and indirect (i.e. associated adverse effects such as loss of weight) damage (Balashov 2007). Prevalence was calculated as number of individuals of the host species infested with a particular parasite group divided by the number of hosts examined x 100 (Poiani 1992; Oorebeek and Kleindorfer 2009).

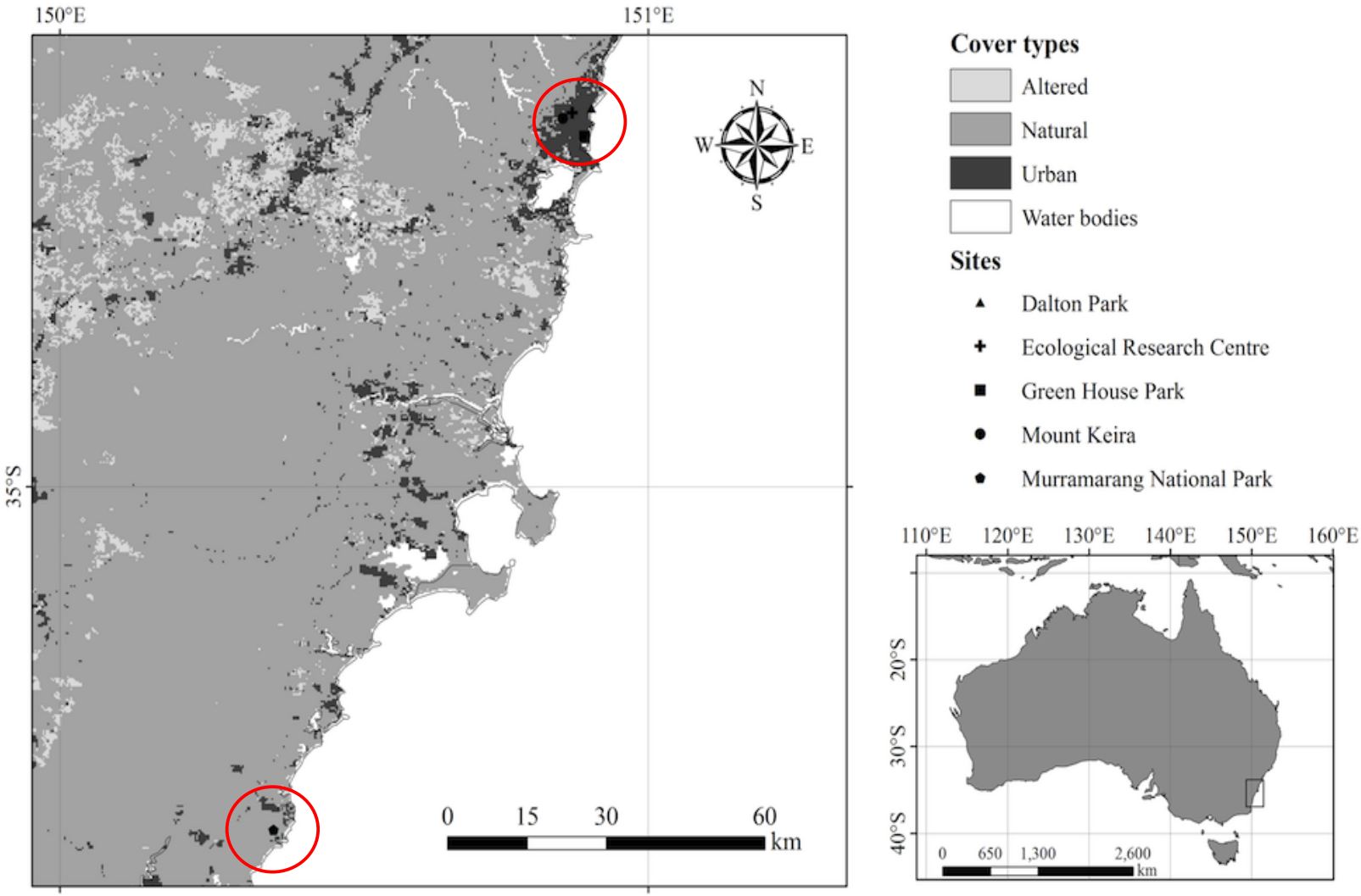
A total of 314 individuals of 8 bird species were examined for ectoparasites: Red-browed finch [RBF (hereafter), n = 90], Superb-fairy-wren [SFW (hereafter), n = 84], Silvereye (n = 3 *Zosterops lateralis*), Eastern yellow robin (n = 20, *Eopsaltria australis*), White-browed scrubwren (n = 46, *Sericornis frontalis*), Grey fantail (n = 1, *Rhipidura fuliginosa*), Eastern spinebill (n = 43, *Acanthorhynchus tenuirostris*), Lewin's honeyeater (n =27, *Meliphaga lewinii*).

Data were analysed separately for each ectoparasite (lice, ticks and louse flies). Logistic regression was used to examine the relationship between parasite infection (presence-absence) and percentage of urbanisation. Individual analysis were undertaken for the Superb-fairy-wren *Malurus cyaneus* and Red-browed finch

Neochima temporalis only since they were the most captured species and occurred at all places sampled along the gradient.

3.3. Results

There was no significant effect of UI on the probability of lice infestation for both species; RBF ($\chi_1^2 = 3.101$, $P = 0.078$) and SFW ($\chi_1^2 = 1.224$, $P = 0.269$) (Figure 3.2a). However, the pattern of tick infestation varied between species. While increasing urbanisation resulted in a significant decline in the probability of tick infestation in RBF ($\chi_1^2 = 7.741$, $P = 0.005$), there was a no clear effect in SFW ($\chi_1^2 = 2.143$, $P = 0.143$) (Figure 3.2b). Finally, louse fly prevalence also changed significantly with degree of urbanisation. The probability of infestation decreased greatly in more urbanised areas in both species ($\chi_1^2 = 28.854$, $P < 0.0001$, and $\chi_1^2 = 12.225$, $P < 0.0005$ for RBK and SFW, respectively) (Figure 3.2c).


150°E
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0 15 30 60
km
Cover types
Altered
Natural
Urban
Water bodies
Sites
Dalton Park
Ecological Research Centre
Green House Park
Mount Keira
Murramarang National Park
110°E
120°E
130°E
140°E
150°E
160°E
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0 650 1,300 2,600
km

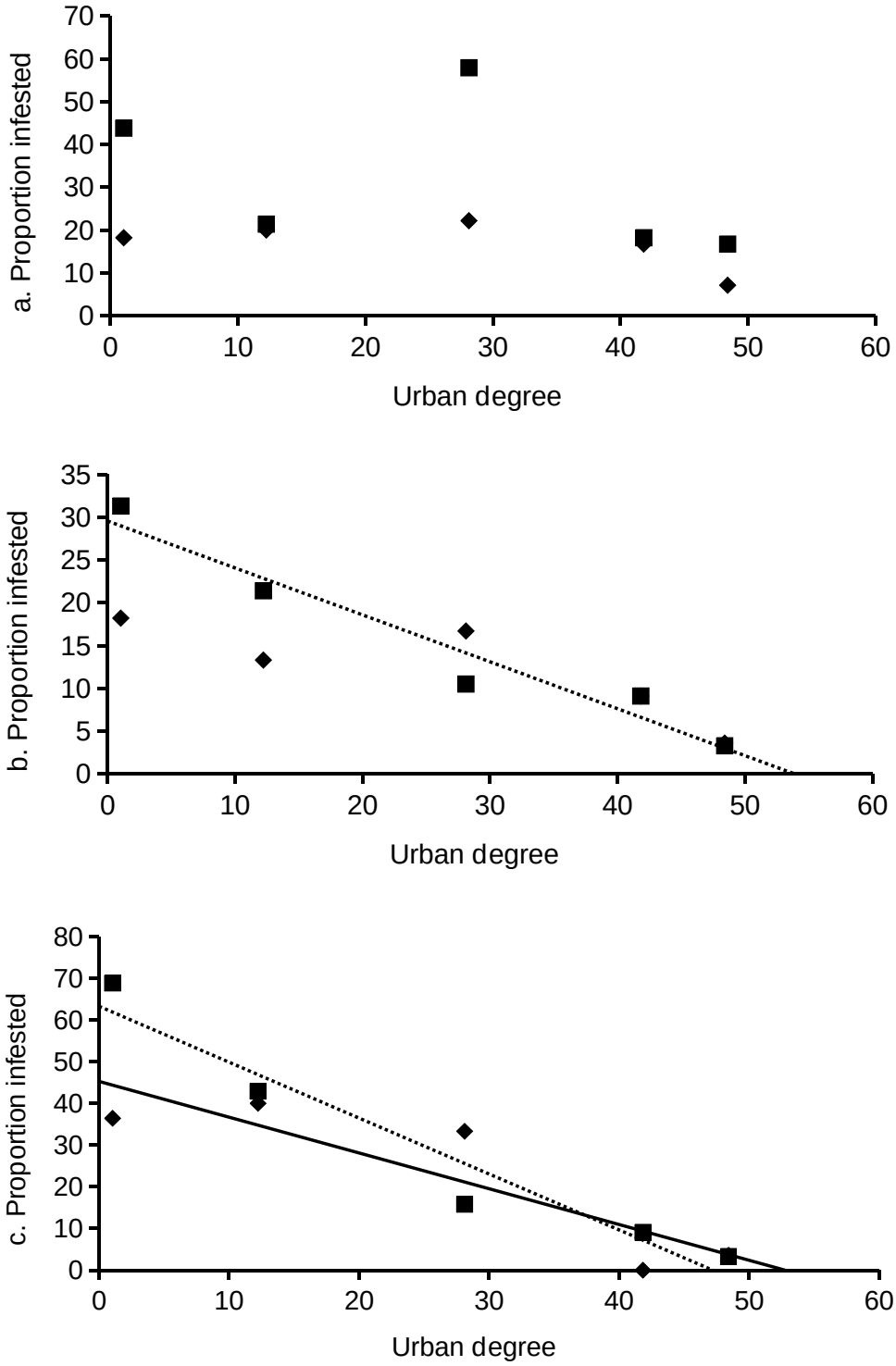

Figure 3.2 a. Lice, **b.** Ticks and c. Louse flies prevalences for Red-browed finch (■), and Superb-fairy wren (♦) along an urban gradient. Best fit lines for RBF (•••) and SFW (—) are depicted when the degree of urbanisation exhibited significant effects.

3.4. Discussion

Prevalence of parasites declined or remained unchanged with urbanisation, rather than increased. While lice were mostly more prevalent in urban fridge habitats (ERC), ticks and louse flies seem to be more sensitive to urbanisation since they were generally more prevalent in areas as urbanisation declined (MNP and MK) and less prevalent in highly urbanised areas (DP and GHP). My results suggest that the urban landscape may create unsustainable areas for ectoparasites which reduce their prevalence.

Although this is the first study to document the relationship between lice loads on birds and urbanisation (Delgado-V. and French 2012), some information is available on ticks and some groups of flies in urban areas. Higher prevalence of ticks in natural areas were also found in Brazil (Arzúa et al. 2003) and Europe (Gregoire et al. 2002; Evans et al. 2009) which compared prevalence in natural, rural areas and ecotone sites with urbanised areas. Lower prevalence of ticks in more urbanised places might be due to higher temperatures, use of pesticides, unsuitable habitats, and variation or fewer intermediate and final hosts (Gregoire et al. 2002; Evans et al. 2009).

Although the hippoboscid flies have not been studied along urban gradients, other Diptera groups vary in their response to urbanisation. Dykstra et al. (2012) report presence of blowflies (Calliphoridae) in rural areas but not in suburban landscapes, but botflies parasitism of *Philornis* (Muscidae) was more prevalent in residential areas (Le Gros et al. 2011). My study found a clear decline in hippoboscid prevalence in more urbanised areas. As blowflies (*Protocalliphora*: Calliphoridae) appear to be affected by pollution (Eeva et al. 1994), the industrial proximities of my industrialised

site might affect the louse flies so they are less frequent compared with other areas.

Urbanisation can affect the presence of wildlife diseases positively and negatively (Bradley and Altizer 2007) since parasite interaction with cities is probably highly dynamic, producing a pattern that includes specific responses based on the type of parasite linage and biology. One of the suggested factors that may be causing the dissimilar responses in these studies is that different species of birds may have different susceptibility to parasite loads. For instance, species which are common in cities may be advantaged by increased immunity whereas species which are now rare in urban environments may be more susceptible (Fokidis et al. 2008). According to this, it has been hypothesized that species sensitive (less frequent or declining) to urbanisation could exhibit a higher parasite infestation in urban areas than natural areas (Delgado-V. and French 2012). I found no evidence to support this hypothesis. Both species I caught in adequate numbers were considered to be species that are affected negatively by urbanisation (Parsons et al. 2003). However, parasitism decreased in urban areas, rather than increased. This raises the possibility that hosts, as well as their parasite fauna, are negatively affected by urbanisation (Nichols and Gómez 2011) regardless of the intrinsic differences in size, nesting and foraging behaviour that RBF and SFW exhibit. However, lice, ticks and louse flies are just a subset of parasites and other kinds of parasites could respond differently under disturbance due to urbanisation. For example, parasites and pathogens with simpler life cycles (such as gastrointestinal parasites) should be studied to detect whether they share common responses to urbanisation.

While lice are obligate parasites that complete their entire life cycle on the same host

and are transmitted by direct physical contact, other ectoparasites such as ticks have several development stages and require different species and substrates for survival and reproduction. Differences in their biology might explain differences in responses along the gradient. For example, while Page et al. (2008) reported a decrease in the intestinal parasite, *Baylisascaris procyonis* in racoons (the final host) with increasing urbanisation, Kellner et al. (2012) found that the prevalence of the same parasite exhibited an opposite trend on small mammals, the intermediate host of the parasite. This suggests that a complete understanding of parasite dynamics requires the sampling of different hosts.

3.5. Conclusions

With so few studies on how parasites might be affected by urbanisation, this study makes an important preliminary contribution. For now, the overall pattern appears to be that urbanisation reduces ectoparasite prevalence in Australian passerine birds. Evidence suggests that parasites can be found either at locations with intermediate development or they can even exhibit higher numbers in natural areas than those that are close to the city. Studies on different urban areas, seasons, parasites and a wider diversity of hosts are needed in order to understand the ecological distribution of parasitism in an urbanised world. In the future, sampling should also be undertaken in the non-breeding seasons of the hosts as parasites are known to change seasonally. They may also interact with other parasite groups (c.f. Kleindorfer et al. 2006) which may influence colonization success when infestation by one parasite compounds the effect of another. Further as infection risk may differ among hosts, additional work is needed to identify how urbanisation influences specific bird/parasite interactions.

CHAPTER FOUR: DIFFERENTIAL INFLUENCE OF URBANISATION ON COCCIDIAN INFECTION IN TWO PASSERINE BIRDS

A modified version of this chapter is published in *Parasitology Research*:

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4.1. Introduction

New cities are created every year and the already established ones expand in area and population (Evans et al. 2009), causing significant impacts on biodiversity through a range of processes. While the effects of urbanisation on some species have been well studied, interactions and relationships amongst species living in cities are virtually unknown (Delgado-V. and French 2012).

Parasite infection has been an important issue of study in natural ecosystem but parasite ecology in urban areas has not received enough attention yet. So that the extension of how urbanisation influences the prevalence and impact of parasites has not been studied in detail (Delgado-V. and French 2012), despite the fact that changes in host-parasite interactions have the potential to be one of the most important consequences of urbanisation (Bradley and Altizer 2007).

Birds are one of the most prevalent animal groups in urban areas. As they are ubiquitous in the urban environment even in highly urbanised areas, they facilitate the study of parasitism and its relation to urban development (Delgado-V. and French 2012). In addition, they are the most studied vertebrate in cities around the world so they offer further possibilities about parasite comparison among urban centres. Even though the same species are not found among cities, comparison might be conducted between biologies, guilts or behaviours.

Passerine birds are host to a variety of parasites which exhibit a diverse array of forms and life history, and where obligate and non-obligate taxa are recognised as vectors of pathogens and diseases. Passerine birds are mainly infected by Coccidia of the genus *Isospora* (Apicomplexa: Coccidia), a cosmopolitan protozoan, which is transmitted via oocysts that are excreted along faeces of the host and infected by ingestion of sporulated oocysts (Dolnik et al. 2010). This is a group of parasites that can reduce weight, affect intestinal nutrient resorption, and reduce fertility (Atkinson et al. 2008). Pathogens as *Isospora* parasites are capable of infecting multiple host species and some pose serious threats to human health and wildlife populations (Atkinson et al. 2008).

Comparative studies on parasites such as ectoparasites and blood parasites have been carried out among natural and urban sites (Delgado-V. and French 2012) but studies that had monitored changes in prevalence of other parasites along urban gradient are scarce (Reperant et al. 2007; Sitko and Zalésny 2014). For example, coccidians parasiting birds has kept virtually understudied in urban areas (Giraudeau et al. 2014)

despite the fact they are relatively easy and inexpensive to monitor.

Delgado-V. and French (submitted) documented a decrease of ticks and louse flies while lice kept unaffected by the degree of urbanisation. However, Evans et al. (2009) hypothesised that avian parasites and pathogens with more simple life cycles, such as some gastrointenstinal parasites, might exhibit a different response. This situation has been recently corroborated in the House finch (*Haemorhous mexicanus*), an urban tolerant bird (Giraudeau et al. 2014) in the US but there is still no information about Coccidian infections in urban sensitive birds or other less urbanised birds.

The proposal of this paper is to document if other parasites may exhibit different responses to urbanisation that those recently found for ectoparasites (Delgado-V. and French submitted). I tested the hypothesis that natural and human-altered locations present different levels of *Isospora* parasite infections on two passerine species which are both sensitive to urbanisation. Particularly, I explored the hypothesis that the overall *Isospora* infestation will be greater in areas with higher urban development.

Parasites are a diverse group which is fundamental in influencing ecological and evolutionary processes, therefore parasitism could contribute greatly to the structure, diversity, conservation, function, and health of biodiversity (Nichols and Gómez 2011). Knowing relative frequency of occurrence of these pathogens in the two species of urban-sensitive passerines with different biology and behaviour is essential for proper epidemiological studies, disease modelling and basic surveys that can subsequently be used as tools for species conservation programs in urban areas.

4.2. Methods

My study focused on two small passerine species known to be less abundant in urban areas (Parsons et al. 2006). Superb-fairy wrens *Malurus cyaneus* (SFW, hereafter) are small (14 cm), sedentary and territorial birds. The species lives in cooperatively breeding groups which usually occupy small (c. 13–24 ha) territories. SFW typically occur in various types of habitat with dense and shrubby understory, including grasslands with scattered shrubs, gardens and urban parks. SFW mainly eats insects (Mulder 1995). Red-browed finch *Neochima temporalis* (RBF, hereafter) is a sociable small estrildid finch (11–12 cm long). It inhabits the east coast of Australia and is found in open and semi-open woodland. This species is sedentary or nomadic. RBF feeds mostly on seeding grasses but it also uses bird feeders. It is found in weedy areas along railway tracks, roads, creek lines and urban parks (Todd 1996).

I chose five sites that differed in their degree of urbanisation. The Urbanisation Index ranged from 1.05%-48.41% based on Gómez et al. (2008) and Hamer et al. (2012) index (described in Chapter 3).

Field work was conducted over a year in breeding and non-breeding seasons. Birds were sampled from February 2012 to March 2013 using mist-nets. Nets were checked for captured birds every 10 to 15 min. Birds were examined for signs of unusual stress upon capture in mist nets (e.g. panting, collapse, extreme shock moult), and any individuals judged to be in extreme distress was immediately released without processing. Birds were individually transported in a clean cotton bag to the nearby processing station where a faecal sample was collected over a twenty minute period. Birds were fitted with

an aluminum leg band (supplied by the Australian Bird and Bat Banding Scheme) and released at the capture site immediately after processing and not included in the analysis if caught again.

Fresh excreta were collected in small vials containing 2.5% potassium dichromate ($K_2Cr_2O_7$) water solution following Dolnik (2006). The samples were stored at room temperature for at least 4 days until analysis. Oocysts of *Isopora* were extracted using the method described in Dolnik (2006). Oocysts were then transferred to a slide and examined under the microscope where I recorded the presence of oocysts. The prevalence of *Isospora* was determined as the percentage of birds infected at each study site.

Data were analysed separately for each host. Logistic regression was used to examine the effects of the degree of urbanisation and season (breeding and non bredding) on parasite prevalence. Interactions were included in the initial models and model simplification carried out by stepwise removal of explanatory factors that did not contribute significantly.

4.3. Results

I sampled 278 birds; 138 SFW and 140 RBF. Overall, there were 55 SFW infected (19.78%) and 66 RBF (47.14%). RBF had a higher prevalence of *Isospora* than SFW (χ^2 = 18.201, df = 9, P = 0.0329).

In RBF, the prevalence of *Isospora* increased significantly in more urbanised areas (χ_1^2

= 4.09, P < 0.005) (Figure 4.1a). Prevalence did not change between breeding and non-breeding seasons (χ_1^2 = 3.16, P = 0.075) but there was a significant interaction between the two (χ_1^2 = 12.09, P = 0.005) (Figure 4.1a). In contrast, in SFW neither degree of urbanisation (χ_1^2 = 0.06, P = 0.8010) nor season exhibited significant effects (χ_1^2 = 0.03, P = 0.9555) on the prevalence of coccidians (Figure 4.1b).

