General introduction and scope of the thesis

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Chapter 1

Introduction

The goal of this section is to introduce the main topics covered in this thesis providing an overview of the expectations of each thesis chapter. The section provides a comparison between temperate and arctic zones, where several studies related to our topics have been carried out, on the one hand and the equatorial tropical environment where many scientific information gaps still exist.

1. Reproduction, growth and immune function in temperate and arctic zones

Temperate and arctic zones have predictable seasonal changes in environmental conditions and day-length is the main driver of the timing of reproduction in birds (Demas and Nelson 1998, Wingfield et al. 1997, Versteegh et al. 2014, Colwell 1974). Birds use day-length to time reproduction (Lambrechts et al. 1996, Nager and van Noordwijk 1995, van Noordwijk et al. 1995) while temperature and food availability serve as supplementary cues to fine tune the timing of reproduction and nestling growth to local environmental conditions (Hau 2001, Lambrechts et al. 1996, Van Noordwijk et al. 1995). This regularity in the timing of reproduction and nestling growth and the predictability in seasonal changes in day-length potentially results in synchronized spring breeding within and among species living at the same location (Nager and van Noordwijk 1995, Lambrechts et al. 1996). Avian growth rate and development is associated with the pace-of-life and nestlings in species and populations from temperate and arctic zones have comparatively faster growth rates compared to those at lower latitudes (Ricklefs 1976, McCarty 2001, Ricklefs and Wikelski 2002, Wikelski et al. 2003, Wiersma et al. 2007). Raising of young in these zones is restricted to the spring season.

Like growth rates, immune function has been hypothesized to vary with the pace-of-life in birds, with reduced investment in the immune function associated with a faster pace-of-life (Ricklefs and Wikelski 2002, Martin et al. 2004, Tieleman et al. 2005). Physiological processes, e.g., reproduction, growth and immune function among others, require resources that are often limited and that come at a fitness cost. Due to the cost associated with the maintenance and use of an immune system, trade-offs have been shown to exist in temperate and arctic zone birds between immune defense and other life-history activities such as reproduction, growth and development, migration, thermoregulation (Sheldon and Verhulst 1996, Lochmiller and Deerenberg 2000, Norris and Evans 2000). In the case of reproduction and immune function, several studies involving increase in reproductive effort in birds have demonstrated reduced immune response when an immune system responds (Moreno et al 1999, Ilmonen et al 2000, Nordling et al 1998, Schmid-Hempel 2003). Birds also cushion increased demands on reproduction by allocating resources from immunity to reproduction in order to avoid reduction in reproductive output (Sheldon and Verhulst 1996). In addition, reduced reproductive effort has been shown to lower infection rates (Ots and Horak 1996) while prevalence and intensity of parasitic infection increase in animals during reproduction (Doreenberg et al 1997). Resource allocation from immunity to reproduction could increase the susceptibility to infection of individuals with increased reproductive investment (Sheldon and Verhulst 1996) leading to reduced fitness. Conversely, an increased investment in immune
function could lead to a correlated unavoidable loss in a fitness component such as reproduction (Stearns 1992).

Just as reproduction in temperate and arctic zone birds is sensitive to environmental variation (Nelson and Demas 1996, Marra and Holberton 1998, Shepherd and Shek 1998, Ruiz et al. 2002, Tieleman et al. 2005), immune function of birds in these zones varies seasonally (Martin et al. 2008), partly due to trade-off between reproduction and immune function (Martin et al. 2008, Buehler et al. 2008, Pap et al. 2010a, Hegemann et al. 2012, Hegemann et al. 2012, Horrocks et al. 2012, Pap et al. 2010b). However, seasonal environmental changes such as temperature, disease risk and food availability have been linked to annual variation in immune function (Nelson et al. 2002). Seasonal changes in disease risk and strengthening of the immune defense in anticipation of disease, based on changes in environmental cues, have also been shown to be a cause for seasonal variation in the immune function in birds (Nelson et al 2002). Reproductive activity and seasonal variation in immune function in the temperate and arctic zone system both being influenced by environmental factors makes it difficult to decouple the effects of reproduction from those of environmental factors on the variation in immune functions.

