Individual Variation in Immune Function in the Purple-crowned Fairy-wren (*Malurus coronatus*)

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Abstract

Animal immune systems are essential for defence against parasitism and disease. Immune functions are costly because they require resources to operate and can cause collateral damage to an organism's own cells. These costs are important when resources are finite because they may result in physiological trade-offs between immune function and other costly processes like reproduction, in order to maximise individual fitness. In theory, there should be some level of optimal immune function, but this could be highly context-dependent, and there is much unexplained variation between individuals. In this thesis I aim to investigate individual variation in constitutive innate immunity (baseline immune defences) and (1) how it can be driven by environmental factors, (2) how it varies with age within and between individuals, and (3) how it can ultimately affect individual fitness outcomes.

My study species, the purple-crowned fairy-wren (*Malurus coronatus*) is a sedentary, resident species of northern Australia. The species lives in social groups occupying stable year-round territories which provides an exceptional opportunity to gather detailed information about the immediate environment, life-histories, and reproductive outcomes for every single studied individual. By quantifying immune function in individuals through repeated captures and sampling, I am able to associate the individual's immune status to the detailed information about of their lives to gather an insightful observational perspective on the possible causes and consequences of individual variation in immune function.

Firstly (chapter 2), I find that social and ecological variables are relatively unimportant compared to short-term climatic variables, which have the strongest relationships with immune function and stress. Immune function remained relatively stable relative to long term seasonal environmental variation. Consequently, immune function may be more susceptible to short-term environmental perturbations, and fairy-wrens may be anticipating predictable seasonal change and moderating their immune investment to maintain baseline function. Secondly (chapter 3), I use multiple repeated measures of immune function from individuals to assess age-related changes over time. I find limited evidence of immunosenescence (a decline in immune function with age), in addition to evidence of immune maintenance of some immune components. These results suggest that self-maintenance roles of immune components beyond parasite defence could become more important with age. In chapter 3, I also demonstrate how longitudinal studies are important for discerning individual and population level trends. Lastly (chapter 4), I find that a higher probability of survival is not related to higher levels of immune function, but I also find some evidence that optimal immunity is context-

dependent. I do not find any evidence of trade-offs between immune function and reproductive output, but instead that individual quality might override trade-offs. I also find that chronic stress and body condition do not strongly relate to fitness outcomes in ways that support hypotheses regarding environmental stressors and resource availability. Maintaining baseline levels of immune components does not appear to be costly for fitness in these fairy-wrens. In the final chapter of my thesis, I discuss the possibility of immune component-specific trade-offs and immunoplasticity emerging from the context-dependency of immune function, in addition to suggested directions for future research.

Publications during enrolment

First author publications

- **M. J. Roast**, A. E. Aulsebrook, M. Fan, N. Hidalgo Aranzamendi, N. Teunissen, A. Peters (2019) Short-term climate variation drives baseline innate immune function and stress in a tropical bird: a reactive scope perspective. *Physiological and Biochemical Zoology.* 92 (2), p140-150. https://doi.org/10.1086/702310

Co-author publications

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- L. B. Martin, B. Addison, A. G. D. Bean, K. L. Buchanan, O. L. Crino, J. R. Eastwood, A. S. Flies, R. Hamede, G. E. Hill, M. Klaassen, R. E. Koch, J. M. Martens, C. Napolitano, E. J. Narayan, L. Peacock, A. J. Peel, A. Peters, N. Raven, A. Risely, **M. J. Roast**, L. A. Rollins, M. Ruiz-Aravena, D. Selechnik, H. S. Stokes, B. Ujvari, L. F. Grogan (2019) Extreme Competence: Keystone Hosts of Infection. *Trends in Ecology and Evolution.* 34 (4), p303-314. https://doi.org/10.1016/j.tree.2018.12.009
- J. R. Eastwood, M. L. Hall, N. Teunissen, S. A. Kingma, N. Hidalgo Aranzamendi, M. Fan, **M. J. Roast**, S. Verhulst, A. Peters (2018) Early-life telomere length predicts lifespan and lifetime reproductive success in a wild bird. *Molecular Ecology.* 28 (5), p1127-1137. https://doi.org/10.1111/mec.15002
- M. Fan, N. Teunissen, M. L. Hall, N. Hidalgo Aranzamendi, S. A. Kingma, **M. J. Roast**, K. Delhey, A. Peters (2018) From ornament to armament or loss of function? Breeding plumage acquisition in a genetically monogamous bird. *Journal Animal Ecology.* 87 (5), p 1274-1285. https://doi.org/10.1111/1365-2656.12855

Thesis including published works declaration

I hereby declare that this thesis contains no material which has been accepted for the award of any other degree or diploma at any university or equivalent institution and that, to the best of my knowledge and belief, this thesis contains no material previously published or written by another person, except where due reference is made in the text of the thesis.

This thesis includes 1 original paper published in peer reviewed journals and 1 submitted publication. The core theme of the thesis is 'Individual Variation in Immune Function'. The ideas, development and writing up of all the papers in the thesis were the principal responsibility of myself, the student, working within the School of Biological Sciences under the supervision of Anne Peters and Matthew Hall.

The inclusion of co-authors reflects the fact that the work came from active collaboration between researchers and acknowledges input into team-based research. In the case of *Chapters 2, 3 and 4,* my contribution to the work involved the following:

Thesis Chapter	Publication Title	Status	Nature and % of student contribution	Co-author name(s) Nature and % of Co-author's contribution*	Co-author(s), Monash student Y/N*
2	Short-term climate variation drives baseline innate immune function and stress in a tropical bird: a reactive scope perspective	Published	65%. Study concept and design, field work, laboratory analysis, data analysis, writing	Anne Peters, 25% Other co-authors 10%	No Yes
3	Does constitutive immune function exhibit senescence in the wild? A longitudinal study	Not submitted	60%. Study concept and design, field work, laboratory analysis, data analysis, writing	Anne Peters, 20% Simon Verhulst, 10% Other co-authors 10%	No No Yes
4	Fitness outcomes in relation to variation in individual constitutive innate immune function	Not submitted	75%. Study concept and design, field work, laboratory analysis, data analysis, writing	Anne Peters, 10% Matthew Hall, 5% Other co-authors 10%	No No Yes

I have renumbered sections of submitted or published papers in order to generate a consistent presentation within the thesis.

Student name: Michael J. Roast

Date: 23/01/2020

I hereby certify that the above declaration correctly reflects the nature and extent of the student's and co-authors' contributions to this work. In instances where I am not the responsible author, I have consulted with the responsible author to agree on the respective contributions of the authors.

Main Supervisor name: Anne Peters

Date: 23/01/2020

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Chapter 1: General Introduction

Background

Ecoimmunology: understanding immune variation

Ecological immunology, or ecoimmunology, is now a maturing field of research that is concerned with bringing together the historically disparate fields of immunology and physiology with ecology and evolution (Schulenburg et al., 2009). From the perspective of Tinbergen's four questions (Tinbergen, 1963), ecoimmunological research aims to link the proximate mechanistic and developmental physiology of immune systems to their ultimate adaptive functionality and evolutionary relevance. The seminal paper by Sheldon & Verhulst (1996), commonly credited with the emergence of the field (Brock et al., 2014; Sadd and Schmid-Hempel, 2008), presents immune systems as costly defences against parasitism that are subject to physiological trade-offs within individuals. These immune defences might be costly purely in terms of energy, or other relevant nutritional or anti-oxidative resources (Hasselquist and Nilsson, 2012). Therefore, where resources are finite, immune function and reproduction (among other processes) are likely to compete for resources and be subject to a trade-off within individuals. Shaped by parasite and pathogen pressure, this trade-off should result in an optimal level of immune function that balances the costs of immunity and reproduction for overall fitness (Sheldon and Verhulst, 1996).

An optimal level of immune function should, in theory, maximise individual fitness (Viney et al., 2005), and it might be expected that natural selection generates individuals in a population with an optimal magnitude and type of immune response (Schoenle et al., 2018). Despite this, there is a huge amount of natural variation in immune function among individuals and at many organisational levels, suggesting that the notion of optimal immune function is far more complicated and context-dependent (Viney et al., 2005). Understanding this context-dependence, and investigating the biotic and abiotic factors that can influence immune variation in free-living organisms is therefore an overarching objective of ecoimmunological research (Martin et al., 2011; Schulenburg et al., 2009). Determining how various contexts shape the proximate physiological trade-offs that are crucial for maximising fitness has become a strong focus in the field of study (French et al., 2009). Continued research in ecoimmunology that bridges the gap between immune physiology and evolutionary ecology will likely have important applications in wildlife research and conservation, livestock management, and even public health (Downs and Stewart, 2014; Schoenle et al., 2018).

Practical ecoimmunology

Key to ecoimmunological research was finding appropriate ways to quantify immune investment to examine trade-offs with other physiological processes and life-history traits. The 'immunocompetence' of an individual – the ability to fight off disease-causing agents – is a conceptually appealing trait that proved difficult to quantify as it oversimplified specific host-parasite interactions and often could not be generalised to all parasite threats (Brock et al., 2014; Martin et al., 2006b). Attempting to encompass immune function by measuring any single immune index is also questionable, as the complexity of the immune system means that trade-offs could occur between immune system components, leading to unexpected correlations between immune indices and other fitness-related traits (Lee, 2006). It is therefore necessary to incorporate a broader range of methodologies and approaches (Boughton et al., 2011; with more methods still being adopted *e.g.* Zylberberg, 2019), that in combination adequately capture an organism's immune status and facilitate interpretation (Adamo, 2004; Bradley and Jackson, 2008; Buehler et al., 2011). Panels of several indices of immune function are now commonly employed in studies to provide a more nuanced overview of the immune status of individuals, as I aim to do through this thesis (Boughton et al., 2011; Demas et al., 2011; Downs and Stewart, 2014). With the breadth of tools now available, publications in the field of research are proliferating (Brock et al., 2014), and consistently demonstrating that the context in which immune measurements are taken appears crucially important.

Traditional immunological studies in a laboratory context have focused specifically on reducing variation in closed and often sterile systems to pinpoint exact immune mechanisms and pathways (Babayan et al., 2011). Nonetheless, there is an appreciation that the laboratory context does not reflect the complexity of wild ecosystems that immune defences have evolved in (Pedersen and Babayan, 2011). The majority of our immunological knowledge derives from humans and rodent models in the laboratory (Maizels and Nussey, 2013), while more diverse immune strategies are likely to exist in other species in the wild. Even within the same mouse species, *Mus musculus*, there are large differences between the immune states of laboratory-reared and wild-caught mice (Abolins et al., 2011), and substantial immunological variation even among different wild populations in different environmental contexts (Abolins et al., 2018). For ecoimmunologists, this natural immunological variation arising in different contexts is an integral part of the question to understand how and why variation is evolutionarily relevant. On a continuum from laboratory-housed to wild and free-living (through domestic, captive, urbanised, managed, and feral degrees; fig. 1, Maizels and Nussey, 2013), only in wild contexts where the driving force of natural selection is greatest are we likely to understand how immune variation affects the health, survival, and fitness of

organisms (Maizels and Nussey, 2013; Pedersen and Babayan, 2011). Although studying wild organisms presents many challenges (*e.g.* capturing organisms, unknown infection histories), overcoming the practical challenges of wild immunological studies is extremely important for understanding the evolutionary causes and consequences of immune variation, and linking proximate and ultimate perspectives on immune function.

The importance of complex immune defences

Parasitism is by far the most prevalent mode of life (Dobson et al., 2008; Martin et al., 2011; Poulin and Morand, 2000), and within any wild ecosystem there are parasites (including in the broader sense pathogenic organisms and viruses) that drive the evolution of complex immune defences (Cooper and Herrin, 2010). Host-specific parasites are expected to evolve more rapidly, with greater adaptive potential than their hosts, as a consequence of relatively faster generation times and larger population sizes (Hafner et al., 1994; Papkou et al., 2016). As a result, strong selective pressures are constantly exerted on hosts to co-evolve, favouring the development of specific and targeted immune functionality on a 'gene-for-gene' or 'matching genotype' basis (Schulenburg et al., 2009; Woolhouse et al., 2002). Greater parasite species richness in the host population will pressure hosts to evolve a greater diversity of immune responses (Bordes and Morand, 2009; Schulenburg et al., 2009). For example, the major histocompatibility complex (MHC) genes – immune genes responsible for coding numerous cell surface proteins which recognise internal parasites – are hypothesised to maintain allelic diversity through antagonistic coevolution with parasites (Kubinak et al., 2012). Parasite species richness has been directly linked to increased MHC genetic polymorphism and diversity in several species of rodent and teleost fish, brushtail possums, and human populations (Kubinak et al., 2012; Morand, 2015). Possessing effective and diverse immune defences is critical to organismal health and functionality in the presence of parasites, and is likely to be evolutionarily advantageous (e.g. Nussey et al., 2014).

Animal immune systems have evolved into complex and multi-faceted systems comprised of many separately acting components that are simultaneously interconnected (Cooper and Herrin, 2010). Classically, the vertebrate immune system has been divided into two branches, innate and adaptive (acquired) immunity, though these systems are tightly linked and not truly independent of one another (Netea et al., 2011; Ochsenbein and Zinkernagel, 2000; Panda and Ding, 2015). Innate components defend non-specifically against any immune challenge, serving as a first line of internal defence (Riera Romo et al., 2016; Schmid-Hempel and Ebert, 2003), while adaptive components specifically target parasites and subsequently form an immunological memory to counter repeated infection

(Litman et al., 2010; Schmid-Hempel and Ebert, 2003). Innate immunity is typically regarded as the common ancestral system shared by both vertebrates and invertebrates, while adaptive immunity is unique to vertebrates; however, invertebrates have continued to evolve rudimentary memory-based immune functions (Kurtz and Armitage, 2006; Milutinović and Kurtz, 2016). Immune system functionality can also be divided into constitutive and induced immune components (Schmid-Hempel and Ebert, 2003). Constitutive components are 'maintained at a certain level irrespective of the disease environment' presenting a state of readiness to detect and combat infection while inducible components are 'activated only in response to a disease challenge' and once deployed by the host, often scale in magnitude (p.566, Martin et al., 2006a). The constitutive-induced and innate-adaptive axes are intersecting and not mutually exclusive, and immune components can be broadly categorised into each combination of these types of defence mechanism (Schmid-Hempel and Ebert, 2003). As a prime example, the inflammatory response is an innate defence and is induced by infection (in addition to trauma/tissue damage; Ashley et al., 2012; Mogensen, 2009). The inflammatory response itself consists of numerous signalling pathways with cellular and humoral effectors that are vital defences, especially for tissue damage and physical breach of integumentary barriers (Galli et al., 1999; Mogensen, 2009). Despite the complexity of immune defences ecoimmunologists have sought indices that characterise individual immune function such as the inflammatory response which are both highly important and costly, therefore allowing the examination of immune trade-offs (Brock et al., 2014; Demas et al., 2011).

Immune function is costly

Central to the concept of fitness trade-offs is that immune defences are costly to operate (Sheldon and Verhulst, 1996). Immune systems are thought to incur physiological costs in several ways: development, maintenance, deployment, collateral damage and immunopathological costs (Ashley et al., 2012; Klasing, 2004; Lee, 2006). The ontogenetic costs of immunity are more substantive in the adaptive compared to the innate branch of the immune system (Palacios et al., 2009) and may come at the expense of growth in early life stages (Brommer, 2004). Although maintenance costs of adaptive immunity are estimated to be relatively low in vertebrates (Fox et al., 2005) while constitutively maintained humoral components may represent the principal ongoing investment (Klasing, 2004), similar to invertebrates (Valtonen et al., 2010). The use and deployment of immune components also incurs physiological costs, both energetically and nutritionally through the proliferation of lymphocytes and innate responses like inflammation and systemic fever, though these demands may be met by internal reallocation of resources (Derting and Compton, 2003; Fox et al., 2005; Klasing, 2004; Martin et al., 2003). Furthermore, at the point of use, collateral

damage is often sustained from immune responses, with inflammation being especially damaging (Ashley et al., 2012) – such that constraining regulatory mechanisms have evolved to limit the damage sustained from inflammation (Barton, 2008; Han and Ulevitch, 2005). Lastly, immunopathology from dysfunctional self-antigen recognition (or absence of self-tolerance, i.e. autoimmunity) can also render self-tissues subject to immune attack (Perl, 2012). Although the physiological costs may not always be purely energetic (Hasselquist and Nilsson, 2012; Lochmiller and Deerenberg, 2000), it is important to differentiate proximate costs of immune functionality (also referred to as: physiological, short-term, direct) from the ultimate costs relating to fitness and survival (also referred to as: evolutionary, long-term, indirect; Hasselquist and Nilsson, 2012; Martin et al., 2003; McKean et al., 2008) as the drivers of individual variation in immune function may have different relevance at proximate and ultimate levels.

Natural variation in immune function

Understanding the drivers of immune variation and how this variation relates to physiological and evolutionary trade-offs generates an integrated and holistic view of how organisms respond to parasitism (Martin et al., 2011; Schulenburg et al., 2009). Immune function varies naturally among species (Hasselquist, 2007; Mendes, 2006; Millet et al., 2007), among geographically separated populations or at opposite ends of a species distribution (Abolins et al., 2018; Adelman et al., 2010; Ardia, 2005a; Martin et al., 2006a), among individuals in the population (Ardia, 2005b; De Coster et al., 2010; Matson et al., 2012) and between the sexes (Forbes, 2007). Within-individuals too, there is observable variation attributable to age, seasonality, circadian rhythm and stress (Martin, 2009; Martin et al., 2008; Peters et al., 2019; Zylberberg, 2015), reiterating the context-dependence of immune function. However, the evolutionary importance of variation may change at different levels of organisation, explaining why some patterns of variation explained at the individual level break down at the species level (Matson et al., 2006). Although substantial genetic variation in immune genes could be involved in variation between individuals (Turner et al., 2011), accounting for environmental context could be crucially important when considering individual level immune variation and trade-offs (Brodin et al., 2015; Sandland and Minchella, 2003; Viney et al., 2005). This raises interesting questions about the potential causes or drivers of immune variation and how it is context-dependent for each individual, and how it may vary over an individual lifetime.

For any individual, the precise balance of immune function that maximises fitness will be a reflection of its current physiological state, intrinsic factors like sex and age, life history, and the environmental context. Therefore, in order to more completely understand the causes

and consequences of individual immune variation, an extremely detailed knowledge of each individual in the study system is required. Gaining this level of detail is difficult in a wild environmental context which is necessary to provide the relevant natural selective forces that govern the fitness consequence of immune variation. The long-term individual-based system that I have studied for my thesis is exceptionally well positioned to provide such detailed information on a large number of wild individuals. Consequently, I am able to address individual immune variation in a way that is seldom possible in the wild, providing new insights into existing ecoimmunological endeavours. By quantifying immune function in individuals through repeated captures and sampling, I am able to associate the individual's constitutive innate immunity to the detailed information about their lives to gather a unique observational perspective on the possible causes and consequences of individual variation in immune function. Constitutive innate immune defences are vital for resistance to novel parasites, so any variation in these indices should reflect individual differences in strategies to deal with parasitism and maximise fitness. Furthermore, as these innate immune components are highly conserved among animals, such wild studies can broadly inform the vulnerability of animals in the presence of environmental variation and emergent disease, while environmental change is occurring at an unprecedented rate.

Thesis Outline

Thesis aims

The overall aim of my thesis is to investigate the possible causes and consequences of individual variation in immune function, focusing on constitutive innate immunity as a comparable baseline of standing immune function. Specifically, in this thesis I aim to:

- Explore different environmental drivers of individual variation in constitutive innate immunity (chapter 2)
- Investigate how constitutive innate immunity varies with age within and between individuals (chapter 3)
- Determine the relationship between individual variation in constitutive innate immunity and fitness-related outcomes (chapter 4)

Model species: the purple-crowned fairy-wren

The purple-crowned fairy-wren (*Malurus coronatus*; fig. 1a & b) is an insectivorous riparian specialist in the Australian tropical wet-dry savannah with a high dependence on river pandanus (*Pandanus aquaticus*; Skroblin and Legge, 2012; van Doorn and Choy, 2009). They are social, sedentary, resident birds. Social groups (2-11 individuals) occupy clearly distinct

year-round territories along river banks with suitable vegetation (Kingma et al., 2011b). Territories are rigorously defended as a valuable resource (Hall and Peters, 2008). Purple-crowned fairy-wrens are also facultative co-operative breeders where in each group, only a dominant male and female will breed (fig. 1c). The dominant pair form a socially and genetically monogamous pair (Kingma et al., 2009), with high fidelity and low extra-pair paternity (~6%, usually to avoid incest; Kingma et al., 2013). Breeding can occur at any time of year in rapid response to rainfall, which stimulates arthropod abundance, with breeding reaching a peak during the wet season from December to March (Hidalgo Aranzamendi et al., 2019). Other subordinate group members commonly become 'helpers' during breeding and contribute to chick rearing (Kingma et al., 2011). This species also undergoes a seasonal moult into sexually dichromatic breeding plumage (fig. 1a & b).

Many of these characteristics make purple-crowned fairy-wrens a useful model species in which to study potential causes of variation in innate immunity. The riparian habitat they occupy is variable with respect to potential parasite vectors such as mosquitoes, ticks and biting insects (e.g. *Reduviidae*; personal observations) which can determine disease prevalence (Sol et al., 2000) and therefore selective pressures for a well-functioning immune system. Social living generates physical contact through affiliative and aggressive interactions between individuals providing opportunity for parasite transmission (Teunissen et al., 2018; fig. 1d); differences in group size should result in variable risk of exposure to parasites between groups (Patterson and Ruckstuhl, 2013). In this climate zone extreme temperatures, rainfall, humidity and aridity are experienced each year which could cause physiological stress and mediate immune investment (Martin, 2009). Because purple-crowned fairy-wrens are a strictly year-round territorial and long-lived species, they are not only continually exposed to these stressors, but these characteristics also facilitate the longitudinal study of variation in immunity through repeated measurements of individuals. Lastly, a detailed understanding of the co-operative breeding system and social structure is useful in demonstrating how immune variation may be consequential for fitness and reproductive success.

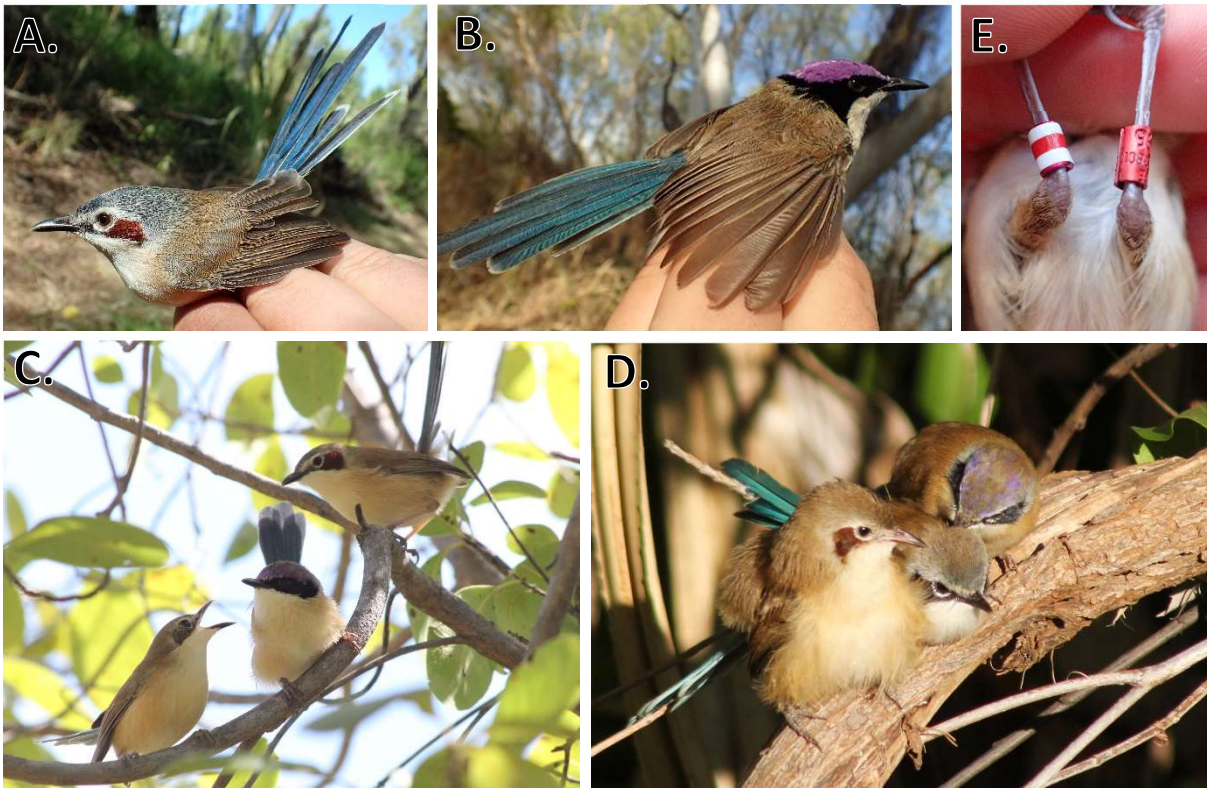

Figure 1: The purple-crowned fairy-wren. **A.** Adult female in complete breeding plumage. **B.** Adult male in complete breeding plumage. **C.** Social group structures; a submissive bill-gape display from a sub-ordinate first-year male (left) to a dominant male (centre), with a dominant female (right). **D.** Affiliative interactions; close 'proximity' behaviour (Teunissen et al. 2018) between a juvenile (left), an adult male in eclipse plumage (centre) and a moulting adult male (right). Allopreening (e.g. between the two pictured males) is sometimes observed in addition to close proximity behaviour. **E.** Example of unique colour band combinations. Images **A-C.** and **E.** Michael Roast/AWC, image **D.** Niki Teunissen/AWC.

The population of purple-crowned fairy-wrens studied for my thesis belongs to the western subspecies (*M. c. coronatus*) and is located in the Kimberley region of northern Western Australia, at the Australian Wildlife Conservancy's (AWC) Mornington Wildlife Sanctuary (126.17°E, -17.57°N). This population has been followed by Anne Peters' research group since 2005 and during this time, all individuals along ~15km of adjacent sections of Annie Creek and Adcock River have been uniquely colour-banded (fig. 1e). Consequently, through long-term observations, detailed knowledge about individual life histories in the population has been acquired that can be associated to measurements of immune function, greatly enabling the study of the causes and consequences of individual immune variation in a wild study system (Clutton-Brock and Sheldon, 2010; Pedersen and Babayan, 2011). Since

2012, the Peters research group has been biannually sampling individuals to quantify levels of constitutive innate immunity that can be linked to this rich dataset collected through detailed field observations and sampling over many years. During the course of my thesis, from September 2015 until May 2017 I have continued to build upon these datasets through my own fieldwork and in collaboration with other members of the research group. In this thesis, I explore this observational individual-based dataset to investigate the possible causes and consequences of individual variation in immune function.

Thesis Chapters

In chapter 2, I aim to evaluate the roles and relative importance of several social, ecological and climatic extrinsic factors that are hypothesised to affect constitutive innate immune function (possibly mediated by stress responses; Martin, 2009). Using an information theoretic approach in combination with multi-model inference (Bartoń, 2018; Burnham et al., 2011), I model the effects of social group size, habitat quality, maximum ambient temperature, temperature variability, and rainfall. I also test for seasonal differences in immune function between the biannual field sampling periods. At the same time, I am able to use detailed individual information to statistically control for intrinsic factors such as sex, social status and breeding activity. I find that social and ecological variables are relatively unimportant despite plausible links to resource availability and parasite pressure. On the other hand, short-term climatic variables appear to have the strongest relationship with immune function and stress, despite remaining relatively stable between the seasons. These findings can be interpreted from a reactive scope model perspective (Romero et al., 2009), where long-term variation is anticipated and an individual maintains stable immune function through predictive homeostasis, but immune function may be more susceptible to short-term environmental perturbations.

In chapter 3, I use multiple repeated measures of constitutive innate immunity collected from individuals as part of the long-term research project. With this longitudinal dataset, I aim to test hypotheses regarding age-related changes in immune function, including the expected immunosenescence and inflammaging with increasing age (Franceschi et al., 2006; Pawelec, 2018). Using a within-subject centring approach (van de Pol and Verhulst, 2006), I differentiate the within- and between-individual patterns to assess if and how population trends emerge from within-individual changes in immune status and between-individual heterogeneity in mortality risk (i.e. selective disappearance). Furthermore, I compare the differences between longitudinal and more commonly used cross-sectional approaches, and show longitudinal studies are important for discerning individual and population level trends. I find only limited

evidence of immunosenescence, in addition to evidence of immune maintenance and putative improvement with old age in some immune components. This latter finding suggests that alternative self-maintenance roles of immune components beyond parasite defence could become more important with age.

In chapter 4 I combine detailed information about survival, reproductive output and social dominance with immune data to assess the possible fitness consequences of variation in immune function. I also test whether measures of chronic stress and body condition relate to fitness outcomes to assess whether environmental stressors or resource availability might mediate any trade-off. I find that the probability of survival is not higher for birds with higher levels of immune indices, but I also find some evidence that optimal levels are context-dependent. Despite the expected trade-off between immune function and reproductive output, I do not find any evidence to support this prediction, but instead that individual quality might override trade-offs, resulting in positive covariation between immune function and reproductive output. I also find that chronic stress and body condition do not strongly relate to fitness outcomes in ways that supported hypotheses regarding environmental stressors and resource availability. Maintaining baseline levels of immune components does not appear to be as costly for fitness as activating induced immune components that are known to invoke trade-offs, and I discuss how trade-offs may be important for certain immune components but not others.

Thesis Organisation

This thesis has been presented as a 'thesis including published works', comprised of a general introduction, three discrete data chapters, followed by a general thesis discussion. Chapter 2 has been published in the journal Physiological and Biochemical Zoology (https://www.journals.uchicago.edu/doi/10.1086/702310). Chapter 3 and Chapter 4 will be submitted to journals for publication. I have significantly contributed to project design, field work, laboratory work, data analyses and the writing of each chapter, however, throughout my thesis data chapters the first person plural is used to reflect the collaborative nature of this research and the long-term field study, and each chapter incorporates the knowledge and expertise of all co-authors.