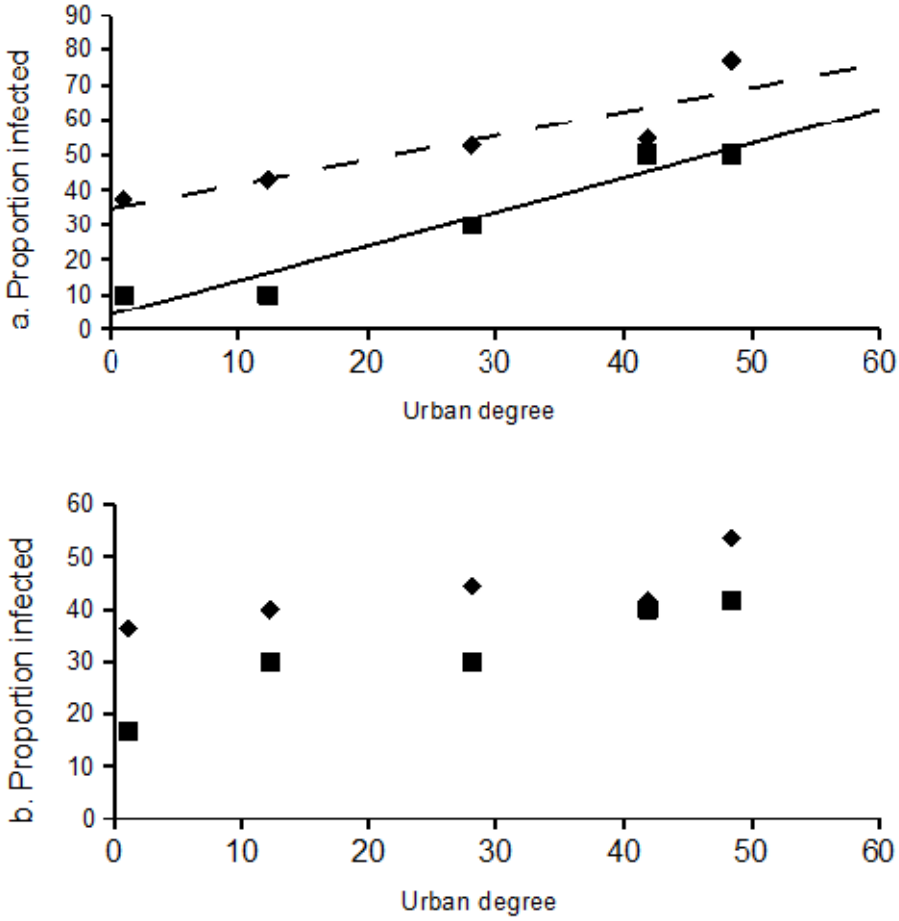

Figure 4.1. Coccidian prevalences for **a.** Red-browed finch and **b.** Superb-fairy wren in breeding (♦), and non-breeding seasons (■), along an urban gradient. Best fit lines for RBF are depicted. Degree of urbanisation exhibited no significant effects in SFW.

4.4. Discussion

In this study, I tested whether the degree of urbanisation affects the occurrence of Coccidian infections in two urban sensitive birds. I found that prevalence was differential between species, and that, contrary to the prediction, prevalence of *Isospora* increased in more urbanised areas on Red-browed finch, in both breeding and non-breeding seasons, but did not significantly change with degree of urbanisation in Superb-fairy wren. Diet, behaviour, and habits, are suspected to be more influential factors on the variation observed between both species, where granivorous and gregarious species being significantly infected.

This study provides the first quantitative baseline information of *Isospora* prevalence along an urban gradient in Oceania. It shows that Coccidians can respond differently depending on the host suggesting that these parasites might be more prevalent in urban areas in some birds but not all. In one species, parasite prevalence in the population increased with urbanisation suggesting that fitness may be affected in birds in more highly urbanised areas. These differences of parasite prevalence may be link to other life history features which need to be further explored.

The prevalence and intensity of infection with Coccidian parasites in wild passerines is highly variable amongst species. In natural areas, Zinke et al. (2004) and Dolnik et al. (2010) found that ground feeder birds have the highest prevalence and intensity of infection, and that these parameters have lower values in insectivore passerines. Also, foraging in flocks may cause an aggregation of faeces at feeding sites, so that social foragers appear to be more exposed to coccidian infections (Giraudeau et al. 2014).

Additionally, infection may also occur during bathing, collection of nest material or pairing where flocks facilitate the transmission of parasites and lead to higher intensities of Coccidians. Many birds foraging at the same area for extended periods of time can result in accumulation of faeces and posterior transmission (Zinke et al. 2004; Dolnik et al. 2010).

My results contrast to other studies which have shown reduced parasite load in urban areas (Gregoire et al. 2002). For example, Geue and Partecke (2008) and Evans et al. (2009) worked on the Eurasian blackbird *Turdus merula* and found a decrease in tick prevalence and intensity in cities. Sitko and Zalésny (2014) found a reduction in the helminth fauna in Eurasian blackbird in urban areas compared with forested sites. Fokidis et al. (2008) found that some urban passerines were less affected by haemoparasites than its rural counterparts in the US desert.

As life histories of parasites vary extensively, responses to urbanisation may also differ. Prevalence and intensity of avian parasites and pathogens with simpler life cycles might be higher in urban areas (Evans et al. 2009) as transmission is easier. Drastic environmental changes associated with urbanisation, habitat conversion, increased temperatures and fragmentation (McCallum and Dobson 1995; Daszak et al. 2000; Bradley and Altizer 2006; Cumming and Vuuren 2006; Calegaro-Marques and Amato 2014) might cause declines in microhabitats, water availability and the abundance of intermediate hosts and vectors which are likely to affect ticks, helminths and blood parasites with complex life histories (Reperant et al. 2007; Evans et al. 2009; Sitko and Zalésny 2014). However, *Isospora* and other parasites, viruses and pathogens might have a different response as transmission pathways are quite distinct.

Isospora infections are passed between hosts by faecal-oral transmission where infective oocysts can contaminate the external environment, including food and water (Dolnik 2006; Dolnik et al. 2010). This suggests that urban aggregations of host species may encourage the spread of parasites that are transmitted by direct contact. For example, Hamer et al. (2012) found the avian exposure to West Nile Virus increased with level of urbanisation. Similarly, Giraudeau et al. (2014) recorded an increase of prevalence of poxvirus associated with urbanisation degree.

My results only partially support this hypothesis as prevalence was only related to urbanisation in one species, not both. Therefore, factors of exposure to parasites, such as diet, behaviour, and environmental factors, need to be investigated to explain the variation seen between RBF and SFW. For example, larger communal roosts or reduced areas for foraging and feeders might provide more transmission opportunities (Giraudeau et al. 2014) facilitating contact rates and potential dispersal and transmission.

Differences in diet may also influence transmission rates. Dolnik et al. (2010) suggested that insects are less likely to be contaminated with sporulated oocysts than berries or seeds, resulting in both a lower probability of exposure and a lower dose for insectivores in comparison with granivores. Ground foraging granivores might be particularly susceptible to infection where birds may encounter faecal material. Granivorous passerines, such as RBF might represent the most vulnerable birds under an eventual chronic prevalence of *Isospora* in urban areas (Giraudeau et al. 2014).

Alternative routes of uptake of transmission of oocysts might also explain differences

in seasonal prevalence. During breeding seasons, nestlings and siblings can be infected by parents during ingestion of contaminated food (Dolnik et al. 2010), causing a higher prevalence of *Isospora* in the breeding season in RBF along the sampled gradient of urbanisation.

4.5. Conclusions

Urban areas are becoming important areas for conserving and managing urban wildlife (Kantsa et al. 2013). Parasites could contribute greatly to the structure, diversity, conservation, function, and health of urban biodiversity, and future studies focusing on the understanding of the transmission dynamics and the ecology of wildlife pathogens in species will corroborate if this pattern of *Isospora* infection in granivorous and insectivore passerines can be applied more generally.

CHAPTER FIVE: PREVALENCE AND LINEAGE DIVERSITY OF AVIAN HAEMOSPORIDIANS FROM URBAN AND NATURAL AREAS IN EASTERN AUSTRALIA

5.1. Introduction

Parasites are a dominant and diverse group which is fundamental in influencing ecological and evolutionary processes. Haemosporidian parasites (Haemosporidia, Apicomplexa) of avian hosts may play important roles in sexual selection and reproductive success in birds (McCallum and Dobson 1995; Atkinson 2008) and are recognised as factors that cause morbidity and mortality in avian species (Palinauskas et al. 2008). However, their pathogenicity is related to factors such as host nutrition, host availability, susceptibility and environmental stress. Additionally, a number of studies documenting the effects of biodiversity on exposure, risk and transmission of diseases in ecological communities have found that a loss of host species diversity could change the infection rate between different hosts (Keesing et al. 2006).

Haemosporidians have been recorded on a wide variety of vertebrates (Santiago-Alarcón et al. 2014) where the most commonly occurring haematozoa in birds are species of *Haemoproteus*, *Leucocytozoon* and *Plasmodium* which are transmitted by a wide range of vectors. Avian Haemosporidians have a broad geographic distribution (Santiago-Alarcón et al. 2012) and are known for causing different impacts on individuals, populations and communities. For instance, avian Haemosporidians may have significant impacts on fitness and reproduction of hosts which may cause decline and extinction of certain species as recorded for some

Hawaiian passerines (Atkinson et al. 1995).

Australian avifauna has a vast number of endemic species with different life-history characteristics associated with a unique evolutionary history with a long period of isolation. Additionally, several avian parasite groups of Australia may well be equally evolutionarily distinct and show unique lineages with different patterns of prevalence to other continents (Clark et al. 2014). However, little is known about bird-parasite relationships and whether they are composed of unique or endemic partners and how they are affected by human-induced changes such as urbanisation. The east coast of the country, a region that has experienced an expanding urban development in recent years, is a critical place where bird-parasite relationships may be changing.

Urban areas are increasing in both extent and number (Evans et al. 2009) which is causing significant impacts on loss of biodiversity. While the effects of urbanisation on biodiversity structure and composition have been well studied, interactions and relationships amongst species are virtually unknown. In new disturbed and anthropogenic environments, such as urban areas, it is predicted that there will be an increase in parasites and pathogens as hosts become susceptible under stressful changes in habitats, as well as from the increased possibility of emerging diseases not experienced in natural undisturbed areas (Reye et al. 2010). Australia, Latin America and many Oceanic islands are regions of major concern (Cumming and Van Vuuren 2006) due to changes in climatic conditions, habitat loss, habitat conversion, increased temperature, fragmentation and a greater presence of human-modified landscapes

(McCallum and Dobson 1995; Daszak et al. 2000; Sutherst 2001; Bradley and Altizer 2006; Cumming and Van Vuuren 2006; Garamszegi et al. 2011; Loiseau et al. 2013).

Identifying and monitoring parasites is important to understand if the risk of parasitism and wildlife diseases caused by parasites increases due to human activity (Bradley and Altizer 2006). However, in contrast to the growing interest in studying factors that can influence the risk of parasitism inherent to the host, such as sex, age, and reproductive status, the number of publications about the habitat-specific factors that promote parasitism is comparatively small (Merilä et al. 1995; Kleindorfer et al. 2006), particularly for urban areas. A recent review (Delgado-V and French 2012), identified a gap in the literature of bird-parasite interactions in urban areas. Studies identifying parasites are more common for American and European urban birds where the parasite fauna is better known, although data is restricted to few bird hosts from a few cities (Delgado-V and French 2012). Understanding of parasite-host relationships has important implications for conservation and management of urban biodiversity, therefore it is crucial to determine which parasites survive in urban areas especially where urbanisation has been increasing (Jansen et al. 2009). Additional studies are urgently needed to increase our understanding of the diversity, prevalence, and distribution of Haemosporidians.

The objectives of the present study are 1. to analyse the prevalence and diversity of Haemosporidian parasites infecting birds in an urban environment of NSW, Australia; 2. to compare the lineage diversity and Haemosporidian prevalence of *Plasmodium*, *Haemoproteus* and *Leucocytozoon* between hosts inhabiting natural

and urban areas in order to detect the influence of urbanisation and, 3. to analyse the results obtained in this study in relation to other areas of Australia and the Southern Hemisphere based on a compilation of references about haemosporidian prevalence. Several excellent literature analyses have been published recently that have compiled research largely from temperate natural areas of the Northern hemisphere (Garamszegi 2011; Lacorte et al. 2013; Clark et al. 2014). As a result my comparison was restricted to 1. natural areas from the Southern Hemisphere, and 2. urban studies all over the world. This decision allowed me to focus my comparison of the situation in natural and disturbed areas of the Southern Hemisphere which is one of regions less explored about its parasite fauna (Delgado-V. and French 2012; Clark et al. 2014).

5.2. Methods

5.2.1. Field sites

The study was conducted from March 2012 to February 2013 in four sites in Wollongong and nearby areas, New South Wales, Australia. Birds were sampled at three sites in urban areas located in the city of Wollongong: 1. Ecological Research Centre at University of Wollongong (hereafter ERC, 34˚ 24’ 17” S 150˚ 52’ 15” E), an urban-fringe area characterised by a woodland edge surrounded by human altered habitats (e.g. urban gardens, edge habitats), where there is a dense, shrubby understorey; 2. Dalton Park (hereafter DP, 34˚ 23’ 47” S 150˚ 53’ 55” E), a city park surrounded by a matrix of house blocks. This park is located at a mosaic of roads, houses and yards with variable amounts of moved grass and ornamental shrubs with occasional large trees; and 3.

Green House Park (hereafter GHP, 34˚ 26’ 31” S 150˚ 53’ 31” E), a small park situated between the industrial and commercial centre of the city surrounded by high traffic streets with scarce trees and bushes. One natural area was also sampled; Murramarang National Park (35˚ 32’ 46” S 150˚ 21’ 49” E) is situated ca. 300 km south of Wollongong and well away from urban expansion.

5.2.2. Bird capture and handling

Birds were caught using mist-nets erected from sunrise until noon. Blood samples were collected from the brachial vein. A drop of blood was immediately placed on a clean glass microscopic slide to prepare a blood smear for microscopic examination. Smears were air-dried in the field and stored until fixation and differential stain using Kwik-Diff stain (Thermo Scientific™) as suggested by the manufacturer. Small dot samples of blood was collected and preserved on Whatman FTA Cards for molecular analysis.

5.2.3. Analysis and measurements of blood parasite infection by microscopy

Blood parasites were detected and determined by microscopic examination (Clark et al. 2009). Blood parasite prevalence was determined by surveying fixed blood smears on microscope slides. Smears were studied using light microscopy at high magnification (100x) under oil immersion and 80 randomly chosen fields with homogeneous distribution of red blood cells were scored for presence/absence of blood parasites. When no parasites were detected after that number of fields, the individual was considered uninfected.

A total of 270 individuals of 17 bird species (representing 10 families) were sampled for blood parasites using blood smears (Table 5.1). All samples were obtained from urban sites (i.e. from ERC, DP and GHP) and no slides were available from the natural site.

5.2.4. Molecular detection

Blood samples from 147 individuals from three passerine host species [the Eastern yellow robin (*Eopsaltria australis*) (n=8, 6 natural and 2 urban), the Superb-fairy wren (*Malurus cyaneus*) (n=74, 46 natural and 28 urban), and the Red-browed finch (*Neochmia temporalis*) (n=65, 45 natural and 20 urban)] were screened using molecular methods. Parasite diversity and prevalence were analysed separately for each host and compared between areas.

DNA was extracted using the Phenol-chloroform method following Sambrook and Russell (2001) with some minor modifications. Nested-polymerase chain reaction (PCR) protocol developed by Hellgren et al. (2004) was used which enables simultaneous detection from the three most common avian blood parasite genera: *Haemoproteus*, *Plasmodium*, and *Leucocytozoon*. The screening method consists of an initial general PCR that allows the amplification of the three genera (primers HaemNFI/HaemNR3). The second PCR amplified *Plasmodium/Haemoproteus* (primers HaemF/HaemR2), and the final one, *Leucocytozoon* (primers HaemFL/HaemR2L). Positive and negative individuals were identified by the presence or absence of a ~500 bp band on 2% agarose gels stained with GelRed, and visualized with a UV light source. Further identification was completed by the sequencing of a fragment of 480-bp of the

cytochrome b following Bensch et al. (2000) and Hellgren et al. (2004). Obtained sequences were aligned and edited using the program Geneious v.8.0.2 (http://www.geneious.com, Kearse et al. 2012). DNA sequences were compared for identification to their closest matches in MalAvi (Bensch et al. 2009) and GenBank.

Natural and urban parasite prevalences for each host species were compared by Fisher Exact Test using Quantitative Parasitology (Reiczigel and Rózsa 2005).

5.2.5. Literature review

For comparison to field data obtained on this thesis with other studies in Australia and overseas, a multi-keyword field literature search including the terms “haemoparasite”, “prevalence”, “parasite” and “passerine” was performed in the databases PubMed, Web of Science, Biological Abstracts, and Summon. Also additional articles were reviewed from initial identified publications in order to expand my literature dataset. Research carried out on introduced, pets, and poultry were excluded from analysis.

Specific data such as geographic locality (tropical or temperate zone, hemisphere, continent, country and natural, rural or urban site), method of sampling, years of survey period, type of Haemosporidian, prevalence, type of bird, number of species and individuals sampled which were infected were extracted.

5.3. Results

5.3.1. Overall urban Haemosporidian prevalence by microscopy screening

Using visual inspection of microscope slides, I only detected two individuals with Haemoparasites. It was not possible to determine the species of any blood parasite due to the low levels of parasitaemia (only one parasite per smear was detected through the screening), however, I was able to assign a genus. Both individuals (from two different species, the Silvereye *Zosterops lateralis* and the Eastern spinebill *Acanthorhynchus tenuirostris*) were infected with *Haemoproteus* (Table 5.1). I did not detect any individual infected with *Leucocytozoon* or *Plasmodium*.

5.3.2. Comparison of prevalence and lineage diversity between urban and natural areas using sequencing

From 147 screened birds, 26 individuals (17.7%) were positive for either *Plasmodium*/*Haemoproteus* or *Leucocytozoon* infections. Irrespective of host species, total prevalence of *Plasmodium*/*Haemoproteus* was 2% and the prevalence of *Leucocytozoon* was 15.7%. No Haemosporidian infection was detected in Eastern yellow robin but mixed infections were found in both Red-browed finch and Super-fairy wren (Table 5.2). Overall prevalence was significantly higher in the natural (23.7%) than urban areas (6%) ($p<0.05$). However, whereas *Leucocytozoon* occurrred in both areas, *Plasmodium*/*Haemoproteus* infection was only found in natural areas.

For Superb-fairy wren, individuals from natural areas exhibited higher total prevalence

of Haemosporidians (21.7%) than urban individuals (7.1%). Similarly, *Plasmodium*/*Haemoproteus* as well as *Leucocytozoon* infection in SFW were both higher in natural areas (Table 5.2) but differences were not significant in any case ($p<0.05$). RBF had a higher prevalence of parasites in natural areas (Table 5.2) and both total Haemosporidians and *Leucocytozoon* were more prevalent in natural areas ($F=0.047$, $p=0.027$, and $F=0.05$, $p=0.03$ respectively).

DNA of 14 individuals was sequenced and four mitochondrial lineages were found which have not been reported in previous studies and are described for the first time here. The new lineages recorded are two *Leucocytozoon* lineages (referred to as lineages MALCYA01 and MALCYA02 hereafter) and two *Haemoproteus* lineages (MALCYA03 and MALCYA04) which matched with no previous sequences available in MalAvi or GenBank (Table 5.3). While MALCYA02 lineages were presented in SFW and RBF hosts, MALCYA01, MALCYA03 and MALCYA04 lineages occurred exclusively in SFW individuals (Table 5.4). Parasite diversity was higher in the natural area than in the urban area. All four lineages were found in natural areas and just one (MALCYA02) was also found in urban areas (Table 5.4).

5.3.3. Literature review

I gathered 26 papers dealing with Southern Hemisphere regions and/or global urban areas. I found 20 papers with 50 estimates of parasite prevalence for natural areas (Table 5.5) and nine papers with 19 prevalence estimates for urban areas (Table 5.6). For natural areas, tropical region from Southern Hemisphere have a higher average prevalence of overall Haemosporidians (17.8%) than temperate region (14.9%) (Table

5.5). Similar patterns are exhibited by particular parasites. For instance, *Haemoproteus* prevalence is higher in tropical zones than temperate areas (22.2% vs 9.2% respectively).

There are differences in prevalence among continents for natural areas. Africa has the highest average prevalence of Haemosporidians (58.5%) followed by South America (19%) and Oceania (15%). A similar pattern is exhibited by particular parasites such as *Plasmodium* which has 58.5%, 16% and 8.8% prevalence from Africa, South America and Oceania in natural areas. Overall, however, Australia appeared to have lower levels of parasitism than all continents.

Cities located in the Southern Hemisphere have similar prevalence of Haemosporidians (16.6%) to cities from Northern hemisphere (16.4%) (Table 5.6). There are some differences among urban centres on different continents. European (33.1%) and South American cities (31.4%) appeared to have higher parasite prevalence than Oceania (11.6%), North American (9.8%) and Asian cities (3.1%). The least frequent Haemosporidian in urban areas is *Leucocytozoon*. This parasite was detected only in four of the 19 studies conducted. *Leucocytozoon* occur in European (49%), Oceanian (22.05%), and Asian cities (4.6%). *Plasmodium* and *Haemoproteus* were the most common parasites, reaching their highest prevalences in urban areas from Europe and North America particularly (Table 5.6).