2. Variation in life history traits: avian reproduction and nestling growth in the context of environmental conditions

a) Variation in environmental conditions in equatorial tropics and avian reproduction

In contrast to the temperate and arctic zones, environmental conditions in equatorial tropical environments are frequently unpredictable and highly variable (Grant and Boag 1980, Boag and Grant 1984, Wrege and Emlen 1991, Conway et al. 2005). Consequently, a diversity of breeding systems and rates of growth exists in this region. In relatively seasonal tropical environments, tropical birds can use tropical photoperiod on a long-term basis and environmental conditions on a short-term basis to time reproduction (Hau et al. 2000). In seasonal tropical environments with predictable changes in wet and dry seasons, birds also breed seasonally and use environmental conditions (rain and food) to time reproduction in much the same way temperate birds do (Wikelski et al. 2000, Hau et al. 2001). Conversely, some tropical birds living in unpredictable climate, breed whenever it rains (Hau 2001), with no physiological preparation for a breeding season, indicating plasticity which allows them to respond to short-term cues and flexibility in their regulation of reproduction.

The high spatiotemporal variability in rainfall and food resources in proximal equatorial tropical environments (Young 1994a, Grant and Boag 1980, Boag and Grant 1984, Wrege and Emlen 1991, Scheuerlein and Gwinner 2002, Stutchbury and Morton 2001, Conway et al. 2005) results in high variability in the timing of reproduction and in nestling growth among and within environments. In such circumstances, birds have to time their reproduction based on short-term and more irregular factors, e.g., rainfall and food resources (Stutchbury and Morton 2001, Dittami and Gwinner 1985), resulting to asynchronous reproduction (Moore et al. 2005). Reproduction in equatorial tropical birds has become more adaptable to their highly unpredictable habitats with large variability in environmental conditions depending on a variety of factors that influence their breeding including rain (Dittami and Gwinner 1985, Dittami 1986, Chapman 1995), food supply (Dittami and Gwinner 1985, Chapman 1995, Komdeur
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1996, Hau et al. 2000, Scheuerlein and Gwinner 2002, Moore et al. 2005) and ambient temperature (Foster 1974, Tye 1991). Others tend to have more flexible breeding schedules breeding opportunistically (Boag and Grant 1984, Young 1994b), yet, in some equatorial tropical locations, birds may breed year-round (Ndithia per obs.). However, there have not been studies carried out to determine factors that may influence the timing of reproduction in opportunistically breeding birds and those that breed all year round. In addition, no studies have investigated several potential factors that may influence the timing of breeding in such species over time (several annual patterns) and comparing over space (different environments with different climatic conditions). It is the goal of this thesis to fill these gaps.

b) Variation in avian growth rates among and within equatorial tropical environments

Variation in avian growth rate and development occurs in equatorial tropical environments with faster growth rate in species and populations at high altitude (Khanna 2005, Scott 2011), in less arid environments (Tieleman et al. 2004, Tieleman 2005) and in early-hatched nestlings (Van Noordwijk 1995, Gebhardt-Henrich and Van Noordwijk 1991, Christians 2002). In addition, patterns of variation in nestling growth and development are hypothesized to reflect adaptation to specific environmental conditions (Starck and Ricklefs 1998, Demas and Nelson 2012). The spatiotemporal variation in climatic conditions associated with proximal equatorial tropical environments provide an opportunity to investigate how nestling growth rates have evolved in response to different within equatorial tropical climates (locations) and to different environmental conditions within the year. Nestling growth is hypothesized to decrease along aridity gradient with cool and wet environments, that are thought to provide more food resources, promoting faster nestling growth while more arid environments, thought to be devoid of food resources, fostering slower growth (Ricklefs 1976, Tieleman 2004). However, investment in growth rate in cool and wet environments is likely to be compromised by the cost associated with thermoregulation (Krijgsveeld et al. 2003). In addition, differences in environmental conditions during the year within an environment with unpredictable and inconsistent patterns of rainfall and food resources is likely to promote variation in nestling growth rates (McCarty 2001) with nestlings raised during wet periods, presumably with food abundance, exhibiting faster growth rate compared to those raised during dry food deficient periods (Emlen and Wrege 1991). Studies that compare growth rates of nestlings in different environments with distinct climatic conditions and in nestlings hatched during different times of the year in unpredictable and highly variable equatorial tropics have not been done. This thesis seeks to fill that gap by providing insights into how variation in environmental conditions shape variation in nestling growth rates.