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Chapter 2: Short-term climate variation drives baseline innate immune function and stress in a tropical bird: a reactive scope perspective

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Abstract

Investment in immune function can be costly and life-history theory predicts trade-offs between immune function and other physiological demands. Environmental heterogeneity may constrain or change the optimal strategy and – possibly mediated by stress responses – thereby alter baseline immune function. We tested several hypotheses relating variation in climatic, ecological, and social environments to chronic stress and levels of baseline innate immunity in a wild, cooperatively breeding bird, the purple-crowned fairy-wren (*Malurus coronatus coronatus*). From samples collected biannually over 5 years, we quantified three indices of constitutive innate immune function (haptoglobin/PIT54, natural antibodies, complement activity) and one index of chronic stress (heterophil-lymphocyte ratio) (n = 513-647). Using an information theoretic and multi-model inference statistical approach, we found that habitat quality and social group size did not affect any immune index, despite hypothesised links to resource abundance and parasite pressure. Rather, short-term variation in temperature and rainfall was related to immune function, while overall differences between seasons were small or absent, despite substantial seasonal variation in climate. Contrary to our expectation, we found no evidence that physiological stress mediated any effects of short-term climatic variables on immune indices and alternative mechanism may be involved. Our results may be interpreted from the perspective of reactive scope models, whereby predictive homeostasis maintains standing immune function relative to long-term demands, while short-term environmental change, being less predictable, has a greater influence on baseline immune function.

Keywords: immunocompetence, ecoimmunology, Maluridae, individual variation, multi-model inference, vertebrate, avian

Introduction

Immunocompetence, the ability to prevent or control infection, is essential for survival (Norris and Evans 2000), but immune defences are nonetheless highly variable within and between individuals. Such variation is thought to arise from the costs of developing, maintaining or deploying a multi-faceted immune system (Lochmiller and Deerenberg 2000; Klasing 2004; Ashley et al. 2012) leading to trade-offs with other physiologically demanding processes (Norris and Evans 2000; Ricklefs and Wikelski 2002; Viney et al. 2005; Lee 2006). As with any life history trade-off, the optimal strategy may depend on environmental conditions (Sandland and Minchella 2003; Ardia 2005; Tschirren and Richner 2006; Tieleman 2018). Identifying which environmental factors account for individual variation in immune function and how, can therefore enhance our understanding of immune trade-offs (Lazzaro and Little 2009).

Environmental factors hypothesised to explain individual variation in immune function include ecological resource availability (food quantity and quality), parasite and pathogen pressure, climate variation, and conspecific interactions (beneficial or detrimental) (reviewed in Maizels and Nussey 2013, fig. 2). These environmental factors could influence immune function through specific nutritional and energetic limitation; diversity and virulence of parasites and pathogens encountered in the environment; and immunosuppressive effects of physiological and social stressors associated with adverse, demanding, or unpredictable conditions (Møller et al. 2001; Bartolomucci 2007; Brzek and Konarzewski 2007; Martin 2009). Chronic stress, in particular, is known to have immunosuppressive effects (Buchanan 2000; Dhabhar 2009; Krams et al. 2012), and may thus link environmental variation and immune function (Mashaly et al. 2004; Bartolomucci 2007; French et al. 2008; Nazar and Marin 2011; Xie et al. 2017). Through some or all of these mechanisms, different environments are likely to shape individual immune function differently.

To assess why and how individual immune defences vary, a real-world context is therefore needed (Maizels and Nussey 2013). Wild organisms are exposed to complex environmental variation, potentially revealing drivers of individual variation obscured in highly controlled environments (Babayan et al. 2011; Pedersen and Babayan 2011). Several studies on wild animals have examined the links between environmental variables and immune function and in each case, environmental context or heterogeneity to some degree explained individual variation in the immune indices measured (Rubenstein et al. 2008; Pigeon et al. 2013; Zylberberg et al. 2013; Vermeulen et al. 2015; Bailly et al. 2016). While these studies often investigated variation in a single environmental variable, components are more likely to act in concert than in isolation (Stahlschmidt et al. 2015). Thus, a need to address the relative

effects of multiple environmental variables on immune function is apparent. This requires an integrative approach combining an assessment of multiple environmental factors with detailed information on study subjects to control for individual differences that may affect life history trade-offs and immune variation (including age, sex, and competing physiological demands such as breeding) and, consequently, large sample sizes.

We examined several non-exclusive hypotheses to assess if and how variation in key environmental variables can account for individual variation in immune function in a free-living tropical songbird. First, we tested the hypothesis that greater resource availability enables individuals to invest more in immune function (Houston et al. 2007) by examining whether higher habitat quality is associated with greater immune function. Second, we evaluated support for two alternative hypotheses regarding social environment (cf. Møller et al. 2001). The first hypothesis, that social stress depresses immune function (Hawley et al. 2006), predicts that individuals in larger group sizes should experience higher levels of social stress, leading to suppressed immunity. The second hypothesis, that individuals in larger groups face increased exposure and risk of infection from parasites through social contact transmission (Patterson and Ruckstuhl 2013), predicts that increasing group size is associated with increasing immune function, without affecting stress levels. Third, we hypothesised that extreme climatic variation (in the context of a tropical climate) would be physiologically stressful, and associated with immunosuppression. Specifically, we tested whether several climatic conditions evoke physiological stress and immune suppression: high daily maximum temperature (cf. acute heat stress; Butler et al. 2013; Xie et al. 2017), high weekly mean maximum temperature (cf. a heatwave; Mashaly et al. 2004; Stahlschmidt et al. 2017), large daily temperature variability (Bozinovic et al. 2013; Briga and Verhulst 2015), and a lack of rainfall (Fair and Whitaker 2008). Fourth, because increased water availability following rain may alleviate the physiological (metabolic) demands of cooling at high temperatures (Wilson et al. 2004), or conversely, high humidity may prevent evaporative cooling at extremely high temperatures and exacerbate physiological stress, (Gerson et al. 2014; El-Tarabany 2016), we tested for an interactive effect between rainfall and maximum temperatures. Fifth, we hypothesised that more long-term environmental conditions would be of greater immunosuppressive consequence (Martin 2009). We predicted that short-term climatic variables, followed by social group size, then habitat quality (representing more enduring environmental variables) will be of progressively greater relative importance for explaining immune variation.

To assess support for these hypotheses, we statistically quantified the relative importance and strength of effects of each environmental variable on baseline levels of three

indices of constitutive innate immunity. In > 500 samples collected over 5 years we measured natural antibodies (NAbs), lytic complement activity (Ca), and haptoglobin-like heme binding protein (Hp), which recognise, eliminate, and mitigate a broad range of pathogens, respectively (Ochsenbein et al. 1999; Rajan et al. 2005; Jayasekera et al. 2007; Rapaka et al. 2010). Additionally, we quantified heterophil-lymphocyte ratio (HL ratio) as an index of chronic (> ~24h) stress (Davis et al. 2008; Davis and Maney 2018). We focused on these three immune indices because their baseline levels are continuously maintained as a first line of defence against novel challenges. Furthermore, they respond non-specifically to any immune challenge (Schmid-Hempel and Ebert 2003; Martin et al. 2006), avoiding problems with immune memory due to unknown prior exposures (Pedersen and Fenton 2007; Hawley and Altizer 2011). Finally, constitutive innate immunity is known to be modulated according to life-history trade-offs (Tieleman et al. 2005).

Methods

Study population

Our study population of purple-crowned fairy-wrens, *Malurus coronatus coronatus* (Gould 1857), is located in the tropical wet-dry Kimberley region of Western Australia at Australian Wildlife Conservancy's (AWC) Mornington Wildlife Sanctuary (126.1°E, -17.5°N) along 15km of waterways on Annie Creek and Adcock River. Studied since 2005, all individuals are uniquely colour-banded to monitor social group composition, territory boundaries, and relatedness (from DNA sampling; Hidalgo Aranzamendi et al. 2016). Purple-crowned fairy-wrens are cooperatively breeding riparian specialists, vocally defending year-round stable, exclusive territories (Hall and Peters 2008). Close proximity and physical contact between individuals within social groups is common (Hall and Peters 2009; Teunissen et al. 2018) but physical interactions between social groups are very rare (pers. obs.). Social groups consist typically of a socially and genetically monogamous dominant breeding pair (Kingma et al. 2009, 2013), with subordinate adults – either previous offspring or unrelated settled dispersers – which may help to rear offspring (Kingma et al. 2010). The majority of breeding occurs in the wet season between December and March, although breeding can occur at any time of the year in response to rainfall (Hidalgo Aranzamendi 2017). This subspecies is dependent on *Pandanus aquaticus* vegetation (fig. S1; Kingma et al. 2011b; Skroblin and Legge 2012), with ~95% of nests built in *P. aquaticus* crowns, and the majority of time spent in *P. aquaticus* vegetation (Kingma et al. 2011a). *P. aquaticus* cover also directly and indirectly enhances reproductive success (Kingma et al. 2010, 2011b; Hidalgo Aranzamendi 2017) and influences female settlement decisions (Hidalgo Aranzamendi et al. 2016).

Capture and blood sampling

Adult birds (> 3 months old) were captured passively in mist-nets between May 2012 and May 2017 on a biannual basis in 'May' (between 29th April and 21st June; post-wet) and 'November' (19th October – 28th November; pre-wet). Birds were typically caught with other social group members and extracted from mist-nets, kept in holding bags, and blood sampled sequentially as quickly as possible (median = 23min after capture, s.d. = 19min) to mitigate handling stress (Davis 2005; Zylberberg 2015), then released. Following brachial venepuncture, up to 100µl of blood was collected in heparinised capillary tubes, which were sealed, and immediately vertically stored on ice. Later that day (median = 3.8h after capture, s.d. = 2h), capillaries were centrifuged at 13,000rpm for 5min, and plasma separated and frozen at -20°C. Within 8 weeks of collection, plasma samples were transferred to -80°C. During sampling, a blood smear was created with whole blood using the wedge-pull method (Campbell 2015). Air-dried blood smears were fixed in absolute methanol for no less than 15min. Samples were collected over 11 consecutive field seasons, however due to laboratory misfortune, the total number of field seasons represented differs (Hp, n = 11, 2012-17; NAbs & Ca, n = 7, 2012-13 & 2016-17; HL ratio, n = 9, 2012-14 & 2016-17).

Immunological analyses

We quantified three components of constitutive innate immunity: natural antibodies (NAbs), lytic complement activity (Ca), and haptoglobin-like heme binding protein (Hp). NAbs are antigen-binding with a generally low specificity, opsonizing foreign cell components for phagocytosis, as well as initiating the lysis complement system via the classic pathway (Juul-Madsen et al. 2014). The lysis complement system is a cascade of sequentially activated proteins that continue the breakdown and elimination of foreign cell components. Both NAbs and Ca were quantified from the same haemolysis-hemagglutination assay (Matson et al. 2005) with minor modifications, where exogenous rabbit red blood cells added to plasma samples are agglutinated by NAbs and lysed by Ca. Assays were run on two clear round-bottomed 96-well plates, and 15 µl of defrosted plasma were added to columns 1 and 2 of the first plate. From column 2 onwards, plasma was serially diluted by 50% with Dulbecco's phosphate-buffered saline (PBS). Then, 15µl of 1.5% rabbit red blood cells in PBS were added to all wells. Plates were covered with Parafilm® and incubated for 90min at 37.5°C. After incubation, plates were tilted at a 45° angle for 20min and then scanned using an Epson Perfection® V370 flat-bed scanner on the 'Positive Film' setting with backlight correction. Plates remained flat at room temperature for a further 70min and scanned a second time. The two scans were then scored blind for agglutination (NAbs) and lysis (Ca) titres respectively. Columns 12 and 24 were left without plasma as negative controls, and on each plate two

samples of chicken plasma (Applied Biological Products Management) were included, with high agglutination (mean = 10.1, n = 247) and high lysis (mean = 3.55, n = 265) scores respectively, resulting in CV = 0.13 and CV = 0.11. Where < 30µl but > 15µl of sample plasma was available, the 100% column 1 was omitted. Where no sign of lysis was observed in column 2, a score of 0.5 was assigned to these samples (n = 83). Of the samples with sufficient plasma, 16% had no evidence of lysis, indicating that the error introduced by this is relatively small.

Haptoglobin (Hp) is a major positive acute phase protein which can increase over 100-fold during an immune challenge and constitutively maintained baseline levels can predict some aspects of the immune response (Matson et al. 2012). Hp and PIT54, a Hp-like functional analogue in some avian orders (Wicher and Fries 2006, 2010), both function primarily as scavengers of toxic heme released by damaged erythrocytes during infection (Quaye 2008; Georgieva 2010; Andersen et al. 2017), providing a valuable index of immune status. Hp/PIT54 (presently undetermined in purple-crowned fairy-wrens) was quantified in this study using a commercial kit based on heme-binding ability (Phase^{TM} Range, TP801; Tri-Delta Development Ltd.), adjusted for small sample volume, using a VersamaxPLUS ROM v1.21 microplate reader. All samples were run in duplicate and where plasma volume was limiting, a 50% dilution was prepared with kit 'Diluent' (n = 168). Rabbit plasma (Monash University Animal Research Platform) in triplicate was used to assess inter-plate variation (CV = 0.24, n = 25 plates). Of 732 samples, 82 fell above the 1.25mg/ml optical saturation threshold of the assay and were excluded from further analyses.

Blood smears were immersed in dilutions of 50% May-Grünwald and 10% Giemsa stain for 15min each, then distilled water for 5min, before air-drying. Differential white blood cell counts were conducted at 1000x magnification to identify heterophils, lymphocytes, basophils, eosinophils and monocytes for the first 100 leukocytes encountered on the blood smear, systematically following the feathered edge as described by Campbell (2015). Scoring was undertaken by 4 scorers, and scorer ID was included in statistical models to account for variation between scorers.

Environmental data

Our sampling seasons, May and November, are wet-dry transitional periods: in May, almost no rain falls, but more residual water persists post-wet season – typically flowing; in November, streams are contracted to free-standing waterholes but there are more frequent heavy rainfall events as the temperature and humidity rises pre-wet season. Daily rainfall information was derived from Mornington station (Bureau of Meteorology, weather station #002076). Temperature information was derived from Fitzroy Crossing Aero station (#003093) located

~93km from the field site. Temperature data have been intermittently recorded at our field site, and recordings were highly correlated with those from Fitzroy Crossing ($r = 0.87$, $p < 0.001$, $n = 577$ daily records). Daily rainfall data were highly zero-inflated and converted to a binary variable for the occurrence of rain in the week preceding capture ($Rain_7$) (figs. S2, S3), which better captures the lasting effects of heavy downpours that can occur on a single day (such as waterhole replenishment; range = 0-59.4mm). Temperature was considered as three separate parameters: maximum temperature the day preceding capture (T_{max}), i.e. the maximum immediately before early morning capture; temperature range the day preceding capture (T_{var}), i.e. maximum of the day preceding capture minus the minimum of the morning of capture; mean of daily temperature maxima in the week preceding capture (T_{max7}).

Given the high dependency of purple-crowned fairy-wrens on *P. aquaticus*, habitat quality was determined using an estimate of *P. aquaticus* cover in each territory (n = 82 territories) as a proxy for habitat quality. Surveys were conducted (annually to 2008, November 2013, November 2015, and May 2017) using the method described in detail by Hidalgo Aranzamendi et al. (2016), which in brief, assigns a *Pandanus* score between 0-20 at 50m intervals along all waterways in the study area. For sampling seasons between *Pandanus* surveys, data were interpolated at even increments or decrements. Survey data were then spatio-temporally matched with territories that individual birds inhabited at the time of capture using QGIS version 2.18.16. Mean territory quality score at each capture was 11.2, with an even distribution of scores across the scale (fig. S4). This proxy of habitat quality is positively correlated with the presence of year-round water availability (remaining waterholes at the end of the 2015 dry season; Adcock River, $r_s = 0.48$, $p < 0.001$, Annie Creek $r_s = 0.45$, $p < 0.001$) and year-round food availability (monthly averages of summed arthropod lengths from sweeps between 2007-2008 (Kingma et al. 2011a), $r = 0.33$, $p = 0.04$). Social group sizes were known from observational data (median group size = 5, range = 1-10, fig. S5) and were positively correlated to the proxy for habitat quality ($r = 0.29$, $p < 0.001$), but not so strongly that it precluded statistical assessment of independent effects.

Statistical analysis

All statistical analyses were done using R-software version 3.4.0 (R Core Team 2017). Distributions of Hp and NAbs values followed a normal distribution. Ca scores were normalised with a natural log-transformation and heterophil-lymphocyte ratios were normalised with a square-root-transformation (all values were between 0 and 1). Outliers beyond ± 2.5 standard deviations of the mean were excluded (2.5% of 2266 observations). Exploratory analyses revealed that there were clear seasonal differences in the distributions of environmental

variables, with limited overlap (fig. S6), and different relationships with immune indices in each season (fig. 1a and b); therefore data from the May and November field seasons were analysed separately, resulting in 8 main analyses (4 response variables across 2 seasons). T-tests were conducted to test for overall seasonal differences in means between the two field seasons.

To test for the effects of environmental parameters on immune indices we combined linear mixed effects models (LMM) using the *lme4* package (Bates et al. 2015) with an information-theoretic approach and multi-model inference (Burnham and Anderson 2002; Grueber et al. 2011). All tested models included Individual ID, Territory ID, Year, and Plate ID (for Hp, NAbs, and Ca) or Scorer ID (for HL ratio) as random intercepts to account for non-independence of observations, repeated sampling structure, and plate or observer effects during measurement. Additionally, we defined a selection of potentially confounding fixed effects known or suspected to have effects on immune parameters, but not of primary interest for this study: time bled – to control for daily fluctuations in immune parameters; time wait – the time delay between initial capture in mist-net to blood sampling to control for handling stress; day of season – to control for any changes during the course of the sampling season; sex; status – as a dominant or subordinate adult within the social group (n = 9 floating individuals were treated as subordinates); breeding activity – categorised as yes or no – to control for breeding-related changes in immunity (Ardia et al. 2003; Davis 2005; Forbes 2007; Cīrule et al. 2012; Hegemann et al. 2012; Zylberberg 2015; Snyder-Mackler et al. 2016). Breeding activity was defined as whether an individual was involved in a breeding attempt within the social group at the time of capture; including for females nest-building, egg-laying, incubation phases, and for all adult birds, offspring provisioning up to 9 weeks post-hatch when offspring start becoming self-sufficient (Hidalgo Aranzamendi 2017). Combined with random intercepts, these fixed parameters formed an unchanging 'null model' that was forced into all models during model selection. An additional potential confounding fixed effect we considered was the presence of active wing moult, to control for moult-related changes in immunity (Moreno 2004; Sanz et al. 2004; Moreno-Rueda 2010). Information on wing moult was available only for a subset of samples so we separately assessed the possibility that it may have affected our immune indices. We conducted ANOVAs comparing the null model in each of the 8 analyses both with and without the addition of wing moult for the subsets of data (n = 132-339). Wing moult had no effect in May or November, in any of the indices (table S1), and was therefore not considered further in the analyses.

Environmental parameters T_{max}, T_{max7}, T_{var}, $Rain_7$, group size and territory quality were included in the global models, as well as the two two-way interactions between T_{max} and T_{max7}

with $Rain_7$ to assess whether rain alters the effect of maximum temperatures. All predictor variables were mean-centred and standardised using the *arm* package (Gelman 2008; Gelman and Su 2016). Variance inflation factors were also calculated to assess for multicollinearity between all modelled variables in each global model (table S2). Model selection was carried out with the *MuMIn* package (Bartoń 2018) comparing the Akaike Information Criterion (AIC) of models with all permutations of environmental parameters, excluding from consideration any models that contained both T_{max} and T_{max7} due to high correlation ($r = 0.89$, $p < 0.001$; each correlated with T_{var}, $|r| < 0.25$), resulting in 64 models per immune response variable (see table S3 for permutations). All models with ΔAIC < 2 from the best-fitting model formed a model set that was then model-averaged to provide inferences of the estimates and relative variable importance of all environmental parameters. Predicted immune index values were obtained using model-averaged estimates, with means of numerical predictors and weighted means of categorical predictors, which were back-transformed as necessary for Ca and HL ratio. Adjusted repeatabilities were calculated from residuals of the null models within individuals and within territories using the *rptR* package (Stoffel et al. 2017).

Results

Over the course of sample collection, a total of 902 blood samples were taken from 387 individuals (56% male captures, of which 45% were dominant individuals; 44% female captures, of which 58% were dominants); 70% during May field seasons and 30% during November seasons. In total, 36% of captures were of individuals involved in breeding activity, and 97% breeding birds were captured during the later breeding stage with already fledged offspring. Final sample sizes were for Hp, n = 647; for NAbs, n = 513; for Ca, n = 517; and for HL ratio, n = 537. Pairwise correlations between immune and stress indices were weak to absent in May (using residuals controlling for parameters within null models, $|r| = 0.01$-0.24, n = 247-300, table S4) and November ($|r| = 0.00$-0.29, n = 111-124, table S4). Adjusted repeatabilities were low to non-detectable within individuals (R_{adj} = 0-0.392, table S5) and within territories (R_{adj} = 0-0.335, table S5).

Model selection for model-averaging

For each of the 8 model sets, a comparison between 64 model permutations was made using AIC (tables S6-13). Models with ΔAIC < 2 from the best fitting model totalled: Hp, n = 11 and 10; NAbs, n = 4 and 15; Ca, n = 4 and 12; HL ratio, n = 6 and 9 for May and November respectively. Comparing the null model to the best fitting model, ΔAIC values for the null

models for May and November respectively were for Hp, ΔAIC = 1.09 and 0; for Nabs, ΔAIC = 7.50 and 0; for Ca, ΔAIC = 36.56 and 0.47; and for HL ratio, ΔAIC = 11.70 and 6.40. For the four analyses with ΔAIC < 2, this implies that no environmental variables could improve the explanatory power of the model, which agrees with the lack of significant effects of any environmental parameters for those four averaged models (Hp in May; and Hp, NAbs and Ca in November; fig. 1, table S14).

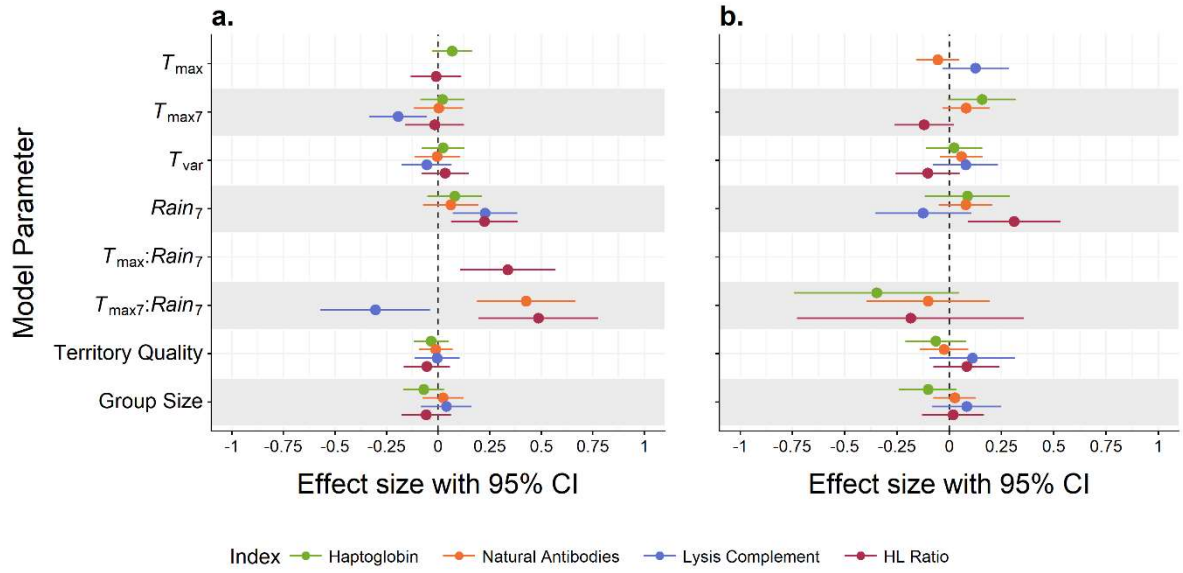

Figure 1: Effects of environmental variables on immune/stress indices for **a.** May and **b.** November. Shown are effect size estimates from models run with standardised (i.e. scaled and centred) immune and stress index response variables, so effects are comparable across models. Model output β-estimates are plotted, with bars spanning 2.5% and 97.5% confidence intervals of β-estimates. Effects with confidence intervals not overlapping zero (dashed vertical line) are statistically significant. Missing values were not among variables in the best fitting model sets, and therefore were not included in averaged models and do not have β-estimates. Sample sizes in May and November respectively were for Hp, n = 453 and 194; NAbs, n = 376 and 137; Ca n = 385 and 132; HL ratio, n = 375 and 162.

Group size and territory quality

Both group size and territory quality had very little statistical influence on any of the immune indices in either May or November (fig. 1). In all analyses, estimated effect sizes were minimal in comparison to other standardised predictors, and 95% confidence intervals (CI) contained zero (fig. 1).

Seasonal differences

There were no overall differences in mean levels of Hp, Ca or HL ratio between May and November (calculated from residuals from the null models; Welch Two-sample T-test for unequal variance: for Hp, $t_{307.81} = 1.52$, $p = 0.13$; for HL ratio, $t_{425.82} = -0.37$, $p = 0.71$; Two-sample T-test, for Ca, $t_{515} = -0.68$, $p = 0.50$; figs. 2c, i and l). The only immune index that exhibited a seasonal difference was NAbs (Welch Two-sample T-test, $t_{360.63} = -3.03$, $p < 0.01$), with slightly lower mean values in May (14.7 ± 2.04 s.e.) compared to November (15.4 ± 1.37 s.e.) (fig. 2f).

Climate variation

In May, climatic variables during the week prior to capture affected immune function and stress. For NAbs, Ca and HL ratio, the interaction between T_{max7} and $Rain_7$ had a clear and significant effect, with high relative importance (fig. 1a, fig. 2, table S14). However, the direction of this interaction varied. When there was rainfall, as T_{max7} increased, the levels of NAbs and HL ratio increased (figs. 2d and j), whereby the main effect of $Rain_7$ was also significant for HL ratio (i.e. HL ratio was higher if there was rain in May). For Ca the interactive effect was opposite: when there was rainfall, with increasing T_{max7} there was a decrease in Ca (figs. 1 and 2g). T_{max} (maximum temperature of the previous day) was of little influence on NAbs or Ca, however for HL ratio, it had a similar and significant effect as T_{max7}, but with lower relative variable importance and smaller effect size (fig. 1a). There was no detectable effect of any climate variable on Hp in May (fig. 1a).

In November, no environmental variables predicted variation in Hp, NAbs, and Ca (fig. 1b). For these immune indices, day of season was significant with moderate effect sizes, negative for Hp and NAbs, and positive for Ca (table S14, fig. S7), so as the November season progressed, Hp and NAbs decreased and Ca increased. Rainfall was linked to an increase in HL ratio, but no effect of temperature (fig. 2k, table S14), indicating an increase in stress after it had rained in the previous week, but no apparent stressful effects of very high temperatures, and no interaction.