Based on analysis of literature, I detected that average prevalence in Australia is higher in natural (17.8%) (Table 5.5) than urban areas (11.6%) but compared to other cities tended to be lower (Table 5.6).

Table 5.1. Haemosporidians found in blood smears from birds from Illawarra urban areas. The list is ordered alphabetically by bird familiy. **N** (number of individuals sampled), **I** (number of individuals infected) (Prevalence in %), **L** (*Leucocytozoon*), **H** (*Haemoproteus*), **P** (*Plasmodium*).

Family	**Bird species**	**N**	**I**	**L**	**H**	**P**
Acanthizidae	Brown thornbill - *Acanthiza pusilla*	1	0			
	Large-billed scrubwren - *Sericornis magnirostra*	1	0			
	White-browed scrubwren - *Sericornis frontalis*	37	0			
	Yellow-throated scrubwren - *Sericornis citreogularis*	2	0			
Estrildidae	Red-browed finch - *Neochmia temporalis*	70	0			
Maluridae	Superb-fairy wren - *Malurus cyaneus*	69	0			
Meliphagidae	New Holland honeyeater - *Phylidonyris novaehollandiae*	1	0			
	Eastern spinebill - *Acanthorhynchus tenuirostris*	35	1 (2.86%)		x	
	Lewin's honeyeater - *Meliphaga lewinii*	13	0			
Monarchidae	Satin flycatcher - *Myiagra cyanoleuca*	1	0			
Muscicapidae	Grey fantail - *Rhipidura albiscapa*	3	0			
	Willie wagtail - *Rhipidura leucophrys*	6	0			
	Bassian thrush - *Zoothera lunulata*	1	0			
Pachycephalidae	Golden whistler - *Pachycephala pectoralis*	4	0			
Pardalotidae	Spotted pardalote - *Pardalotus punctatus*	1	0			
Petroicidae	Eastern yellow robin - *Eopsaltria australis*	16	0			
Zosteropidae	Silvereye - *Zosterops lateralis*	9	1 (11.11%)		x	
Total		**270**	**2 (0.7%)**			

Table 5.2. Prevalence (% of birds with infection) of Haemosporidians with no specific parasite discrimination (**TH**), *Plasmodium*/*Haemoproteus* (**P/H**), and *Leucocytozoon* (**L**) infections for Superb-fairy wrens and Red-browed finches in urban and natural sampling sites.

Sampling area	TH	P/H	L
Superb-fairy wren			
Urban area (n=28)	7.1	0	7.1
Natural area (n=46)	21.7	4.3	17.4
Red-browed finch			
Urban area (n=20)	**5***	0	**5***
Natural area (n=45)	**28.9***	2.2	**26.7***

Table 5.3. MalAvi accession numbers of lineages identified in this study.

Parasite	Lineage	MalAvi Accession number
Leucocytozoon sp.	MALCYA01	To be determined
Leucocytozoon sp.	MALCYA02	To be determined
Haemoproteus sp.	MALCYA03	To be determined
Haemoproteus sp.	MALCYA04	To be determined

Table 5.4. Area and number of individuals of Superb-fairy wren (SFW) and Red-browed finch (RBF) infected by each mitochondrial lineage of *Haemoproteus* and *Leucocytozoon* parasite.

Host	Area	Lineages			
		MALCYA01 *Leucocytozoon* sp.	MALCYA02 *Leucocytozoon* sp.	MALCYA03 *Haemoproteus* sp.	MALCYA04 *Haemoproteus* sp.
SFW	Natural	1	3	1	3
	Urban	-	1	-	-
RBF	Natural	-	5	-	-
	Urban	-	-	-	-
Total		1	9	1	3

Table 5.5. Haemosporidians prevalence data obtained for natural sites located in the Southern Hemisphere recovered from the literature review. Abbreviations include: Type of Haemosporidian (P: *Plasmodium*, H: *Haemoproteus*, L: *Leucocytozoon*) and P (Prevalence in %).

Continent	Country	Date	Haemosporidian	Type of bird	N sampled	P (%)	Reference
MICROSCOPY							
Tropical							
Africa	South Africa	2004	P	Passerines	104	58.5	Garamszegi 2011
Oceania	American Samoa	1996	P	Passerines	214	0.0	Garamszegi 2011
Oceania	American Samoa	2001-2005	P	Passerines	857	1.0	Atkinson et al. 2006
Oceania	Australia	1975	P, H	Non passerines	316	7.0	Bennett et al. 1975
Oceania	Australia	1997-2001	L	Passerines & non passerines	3059	51.1	Adlar et al. 2004
Oceania	Australia	1997-2001	H	Passerines & non passerines	3059	31.4	Adlar et al. 2004
Oceania	Australia	1997-2001	H	Passerines & non passerines	3059	10.9	Adlar et al. 2004
South America	Bolivia	1988	P	Passerines	640	0.8	Garamszegi 2011

South America	Bolivia	1988	P, H	Passerines & non passerines	641	5.1	Benett et al. 1991
South America	Brazil	2000-2001	P, H	Passerines	436	17.7	Sebaio et al. 2010
South America	Brazil	2000-2001	P, H	Passerines	489	13.9	Sebaio et al. 2010
South America	Brazil	2000-2001	P	Passerines	925	9.2	Sebaio et al. 2010
South America	Brazil	2000-2001	H	Passerines	925	3.2	Sebaio et al. 2010
South America	Brazil	2000	P	Passerines	246	23.2	Garamszegi 2011
South America	Brazil	2005-2009	H	Passerines & non passerines	772	7.1	Fecchio et al. 2011
South America	Brazil	2005-2009	P	Passerines & non passerines	772	3.6	Fecchio et al. 2011
South America	Colombia	1999	P	Passerines	159	0.4	Garamszegi 2011
South America	Colombia	1999	P	Passerines	315	1.1	Garamszegi 2011
Temperate							
Oceania	Australia	2004	H	Passerines	110	9.1	Kleindorfer et al. 2006

MOLECULAR							
Tropical							
Oceania	Australia	2002, 2003	H	Passerines	219	28.0	Beadell et al. 2004
Oceania	Australia	2002, 2003	P	Passerines	219	14.0	Beadell et al. 2004
Oceania	Australia	2005-2006	P, L, H	Passerines	403	32.3	Zamora-Vilchis et al. 2012
Oceania	Australia	2005-2006	H	Passerines	403	19.9	Zamora-Vilchis et al. 2012
Oceania	Australia	2005-2006	L	Passerines	403	6.2	Zamora-Vilchis et al. 2012
Oceania	Australia	2005-2006	P	Passerines	403	1.7	Zamora-Vilchis et al. 2012
Oceania	Australia	2004, 2005, 2006	H	Passerines	188	10.6	Collombelli-Négret and Kleindorfer 2008
Oceania	Australia	2011	P	Passerines	299	12.7	Laurance et al. 2013
Oceania	Australia	2011	H	Passerines	299	27.8	Laurance et al. 2013
Oceania	Australia	2005	P	Passerines	165	8.5	Beadell et al. 2007
Oceania	Australia	2005	H	Passerines	165	39.1	Beadell et al. 2007
Oceania	Australia	2005	L	Passerines	165	1.5	Beadell et al. 2007
Oceania	Australia	2008-2011	H	Passerines	1005	3.2	Balasubramaniam et al. 2013

Oceania	Australia	2011-2013	P, H, L	Passerines	97	23.7	This study
South America	Brazil	2000-2006, 2010	P, H	Passerines	1545	35.3	Lacorte et al. 2013
South America	Brazil	2007-2009	P, H	Passerines	676	42.2	Belo et al. 2011
Oceania	French Polynesia	2005	P	Passerines	174	1.9	Beadell et al. 2007
South America	Guyana	1994-2000	P	Passerines	195	42.7	Durrant et al. 2006
South America	Guyana	1994-2000	H	Passerines	195	23.2	Durrant et al. 2006
Oceania	Papua Guinea	2002, 2003	H	Passerines	209	31.0	Beadell et al. 2004
Oceania	Papua Guinea	2002, 2003	P	Passerines	209	10.0	Beadell et al. 2004
Temperate							
South America	Chile	2003-2005	P, H, L	Passerines	760	15.4	Merino et al. 2008
Oceania	New Zealand	2010	P	Passerines	50	2.0	Baillie and Brunton 2011
Oceania	New Zealand	2010	P	Passerines	145	16.0	Baillie and Brunton 2011
Oceania	New Zealand	2010	P	Passerines	116	23.0	Baillie and Brunton 2011
Oceania	New Zealand	2010	P	Passerines	196	20.0	Baillie and Brunton 2011
Oceania	New Zealand	2010	P	Passerines	44	3.0	Baillie and Brunton 2011

Oceania	New Zealand	2010	P	Passerines	41	3.0	Baillie and Brunton 2011
South America	Uruguay	2002-2003	P	Passerines	322	61.5	Durrant et al. 2006
South America	Uruguay	2002-2003	H	Passerines	322	14.1	Durrant et al. 2006
South America	Venezuela	2004-2005	P, H	Passerines	527	41.0	Belo et al. 2012

Table 5.6. Haemosporidian prevalence data of urban areas around the world recovered from selected references. Abbreviations include: Type of Haemosporidian (P: *Plasmodium*, H: *Haemoproteus*, L: *Leucocytozoon*) and P (Prevalence in %).

Continent	Country	Method	Date	Haemosporidian	P (%)	Type of bird	N sampled	Reference
Oceania	Australia	Molecular	2011-2013	P/H	4.3	Superb-fairy wren	46	This study
Oceania	Australia	Molecular	2011-2013	L	17.4	Superb-fairy wren	46	This study
Oceania	Australia	Molecular	2011-2013	P/H	2.2	Superb-fairy wren	46	This study
Oceania	Australia	Molecular	2011-2013	L	26.7	Superb-fairy wren	46	This study
South America	Brazil	Molecular & microscopy	2007-2009	P/H	56.2	Passerines	676	Belo et al. 2011
South America	Brazil	Molecular	2007	P/H	6.7	Passerines	119	Lima et al. 2010
Europe	Germany	Microscopy	2000	L	49.0	Eurasian blackbird	106	Geue and Partecke 2008
Europe	Germany	Microscopy	2000	H	16.0	Eurasian blackbird	106	Geue and Partecke 2008

Europe	Germany	Microscopy	2000	P	6.6	Eurasian blackbird	106	Geue and Partecke 2008
Asia	Japan	Microscopy	1988-2001	P	1.7	Passerines and non passerines	701	Murata 2002
Asia	Japan	Microscopy	1988-2001	L	4.6	Passerines and non passerines	701	Murata 2002
Oceania	New Zealand	Molecular	2010	P	3.0	Passerines	46	Baillie and Brunton 2011
Europe	Tunisia, Spain, Netherlands, UK, Germany, Poland, Czech Republic, Latvia, Estonia	Molecular	2006, 2007	P/H	61.0	Eurasian blackbird	NA	Evans et al. 2009
North America	USA	Microscopy	2003	H	8.0	House finch	757	Hartup et al. 2008
North America	USA	Microscopy	2003	P	5.0	House finch	757	Hartup et al. 2008
North America	USA	Microscopy	2001	H	3.0	House finch	282	Hartup et al. 2008
North America	USA	Microscopy	2001	P	3.0	House finch	282	Hartup et al. 2008

North America	USA	Microscopy	2006	H	22.0	Northern Mockingbird	23	Fokidis et al. 2008
North America	USA	Microscopy	2006	H	18.0	Curve-billed thrasher	23	Fokidis et al. 2008

5.4. Discussion

5.4.1. Are Australian Haemosporidians distinct?

Haemosporidians have been recorded on a wide variety of Australian birds (MacKerras and MacKerras 1960; Beadell et al. 2007; Averis et al. 2009; Zamora-Vilchis et al. 2012; Laurance et al. 2013) where the most commonly occurring haematozoa are species of *Haemoproteus*, *Leucocytozoon* and *Plasmodium*. These have a broad geographic distribution and infect a wide range of vertebrate hosts world-wide (Santiago-Alarcón et al. 2012), however information for Australia is still limited (Clark et al. 2014). My results suggest that parasite loads of this parasitic group is low compared to other parts of the world, although more extensive sampling is needed to fully explore this point.

This study provides evidence of the presence of avian Haemosporidians in natural and urban areas of eastern Australia. However, the four new lineages here reported suggest that the diversity of Haemosporidians is currently underestimated and together with other published research, suggests that further sampling ought to be undertaken to elucidate the evolutionary distinctiveness of Australian Haemosporidian parasites in a global context (Clark et al. 2014). Australian studies carried out in tropical (Beadell et al. 2004; Zamora-Vilchis et al. 2012) and temperate areas of the country (Balasubramaniam et al. 2013) have also revealed several unreported lineages of Haemosporidians as in this study. Four linages were detected in this study but their occurrence is differential. While SFW and RBF had

a higher prevalence of *Leucocytozoon* in natural areas, *Haemoproteus* was absent in urban areas (Table 5.2).

The absence of enough molecular research of Australian Haemosporidians does not allow us to do further analysis about the diversity of the group. For example, it is unknown if the diversity of lineages found in this study are exclusive to the eastern Australia because this seems to be the first molecular research of avian Haemosporidians in the region, so geographic distributions are unknown. However, at the present none of the *Leucocytozoon* or *Haemoproteus* lineages here reported have been found occurring in RBF from Tropical Australia mainland or Pacific islands (Beadell et al. 2007). Studies in tropical areas from Oceania have reported around 20 different lineages occurring in this region and perhaps no more than 10 lineages in temperate zones of the continent (Baillie and Brunton 2011; Balasubramaniam et al. 2013).

In Australia, most studies of avian haemosporidians have been carried out in natural and/or rural environments (Lawrence et al. 1946; MacKerras and MacKerras 1960; Adlar and O'Donoghue 1998; Adlard et al. 2004; Beadell et al. 2004; Beadell et al. 2007; Collombelli-Négrel and Kleindorfer 2008; Averis et al. 2009; Zamora-Vilchis et al. 2012; Laurance et al. 2013). However new descriptions, new host records and biogeographic extensions of bird haemosporidian parasites still take place (Adlar et al. 2002), suggesting the field is in its infancy. And, as shown in this study, even urban areas actually need better sampling.

5.4.2. Microscopy versus Molecular techniques for sampling Haemosporidians

While microscopy has been used for Haemoporidian studies and has been considered a powerful tool (Braga et al. 2011), other sensitive techniques are likely to improve the accuracy of prevalence and diversity estimates in natural and urban areas (see Delgado-V. and French 2012). For instance, PCR methods can provide a more sensible means of detecting prevalence and diversity of parasite lineages than microscopy and are particularly important in cases when there are low levels of parasitaemia or when the quality of smears is poor. Low correspondence between microscopy and molecular methods was detected in my study. Whereas the prevalence of SFW and RBF in urban areas was not detected by microscopy, it was 7.1% and 5% respectively using molecular analysis. Additionally, molecular screening in natural areas allowed the detection of *Leucocytozoon* and *Haemoproteus* in SFW and RBF. In Australia, the early research involved microscopy screening to detect haemosporidians (Lawrence 1946), however, the molecular approach has provided a significant expansion in our understanding with new unrecorded lineages in several passerines (Balasubramaniam et al. 2013, this study).

5.4.3. Are prevalence levels similar to other parts of the world?

The literature review found fewer studies in the Southern Hemisphere compared to the Northern Hemisphere (Balasubramaniam et al. 2013), especially in Australia (Clark et al. 2014), and this review brings this limited work together and complements other reviews

(Garamszegi 2011; Lacorte et al. 2013) carried out with Northern Hemisphere emphasis. The review identified that Australia has some of the lowest levels of parasitism, even in cities. There is no clear reason for this scenario since differences between continents (due to for example the composition and the occurrence of endemic hosts as is experienced in Australia) and inadequate baseline information on parasites from Austral regions may hamper our understanding of patterns of host infestation and parasite diversity.

Continentally, the prevalence of Haemosporidians in natural areas of Oceania seems to follow the same global pattern where prevalence decreases from tropical to temperate regions (Table 5.5). Similarly urban areas with tropical cities have more individuals with Haemosporidians than those sampled in temperate cities (Table 5.6). It has been suggested that this may be a result of a low abundance of vectors in temperate locations (Balasubramaniam et al. 2013).

5.4.4. Do urban areas have greater prevalence of Haemosporidians?

I detected a lower prevalence of Haemosporidians in urban areas compared to natural sites in Australia, a pattern that is corroborated in other areas around the world (e.g. Evans et al. 2009). What this study suggests is that cities are not areas where birds become seriously infected with parasites. In fact, they may be rather sterile environments. I suspect that changes in prevalence may be due to changes in abundance of vectors to spread these parasites in cities. Blood-sucking dipteran insects are the vectors of the haemosporidians. The primary vectors of avian

Plasmodium spp. are mosquitoes belonging among others to the genera *Culex*, *Aedes*, *Culiseta*, *Anopheles* and *Mansonia* whereas *Haemoproteus* parasites are transmitted by blood-sucking biting midges (Culicoides) and louse-flies, and *Leucocytozoon* uses blackflies (*Simulium* spp), or biting midges (*Culicoides* spp) (Santiago-Alarcón et al. 2012). I have found a significant lower prevalence of louse-flies in more urbanised areas, a group of flies that is recognised as one of the main vectors of *Haemoproteus* (Santiago-Alarcón et al. 2012; Delgado-V. and French unpub data). Therefore, it is imperative to extend sampling efforts for diversity, distribution and abundance of the insect vectors and a concomitant study of infections in both final hosts and vectors in different microhabitats, particularly near water, where the development of vectors might contribute to greater opportunities to be parasitised (Jansen et al. 2009; Balasubramaniam et al. 2013).

In natural areas, small passerines such as Superb-fairy wren, Red-browed finch, Eastern spinebill and Silvereye were found to be infected with haemosporidians confirming other studies (e.g. Adlar et al. 2004; Collombelli-Négrel and Kleindorfer 2008; Zamora-Vilchis et al. 2012). Therefore I recognise these birds as potential bird models to study long-term (seasonal) parasitological studies comparing urban and natural areas. Furthermore, other host species sampled and analysed by microscopy in this study need further work to determine if the low prevalence recorded is a result of the poor performance of microscopy or whether these species have low prevalence of parasitic fauna. For example, Kleindorfer et al. (2006) and Laurance et al. (2013) reported *Haemoproteus* in New holland honeyeaters and Golden whistler, respectively. Absence of blood parasites in these

two species in urban areas might be interpretable by the low number of individuals sampled, an actual low prevalence or a result of the use of microscopy to detect presence.

Leucocytozoon in Australia has been found in natural and rural areas (Adlard et al. 2004) in some of the bird species sampled here. Recently, Beadell et al. (2007) found *Leucocytozoon* parasiting Red-browed finch and Zamora-Vilchis et al. (2012) reported it infecting Lewin's honeyeater. During this study, RBF and SFW were the only hosts found parasitized by *Leucocytozoon* in urban areas (Table 5.2), however we need further studies with bigger samples to determine if Haemosparasites of the genus *Leucocytozoon* are really absent in urban individuals of most passerines.

5.5. Conclusions

Human-induced changes may threaten wild populations not only through habitat loss, but by disturbing inter and intraspecific interactions. For this reason, it is essential to understand how anthropogenic habitats affect host-parasite dynamics and define the underlying factors that may act to modifying these interactions. With cities growing globally, and urban diversity becoming important in the conservation of biodiversity (Kantsa et al. 2013), a better understanding of the types of parasites, the mechanisms that influence their prevalence and their impact is needed. Understanding the distributions of parasites in an urbanised world can reveal patterns of parasite prevalence within particular habitats and in comparison

to other habitats of different habitat structure. Such differences could shed light on landscape- or patch-level biotic or abiotic factors that might influence exposure to these blood parasites.

CHAPTER SIX: SPATIAL CHANGES IN HEALTH PARAMETERS AND BODY CONDITION OF URBAN SENSITIVE BIRDS ALONG AN URBAN GRADIENT

6.1. Introduction

The diversity of wildlife is negatively correlated with human activities and infrastructure derived from urban development (Gaston et al. 2010). However, while some bird species survive and prosper in urbanised areas (Urban Tolerant Birds, UTB hereafter), others are affected by a wide range of anthropogenic activities and infrastructure (Urban Sensitive Birds, USB hereafter) (Parsons et al. 2006). An increase in some kinds of food and water, loss of suitable vegetation, reduced presence of predators, fewer or limited competitive interactions, and a changed microclimate are some of the most cited factors potentially contributing to the inclusion or exclusion of birds in cities (Fokidis et al. 2008), however, they do not explain (or characterise) fully the presence, establishment and success of some native species that thrive in urban environments. A number of other factors that are more cryptic and unstudied may also be important. For example, parasite and pathogen exposure (see previous chapters, Bradley and Altizer 2006) and susceptibility are factors that may influence urban wildlife (Delgado-V. and French 2012).