3. Ecological immunology

The immune system is critical in the protection of any organism from disease-causing agents and works to increase fitness in animals. The immune system is highly complex and is as costly as it is beneficial to animals. Development, maintenance and use of an immune system is costly in terms of energy, e.g. nutrients required for the maintenance of lymphoid tissue, turnover of leukocytes, and time (Schmid-Hempel and Ebert 2003). This cost is paid whether
an individual is attacked or not (Kraaijeveld et al 2001). Animals also incur immunopathological costs due to the damage to the body’s own healthy cells as a result of an immune response. Increased formation of detrimental waste products causes damage to tissue, including the immune system as a result of high metabolic rate due to increased wear and tear of the body (Råberg et al. 1998). It is also costly to mount an immune response during infection or challenge (Råberg et al 1998, Schmid-Hempel 2003, Kraaijeveld et al 2001). These factors result in differences in immune responses depending on the magnitude of disease threat and resource availability based on the extent of investment in a defense mechanism by an individual (Sheldon and Verhulst 1996, Kraaijeveld et al 2001), all of which are regulated by ecological factors. The central goal of ecological immunology is to understand and explain immunological variation among and within species, typically in free-living animals, and uses immune measures to test ecological and evolutionary hypothesis (Sheldon and Verhulst 1996).

The innate immune system: a synopsis

All animals have natural enemies in the form of parasites and pathogens; a defense mechanism is therefore critical to fight these foreign bodies. Foreign bodies can either be non-biological or biological (antigens). Non-biological foreign substances are less harmful and are dealt with by the liver and kidneys (Clark 2008). Antigens are microbes found in the system of animals that live and reproduce in hosts and produce biologically-active molecules that are released into the body of the host (Clark 2008). The vertebrate immune system is composed of innate and adaptive immune systems.

Innate immune system

The innate immune system has evolved together with an organism over evolutionary times; its proteins are encoded in genes in the DNA passed from generation to generation in a species. Innate immune system responds to infection through two interconnected mechanisms.

a) Microbial pattern recognition (MPR) is based on the fact that microbes have certain structural features that they cannot change without losing their ability to survive and function and which become prime target for a chemically-based innate immune defense (Clark 2008). MPR have genetically encoded proteins that are able to recognize and bind to those unique, unalterable foreign microbial structural patterns called pathogen-associated molecular patterns (PAMPs); the innate immune system uses pattern recognition receptors (PRR) to interact with PAMPs in a similar way antibodies interact with antigens (Clark 2008).