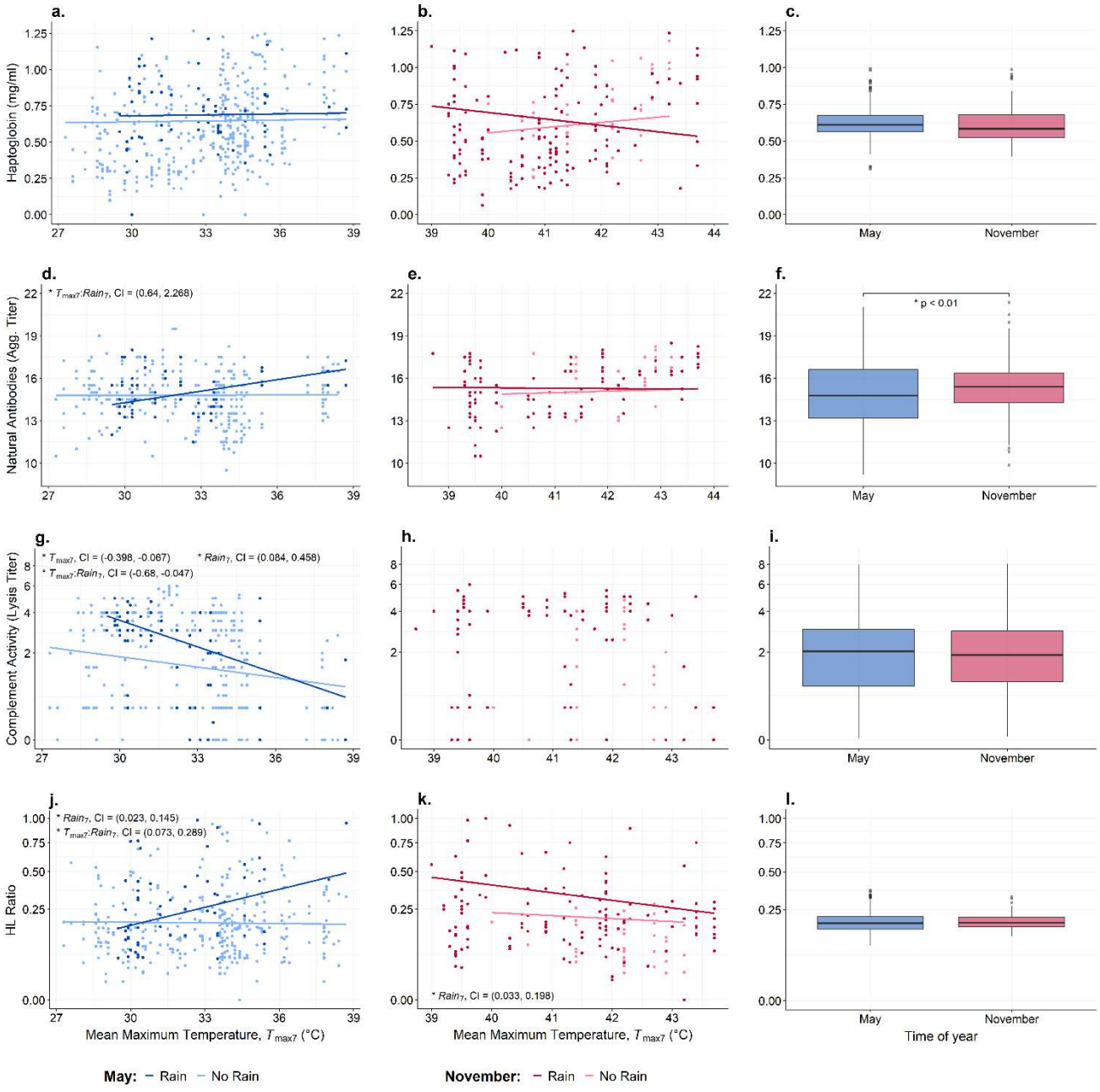
a.
b.
c.
d.
e.
f.
g.
h.
i.
j.
k.
l.
Haptoglobin (mg/ml)
Natural Antibodies (Agg. Titer)
Complement Activity (Lysis Titer)
HL Ratio
* T_{max7}:$Rain_7$, CI = (0.64, 2.268)
* p < 0.01
* T_{max7}, CI = (-0.398, -0.067)
* $Rain_7$, CI = (0.084, 0.458)
* T_{max7}:$Rain_7$, CI = (-0.68, -0.047)
* $Rain_7$, CI = (0.023, 0.145)
* T_{max7}:$Rain_7$, CI = (0.073, 0.289)
* $Rain_7$, CI = (0.033, 0.198)
May
November
Time of year
Mean Maximum Temperature, T_{max7} (°C)
May: Rain No Rain
November: Rain No Rain

Figure 2: Complex relationships between temperature and rain in the week before sampling (T_{max7}, $Rain_7$) and immune and stress indices. Panel of plots in row **a-c.** haptoglobin, **d-f.** natural antibodies, **g-i.** complement activity, and **j-l.** HL ratio. In first column, May season, and in second column, November season, data are plotted as raw immune index scores against mean maximum temperatures in the week before capture (T_{max7}), with fitted lines of predicted values from model-averaged β-estimates (table S14). Predictions could not be made for plot **h.** as T_{max7} β-estimates were not obtained after model selection and averaging. Black lines and points are with rainfall ($Rain_7$: Yes), grey lines and points are without rainfall ($Rain_7$: No). Plots **d.**, **g.**, **j.** and **k.** are annoted with 'significant' main effects and interactions, ascribed in each case where 95% confidence intervals of model-averaged β-estimates do not contain zero (fig. 2, table S14). In third column, boxplots of the residual values from the null models (i.e. corrected raw scores) indicate seasonal median values and interquartile ranges (boxes), and maximally 1.5x interquartile ranges (whiskers). Outliers are individually plotted; in boxplots **f.** and **l.** in May the were 17 and 16 omissions respectively, beyond the plotted y-axis scale. Plot **f.** p-value is from Welch two-sample t-test.

Discussion

No long-term environmental effects

We found no support for the hypothesis that longer-term stable environmental variation is associated with greater differences in immune indices: neither habitat quality nor social environment affected immune or stress indices. We predicted that greater resource availability, reflected by higher habitat quality, should enable birds to invest more in immune function, but this was not supported. Possibly, costs of maintaining constitutive innate immunity may not be large enough to impose on other bodily demands. Alternatively, although our proxy of habitat quality is correlated with aspects of food and water availability and predicts important fitness-related traits (Kingma et al. 2010, 2011a; Hidalgo Aranzamendi et al. 2016; Hidalgo Aranzamendi 2017), it may not reflect the aspects of resource availability that mechanistically relate to immune function. Perhaps fine-scale variation in habitat quality between territories was lower than what is typically important for immune variation (e.g. land use, degradation, urbanization, pollution; Fokidis et al. 2008; Eeva and Klemola 2013; Pigeon et al. 2013; Bailly et al. 2016).

Like habitat quality, group size also did not relate to immune or stress indices, contrary to expectation. We hypothesised that group size could affect immune function in this group living species in two opposing ways – indirectly via social stress and competition or directly as a result of parasite exposure. These hypotheses are not mutually exclusive, and support for both has been found in other birds, including songbirds (Møller et al. 2006, 2001; Spottiswoode

2008; but see Tella et al. 2001; Hawley et al. 2006). Conspecific interactions within groups of purple-crowned fairy-wrens tend to be mostly affiliative (e.g. allopreening) and presumably beneficial (Teunissen et al. 2018). As a consequence, we expected that elevated parasite pressure from close contact with group members would be more important than social stress. Nonetheless, simultaneous but opposing effects of social stress and parasite pressure may have obscured each independent effect. However, as we did not see an increase in HL ratio in larger groups (i.e. higher chronic stress), as was expected under the social stress hypothesis, it seems most probable that neither of these hypothesised costs of social living are related to standing immune variation.

Congruent with the lack of effects of long-term environmental variation, there were limited overall seasonal differences in immune and stress indices – only levels of NAbs were slightly higher in November (fig. 2f) – despite substantial differences in ambient temperature (fig. S6). In November, warmer and to some extent wetter conditions are likely to result in a proliferation of diverse microbial threats (Horrocks et al. 2012, 2015), as well as vectors (Ortego and Espada 2007; Wood et al. 2007; Nkuo-Akenji et al. 2008). Increased levels of natural antibodies enhance a host's ability to detect novel pathogens and an increase in NAbs during this season could be advantageous (Matson et al. 2005; Schulenburg et al. 2009). The observed increase in NAbs in May with increasing temperature when rainfall occurs (fig. 2d) is consistent with the interpretation that NAbs might be upregulated when warm and wet conditions are favourable for proliferation of vectors and parasites. The lack of a similar within-season effect in November may indicate that parasite pressure plateaus at very high temperatures.

Complex short-term climatic effects

We hypothesised that higher maximum temperatures, larger daily temperature variability, and a lack of rainfall represent more physiologically stressful environmental conditions that therefore would be associated with immunosuppression (cf. Martin 2009). The short-term climatic variables in our study were indeed found to predict some variation in immune and stress indices but these relationships were complex, in differing directions, and not as we predicted. Our hypotheses that climate-associated physiological stress mediates immunosuppression were not supported, as we found no environmental variable related to a decrease in an immune index at the same time as an increase in HL ratio. The short-term environmental variables did, however, account for individual variation in some immune and stress indices (fig. 1).

There was no evidence of heat related immunosuppression in November, when temperatures are hottest (mean maximum = 41.5°C). However, we did observe an increase in Ca (complement activity) with decreasing temperatures in May, the cooler season (fig. 2g). This is reminiscent of predictions from the 'winter-immunoenhancement' hypothesis that immune function is upregulated in response to decreasing day length in anticipation of increased risk of sickness during winter (Nelson et al. 1995, 2002; Nelson and Demas 2004; Walton et al. 2011). Ca is an aggressive constitutive innate immune component (Klasing 2004; Trouw and Daha 2011) and is therefore likely to be tightly regulated according to (seasonal) variation in the balance of benefits and costs (Buehler et al. 2008). In agreement with this, Ca also increased as day length shortened in May (fig. S7, table S14), providing some support for winter-immunoenhancement even in this tropical species, where the cold season is much milder and photoperiod less variable than in temperate regions (May mean minimum = 15.3°C, lowest recorded = 5.9°C; Schultz et al. 2017).

The only consistent effect across both seasons was a positive relationship between $Rain_7$ and HL ratio (figs. 1a and 1b), with recent rainfall associated with increased chronic stress (hours/days). Rainfall is the critical short-term cue for the initiation of breeding in purple-crowned fairy-wrens and even small quantities of rain can stimulate breeding at any time for individuals not with eggs/dependent young (Hidalgo Aranzamendi 2017). Chronic stress, as measured with HL ratio, has been shown in other species to increase during breeding attempts (Ots and Horak 1996; Hanssen et al. 2003; Ilmonen et al. 2003). Although the late stages of breeding were not associated with increased HL ratio (table S14), our data suggest that preparing for breeding initiation, as triggered by rain, may invoke some stress. Furthermore, this may be compounded by elevated temperature; in May, HL ratio increased with temperature (up to ~38˚C), and in November, when temperatures are always high (above ~38˚C), HL ratio was consistently a little higher (dark lines, figs. 2j and k). This could suggest that initiating breeding at higher temperatures represents a more stressful event for purple-crowned fairy-wrens.

A reactive scope perspective

Only short-term climatic variables were identified as important predictors of individual immune variation, whereas immune and stress indices did not vary with longer-term (months, seasonal or permanent) environmental variation. Given that the latter are stable or to a large extent predictable, whereas the former are unpredictable, these results are consistent with reactive scope models and reactive homeostasis (Romero et al. 2009; *sensu* allostasis, McEwen and Wingfield 2003). These models propose that predictive homeostasis manages investment into

physiological processes in anticipation of predictable change (Romero et al. 2009). If immune function is crucial to maintain, then investment in seasonally predictable reproduction, moult or other physiological processes may be modified to avoid conflict with immune function. Consequently, immune variation would not relate to longer-term environmental variation (Cyr et al. 2007). However, when shorter-term (days or weeks), unpredictable environmental variation impacts physiological demands, many processes, such as reproduction and moult, cannot easily be modified once initiated (Cyr and Romero 2007). This may be resolved by modifying investment in immune function instead (DuRant et al. 2016; Gormally et al. 2018). Consequently, according to this scenario, the only environmental parameters that might be expected to correlate with immune function would be unpredictable short-term changes in conditions that could push individuals into reactive homeostasis and possibly homeostatic overload - which may account for the results we found.

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Supplementary Materials

Figure S1: An example of the variation in habitat quality. **A**. Good quality habitat for *M. coronatus* with a high density of *P. aquaticus* along the riverbank. This territory scored highly (18/20) in the territory quality survey. **B**. Low quality habitat for *M. coronatus* within the study area with no *P. aquaticus* (0/20). This area was unoccupied by purple-crowned fairy-wrens. Images: M. Roast/AWC.

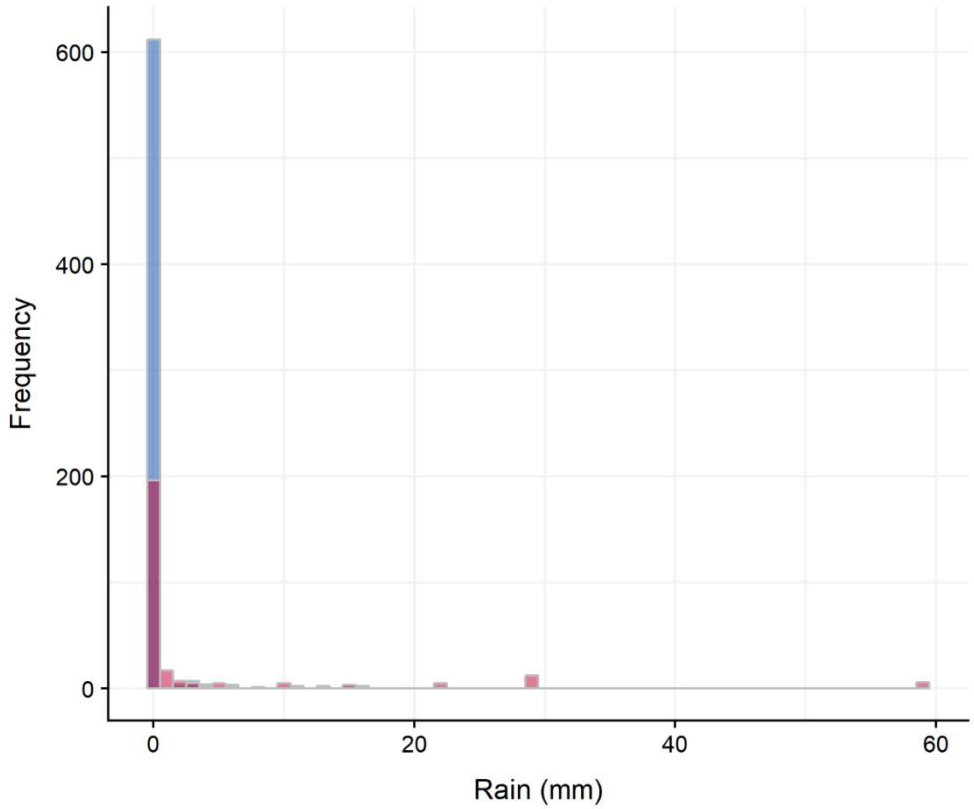

Figure S2: Zero-inflation of rainfall data associated with captures. Typically there was 0mm of rain, however when it did rain, up to 59mm fell in a single downpour. Transparent May (blue) and November (red) bars show overlapping bars in purple.

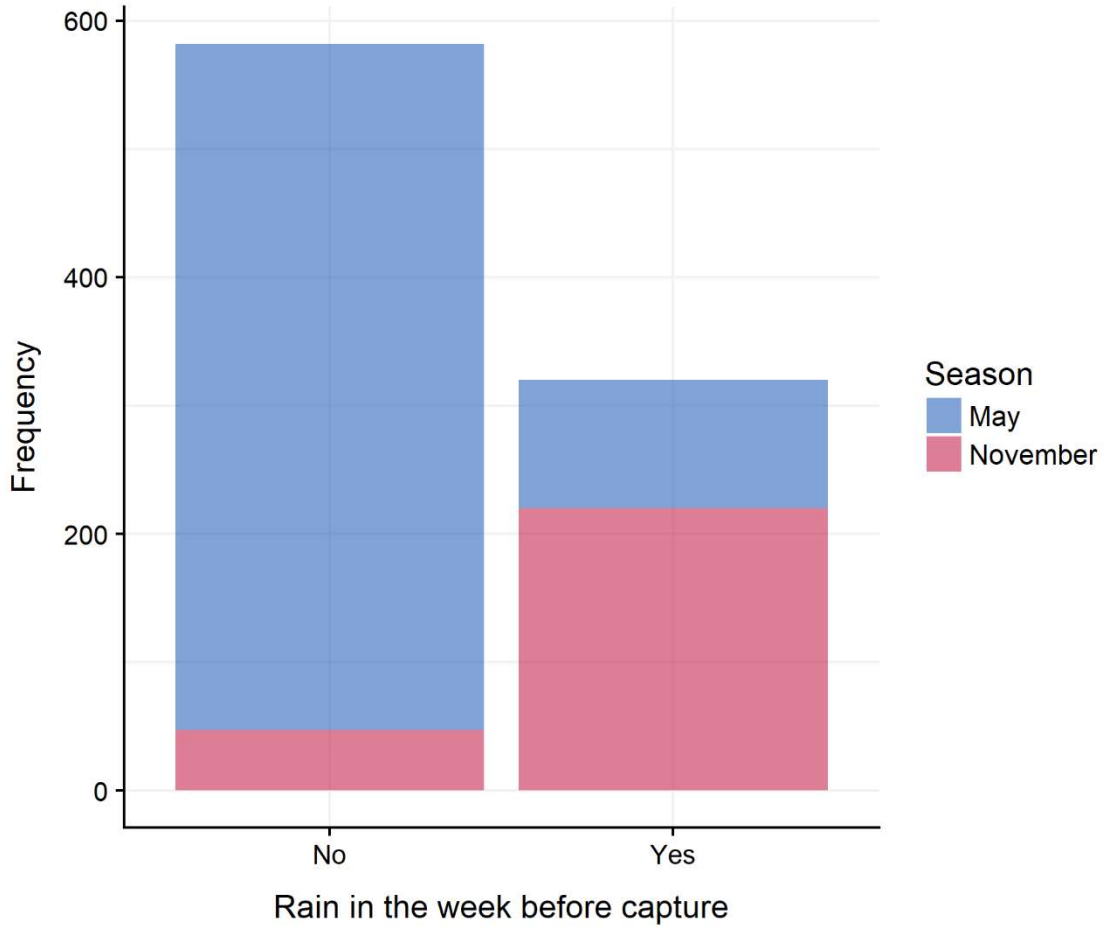

Figure S3: Histogram of recoded rainfall data. Proportionally fewer May captures were preceded by rainfall than November captures, illustrating a distinct difference between May and November data sets.

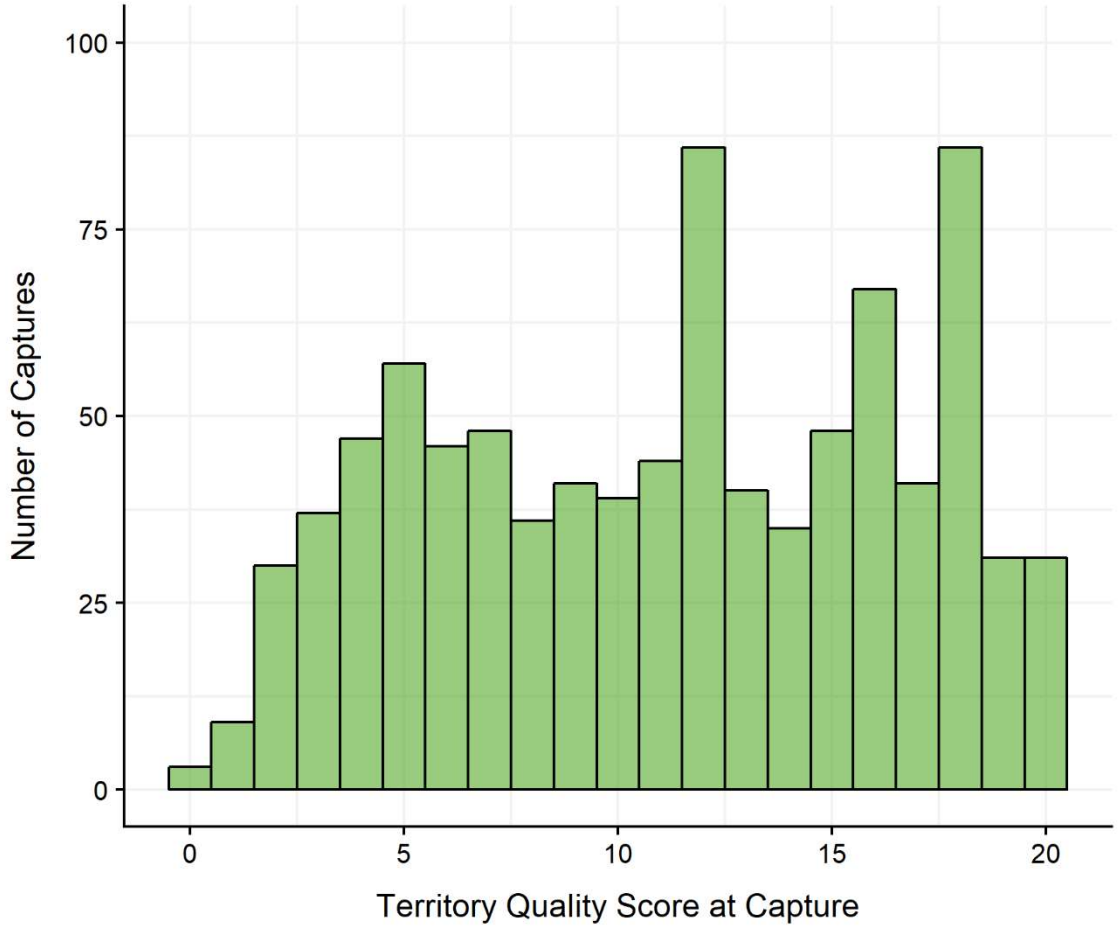

Figure S4: Histogram of the frequency of territory quality scores assigned to the time of capture. Only one single territory with a territory quality score of 0 was ever recorded, and all captures are repeated measures of the dominant breeding pair from the territory.

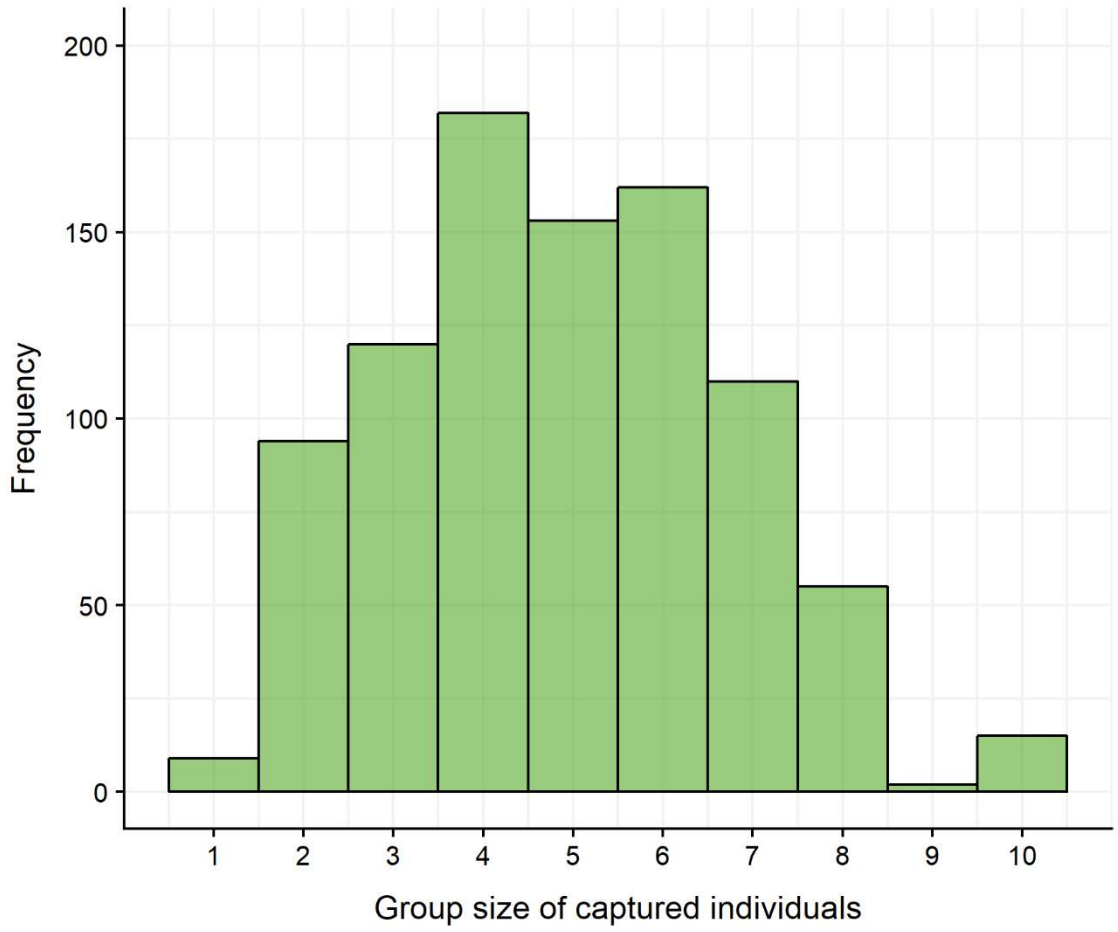

Figure S5: Histogram of the frequency of individual captured from groups of different sizes. Group size ranged from 1-10 individuals. 'Groups' with 1 individual typically refer to cases where a new territory with suitable habitat became occupied by a settled bird, but a new social partner had not yet arrived to join the first bird. Occasionally, group size of 1 refers to cases of lost social partners. Groups of up to 11 birds have been recorded in other population of this species.

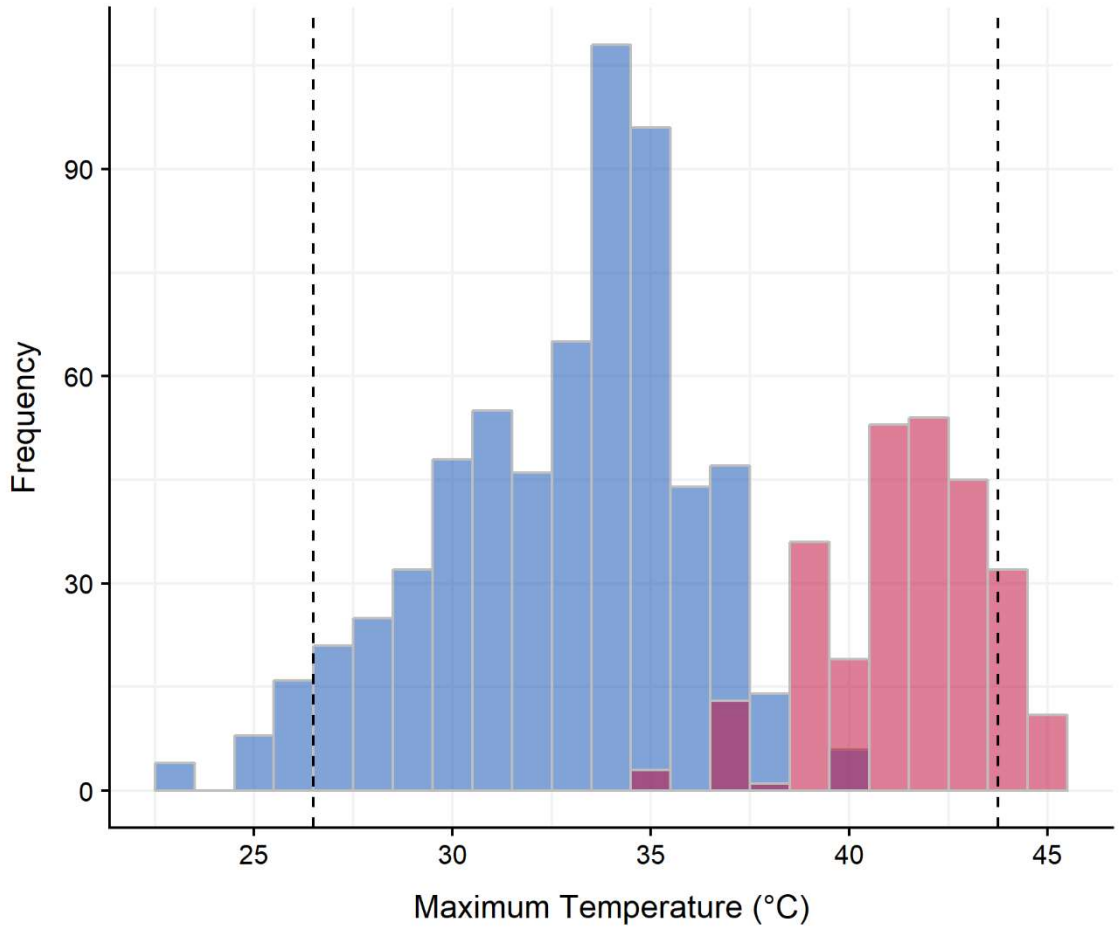

Figure S6: Histogram of the frequency of maximum temperatures on capture day, T_{max}. Dashed lines indicate the 2.5th and 97.5th percentiles, below and above which can be considered extreme cold and hot days. Transparency of May (blue) and November (red) bars indicates little overlap (purple) in seasonal temperatures.

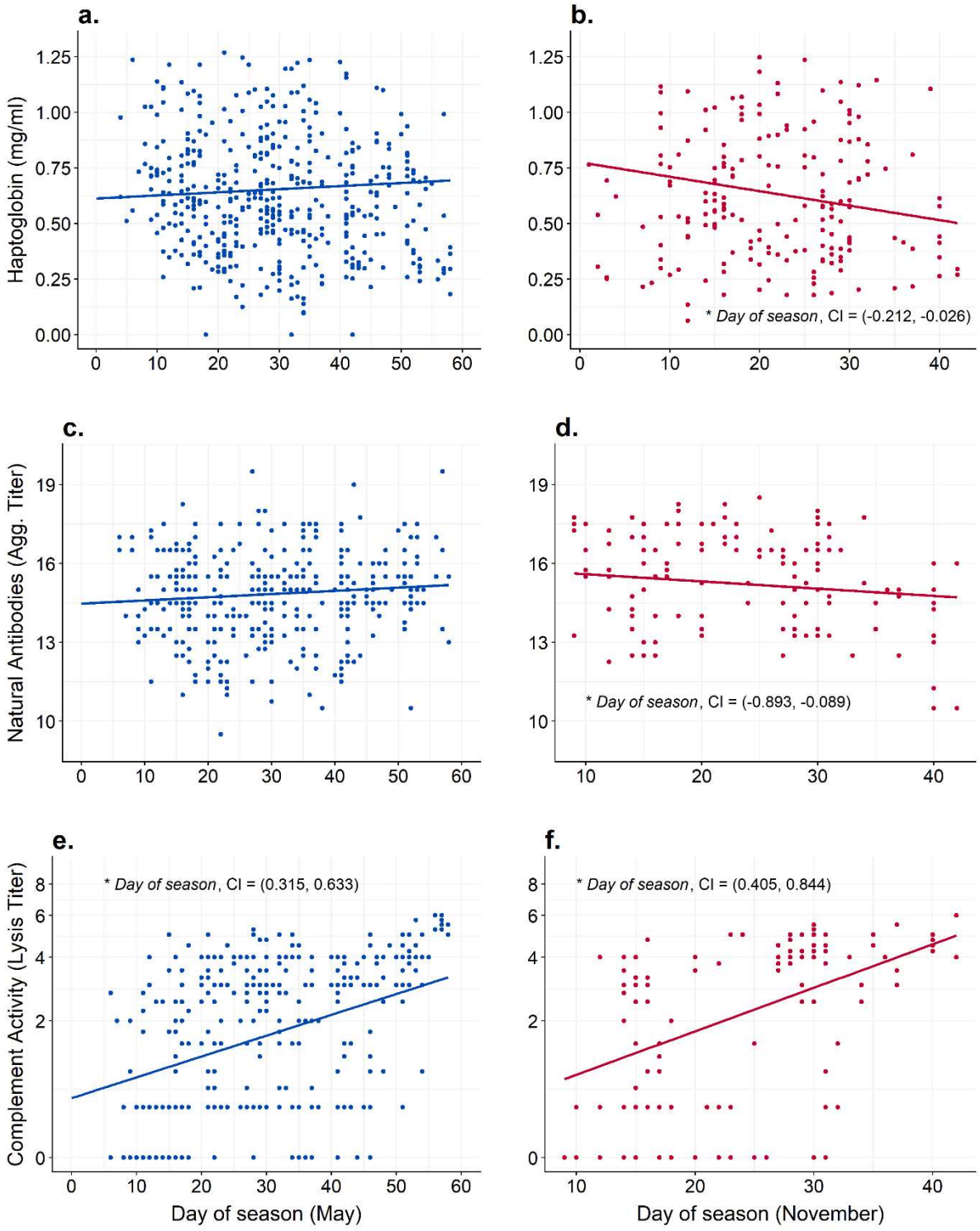

Figure S7: Temporal changes in immune function: possible preparation for the wet season breeding peak and winter immunoenhancement. Panel of **a.** haptoglobin, **c.** natural antibodies, and **e.** complement activity in May field season (blue); and **b.** haptoglobin, **d.** natural antibodies, and **f.** complement activity in November field season (red). Raw immune index scores plotted against date through the field season. Fitted lines of predicted values from model-averaged β estimates. Annotated with 'significant'effects, ascribed in each case where 95% confidence intervals (CI) of model-averaged β estimates do not contain zero (table 1).