Urban habitat fragmentation and associated anthropogenic pollution may make an environment more stressful for wildlife. Human disturbance (Fernández- Juricic 2002), introduced species (Klotz and Kühn 2010), road networks (Delgado-V. 2007, 2014), residential developments (Lussier et al. 2006), and pollution (Burger et al. 2004) are

anthropogenic pressures which affect wildlife. Prolonged or frequent increases in the number of stressors in the urban environment could result in lowering of health (such as haematological measures and body condition) which can exacerbate the susceptibility to parasite infections and immune-mediated diseases, resulting in reproductive and behavioural problems (Ruiz et al 2002; Martin and Boruta 2013).

Haematological measures provide useful indicators of physiological status and have long been used as indicators of metabolic activity, disease, stress and nutritional status. Haematological parameters have been successfully used in combination with other condition measures to document wildlife health differences in altered environments (Stevenson and Woods 2006). When combined with health indices, haematological condition and morphological measures are strong parameters of relative health status of individuals and populations, and can be used as an indicator of the physiological status of individual birds and how this varies with habitat and behaviour (Brown 1996).

Measuring changes in haematological and body condition are useful in assessing whether birds are under increased stress. Such changes are likely to result in reduced success and persistence of individuals and local populations (Romero 2004). Chronic stress, health and body condition can potentially reveal important patterns in risk of mortality (Romero 2002). Therefore, to understand the decline of birds as a result of urbanisation it is crucial to collect and monitor the health and condition of individuals and populations (Varela et al. 2006), although this has been poorly studied (Delgado-V. and French 2012).

The relationship between habitat fragmentation and health remains largely unexplored

even though some recent research has shown that bird health does change with land use (Maute et al. 2013). No study, to date, has investigated health in passerines occupying Australian cities. This chapter seeks to describe the body condition along an urban gradient for two USB in Australia: the Red-browed finch and the Superb-fairy wren.

I monitored six haematological and body condition parameters to check if immune system investment and body condition decrease in more urbanised areas for both birds. Specifically, I tested the hypothesis that since urban areas are considered to offer less optimal conditions, birds in more urbanised areas (such as industrial areas) would have higher leukocyte counts, haematocrit, and H/L ratio. Additionally, I tested if individuals inhabiting less urban developed sites (such as natural national parks) have a higher body mass and haemoglobin concentrations.

6.2. Methods

6.2.1. Field sites

I caught birds at five sites along a gradient of urbanisation within and around Wollongong, NSW, Australia. Field work was carried out in the Illawarra region, New South Wales. The Illawarra includes the coastal land mass east of the escarpment between southern Sydney and the Shoalhaven River where four study sites were selected for having different levels of urbanisation (urbanisation index UI based on Gómez et al. (2008) and Hamer et al. (2012) index (described in chapter 3):

1. Heavily urbanised and industrial areas. These areas are situated in the city and the

surrounding industrialised zone. This area was Green House Park (hereafter GHP, 34˚ 26’ 31” S 150˚ 53’ 31” E), a small park situated between the industrial and commercial centre of the city which exhibits high traffic streets and scarce trees and bushes, UI = 48.41%.

2. Residential areas and urban parks. These are suburban areas where houses are surrounded by gardens and street trees. Dalton Park (hereafter DP, 34˚ 23’ 47” S 150˚ 53’ 55” E) was a city park surrounded by a matrix of house blocks with variable amounts of mowed grass and ornamental shrubs with occasional large trees, UI = 41.83%.

3. Woodland edge. Ecological Research Centre (hereafter ERC, 34˚ 24’ 17” S 150˚ 52’ 15” E) was a woodland edge surrounded by human altered habitats (e.g. urban gardens, paddocks and edge habitats), where there is a dense, shrubby understorey, UI = 28.12%;

4. Sub-natural and Natural areas. These include extensive vegetation with low levels of intervention located in National Parks, Nature Reserves, and State Recreation Areas. A Natural area was the Murramarang National Park (hereafter MNP, 35˚ 32’ 46” S 150˚ 21’ 49” E), which is situated ca. 300 km (UI = 1.05%); and Mount Keira (hereafter MK, 34˚ 24’ 48” S 150˚ 51’ 19” E) was a sub-natural area (UI = 12.23%).

I sampled Red-browed finches *Neochima temporalis* (RBF, hereafter) and Superb-fairy wrens *Malurus cyaneus* (SFW, hereafter) which were captured along the urban gradient (see Chapter 4 for details about the biology and natural history of the birds).

6.2.2. Sampling

Field work was conducted over two years in breeding and non-breeding seasons from January 2012 to August 2013. Birds were caught using mist-nets and transported in a clean cotton bag to a nearby processing station where they were measured and a blood sample was taken.

6.2.3. Body condition

Individual right tarsus length was divided by individual weight and this result was used as an indicator of condition (Stevenson and Woods 2006). Tarsus length is considered as a general estimate of an individual's size and individuals with a greater amount of body mass for a given size are thought to have greater energy reserves and better body condition (Green 2001).

Bill and head length is the size of the bird’s head and bill taken from the tip of the bill until the base of head. It was measured with a caliper. The bird’s age and sex were recorded if identifiable.

6.2.4. Haematological condition

A blood sample was taken by venipuncture of the brachial vein with a 26 gauge needle. The quantity of blood taken varied with bird size but it was no more than 2% of the weight of the bird and depending on quantity and quality it was used for analysing haematocrit, haemoglobin, H/L ratio and/or TLC.

6.2.4.1. Haematocrit levels and Haemoglobin concentrations

Up to 50 microliters was collected into a heparinised tube. At the end of each day of field sampling, blood contained in 50 microliter capillaries was centrifuged for 5 min at 13000 rev/min. Haematocrit was expressed as the percentage of the tube containing packed red cells and it is a measure of the efficiency of oxygen exchange in an individual. It can identify individuals who may be anaemic (Booth and Elliot 2003) and is therefore a useful measure of a bird's nutritional state.

A portion of blood sample was also used to measure haemoglobin concentration using Portable HemoCue Hb 201+ (Hemocue, Angelholm, Sweden). High Hb can indicate a large blood oxygen carrying capacity, which could enhance metabolic performance (Lill et al. 2013).

6.2.4.2. Heterophil:Lymphocyte ratio and total leucocyte count

Blood was smeared onto a microscope slide and white blood cells identified and counted. These identify the strength of the immune system (Davis et al. 2008). Heterophyll:Lymphocyte is used as an index of chronic stress (Fokidis et al. 2008) and has been shown to both correlate with corticosteroid levels and reliably reflect chronic stress in various bird species (Davis et al. 2008; Dehnhard et al. 2011). Heterophylls are the primary phagocytic leukocytes which proliferate in response to infections, inflammation and stress, while leukocyte are involved in a variety of immunological functions such as immune defence (Davis et al. 2008). The ratio was calculated by

dividing the number of heterophils seen at 80 fields of view under a microscope by the number of lymphocytes seen at the same number of fields. Heterophils and Lymphocytes were identified according to the criteria of Clarck et al. (2009). Total Leucocyte Count (TLC) was calculated by counting the sum of all leukocytes observed in 80 fields of view. It informs about health and strength of the immune system in an individual (Davis et al. 2008).

6.2.5. Statistical analysis

Data were tested for normality (Shapiro-Wilk test) and homogeneity of variance (Levene's test). I normalised Haematocrit levels using log transformations. Body and blood conditions that were not normally distributed even after transformation were compared for each species separately using non-parametric tests (Kruskall-Wallis). Post-hoc comparisons between sites were multiple compared using Bonferroni correction. In the few cases where conditions were found to be normally distributed (such as RBF Haematocrit levels in breeding season), parametric tests (such as ANOVA or t-student tests) were used for analysing differences among sites. Wilcoxon rank sum test (or ANOVA if normally distributed) was used to test the influence of season and sex. If no differences were found, all data were pooled and compared along the urbanisation gradient with no discrimination between season or sex. Differences between sexes could not be distinguished in the Red-browed finch so only season and site were included for this species.

6.3. Results

6.3.1. Body condition

6.3.1.1. Body mass

In RBF, body mass was lower in GHP (p value adjustment method: bonferroni $p<0.05$) (Figure 6.1a). In SFW, however, no difference was detected among sites (Kruskal-Wallis rank sum test, Kruskal-Wallis chi-squared = 4.7991, df = 4, p-value = 0.3085) (Figure 6.1b).

6.3.1.2. Head and bill length

RBF exhibited higher values of head and bill length at the area with less urban influence (MNP). These values were significant higher than in industrial areas (GHP) ($p<0.01$) and even higher than in subnatural areas (MK) ($p<0.01$) (Figure 6.2a). Contrary, SFW had no differences among sites (Kruskal-Wallis chi-squared = 3.7406, df = 4, p-value = 0.4422) (Figure 6.2b).

6.3.2. Haematological condition

6.3.2.1. Haemoglobin concentrations

RBF had the highest levels of Haemoglobin in natural areas (MNP) and the lowest in the most urbanised area (GHP); these sites were significant different to other sites along

the urbanisation gradient ($p<0.05$) (Figure 6.3a). No differences among sites for SFW were detected (Kruskal-Wallis chi-squared = 9.1057, df = 4, p-value = 0.05851) (Figure 6.3b).

6.3.2.2. Haematocrit

There were significant differences between seasons (higher in breeding season) for RBF (t = 2.9242, df = 160, p-value = 0.0040), however, there were no differences among sites within each season (breeding season: ANOVA F= 0.316, p-value= 0.867 [Figure 6.4a], and non breeding season: Kruskal-Wallis chi-squared = 0.912, df = 4, p-value = 0.9228 [Figure 6.4b]). Differences were not found for SFW (Kruskal-Wallis chi-squared = 0.912, df = 4, p-value = 0.9228 [Figure 6.4c]).

6.3.2.3. H/L ratio

RBFs captured in the industrial area (GHP) had the highest values of H/L ratio, which were significantly different than the average condition in finches from the natural area (MNP) ($p<0.005$) (Figure 6.5a). For SFW, there were no differences between sex in non-breeding season (W = 1042.5, p-value = 0.602), however, some significant differences were detected for breeding season. For example, females (Figure 6.5b) and males (Figure 6.5c) presented the highest H/L ratio values in GHP which is significant different from other places such as MK, ERC, and DP ($p<0.05$).

6.3.2.4. Total Leukocyte Count

In RBF, one of the lowest values was found at the natural area (MNP) which is significant different with the ones recorded in other study sites (Figure 6.6a). No differences among sites for SFW were detected (Kruskal-Wallis chi-squared = 4.6494, df = 4, p-value = 0.3252) (Figure 6.6b).

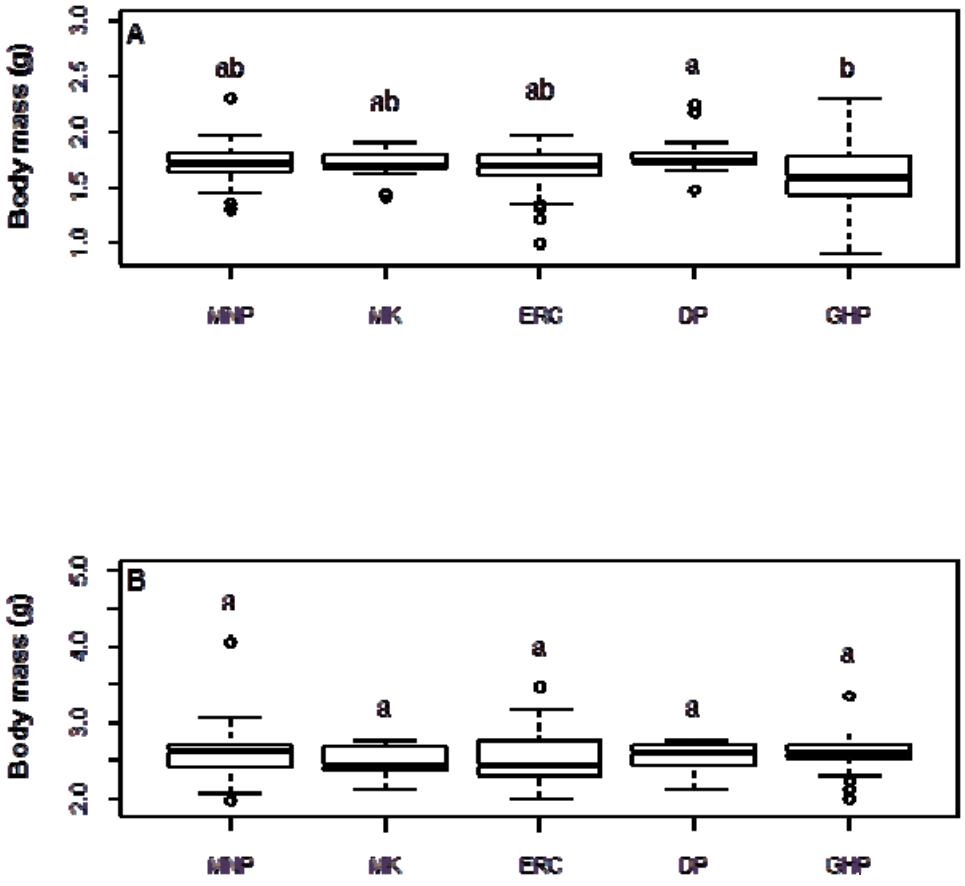

Figure 6.1. Variation of average body mass of **a.** Red-browed finch and **b.** Superb-fairy wren along sites with different degrees of urbanisation development. Different letters indicate significant differences ($p<0.05$).

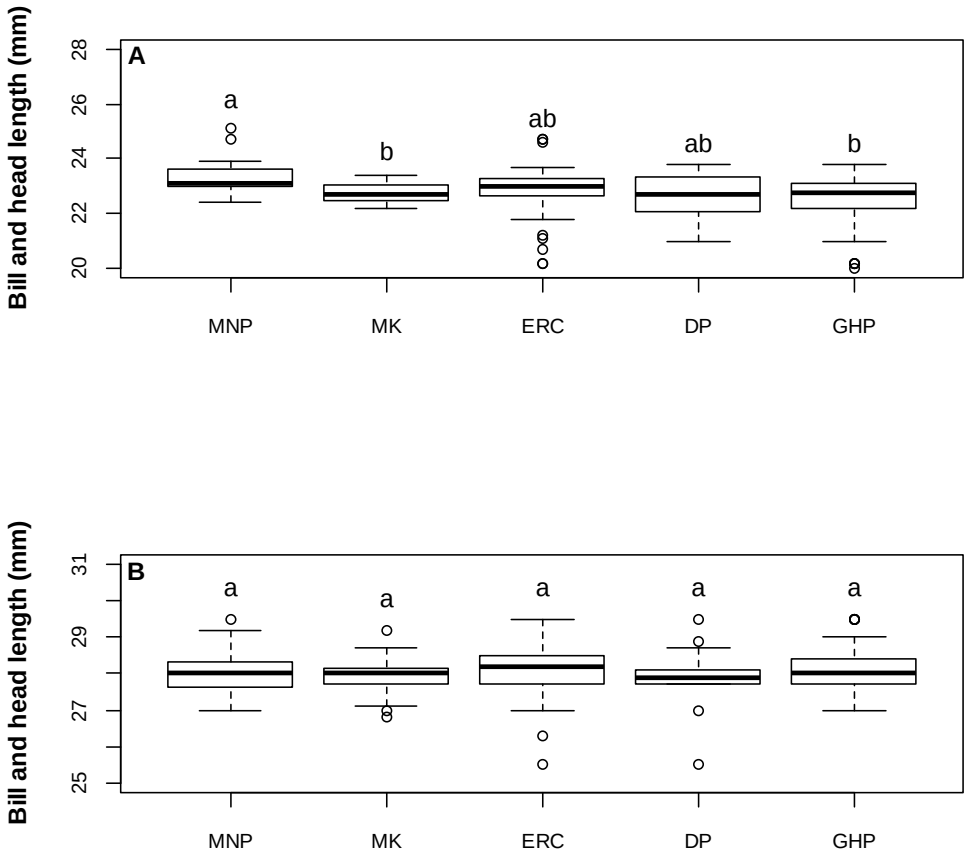

Figure 6.2. Variation of average head and bill length of **a.** Red-browed finch and **b.** Superb-fairy wren along sites with different degrees of urbanisation development. Different letters indicate significant differences ($p<0.05$).

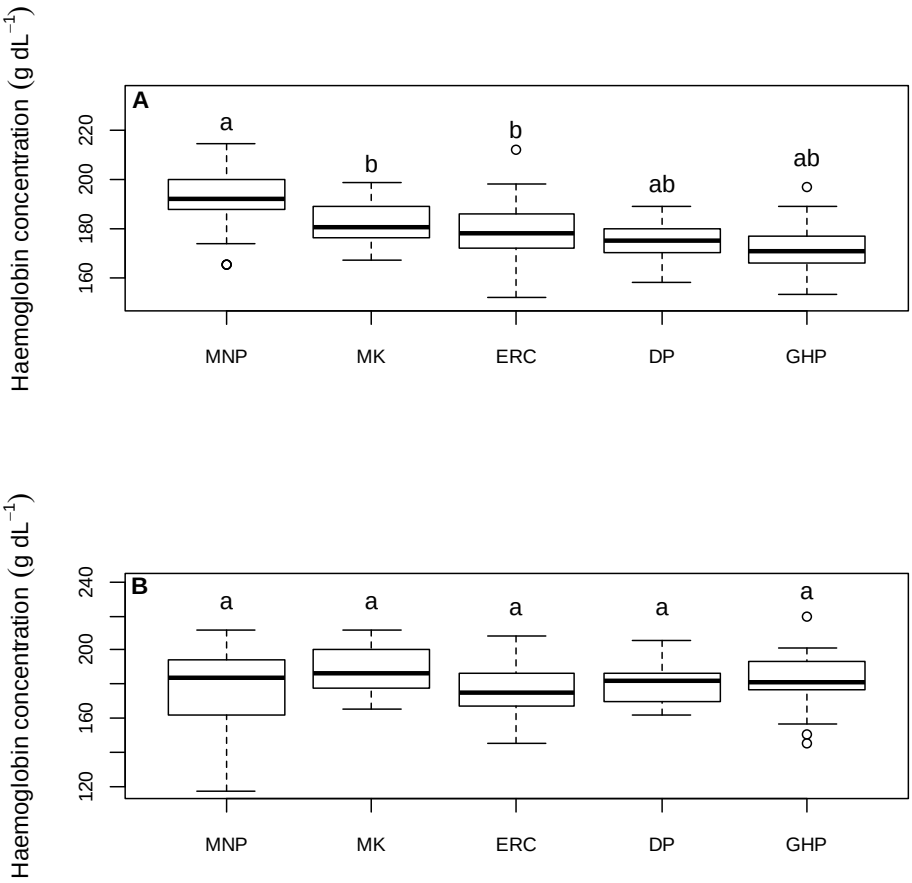

Figure 6.3. Variation of average Haemoglobin concentration (g dL^{-1}) of **a.** Red-browed finch and **b.** Superb-fairy wren along sites with different degrees of urbanisation development. Different letters indicate significant differences ($p<0.05$).

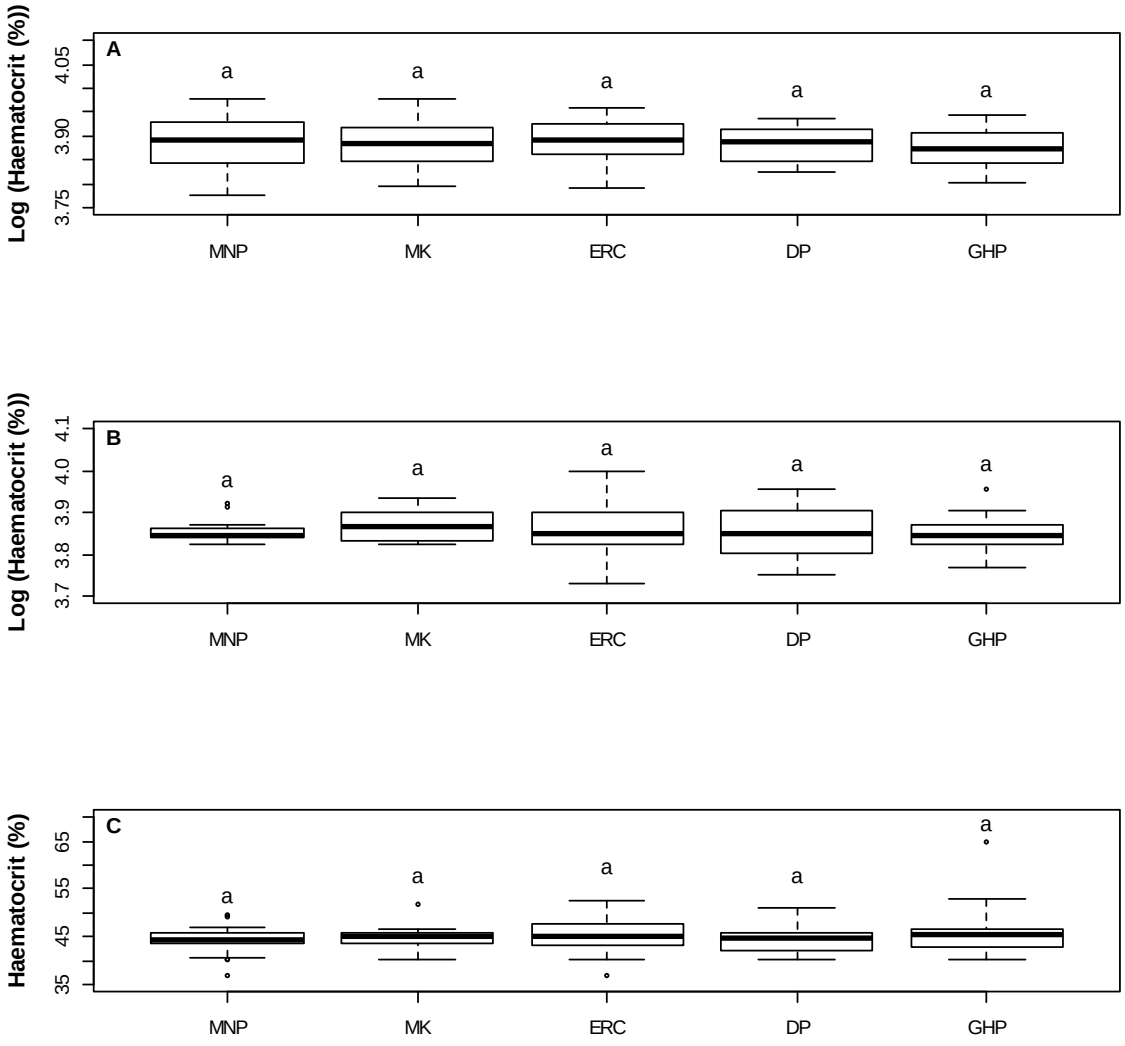

Figure 6.4. Average Haematocrit level of Red-browed finch in **a.** Breeding and **b.** Non Breeding season, and **c.** Superb-fairy wren along sites with different degrees of urbanisation development. Different letters indicate significant differences ($p<0.05$).