b) Inflammation involves changes in blood flow, release of cytokines and altered white blood cell flow (Clark 2008). When white blood cells encounter microbes, the cells become activated, release pro-inflammatory cytokines and kick off an inflammatory reaction which involve redness and heat (caused by blood rushing to affected site), swelling and pain (caused by accumulation of lymph as a result of pressure on local nerves) (Clark 2008).
Complement and macrophages are components of the innate immune system. Complement helps antibodies kill microbes. Antibodies do not kill antigens, but only bind them causing agglutination (Clark 2008). When two antibody molecules bind close enough together on the same cell, their protruding antigen combining site (Fc tails) serve as an anchoring site for an assembly of a complement complex at that spot on the cell; the complement complex form pore structures, punch a hole in the cell membrane of microbe and allow water to enter the cell, killing it through osmotic rupture (Clark 2008). Macrophages are huge microbicidal cells which kill microbes by engulfing (Clark 2008). Macrophages have pattern recognition receptors (PRR) and therefore are PAMP-recognizing, and kill bacteria, fungi, and protozoan parasites through phagocytosis (Clark 2008). To improve their efficiency, macrophages use their Fc receptors and work together with antibodies - antibodies tag many microbial cells through its antigen-combining sites, macrophages kill them through phagocytosis in their lysosomes, therefore killing hundreds of times more efficiently (Clark 2008).

Adaptive immune function
The adaptive (also acquired) immune function, which mainly consists of B and T cells, uses ‘memory’ pool which acts as a record of the antigens the immune system has encountered in the past, and is thus able to compete genetically with the rapidly reproducing microbes (Clark 2008). Adaptive immune system focuses and intensifies the innate immune mechanisms and therefore provides synergistic interaction with the innate immune function (Clark 2008, Ochsenbein and Zinkernagel 2000, Matson et al. 2005). Innate and adaptive immunity are equal ‘partners’ and work together in a coordinated response mounted against microbial infection. However, we only focus on the innate immune function in this thesis because it is the only defense system present to protect organisms against pathogen and parasite exposure before the acquired immune response develops (Matson et al. 2005, Starck and Ricklefs 1998, Mauck et al. 2005, Pihlaja et al. 2004, Schmid-Hempel 2003, Clark 2008).

4. Variation in immune function

Inter-and-intra-tropical variation in immune function
In the face of the large variation in environmental conditions in equatorial tropical environments, the ontogeny of the immune function may vary widely among conspecifics living in different environments with distinct climate and within populations of conspecifics over time. These variations reflect adaptation to specific environmental conditions (Starck and Ricklefs 1998, Demas and Nelson 2012). Hypothesized to be related to the pace-of-life (Tella et al. 2002, Lee et al. 2006, Lee et al. 2008) innate immune function of nestlings is expected to match the parasitic and pathogen pressure exerted by different environments and at different periods of the year through the maternally-derived antibodies (Starck and Ricklefs 1998, Mauck et al. 2005, Stambaugh et al. 2011). Besides, nestlings raised under favourable environmental conditions, e.g., wet conditions with presumably more abundant food resources, are expected to have better innate immune function through better diet and by parent females depositing
high concentrations of maternal antibodies and defences compared to those raised under resource-strenuous conditions.

Unlike in the temperate and arctic zones, equatorial tropical environments that are in close proximity to each other are characterized by large inter-and-intra-annual variations in rainfall and food supply (Dittami and Gwinner 1985, Conway et al. 2005). Immune responses can be sensitive to environmental variation (Nelson and Demas1996, Marra and Holberton 1998, Shepherd and Shek 1998, Ruiz et al. 2002, Tieleman et al. 2005), which in equatorial tropical environments can vary within an environment, leading to variation in the occurrence, distribution, abundance and diversity of pathogens and parasites. This variation in environmental conditions and disease risk within an environment may result in sympatric species occupying different ecological niches within the same environment and/or having different reproductive strategies, leading to differences in immune responses. The co-occurrence of two study species in such a set up provides an opportunity (unique to this region) for interspecific comparison of reproduction-induced variation in immune function.