Table S1: Results from analysis of variance testing the difference between null models including and excluding wing moult as an additional fixed effect. This was conducted on a subset of data where wing moult scores were available. No result showed any effect of wing moult.

Index-Season	**χ^2**	**df**	**p**	**n**
Hp-May	0.013	1	0.911	339
Hp-November	0.386	1	0.535	194
NAbs-May	2.764	1	0.096	252
NAbs-November	1.583	1	0.208	137
Ca-May	0.162	1	0.688	266
Ca-November	0.018	1	0.892	132
HLR-May	0.618	1	0.432	263
HLR-November	0.213	1	0.644	132

Table S2: Variance inflation factors (VIFs) testing for multicollinearity between modelled variables. VIFs for each variable from each of the 8 global models were calculated, and VIFs were < 5 (considered the threshold for multicollinearity) in all cases, with 2 exceptions (**bold** typeface). In the November Ca and HL ratio models, $Rain_7$ generated VIFs of 5.22 and 6.74 respectively. This collinearity was with the $Rain_7$:T_{max} interaction term included in the model – which had high VIFs of 4.39 and 4.64, respectively – which is to be expected with main effect and interaction terms, and was disregarded as problematic.

Season	**May**				**November**			
Environmental Variable	**Hp**	**NAbs**	**Ca**	**HL ratio**	**Hp**	**NAbs**	**Ca**	**HL ratio**
Breeding activity	1.26	1.42	1.36	1.33	1.33	1.39	1.38	1.4
Day of season	2.39	2.47	2.47	2.65	1.54	1.37	1.3	1.4
Sex	1.04	1.04	1.03	1.05	1.08	1.05	1.06	1.04
Status	1.23	1.26	1.19	1.29	1.25	1.22	1.18	1.27
Time bled	1.05	1.14	1.11	1.24	1.13	1.38	1.32	1.3
Time wait	1.13	1.14	1.15	1.37	1.27	1.32	1.28	1.66
T_{max}	2.26	3.34	3.58	3.03	2.16	3.56	4.49	3.87
T_{max7}	2.82	3.56	4.16	4.34	1.59	1.82	2.12	2.26
$Rain_7$	1.66	2.04	2.08	2.2	2.48	4.97	**5.22**	**6.74**
T_{max}:$Rain_7$	1.73	2.02	2.27	2.15	2.26	3.91	4.39	4.64
T_{max7}:$Rain_7$	1.56	1.46	1.48	1.55	1.74	1.82	2.24	2.13
T_{var}	1.47	1.71	1.88	1.92	1.63	1.83	1.99	2.08
Group Size	1.68	1.79	1.58	1.8	1.7	1.64	1.5	1.51
Territory Quality	1.2	1.17	1.12	1.19	1.41	1.61	1.39	1.54

Table S3: All 64 model permutations of variables used in the main analyses. In each row for a single model, 1 denotes inclusion of the variable into the model, 0 denotes exclusion of the variable. All variables in the null model were forced into every model (all marked with 1), and models which contained T_{max} and T_{max7} together were not considered.

	Global Model													
	Null Model						**Permuted Variables**							
Model	Breeding Activity	Day of Season	Sex	Status	Time bled	Time wait	T_{max}	T_{max7}	$Rain_7$	T_{max}:$Rain_7$	T_{max7}:$Rain_7$	T_{var}	Group size	Territory quality
1	1	1	1	1	1	1	0	0	1	0	0	1	1	1
2	1	1	1	1	1	1	1	0	0	0	0	1	1	1
3	1	1	1	1	1	1	0	0	0	0	0	1	1	1
4	1	1	1	1	1	1	0	0	1	0	0	0	1	1
5	1	1	1	1	1	1	1	0	0	0	0	0	1	1
6	1	1	1	1	1	1	0	0	0	0	0	0	1	1
7	1	1	1	1	1	1	0	0	1	0	0	1	0	1
8	1	1	1	1	1	1	1	0	0	0	0	1	0	1
9	1	1	1	1	1	1	0	0	0	0	0	1	0	1
10	1	1	1	1	1	1	0	0	1	0	0	0	0	1
11	1	1	1	1	1	1	1	0	0	0	0	0	0	1
12	1	1	1	1	1	1	0	0	0	0	0	0	0	1
13	1	1	1	1	1	1	0	0	1	0	0	1	1	0
14	1	1	1	1	1	1	1	0	0	0	0	1	1	0
15	1	1	1	1	1	1	0	0	0	0	0	1	1	0
16	1	1	1	1	1	1	0	0	1	0	0	0	1	0
17	1	1	1	1	1	1	1	0	0	0	0	0	1	0
18	1	1	1	1	1	1	0	0	0	0	0	0	1	0
19	1	1	1	1	1	1	0	0	1	0	0	1	0	0
20	1	1	1	1	1	1	1	0	0	0	0	1	0	0
21	1	1	1	1	1	1	0	0	0	0	0	1	0	0
22	1	1	1	1	1	1	0	0	1	0	0	0	0	0
23	1	1	1	1	1	1	1	0	0	0	0	0	0	0
24	1	1	1	1	1	1	0	0	0	0	0	0	0	0
25	1	1	1	1	1	1	1	0	1	1	0	1	1	1
26	1	1	1	1	1	1	1	0	1	1	0	0	1	1
27	1	1	1	1	1	1	1	0	1	1	0	1	0	1
28	1	1	1	1	1	1	1	0	1	1	0	0	0	1
29	1	1	1	1	1	1	1	0	1	1	0	1	1	0
30	1	1	1	1	1	1	1	0	1	1	0	0	1	0

31	1	1	1	1	1	1	1	0	1	1	0	1	0	0
32	1	1	1	1	1	1	1	0	1	1	0	0	0	0
33	1	1	1	1	1	1	1	0	1	0	0	1	1	1
34	1	1	1	1	1	1	1	0	1	0	0	0	1	1
35	1	1	1	1	1	1	1	0	1	0	0	1	0	1
36	1	1	1	1	1	1	1	0	1	0	0	0	0	1
37	1	1	1	1	1	1	1	0	1	0	0	1	1	0
38	1	1	1	1	1	1	1	0	1	0	0	0	1	0
39	1	1	1	1	1	1	1	0	1	0	0	1	0	0
40	1	1	1	1	1	1	1	0	1	0	0	0	0	0
41	1	1	1	1	1	1	0	1	0	0	0	1	1	1
42	1	1	1	1	1	1	0	1	0	0	0	0	1	1
43	1	1	1	1	1	1	0	1	0	0	0	1	0	1
44	1	1	1	1	1	1	0	1	0	0	0	0	0	1
45	1	1	1	1	1	1	0	1	0	0	0	1	1	0
46	1	1	1	1	1	1	0	1	0	0	0	0	1	0
47	1	1	1	1	1	1	0	1	0	0	0	1	0	0
48	1	1	1	1	1	1	0	1	0	0	0	0	0	0
49	1	1	1	1	1	1	0	1	1	0	1	1	1	1
50	1	1	1	1	1	1	0	1	1	0	1	0	1	1
51	1	1	1	1	1	1	0	1	1	0	1	1	0	1
52	1	1	1	1	1	1	0	1	1	0	1	0	0	1
53	1	1	1	1	1	1	0	1	1	0	1	1	1	0
54	1	1	1	1	1	1	0	1	1	0	1	0	1	0
55	1	1	1	1	1	1	0	1	1	0	1	1	0	0
56	1	1	1	1	1	1	0	1	1	0	1	0	0	0
57	1	1	1	1	1	1	0	1	1	0	0	1	1	1
58	1	1	1	1	1	1	0	1	1	0	0	0	1	1
59	1	1	1	1	1	1	0	1	1	0	0	1	0	1
60	1	1	1	1	1	1	0	1	1	0	0	0	0	1
61	1	1	1	1	1	1	0	1	1	0	0	1	1	0
62	1	1	1	1	1	1	0	1	1	0	0	0	1	0
63	1	1	1	1	1	1	0	1	1	0	0	1	0	0
64	1	1	1	1	1	1	0	1	1	0	0	0	0	0

Table S4: Pairwise correlations between null model residuals of immune and stress indices. Ca values are normalised by natural log-transformation and HL ratio by square-root-transformation.

	May			November		
Index-index	**r**	**p**	**n**	**r**	**p**	**n**
Hp-NAbs	0.24	<0.001	256	0.29	0.001	118
Hp-Ca	-0.01	0.812	259	-0.17	0.070	113
Hp-HLR	-0.05	0.390	247	0.00	0.959	112
NAbs-Ca	-0.08	0.185	298	-0.22	0.012	124
NAbs-HLR	-0.03	0.608	247	-0.08	0.376	114
Ca-HLR	0.03	0.633	248	0.13	0.157	111

Table S5: Intra-grouping adjusted repeatabilities within individuals and within territories, calculated from null models in each analysis. Corrected complement activity scores in November showed within-territory repeatability significantly different from 0 (*); corrected HL ratio in November showed within-individual repeatability significantly different from 0 (*).

	Within-individual Repeatability (Individual ID)			Within-territory Repeatability (Territory ID)		
Index-Season	R_{adj}	**SE**	**95% CI**	R_{adj}	**SE**	**95% CI**
Hp-May	0	0.025	0, 0.084	0.034	0.021	0, 0.080
Hp-November	0	0.097	0, 0.320	0.034	0.039	0, 0.135
NAbs-May	0	0.029	0, 0.096	0.026	0.020	0, 0.076
NAbs-November	0	0.110	0, 0.419	0.037	0.062	0, 0.232
Ca-May	0	0.053	0, 0.176	0.182	0.054	0, 0.292
Ca-November	0	0.206	0, 0.717	0.335	0.131	0.056, 0.585*
HLR-May	0.112	0.071	0, 0.273	0.058	0.039	0, 0.146
HLR-November	0.392	0.154	0.122, 0.758*	0	0.042	0, 0.147

Table S6: Model selection and comparison of model permutations for Hp in May.

Model Rank	Model ID	Intercept	Breeding activity : Yes	Day of season	Sex : M	Status : Subordinate	Time bled	Time wait	T_{max}	T_{max7}	$Rain_7$: Yes	T_{max} x $Rain_7$: Yes	T_{max7} x $Rain_7$: Yes	T_{var}	Group size	Territory quality	df	logLik	AIC	ΔAIC	weight
1	1566	0.660	-0.043	0.030	-0.020	0.030	0.002	-0.015	NA	NA	NA	NA	NA	NA	NA	NA	12	73.742	-123.48	0.00	0.062
2	1598	0.658	-0.031	0.029	-0.020	0.040	0.002	-0.007	NA	NA	NA	NA	NA	NA	-0.035	NA	13	74.599	-123.20	0.29	0.053
3	1664	0.657	-0.028	0.059	-0.017	0.041	0.003	-0.005	0.044	NA	0.052	NA	NA	NA	-0.039	NA	15	76.459	-122.92	0.57	0.046
4	1630	0.659	-0.041	0.039	-0.020	0.029	0.004	-0.016	0.027	NA	NA	NA	NA	NA	NA	NA	13	74.369	-122.74	0.75	0.043
5	1632	0.660	-0.043	0.058	-0.018	0.030	0.003	-0.014	0.040	NA	0.048	NA	NA	NA	NA	NA	14	75.349	-122.70	0.79	0.042
6	1662	0.657	-0.028	0.038	-0.019	0.039	0.004	-0.008	0.029	NA	NA	NA	NA	NA	-0.036	NA	14	75.330	-122.66	0.82	0.041
7	1568	0.661	-0.045	0.039	-0.019	0.032	0.001	-0.013	NA	NA	0.029	NA	NA	NA	NA	NA	13	74.152	-122.30	1.18	0.034
8	1600	0.659	-0.032	0.038	-0.019	0.042	0.001	-0.005	NA	NA	0.031	NA	NA	NA	-0.036	NA	14	75.056	-122.11	1.37	0.031
9	3614	0.659	-0.043	0.029	-0.020	0.033	0.003	-0.015	NA	NA	NA	NA	NA	NA	NA	-0.018	13	74.054	-122.11	1.38	0.031
10	1822	0.661	-0.043	0.027	-0.020	0.030	0.002	-0.014	NA	NA	NA	NA	NA	0.013	NA	NA	13	73.846	-121.69	1.79	0.025
11	1694	0.659	-0.042	0.035	-0.020	0.030	0.002	-0.015	NA	0.011	NA	NA	NA	NA	NA	NA	13	73.813	-121.63	1.86	0.024
12	3646	0.657	-0.032	0.028	-0.020	0.041	0.003	-0.008	NA	NA	NA	NA	NA	NA	-0.031	-0.012	14	74.722	-121.44	2.04	0.022
13	3680	0.658	-0.043	0.058	-0.018	0.033	0.003	-0.014	0.039	NA	0.050	NA	NA	NA	NA	-0.018	15	75.678	-121.36	2.13	0.021
14	1854	0.659	-0.031	0.027	-0.020	0.040	0.002	-0.007	NA	NA	NA	NA	NA	0.010	-0.034	NA	14	74.666	-121.33	2.15	0.021
15	1726	0.658	-0.030	0.033	-0.020	0.040	0.002	-0.007	NA	0.008	NA	NA	NA	NA	-0.034	NA	14	74.641	-121.28	2.20	0.021
16	3678	0.657	-0.042	0.038	-0.020	0.032	0.004	-0.016	0.026	NA	NA	NA	NA	NA	NA	-0.016	14	74.624	-121.25	2.24	0.020
17	3712	0.657	-0.030	0.059	-0.017	0.043	0.003	-0.005	0.043	NA	0.053	NA	NA	NA	-0.036	-0.011	16	76.569	-121.14	2.35	0.019
18	1696	0.660	-0.042	0.057	-0.020	0.031	0.000	-0.012	NA	0.029	0.044	NA	NA	NA	NA	NA	14	74.553	-121.11	2.38	0.019
19	3616	0.660	-0.045	0.039	-0.020	0.035	0.002	-0.013	NA	NA	0.032	NA	NA	NA	NA	-0.020	14	74.529	-121.06	2.43	0.018
20	5760	0.658	-0.029	0.057	-0.017	0.041	0.002	-0.004	0.041	NA	0.051	0.015	NA	NA	-0.038	NA	16	76.494	-120.99	2.50	0.018
21	1920	0.657	-0.028	0.059	-0.017	0.041	0.003	-0.004	0.043	NA	0.052	NA	NA	0.002	-0.039	NA	16	76.460	-120.92	2.56	0.017
22	5728	0.660	-0.042	0.055	-0.018	0.030	0.002	-0.013	0.036	NA	0.047	0.023	NA	NA	NA	NA	15	75.432	-120.86	2.62	0.017
23	3710	0.656	-0.029	0.038	-0.019	0.040	0.004	-0.008	0.028	NA	NA	NA	NA	NA	-0.034	-0.009	15	75.405	-120.81	2.68	0.016
24	1728	0.658	-0.030	0.055	-0.019	0.041	0.000	-0.004	NA	0.027	0.045	NA	NA	NA	-0.034	NA	15	75.396	-120.79	2.69	0.016
25	1886	0.659	-0.041	0.038	-0.020	0.029	0.004	-0.016	0.026	NA	NA	NA	NA	0.003	NA	NA	14	74.373	-120.75	2.74	0.016
26	1888	0.660	-0.043	0.057	-0.018	0.030	0.003	-0.013	0.039	NA	0.049	NA	NA	0.006	NA	NA	15	75.368	-120.74	2.75	0.016
27	1824	0.663	-0.045	0.036	-0.019	0.031	0.000	-0.012	NA	NA	0.033	NA	NA	0.018	NA	NA	14	74.351	-120.70	2.78	0.015
28	1918	0.656	-0.028	0.039	-0.019	0.040	0.004	-0.008	0.030	NA	NA	NA	NA	-0.001	-0.036	NA	15	75.331	-120.66	2.82	0.015
29	3648	0.658	-0.033	0.038	-0.019	0.043	0.002	-0.006	NA	NA	0.032	NA	NA	NA	-0.032	-0.013	15	75.221	-120.44	3.04	0.013
30	1856	0.661	-0.032	0.036	-0.019	0.041	0.000	-0.004	NA	NA	0.034	NA	NA	0.016	-0.035	NA	15	75.206	-120.41	3.07	0.013
31	3870	0.660	-0.043	0.026	-0.021	0.033	0.002	-0.015	NA	NA	NA	NA	NA	0.015	NA	-0.019	14	74.196	-120.39	3.09	0.013
32	3742	0.658	-0.042	0.033	-0.021	0.033	0.002	-0.015	NA	0.009	NA	NA	NA	NA	NA	-0.017	14	74.107	-120.21	3.27	0.012
33	9888	0.662	-0.042	0.047	-0.020	0.033	-0.001	-0.012	NA	0.017	0.036	NA	0.067	NA	NA	NA	15	75.077	-120.15	3.33	0.012
34	9920	0.661	-0.030	0.045	-0.019	0.043	-0.001	-0.004	NA	0.015	0.037	NA	0.068	NA	-0.034	NA	16	75.933	-119.87	3.62	0.010
35	3744	0.658	-0.043	0.057	-0.020	0.035	0.001	-0.012	NA	0.028	0.046	NA	NA	NA	NA	-0.019	15	74.911	-119.82	3.66	0.010
36	1950	0.660	-0.042	0.032	-0.020	0.030	0.001	-0.015	NA	0.010	NA	NA	NA	0.012	NA	NA	14	73.901	-119.80	3.68	0.010
37	3902	0.658	-0.032	0.026	-0.020	0.041	0.002	-0.008	NA	NA	NA	NA	NA	0.012	-0.030	-0.013	15	74.812	-119.62	3.86	0.009
38	3872	0.661	-0.045	0.036	-0.020	0.035	0.001	-0.012	NA	NA	0.036	NA	NA	0.021	NA	-0.022	15	74.797	-119.59	3.89	0.009
39	7776	0.659	-0.043	0.054	-0.018	0.034	0.003	-0.013	0.034	NA	0.049	0.026	NA	NA	NA	-0.019	16	75.785	-119.57	3.91	0.009
40	3774	0.657	-0.031	0.032	-0.020	0.041	0.002	-0.008	NA	0.008	NA	NA	NA	NA	-0.031	-0.011	15	74.756	-119.51	3.97	0.008
41	1952	0.661	-0.042	0.054	-0.020	0.031	-0.001	-0.011	NA	0.029	0.048	NA	NA	0.017	NA	NA	15	74.740	-119.48	4.01	0.008
42	3936	0.659	-0.043	0.055	-0.018	0.033	0.003	-0.013	0.037	NA	0.051	NA	NA	0.009	NA	-0.019	16	75.722	-119.44	4.04	0.008
43	1982	0.659	-0.030	0.030	-0.020	0.040	0.001	-0.007	NA	0.007	NA	NA	NA	0.010	-0.033	NA	15	74.698	-119.40	4.09	0.008
44	3934	0.658	-0.042	0.036	-0.020	0.032	0.004	-0.016	0.024	NA	NA	NA	NA	0.005	NA	-0.017	15	74.640	-119.28	4.20	0.008
45	7808	0.657	-0.030	0.056	-0.018	0.042	0.003	-0.005	0.039	NA	0.052	0.017	NA	NA	-0.035	-0.011	17	76.617	-119.23	4.25	0.007
46	3968	0.657	-0.030	0.058	-0.018	0.042	0.003	-0.005	0.042	NA	0.053	NA	NA	0.004	-0.036	-0.011	17	76.577	-119.15	4.33	0.007
47	3776	0.657	-0.032	0.055	-0.019	0.043	0.000	-0.005	NA	0.026	0.046	NA	NA	NA	-0.031	-0.013	16	75.553	-119.11	4.38	0.007
48	1984	0.659	-0.030	0.053	-0.019	0.041	-0.001	-0.004	NA	0.026	0.048	NA	NA	0.015	-0.033	NA	16	75.536	-119.07	4.41	0.007
49	6016	0.658	-0.029	0.057	-0.017	0.041	0.003	-0.005	0.041	NA	0.051	0.015	NA	-0.001	-0.039	NA	17	76.494	-118.99	4.50	0.007
50	5984	0.661	-0.043	0.054	-0.018	0.030	0.002	-0.013	0.036	NA	0.047	0.021	NA	0.002	NA	NA	16	75.435	-118.87	4.61	0.006
51	3904	0.660	-0.034	0.036	-0.019	0.043	0.001	-0.005	NA	NA	0.036	NA	NA	0.018	-0.030	-0.015	16	75.417	-118.83	4.65	0.006
52	10144	0.665	-0.042	0.042	-0.020	0.032	-0.002	-0.011	NA	0.015	0.040	NA	0.077	0.023	NA	NA	16	75.408	-118.82	4.67	0.006
53	3966	0.656	-0.029	0.038	-0.019	0.040	0.004	-0.008	0.028	NA	NA	NA	NA	0.000	-0.034	-0.009	16	75.405	-118.81	4.67	0.006
54	11936	0.661	-0.042	0.048	-0.020	0.035	0.000	-0.012	NA	0.018	0.039	NA	0.060	NA	NA	-0.016	16	75.317	-118.63	4.85	0.005
55	3998	0.659	-0.043	0.030	-0.021	0.033	0.002	-0.015	NA	0.008	NA	NA	NA	0.014	NA	-0.019	15	74.232	-118.46	5.02	0.005
56	10176	0.663	-0.030	0.040	-0.019	0.042	-0.002	-0.004	NA	0.013	0.040	NA	0.077	0.021	-0.033	NA	17	76.203	-118.41	5.08	0.005
57	4000	0.660	-0.043	0.053	-0.020	0.035	0.000	-0.012	NA	0.028	0.050	NA	NA	0.020	NA	-0.021	16	75.162	-118.32	5.16	0.005
58	11968	0.660	-0.031	0.046	-0.019	0.044	-0.001	-0.005	NA	0.015	0.038	NA	0.063	NA	-0.032	-0.009	17	76.010	-118.02	5.46	0.004
59	4030	0.658	-0.032	0.029	-0.020	0.041	0.002	-0.008	NA	0.006	NA	NA	NA	0.011	-0.030	-0.012	16	74.836	-117.67	5.81	0.003
60	8032	0.659	-0.043	0.053	-0.018	0.034	0.002	-0.013	0.034	NA	0.049	0.023	NA	0.005	NA	-0.019	17	75.799	-117.60	5.89	0.003
61	4032	0.658	-0.032	0.052	-0.019	0.042	0.000	-0.005	NA	0.026	0.050	NA	NA	0.017	-0.029	-0.015	17	75.738	-117.48	6.01	0.003
62	12192	0.663	-0.042	0.042	-0.020	0.035	-0.002	-0.011	NA	0.015	0.043	NA	0.070	0.025	NA	-0.017	17	75.700	-117.40	6.08	0.003
63	8064	0.657	-0.030	0.056	-0.018	0.042	0.003	-0.005	0.039	NA	0.052	0.017	NA	0.001	-0.035	-0.012	18	76.618	-117.24	6.25	0.003
64	12224	0.662	-0.032	0.041	-0.019	0.043	-0.002	-0.004	NA	0.013	0.042	NA	0.072	0.023	-0.030	-0.011	18	76.312	-116.62	6.86	0.002

Table S7: Model selection and comparison of model permutations for Hp in November.