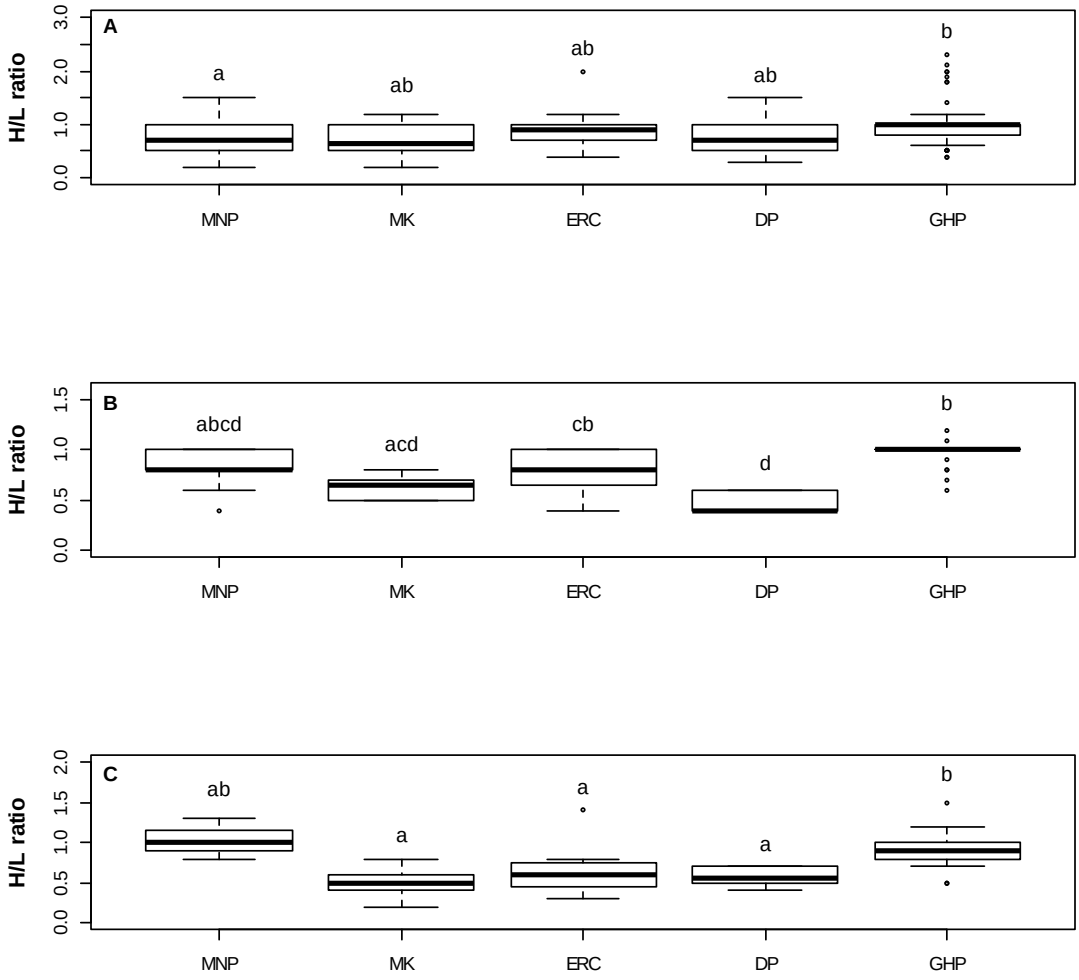

Figure 6.5. H/L ratio of **a.** Red-browed finch and **b.** Superb-fairy wren females and **c.** males in Breeding season along sites with different degrees of urbanisation development. Different letters indicate significant differences ($p<0.05$).

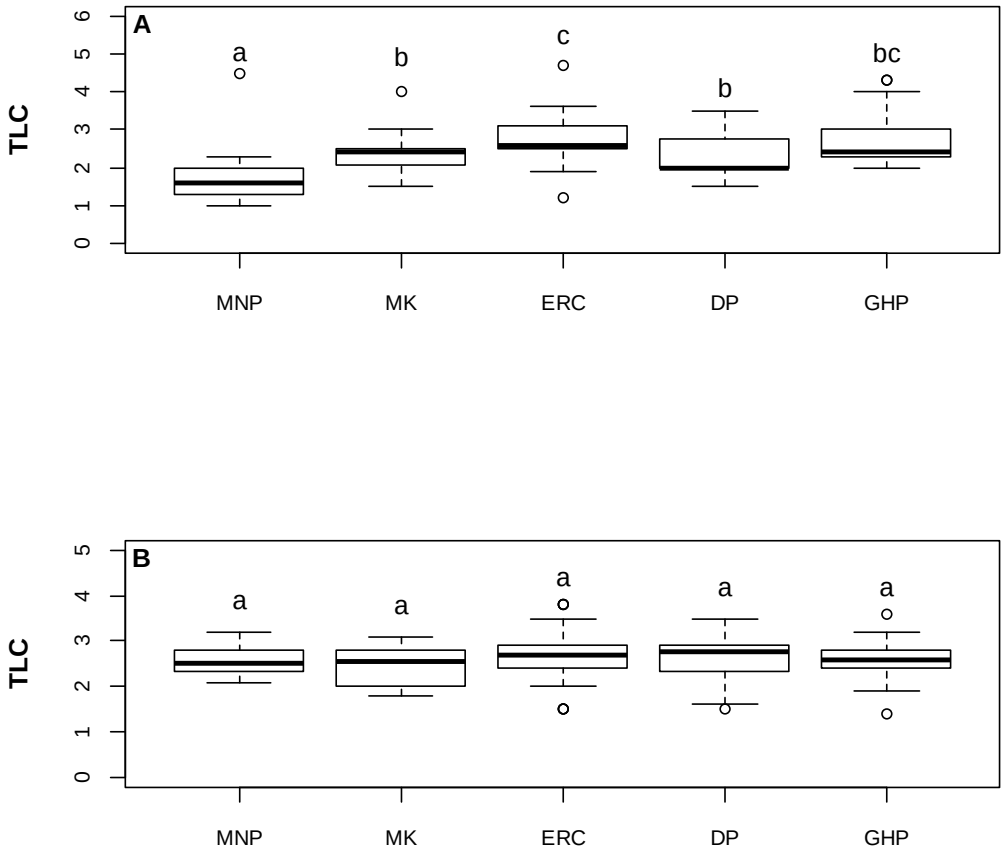

Figure 6.6. Variation of Total Leukocyte Count of **a.** Red-browed finch and **b.** Superb-fairy wren along sites with different degrees of urbanisation development. Different letters indicate significant differences ($p<0.05$).

6.4. Discussion

In this study, I examined the relationships of urban development with health status and body condition to explore how these mechanisms might be involved in the decline of some small passerines in urban areas from Australia. I had predicted that if urban areas are contributing to the decline of small passerine birds, evidence of a negative relationship between urban development and health and body condition should be found. Although I sampled several different measures that are commonly used, I only detected significant differences for Red-browed finch. Excepting Haematocrit, I found significant differences in body and blood conditions for 5 out of 6 considered measurements for RBF. In this species, I particularly found a significant decrease in Haemoglobin concentration as well as a higher H:L ratio in more urbanised areas compared to natural areas, and a general increase of Total Leukocyte Count in more urbanised locations. Contrary, significant differences for SFW were found in just one condition (i.e. H/L ratio) and restricted to breeding season.

Changes in leucocyte profiles and haemoglobin levels can indicate stress that has detrimental effects in some animals living in cities. For example, body and haematological conditions such as Haemoglobin concentrations, can change due to effects of environmental variation, including habitat fragmentation and habitat quality on individual condition in wild passerines (Davis et al. 1998; Hõrak et al. 1998) and small mammals (Johnstone et al. 2011), which can be connected to individual persistence (Dubiec and Cichon 2001) and breeding success (Gustafsson et al. 1994). Therefore, while some animals seem to adapt to live in disturbed landscapes, others often decline and are less likely to persist in urban landscapes.

Previous research on Australian mammals has shown that chronic stress due to anthropogenic activity could contribute to a decline of certain mammals since they can cause physiological stress and make them more susceptible to pathogens, diseases and parasites (Johnstone et al. 2010). These authors also found that anthropogenic habitat fragmentation has detrimental effects on several indicators of population health in the agile antechinus (*Antechinus agilis*) in urban environments. Brearley et al. (2012) found that individual squirrel gliders (*Petaurus norfolcensis*) had lower hair cortisol levels in forest interiors compared to those living in habitats adjacent to urban edges. These results are consistent with previous studies on other mammals in relation to other non-urban activities and influence such as those carried out in howler monkeys (*Aloutta pigra*) (Martínez-Mota et al. 2007). In birds, however, studies of the relationship between urbanisation and individual condition have suggested that health parameter might be more variable and complex.

Some studies have reported patterns where urban individuals have lower condition than rural ones (e.g. House sparrow, Liker et al. 2008). Similarly, urban European blackbirds had lower fat reserves than rural birds (Partecke et al. 2005), and rural American crows were substantially larger and presumed healthier than urban crows (Heiss et al. 2009). Additionally, Ots et. al. (1998) found lower haematocrit levels for birds breeding in urban versus rural habitats. In addition, in urban areas, Rufous-collared sparrows have lower body weight, higher blood glucose concentration, higher proportion of heterophils, lower proportion of lymphocytes, and consequently, a larger H:L stress index, than rural ones (Ruiz et al. 2002). In comparisons with natural habitats, some variable responses have also been shown. Ovenbirds (*Seiurus aurocapillus)* nesting in

continuous forest habitat had higher mass and haematocrit and normal immune system activity that individual in fragmented forests which were lighter, had lower haematocrits, and may have had depressed immune responses (Mazerolle and Hobson 2002). Contrary, others have found that urban individuals are in better condition than their natural or rural conspecifics (Cypher and Frost 1999) or have documented no significant differences in birds between intact and altered habitats (Gavett and Wakeley 1986) suggesting that these effects might vary from species to species according to the energetic and/or social needs of organisms.

One of the factors that may be causing contrasting results is the kind of species investigated. For instance, species which are common in cities with relatively stable or increasing population sizes may be advantaged by increased immunity whereas species which are now rare in urban environments and declining in abundance may be more susceptible. Thus different species of birds may have different susceptibility to parasite load thus influencing their ability to occupy urban centres (Bonier 2012). This research suggests that responses might be variable, even within the group of urban sensitive birds. For example, Chávez-Zichinelli et al. (2013) studied the Canyon Towhee (*Melozone fusca*) and Inca Dove (*Columbina inca*) and found that physiological variables differed between the species and habitat condition. Similarly, authors found that immunoglobulin concentration in *C. inca* was higher at urban sites, whereas it was lower in *M. fusca,* therefore they might exhibit differential health effects due to urbanisation processes.

RBF and SFW are considered not to be abundant in urban areas and affected by urbanisation processes. Parsons et al. (2006) found that RBF is about half as common as

SFW in urban areas. While SFW occurs in about 25% of gardens, RBF were only recorded in about 11-12%. Data obtained from my research suggests that this may be associated with RBFs being more stressed and having lower body condition.

Health differences recorded in this study might depend on species and life history stage, particularly on their type of diet and foraging strategy. A lower Hb concentration and a higher H/L ratio and TLC values in more urbanised areas for RBF may result from the higher energy demands imposed by the urban habitat. For instance, as RBF mostly feeds on grass seeds, the fragmentation of the habitat in urban zones, as well as the increased area of pavement, infrastructure, and residences in the urban environment will lower food resources and should impose a greater metabolic pressure in these birds. Therefore in urban areas, food supplies are possibly more commonly low for RBF than for SFW, so RBF are restricted to parts of the city where there is adequate supplies of grass seeds.

The idea that urban birds are stressed by food shortage has been hypothesised for other granivorous birds. For example, Chilean rufous-collared sparrows (*Zonotrichia capensis*) from urban localities had body weights significantly lower than that of rural ones (Ruiz et al. 2002). However, factors other than differences in food availability may account for the changes. An additional factor that may help to explain differences in body mass and health parameters between SFW and RBF is the effect of the presence of some parasites. While I found that Coccidian parasites are more prevalent in urban RBF while urbanisation increases, prevalence is not significantly different along urban gradients for SFW (see Chapter 4).

6.5. Conclusions

According to the results, the direction and magnitude of these differences indicate that that while SFW might have a greater resistance to parasite infections, pathogens and diseases in urban areas, the immune system of urban RBF might not have the optimal condition to respond successfully to urban pressures in cities. Also I consider that health parameters make suspect that RBF is more prone to have higher infestation by parasites since it is in poorer health and body conditions in the urban populations than SFW.

CHAPTER SEVEN: CONCLUSION, GENERAL DISCUSSION AND FUTURE DIRECTIONS

Changes in host-parasite interactions have the potential to be one of the most important consequences of urbanisation. However, this research found that bird-parasite interactions in urban areas are one of the relationships less studied globally, particularly in Australia (Chapter 1), a territory that exhibits a unique evolutionary history of isolation. Consequently there may be unique lineages of parasites with different patterns of prevalence compared to the rest of the world.

In order to increase our understanding of diversity, prevalence, and distribution of parasites in Australia and Oceania, and how bird-parasite relationships may be affected by human induced changes along an urbanisation gradient in New South Wales, this thesis focused on trying to understand a possible pattern of spatial dynamics of three types of parasites (ectoparasites, Coccidians and Haemosporidians). These parasites have different biologies and ways of transmission. Additionally, this research sought to describe body and blood conditions, as well as susceptibility along the gradient of their passerine hosts.

The results found that some parasites, such as louse flies and ticks (Chapter 3) as well as haemosporidians (Chapter 5), are less prevalent (or even disappear) while others might be either more prevalent (e.g. Coccidian parasites, Chapter 4) or stable in urban areas (e.g. lice, Chapter 3). However, this study was unable to detect a general pattern of loss of body or blood condition associated with urban development for the species sampled (Chapter 6), suggesting that urbanisation effects are complex and might be both site-

parasite- and host-specific related. Thus, future avian health surveys and disease controls should be implemented as strong variation and pathogen pressures are known to fluctuate.

As corroborated in this thesis (four new lineages of haemosporidians were found indicating the great potential for Australian bird parasites to be fundamentally unique, Chapter 5), the insufficient knowledge on Australian parasite fauna urges inventories and systematic monitoring schemes. It is also important to conduct detection of vectors and intermediate hosts since this information does not exist for many of the parasites recorded herein.

Moreover, parasites sampled during this research are not the only parasites that infest bird hosts: there are other groups that should be considered in the future which are more likely to respond differently due to variable environmental conditions (e.g. Trichomonas). Certainly, the inclusion of molecular diagnosis should be incorporated in urban screening, since intensity of parasite infection could be below the limit of detection via regular techniques.

There is a growing number of tests used to measure variation and possible effects of parasites on avian hosts (Boughton et al. 2011). The list includes behavioural, immunological and physiological tests. In order to gain a comprehensive idea about body condition, immune responses and differential parasitism, a diverse selection of tests should be considered (Pedersen and Fenton 2006).

Urban ecology should articulate its knowledge on species with the study, understanding

and conservation of biotic relationships. However, while studies of urbanisation on biodiversity structure and composition have been numerous in recent years, few of them emphasise the effects of urbanisation on ecological and evolutionary processes and how they maintain and conserve biodiversity within urban environments. What it is needed in the future is an integrative field of study stemming from ecological and physiological approaches, using distribution of parasites along urbanisation gradients, and exploring the potential role of parasites and immune status in determining the structure of urban-bird communities.

REFERENCES

1. Adlard, R.D., Peirce, M.A., Lederer, R., 2002. New species of *Leucocytozoon* from the avian families Otidae, Podargidae, and Threskiornithidae. Journal of Natural History. 36, 1261-1267.
2. Adlar R.D., O'Donoghue, P.J., 1998. Perspectives on the biodiversity of parasitic protozoa in Australia. International Journal of Parasitology. 28, 887-897.
3. Adlard, R.D., Peirce, M.A., Lederer, R., 2004. Blood parasites of birds from south-east Queensland. Emu 104, 191-196.
4. Alekseev, A.N., 2006. The effects of global climatic changes on bloodsucking ectoparasites and pathogens they transmit. Vestn. Ross. Akad. Med. Nauk. 3, 21-25.
5. Alvey, A.A., 2006. Promoting and preserving biodiversity in the urban forest. Urban Forestry and Urban Greening. 5, 195-201.
6. Aronstein, K.A., Saldivar, E., Vega, R., Westmiller, S., Douglas, A.E., 2012. How Varroa Parasitism affects the immunological and nutritional status of the Honey Bee, *Apis mellifera*. Insects. 3, 601-615.
7. Arzúa M., Barros-Battesti, D.M., 1999. Parasitism of *Ixodes* (*Multidentatus*) *auritulus* Neumann (Acari: Ixodidae) on birds from the city of Curitiba, State of Paraná, southern Brazil. Memórias do Instituto Oswaldo Cruz. 94, 597-603.
8. Arzúa, M., Navarro da Silva, M.A., Famadas, K.M., Beati, L.,Barros-Battesti, D.M., 2003. Amblyomma aureolatum and *Ixodes auritulus* (Acari: Ixodidae) on birds in southern Brazil, with notes on their ecology. Experimental and Applied Acarology. 31, 283–296.
9. Atkinson, C.T., Thomas, N.J., Hunter, D.B. 2008. Parasitic diseases of wild

birds. Blackwell, Iowa.

10. Atkinson, C.T., Utzurrum, R.C., Seamon, J.O., Savage, A.F., Lapointe, D.A., 2006. Heamatozoa of forest birds in American Samoa – evidence for a diverse, indigenous parasite fauna from South Pacific. Pacific Conservation Biology. 12, 229-237.
11. Atkinson, C.T., Woods, K.L., Dusek, R.J., Sileo, L.S., Iko, W.M., 1995. Wildlife disease and conservation in Hawaii: pathogenicity of avian malaria (*Plasmodium relictum*) in experimentally infected Iiwi (*Vestiaria coccinea*). Parasitology. 111, 59-69.
12. Averis, S., Thompson, R.C.A., Lymbery, A.J., Wayne, A.F., Morris, K.D., Smith, A., 2009. The diversity, distribution and host parasite associations of trypanosomes in western Australian wildlife. Parasitology. 136, 1269-1279.
13. Baillie, S.M., Brunton, D.H., 2011. Diversity, distribution and biogeographical origins of *Plasmodium* parasites from the New Zealand bell bird (*Anthornis melanura*). Parasitology. 138, 1843-1851.
14. Bairlein, F., 1985. Body weights and fat deposition of paleartic passerine migrants in the central Sahara. Oecologia. 66, 141-146.
15. Balasubramaniam, S., Mulder, R.A., Sunnuckes, P., Pavlova, A., Amos, J.N., Melville, J., 2013. Prevalence and diversity of avian haematozoa in three species of Australian passerine. Emu. 113, 353-358.
16. Bar, M.E., Oscherov, E.B., Damborsky, M.P., 1993. Presencia de Triatoma sordida Stal, 1859 en ecotopos urbanos de la ciudad de Corrientes, Argentina (Presence of *Triatoma sordida* Stal, 1859 in Corrientes city urban ecotopes). Revista de Saude Publica. 27, 117-122. [in Spanish]
17. Bardach, H., 1981. Acariasis due to *Dermanyssus gallinae gamasoidosis* in

Vienna, Austria. Zeitschrift fuer Hautkrankheiten. 56, 21-26.