Additionally, just like disease risk, immune function may also vary with the climatic conditions of the environment of the host (Horrocks 2015, Horrocks 2012b, Zamora-Vilchis et al. 2012, Sehgal et al. 2011, Rubenstein et al 2008), and weather conditions of their environment during the year, resulting in differences in immune responses of conspecific species depending on disease threat in their geographical location (Ardia 2007). Conspecifics may also vary in their immune responses based on their sex due to differences in the roles males and females play during reproduction (Emerson and Hess 1996, Sossinka 1980, Hau et al. 2004, Möller et al. 2003). In Red-capped Larks, only females build nests and incubate but both sexes feed nestlings, with expected immunosuppression in females compared to males. In addition, immune responses of males may be selectively suppressed due to activation of reproductive hormones (Nunn et al. 2009). Immune responses may also vary due to fundamental differences in male and female life-histories, for instance body size differences (upregulated in males) (Nunn et al. 2009, Hasselquist 2007, Zuk 1996). Yet, factors influencing variation in immune function in equatorial tropical birds have not been studied. Some equatorial tropical bird species breed year round but it is unknown whether immune function of such year-round breeding equatorial species varies with reproduction and how that happens. In our lark species reproduction occurs year-round, while their environment(s) are characterized by varying environmental conditions across the year among and within environments. These larks are potentially opportunistic breeders whose timing of reproduction is potentially not related to biotic and abiotic environmental factors. Equatorial tropical system provides the unique opportunity to investigate the effects of breeding behaviour on immunity, while excluding effects of the environment. By confirming that environmental conditions do not differ between periods at which we sampled breeding and non-breeding birds, we are sure that any differences in immune function between breeding and non-breeding birds do not result from environmental variation.
5. The study system and the lark family

Birds have been used for a variety of ecological studies and can be used to assess what the drivers of patterns in ecosystems are (Rahbeck 1997, Sanders et al. 2003) because they are well-studied, are taxonomically stable, are easily surveyed and are widely-distributed across many habitats. The larks family Alaudidae has closely related species that occur in a broad range of climatic and environmental conditions (Alström et al. 2013, Zimmermann et al. 1999). This makes the family an ideal choice as model to investigate spatiotemporal variations in physiology and life-history (Alström et al. 2013). The larks family has been used to investigate interspecific variation in immune function along climate gradient as proxy for environmental disease risk (Horrocks et al. 2015, Horrocks et al. 2012, Williams and Tieleman 2005) and to provide an ecological perspective on microbes and immune defense in eggs (Grizard et al. 2015). These evaluations using the lark family have provided a perfect study system for comparative inter-and-intra-specific variation in immune indices, reproductive strategies and growth patterns particularly in an equatorial tropical region with high intra-and-inter-tropical variation in environmental conditions.

Our study system uses the Red-capped Calandrella cinerea and the Rufous-naped Larks Mirafra africana, two sedentary species living and breeding simultaneously in different environments with differing climatic conditions in Kenya: cool and wet South Kinangop; warm and wet North Kinangop and warm and dry Kedong. South Kinangop receives on average 939 ±132.7 (SD) mm of rain per year, and experiences variation in monthly mean T_{min} between 3.0 and 8.2ºC, and monthly mean T_{max} between 21.2 – 30.0ºC. North Kinangop receives on average 584 ± 62.6 (SD) mm of rain per year, and experiences variation in monthly mean T_{min} between 3.0 and 13.7ºC, and monthly mean T_{max} between 22.1 and 30.5ºC, while Kedong receives on average 419 ± 96.8 (SD) mm of rain per year, and experiences variation in monthly mean T_{min} between 6.2 – 15.7ºC, and monthly mean T_{max} between 25.3 – 34.9ºC (for details of climatic conditions, see Ndithia et al. 2017a). Variation in climatic conditions in these environments help to comparatively examine environmentally induced adaptations of reproduction and physiology, and is important in understanding how environmental conditions of different locations shape the reproductive strategies of different populations of the same species. In addition, as a result of the variation of environmental conditions of an environment during the year, this set up allows for the evaluation of across-species within-location variation in costs and benefits of different reproductive strategies and immune response to understand why species have certain reproductive strategies/immune response compared to others.