Model Rank	Model ID	Intercept	Breeding activity : Yes	Day of season	Sex : M	Status : Subordinate	Time bled	Time wait	T_{max}	T_{max7}	$Rain_7$: Yes	T_{max} x $Rain_7$: Yes	T_{max7} x $Rain_7$: Yes	T_{var}	Group size	Territory quality	df	logLik	AIC	ΔAIC	weight
1	1694	0.646	-0.108	-0.114	0.011	-0.077	-0.005	0.022	NA	0.080	NA	NA	NA	NA	NA	NA	13	4.914	16.17	0.00	0.072
2	1726	0.644	-0.092	-0.123	0.014	-0.060	-0.013	0.024	NA	0.079	NA	NA	NA	NA	-0.051	NA	14	5.767	16.47	0.29	0.062
3	9920	0.633	-0.099	-0.146	0.010	-0.058	-0.006	0.014	NA	0.104	0.069	NA	-0.202	NA	-0.066	NA	16	7.640	16.72	0.55	0.055
4	1566	0.645	-0.091	-0.093	0.018	-0.078	-0.003	0.034	NA	NA	NA	NA	NA	NA	NA	NA	12	3.370	17.26	1.09	0.042
5	1598	0.642	-0.076	-0.103	0.020	-0.060	-0.011	0.036	NA	NA	NA	NA	NA	NA	-0.053	NA	13	4.256	17.49	1.32	0.037
6	3742	0.645	-0.106	-0.110	0.012	-0.074	-0.011	0.026	NA	0.080	NA	NA	NA	NA	NA	-0.036	14	5.241	17.52	1.35	0.037
7	9888	0.637	-0.120	-0.126	0.007	-0.079	0.004	0.011	NA	0.101	0.048	NA	-0.178	NA	NA	NA	15	6.226	17.55	1.38	0.036
8	1696	0.646	-0.104	-0.121	0.011	-0.078	-0.006	0.024	NA	0.084	0.019	NA	NA	NA	NA	NA	14	4.974	18.05	1.88	0.028
9	1950	0.647	-0.107	-0.114	0.011	-0.077	-0.007	0.022	NA	0.075	NA	NA	NA	0.013	NA	NA	14	4.969	18.06	1.89	0.028
10	1728	0.645	-0.084	-0.137	0.013	-0.060	-0.017	0.029	NA	0.085	0.034	NA	NA	NA	-0.055	NA	15	5.960	18.08	1.91	0.028
11	1982	0.645	-0.092	-0.124	0.014	-0.059	-0.015	0.024	NA	0.073	NA	NA	NA	0.015	-0.052	NA	15	5.845	18.31	2.14	0.025
12	3774	0.644	-0.093	-0.121	0.014	-0.060	-0.015	0.026	NA	0.079	NA	NA	NA	NA	-0.045	-0.016	15	5.825	18.35	2.18	0.024
13	10176	0.634	-0.100	-0.144	0.009	-0.056	-0.009	0.013	NA	0.095	0.065	NA	-0.211	0.021	-0.067	NA	17	7.790	18.42	2.25	0.023
14	1822	0.647	-0.092	-0.097	0.015	-0.076	-0.008	0.032	NA	NA	NA	NA	NA	0.032	NA	NA	13	3.763	18.47	2.30	0.023
15	1854	0.645	-0.077	-0.108	0.018	-0.058	-0.016	0.034	NA	NA	NA	NA	NA	0.034	-0.054	NA	14	4.689	18.62	2.45	0.021
16	3614	0.644	-0.090	-0.089	0.019	-0.074	-0.009	0.038	NA	NA	NA	NA	NA	NA	NA	-0.036	13	3.688	18.62	2.45	0.021
17	11968	0.633	-0.100	-0.143	0.010	-0.058	-0.008	0.015	NA	0.104	0.067	NA	-0.201	NA	-0.061	-0.013	17	7.679	18.64	2.47	0.021
18	11936	0.635	-0.119	-0.121	0.008	-0.075	-0.002	0.015	NA	0.100	0.045	NA	-0.182	NA	NA	-0.040	16	6.646	18.71	2.54	0.020
19	1918	0.648	-0.063	-0.112	0.023	-0.062	-0.018	0.035	-0.057	NA	NA	NA	NA	0.059	-0.061	NA	15	5.590	18.82	2.65	0.019
20	1662	0.643	-0.069	-0.103	0.024	-0.063	-0.010	0.037	-0.027	NA	NA	NA	NA	NA	-0.056	NA	14	4.495	19.01	2.84	0.017
21	1630	0.645	-0.087	-0.093	0.020	-0.080	-0.002	0.034	-0.019	NA	NA	NA	NA	NA	NA	NA	13	3.493	19.01	2.84	0.017
22	1568	0.645	-0.092	-0.092	0.018	-0.078	-0.003	0.033	NA	NA	-0.003	NA	NA	NA	NA	NA	13	3.371	19.26	3.09	0.015
23	10144	0.638	-0.121	-0.125	0.007	-0.079	0.002	0.010	NA	0.093	0.044	NA	-0.185	0.019	NA	NA	16	6.345	19.31	3.14	0.015
24	1886	0.650	-0.084	-0.100	0.019	-0.082	-0.008	0.033	-0.047	NA	NA	NA	NA	0.053	NA	NA	14	4.340	19.32	3.15	0.015
25	3646	0.642	-0.077	-0.100	0.020	-0.060	-0.013	0.037	NA	NA	NA	NA	NA	NA	-0.047	-0.016	14	4.307	19.39	3.22	0.014
26	3998	0.646	-0.106	-0.111	0.012	-0.073	-0.013	0.026	NA	0.074	NA	NA	NA	0.012	NA	-0.036	15	5.295	19.41	3.24	0.014
27	3744	0.646	-0.103	-0.117	0.012	-0.074	-0.012	0.028	NA	0.083	0.016	NA	NA	NA	NA	-0.035	15	5.289	19.42	3.25	0.014
28	1600	0.642	-0.074	-0.107	0.020	-0.060	-0.012	0.037	NA	NA	0.009	NA	NA	NA	-0.054	NA	14	4.271	19.46	3.29	0.014
29	3870	0.647	-0.091	-0.094	0.016	-0.073	-0.014	0.036	NA	NA	NA	NA	NA	0.032	NA	-0.036	14	4.075	19.85	3.68	0.011
30	1952	0.647	-0.105	-0.120	0.010	-0.077	-0.008	0.024	NA	0.079	0.016	NA	NA	0.010	NA	NA	15	5.009	19.98	3.81	0.011
31	3776	0.645	-0.085	-0.134	0.013	-0.060	-0.019	0.030	NA	0.085	0.032	NA	NA	NA	-0.050	-0.013	16	5.997	20.01	3.84	0.011
32	1984	0.646	-0.085	-0.136	0.013	-0.059	-0.018	0.029	NA	0.081	0.031	NA	NA	0.010	-0.055	NA	16	5.996	20.01	3.84	0.011
33	3678	0.644	-0.085	-0.088	0.022	-0.077	-0.008	0.039	-0.026	NA	NA	NA	NA	NA	NA	-0.041	14	3.907	20.19	4.01	0.010
34	4030	0.645	-0.093	-0.121	0.014	-0.059	-0.017	0.026	NA	0.073	NA	NA	NA	0.015	-0.046	-0.016	16	5.900	20.20	4.03	0.010
35	3934	0.649	-0.081	-0.095	0.021	-0.078	-0.015	0.037	-0.052	NA	NA	NA	NA	0.054	NA	-0.043	15	4.846	20.31	4.14	0.009
36	12224	0.634	-0.102	-0.141	0.009	-0.056	-0.010	0.014	NA	0.095	0.062	NA	-0.210	0.021	-0.062	-0.013	18	7.831	20.34	4.17	0.009
37	6016	0.635	-0.090	-0.113	0.018	-0.061	-0.009	0.025	-0.040	NA	0.022	-0.204	NA	0.073	-0.062	NA	17	6.816	20.37	4.20	0.009
38	12192	0.636	-0.121	-0.119	0.008	-0.074	-0.004	0.014	NA	0.091	0.041	NA	-0.190	0.020	NA	-0.040	17	6.777	20.45	4.27	0.009
39	1824	0.647	-0.094	-0.095	0.016	-0.076	-0.007	0.031	NA	NA	-0.008	NA	NA	0.033	NA	NA	14	3.775	20.45	4.28	0.008
40	3902	0.645	-0.078	-0.105	0.018	-0.058	-0.018	0.036	NA	NA	NA	NA	NA	0.033	-0.049	-0.015	15	4.735	20.53	4.36	0.008
41	1920	0.649	-0.068	-0.104	0.025	-0.063	-0.016	0.031	-0.067	NA	-0.027	NA	NA	0.066	-0.058	NA	16	5.711	20.58	4.41	0.008
42	3616	0.644	-0.091	-0.087	0.019	-0.074	-0.009	0.037	NA	NA	-0.005	NA	NA	NA	NA	-0.037	14	3.694	20.61	4.44	0.008
43	1856	0.645	-0.076	-0.109	0.018	-0.058	-0.017	0.035	NA	NA	0.004	NA	NA	0.033	-0.054	NA	15	4.692	20.62	4.44	0.008
44	3966	0.647	-0.063	-0.108	0.023	-0.062	-0.020	0.037	-0.057	NA	NA	NA	NA	0.059	-0.053	-0.019	16	5.684	20.63	4.46	0.008
45	3710	0.643	-0.070	-0.099	0.025	-0.063	-0.013	0.039	-0.029	NA	NA	NA	NA	NA	-0.048	-0.021	15	4.585	20.83	4.66	0.007
46	1888	0.650	-0.090	-0.089	0.022	-0.083	-0.005	0.027	-0.061	NA	-0.038	NA	NA	0.063	NA	NA	15	4.566	20.87	4.70	0.007
47	1632	0.645	-0.089	-0.089	0.021	-0.081	-0.001	0.033	-0.022	NA	-0.012	NA	NA	NA	NA	NA	14	3.515	20.97	4.80	0.007
48	5984	0.639	-0.111	-0.096	0.015	-0.082	0.001	0.021	-0.035	NA	0.007	-0.192	NA	0.070	NA	NA	16	5.508	20.98	4.81	0.006
49	1664	0.643	-0.069	-0.103	0.024	-0.063	-0.010	0.037	-0.027	NA	-0.001	NA	NA	NA	-0.056	NA	15	4.495	21.01	4.84	0.006
50	5760	0.631	-0.088	-0.111	0.018	-0.061	-0.004	0.032	0.001	NA	0.045	-0.182	NA	NA	-0.059	NA	16	5.340	21.32	5.15	0.005
51	4000	0.647	-0.104	-0.116	0.011	-0.074	-0.013	0.027	NA	0.078	0.013	NA	NA	0.010	NA	-0.035	16	5.325	21.35	5.18	0.005
52	3648	0.642	-0.075	-0.103	0.020	-0.060	-0.014	0.038	NA	NA	0.007	NA	NA	NA	-0.048	-0.015	15	4.316	21.37	5.20	0.005
53	5728	0.635	-0.107	-0.096	0.015	-0.080	0.005	0.028	0.005	NA	0.031	-0.171	NA	NA	NA	NA	15	4.236	21.53	5.36	0.005
54	3936	0.649	-0.088	-0.080	0.024	-0.078	-0.013	0.030	-0.069	NA	-0.048	NA	NA	0.066	NA	-0.048	16	5.210	21.58	5.41	0.005
55	8032	0.637	-0.110	-0.087	0.017	-0.077	-0.006	0.024	-0.045	NA	-0.002	-0.191	NA	0.073	NA	-0.050	17	6.190	21.62	5.45	0.005
56	3872	0.646	-0.094	-0.090	0.017	-0.072	-0.013	0.034	NA	NA	-0.011	NA	NA	0.033	NA	-0.037	15	4.096	21.81	5.64	0.004
57	4032	0.646	-0.086	-0.133	0.013	-0.059	-0.020	0.030	NA	0.080	0.029	NA	NA	0.010	-0.050	-0.013	17	6.034	21.93	5.76	0.004
58	8064	0.635	-0.092	-0.105	0.018	-0.062	-0.011	0.026	-0.043	NA	0.015	-0.203	NA	0.074	-0.052	-0.025	18	6.971	22.06	5.89	0.004
59	3680	0.644	-0.088	-0.082	0.024	-0.077	-0.007	0.036	-0.031	NA	-0.018	NA	NA	NA	NA	-0.044	15	3.963	22.07	5.90	0.004
60	3968	0.647	-0.070	-0.096	0.025	-0.063	-0.018	0.032	-0.069	NA	-0.034	NA	NA	0.067	-0.048	-0.025	17	5.866	22.27	6.10	0.003
61	3904	0.645	-0.078	-0.106	0.018	-0.058	-0.018	0.036	NA	NA	0.002	NA	NA	0.033	-0.049	-0.014	16	4.735	22.53	6.36	0.003
62	7776	0.634	-0.107	-0.089	0.018	-0.077	0.000	0.032	-0.005	NA	0.024	-0.166	NA	NA	NA	-0.043	16	4.683	22.63	6.46	0.003
63	3712	0.643	-0.071	-0.098	0.025	-0.063	-0.012	0.038	-0.030	NA	-0.005	NA	NA	NA	-0.047	-0.021	16	4.589	22.82	6.65	0.003
64	7808	0.631	-0.090	-0.106	0.019	-0.061	-0.005	0.033	-0.002	NA	0.041	-0.178	NA	NA	-0.052	-0.019	17	5.415	23.17	7.00	0.002

Table S8: Model selection and comparison of model permutations for NAbs in May.

Model Rank	Model ID	Intercept	Breeding activity : Yes	Day of season	Sex : M	Status : Subordinate	Time bled	Time wait	T_{max}	T_{max7}	$Rain_7$: Yes	T_{max} x $Rain_7$: Yes	T_{max7} x $Rain_7$: Yes	T_{var}	Group size	Territory quality	df	logLik	AIC	ΔAIC	weight
1	9888	14.681	0.091	0.344	0.038	0.171	0.286	0.175	NA	0.006	0.209	NA	1.457	NA	NA	NA	15	-615.226	1260.45	0.00	0.342
2	9920	14.683	0.055	0.342	0.036	0.146	0.285	0.154	NA	0.006	0.205	NA	1.454	NA	0.082	NA	16	-615.114	1262.23	1.78	0.141
3	11936	14.678	0.092	0.343	0.039	0.178	0.290	0.174	NA	0.007	0.214	NA	1.449	NA	NA	-0.039	16	-615.188	1262.38	1.92	0.131
4	10144	14.680	0.091	0.345	0.038	0.172	0.287	0.175	NA	0.006	0.204	NA	1.450	-0.016	NA	NA	16	-615.223	1262.45	1.99	0.126
5	11968	14.681	0.048	0.341	0.037	0.151	0.291	0.149	NA	0.008	0.212	NA	1.441	NA	0.098	-0.058	17	-615.036	1264.07	3.62	0.056
6	10176	14.683	0.055	0.343	0.036	0.146	0.286	0.154	NA	0.006	0.202	NA	1.449	-0.011	0.081	NA	17	-615.113	1264.23	3.77	0.052
7	12192	14.678	0.092	0.344	0.039	0.178	0.291	0.174	NA	0.007	0.210	NA	1.445	-0.010	NA	-0.039	17	-615.187	1264.37	3.92	0.048
8	12224	14.680	0.048	0.341	0.037	0.151	0.291	0.149	NA	0.008	0.211	NA	1.441	-0.002	0.098	-0.058	18	-615.036	1266.07	5.62	0.021
9	1566	14.640	0.032	0.336	-0.005	0.157	0.346	0.197	NA	NA	NA	NA	NA	NA	NA	NA	12	-621.975	1267.95	7.50	0.008
10	1822	14.633	0.039	0.369	-0.007	0.163	0.360	0.198	NA	NA	NA	NA	NA	-0.217	NA	NA	13	-621.300	1268.60	8.15	0.006
11	1568	14.639	0.039	0.395	0.003	0.158	0.351	0.197	NA	NA	0.219	NA	NA	NA	NA	NA	13	-621.308	1268.62	8.16	0.006
12	1598	14.642	-0.012	0.335	-0.008	0.125	0.345	0.170	NA	NA	NA	NA	NA	NA	0.100	NA	13	-621.809	1269.62	9.17	0.003
13	1630	14.642	0.024	0.311	-0.003	0.160	0.338	0.199	-0.074	NA	NA	NA	NA	NA	NA	NA	13	-621.834	1269.67	9.22	0.003
14	3614	14.637	0.033	0.332	-0.003	0.168	0.351	0.195	NA	NA	NA	NA	NA	NA	NA	-0.059	13	-621.887	1269.77	9.32	0.003
15	1694	14.641	0.023	0.302	-0.004	0.159	0.351	0.194	NA	-0.070	NA	NA	NA	NA	NA	NA	13	-621.888	1269.78	9.32	0.003
16	1824	14.634	0.043	0.404	-0.001	0.162	0.361	0.198	NA	NA	0.164	NA	NA	-0.163	NA	NA	14	-620.971	1269.94	9.49	0.003
17	1600	14.641	-0.002	0.392	0.000	0.128	0.351	0.173	NA	NA	0.215	NA	NA	NA	0.091	NA	14	-621.170	1270.34	9.89	0.002
18	3616	14.636	0.040	0.391	0.005	0.171	0.358	0.196	NA	NA	0.228	NA	NA	NA	NA	-0.074	14	-621.171	1270.34	9.89	0.002
19	1854	14.635	0.000	0.366	-0.010	0.135	0.359	0.176	NA	NA	NA	NA	NA	-0.210	0.087	NA	14	-621.177	1270.35	9.90	0.002
20	3870	14.630	0.040	0.365	-0.006	0.171	0.364	0.197	NA	NA	NA	NA	NA	-0.212	NA	-0.047	14	-621.246	1270.49	10.04	0.002
21	1696	14.638	0.051	0.444	0.004	0.156	0.348	0.201	NA	0.073	0.269	NA	NA	NA	NA	NA	14	-621.247	1270.49	10.04	0.002
22	1950	14.633	0.034	0.351	-0.007	0.164	0.362	0.197	NA	-0.034	NA	NA	NA	-0.210	NA	NA	14	-621.280	1270.56	10.11	0.002
23	1886	14.633	0.038	0.365	-0.007	0.163	0.359	0.198	-0.009	NA	NA	NA	NA	-0.212	NA	NA	14	-621.298	1270.60	10.14	0.002
24	1632	14.638	0.042	0.405	0.003	0.157	0.354	0.197	0.019	NA	0.233	NA	NA	NA	NA	NA	14	-621.301	1270.60	10.15	0.002
25	3646	14.639	-0.022	0.328	-0.006	0.133	0.352	0.162	NA	NA	NA	NA	NA	NA	0.123	-0.082	14	-621.648	1271.30	10.84	0.002
26	1662	14.644	-0.021	0.310	-0.006	0.127	0.338	0.172	-0.075	NA	NA	NA	NA	NA	0.100	NA	14	-621.667	1271.33	10.88	0.001
27	1726	14.643	-0.021	0.302	-0.007	0.127	0.350	0.167	NA	-0.067	NA	NA	NA	NA	0.098	NA	14	-621.728	1271.46	11.00	0.001
28	3678	14.639	0.025	0.307	-0.002	0.170	0.343	0.197	-0.074	NA	NA	NA	NA	NA	NA	-0.058	14	-621.748	1271.50	11.04	0.001
29	3742	14.638	0.023	0.296	-0.003	0.170	0.356	0.192	NA	-0.073	NA	NA	NA	NA	NA	-0.061	14	-621.794	1271.59	11.14	0.001
30	1856	14.636	0.005	0.401	-0.004	0.135	0.360	0.177	NA	NA	0.162	NA	NA	-0.157	0.084	NA	15	-620.857	1271.71	11.26	0.001
31	3872	14.631	0.043	0.400	0.001	0.173	0.366	0.197	NA	NA	0.174	NA	NA	-0.153	NA	-0.062	15	-620.880	1271.76	11.31	0.001
32	1888	14.631	0.052	0.439	-0.002	0.161	0.370	0.197	0.064	NA	0.203	NA	NA	-0.184	NA	NA	15	-620.905	1271.81	11.36	0.001
33	1952	14.633	0.055	0.451	0.000	0.161	0.358	0.201	NA	0.071	0.212	NA	NA	-0.162	NA	NA	15	-620.916	1271.83	11.38	0.001
34	3648	14.638	-0.013	0.387	0.002	0.138	0.359	0.164	NA	NA	0.225	NA	NA	NA	0.119	-0.096	15	-620.951	1271.90	11.45	0.001
35	3902	14.633	-0.008	0.360	-0.009	0.141	0.364	0.169	NA	NA	NA	NA	NA	-0.202	0.107	-0.068	15	-621.072	1272.14	11.69	0.001
36	3744	14.634	0.052	0.441	0.006	0.170	0.354	0.199	NA	0.075	0.279	NA	NA	NA	NA	-0.075	15	-621.108	1272.22	11.76	0.001
37	1728	14.640	0.010	0.441	0.002	0.127	0.347	0.177	NA	0.073	0.264	NA	NA	NA	0.091	NA	15	-621.110	1272.22	11.77	0.001
38	1982	14.636	-0.004	0.349	-0.010	0.136	0.361	0.174	NA	-0.033	NA	NA	NA	-0.203	0.086	NA	15	-621.159	1272.32	11.87	0.001
39	3680	14.635	0.043	0.404	0.005	0.171	0.361	0.195	0.025	NA	0.246	NA	NA	NA	NA	-0.075	15	-621.160	1272.32	11.87	0.001
40	1664	14.641	0.000	0.401	0.000	0.128	0.353	0.173	0.016	NA	0.226	NA	NA	NA	0.091	NA	15	-621.165	1272.33	11.88	0.001
41	1918	14.636	-0.002	0.361	-0.010	0.135	0.357	0.176	-0.012	NA	NA	NA	NA	-0.204	0.087	NA	15	-621.174	1272.35	11.90	0.001
42	3998	14.631	0.034	0.345	-0.006	0.172	0.366	0.195	NA	-0.037	NA	NA	NA	-0.204	NA	-0.049	15	-621.222	1272.44	11.99	0.001
43	3934	14.631	0.038	0.360	-0.006	0.171	0.363	0.197	-0.011	NA	NA	NA	NA	-0.206	NA	-0.047	15	-621.243	1272.49	12.03	0.001
44	5728	14.643	0.042	0.389	0.003	0.156	0.355	0.196	-0.001	NA	0.228	0.090	NA	NA	NA	NA	15	-621.266	1272.53	12.08	0.001
45	3710	14.641	-0.030	0.303	-0.005	0.135	0.345	0.164	-0.075	NA	NA	NA	NA	NA	0.124	-0.081	15	-621.507	1273.01	12.56	0.001
46	3774	14.640	-0.031	0.293	-0.006	0.135	0.357	0.159	NA	-0.071	NA	NA	NA	NA	0.122	-0.084	15	-621.559	1273.12	12.67	0.001
47	5984	14.641	0.055	0.407	-0.002	0.160	0.376	0.194	0.023	NA	0.179	0.235	NA	-0.237	NA	NA	16	-620.701	1273.40	12.95	0.001
48	3904	14.634	-0.005	0.396	-0.002	0.142	0.366	0.169	NA	NA	0.175	NA	NA	-0.142	0.108	-0.083	16	-620.702	1273.40	12.95	0.001
49	1920	14.634	0.015	0.434	-0.004	0.135	0.368	0.176	0.059	NA	0.198	NA	NA	-0.177	0.081	NA	16	-620.800	1273.60	13.15	0.000
50	1984	14.636	0.017	0.449	-0.002	0.134	0.356	0.180	NA	0.070	0.210	NA	NA	-0.156	0.084	NA	16	-620.802	1273.60	13.15	0.000
51	3936	14.628	0.053	0.437	0.000	0.172	0.375	0.195	0.066	NA	0.215	NA	NA	-0.175	NA	-0.064	16	-620.809	1273.62	13.17	0.000
52	4000	14.630	0.055	0.449	0.002	0.171	0.363	0.200	NA	0.072	0.224	NA	NA	-0.151	NA	-0.063	16	-620.822	1273.64	13.19	0.000
53	3776	14.637	-0.001	0.437	0.003	0.136	0.355	0.168	NA	0.075	0.276	NA	NA	NA	0.119	-0.097	16	-620.888	1273.78	13.32	0.000
54	3712	14.637	-0.010	0.399	0.002	0.137	0.362	0.164	0.022	NA	0.241	NA	NA	NA	0.118	-0.097	16	-620.942	1273.88	13.43	0.000
55	4030	14.634	-0.014	0.340	-0.008	0.142	0.366	0.167	NA	-0.038	NA	NA	NA	-0.194	0.107	-0.069	16	-621.048	1274.10	13.64	0.000
56	3966	14.634	-0.011	0.354	-0.008	0.141	0.362	0.169	-0.015	NA	NA	NA	NA	-0.194	0.107	-0.068	16	-621.067	1274.13	13.68	0.000
57	5760	14.646	-0.002	0.381	0.000	0.125	0.354	0.170	-0.009	NA	0.219	0.111	NA	NA	0.097	NA	16	-621.113	1274.23	13.77	0.000
58	7776	14.640	0.044	0.387	0.005	0.171	0.362	0.194	0.003	NA	0.240	0.100	NA	NA	NA	-0.078	16	-621.117	1274.23	13.78	0.000
59	6016	14.645	0.014	0.399	-0.005	0.132	0.375	0.170	0.015	NA	0.171	0.251	NA	-0.233	0.091	NA	17	-620.567	1275.13	14.68	0.000
60	8032	14.638	0.056	0.404	0.000	0.171	0.382	0.192	0.024	NA	0.190	0.237	NA	-0.228	NA	-0.065	17	-620.600	1275.20	14.75	0.000
61	3968	14.631	0.005	0.430	-0.002	0.142	0.375	0.168	0.061	NA	0.213	NA	NA	-0.162	0.105	-0.084	17	-620.642	1275.28	14.83	0.000
62	4032	14.633	0.007	0.445	0.000	0.141	0.363	0.172	NA	0.072	0.225	NA	NA	-0.141	0.108	-0.084	17	-620.643	1275.29	14.83	0.000
63	7808	14.644	-0.013	0.376	0.002	0.135	0.363	0.160	-0.007	NA	0.233	0.130	NA	NA	0.126	-0.101	17	-620.871	1275.74	15.29	0.000
64	8064	14.643	0.003	0.393	-0.003	0.139	0.382	0.162	0.015	NA	0.185	0.259	NA	-0.219	0.117	-0.088	18	-620.395	1276.79	16.34	0.000

Table S9: Model selection and comparison of model permutations for NAbs in November.

Model Rank	Model ID	Intercept	Breeding activity : Yes	Day of season	Sex : M	Status : Subordinate	Time bled	Time wait	T_{max}	T_{max7}	$Rain_7$: Yes	T_{max} x $Rain_7$: Yes	T_{max7} x $Rain_7$: Yes	T_{var}	Group size	Territory quality	df	logLik	AIC	ΔAIC	weight
1	1566	15.191	-0.178	-0.436	-0.086	-0.145	0.032	-0.038	NA	NA	NA	NA	NA	NA	NA	NA	12	-188.544	401.09	0.00	0.060
2	1694	15.210	-0.245	-0.512	-0.110	-0.149	0.021	-0.067	NA	0.283	NA	NA	NA	NA	NA	NA	13	-187.566	401.13	0.05	0.058
3	1696	15.196	-0.197	-0.591	-0.112	-0.136	-0.008	-0.010	NA	0.319	0.306	NA	NA	NA	NA	NA	14	-186.638	401.28	0.19	0.054
4	1822	15.218	-0.230	-0.441	-0.100	-0.141	-0.032	-0.039	NA	NA	NA	NA	NA	0.212	NA	NA	13	-187.770	401.54	0.45	0.048
5	1886	15.192	-0.150	-0.461	-0.100	-0.149	-0.012	-0.031	-0.248	NA	NA	NA	NA	0.305	NA	NA	14	-186.854	401.71	0.62	0.044
6	1568	15.175	-0.129	-0.497	-0.082	-0.135	0.007	0.013	NA	NA	0.254	NA	NA	NA	NA	NA	13	-187.906	401.81	0.72	0.042
7	1950	15.226	-0.273	-0.507	-0.114	-0.148	-0.029	-0.064	NA	0.237	NA	NA	NA	0.164	NA	NA	14	-187.139	402.28	1.19	0.033
8	1630	15.172	-0.125	-0.444	-0.084	-0.151	0.059	-0.033	-0.133	NA	NA	NA	NA	NA	NA	NA	13	-188.243	402.49	1.40	0.030
9	1824	15.200	-0.181	-0.490	-0.093	-0.133	-0.043	0.002	NA	NA	0.203	NA	NA	0.179	NA	NA	14	-187.374	402.75	1.66	0.026
10	1598	15.199	-0.203	-0.413	-0.092	-0.173	0.052	-0.047	NA	NA	NA	NA	NA	NA	0.100	NA	13	-188.399	402.80	1.71	0.025
11	9888	15.173	-0.219	-0.580	-0.112	-0.142	0.021	-0.039	NA	0.369	0.368	NA	-0.365	NA	NA	NA	15	-186.408	402.82	1.73	0.025
12	3614	15.180	-0.166	-0.426	-0.079	-0.132	0.008	-0.025	NA	NA	NA	NA	NA	NA	NA	-0.099	13	-188.436	402.87	1.78	0.024
13	1726	15.215	-0.264	-0.492	-0.114	-0.173	0.037	-0.075	NA	0.277	NA	NA	NA	NA	0.084	NA	14	-187.463	402.93	1.84	0.024
14	1952	15.208	-0.219	-0.577	-0.115	-0.135	-0.036	-0.015	NA	0.281	0.270	NA	NA	0.104	NA	NA	15	-186.472	402.94	1.86	0.024
15	3742	15.201	-0.234	-0.499	-0.105	-0.137	0.003	-0.056	NA	0.273	NA	NA	NA	NA	NA	-0.079	14	-187.499	403.00	1.91	0.023
16	3934	15.170	-0.123	-0.447	-0.090	-0.128	-0.052	-0.009	-0.273	NA	NA	NA	NA	0.317	NA	-0.163	15	-186.558	403.12	2.03	0.022
17	1728	15.201	-0.214	-0.573	-0.116	-0.156	0.005	-0.018	NA	0.313	0.299	NA	NA	NA	0.067	NA	15	-186.570	403.14	2.05	0.021
18	3744	15.191	-0.192	-0.584	-0.109	-0.130	-0.019	-0.006	NA	0.315	0.300	NA	NA	NA	NA	-0.044	15	-186.616	403.23	2.14	0.020
19	3870	15.206	-0.218	-0.430	-0.093	-0.126	-0.059	-0.026	NA	NA	NA	NA	NA	0.214	NA	-0.105	14	-187.644	403.29	2.20	0.020
20	1854	15.222	-0.246	-0.425	-0.103	-0.162	-0.015	-0.046	NA	NA	NA	NA	NA	0.204	0.076	NA	14	-187.685	403.37	2.28	0.019
21	1600	15.182	-0.153	-0.476	-0.087	-0.160	0.025	0.003	NA	NA	0.246	NA	NA	NA	0.087	NA	14	-187.795	403.59	2.50	0.017
22	1888	15.190	-0.145	-0.471	-0.099	-0.146	-0.017	-0.022	-0.226	NA	0.048	NA	NA	0.289	NA	NA	15	-186.838	403.68	2.59	0.016
23	1918	15.194	-0.159	-0.453	-0.102	-0.157	-0.006	-0.034	-0.242	NA	NA	NA	NA	0.299	0.031	NA	15	-186.840	403.68	2.59	0.016
24	3616	15.167	-0.122	-0.488	-0.077	-0.126	-0.011	0.020	NA	NA	0.245	NA	NA	NA	NA	-0.074	14	-187.845	403.69	2.60	0.016
25	1632	15.168	-0.111	-0.493	-0.082	-0.140	0.023	0.008	-0.063	NA	0.219	NA	NA	NA	NA	NA	14	-187.849	403.70	2.61	0.016
26	3678	15.154	-0.103	-0.432	-0.075	-0.135	0.030	-0.016	-0.150	NA	NA	NA	NA	NA	NA	-0.129	14	-188.062	404.12	3.04	0.013
27	3998	15.217	-0.261	-0.492	-0.110	-0.134	-0.048	-0.052	NA	0.225	NA	NA	NA	0.165	NA	-0.085	15	-187.063	404.13	3.04	0.013
28	1982	15.230	-0.288	-0.491	-0.117	-0.168	-0.014	-0.071	NA	0.233	NA	NA	NA	0.158	0.070	NA	15	-187.067	404.13	3.05	0.013
29	3646	15.183	-0.199	-0.383	-0.084	-0.167	0.021	-0.032	NA	NA	NA	NA	NA	NA	0.157	-0.168	14	-188.128	404.26	3.17	0.012
30	1662	15.180	-0.151	-0.424	-0.090	-0.174	0.073	-0.041	-0.123	NA	NA	NA	NA	NA	0.083	NA	14	-188.144	404.29	3.20	0.012
31	10144	15.186	-0.240	-0.567	-0.116	-0.141	-0.005	-0.043	NA	0.333	0.331	NA	-0.356	0.100	NA	NA	16	-186.254	404.51	3.42	0.011
32	3774	15.203	-0.257	-0.460	-0.108	-0.166	0.014	-0.060	NA	0.258	NA	NA	NA	NA	0.131	-0.137	15	-187.284	404.57	3.48	0.010
33	3872	15.191	-0.174	-0.479	-0.088	-0.122	-0.064	0.010	NA	NA	0.192	NA	NA	0.183	NA	-0.085	15	-187.293	404.59	3.50	0.010
34	1856	15.204	-0.197	-0.474	-0.097	-0.152	-0.027	-0.006	NA	NA	0.199	NA	NA	0.173	0.069	NA	15	-187.304	404.61	3.52	0.010
35	11936	15.162	-0.212	-0.568	-0.106	-0.132	0.007	-0.035	NA	0.368	0.364	NA	-0.400	NA	NA	-0.073	16	-186.348	404.70	3.61	0.010
36	9920	15.178	-0.230	-0.568	-0.115	-0.156	0.030	-0.043	NA	0.362	0.359	NA	-0.342	NA	0.049	NA	16	-186.372	404.74	3.66	0.010
37	1984	15.211	-0.232	-0.562	-0.118	-0.152	-0.022	-0.021	NA	0.278	0.265	NA	NA	0.099	0.058	NA	16	-186.421	404.84	3.75	0.009
38	3902	15.207	-0.240	-0.396	-0.096	-0.156	-0.044	-0.032	NA	NA	NA	NA	NA	0.201	0.131	-0.162	15	-187.426	404.85	3.76	0.009
39	4000	15.202	-0.213	-0.568	-0.111	-0.128	-0.050	-0.009	NA	0.275	0.261	NA	NA	0.108	NA	-0.055	16	-186.438	404.88	3.79	0.009
40	3966	15.172	-0.144	-0.421	-0.092	-0.149	-0.042	-0.014	-0.260	NA	NA	NA	NA	0.303	0.096	-0.202	16	-186.442	404.88	3.80	0.009
41	3776	15.192	-0.211	-0.551	-0.110	-0.153	-0.010	-0.012	NA	0.302	0.284	NA	NA	NA	0.099	-0.089	16	-186.492	404.98	3.90	0.009
42	5984	15.274	-0.114	-0.463	-0.109	-0.156	-0.040	-0.004	-0.364	NA	-0.231	0.681	NA	0.311	NA	NA	16	-186.494	404.99	3.90	0.008
43	3936	15.170	-0.124	-0.445	-0.090	-0.128	-0.052	-0.011	-0.277	NA	-0.008	NA	NA	0.320	NA	-0.164	16	-186.558	405.12	4.03	0.008
44	3648	15.171	-0.152	-0.447	-0.081	-0.156	0.002	0.011	NA	NA	0.225	NA	NA	NA	0.133	-0.134	15	-187.624	405.25	4.16	0.007
45	5728	15.230	-0.088	-0.483	-0.089	-0.145	0.010	0.023	-0.158	NA	0.023	0.502	NA	NA	NA	NA	15	-187.672	405.34	4.26	0.007
46	3680	15.155	-0.095	-0.479	-0.075	-0.129	0.005	0.016	-0.083	NA	0.197	NA	NA	NA	NA	-0.095	15	-187.753	405.51	4.42	0.007
47	1664	15.176	-0.136	-0.473	-0.087	-0.162	0.037	0.000	-0.054	NA	0.216	NA	NA	NA	0.081	NA	15	-187.753	405.51	4.42	0.007
48	3710	15.160	-0.140	-0.390	-0.081	-0.168	0.042	-0.023	-0.140	NA	NA	NA	NA	NA	0.146	-0.190	15	-187.795	405.59	4.50	0.006
49	1920	15.192	-0.154	-0.464	-0.100	-0.155	-0.010	-0.024	-0.219	NA	0.052	NA	NA	0.282	0.034	NA	16	-186.822	405.64	4.56	0.006
50	4030	15.217	-0.278	-0.457	-0.113	-0.158	-0.035	-0.056	NA	0.212	NA	NA	NA	0.155	0.116	-0.138	16	-186.893	405.79	4.70	0.006
51	3904	15.193	-0.196	-0.446	-0.091	-0.148	-0.051	0.003	NA	NA	0.178	NA	NA	0.173	0.116	-0.136	16	-187.125	406.25	5.16	0.005
52	12192	15.174	-0.233	-0.552	-0.110	-0.130	-0.023	-0.038	NA	0.329	0.325	NA	-0.396	0.105	NA	-0.083	17	-186.177	406.35	5.27	0.004
53	8032	15.256	-0.095	-0.435	-0.100	-0.137	-0.076	0.006	-0.415	NA	-0.290	0.685	NA	0.342	NA	-0.162	17	-186.205	406.41	5.32	0.004
54	10176	15.189	-0.248	-0.557	-0.118	-0.153	0.002	-0.046	NA	0.328	0.325	NA	-0.337	0.096	0.041	NA	17	-186.229	406.46	5.37	0.004
55	11968	15.165	-0.228	-0.540	-0.108	-0.152	0.013	-0.038	NA	0.353	0.346	NA	-0.377	NA	0.086	-0.110	17	-186.254	406.51	5.42	0.004
56	4032	15.203	-0.230	-0.538	-0.112	-0.149	-0.040	-0.014	NA	0.265	0.247	NA	NA	0.103	0.092	-0.097	17	-186.331	406.66	5.57	0.004
57	3968	15.172	-0.145	-0.418	-0.092	-0.150	-0.041	-0.016	-0.266	NA	-0.012	NA	NA	0.307	0.096	-0.204	17	-186.441	406.88	5.80	0.003
58	6016	15.274	-0.120	-0.459	-0.109	-0.161	-0.036	-0.006	-0.358	NA	-0.225	0.672	NA	0.306	0.020	NA	17	-186.488	406.98	5.89	0.003
59	3712	15.160	-0.128	-0.438	-0.079	-0.158	0.017	0.008	-0.080	NA	0.179	NA	NA	NA	0.132	-0.154	16	-187.536	407.07	5.99	0.003
60	7776	15.217	-0.075	-0.470	-0.083	-0.134	-0.007	0.030	-0.174	NA	0.005	0.492	NA	NA	NA	-0.090	16	-187.583	407.17	6.08	0.003
61	5760	15.235	-0.112	-0.466	-0.093	-0.168	0.024	0.014	-0.147	NA	0.026	0.486	NA	NA	0.076	NA	16	-187.585	407.17	6.08	0.003
62	12224	15.176	-0.247	-0.527	-0.111	-0.148	-0.016	-0.041	NA	0.317	0.309	NA	-0.374	0.101	0.080	-0.117	18	-186.096	408.19	7.10	0.002
63	8064	15.252	-0.114	-0.413	-0.101	-0.156	-0.066	0.000	-0.399	NA	-0.279	0.650	NA	0.329	0.080	-0.195	18	-186.121	408.24	7.16	0.002
64	7808	15.217	-0.106	-0.431	-0.086	-0.164	0.005	0.020	-0.167	NA	0.000	0.460	NA	NA	0.125	-0.145	17	-187.383	408.77	7.68	0.001

Table S10: Model selection and comparison of model permutations for Ca in May.