18. Barros-Battesti D.M., Arzua M., Pichorim M., Keirans J.E., 2003. *Ixodes* (Multidentatus) *paranaensis* n. sp. (Acari: Ixodidae) a parasite of *Streptoprocne biscutata* (Sclater, 1865) (Apodiformes: Apodidae) birds in Brazil. Memórias do Instituto Oswaldo Cruz. 98, 93–102.
19. Bart, A., Wentink-Bonnena, E.M., Heddema, E.R., Buijs, J., Gool, T., 2008. Frequent occurrence of human-associated microsporidia in fecal droppings of urban pigeons in Amsterdam, The Netherlands. Applied and Environmental Microbiology. 74, 7056-7058.
20. Basto, N., Rodríguez, O.A., Marinkelle, C.J., Gutiérrez, R., Matta, N.E., 2006. Haematozoa in birds from La Macarena National Park (Colombia). Caldasia. 28, 371-377.
21. Beadell, J.S., Gering, E., Austin, J., Dumbacher, J.P., Peirce, M.A., Pratt, T.K, Atkinson, C.T., Fleischer, R.C., 2004. Prevalence and differential host-specificity of two avian blood parasite genera in the Australo-Papuan region. Molecular Ecology. 13, 3829-3844.
22. Beadell, J.S., Atkins, C., Cashion, E., Jonker, M., Fleischer, R.C., 2007. Immunological change in a parasite-impoverished environment: divergent signals from four island taxa. PLoS One. 9, 1-10.
23. Belo, N.O., Pinheiro, R.T., Reis, E.S., Ricklefs, R.E., Braga, E.M., 2011. Prevalence and lineage diversity of avian haemosporidians from three distinct cerrado habitats in Brazil. PLoS One. 6, 1-8.
24. Belo, N.O., Rodríguez-Ferraro, A., Braga, E.M., Ricklefs, R.E., 2012. Diversity of avian haemosporidians in arid zones of northern Venezuela. Parasitology. 139, 1021-1028.

25. Benedikt, V., Barus, V., Capek, M., Havlicek, M., Literak, I., 2009. Blood parasites (*Haemoproteus* and microfilariae) in birds from the Caribbean slope of Costa Rica). Acta Parasitologica. 54, 197-204.

26. Bennett, G.F., Garvin, M., Bates, J.M., 1991. Avian hematozoa from west-central Bolivia. Journal of Parasitology. 77, 207-211.

27. Bennett, G.F., Greiner, E.C., Whiteley, P.L., Norman, F.I., 1977. Blood parasites of some waterfowl from Victoria, Australia. Journal of Wildlife Diseases. 13, 202-204.

28. Bensch, S., Hellgren, O., Perez-Tris, J., 2009. MalAvi: a public database of malaria parasites and related haemosporidians in avian hosts based on mitochondrial cytochrome b lineages. Molecular Ecology Resources. 9, 1353-1358.

29. Bentz, S., Rigaud, T., Barroca, M., Martin-Laurent, F., Bru, D., Moreau, J., Faivre, B., 2006. Sensitive measure of prevalence and parasitaemia of haemosporidia from European blackbird (*Turdus merula*) populations: value of PCR-RFLP and quantitative PCR. Parasitology. 133, 685-692.

30. Biamonte, E., Sandoval, L., Chacón, E., Barrantes, G., 2011. Effect of urbanization on the avifauna in a tropical metropolitan area. Lanscape Ecology. 26, 183-194.

31. Blair, R.B., 1996. Land use and avian species diversity along an urban gradient. Ecological Applications. 6, 506-519.

32. Boal, C.W., Mannan, R.W., Hudelson, K.S., 1998. Trichomoniasis in Cooper's hawks from Arizona. Journal of Wildlife Diseases. 34, 590-593.

33. Boid, E.M., 1951. The external parasites of birds. The Wilson Bulletin 63, 363-369.

34. Bonier, F. 2012. Hormones in the city: endocrine ecology of urban birds. Hormones and Behavior. 61, 763-772.

35. Booth, C.E., Elliott, P.F., 2002. Hematological responses to hematozoa in North American and neotropical songbirds. Comparative Biochemistry and Physiology - Part A: Molecular & Integrative Physiology. 133, 451-467.

36. Boughton, R.K., Joop, G., Armitage, S.A.O. 2011. Outdoor immunology: methodological considerations for ecologists. Functional Ecology. 25, 81-100.

37. Bouttefroy, A., Rosset, A., Lemaître, J.P., 1997. Prevalence of *Listeria* sp. in droppings from urban rooks (*Corvus frugilegus*). Journal of Applied Microbiology. 82, 641-647.

38. Bradley, C.A., Altizer, S., 2006. Urbanization and the ecology of wildlife diseases. Trends in Ecology and Evolution. 22, 95-102.

39. Bradley, C.A., Gibbs, S.E.J., Altizer, S., 2008. Urban land use predicts West Nile Virus exposure in songbirds. Ecological Applications. 18, 1083-1092.

40. Brearley, G., McAlpine, C., Bell, S., Bradley, A., 2012. Influence of urban edges on stress in an arboreal mammal: a case study of squirrel gliders in southeast Queensland, Australia. Landscape Ecology. 27, 1407-1419.

41. Braga, E.M., Silveira, P., Belo, N.O., Valkiunas, G. 2011. Recent advances in the study of avian malaria: an overview with an emphasis on the distribution of *Plasmodium* spp in Brazil. Memórias do Instituto Oswaldo Cruz. 106, 3-11.

42. Brown, M.E., 1996. Assessing body condition in birds. Pages 67-135 in V. J. Nolan, and E. D. Ketterson, editors. Current ornithology. Plenum Press, New York.

43. Bunbury, N., Bell, D., Jones, C., Greenwood, A., Hunter, P. 2005. Comparison of the InPouch culture system and wet-mount microscopy for diagnosis of

Trichomonas gallinae infections in the Pink pigeon *Columba mayeri*. Journal of Clinical Microbiology. 43, 1005-1006.

44. Burger, J., Bowman, R., Woolfenden, G.E., Gochfeld, M., 2004. Metal and metalloid concentrations in the eggs of threatened Florida scrub-jays in suburban habitat from south-central Florida. Science of the Total Environment. 328, 185-193.
45. Calegaro-Marques, C., Amato, S.Z., 2010. Helminths of introduced house sparrows (*Passer domesticus*) in Brazil: does population age affect parasite richnes? Iheringia Série Zoologia. 100, 73-78.
46. Calegaro-Marques, C., Amato, S.B., 2014. Urbanization breaks up host-parasite interactions: a case study on parasite community ecology of Rufoues-bellied thrushes (*Turdus rufiventris*) along a rural-urban gradient. PLoS One. 9, 1-8.
47. Carlson, J.C., Martínez-Gómez, J.E., Valkiunas, G., Loiseau, C., Bell, D.A., Sehgal, R.N.M., 2013. Diversity and phylogenetic relationships of hemosporidian parasites in birds of Socorro Island, México, and their role in the re-introduction of the Socorro dove (*Zenaida graysoni*). Journal of Parasitology. 99, 270-276.
48. Carrillo, C.M., Valera, F., Barbosa, A., Moreno, E., 2007. Thriving in an arid environment: high prevalence of avian lice in low humidity conditions. Ecoscience. 14, 241-249.
49. Chasar, A., Loiseau, C., Valkiunas, G., Iezhova, T., Smith, T.B., Sehgal, R.N.M., 2009. Prevalence and diversity patterns of avian blood parasites in degraded African rainforest habitats. Molecular Ecology. 18, 4121-4133.
50. Chace, J.F., Walsh, J.J., 2006. Urban effects on native avifauna: a review. Landscape Urban and Planning. 74, 46-69.

51. Chávez-Zichinelli, C., MaCGreGor-Fors, I., Quesada, J., Rohana, P.T., Romano, M.C., Valdéz, R., Schondube, J.E., 2013. How stressed are birds in an urbanizing landscape? Relationships between the physiology of birds and three levels of habitat alteration. The Condor. 115, 84-92.

52. Cheptou, P.O., Carrue, O., Rouifed, S., Catarel, A., 2008. Rapid evolution of seed dispersal in an urban environment in the weed *Crepis sancta*. Proccedings of the National Academy of Science. 105, 3796-3799.

53. Christie, F.J., Hochuli, D.F., 2009. Responses of wasp communities to urbanization: effects on community resilience and species diversity. Journal of Insect Conservation. 13, 213-221.

54. Clark, N., Clegg, S.M., Lima, M.R., 2014. A review of global diversity in avian haemosporidians (*Plasmodium* and *Haemoproteus*: Haemospodida): new insights from molecular data. International Journal for Parasitology. 44, 329-338.

55. Clarck, P., Boardman, W., Raidal, S., 2009. Atlas of clinical avian hematology. Wiley-Blackwell. Oxford UK.

56. Clark, G.W., Swinehart, B., 1966. Blood protozoa of passerine birds of the Sacramento (California) region. Bulletin of Wildlife Disease Association. 2, 53-54.

57. Clayton, D.H., Drown, D.M., 2001. Critical evaluation of five methods for quantifying chewing lice (Insecta: Phtiraptera). Journal of Parasitology. 87, 1291-1300.

58. Clayton, D.H., Koop, J.A.H., Harbison, C.W., Moyer, B.R., and Bush, S.E., 2010. How birds combat ectoprasites. The Open Ornithology Journal. 3, 41-71.

59. Clayton, D.H., Walther, B.A., 1997. Collection and quantification of arthropod parasites of birds, in: Clayton, D.H., Moore, J. (Eds.), Host-parasite evolution:

general principles and avian models. Oxford University Press, Oxford, pp. 419-440.

60. Collombelli-Négrel, D., Kleindorfer, S. 2008. In Superb-fairy wrens (*Malurus cyaneus*), nuptial males have more blood parasites and higher haemoglobin concentration than eclipsed males. Australian Journal of Zoology. 56, 117-121.
61. Coimbra, M.A., Mascarenhas, C.S., Krüger, C., Muller, G., 2009. Helminths Parasitizing *Columbina picui* (Columbiformes: Columbidae) in Brazil. Journal of Parasitology. 95, 1011-1012
62. Combes, C., 2001. Parasitism: the ecology and evolution of intimate interactions. The University Press, Chicago.
63. Conner, M.M. & M.W. Miller. 2005. Human land use influences chronic wasting disease prevalence in mule deer. Ecological Applications. 15: 119-126.
64. Cumming, G.S., Shepard, E., Okanga, S., Caron, A., Ndlovu, M., Peters, J.L., 2013. Host associations, biogeography, and phylogenetics of avian malaria in southern african waterfowl. Parasitology. 140, 193-201.
65. Cumming, G.S., Van Vuuren, S.P., 2006. Will climate change affect ectoparasite species range? Global Ecology and Biogegraphy. 15, 486-497.
66. Cypher, B.L., Frost, N., 1999. Condition of San Joaquin kit foxes in urban and exurban habitats. Journal of Wildlife Management. 63, 930-938.
67. Daszak, P., Cunningham, A.A., Hyatt, A.D., 2000. Emerging infectious diseases of wildlife--threats to biodiversity and human health. Science. 287, 443-449.
68. Dautel, H., Kahl, O., Knuelle, W., 1991. The soft tick *Argas reflexus* f. Acari Argasidae in urban environments and its medical significance in Berlin West, Germany. Journal of Applied Entomology. 111, 380-390.
69. Davis, A.K., Hood, W.R., Hill, G.E., 2013. Prevalence of blood parasites in

eastern versus western house finches: are eastern birds resistant to infection? EcoHealth. 10, 290-297.

70. Davis, A.K., Maney, D.L., Maerz, J.C., 2008. The use of leukocyte profiles to measure stress in vertebrates: a review fro ecologists. Functional Ecology. 22, 760-772.

71. DeGroote, L.W., Rodewald, P.G., 2010. Blood parasites in migrating wood-warblers (Parulidae): effects on refueling, energetic condition, and migration timing. Journal of Avian Biology. 41, 147-153.

72. Delgado-V., C.A., 2007. Roadway mortality of mammals on the Escobero road, Envigado (Antioquia), Colombia. Actualidades Biológicas. 29, 235-239. [in Spanish]

73. Delgado-V., C.A., 2014. Adiciones al atropellamiento vehicular de mamíferos en la vía del Escobero, Envigado (Antioquia), Colombia. Revista Escuela Ingeniería de Antioquia. 11, 145-151. [in Spanish]

74. Delgado-V., C.A., French, K., 2012. Parasite-bird interactions in urban areas: Current evidence and emerging questions. Landscape and Urban Planning. 105, 5-14.

75. Delgado-V., C.A., French, K., In press. Differential influence of urbanisation on Coccidian infection in two passerine birds. Parasitology Research. DOI: 10.1007/s00436-015-4414-2

76. Dehnhard, N., Quillfeldt, P., Hennicke, J.C., 2011. Lecucyte profiles and H/L ratios in chicks of Red-tailed tropicbirds reflect the ontogeny of the immune system. Journal of Comparative Physiology B. 181, 641-648.

77. de Lima, V.Y., Langoni, H., da Silva, A.V., Pezerico, S.B., de Castro, A.P., da Silva, R.C., Ara, J.P. Jr., 2011. *Chlamydophila psittaci* and *Toxoplasma gondii*

infection in pigeons (*Columba livia*) from Saõ Paulo State, Brazil. Veterinary Parasitology. 175, 9-14.

78. Dement S.H., Chrisolm J.Jr, Barber J..C., Strandberg J.D., 1986. Lead exposure in an urban Peregrine falcon *Falco peregrinus* and its avian prey. Journal of Wildlife Diseases. 22, 238-244.

79. de Sousa, E., Berchieri, A.J., Pinto, A.A., Machado, R.Z., de Carrasco, A.O., Werther, K., 2010. Prevalence of *Salmonella* spp. antibodies to *Toxoplasma gondii*, and Newcastle disease virus in feral pigeons (*Columba livia*) in the city of Jaboticabal, Brazil. Journal of Zoo and Wildlife Medicine. 41, 603-607.

80. Deviche, P., Fokidis, H.B., Lerbour, B., Greiner, E., 2010. Blood parasitemia in a high latitude flexible breeder, the white-winged crossbill, *Loxia leucoptera*: contribution of seasonal relapse versus new inoculations. Parasitology. 137, 261-273.

81. Diuk-Wasser, M.A., Molaei, G., Simpson, J.E., Folson-O'Keefe, Armstrong, P.M., Andreadis, T.G., 2010. Avian communal roosts as amplification foci for West Nile virus in urban areas in northeastern United States. American Journal of Tropical Medicine and Hygiene. 82, 337-343.

82. Dobson, A.P., May, R.M., 1986. Disease and conservation. In: Soulé, M.E. (ed.). Conservation Biology. Sinaeur Associates, Sunderland, pp. 345-365.

83. Dolnik, O., 2006. The relative stability of chronic Isospora sylvianthina (Protozoa: Apicomplexa) infection in blackcaps (Sylvia atricapilla): evaluation of a simplified method of estimating *Isospora* infection intensity in passerine birds. Parasitology Research. 100, 155-160.

84. Dolnik, O.V., Dolnik, V.R., Bairlein, F., 2010. The effect of host foraging

ecology on the prevalence and intensity of coccidian infection in wild passerine birds. Ardea. 98, 97-103.

85. Dubiec A., Cichon M., 2001. Seasonal decline in health status of Great Tit (*Parus major*) nestlings. Canadian Journal of Zoology. 79, 1829-1833.

86. Dubska, L., Literak, I., Kocianova, E., Taragelova, V., Sychra, O., 2009. Differential role of passerine birds in distribution of *Borrelia spirochetes*, based on data from ticks collected from birds during the postbreeding migration period in Central Europe. Applied and Environmental Microbiology. 75, 596-602.

87. Durrant, K.L., Beadell., J.S., Ishtiaq, F., Graves, G.R., Olson, S.L., Gering, E., Pierce, M.A., Milensky, C.M., Schmidt, B.K., Gebhard, C., Fleischer, R.C., 2006. Avian hematozoa in South America: a comparison of temperate and tropical zones. Ornithological Monographs. 60, 98-111.

88. Eens, M., Pinxten, R., Verheyen, R.F., Blust, R., Bervoets, L., 1999. Great and blue tits as indicators of heavy metal contamination in terrestrial ecosystems. Ecotoxicology and Environmental Safety. 44, 81-85.

89. Epstein, J.H., McKee, J., Shaw, P., Hicks, V., Micalizzi, G., Dasak, P., Kilpatrick, A.M., Micalizzi, G., Kaufman, G., 2007. The Australian White Ibis (*Threskiornis molucca*) as a Reservoir of Zoonotic and Livestock Pathogens. Ecohealth. 3, 290-298.

90. Evans, K.L., 2010. Individual species and urbanisation, in: Gaston, K.J. (Ed.). Urban Ecology. Cambridge University Press, Cambridge, pp. 53-87.

91. Evans, K.L., Chamberlain, D.E., Hatchwell, B.J., Gregory, R.D., Gaston, K.J., 2011. What makes an urban bird? Global Change Biology. 17, 32-44.

92. Evans, K.L., Gaston, K.J., Sharp, S.P., McGowan, A., Simmeoni M., Hatchwell, B.J., 2009. Effects of urbanisation on disease prevalence and age structure in blackbird *Turdus merula* populations. Oikos. 118, 774-782.

93. Faeth, S.H., Warren, P.S., Schochat, E., Marussich, W.A., 2005. Trophic Dynamics in Urban Communities. Bioscience. 55, 399-407.

94. Faeth, S.H., Bang, C., Saari, S., 2011. Urban biodiversity: patterns and mechanisms. Annals of the New York Academy of Science. 1223, 69-81.

95. Fairn, E.R., Schulte-Hostedde, A.I., Yves, A., 2008. Water mite parasitism is associated with body condition and sex of the whirligig beetle *Dineutus nigrior* (Coleoptera: Gyrinidae). Ecoscience. 15, 327-331.

96. Fakhar, M., Kalani, H., Rahimi-Esboei, B., Armat, S., 2013. Hemoprotozoa in free-ranging birds from rural areas of Mazandaran provice, northern Iran. Comparative Clinical Pathology. 22, 509-512.

97. Fecchio, A., Lima, M.R., Silveira, P., Braga, E.M., Marini, M.A., 2011. High prevalence of blood parasites in social birds from a neotropical savanna in Brazil. Emu. 111, 132-138.

98. Fernández-Juricic, E., 2002. Can human disturbance promote nestedness? A case study with birds in an urban fragmented landscape. Oecologia. 13, 269-278.

99. Fokidis, H.B., Greiner, E.C., Deviche, P., 2008. Interspecific variation in avian bood parasites and haematology associated with urbanization in a desert habitat. Journal of Avian Biology. 39, 300-310.

100. Ford, H.A., Barrett, G.W., Saunders, D.A., and Recher, H.F., 2001. Why have birds in the woodlands of Southern Australia declined? Biological Conservation. 97, 71-88.

101. Foronda, P., Valladares, B., Rivera-Medina, J.A., Abreau, N., Casanova,

J.C., 2004. Parasites of *Columba livia* (Aves: Columbiformes) in Tenerife (Canary Islands) and their role in the conservation biology of the Laurel pigeons. Parasite. 11, 311-316.

102. Forrester, D.J., Spalding, M.G., 2003. Parasites and diseases of wild birds in Florida. University Press of Florida, Gainesville, Florida.

103. Fox, J.G., Taylor, N.S., Howe, S., Tidd, M, Xu, S., Paster, B.J., Dewhirst, F.E., 2006. *Helicobacter anseris* sp. nov. and *Helicobacter brantae* sp. nov., isolated from feces of resident Canada geese in the greater Boston area. Applied and Environmental Microbiology. 72, 4633-4637.

104. Freed, L.A., Medeiros, M.C., Bodner, G.R., 2008. Explosive increase in ectoparasites in Hawaiian forest birds. Journal of Parasitology. 94, 1009-1021.

105. French, S.S., Fokidis, H.B., Moore, M.C., 2008. Variation in stress and innate immunity in the tree lizard (*Urosaurus ornatus*) across an urban-rural gradient. Journal of Comparative Physiology B. 178, 997-1005.

106. Garamszegi, L.Z., 2011. Climate change increases the risk of malaria in birds. Global Change Biology. 17, 1751-1759.

107. Garvin, M.C., Remsen, J.V., Bishop, M.A., Bennett, G.F., 1993. Hematozoa from passeriform birds in Louisiana. Journal of Parasitology. 79, 318-321.

108. Garvin, M.C., Szell, C.C., Moore, F.R., 2006. Blood parasites on the Neartic-Neotropical migrant passerine birds durirg spring Trans-gulf migration: impact on host body condition. Journal of Parasitology. 92, 990-996.

109. Gaston, K.J., 2010. Urbanisation, in: Gaston, K.J. (Ed.). Urban Ecology. Cambridge University Press, Cambridge, pp. 10-34.

110. Gavett, A.P., Wakeley, J.S., 1986. Blood constituents and their relation to diet in urban and rural House sparrows. The Condor. 88, 179-284.

111. Geens, A., Dauwe, T., Bervoets, L., Blust, R., Eens, M., 2010. Haematological status of wintering great tits (*Parus major)* along a metal pollution gradient. Science of the toal environment. 408, 1174-1179.

112. Geue, D., Partecke, J., 2008. Reduced parasite infestation in urban Eurasian blackbirds (*Turdus merula*): a factor favouring urbanization? Canadian Journal of Zoology. 86, 1419-1425.

113. Giraudeau, M., Mousel, M., Earl, S., McGraw., K., 2014. Parasites in the city: degree of urbanization predicts Poxvirus and Coccidian infections in House finches (Haemorhous mexicanus). PlosONE. 9, 1-8.