This comparative study benefits from the fact that the two lark species have similar ecological traits therefore avoiding the complications that arise from different evolutionary histories. Both species are ground-nesters, build open-cuped nests, breed year-round, have similar clutch size and incubation patterns, consume similar diet and have similar foraging behaviour. Despite both species inhabiting open grasslands, they use different micro-habitats occasioned by variation in weather conditions within an environment, and with potentially different disease risk and immune responses. However, up to now, factors that influence variation in immune function among sympatric species during the year and factors that influence variation in immune function within a single species living in different environments differing in climatic conditions are unknown in an equatorial tropical region. Overall, based on
existing knowledge on their behaviour, physiology, life history and environmental conditions, these two lark species and the associated spatiotemporal differences in climatic conditions of their environments provide a perfect and unique model for investigating and understanding the ecology and evolution of immune function with respect to reproduction and environmental conditions in an equatorial tropical region.

6. General objectives and thesis outline

Factors that influence the timing of reproduction, variation in growth and immune function among and within environments in equatorial tropical birds are largely unknown. This is the general objective of this thesis. The unpredictability and high variability of environmental conditions in equatorial tropical environments results in different factors playing the role of influencing reproduction in birds among and within environments in this region. To understand environmental conditions of equatorial tropics in order to evaluate how they may influence the ecology and physiology of birds living in this region, we started in Chapter 2 by quantifying and examining (to understand the patterns) intra-annual variation in rainfall, average minimum ($T_{\text{min}}$) and average maximum ($T_{\text{max}}$) temperatures and invertebrate abundance and occurrence and intensity of breeding in Red-capped Larks within each of our three climatically distinct environments. We had expected a bimodal pattern of rainfall which subsequently could influence pattern of food availability and breeding in larks. We consequently related breeding in each environment with these biotic and abiotic factors to determine which among them influenced breeding. Essentially, we investigated the hypotheses that in equatorial tropical environments, birds time their breeding to match periods of high rainfall and temperature (Hau 2001, Tieleman and Williams 2005, Foster 1974, Skutch 1950, Tye 1991) and/or peak periods of food supply (Tieleman and Williams 2005, Young 1994b, Boag and Grant 1984, Poulin et al. 1992, Skutch 1950) using larks as a model. Subsequently among these environments, we compared these biotic and abiotic factors and the occurrence and intensity of breeding to determine their differences, and whether reproduction in Red-capped Larks is influenced by the same factors in different environments, in which case it would point to an evolutionary link of reproduction to environmental conditions.

Variation in environmental conditions is hypothesized to cause variation in patterns of growth and development and variation in the ontogeny of immune function among and within populations (Starck and Ricklefs 1998, Demas and Nelson 2012). We exploited the spatiotemporal variation in climatic conditions in our three climatically-distinct study environments to understand variation in life-history strategies among and within tropical locations which formed the basis of our study in Chapter 3 of this thesis. We compared growth and immune function in lark nestlings from South Kinangop, North Kinangop and Kedong and studied growth and immune function as a function of year-round variation in breeding intensity and rain within Kedong. We proposed that differences of our three study locations in orography and in altitude, and the fluctuating and inconsistent patterns of rainfall and food availability within and between years in Kedong (see chapter 2 of this thesis) may have consequences on growth. In addition, we hypothesized that the large environmental variation among equatorial environments is likely to cause variation in immune function in nesting birds, variations that may reflect the local pathogen pressures exerted by different environments through the
maternally derived antibodies (Sheldon and Verhulst 1996, Norris and Evans 2000). Again, breeding under favorable environmental conditions during the year allows females to access better diet and are thus able to deposit higher concentrations of maternal antibodies and defenses. Females are therefore able to give rise to nestlings with better immune function compared to those bred during periods with poor environmental conditions. We investigated how nestling growth rates and variation in immune function have evolved in response to differences in environmental conditions among locations and during different periods of the year respectively, questions that are still unexplored in equatorial tropical environments.