Model Rank	Model ID	Intercept	Breeding activity : Yes	Day of season	Sex : M	Status : Subordinate	Time bled	Time wait	T_{max}	T_{max7}	$Rain_7$: Yes	T_{max} x $Rain_7$: Yes	T_{max7} x $Rain_7$: Yes	T_{var}	Group size	Territory quality	df	logLik	AIC	ΔAIC	weight
1	9888	0.984	-0.069	0.475	0.026	-0.091	0.037	-0.028	NA	-0.229	0.281	NA	-0.361	NA	NA	NA	15	-264.895	559.79	0.00	0.256
2	10144	0.983	-0.068	0.468	0.023	-0.088	0.037	-0.025	NA	-0.244	0.238	NA	-0.371	-0.066	NA	NA	16	-264.504	561.01	1.22	0.139
3	9920	0.987	-0.089	0.476	0.026	-0.101	0.035	-0.036	NA	-0.228	0.281	NA	-0.359	NA	0.047	NA	16	-264.700	561.40	1.61	0.114
4	11936	0.984	-0.070	0.475	0.026	-0.090	0.037	-0.028	NA	-0.229	0.281	NA	-0.362	NA	NA	-0.007	16	-264.889	561.78	1.99	0.095
5	10176	0.986	-0.088	0.469	0.024	-0.100	0.035	-0.033	NA	-0.243	0.238	NA	-0.370	-0.067	0.049	NA	17	-264.295	562.59	2.80	0.063
6	1696	0.993	-0.075	0.445	0.026	-0.077	0.032	-0.046	NA	-0.263	0.265	NA	NA	NA	NA	NA	14	-267.326	562.65	2.86	0.061
7	12192	0.983	-0.068	0.468	0.023	-0.088	0.037	-0.025	NA	-0.244	0.238	NA	-0.371	-0.066	NA	0.002	17	-264.504	563.01	3.22	0.051
8	11968	0.986	-0.091	0.475	0.026	-0.101	0.036	-0.037	NA	-0.229	0.280	NA	-0.361	NA	0.052	-0.018	17	-264.668	563.34	3.55	0.043
9	1952	0.992	-0.073	0.440	0.025	-0.075	0.033	-0.044	NA	-0.273	0.234	NA	NA	-0.053	NA	NA	15	-267.080	564.16	4.37	0.029
10	1728	0.996	-0.097	0.445	0.027	-0.089	0.029	-0.054	NA	-0.263	0.264	NA	NA	NA	0.053	NA	15	-267.086	564.17	4.38	0.029
11	12224	0.985	-0.089	0.469	0.024	-0.099	0.036	-0.034	NA	-0.243	0.238	NA	-0.371	-0.066	0.051	-0.008	18	-264.288	564.58	4.79	0.023
12	3744	0.993	-0.075	0.445	0.026	-0.077	0.031	-0.046	NA	-0.263	0.265	NA	NA	NA	NA	0.001	15	-267.326	564.65	4.86	0.022
13	1984	0.995	-0.095	0.441	0.026	-0.087	0.030	-0.053	NA	-0.272	0.234	NA	NA	-0.053	0.053	NA	16	-266.839	565.68	5.89	0.013
14	4000	0.992	-0.073	0.441	0.025	-0.076	0.032	-0.044	NA	-0.272	0.234	NA	NA	-0.055	NA	0.010	16	-267.069	566.14	6.35	0.011
15	3776	0.996	-0.098	0.445	0.027	-0.088	0.029	-0.054	NA	-0.264	0.263	NA	NA	NA	0.055	-0.009	16	-267.077	566.15	6.36	0.011
16	1950	0.990	-0.092	0.336	0.020	-0.075	0.036	-0.050	NA	-0.391	NA	NA	NA	-0.119	NA	NA	14	-269.508	567.02	7.23	0.007
17	1694	0.995	-0.102	0.312	0.021	-0.078	0.029	-0.052	NA	-0.412	NA	NA	NA	NA	NA	NA	13	-270.834	567.67	7.88	0.005
18	4032	0.995	-0.095	0.441	0.026	-0.087	0.030	-0.053	NA	-0.272	0.234	NA	NA	-0.053	0.053	-0.001	17	-266.839	567.68	7.89	0.005
19	1982	0.994	-0.114	0.337	0.021	-0.088	0.033	-0.059	NA	-0.390	NA	NA	NA	-0.118	0.055	NA	15	-269.249	568.50	8.71	0.003
20	3998	0.992	-0.092	0.338	0.020	-0.077	0.035	-0.050	NA	-0.390	NA	NA	NA	-0.122	NA	0.018	15	-269.474	568.95	9.16	0.003
21	1726	0.999	-0.125	0.313	0.022	-0.092	0.026	-0.061	NA	-0.410	NA	NA	NA	NA	0.059	NA	14	-270.546	569.09	9.30	0.002
22	1568	0.990	-0.033	0.619	0.032	-0.086	0.018	-0.032	NA	NA	0.427	NA	NA	NA	NA	NA	13	-271.717	569.43	9.65	0.002
23	3742	0.994	-0.102	0.312	0.021	-0.078	0.029	-0.052	NA	-0.412	NA	NA	NA	NA	NA	0.000	14	-270.834	569.67	9.88	0.002
24	4030	0.995	-0.113	0.338	0.021	-0.088	0.032	-0.058	NA	-0.390	NA	NA	NA	-0.120	0.053	0.007	16	-269.244	570.49	10.70	0.001
25	1600	0.994	-0.057	0.619	0.033	-0.101	0.015	-0.042	NA	NA	0.427	NA	NA	NA	0.059	NA	14	-271.415	570.83	11.04	0.001
26	3774	0.998	-0.126	0.312	0.022	-0.092	0.027	-0.062	NA	-0.411	NA	NA	NA	NA	0.061	-0.012	15	-270.532	571.06	11.27	0.001
27	1632	0.990	-0.028	0.632	0.033	-0.087	0.020	-0.031	0.027	NA	0.443	NA	NA	NA	NA	NA	14	-271.641	571.28	11.49	0.001
28	3616	0.992	-0.032	0.619	0.033	-0.088	0.017	-0.033	NA	NA	0.426	NA	NA	NA	NA	0.017	14	-271.685	571.37	11.58	0.001
29	1824	0.990	-0.033	0.619	0.032	-0.086	0.018	-0.032	NA	NA	0.426	NA	NA	-0.002	NA	NA	14	-271.717	571.43	11.64	0.001
30	5728	0.983	-0.022	0.663	0.033	-0.089	0.010	-0.020	0.063	NA	0.444	-0.181	NA	NA	NA	NA	15	-270.783	571.57	11.78	0.001
31	1664	0.994	-0.052	0.631	0.034	-0.101	0.017	-0.041	0.024	NA	0.442	NA	NA	NA	0.058	NA	15	-271.355	572.71	12.92	0.000
32	3648	0.995	-0.056	0.619	0.034	-0.101	0.015	-0.042	NA	NA	0.427	NA	NA	NA	0.058	0.005	15	-271.412	572.82	13.04	0.000
33	1856	0.994	-0.057	0.619	0.033	-0.100	0.015	-0.041	NA	NA	0.424	NA	NA	-0.005	0.060	NA	15	-271.413	572.83	13.04	0.000
34	5984	0.982	-0.021	0.667	0.036	-0.094	0.012	-0.023	0.073	NA	0.476	-0.236	NA	0.059	NA	NA	16	-270.539	573.08	13.29	0.000
35	5760	0.987	-0.044	0.661	0.034	-0.102	0.008	-0.029	0.061	NA	0.443	-0.176	NA	NA	0.053	NA	16	-270.542	573.08	13.29	0.000
36	3680	0.991	-0.027	0.633	0.034	-0.089	0.019	-0.032	0.027	NA	0.443	NA	NA	NA	NA	0.018	15	-271.606	573.21	13.42	0.000
37	1888	0.990	-0.028	0.633	0.033	-0.087	0.019	-0.031	0.027	NA	0.441	NA	NA	-0.003	NA	NA	15	-271.640	573.28	13.49	0.000
38	3872	0.992	-0.032	0.619	0.033	-0.088	0.017	-0.032	NA	NA	0.424	NA	NA	-0.005	NA	0.018	15	-271.683	573.37	13.58	0.000
39	7776	0.985	-0.021	0.665	0.033	-0.092	0.008	-0.021	0.065	NA	0.443	-0.185	NA	NA	NA	0.027	16	-270.707	573.41	13.62	0.000
40	1920	0.994	-0.052	0.632	0.034	-0.101	0.017	-0.040	0.025	NA	0.438	NA	NA	-0.007	0.058	NA	16	-271.350	574.70	14.91	0.000
41	3712	0.994	-0.051	0.631	0.034	-0.102	0.017	-0.041	0.024	NA	0.442	NA	NA	NA	0.056	0.006	16	-271.351	574.70	14.91	0.000
42	6016	0.986	-0.041	0.665	0.036	-0.105	0.010	-0.031	0.069	NA	0.471	-0.225	NA	0.052	0.047	NA	17	-270.351	574.70	14.91	0.000
43	3904	0.995	-0.056	0.619	0.033	-0.101	0.015	-0.041	NA	NA	0.423	NA	NA	-0.006	0.058	0.006	16	-271.409	574.82	15.03	0.000
44	8032	0.984	-0.019	0.669	0.037	-0.096	0.011	-0.024	0.075	NA	0.474	-0.238	NA	0.056	NA	0.022	17	-270.489	574.98	15.19	0.000
45	7808	0.988	-0.041	0.663	0.034	-0.103	0.008	-0.029	0.062	NA	0.443	-0.180	NA	NA	0.048	0.016	17	-270.514	575.03	15.24	0.000
46	3936	0.991	-0.027	0.634	0.033	-0.089	0.018	-0.031	0.028	NA	0.439	NA	NA	-0.007	NA	0.019	16	-271.602	575.20	15.42	0.000
47	8064	0.986	-0.039	0.666	0.037	-0.105	0.010	-0.031	0.070	NA	0.471	-0.227	NA	0.051	0.044	0.013	18	-270.334	576.67	16.88	0.000
48	3968	0.994	-0.051	0.632	0.034	-0.102	0.017	-0.040	0.025	NA	0.437	NA	NA	-0.008	0.056	0.008	17	-271.345	576.69	16.90	0.000
49	1886	0.986	-0.057	0.499	0.025	-0.089	0.007	-0.037	-0.110	NA	NA	NA	NA	-0.119	NA	NA	14	-282.094	592.19	32.40	0.000
50	1630	0.990	-0.071	0.470	0.026	-0.092	-0.002	-0.040	-0.150	NA	NA	NA	NA	NA	NA	NA	13	-283.312	592.62	32.83	0.000
51	1822	0.983	-0.041	0.545	0.026	-0.095	0.018	-0.036	NA	NA	NA	NA	NA	-0.159	NA	NA	13	-283.467	592.93	33.14	0.000
52	1918	0.991	-0.088	0.498	0.025	-0.108	0.003	-0.049	-0.114	NA	NA	NA	NA	-0.118	0.077	NA	15	-281.622	593.24	33.45	0.000
53	1662	0.995	-0.104	0.469	0.026	-0.111	-0.007	-0.053	-0.154	NA	NA	NA	NA	NA	0.082	NA	14	-282.782	593.56	33.78	0.000
54	3934	0.989	-0.055	0.503	0.025	-0.094	0.005	-0.037	-0.107	NA	NA	NA	NA	-0.127	NA	0.041	15	-281.927	593.85	34.07	0.000
55	1854	0.988	-0.069	0.545	0.027	-0.112	0.015	-0.047	NA	NA	NA	NA	NA	-0.161	0.069	NA	14	-283.082	594.16	34.37	0.000
56	3870	0.986	-0.040	0.548	0.027	-0.100	0.015	-0.037	NA	NA	NA	NA	NA	-0.167	NA	0.048	14	-283.232	594.46	34.67	0.000
57	3678	0.991	-0.071	0.471	0.026	-0.094	-0.003	-0.040	-0.150	NA	NA	NA	NA	NA	NA	0.021	14	-283.269	594.54	34.75	0.000
58	3966	0.993	-0.085	0.500	0.026	-0.109	0.002	-0.048	-0.112	NA	NA	NA	NA	-0.123	0.070	0.026	16	-281.555	595.11	35.32	0.000
59	3710	0.996	-0.103	0.469	0.026	-0.111	-0.007	-0.053	-0.154	NA	NA	NA	NA	NA	0.080	0.005	15	-282.780	595.56	35.77	0.000
60	3902	0.990	-0.064	0.548	0.027	-0.113	0.013	-0.046	NA	NA	NA	NA	NA	-0.166	0.060	0.036	15	-282.955	595.91	36.12	0.000
61	1566	0.986	-0.057	0.524	0.030	-0.102	0.017	-0.047	NA	NA	NA	NA	NA	NA	NA	NA	12	-286.175	596.35	36.56	0.000
62	1598	0.991	-0.085	0.524	0.031	-0.119	0.013	-0.057	NA	NA	NA	NA	NA	NA	0.070	NA	13	-285.799	597.60	37.81	0.000
63	3614	0.987	-0.057	0.525	0.030	-0.103	0.016	-0.047	NA	NA	NA	NA	NA	NA	NA	0.019	13	-286.139	598.28	38.49	0.000
64	3646	0.991	-0.084	0.524	0.031	-0.119	0.013	-0.057	NA	NA	NA	NA	NA	NA	0.068	0.006	14	-285.795	599.59	39.80	0.000

Table S11: Model selection and comparison of model permutations for Ca in November.

Model Rank	Model ID	Intercept	Breeding activity : Yes	Day of season	Sex : M	Status : Subordinate	Time bled	Time wait	T_{max}	T_{max7}	$Rain_7$: Yes	T_{max} x $Rain_7$: Yes	T_{max7} x $Rain_7$: Yes	T_{var}	Group size	Territory quality	df	logLik	AIC	ΔAIC	weight
1	1630	1.234	-0.001	0.617	-0.017	0.087	-0.051	0.012	0.175	NA	NA	NA	NA	NA	NA	NA	13	-88.328	202.66	0.00	0.066
2	1566	1.206	0.094	0.593	-0.015	0.080	-0.020	0.005	NA	NA	NA	NA	NA	NA	NA	NA	12	-89.560	203.12	0.47	0.052
3	3678	1.257	-0.036	0.616	-0.015	0.075	-0.020	-0.001	0.191	NA	NA	NA	NA	NA	NA	0.177	14	-87.627	203.25	0.60	0.049
4	1662	1.245	-0.028	0.663	-0.015	0.061	-0.037	0.002	0.192	NA	NA	NA	NA	NA	0.136	NA	14	-87.656	203.31	0.66	0.047
5	1568	1.222	0.057	0.626	-0.011	0.077	-0.003	-0.028	NA	NA	-0.189	NA	NA	NA	NA	NA	13	-88.817	203.63	0.98	0.040
6	1822	1.226	0.059	0.612	-0.025	0.082	-0.058	0.016	NA	NA	NA	NA	NA	0.120	NA	NA	13	-88.909	203.82	1.16	0.037
7	1824	1.248	0.011	0.655	-0.022	0.077	-0.045	-0.020	NA	NA	-0.220	NA	NA	0.141	NA	NA	14	-87.915	203.83	1.18	0.036
8	1632	1.238	-0.004	0.631	-0.014	0.084	-0.036	-0.008	0.143	NA	-0.107	NA	NA	NA	NA	NA	14	-88.127	204.25	1.60	0.029
9	3614	1.223	0.070	0.592	-0.014	0.070	0.007	-0.005	NA	NA	NA	NA	NA	NA	NA	0.140	13	-89.128	204.26	1.60	0.029
10	1598	1.212	0.080	0.628	-0.013	0.059	-0.006	-0.004	NA	NA	NA	NA	NA	NA	0.108	NA	13	-89.139	204.28	1.62	0.029
11	1886	1.240	-0.004	0.623	-0.022	0.087	-0.065	0.016	0.148	NA	NA	NA	NA	0.059	NA	NA	14	-88.196	204.39	1.74	0.027
12	3710	1.260	-0.049	0.649	-0.014	0.058	-0.016	-0.005	0.201	NA	NA	NA	NA	NA	0.102	0.135	15	-87.281	204.56	1.91	0.025
13	1600	1.229	0.042	0.662	-0.009	0.054	0.012	-0.036	NA	NA	-0.191	NA	NA	NA	0.110	NA	14	-88.371	204.74	2.09	0.023
14	1694	1.210	0.075	0.588	-0.021	0.082	-0.023	0.002	NA	0.069	NA	NA	NA	NA	NA	NA	13	-89.385	204.77	2.12	0.023
15	3616	1.236	0.037	0.623	-0.010	0.067	0.021	-0.035	NA	NA	-0.179	NA	NA	NA	NA	0.128	14	-88.444	204.89	2.23	0.021
16	1664	1.248	-0.031	0.675	-0.012	0.058	-0.023	-0.016	0.162	NA	-0.098	NA	NA	NA	0.133	NA	15	-87.483	204.97	2.31	0.021
17	3870	1.241	0.038	0.609	-0.024	0.072	-0.032	0.006	NA	NA	NA	NA	NA	0.116	NA	0.135	14	-88.505	205.01	2.35	0.020
18	3680	1.259	-0.037	0.627	-0.013	0.073	-0.010	-0.015	0.166	NA	-0.081	NA	NA	NA	NA	0.167	15	-87.510	205.02	2.36	0.020
19	3934	1.261	-0.037	0.620	-0.019	0.075	-0.032	0.003	0.170	NA	NA	NA	NA	0.045	NA	0.171	15	-87.550	205.10	2.45	0.019
20	1856	1.253	0.000	0.684	-0.020	0.058	-0.030	-0.028	NA	NA	-0.220	NA	NA	0.132	0.097	NA	15	-87.571	205.14	2.49	0.019
21	3872	1.261	-0.006	0.650	-0.021	0.069	-0.021	-0.028	NA	NA	-0.210	NA	NA	0.137	NA	0.122	15	-87.575	205.15	2.50	0.019
22	1854	1.230	0.049	0.642	-0.023	0.062	-0.044	0.008	NA	NA	NA	NA	NA	0.111	0.096	NA	14	-88.579	205.16	2.50	0.019
23	1918	1.248	-0.028	0.664	-0.018	0.062	-0.046	0.005	0.174	NA	NA	NA	NA	0.037	0.129	NA	15	-87.606	205.21	2.56	0.018
24	1696	1.226	0.041	0.621	-0.016	0.078	-0.006	-0.030	NA	0.062	-0.184	NA	NA	NA	NA	NA	14	-88.676	205.35	2.70	0.017
25	1888	1.250	-0.010	0.650	-0.021	0.081	-0.051	-0.011	0.076	NA	-0.168	NA	NA	0.104	NA	NA	15	-87.778	205.56	2.90	0.015
26	1950	1.226	0.056	0.610	-0.026	0.082	-0.057	0.014	NA	0.018	NA	NA	NA	0.113	NA	NA	14	-88.899	205.80	3.14	0.014
27	1952	1.249	0.011	0.655	-0.022	0.077	-0.045	-0.019	NA	-0.004	-0.220	NA	NA	0.142	NA	NA	15	-87.915	205.83	3.17	0.013
28	3646	1.223	0.065	0.618	-0.013	0.056	0.011	-0.008	NA	NA	NA	NA	NA	NA	0.080	0.105	14	-88.917	205.83	3.18	0.013
29	3742	1.228	0.050	0.586	-0.019	0.071	0.004	-0.009	NA	0.073	NA	NA	NA	NA	NA	0.144	14	-88.929	205.86	3.20	0.013
30	1726	1.216	0.063	0.623	-0.018	0.061	-0.010	-0.006	NA	0.064	NA	NA	NA	NA	0.105	NA	14	-88.989	205.98	3.32	0.012
31	5728	1.269	0.007	0.631	-0.012	0.084	-0.047	0.000	0.090	NA	-0.228	0.303	NA	NA	NA	NA	15	-87.990	205.98	3.32	0.012
32	3712	1.262	-0.050	0.660	-0.012	0.056	-0.006	-0.019	0.176	NA	-0.080	NA	NA	NA	0.101	0.125	16	-87.164	206.33	3.67	0.010
33	3648	1.237	0.030	0.651	-0.009	0.052	0.026	-0.040	NA	NA	-0.183	NA	NA	NA	0.086	0.091	15	-88.201	206.40	3.75	0.010
34	3966	1.262	-0.048	0.651	-0.017	0.059	-0.025	-0.002	0.185	NA	NA	NA	NA	0.031	0.097	0.133	16	-87.243	206.49	3.83	0.010
35	1728	1.232	0.028	0.656	-0.014	0.056	0.009	-0.038	NA	0.056	-0.186	NA	NA	NA	0.107	NA	15	-88.254	206.51	3.85	0.010
36	3744	1.240	0.020	0.617	-0.015	0.068	0.019	-0.038	NA	0.065	-0.174	NA	NA	NA	NA	0.132	15	-88.285	206.57	3.91	0.009
37	3936	1.266	-0.039	0.642	-0.019	0.072	-0.025	-0.018	0.111	NA	-0.132	NA	NA	0.083	NA	0.150	16	-87.292	206.58	3.93	0.009
38	1920	1.256	-0.032	0.684	-0.018	0.059	-0.036	-0.017	0.110	NA	-0.145	NA	NA	0.078	0.118	NA	16	-87.294	206.59	3.93	0.009
39	3902	1.241	0.035	0.631	-0.022	0.060	-0.027	0.002	NA	NA	NA	NA	NA	0.111	0.068	0.106	15	-88.352	206.70	4.05	0.009
40	5760	1.275	-0.020	0.673	-0.011	0.060	-0.033	-0.009	0.117	NA	-0.202	0.260	NA	NA	0.129	NA	16	-87.381	206.76	4.11	0.008
41	7776	1.288	-0.025	0.627	-0.011	0.074	-0.021	-0.008	0.116	NA	-0.195	0.287	NA	NA	NA	0.165	16	-87.382	206.76	4.11	0.008
42	3904	1.261	-0.011	0.673	-0.020	0.056	-0.016	-0.032	NA	NA	-0.213	NA	NA	0.132	0.073	0.091	16	-87.400	206.80	4.14	0.008
43	3998	1.242	0.034	0.606	-0.025	0.072	-0.030	0.004	NA	0.024	NA	NA	NA	0.107	NA	0.137	15	-88.486	206.97	4.32	0.008
44	5984	1.300	0.005	0.653	-0.020	0.081	-0.073	0.000	-0.018	NA	-0.362	0.450	NA	0.128	NA	NA	16	-87.496	206.99	4.34	0.007
45	1984	1.253	0.001	0.685	-0.019	0.058	-0.030	-0.028	NA	-0.006	-0.221	NA	NA	0.135	0.097	NA	16	-87.570	207.14	4.48	0.007
46	1982	1.230	0.047	0.639	-0.024	0.063	-0.042	0.006	NA	0.017	NA	NA	NA	0.105	0.096	NA	15	-88.570	207.14	4.49	0.007
47	4000	1.261	-0.007	0.650	-0.021	0.069	-0.021	-0.028	NA	0.001	-0.210	NA	NA	0.137	NA	0.122	16	-87.575	207.15	4.50	0.007
48	9888	1.230	0.044	0.621	-0.015	0.079	-0.010	-0.025	NA	0.049	-0.200	NA	0.077	NA	NA	NA	15	-88.660	207.32	4.66	0.006
49	3774	1.228	0.046	0.611	-0.018	0.058	0.008	-0.012	NA	0.069	NA	NA	NA	NA	0.076	0.111	15	-88.741	207.48	4.83	0.006
50	10144	1.258	0.016	0.657	-0.020	0.079	-0.054	-0.011	NA	-0.029	-0.249	NA	0.139	0.147	NA	NA	16	-87.861	207.72	5.07	0.005
51	3968	1.267	-0.050	0.669	-0.017	0.057	-0.019	-0.021	0.130	NA	-0.122	NA	NA	0.067	0.091	0.116	17	-87.023	208.05	5.39	0.004
52	8032	1.311	-0.024	0.646	-0.018	0.073	-0.046	-0.007	0.024	NA	-0.310	0.410	NA	0.106	NA	0.144	17	-87.052	208.10	5.45	0.004
53	7808	1.288	-0.039	0.659	-0.010	0.057	-0.017	-0.012	0.130	NA	-0.183	0.257	NA	NA	0.098	0.125	17	-87.062	208.12	5.47	0.004
54	3776	1.241	0.015	0.644	-0.014	0.054	0.023	-0.042	NA	0.060	-0.178	NA	NA	NA	0.082	0.097	16	-88.064	208.13	5.47	0.004
55	6016	1.298	-0.017	0.684	-0.017	0.061	-0.055	-0.007	0.028	NA	-0.311	0.381	NA	0.100	0.108	NA	17	-87.091	208.18	5.53	0.004
56	9920	1.241	0.033	0.659	-0.011	0.057	0.002	-0.030	NA	0.033	-0.214	NA	0.140	NA	0.113	NA	16	-88.200	208.40	5.75	0.004
57	11936	1.253	0.026	0.618	-0.013	0.070	0.012	-0.028	NA	0.037	-0.207	NA	0.171	NA	NA	0.145	16	-88.206	208.41	5.76	0.004
58	4030	1.241	0.031	0.627	-0.024	0.060	-0.025	0.000	NA	0.023	NA	NA	NA	0.102	0.068	0.108	16	-88.337	208.67	6.02	0.003
59	4032	1.261	-0.010	0.673	-0.019	0.056	-0.016	-0.032	NA	-0.001	-0.213	NA	NA	0.132	0.073	0.091	17	-87.400	208.80	6.14	0.003
60	12192	1.277	0.000	0.651	-0.018	0.071	-0.032	-0.015	NA	-0.037	-0.254	NA	0.219	0.142	NA	0.137	17	-87.444	208.89	6.23	0.003
61	10176	1.266	0.007	0.689	-0.017	0.059	-0.041	-0.016	NA	-0.040	-0.261	NA	0.191	0.140	0.104	NA	17	-87.469	208.94	6.28	0.003
62	8064	1.307	-0.035	0.670	-0.016	0.059	-0.038	-0.011	0.050	NA	-0.282	0.366	NA	0.089	0.082	0.113	18	-86.833	209.67	7.01	0.002
63	11968	1.255	0.020	0.647	-0.011	0.055	0.015	-0.031	NA	0.029	-0.216	NA	0.194	NA	0.086	0.109	17	-87.963	209.93	7.27	0.002
64	12224	1.279	-0.004	0.676	-0.016	0.057	-0.027	-0.018	NA	-0.043	-0.261	NA	0.239	0.138	0.077	0.105	18	-87.244	210.49	7.83	0.001

Table S12: Model selection and comparison of model permutations for HL ratio in May.