114. Githeko, A.K., Lindsay, S.W., Confalonier, V.E., Patz, J.A., 2000. Climate change and vector-borne diseases: a regional analysis. Bulletin of the World Health Organization. 78, 1136-1147.

115. Gomercic, T., Vlahovic, K., Horvatek, D., Dovc, A. Prukner-Radovcic, E., Pavlak, M., Rumiha, Z., 2010. Bacterial and fungal flora in fecal samples from rooks (*Corvus frugilegus)* in the city of Zagreb, Croatia. Veterinarski Arhiv. 80, 81.

116. Gómez A., Kilpatrick, A.M., Kramer, L.D., Dupuis, A.P., Maffei, J.G., Goetz, S.J., Marra, P.P., Daszak, P., Aguirre, A., 2008. Land use and West Nile Virus seroprevalence in wild mammals. Emerging Infectious Diseases. 14, 962-965.

117. Gondim, L.S.Q., Abe-Sandes, K., Uzêda, R.S., Silva, M.S.A., Santos, S.L., Mota, R.A., Vilela, M.O., Gondim, L.F.P., 2010. *Toxoplasma gondii* and *Neospora caninum* in sparrows (*Passer domesticus*) in the northeast of Brazil. Veterinary Parasitology. 168, 121-124.

118. Green, A.J., 2001. Mass/length residuals: measures of body condition or

generators of spurious results? Ecology. 82, 1473-1483.

119. Gregoire, A., Faivre, B., Heeb, P., Cezilly, F., 2002. A comparison of infestation patterns by *Ixodes* ticks in urban and rural populations of the Common Blackbird *Turdus merula*. Ibis. 144, 640-645.

120. Greiner, E.C., Bennett, G.F., White, E.M., Coombs, R.F., 1975. Distribution of the avian hematozoa of North America. Canadian Journal of Zoology. 53, 1762-1787.

121. Gronesová, P., Mizáková, P., Betáková, T., 2009. Determination of hemaglutinin and neuraminidase subtypes of avian influenza A viruses in urban pigeons by a new nested RT-PCR. Acta Virologica. 53, 213-216.

122. Gustafsson, L., Nordling, D., Andersson, M., Sheldon, B., Qvarnström A., 1994. Infectious diseases, reproductive effort and the cost of reproduction in birds. Philosophical Transaction of the Royal Society Series B. 346, 323-331.

123. Haag-Wackernagle., D., 2005. Parasites from feral pigeons as a health hazard for humans. Annals of Applied Biology. 147, 203-210.

124. Hamer, A.J., McDonell, M.J., 2008. Amphibian ecology and conservation in the urbanising world: a review. Biological Conservation. 141, 2432-2449.

125. Hart, B.L., 2000. Role of grooming in biological control of ticks. Trop. Vet. Diseas. 916, 565-569.

126. Harkinezhad, T., Geens, T., Vanrompay, D., 2009. *Chlamydophila psittaci* infections in birds: a review with emphasis on zoonotic consequences. Veterinary Microbiology. 135, 68-77.

127. Haro, M., Izquierdo, F., Henriques-Gil, N., Andrés, I., Alonso, F., Fenoy S, del Aguila, C., 2005. First detection and genotyping of human-associated microsporidia in pigeons from urban parks. Applied and Environmental

Microbiology. 71, 3153-3157.

128. Hartup, B.K., Bickal, J.M., Dhondt, A.A., Ley, D.H., Kollias, G.V., 2001. Dynamics of conjunctivitis and *Mycoplasma gallisepticum* infections in House Finches. Auk. 118, 327-333.

129. Hartup, B.K., Oberc, A., Stott-Messick, B., Davis, A.K., Swarthout, E.C.H., 2008. Blood parasites of house finches (*Carpodacus mexicanus*) from Georgia and New York. Journal of Wildlife Diseases. 44, 469-474.

130. Hauptmanová, K., Benedikt, V., Literák, I., 2006. Blood parasites in passerine birds in Slovakian east Carpathians. Acta Protozoologica. 45, 105-109.

131. Hedema, E.R., Sluis, S., Buys, J.A., Vanderbroucke,-Grauls, C.M.J.E., van Wjen, J.H., Visser, C.E., 2006. Prevalence of *Chlamydophila psittaci* in fecal droppings from feral pigeons in Amsterdam, The Netherlands. Applied and Environmental Microbiology. 72, 4423-4425.

132. Heins, D.C., Baker, J.A., 2014. Fecundity compensation and fecundity reduction among populations of the three-spined stickleback infected by Schistocephalus solidus in Alaska. Parasitology. 141, 1088-1096.

133. Heiss, R.S., Clark, A.B., McGowan, K.J., 2009 Growth and nutritional state of American crow nestlings vary between urban and rural habitats. Ecological Application. 19, 829-839.

134. Helms, C.W., Drudy, W.H., 1960. Winter and migratory weight and fat field studies on some North American buntings. Bird banding. 31, 1-40.

135. Hirata, A., Yonemaru, K., Kubo, M., Murakami, M., Sakai, H., Yanai, T., Masegi, T., 2010. Frequent Development of inflammatory lesions and Lymphoid oci in the kidneys of Japanese wild crows (*Corvus macrorhynchos* and *Corvus corone*) as a result of the entry of causal agents via the renal portal blood. The

Journal of Veterinary Medical Science. 72, 327-332.

136. Hõrak, P., Ots, I., Murumagi, A., 1998. Haematological health state indices of reproducing Great Tits: a response to brood size manipulation Functional Ecology. 12, 750-756.

137. Hõrak, P., Ots, I., Vellau, H., Spottiswoode, C., Møller, A.P., 2001. Carotenoid-based plumage coloration reflects hemoparasite infectation and local survival in breeding great tits. Oecologia. 126, 166-173.

138. HubáleK, Z., 2004. An annotated checklist of pathogenic microorganisms associated with migratory birds. Journal of Wildlife Diseases. 40, 639–659.

139. Hubalek, Z., Anderson, J.F., Halouzka, J., Hajek, V., 1996. Borreliae in immature *Ixodes ricinus* (Acari: Ixodidae) ticks parasitizing birds in the Czech Republic. Journal of Medical Entomology. 33, 766-771.

140. Hudson, P., Dobson, A.P., Lafferty, K.D., 2006. Is a healthy ecosystem one that is rich in prasites? Trends in Ecology and Evolution. 21, 381-385.

141. Ives, C.D., Hose, G.C., Nipperess, D.A., Taylor, M.P., 2011. Environmental and landscape factors influencing ant and plant diversity in suburban riparian corridors. Landscape and Urban Planning. 103, 372–382.

142. Iwasa, M, Hori, K., Aoki, N., 1995. Fly fauna of bird nests in Hokkaido, Japan. Canadian Entomologist. 127, 613-621.

143. Jacquin, L., Haussy, L.C., Ducatez, S., Gasparini, J., 2011. Melanin-based coloration is related to parasite intensity and cellular immune response in an urban free living bird: the feral pigeon *Columba livia*. Journal of Avian Biology. 42, 11-15.

144. Jansen, C., Webb, C.E., Graham, G.C., Craig, S.B., Zborowski, P., Ritchie, S.A., Rusell, R.C., van den Hurk, A.F., 2009. Blood sources of mosquitoes

collected from urban and peri-urban environments in eastern Australia with species-specific molecular analysis of avian blood meals. American Journal of Tropical Medicine and Hygiene. 81, 849-857.

145. Johnson, D.M, Stewart, G.L., Corley, M., Ghrist, R., Hagner, J., Ketterer, A., McDonnell, B., Newson, W., Owen, E., Samuels P., 1980. Brown-headed cowbird *Molothrus ater* mortality in an winter urban roost. The Auk. 97, 299-320.

146. Johnstone, C.P., Reina, R.D., Lill, A., 2010. Impact of anthropogenic habitat fragmentation on population health in a small, carnivorous marsupial. Journal of Mammalogy. 91, 1332-1341.

147. Jones, D.N., Reynolds, J., 2008. Feeding birds in our towns and cities: a global research opportunity. Journal of Avian Biology. 39, 265-271.

148. Kantsa, A., Tschulin, T., Junker, R.R., Petanidou, T., 2013. Urban biodiversity hotspots wait to get discovered: the example of the city of Ioannina, NW Greece. Landscape and Urban Planning. 120, 129-137.

149. Kark, S., Iwaniuk, S., Schalimtzek, A., Banker, E., 2007. Living in the city: can anyone become an 'urban exploiter'? Journal of Biogeography. 34, 638-651.

150. Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S., Duran, C., Thierer, T., Ashton, B., Mentjies, P., Drummond, A., 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics. 28, 1647-1649.

151. Keiss, R.S., Clark, A.B., McGowan, K.J., 2009. Growth and nutritional state of American crow nestlings vary between urban and rural habitats. Ecological Applications. 19, 829-839.

152. Keesing, F., Holt, R.D., Ostfeld, R.S. 2006. Effects of species diversity on disease risk. Ecology Letters. 9, 485-498.

153. Keesing, F., Belden, L.K., Daszak, P., Dobson, A., Harvell, C.D., Ostfeld, R.S., Holt., R.D., Hudson, P., Jolles, A., Mitchell, C.E., Myers, S.S., Bogich, T., Ostfield, R.S., 2010. Impacts of biodiversity on the emergence and transmission of infectious diseases. Nature. 468, 647-652.

154. Kirkpatrick C.E., Colvin, B.A., 1989. Ectoparasitic fly *Carnus hemapterus* (Diptera, Carnidae) in a nesting population of common barn owls (Strigiformes, Tytonidae). Journal of Medical Entomology. 26, 109-112.

155. Kirkpatrick, C.E., Colvin, B.A., Dubey, J.P., 1990. *Toxoplasma gondii* antibodies in common barn-owls (*Tyto alba*) and pigeons (*Columba livia*) in New Jersey. Veterinary Parasitology. 36, 177-180.

156. Kirkpatrick. C.E., Sluthers, H.B., 1988. Epizootiology of blood parasite infections in passerine birds from central New Jersey USA. Canadian Journal of Zoology. 66, 2374-2382.

157. Kleindorfer, S., Lambert, S., Paton, D.C., 2006. Ticks (*Ixodes* sp.) and blood parasites (*Haemoproteus* spp.) in New Holland Honeyeaters (*Phylidonyris novaehollandie*): evidence for site specificity and fitness costs. Austral Ornithology. 106, 113-118.

158. Klotz, S., Kühn, I., 2010. Urbanisation and alien invasion. in: Gaston, K.J. (Ed.). Urban Ecology. Cambridge University Press, Cambridge, pp. 120-133

159. Koenig, W.D., Hochachka, W.M., Zuckerberg, B., Dickinson, J.L., 2010. Ecological determinants of American crow mortality due to West Nile virus during its North American sweep. Oecologia. 163, 903-909.

160. Krone, O., Altenkamp, R., Kenntner, N., 2005. Prevalence of *Trichomonas gallinae* in northern goshawks from the Berlin area of northeastern Germany. Journal of Wildlife Diseases. 41, 304-309.

161. Krone, O., Waldenstruöm, J., Valkiunas, G., Lessow, O., Müller, K., Lezhova, T.A., Fickel, J., Bensch, S., 2008. Haemospodirian blood parasites in european birds of prey and owls. Journal of Parasitology. 94, 709-715.

162. Kullas, H., Coles, M., Rhyan, J., Clark, L., 2002. Prevalence of Escherichia coli serogroups and human virulence factors in faeces of urban Canada geese (*Branta canadensis)*. International Journal of Environmental Health Research. 12, 153-162.

163. Lacorte, G.A., Félix, G.M.F., Pinheiro, R.R.B., Chaves, A.V., Almeida-Neto, G., Neves, F.S., Leite, L.O., Santos, F.R., Braga, E.M., 2013. Exploring the diversity and distribution of Neotropical avian malaria parasites – a molecular survey from Southeast Brazil. PLoS One. 8, 1-9.

164. Lafferty, K.D., Morris, A.K., 1996. Altered behavior of parasitized killifish increases susceptibility to predation by bird final hosts. Ecology. 77, 1390-1397.

165. Laurance, S.G.W., Jones, D., Westcott, D., Mckeown, A., Harrington, G., Hilbert, D.W., 2013. Habitat Fragmentation and Ecological Traits Influence the Prevalence of Avian Blood Parasites in a Tropical Rainforest Landscape. PLoS One. 8, 1-8.

166. Latta, S.C., Ricklefs, R.E., 2010. Prevalence patterns of avian haemosporida on Hispaniola. Journal of Avian Biology. 41, 25-33.

167. Lawrance, J., 1946. Some observations on the Plasmodia and other blood parasites of sparrows. Proceedings of the Linnean Society of NSW. 71, 1-5.

168. Lawson, B., Robinson, R.A., Neimanis, A., Handeland, K., Isomursu, M., Agren, E.O., Hamnes, I.S., Tyler, K.V., Chantrey, J., Hughes, L.A., Pennycott, T.W., Simpson, V.R., John, S.K., Peck, K.M., Toms, M.P., Bennet, M., Kirkwood, J.K., Cunningham, A.A., 2011. Evidence of spread of the Emerging Infectious Disease, finch Trichomoniasis, by migrating birds. Ecohealth. 8, 143-153.

169. Lehikoinen, A., Lehikoinen, E., Valkama, J., Väisänen, R.A., 2011. Effects of Trichomonas on Finnish greenfich population. Linnut Vuosikirja. 110-116. [in Finnish]

170. Lehrer, E.W., Fredebaugh, S.L., Schooley, R.L., Mateus-Pinilla, N.E., 2010. Prevalence of antibodies to *Toxoplasma gondii* in woodchucks across an urban-rural gradient. Journal of Wildlife Diseases. 46, 977-980.

171. Levin, I.I., Parker, P.G., 2012. Prevalence of Hemoproteus iwa in Galapagos Great frigatebirds (*Fregata minor*) and their obligate fly ectoparasite (*Olfersia spinifera*). Journal of Parasitology. 98, 924-929.

172. Liker, A., Papp, Z., Bókony, V., Lendvai, A.Z., 2008. Lean birds in the city: body size and condition of house sparrows along the urbanization gradient. Journal of Animal Ecology 77, 789-795.

173. Lill, A., Rajchl, K., Yachou-Wos, L., Johnstone, C.P., 2013. Are haematocrit and haemoglobin concentration reliable body condition indicators in nestlings: the Welcome Swallow as a case study. Avian Biology Research. 6, 1-10.

174. Lim, H.C., Sodhi, N.S., 2004. Responses of avian guilds to urbanisation in a tropical city. Landscape and Urban Planning. 66, 199-215.

175. Lima, M.R., Simpson, L., Fecchio, A., Kyaw, C.M., 2010. Low prevalence

of haemosporidian parasites in the introduced house sparrow (*Passer domesticus*) in Brazil. Acta Parasitologica. 55, 297-303.

176. Loss, S.R., Hamer, G.L., Goldberg, T.L., Ruiz, M.O., Kitron, U.D., Brawn, J.D., 2009. Nestling passerines are not important hosts for amplification of West Nile Virus in Chicago, Illinois. Vector Borne Zoonotic Diseases. 9, 13-17.

177. Loiseau, C., Harrigan, R. J., Bichet, C., Julliard, R., Garnier, S., Lendvai, A.Z., Chastel, O., Sorci, G., 2013. Predictions of avian *Plasmodium* expansion under climate change. Scientific Reports. 3, 1-6.

178. Loiseau, C., Iezhova, T., Valkiunas, G., Chasar, A., Hutchinson, A., Buermann, W., Smith, T.B., Shegal, R.N.M., 2010. Spatial variation of haemosporidian parasite infection in African rainforest bird species. Journal of Parasitology. 96, 21-29.

179. Lussier, S.M., Enser, R.W., Dasilva, S.N., Charpentier, M., 2006. Effects of habitat disturbance from residential development on breeding bird communities in riparian corridors. Environmental Management. 38, 504-521.

180. Lüdtke, B., Moser, I., Santiago-Alarcón, D., Fischer, M., Kalko, E.K.V., Schaefer, H.M., Suárez-Rubio, M., Tschapka, M., Renner, S.C., 2013. Associations of forest type, parasitism and body condition of two European passerines, *Fringillia coelebs* and *Sylvia atricapilla*. PloS One. 8: 1-9.

181. Ludwig, A., Bigras-Poulin, M., Michel, P., Bélnger, D., 2010. Risk factors associated with West Nile virus mortality in American Crow populations in Southern Quebec. Journal of Wildlife Diseases. 46, 195-208.

182. MacGregor-Fors, I.L., Morales-Pérez, J.E. Schondube, R., 2010. Migrating to the city: responses of neotropical migrant bird communities to urbanization. Condor. 112, 711-717.

183. MacKerras, M.J., MacKerras, I.M., 1960. The haematozoa of Australian birds. Australian Journal of Zoology. 9, 123-140.

184. Maetzel, D., Maier, W.A., Kampen, H., 2005. *Borrelia burgdorferi* infection prevalences in questing *Ixodes ricinus* ticks (Acari: Ixodidae) in urban and suburban Bonn, western Germany. Parasitology Research. 95, 5-12.

185. Mannelli, A., Kitron, U., Jones, C.J. Slajchert, T.L., 1993. *Ixodes dammini* (Acari: Ixodidae) infestation on medium-sized mammals and blue jays in northwestern Illinois. Journal of Medical Entomology. 30, 950-952.

186. Marques, S.M.T., De Quadros, R.M., Da Silva, C.J., Baldo, M., 2007. Parasites of pigeons (*Columba livia*) in urban areas of Lages, Southern Brazil. Parasitología Latinoamericana. 62, 183-187.

187. Marrow, J., Whittington, J.K., Mitchell, M. Hoyer, L.L., Maddox, C., 2009. Prevalence and antibiotic-resistance characteristics of *Enterococcus* spp. Isolated from free-living and captive raptors in Central Illinois. Journal of Wildlife Diseases. 45, 302-313.

188. Martin, L.B., Boruta, M., 2014. The impacts of urbanization on avian disease transmission and emergence. In 'Avian Urban Ecology'. (Eds. D Gil and H. Brumm) pp.116-128. (Oxford University Press, U.K.)

189. Martínez-Mota, R., Valdespino, C., Sánchez-Ramos, M.A., Serio-Silva, J.C., 2007. Effects of forest fragmentatio on the physiological stress response of black howler monkeys. Animal Conservation. 10, 374-379.

190. Marzal, A., Ricklefs, R.E., Valkiünas, G., Albayrak, T., Arriero, E., Bonneaud, C., Czirják, G.A., Ewen, J., Hellgren, O., Horákova, D., Iezhova, T.A., Jensen, H., Krizanauskiené, A., Lima, M.R., Lope, F., Magnussen, E., Martin, L.B., Møller, A.P., Palinauskas, V., Pap, P.L., Pérez-Tris, J., Sehgal,

R.N.M., Soler, M., Szöllõsi, Westerdahl, H., Zetindjiev, P., Bensch, S., 2011. Diversity, loss, and gain of malaria parasites in a globally invasive bird. PLoS One. 6, 1-8.

191. Maute, K.L., French, K., Legge, S., Astheimer, L., 2013. Seasonal stress physiology and body condition differ among co-ocurring tropical finch species. Journal of Comparative Physiology B. 183, 1023-1037.

192. Mazerolle, D.F., Hobson, K.A. 2002. Physiological ramifications of habitat selection in territorial male ovenbirds: consequences of landscape fragmentation. Oecologia. 130, 356-363.

193. McCallum, H., Dobson, A., 1995. Detecting disease and parasite threats to endangered species and ecosystems. Trends in Ecology and Evolution. 10, 190-194.

194. McFarland, D.C., 1986. Presence of ticks on the heads of Honeyeaters in New England National Park. Corella. 10, 25-28.

195. McKinney, M.L., 2006. Urbanization as a major cause of biotic homogenization. Biological Conservation. 127, 247-260.

196. McKinney, M.L., 2008. Effects of urbanization on species richness: a review of plant and animals. Urban Ecosystems. 11, 161-176.

197. McLean, R.G., 2006. West Nile Virus in North American birds. Ornithological Monographs. 60123, 44-64.

198. McLean, R.G., Mullenix, J., Kerschner, J., Hamm, J., 1983. The house sparrow (*Passer domesticus*) as a sentinel for St. Louis encephalitis virus. American Journal of Tropical Medicine and Hygiene. 32, 1120-1129.

199. Merilä, J., Bjorklund, M., Bennett, G. F., 1995. Geographic and individual variation in haematozoan infections in the greenfinch, *Carduelis chloris*.

Canadian Journal of Zoology. 73, 1798–1804.

200. Merino, S., Moreno, J., Vásquez, R., Martínez, J., Sánchez-Mosnalve, I., Estades, C., Ippi, S., Sabat, P., Rozzi, R., McGehee, S., 2008. Haematozoa in forest birds from Southern Chile: latitudinal gradients in prevalence and parasite lineage richness. Austral Ecology. 33, 319-340.

201. Minchella, D.J., Scott, M.E., 1991. Parasitism: a cryptic determinant of animal community structure. Trends in Ecology and Evolution. 6, 250-254.

202. Møller, A.P., Soler, J.J., Vivaldi, M.M., 2010. Spatial heterogeneity in distribution and ecology of Western Palearctic birds. Ecology. 91, 2769-2782.

203. Montenegro, H., Paula, C.R., 2000. Environmental isolation of *Cryptococcus neoformans* var. *gattii* and *C. neoformans* var. *neoformans* in the city of Saõ Paulo, Brazil. Medical Mycology. 38: 385-390.