Reproduction is reported in multiple studies to trade-off with immune function; they are both costly physiological processes that compete for allocation of the often limited resources (Sheldon and Verhulst 1996, Lochmiller and Deerenberg 2000, Norris and Evans 2000). At the same time, immune function can be sensitive to environmental variations (Nelson and Demas 1996, Marra and Holberton 1998, Shepherd and Shek 1998, Ruiz et al. 2002, Tieleman et al. 2005). However, our study species breed year-round as opposed to temperate and arctic zone birds which breed only in the spring hence the effect of reproduction on immune function can also be confounded by the effects of environmental conditions. In Chapter 4, we exploited this unique system of equatorial tropical environments by examining the role of reproduction in the variation of immune function and mass while controlling for the effects of environmental conditions in two sympatric species in North Kinangop, the Red-capped Calandrella cinerea and the Rufous-naped Larks Mirafræ africana. Variation in environmental condition is likely to result in different ecological niches within a location (with potentially different pathogen pressure), leading to sympatric species occupying these different niches or exhibiting different reproductive strategies. We investigated whether our two species differed in their immune responses and mass during breeding and non-breeding periods, and whether males and females of each of these species differed in their immune response as a result of the different roles they play during reproduction (Emerson and Hess 1996, Sossinka 1980, Hau et al. 2004, Møller et al. 2003) or due to fundamental differences in male and female life-histories (Nunn et al. 2009, Hasselquist 2007, Zuk 1996). We expected non-breeding birds to generally have more robust immune function compared to breeding ones (Nelson and Demas 1996, Bentley et al. 1998, Martin et al. 2008). We checked if environmental conditions differed between periods at which we sampled breeding and non-breeding birds. We also did not expect environmental variables to differ between breeding and non-breeding birds in any of the two species. This study of the influence of reproduction on immune function while excluding the effects of environmental conditions is novel in equatorial tropics.

Variation in immune function has been attributed to life history trade-offs and to variation in environmental conditions (Sheldon and Verhulst 1996, Tieleman 2018), the latter of which may reflect pathogen pressure in an environment. In Chapter 5 of this thesis, we investigated the role of reproduction in the variation of immune function by exploiting a unique study system of three populations of year-round breeding Red-capped Larks living and breeding simultaneously in environments with large intra-and inter-annual variations and unpredictable temporal patterns of rainfall and food availability. We investigated if immune function and body mass differed among chick-feeding and non-breeding (males and females), and incubation (females only) from three climatically distinct environments that are generally permissive of year-round breeding (Ndithia et al. 2017a). Based on resource trade-offs, within
each location, we expected non-breeding birds to generally have more robust immune function (Bentley et al. 1998, Nelson and Demas 1996, Martin et al. 2008) and higher body mass (Moreno 1989) compared to breeding ones. Based on the antigen exposure hypothesis which predicts reduced microbial abundance in arid environments (Horrocks et al. 2012), we expected immune function to decrease along a gradient of aridity from South Kinangop to North Kinangop and Kedong. We checked if environmental conditions differed between periods at which we sampled breeding and non-breeding birds, therefore excluding the potential role of environmental condition in influencing variation in immune function. We expected that environmental conditions would not differ according to breeding stages and hence could be excluded as confounding factors in explaining any reproduction-associated variation in immune function. Again, like in Chapter 4 above, we explored the role of sex in the variation of immune function and expected that during breeding, females would have depressed immune function due to their higher reproductive effort (nest building, incubating and chick-feeding as opposed to males’ chick-feeding only).

Chapter 6 synthesizes the findings of each individual chapter in this thesis putting the findings in the context of expected and/or predicted results and compare/contrast with what is known from literature.
Chapter 2

Year-round breeding equatorial Larks from three climatically-distinct populations do not use rainfall, temperature or invertebrate biomass to time reproduction.

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