Model Rank	Model ID	Intercept	Breeding activity : Yes	Day of season	Sex : M	Status : Subordinate	Time bled	Time wait	T_{max}	T_{max7}	$Rain_7$: Yes	T_{max} x $Rain_7$: Yes	T_{max7} x $Rain_7$: Yes	T_{var}	Group size	Territory quality	df	logLik	AIC	ΔAIC	weight
1	9888	0.422	0.032	-0.017	0.025	-0.057	0.089	-0.053	NA	-0.006	0.083	NA	0.184	NA	NA	NA	15	142.494	-254.99	0.00	0.167
2	9920	0.421	0.041	-0.018	0.026	-0.049	0.089	-0.048	NA	-0.008	0.081	NA	0.183	NA	-0.022	NA	16	142.928	-253.86	1.13	0.095
3	5728	0.424	0.031	-0.014	0.023	-0.058	0.091	-0.049	-0.004	NA	0.085	0.127	NA	NA	NA	NA	15	141.863	-253.73	1.26	0.089
4	7776	0.425	0.030	-0.012	0.023	-0.052	0.091	-0.049	-0.003	NA	0.087	0.125	NA	NA	NA	-0.028	16	142.750	-253.50	1.49	0.079
5	11936	0.422	0.031	-0.017	0.025	-0.053	0.089	-0.053	NA	-0.008	0.081	NA	0.175	NA	NA	-0.013	16	142.697	-253.39	1.59	0.075
6	10144	0.422	0.030	-0.015	0.026	-0.056	0.088	-0.054	NA	-0.003	0.090	NA	0.180	0.012	NA	NA	16	142.640	-253.28	1.71	0.071
7	5760	0.424	0.039	-0.013	0.024	-0.052	0.090	-0.044	-0.004	NA	0.085	0.126	NA	NA	-0.019	NA	16	142.200	-252.40	2.59	0.046
8	5984	0.424	0.033	-0.016	0.023	-0.058	0.090	-0.047	-0.006	NA	0.078	0.147	NA	-0.017	NA	NA	16	142.106	-252.21	2.78	0.042
9	10176	0.422	0.039	-0.015	0.026	-0.048	0.088	-0.048	NA	-0.004	0.090	NA	0.179	0.013	-0.022	NA	17	143.101	-252.20	2.79	0.042
10	11968	0.422	0.039	-0.018	0.026	-0.048	0.089	-0.048	NA	-0.009	0.081	NA	0.178	NA	-0.019	-0.009	17	143.016	-252.03	2.96	0.038
11	12192	0.423	0.029	-0.014	0.025	-0.052	0.089	-0.053	NA	-0.003	0.091	NA	0.169	0.016	NA	-0.016	17	142.942	-251.88	3.10	0.035
12	7808	0.424	0.034	-0.011	0.023	-0.048	0.091	-0.046	-0.003	NA	0.087	0.124	NA	NA	-0.013	-0.025	17	142.890	-251.78	3.21	0.034
13	8032	0.425	0.030	-0.013	0.022	-0.052	0.091	-0.048	-0.005	NA	0.083	0.136	NA	-0.011	NA	-0.026	17	142.848	-251.70	3.29	0.032
14	6016	0.424	0.040	-0.015	0.023	-0.052	0.089	-0.043	-0.006	NA	0.079	0.143	NA	-0.014	-0.018	NA	17	142.378	-250.76	4.23	0.020
15	12224	0.423	0.037	-0.014	0.026	-0.047	0.088	-0.049	NA	-0.004	0.091	NA	0.171	0.016	-0.019	-0.012	18	143.259	-250.52	4.47	0.018
16	8064	0.424	0.035	-0.012	0.023	-0.048	0.091	-0.045	-0.004	NA	0.083	0.134	NA	-0.010	-0.012	-0.024	18	142.973	-249.95	5.04	0.013
17	3616	0.421	0.023	-0.003	0.024	-0.051	0.091	-0.050	NA	NA	0.080	NA	NA	NA	NA	-0.030	14	138.742	-249.48	5.50	0.011
18	1568	0.421	0.024	-0.005	0.026	-0.057	0.089	-0.050	NA	NA	0.078	NA	NA	NA	NA	NA	13	137.700	-249.40	5.59	0.010
19	3680	0.420	0.024	0.010	0.024	-0.050	0.092	-0.049	0.025	NA	0.096	NA	NA	NA	NA	-0.031	15	139.323	-248.65	6.34	0.007
20	1600	0.420	0.033	-0.004	0.026	-0.049	0.089	-0.044	NA	NA	0.079	NA	NA	NA	-0.024	NA	14	138.206	-248.41	6.58	0.006
21	1632	0.420	0.025	0.007	0.025	-0.057	0.090	-0.049	0.022	NA	0.093	NA	NA	NA	NA	NA	14	138.184	-248.37	6.62	0.006
22	3872	0.421	0.023	-0.003	0.024	-0.051	0.090	-0.050	NA	NA	0.087	NA	NA	0.021	NA	-0.032	15	139.132	-248.26	6.72	0.006
23	3648	0.421	0.030	-0.002	0.025	-0.045	0.091	-0.046	NA	NA	0.080	NA	NA	NA	-0.017	-0.027	15	138.993	-247.99	7.00	0.005
24	1824	0.421	0.024	-0.006	0.026	-0.058	0.089	-0.050	NA	NA	0.084	NA	NA	0.017	NA	NA	14	137.974	-247.95	7.04	0.005
25	3744	0.421	0.023	-0.007	0.024	-0.050	0.091	-0.050	NA	-0.006	0.076	NA	NA	NA	NA	-0.030	15	138.758	-247.52	7.47	0.004
26	1696	0.421	0.024	-0.010	0.026	-0.057	0.090	-0.050	NA	-0.008	0.073	NA	NA	NA	NA	NA	14	137.731	-247.46	7.53	0.004
27	1664	0.420	0.034	0.008	0.026	-0.049	0.090	-0.043	0.022	NA	0.093	NA	NA	NA	-0.024	NA	15	138.691	-247.38	7.61	0.004
28	3712	0.420	0.030	0.011	0.025	-0.045	0.092	-0.044	0.024	NA	0.096	NA	NA	NA	-0.017	-0.028	16	139.567	-247.13	7.85	0.003
29	3936	0.421	0.024	0.008	0.024	-0.051	0.091	-0.048	0.021	NA	0.099	NA	NA	0.017	NA	-0.033	16	139.559	-247.12	7.87	0.003
30	1856	0.421	0.033	-0.005	0.026	-0.050	0.088	-0.044	NA	NA	0.085	NA	NA	0.018	-0.025	NA	15	138.501	-247.00	7.99	0.003
31	3904	0.421	0.030	-0.003	0.025	-0.046	0.090	-0.046	NA	NA	0.087	NA	NA	0.021	-0.017	-0.029	16	139.382	-246.76	8.22	0.003
32	1888	0.420	0.025	0.005	0.025	-0.058	0.090	-0.049	0.020	NA	0.095	NA	NA	0.014	NA	NA	15	138.349	-246.70	8.29	0.003
33	1728	0.420	0.033	-0.010	0.026	-0.049	0.090	-0.044	NA	-0.009	0.073	NA	NA	NA	-0.025	NA	15	138.250	-246.50	8.49	0.002
34	4000	0.421	0.023	-0.003	0.024	-0.051	0.090	-0.050	NA	0.000	0.087	NA	NA	0.021	NA	-0.032	16	139.132	-246.26	8.72	0.002
35	3776	0.421	0.029	-0.007	0.025	-0.045	0.091	-0.046	NA	-0.007	0.076	NA	NA	NA	-0.018	-0.027	16	139.017	-246.03	8.95	0.002
36	1952	0.421	0.024	-0.007	0.026	-0.058	0.089	-0.050	NA	-0.003	0.082	NA	NA	0.017	NA	NA	15	137.977	-245.95	9.03	0.002
37	1920	0.420	0.034	0.006	0.026	-0.050	0.089	-0.042	0.020	NA	0.096	NA	NA	0.015	-0.025	NA	16	138.869	-245.74	9.25	0.002
38	3968	0.420	0.030	0.009	0.025	-0.046	0.091	-0.044	0.021	NA	0.099	NA	NA	0.017	-0.017	-0.030	17	139.801	-245.60	9.39	0.002
39	1984	0.421	0.033	-0.007	0.026	-0.049	0.089	-0.044	NA	-0.004	0.082	NA	NA	0.018	-0.025	NA	16	138.509	-245.02	9.97	0.001
40	4032	0.421	0.030	-0.004	0.025	-0.046	0.090	-0.046	NA	-0.001	0.086	NA	NA	0.021	-0.018	-0.029	17	139.383	-244.77	10.22	0.001
41	1694	0.422	0.021	-0.048	0.025	-0.058	0.095	-0.056	NA	-0.047	NA	NA	NA	NA	NA	NA	13	135.349	-244.70	10.29	0.001
42	3742	0.422	0.021	-0.046	0.024	-0.052	0.096	-0.055	NA	-0.047	NA	NA	NA	NA	NA	-0.028	14	136.227	-244.45	10.53	0.001
43	1726	0.421	0.031	-0.048	0.026	-0.050	0.095	-0.049	NA	-0.048	NA	NA	NA	NA	-0.025	NA	14	135.898	-243.80	11.19	0.001
44	1566	0.421	0.026	-0.022	0.023	-0.063	0.090	-0.051	NA	NA	NA	NA	NA	NA	NA	NA	12	133.646	-243.29	11.70	0.000
45	3774	0.422	0.028	-0.046	0.025	-0.047	0.096	-0.050	NA	-0.048	NA	NA	NA	NA	-0.019	-0.024	15	136.522	-243.04	11.94	0.000
46	3614	0.421	0.025	-0.020	0.022	-0.056	0.091	-0.051	NA	NA	NA	NA	NA	NA	NA	-0.028	13	134.479	-242.96	12.03	0.000
47	1950	0.422	0.021	-0.047	0.025	-0.058	0.095	-0.055	NA	-0.047	NA	NA	NA	-0.003	NA	NA	14	135.360	-242.72	12.27	0.000
48	3998	0.422	0.021	-0.046	0.024	-0.052	0.096	-0.055	NA	-0.047	NA	NA	NA	0.000	NA	-0.028	15	136.227	-242.45	12.53	0.000
49	1598	0.421	0.035	-0.021	0.024	-0.055	0.090	-0.045	NA	NA	NA	NA	NA	NA	-0.023	NA	13	134.086	-242.17	12.82	0.000
50	1982	0.421	0.031	-0.047	0.026	-0.050	0.095	-0.049	NA	-0.048	NA	NA	NA	-0.003	-0.025	NA	15	135.905	-241.81	13.18	0.000
51	1630	0.421	0.025	-0.025	0.024	-0.062	0.090	-0.052	-0.008	NA	NA	NA	NA	NA	NA	NA	13	133.730	-241.46	13.53	0.000
52	3646	0.421	0.031	-0.020	0.023	-0.052	0.091	-0.047	NA	NA	NA	NA	NA	NA	-0.017	-0.024	14	134.697	-241.39	13.59	0.000
53	1822	0.421	0.026	-0.022	0.023	-0.063	0.090	-0.050	NA	NA	NA	NA	NA	-0.002	NA	NA	13	133.650	-241.30	13.69	0.000
54	3678	0.422	0.024	-0.024	0.023	-0.056	0.091	-0.052	-0.008	NA	NA	NA	NA	NA	NA	-0.027	14	134.559	-241.12	13.87	0.000
55	4030	0.422	0.028	-0.046	0.025	-0.047	0.096	-0.050	NA	-0.048	NA	NA	NA	0.000	-0.019	-0.024	16	136.522	-241.04	13.94	0.000
56	3870	0.421	0.025	-0.020	0.022	-0.056	0.091	-0.051	NA	NA	NA	NA	NA	0.001	NA	-0.028	14	134.480	-240.96	14.03	0.000
57	1662	0.421	0.034	-0.025	0.024	-0.054	0.089	-0.046	-0.009	NA	NA	NA	NA	NA	-0.023	NA	14	134.179	-240.36	14.63	0.000
58	1854	0.421	0.035	-0.021	0.024	-0.055	0.090	-0.045	NA	NA	NA	NA	NA	-0.001	-0.023	NA	14	134.086	-240.17	14.82	0.000
59	3710	0.421	0.030	-0.023	0.023	-0.051	0.091	-0.047	-0.008	NA	NA	NA	NA	NA	-0.017	-0.024	15	134.784	-239.57	15.42	0.000
60	1886	0.421	0.025	-0.025	0.024	-0.062	0.090	-0.052	-0.008	NA	NA	NA	NA	0.000	NA	NA	14	133.730	-239.46	15.53	0.000
61	3902	0.421	0.031	-0.020	0.023	-0.052	0.091	-0.047	NA	NA	NA	NA	NA	0.002	-0.017	-0.025	15	134.699	-239.40	15.59	0.000
62	3934	0.422	0.024	-0.024	0.023	-0.056	0.091	-0.052	-0.009	NA	NA	NA	NA	0.003	NA	-0.028	15	134.570	-239.14	15.85	0.000
63	1918	0.421	0.034	-0.025	0.024	-0.054	0.089	-0.046	-0.009	NA	NA	NA	NA	0.001	-0.023	NA	15	134.180	-238.36	16.63	0.000
64	3966	0.421	0.030	-0.024	0.023	-0.051	0.091	-0.048	-0.009	NA	NA	NA	NA	0.004	-0.017	-0.025	16	134.797	-237.59	17.39	0.000

Table S13: Model selection and comparison of model permutations for HL ratio in November.

Model Rank	Model ID	Intercept	Breeding activity : Yes	Day of season	Sex : M	Status : Subordinate	Time bled	Time wait	T_{max}	T_{max7}	$Rain_j$: Yes	T_{max} x $Rain_j$: Yes	T_{max7} x $Rain_j$: Yes	T_{var}	Group size	Territory quality	df	logLik	AIC	ΔAIC	weight
1	1696	0.432	0.046	-0.010	0.032	-0.005	0.130	-0.054	NA	-0.049	0.104	NA	NA	NA	NA	NA	14	70.562	-113.12	0.00	0.099
2	1824	0.432	0.042	-0.018	0.033	-0.002	0.140	-0.048	NA	NA	0.126	NA	NA	-0.047	NA	NA	14	70.264	-112.53	0.60	0.073
3	3744	0.432	0.050	-0.013	0.031	-0.008	0.138	-0.060	NA	-0.052	0.115	NA	NA	NA	NA	0.031	15	71.107	-112.21	0.91	0.063
4	1952	0.432	0.049	-0.014	0.033	-0.006	0.136	-0.049	NA	-0.035	0.114	NA	NA	-0.030	NA	NA	15	71.075	-112.15	0.98	0.061
5	9888	0.428	0.045	-0.010	0.032	-0.007	0.132	-0.059	NA	-0.038	0.118	NA	-0.069	NA	NA	NA	15	70.787	-111.57	1.55	0.045
6	1568	0.435	0.035	-0.013	0.032	-0.002	0.127	-0.050	NA	NA	0.111	NA	NA	NA	NA	NA	13	68.733	-111.47	1.66	0.043
7	3872	0.435	0.049	-0.023	0.032	-0.008	0.145	-0.047	NA	NA	0.134	NA	NA	-0.044	NA	0.031	15	70.656	-111.31	1.81	0.040
8	1728	0.432	0.044	-0.008	0.032	-0.008	0.130	-0.055	NA	-0.048	0.104	NA	NA	NA	0.007	NA	15	70.592	-111.18	1.94	0.037
9	4000	0.432	0.053	-0.017	0.032	-0.008	0.144	-0.055	NA	-0.039	0.123	NA	NA	-0.029	NA	0.030	16	71.586	-111.17	1.95	0.037
10	1888	0.433	0.041	-0.020	0.035	-0.001	0.139	-0.045	0.020	NA	0.140	NA	NA	-0.060	NA	NA	15	70.431	-110.86	2.26	0.032
11	1856	0.433	0.038	-0.015	0.033	-0.008	0.141	-0.048	NA	NA	0.125	NA	NA	-0.047	0.014	NA	15	70.385	-110.77	2.35	0.030
12	3616	0.438	0.043	-0.019	0.031	-0.010	0.135	-0.048	NA	NA	0.121	NA	NA	NA	NA	0.037	14	69.351	-110.70	2.42	0.029
13	11936	0.428	0.049	-0.013	0.031	-0.009	0.140	-0.064	NA	-0.042	0.129	NA	-0.068	NA	NA	0.031	16	71.325	-110.65	2.47	0.029
14	10144	0.429	0.047	-0.015	0.033	-0.007	0.138	-0.054	NA	-0.026	0.127	NA	-0.066	-0.029	NA	NA	16	71.281	-110.56	2.56	0.027
15	1984	0.432	0.046	-0.012	0.033	-0.010	0.137	-0.050	NA	-0.034	0.113	NA	NA	-0.030	0.009	NA	16	71.126	-110.25	2.87	0.023
16	3776	0.432	0.050	-0.013	0.031	-0.008	0.138	-0.060	NA	-0.052	0.115	NA	NA	NA	0.000	0.031	16	71.107	-110.21	2.91	0.023
17	3936	0.436	0.051	-0.027	0.034	-0.008	0.145	-0.042	0.030	NA	0.156	NA	NA	-0.062	NA	0.039	16	71.015	-110.03	3.10	0.021
18	1632	0.434	0.037	-0.012	0.031	-0.003	0.131	-0.053	-0.017	NA	0.104	NA	NA	NA	NA	NA	14	68.911	-109.82	3.30	0.019
19	9920	0.428	0.043	-0.009	0.033	-0.009	0.132	-0.059	NA	-0.038	0.118	NA	-0.067	NA	0.005	NA	16	70.804	-109.61	3.52	0.017
20	12192	0.429	0.051	-0.018	0.032	-0.010	0.146	-0.059	NA	-0.030	0.137	NA	-0.065	-0.028	NA	0.030	17	71.787	-109.57	3.55	0.017
21	1600	0.434	0.031	-0.010	0.032	-0.005	0.129	-0.053	NA	NA	0.112	NA	NA	NA	0.009	NA	14	68.770	-109.54	3.58	0.016
22	3904	0.434	0.047	-0.022	0.032	-0.009	0.145	-0.048	NA	NA	0.134	NA	NA	-0.045	0.003	0.029	16	70.658	-109.32	3.81	0.015
23	1920	0.433	0.036	-0.016	0.035	-0.007	0.141	-0.046	0.023	NA	0.141	NA	NA	-0.062	0.017	NA	16	70.613	-109.23	3.90	0.014
24	5984	0.425	0.038	-0.021	0.033	-0.001	0.142	-0.048	0.033	NA	0.171	-0.082	NA	-0.060	NA	NA	16	70.598	-109.20	3.93	0.014
25	4032	0.432	0.052	-0.016	0.032	-0.009	0.144	-0.055	NA	-0.039	0.123	NA	NA	-0.029	0.003	0.030	17	71.590	-109.18	3.94	0.014
26	1694	0.432	0.033	0.017	0.034	-0.004	0.137	-0.087	NA	-0.058	NA	NA	NA	NA	NA	NA	13	67.499	-109.00	4.13	0.013
27	3648	0.439	0.049	-0.024	0.031	-0.007	0.134	-0.045	NA	NA	0.123	NA	NA	NA	-0.013	0.044	15	69.422	-108.84	4.28	0.012
28	3680	0.437	0.044	-0.018	0.030	-0.010	0.136	-0.050	-0.009	NA	0.117	NA	NA	NA	NA	0.035	15	69.401	-108.80	4.32	0.011
29	11968	0.428	0.050	-0.014	0.031	-0.008	0.140	-0.064	NA	-0.042	0.129	NA	-0.068	NA	-0.001	0.031	17	71.327	-108.65	4.47	0.011
30	10176	0.429	0.045	-0.013	0.033	-0.010	0.139	-0.054	NA	-0.025	0.126	NA	-0.063	-0.030	0.007	NA	17	71.315	-108.63	4.49	0.010
31	8032	0.429	0.046	-0.027	0.033	-0.007	0.147	-0.045	0.041	NA	0.183	-0.072	NA	-0.063	NA	0.038	17	71.137	-108.27	4.85	0.009
32	5728	0.426	0.032	-0.012	0.029	-0.002	0.134	-0.058	-0.007	NA	0.131	-0.070	NA	NA	NA	NA	15	69.020	-108.04	5.09	0.008
33	3968	0.436	0.051	-0.027	0.034	-0.008	0.145	-0.042	0.030	NA	0.156	NA	NA	-0.062	0.000	0.039	17	71.015	-108.03	5.10	0.008
34	1664	0.432	0.032	-0.008	0.031	-0.005	0.133	-0.058	-0.017	NA	0.105	NA	NA	NA	0.011	NA	15	68.961	-107.92	5.20	0.007
35	6016	0.424	0.032	-0.017	0.033	-0.009	0.144	-0.049	0.040	NA	0.178	-0.097	NA	-0.063	0.020	NA	17	70.840	-107.68	5.44	0.006
36	12224	0.429	0.051	-0.017	0.032	-0.010	0.146	-0.059	NA	-0.030	0.137	NA	-0.065	-0.028	0.001	0.030	18	71.788	-107.58	5.55	0.006
37	1950	0.432	0.033	0.016	0.035	-0.004	0.140	-0.086	NA	-0.053	NA	NA	NA	-0.012	NA	NA	14	67.578	-107.16	5.97	0.005
38	3742	0.432	0.034	0.017	0.034	-0.004	0.140	-0.090	NA	-0.059	NA	NA	NA	NA	NA	0.011	14	67.565	-107.13	6.00	0.005
39	1726	0.432	0.030	0.019	0.034	-0.007	0.138	-0.088	NA	-0.057	NA	NA	NA	NA	0.009	NA	14	67.551	-107.10	6.02	0.005
40	7776	0.432	0.041	-0.018	0.029	-0.009	0.138	-0.052	-0.001	NA	0.137	-0.055	NA	NA	NA	0.034	16	69.472	-106.94	6.18	0.004
41	3712	0.439	0.049	-0.023	0.030	-0.007	0.135	-0.046	-0.008	NA	0.119	NA	NA	NA	-0.012	0.041	16	69.460	-106.92	6.21	0.004
42	1566	0.439	0.020	0.013	0.035	-0.004	0.135	-0.077	NA	NA	NA	NA	NA	NA	NA	NA	12	65.364	-106.73	6.40	0.004
43	8064	0.424	0.035	-0.020	0.032	-0.008	0.150	-0.052	0.044	NA	0.192	-0.100	NA	-0.066	0.016	0.026	18	71.191	-106.38	6.74	0.003
44	1630	0.427	0.021	0.011	0.032	-0.006	0.141	-0.075	-0.034	NA	NA	NA	NA	NA	NA	NA	13	66.164	-106.33	6.80	0.003
45	5760	0.425	0.028	-0.009	0.029	-0.006	0.136	-0.061	-0.004	NA	0.136	-0.082	NA	NA	0.013	NA	16	69.122	-106.24	6.88	0.003
46	1822	0.436	0.023	0.013	0.035	-0.003	0.143	-0.080	NA	NA	NA	NA	NA	-0.030	NA	NA	13	65.932	-105.86	7.26	0.003
47	1982	0.432	0.030	0.018	0.035	-0.008	0.141	-0.087	NA	-0.052	NA	NA	NA	-0.013	0.011	NA	15	67.643	-105.29	7.84	0.002
48	3998	0.432	0.034	0.016	0.034	-0.005	0.143	-0.089	NA	-0.055	NA	NA	NA	-0.011	NA	0.010	15	67.633	-105.27	7.86	0.002
49	3774	0.432	0.031	0.018	0.034	-0.007	0.141	-0.090	NA	-0.058	NA	NA	NA	NA	0.007	0.009	15	67.595	-105.19	7.93	0.002
50	7808	0.434	0.045	-0.022	0.029	-0.008	0.137	-0.049	-0.002	NA	0.134	-0.043	NA	NA	-0.009	0.039	17	69.498	-105.00	8.13	0.002
51	3614	0.440	0.022	0.011	0.034	-0.007	0.139	-0.077	NA	NA	NA	NA	NA	NA	NA	0.015	13	65.473	-104.95	8.18	0.002
52	1598	0.424	0.010	0.015	0.035	-0.006	0.135	-0.072	NA	NA	NA	NA	NA	NA	0.006	NA	13	65.431	-104.86	8.26	0.002
53	1886	0.428	0.022	0.012	0.033	-0.005	0.144	-0.077	-0.026	NA	NA	NA	NA	-0.017	NA	NA	14	66.303	-104.61	8.52	0.001
54	3678	0.437	0.028	0.010	0.031	-0.008	0.143	-0.079	-0.034	NA	NA	NA	NA	NA	NA	0.009	14	66.210	-104.42	8.70	0.001
55	1662	0.429	0.020	0.014	0.032	-0.007	0.142	-0.079	-0.035	NA	NA	NA	NA	NA	0.007	NA	14	66.185	-104.37	8.75	0.001
56	1854	0.434	0.018	0.017	0.035	-0.007	0.146	-0.086	NA	NA	NA	NA	NA	-0.032	0.015	NA	14	66.019	-104.04	9.09	0.001
57	3870	0.424	0.016	0.012	0.035	-0.007	0.145	-0.072	NA	NA	NA	NA	NA	-0.027	NA	0.011	14	66.019	-104.04	9.09	0.001
58	4030	0.432	0.031	0.018	0.034	-0.008	0.143	-0.089	NA	-0.053	NA	NA	NA	-0.012	0.009	0.008	16	67.676	-103.35	9.77	0.001
59	3646	0.440	0.022	0.011	0.034	-0.007	0.139	-0.077	NA	NA	NA	NA	NA	NA	-0.001	0.016	14	65.473	-102.95	10.18	0.001
60	1918	0.433	0.022	0.016	0.033	-0.007	0.147	-0.088	-0.027	NA	NA	NA	NA	-0.020	0.013	NA	15	66.368	-102.74	10.39	0.001
61	3934	0.436	0.028	0.010	0.032	-0.007	0.146	-0.080	-0.026	NA	NA	NA	NA	-0.016	NA	0.007	15	66.330	-102.66	10.47	0.001
62	3710	0.436	0.027	0.011	0.031	-0.008	0.143	-0.079	-0.034	NA	NA	NA	NA	NA	0.002	0.008	15	66.212	-102.42	10.70	0.000
63	3902	0.427	0.015	0.015	0.035	-0.008	0.146	-0.077	NA	NA	NA	NA	NA	-0.030	0.009	0.006	15	66.043	-102.09	11.04	0.000
64	3966	0.432	0.021	0.017	0.033	-0.007	0.147	-0.088	-0.027	NA	NA	NA	NA	-0.020	0.015	-0.004	16	66.375	-100.75	12.37	0.000

Table S14: Model-averaged model estimates for the four indices in May and November. Global models include all parameters fitted during model selection; T_{max} and T_{max7} were not included together in any single model during model selection. Null model indicates parameters forced into all models during model selection and therefore have a forced relative variable importance (RVI) of 1. Models with ΔAIC < 2 from the best fitting model during model selection were model-averaged and model estimates are presented here; parameters with blanks were not in any of the ΔAIC < 2 models. Reference category is a non-breeding, dominant, female, caught with no rainfall in the week of capture. SE is the unconditional standard error, which incorporates model selection uncertainty (Grueber et al., 2011). Estimates (β) are standardised by predictors, therefore mean-centred and scaled to a SD of 0.5. Highlighted in bold are all parameters whose confidence intervals did not contain 0 (excluding intercepts) which are interpreted as significant. Lysis estimates are natural log-transformed and heterophil-lymphocyte ratio estimates are square-root-transformed. From left to right, samples sizes in May were n = 453, n = 376, n = 385, n = 375, and in November n = 194, n = 137, n = 132, n = 162.