204. Morand, S., Poulin, R., 1998. Density, body mass and parasite species richness of terrestrial mammals. Evolutionary Ecology. 12, 717–727.

205. Moreno, J., Briones, V., Merino, S., Ballesteros, C., Sanz, J.J., Tomás, G., 2003. Beneficial effects of cloacal bacteria on growth and fledging size in nestling Pied flycatchers (*Ficedula hypoleuca*) in Spain. Auk. 120, 784-790.

206. Mouritsen, K.N., Poulin, R., 2002. Parasitism, community structure and biodiversity in intertidal ecosystems. Parasitology. 124, 101-117.

207. Movila, A., Gatewood, A., Toderas, I., Duca, M., Papero, M., Uspenskaia, I., Conovalov, J., Fish, D. 2008. Prevalence of *Borrelia burgdorferi* sensu lato in *Ixodes ricinus* and I. *lividus* ticks collected from wild birds in the Republic of Moldova. International Journal of Medical Microbiology. 298, 149-153.

208. Moyer, B.R., and Clayton, D.H., 2004. Avian defence against ectoparasites. In 'Insect and Bird Interactions'. (Eds H. F. van Emden and M. Rothschild) pp.

241-257. (Intercept: Andover, U.K.)

209. Moyer, B.R., Drown, D.M., Clayton, D.H., 2002. Low humidity reduces ectoparasite pressure: implications for host life history evolution. Oikos. 97, 223-228.

210. Mulder, R.A., 1995. Natal and breeding dispersal in a cooperative, extra-group mating bird. Journal of Avian Biology. 26, 234-240.

211. Muñoz, M.J., Neves, E., Ballesteros, C, López, J., Vásquez, E., Esperón, F., 2010. Screening for several potential pathogens in feral pigeons (*Columba livia*) in Madrid. Acta Veterinaria Scandinavica. 52, 45.

212. Murata, K., 2002. Prevalence of blood parasites in Japanese wild birds. Journal of Veterinary Medical Science. 64, 785-790.

213. Murata, K., Nii, R., Yui, S., Sasaki, E., Isuikawa, S., Sato, Y., Matsui, S., Horie, S., Akatani, K., Takagi, M., Sawabe, K., Tsuda, Y., 2008. Avian haemosporidian parasites infection in wild birds inhabiting Minami-Daito Island of the northwest pacific, Japan. Journal of Veterinary Medicine Science. 70, 501-503.

214. Neil, K., Wu, J., 2006. Effects of urbanization on plant flowering phenology: a review. Urban Ecosystems. 9, 243-257.

215. Nichols, E., Gómez, A., 2011. Conservation education needs more parasites. Biological Conservation. 144, 937-941.

216. Ogrzewalska, M., Uezu, A., Labruna, M.B., 2010. Ticks (Acari: Ixodidae) infesting wild birds in the eastern Amazon, norhtern Brazil, with notes on rickettsial infection in ticks. Parasitology Research. 106: 809-816.

217. Oorebeek, M., Kleindorfer, S., 2009. The prevalence and intensity of tick infestation in passerines for South Australia. Austral Ornithology 109, 121-125.

218. Ortega-Álvarez, R., MacGregor-Fors, I. 2011. Dusting-off the file: A review of knowledge on urban ornithology in Latin America. Landscape and Urban Planning. 101, 1-10.

219. Ots, I., Hõrak, P., 1998. Health impact of blood parasites in breeding great tits. Oecologia. 116, 441-448.

220. Ots, I., Murumagi, A., Hõrak, P., 1998. Haematological health state indices of reproducing great tits: methodology and sources of natural variation. Functional Ecology. 12, 700-707.

221. Owen, J.V., 2011. Collecting, processing, and storing avian blood: a review. Journal Field Ornithology. 82, 339-354.

222. Owen, J.C., Moore, F.R., 2006. Seasonal differences in immunological condition of three species of thrushes. The Condor. 108, 389-398.

223. Palinauskas, V., Valkiunas, V., Bolshakov, C.V., Bensch, S., 2008. *Plasmodium relictum* (linage P-SGS1): effects on experimentally infected passerine birds. Experimental Parasitology. 120, 372-380.

224. Parsons, H., French, K., Major, R.E., 2003. The influence of remnant bushland on the composition of suburban bird assemblages in Australia. Landscape and Urban Planning. 66, 43-56.

225. Parsons, H., Major, R.E., French, K., 2006. Species interactions and habitat associations of birds inhabiting urban areas of Sydney, Australia. Austral Ecology. 31, 217-227.

226. Partecke, J., Hof, J., Gwinner, V., 2013. Underlying physiological control of reproduction in urban and forest-dwelling European Blackbirds *Turdus merula*. Journal of Avian Biology. 36, 295-305.

227. Partecke, J., Schwabl, I., Gwinner, E., 2006. Stress and the city: urbanization

and its effects on the stress physiology in European black-birds. Ecology. 87, 1945-1952.

228. Patz, J.A., Daszak, P., Tabor, G.M., Aguirre, A.A., Pearl, M., Epstein, J., Wolfe, N.D., Kilpatrick, A.M., Foufopoulos, J., Molyneux, D., Bradly, D.J., Members of the Working Group on Land Use Change and Disease Emergence. 2004. Unhealthy landscapes: policy recommendations on land use change and infectious disease emergence. Environmental Health Perspectives. 112, 1092-1098.

229. Peach, W., Fowler, J., Hay, J., 1989. Incidence of Toxoplasma infection in a population of European starlings *Sturnus vulgaris* from Central Englad UK. Annals of Tropical Medicine and Parasitology. 83, 173-178.

230. Pedersen, A.B, Babayan, S.A., 2011. Wild immunology. Molecular Ecology. 20, 872-880.

231. Pedersen, A.B. Fenton, A., 2006. Emphasizing the ecology in parasite community ecology. Trends in Ecology and Evolution. 22, 133-139.

232. Pedersen, K., Clark, L., Andelt, W.F., Salman, M.D. 2006. Prevalence of Shiga toxin-producing *Escherichia coli* and *Salmonella enterica* in rock pigeons captured in Fort Collins, Colorado. Journal of Wildlife Diseases. 42, 46-55.

233. Poiani, A., 1992. Ectoparasitism as a possible cost of social life: a comparative analysis using Australian Passerines (Passeriformes). Oecologia. 92, 429-441.

234. Poiani, A., 1993. Small clutch sizes as a possible adaptation against ectoparasitism: a comparative analysis. Oikos. 68, 455-462.

235. Poiani, A., Goldsmith, A.R., Evans, M.R., 2000. Ectoparasites of house sparrows (*Passer domesticus*): and experimental test of the immunocompetence

handicap hypothesis and a new model. Behavioral Ecology and Sociobiology. 47, 230-242.

236. Polley, L., 2005. Navigating parasite webs and parasite flow: Emerging and re-emerging parasitic zoonoses of wildlife origin. International Journal of Parasitology. 35, 1279-1294.

237. Pongsiri, M.J., Roman, J., Ezenwa, V.A., Goldberg, T.L., Koren, H.S., Newbold, S.C., Ostfeld, R.S., Pattanayak, S.K., Salkeld, D.J., 2009. Biodiversity loss affects global disease ecology. Bioscience. 59, 945-954.

238. Prukner-Radovcic, E., Horvatek, D., Gottstein, Z., Ciglar-Grozdanic, I., Mazija, H., 2005. Epidemiological investigation of *Chlamydophila pssitaci* in pigeons and free-living birds in Croatia. Veterinary Research Communications. 29, 17-21.

239. Quillfeldt, P., Arriero, E., Martinez, J., Masello, J.F., Merino, S., 2011. Prevalence of blood parasites in seabirds- a review. Frontiers in Zoology. 8, 26. DOI: 10.1186/1742-9994-8-26

240. Rehacek, J., Kocianova, E., Brezina, R., 1984. The possible significance of urban populations of the pigeons Columba livia f. domestica in propagating *Coxiella burnetii* and *Chlamydia psittaci* in Bratislava Czechoslovakia. Biologia (Bratislava). 39, 293-300.

241. Reiczigel, J., Rózsa, L. 2005., Quantitative Parasitology 3.0. Budapest. Distributed by the authors.

242. Reisen, W.K., Martinez, V.M., Fang, Y., Garcia, S., Ashtari, S., Wheeler, S.S., Caroll, B.D., 2006. Role of California (*Callipepla californica*) and Gambel's (*Callipepla gambelii*) quail in the ecology of mosquito-borne encephalitis viruses in California, USA. Vector Borne and Zoonotic Diseases. 6,

248-260.

243. Reperant, L.A., Hegglin, D., Fischer, C., Kohler, L., Weber, J.M., Deplazes, P., 2007. Influence of urbanization on the epidemiology of intestinal helminths of the red fox (*Vulpes vulpes*) in Geneva, Switzerland. Parasitology Research. 101, 605-611.

244. Reye, A.L., Hübschen, J.M., Sausy, A., Muller, C.P., 2010. Prevalence and Seasonality of Tick-Borne Pathogens in Questing *Ixodes ricinus* Ticks from Luxembourg. Applied and Environmental Microbiology. 76, 2923-2931.

245. Ricklefs, R.E., Gray, J.D., Latta, S.C., Svensson-Coelho, M., 2011. Distribution anomalies in avian haemosporidian parasites in the southern Lesser Antilles. Journal of Avian Biology. 42, 570-584.

246. Riley, S.P.D., Serieys, L.E.K., Moriarty, J.G., 2014. Infectious disease and contaminants in urban wildlife: unseen and often overlooked threats. In 'Urban Wildlife Conservation'. (Eds. R.A. McCleery, C.E. Moorman and M.N. Peterson) pp. 175-215. (Springer, U.K.)

247. Robinson, R.A., Lawson, B., Toms, M.P., Peck, K.M., Kirkwood, J.K., Chantrey, J., Clatworthy, I.R., Evans, A.D., Hughes, L.A., Hutchinson, O.C., John, S.K., Pennycott, T.W., Perkins, M.W., Rowley, P.S., Simpson, V.R., Tyler, K.M., Cunningham, A.A., 2010. Emerging infectious disease leads to rapid population declines of common british birds. PLoS One. 5, 1-12.

248. Rojas, R., Marini, M.Â., Zanatta-Coutinho, M.T., 1999. Wild birds as hosts of *Amblyomma cajennense* (Fabricius, 1787) (Acari: Ixodidae). Memórias do Instituto Oswaldo Cruz. 94, 315-322.

249. Romero, L.M., 2002. Seasonal changes in plasma glucocorticoid concentrations in free-living vertebrates. General and Comparative

Endocrinology. 128, 1-24.

250. Romero, L.M., 2004. Physiological stress in ecology: lessons from biomedical research. Trends in Ecology and Evolution. 19, 249-255.

251. Rościszewska M, Sciszewska, M., Bonczar, Z, & Pospiech, N. nczar Z, Pośpiech N. Petryszak, A., Ros, A., 2000. Analyses of the population structure of Mallophaga infesting urban pigeons. Wiadomosci Parazytologiczne. 46: 75-85.

252. Rosenfield, R.N., Taft, S.J., Stout, W.E., Driscoll, T.G., Evans, D.L., Bozek, M.A., 2009. Low prevalence of *Trichomonas gallinae* in urban and migratory cooper's hawks in northcentral North America. The Journal Wilson of Ornithology. 121, 641-644.

253. Ruiz, G., Rosenmann, M., Novoa, F.F., Sabat, P., 2002. Hematological parameters and stress index in Rufous-collared Sparrows dwelling in urban environments. The Condor. 104, 162-166.

254. Sambrook, J., Russell, D.W., 2001. Molecular cloning: a laboratory manual. Cold Spring Harbor: Cold Spring Harbor Laboratory

255. Santaniello, A., Gargiulo, A., Borrelli, L., Pipineto, L., Cuomo, A., Sensale, M., Fontanella, M., Calabria, M., Musella, V., Menna, L.C., Fioretti, A., 2007. Survey of Shiga toxin-producing in urban pigeons (*Columba livia*) in the city of Napoli, Italy. Italian Journal of Animal Science. 6, 313-316.

256. Santiago-Alarcón, D., Palinauskas, V., Schaefer, H.M., 2012. Diptera vectors of avian Haemosporidians parasites: untangling parasite life cycles and their taxonomy. Biological Reviews. 87, 928-964.

257. Santiago-Alarcón, D., Rodríguez-Ferraro, A., Parker, P.G., Ricklefs, R.E., 2014. Different meal, same flavor: coespeciation and host switching of haemosporidian parasites in some non-passerine birds. Parasites and Vectors. 7,

1-9.

258. Schochat, E., Warren, P.S., Faeth, S.H., Hope, D. 2006. From patterns to emerging process in mechanistic urban ecology. Trends in Ecology and Evolution. 21, 186-191.

259. Scheuerlein, A., Ricklefs, R.E., 2004. Prevalence of blood parasites in European passeriform birds. Proceedings of the Royal Society of London B: Biological Sciences. 271, 1363–1370.

260. Schmidt, K.A., Whelan, C.J., 1999. The relative impacts of nest predation and brood parasitism on seasonal fecundity in songbirds. Conservation Biology. 13, 46-57.

261. Sebaio, F., Braga, E.M., Branquinho, F., Manica, L.T., Marini, M.A., 2010. Blood parasites in Brazilian Atlantic Forest birds: effect of fragment size and habitat dependency. Bird Conservation International. 20, 432-439.

262. Sehgal, R.N.M., 2010. Deforestation and avian infectious diseases. The Journal of Experimental Biology. 15, 955-960.

263. Seneviratna, P., Edward, A.G., DeGiusti, D.L., 1975. Frequency of *Sarcocystis* spp. in Detroit, metropolitan area, Michigan. American Journal of Veterinary Research. 36, 337-339.

264. Shehata, C.; Freed, L., Cann, R.L., 2001. Changes in native and introduced bird populations on O'ahu: Infectious diseases and species replacement. Studies in Avian Biology. 22, 264-273.

265. Shurulinkov, P., Ilieva, M., 2009. Spatial and temporal differences in the blood parasite fauna of passerine birds during the spring migration in Bulgaria. Parasitology Research. 104, 1453-1458.

266. Sitko, J., Zalésny, G., 2014. The effects of urbanization on helminth

communities in the Eurasian blackbird (Turdus merula L.) from the eastern part of the Czech Republic. Journal of Helminthology. 88, 97-104.

267. Skoracki, M., Buczek, A., Hofman, T., Sikora, B., Michalik, J., 2003. *Borrelia burgdorfesi* s.l. in *Ixodes ricinus* (Acari: Ixodidae) ticks collected from vegetation and small rodents in recreational areas of the City Poznan. Journal of Medical Entomology. 40, 690-697.

268. Sol, D., Jovani, R., Torres, J., 2003. Parasite mediated mortality and host immune response explain age-related differences in blood parasitism in birds. Oecologia. 135, 542-547.

269. Sorci, G., Cornet, S., 2010. Immunity and virulence in bird-parasite interactions. Open Ornithology Journal. 3: 33-40.

270. Stevenson, R.D., Woods, W.A, Jr,. 2006. Condition indices for conservation: new uses for evolving tools. Integrative Comparative Biology. 46, 1169-1190.

271. Sumoski, S.E., Johncox, A.J., Althoff, D.M., Segraves, K.A., 2009. Impact of urbanization of tri-trophic interactions in an endemic scrub community. The Florida Entomologist. 92, 582-587.

272. Sutherst, R.W., 2001. The vulnerability of animal and human health to parasites under global change. International Journal for Parasitology. 31, 933-948.

273. Swaddle, J.P., Calos, S.E., 2008. Increased avian diversity is associated with lower incidence of human West Nile Virus infection: observation of Dilution Effects. PloS One. 3, 1-8.

274. Tarsitano, E., Greco, G., Decaro, N., Nicassio, F., Lucente, M.S., Buonavoglia, C., Tempesta, M., 2010. Environmental Monitoring and Analysis of Faecal Contamination in an Urban Setting in the City of Bari (Apulia Region,

Italy): Health and Hygiene Implications. International Journal of Environmental Research and Public Health. 7, 3972-3986.

275. Tauro, L.B., Díaz, L.A., Almirón, W.R., Contigiani. M.S., 2009. Infection by Bunyamwera virus (*Orthobunyavirus*) in free ranging birds of Cordoba city (Argentina). Veterinary Microbiology. 139, 153-155.

276. Todd, M.K., 1996. Diet and foraging behaviour of Red-browed finches *Neochmia temporalis* near Newcastle, New South Wales. Emu. 96, 245-249.

277. Toro, H., Saucedo, C., Borie, C., Gough, R.E., Alcaino, H., 1999. Health status of free-living pigeons in the city of Santiago. Avian Pathology. 28, 619-623.

278. Tratalos, J., Fuller, R.A., Evans, K.L., Davies, R.G., Newson, S.E., Greenwood, J.J.D., Gaston, K.J., 2007. Bird densities are associated with household densities. Global Change Biology. 13, 1685-1695.

279. Tsiodras, S., Kelesidis, T., Kelesidis, L., Bauchinger, U., Falagas, M.E., 2008. Human infections associated with wild birds. Journal of Infection. 56, 83-98.

280. Valera, F., Hoi, H., Kristin, A., 2006. Parasite pressure and its effects on blood parameters in a stable and dense population of the endangered Lesser grey shrike. Biodiversity and Conservation. 15, 2187-2195.

281. Vallve, S.L., Rojo, H., Wisniveksy-Colli, C., 1995. Ecología urbana de Triatoma infestans en Argentina: asociación entre *Triatoma infestans* y palomares (*Triatoma infestans* urban ecology in Argentina: Association between *Triatoma infestans* and pigeon cotes). Revista Saude Publica. 29, 192-198. [in Spanish]

282. Ventim, R., Mendes, L., Ramos, J.A., Cardoso, H., Pérez-Tris, J., 2012.

Local haemoparasites in introduced wetland passerines. Journal of Ornithology. 153, 1253-1259.

283. Ventim, R., Morais, J., Pardal, S., Mendes, L., Ramos, J.A., Pérez-Tris, J., 2012. Host-parasite associations and host-specificity in haemoparasites of reed bed passerines. Parasitology. 139, 310-316.

284. Ventim, R., Tenreiro, P., Grade, N., Encarnacão, P., Araújo, M., Mendes, L., Ramos, J.A., Pérez-Tris, J., Ramos, J.A., 2012. Characterization of haemosporidian infections in warblers and sparrows at south-western European reed beds. Journal of Ornithology. 153, 505-512.

285. Vinkler, M., Schnitzer, J., Munclinger, P., Votypka, Albrecht, T., 2010. Haematological health assessment in a passerine with extremely high proportion of basophils in peripheral blood. Journal of Ornithology. 151, 841-849.

286. Waap, H., Vilares, A., Rebelo, E., Gomes, S., Angelo, H., 2008. Epidemiological and genetic characterization of *Toxoplasma gondii* in urban pigeons from the area of Lisboa (Portugal). Veterinary Parasitology. 157, 306-309.

287. Waldenström, J., Bensch, S., Kiboi, S., Hasselquist, D., Ottosson, U., 2002. Cross-species infection of blood parasites between resident and migratory song birds in Africa. Molecular Ecology. 11, 1545-1554.

288. Walther, B.A. and Clayton, D.H. (1997). Dust-ruffling: a simple method for quantifying ectoparasite loads of live birds. Journal of Field Ornithology. 68, 509-518.

289. Ward, M.P., Raim, A., Yaremych-Hamer, S., Lampman, R., Novak, R.J. 2006. Does the roosting behavior of birds affect transmission dynamics of West Nile virus? American Journal of Tropical Medicine and Hygiene. 75, 350-355.

290. White, M., Greiner, E.C., Bennett, G.F., Herman, C.M., 1978. Distribution of the hematozoa of Neotropical birds. Revista de Biología Tropical. 26, 43-102.

291. Wood, M.J., Cosgrove, C.L., Wilkin, T.A., Knowles, S.C., Day, K.P., Sheldon, B.C., 2007. Within-population variation in prevalence and linage distribution of avian malaria in blue tits, *Cyanistes caeruleus*. Molecular Ecology. 16, 3263-3273.

292. Yanga, S., Martínez-Gómez, J.E., Sehgal, R.N.M., Escalante, P., Camacho, F.C., Bell, D.A., 2011. A preliminary survey for avian pathogens in Columbiform birds on Socorro Island, Mexico. Pacific Conservation Biology. 17, 11-21.

293. Yong, L.H.,, Ambu S., Devi, S., Maung, M. 2008. Detection of protozoan and bacterial pathogens of public health importance in faeces of *Corvus* spp. (large-billed crow). Tropical Biomedicine. 25, 134-139.

294. Zamora-Vichis, I., Williams, S.E., Johnson, C.N., 2012. Environmental temperature affects prevalence of blood parasites of birds on an elevation gradient: implications for disease in a warming climate. PLoS One. 7, 1-8.

295. Zarrin, M., Jorfi, M., Amirrajab, N., Rostami, M., 2010. Isolation of *Cryptococcus neoformas* from pigeon droppings in Ahwaz, Iran. Turkish Journal of Medical Science. 40, 313-316.

296. Zinke, A., Schnebel, B., Dierchke, V. 2004. Prevalence and intensity of excretion of coccidial oocysts in migrating passerines on Helgoland. Journal of Ornithology. 145, 74-78.