			Haptoglobin				Natural antibodies				Complement activity				Heterophil:Lymphocyte ratio			
		Parameter	β	SE	95% CI	RVI	β	SE	95% CI	RVI	β	SE	95% CI	RVI	β	SE	95% CI	RVI
May global model	Null model	*Intercept*	0.659	0.055	(0.551, 0.767)	-	14.681	0.433	(13.829, 15.532)	-	0.984	0.050	(0.887, 1.082)	-	0.423	0.025	(0.373, 0.472)	-
		Breeding activity : Yes	-0.037	0.024	(-0.084, 0.009)	1	0.085	0.148	(-0.207, 0.376)	1	-0.073	0.060	(-0.191, 0.045)	1	0.033	0.019	(-0.005, 0.07)	1
		Day of season	0.039	0.027	(-0.014, 0.092)	1	0.344	0.196	(-0.043, 0.73)	1	**0.474**	**0.081**	**(0.315, 0.633)**	**1**	-0.016	0.026	(-0.067, 0.036)	1
		Sex : M	-0.019	0.019	(-0.057, 0.018)	1	0.038	0.118	(-0.195, 0.271)	1	0.025	0.049	(-0.07, 0.121)	1	0.025	0.019	(-0.012, 0.061)	1
		Status : Subordinate	0.035	0.021	(-0.006, 0.075)	1	0.168	0.127	(-0.082, 0.418)	1	-0.092	0.053	(-0.196, 0.012)	1	**-0.054**	**0.019**	**(-0.092, -0.017)**	**1**
		Time bled	0.002	0.020	(-0.038, 0.043)	1	**0.287**	**0.131**	**(0.029, 0.545)**	**1**	0.037	0.056	(-0.073, 0.147)	1	**0.089**	**0.019**	**(0.052, 0.126)**	**1**
		Time wait	-0.011	0.022	(-0.054, 0.032)	1	0.171	0.142	(-0.108, 0.449)	1	-0.029	0.057	(-0.141, 0.083)	1	**-0.051**	**0.020**	**(-0.09, -0.013)**	**1**
		T_{max}	0.035	0.024	(-0.015, 0.086)	0.4	-	-	-	-	-	-	-	-	-0.004	0.013	(-0.05, 0.042)	0.29
		T_{max7}	0.011	0.007	(-0.045, 0.067)	0.06	0.006	0.206	(-0.399, 0.412)	1	**-0.232**	**0.084**	**(-0.398, -0.067)**	**1**	-0.006	0.023	(-0.06, 0.047)	0.71
		$Rain_{7}$ *: Yes*	0.042	0.029	(-0.027, 0.111)	0.35	0.208	0.234	(-0.252, 0.668)	1	**0.271**	**0.095**	**(0.084, 0.458)**	**1**	**0.084**	**0.031**	**(0.023, 0.145)**	**1**
		T_{max} *x* $Rain_{7}$ *: Yes*	-	-	-	-	-	-	-	-	-	-	-	-	**0.126**	**0.062**	**(0.04, 0.213)**	**0.29**
		T_{max7} *x* $Rain_{7}$ *: Yes*	-	-	-	-	**1.454**	**0.414**	**(0.64, 2.268)**	**1**	**-0.363**	**0.161**	**(-0.68, -0.047)**	**1**	**0.181**	**0.094**	**(0.073, 0.289)**	**0.71**
		T_{var}	0.013	0.007	(-0.042, 0.067)	0.06	-0.016	0.080	(-0.397, 0.366)	0.17	-0.066	0.045	(-0.21, 0.079)	0.23	0.012	0.009	(-0.03, 0.055)	0.12
		Group size	-0.036	0.024	(-0.088, 0.015)	0.4	0.082	0.082	(-0.258, 0.422)	0.19	0.047	0.037	(-0.1, 0.194)	0.19	-0.022	0.012	(-0.067, 0.024)	0.16
		Territory quality	-0.018	0.008	(-0.062, 0.026)	0.07	-0.039	0.062	(-0.319, 0.24)	0.18	-0.007	0.026	(-0.138, 0.124)	0.16	-0.020	0.014	(-0.062, 0.021)	0.27
November global model	Null model	*Intercept*	0.643	0.072	(0.502, 0.784)	-	15.198	0.603	(14.005, 16.391)	-	1.234	0.237	(0.765, 1.703)	-	0.432	0.014	(0.405, 0.46)	-
		Breeding activity : Yes	-0.099	0.061	(-0.219, 0.021)	1	-0.199	0.229	(-0.652, 0.254)	1	0.020	0.162	(-0.299, 0.34)	1	0.046	0.031	(-0.016, 0.108)	1
		Day of season	**-0.119**	**0.047**	**(-0.212, -0.026)**	**1**	**-0.491**	**0.203**	**(-0.893, -0.089)**	**1**	**0.624**	**0.111**	**(0.405, 0.844)**	**1**	-0.014	0.027	(-0.068, 0.04)	1
		Sex : M	0.013	0.033	(-0.052, 0.078)	1	-0.100	0.138	(-0.373, 0.173)	1	-0.017	0.074	(-0.163, 0.13)	1	0.032	0.027	(-0.021, 0.085)	1
		Status : Subordinate	-0.070	0.036	(-0.14, 0.001)	1	-0.145	0.149	(-0.44, 0.15)	1	0.076	0.083	(-0.089, 0.24)	1	-0.006	0.026	(-0.058, 0.046)	1
		Time bled	-0.007	0.036	(-0.078, 0.063)	1	0.005	0.169	(-0.33, 0.34)	1	-0.030	0.114	(-0.256, 0.195)	1	**0.135**	**0.027**	**(0.081, 0.189)**	**1**
		Time wait	0.024	0.041	(-0.057, 0.104)	1	-0.035	0.177	(-0.384, 0.315)	1	-0.001	0.112	(-0.222, 0.221)	1	-0.053	0.029	(-0.111, 0.005)	1
		T_{max}	-	-	-	-	-0.201	0.097	(-0.57, 0.167)	0.14	0.177	0.121	(-0.047, 0.402)	0.52	-	-	-	-
		T_{max7}	0.086	0.053	(-0.004, 0.177)	0.81	0.292	0.199	(-0.111, 0.695)	0.45	-	-	-	-	-0.045	0.030	(-0.098, 0.009)	0.69
		$Rain_{7}$ *: Yes*	0.048	0.040	(-0.065, 0.16)	0.34	0.281	0.185	(-0.178, 0.741)	0.32	-0.177	0.108	(-0.501, 0.148)	0.23	**0.116**	**0.042**	**(0.033, 0.198)**	**1**
		T_{max} *x* $Rain_{7}$ *: Yes*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		T_{max7} *x* $Rain_{7}$ *: Yes*	-0.192	0.094	(-0.411, 0.026)	0.21	-0.365	0.139	(-1.421, 0.69)	0.05	-	-	-	-	-0.069	0.037	(-0.272, 0.133)	0.09
		T_{var}	0.013	0.010	(-0.062, 0.088)	0.06	0.207	0.144	(-0.164, 0.578)	0.32	0.111	0.069	(-0.109, 0.33)	0.22	-0.038	0.027	(-0.095, 0.019)	0.42
		Group size	-0.057	0.038	(-0.133, 0.02)	0.42	0.092	0.061	(-0.271, 0.455)	0.09	0.119	0.074	(-0.115, 0.354)	0.22	0.007	0.008	(-0.049, 0.062)	0.08
		Territory quality	-0.036	0.016	(-0.116, 0.044)	0.08	-0.089	0.068	(-0.508, 0.329)	0.09	0.156	0.094	(-0.132, 0.444)	0.22	0.031	0.021	(-0.028, 0.09)	0.28

Chapter 3: Does constitutive immune function exhibit senescence in the wild? A longitudinal study

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Abstract

1. Advancing age underlies physiological performance declines, and consequently fitness. Age-related decline in immune defence is likely an important component of age-related decline in survival. In older age classes the adaptive (memory-based) responses appear impaired, with a lesser decline in innate (non-specific) immune responses, and increased inflammation. However, longitudinal studies of within-individual changes in immune functions are rare in wild animals, yet are needed to understand how immune components senesce under evolutionarily relevant conditions.
2. Using longitudinal data from a long-studied population of a tropical passerine (*Malurus coronatus*), we investigate if and how population trends emerge from between-individual heterogeneity (*e.g.* selective disappearance) and within-individual changes in immune status. We quantified four commonly assessed constitutive immune indices (haptoglobin, natural antibodies, complement activity, and heterophil-lymphocyte ratio – also an index of chronic stress; n = 505-631), from 849 measures of 372 individuals, 0-12 years old, sampled 1-7 times over 5 years.
3. Population-level cross-sectional analyses provided evidence for age-related decline in natural antibodies – important for pathogen recognition and front-line defences. Additionally, heterophil-lymphocyte ratio increased with age, consistent with relatively greater senescence of adaptive (lymphocytes) *vs.* innate (heterophils) cellular immunity. Contrary to expectation, we found no age-related change in baseline haptoglobin (an inflammatory marker) or lytic complement activity.
4. However, within-individual longitudinal analyses suggest that cross-sectional trends are driven by differences between individuals, possibly due to heterogeneity in mortality risk, with limited evidence for age-related decline within individuals (immunosenescence).

5. We highlight that extrapolating from demographic patterns to within-individual processes (senescence) is not straightforward for physiological parameters plausibly associated with selective disappearance from the population.

Keywords: ageing, aging, ecoimmunology, gerontology, HLHA assay, immunosenescence, inflammaging

Introduction

Like humans, animals exhibit *senescence* – a decline in physiological performance and fitness with advancing age across a diverse range of life-history strategies (Baudisch, 2011; Jones et al., 2014, 2008). Senescence in the wild was once considered rare as a result of intense selection from extrinsic sources of mortality against senescent individuals before any decline could be observed (Lemaître and Gaillard, 2017; Nussey et al., 2013). Intrinsic sources of mortality are still evident in wild populations, although they may interact with environmental sources of mortality, and therefore some underlying decline in physiological processes must increase the probability of death with advancing age (Ricklefs, 2008, 2000). Consequently, a decline in survival probability with age – *actuarial senescence* – is a focal point for aging studies in wild organisms particularly (Brunet-Rossinni and Austad, 2005; Gaillard et al., 2017; Nussey et al., 2013), as aging and individual physiological decline ultimately contribute to overall survival (Ricklefs, 2008). In addition, there is good evidence for a decline in reproductive performance with age – *reproductive senescence* – in wild animals (Lemaître and Gaillard, 2017; Nussey et al., 2013). However, age-related changes in physiological traits and the processes underlying decreased survival and fecundity with advancing age, are relatively under-studied in the wild.

Senescent decline in immune function – *immunosenescence* (Pawelec, 2018) – is likely to be particularly consequential for survival and reproduction in wild organisms (Cheynel et al., 2017; Froy et al., 2019; Sadd and Schmid-Hempel, 2008), where infection may exacerbate the threat of predation (Møller and Erritzøe, 2000), or reduce competitiveness for critical resources (Verhulst et al., 2014). In humans, the aging immune system renders the elderly more susceptible to contracting and succumbing to infectious disease, especially novel infections (Pawelec et al., 2010; Pera et al., 2015). A few consistent patterns are emerging from wild animal studies suggesting there may be congruent age-related changes in immunity in humans and wild animals (Froy et al., 2019; Peters et al., 2019). In vertebrates, evolutionarily ancient innate immune components rapidly deal with novel infections, while

adaptive immune responses deal with repeated infections through specific (acquired, memory-based) immune responses (Litman et al., 2010; Riera Romo et al., 2016). Although some senescence of innate immunity has been observed in vertebrates and invertebrates alike (Müller et al., 2013), there is much stronger and consistent evidence for senescence of adaptive immunity in vertebrates, primarily through thymic involution and depletion of naïve T-cells (Müller et al., 2013; Shanley et al., 2009) – the cells responsible for generating new memory-based immune repertoire (Dowling and Hodgkin, 2009). The balance between innate and adaptive immunity could therefore change with advancing age (McDade et al., 2016), and result in a 'remodelling' of the immune system (Fulop et al., 2018; Müller et al., 2013). Despite detailed knowledge of human aging, humans are exceptionally long-lived post-reproduction (Ellis et al., 2018), and a better understanding of senescence of immune components in different species is needed to refine our evolutionary perspectives on how physiological aging processes underpin diverse life-histories, and actuarial and reproductive senescence.

Senescence, in any trait, is a progressive decline within individuals resulting in reduced function in older age classes. However, the overall differences observed between age classes in a population can also (partly) result from individual heterogeneity in trait values associated with disappearance from the population (e.g. mortality risk, Froy et al., 2019). Therefore, studies that can distinguish these within-individual and between-individual processes are necessary to demonstrate senescence (Nussey et al., 2008). Only in longitudinal studies can age-related changes be partitioned statistically to identify independent trends both within and between individuals that comprise cross-sectional population demographic patterns (van de Pol and Wright, 2009). Inter-individual-level phenomena such as heterogeneity in mortality risk (Vaupel et al., 1979), *i.e.* selective disappearance of individuals with particular trait values, may cancel out, strengthen or mimic individual-level effects, hampering interpretation of cross-sectional studies, in particular for traits linked to survival. However, the difficulties of repeated capture and measurement of wild individuals has meant that a paucity of longitudinal studies of immunity in the wild has limited our understanding of immunosenescence (reviewed in Peters et al., 2019; also see Froy et al., 2019). Consequently, more long-term longitudinal studies of animals are required to decipher the evolutionary role of senescence of immune components in a natural ecological context (Abolins et al., 2018; Kowald and Kirkwood, 2015; Maizels and Nussey, 2013; Nussey et al., 2008), and affirm findings of previous cross-sectional studies (Peters et al., 2019).

To examine longitudinal patterns of immune function, we repeatedly captured individuals from a long-term individual-based study of a wild tropical passerine, the purple-crowned fairy-wren (*Malurus coronatus*). We addressed two main questions, **1)** what is the

evidence for age-related decline in immunity and **2)** do age-related trends emerge from longitudinal within-individual change and/or between-individual processes (individual heterogeneity such as selective disappearance)? To do this, we quantified commonly used indices of constitutive immune function from > 500 captures of > 280 individuals over 5 years, including: natural antibodies, lytic complement activity (both innate), haptoglobin-like protein (an inflammatory marker), and heterophil-lymphocyte ratio (ratio of the primary cell types of innate (heterophil) and adaptive (lymphocyte) cell-mediated immunity; also a marker of chronic stress). Comparing both cross-sectional and longitudinal analyses to distinguish within individual senescence from population trends, we tested whether the innate immune indices exhibited a decline with age, as predicted if they showed immunosenescence. Although innate immunity initially appeared less prone to age-related decline, age-dependent dysregulation of innate immunity is now considered quite central to immune aging (Shaw et al., 2013), especially inflammaging, the chronic low-grade inflammatory activity that characterises elderly humans (Bruunsgaard et al., 2001; Franceschi et al., 2006), and possibly older animals (Peters et al., 2019). As a marker of inflammation, haptoglobin can thus be predicted to increase with age if there is 'inflammaging' (Peters et al., 2019). Lastly, because senescence in adaptive immunity is expected to be more pronounced than for innate immune functions (Peters et al., 2019; Shanley et al., 2009), we predicted that HL ratio would increase with age.

Methods

Study population

Purple-crowned fairy-wrens are riparian habitat specialists in the tropical wet-dry savannah of northern Australia. This cooperatively breeding species defends year-round stable territories (Kingma et al., 2011), with social groups comprised of a dominant breeding pair, usually with subordinate adults, and any offspring (Hall and Peters, 2008; Kingma et al., 2010). Our study population resides along 15km of Annie Creek and Adcock River at the Australian Wildlife Conservancy's (AWC) Mornington Wildlife Sanctuary (126.1°E, -17.5°N). Since 2005, all individuals have been uniquely colour-banded to monitor social group composition, territory boundaries, individual movements, dispersal within the population, and survival. During this study, the estimated census detection rate in the core population was 98% (28 birds were initially presumed dead but subsequently rediscovered). The reliability of survival estimates is strengthened further as this species is known to only disperse along waterways, and ~95km of waterways with suitable habitat in the wider catchment (up to 60km away) are surveyed annually using a 90% successful detection survey technique, discovering emigrants that dispersed from the core banded population (Hidalgo Aranzamendi et al., 2016).

Aging, capture and sampling

All individuals were aged at first capture, either as nestlings, or based on behavioural, morphological and plumage traits of known age of acquisition. Ages of captured adults included in this study range 91d-11y 218d (mean = 1y 321d, median = 364d, see supplementary materials, figs. S1 and S2 for age structure of final samples). The oldest bird recorded in the population was 13y 249d ± 195d (estimated from immature plumage at first capture). Birds reach independence at ~90d, so birds younger than 91d at capture were excluded from analyses because early age-related changes in immune indices were expected to be related to maturation (Killpack et al., 2013), rather than senescence. We excluded birds banded as adults at the start of the study because they were few in number (10-12 samples), and had high (> 5 years) uncertainty in age estimate. Ages of immigrants were also not precisely known, but were based on the age at which emigrants were last seen in the core population before dispersal into the wider catchment (median = 252d). The majority (74%) of dispersal occurs in the first year of life and always < 4 years of age (table S1).

From April 2012 to June 2017, in total 849 samples of 372 individuals contributed to the final dataset, although not all immune indices could be analysed from all samples (final sample sizes for each index can be found in the statistical methods). Captures were made during two annual sampling periods from mid-April to mid-June and from mid-October to late November each year, before and after the main breeding peak (Hidalgo Aranzamendi et al., 2019). Birds were captured in mist-nets and kept in holding bags until blood sampling as quickly as possible (median = 23min, s.d. = 19.6min) to minimise handling stress known to influence some immunological indices (Davis, 2005; Zylberberg, 2015). Following brachial venepuncture, blood was collected in heparinised capillary tubes that were sealed, stored on ice, and centrifuged at 16,060g for 5min later that day. Plasma was frozen at -20°C and transferred to -80°C within 8 weeks. At capture, a blood smear was created using the wedge-pull method (Campbell, 2015a), air-dried and fixed in methanol for at least 15min.

Immune indices

We quantified innate immune indices that form integral components of immune surveillance and initial defence against infection: (1) Natural antibodies (NAbs) are key factors in non-specifically identifying foreign antigenic components and opsonising them for phagocytosis (Matson et al., 2005; Ochsenbein et al., 1999), as well as linking the innate and adaptive immune systems (Panda and Ding, 2015). In addition, NAbs can initiate (2) Complement activity (Ca) via the classic pathway (Panda and Ding, 2015), which lyses and breaks down foreign bodies towards elimination of infection through the protein cascade of the complement

system (Trouw and Daha, 2011). (3) Haptoglobin-like haem-binding (Hp) scavengers mitigate damage incurred from reactive oxidative haem groups released by cells that are damaged by infection or inflammation (Andersen et al., 2017; Quaye, 2008). These scavengers are major positive acute phase proteins that are tightly linked to inflammatory responses, with baseline levels to some extent predictive of immune responsiveness (Matson et al., 2012). Lastly, (4) the heterophil-lymphocyte (HL) ratio, is comprised of heterophils that exhibit bactericidal and phagocytotic ability important for cellular innate immunity (Genovese et al., 2013), and lymphocytes that secrete antibodies crucial to the adaptive immune response (Sharma, 1991). Additionally, the HL ratio is an indicator of chronic stress (Davis and Maney, 2018), but can also be predictive of immune responsiveness (Krams et al., 2012). The specific assay methods for these constitutive immune indices are outlined here and described fully in Chapter 2 (Roast et al., 2019). Within-individual repeatability is low across all indices in purple-crowned fairy-wrens, with HL ratio the most repeatable index (table S5 in Chapter 2).

(1) NAbs and (2) Ca were both quantified using the haemolysis-haemagglutination assay (Matson et al., 2005) with minor modifications as in Chapter 2. Inter-plate standards were for both agglutination (mean = 10.1, n = 247 standards) and lysis (mean = 3.55, n = 265 standards) titres respectively, resulting in CV = 0.13 and CV = 0.11.

(3) Baseline Hp was assayed using a commercial kit (Phase^{TM} Range, TP801; Tridelta Development Ltd.) and micro-plate reader, with a modified protocol from Matson et al. (2012); details in Chapter 2. All samples were run in duplicate; standards run in triplicate were used to assess inter-plate variation (CV = 0.24, n = 25 plates). Of all samples initially assayed, the majority (89%) formed a normal distribution (mean = 0.62mg/ml, s.d. = 0.27) falling below the 1.25mg/ml optical saturation threshold of the assay. For 82 individuals, Hp levels fell above this threshold, assumed to represent an extreme distribution tail of individuals undergoing an acute phase response, which were excluded from further Hp analyses.

(4) Stained blood smears were examined at 1000x magnification and heterophils, lymphocytes, basophils, eosinophils and monocytes were counted for the first 100 leukocytes observed. The majority of all cells identified (87%) were heterophils or lymphocytes, with other cell types observed infrequently among individuals or in low number. As proportions, heterophils and lymphocytes were highly negatively correlated ($r = -0.78$, $p < 0.001$) and therefore analysed as a single composite metric, the heterophil-lymphocyte ratio. Scoring was undertaken by 4 individual scorers following (Campbell, 2015b). Scorer ID was included in all statistical models to account for variation between individuals; all further details in Chapter 2.

Statistical Analysis

All statistical analyses were performed using R software v3.4.0 (R Core Team, 2017). Hp and NAbs immune indices were normally distributed, while Ca titre scores were natural-log-transformed and HL ratios square-root-transformed (all values were > 0 and < 1) to normalise distributions. Outlying observations beyond ± 2.5 standard deviations of the mean were excluded (Hp, n = 2; NAbs and HL ratio, n = 11; Ca, n = 0).

1) To assess population level age-related changes in immune indices, univariate linear mixed-effects models (LMMs) were created with immune indices as response variables and age-at-sampling as an explanatory variable (Equation 1; van de Pol and Wright, 2009). Each cross-sectional model contained a combination of fixed effects and random intercepts that controlled for sources of methodological quantification error, biological variation not of primary interest, and non-independent sampling structure, providing a 'null model'. These fixed effects and random intercepts were applied to relevant models (see table S2 for specific model structures) and included: sex; time bled – the time of day relative to sunrise; time wait – the delay between capture and sampling; plate standard or plate ID – to control for assay inter-plate variation; scorer ID; individual ID; field season – a multilevel factor of each field sampling period. Being an immigrant did not affect any immune index, so immigrant samples were retained (see table S3 for models including immigrants). Final sample size was for Hp, n = 631; NAbs, n = 506; Ca, n = 505; and HL ratio, n = 521, including both single and multiple repeated measures of individuals (repeated measures formed 75% and 70% of the final sample with up to 7 and 5 measures per individual for Hp and all other indices, respectively; see fig. S3 for repeated measure structure of sample). In exploratory analyses, cohort was initially included in models as a random factor, but explained very little (< 0.2%) variance and was therefore removed from analyses. Social status (dominant vs subordinate) was significant in Ca models as a main effect only, but including it did not change the estimates of senescence in any model – i.e. there was no interaction with age – and given the correlation with age (as subordinates only progress to become older dominants), status was excluded from further models (see table S4 for models with 'status').

We additionally investigated the potential for non-linear changes in immune parameters with advancing age. First, we used an information theoretic approach to compare several common age functions (linear age; quadratic age + linear age; log age; and factor age – binned yearly) which were added to the null models and to assess support for alternative shapes of age-related change in each response variable. Akaike's Information Criterion (AIC) was calculated for each model fitted by maximum likelihood, along with the ΔAIC from the best-fitting model in the set, Akaike weights or model probabilities (ω), and evidence ratios

(ER) (Burnham et al., 2011; Garamszegi et al., 2009). For similarly well-supported models with ΔAIC < 2, standardised β-estimate effect sizes with 95% confidence intervals were calculated by standardising all model variables by two standard deviations using the '*standardize'* function of the *arm* R package (Gelman and Su, 2016) to allow further comparison of effect sizes of different age function variables (see table S5 for complete model comparisons). Second, general additive mixed models (GAMMs) were run for all response variables to assess broader non-linear functions, using the *gamm4* package, with similar model structures to LMMs (table S2), and with linear age included as a smooth function. These GAMMs indicated no support for non-linearity (table S6), and so only LMMs were used further for direct comparison with the longitudinal analysis method which required linear models.

2) To disentangle within-individual change and between-individual selection, we used a longitudinal within-subject centring method (Equation 2; van de Pol and Wright, 2009). Between-individual (β_B) and within-individual (β_W) effects were estimated by replacing age at capture in each model with mean (μ) age of each individual across all captures and delta (Δ) age at capture (age at capture – mean age); a β_B effect shows how immune function changes between individuals in the population as a function of individual mean age, while the β_W effect shows the overall age-related change that occurs within individuals captured repeatedly at different ages (senescence). Single measures (making up 25-30% of the sample, fig. S3) were included with Δ = 0, and μ = the age at capture. The linear and non-linear age variables (see above) were split into their corresponding within- and between-individual components (i.e. Δ linear age + μ linear age; Δ quadratic age + μ quadratic age + Δ linear age*μ linear age; Δ log age + μ log age) and run in separate models. The relative fit of these models was compared using ΔAIC, ω, and ER. In all best-fitting models where 95% confidence intervals for any standardised age effect did not include zero, the difference between β_B and β_W parameter slopes was formally tested (Equation 3; van de Pol and Wright, 2009; table S7). Lastly, many individuals survived but were not recaptured after their final repeated measurement which may resemble selective disappearance in the dataset despite their longer-term survival. To assess the impact of this, whether or not an individual survived at least 3 months after the previous sample (post-capture survival > 3 months) was included as a binary variable to best-fitting longitudinal models (see table S8 for models including this survival parameter).

Results

Cross-sectional age-related change

Of the immune indices, NAbs was lower, and HL ratio was higher, in older individuals, while Ca and Hp showed no evidence for age-related changes (fig. 1). Age-related changes in NAbs and HL ratio were best explained by linear age models which were many times more probable than the null models for NAbs and HL ratio (ER = 503.81 and ER = 17.56 respectively; table 1), with clear age effects bound by 95% confidence intervals not containing zero (table 1; *sensu* Dushoff et al., 2019). While best-explained by log age, there was only a weak age effect for Ca (β(Std) = 0.075, CI = -0.017, 0.166; table 1), whereby the log age model was almost equally as probable as the null model (ER = 1.32; table 1). For haptoglobin, the null model was the best-fitting model, followed by log age (ΔAIC = 1.57; table S5), with a negligible effect size (β(Std) = 0.022, CI = -0.044, 0.089; table 1). GAMMS analyses indicated no support for complex non-linear age-related change (table S6).

Table 1: An overview of best-fitting cross-sectional and longitudinal age models. Bold typeface indicates standardised β-estimates with 95% confidence intervals not containing zero which are interpreted as showing a clear effect. Evidence ratios (ER) expressed as the model probability of the best-fitting age model relative to the null model. For each response, the same age function was best supported in both simple and within-subject-centred analyses separating within- (Δ age) and between- (μ age) individual effects.

	Best-fitting Age Models							
		Cross-sectional			Longitudinal			
Response variable	**Age Function**	**ER**	**β (Std)**	**CI (Std)**	**ER**	**μ/Δ**	**β (Std)**	**CI (Std)**
Hp	Log	0.46	0.022	(-0.044, 0.089)	0.52	μ	-0.003	(-0.066, 0.061)
						Δ	0.061	(-0.012, 0.134)
NAbs	**Linear**	**503.81**	**-0.141**	**(-0.213, -0.069)**	**261.89**	**μ**	**-0.134**	**(-0.203, -0.065)**
						Δ	-0.040	(-0.122, 0.042)
Ca	Log	1.32	0.075	(-0.017, 0.166)	1.01	μ	0.040	(-0.049, 0.129)
						Δ	0.087	(-0.006, 0.181)
HL ratio	**Linear**	**17.56**	**0.128**	**(0.038, 0.217)**	**6.46**	**μ**	**0.110**	**(0.023. 0.197)**
						Δ	0.065	(-0.027, 0.155)

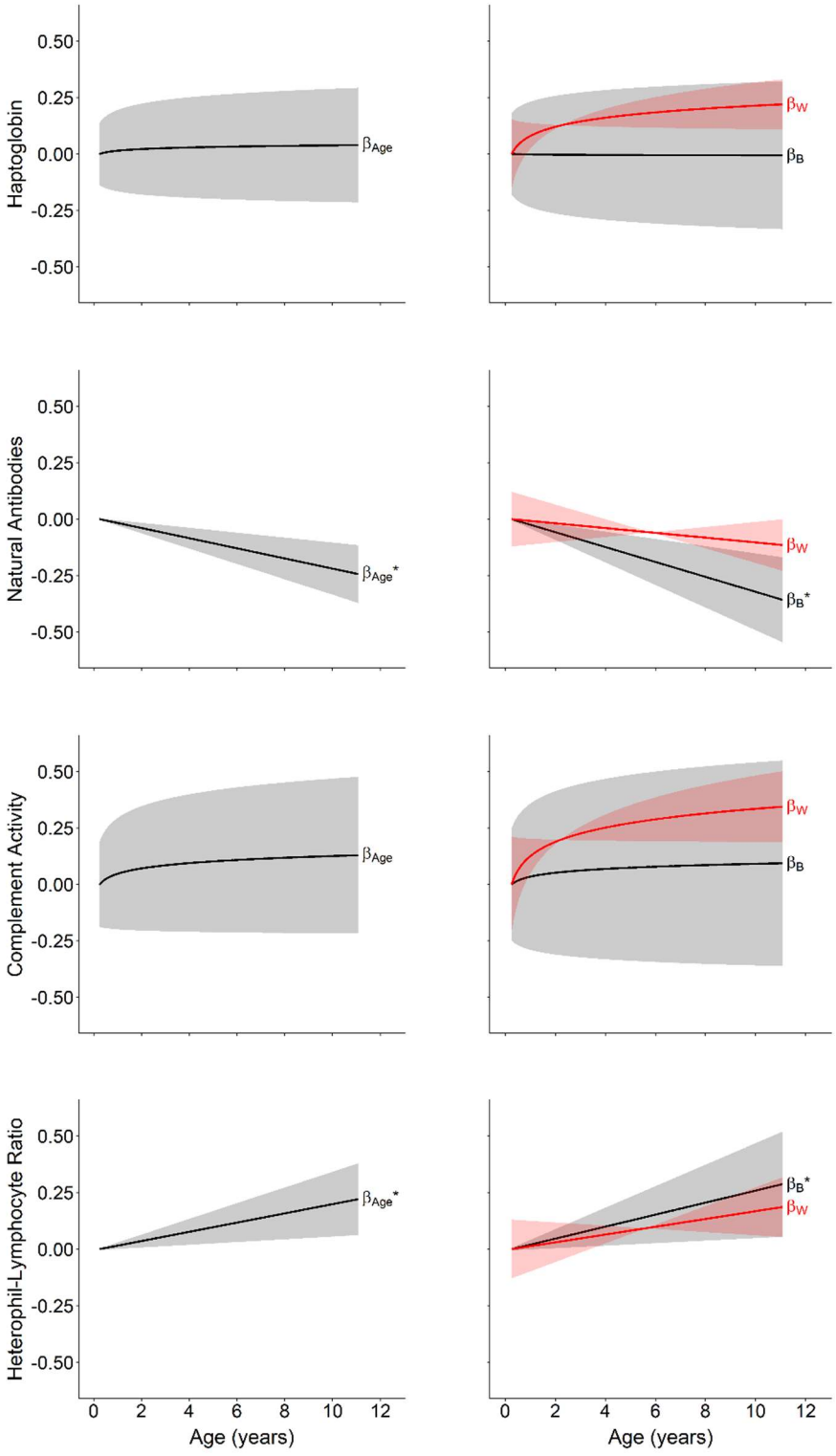
Haptoglobin
Natural Antibodies
Complement Activity
Heterophil-Lymphocyte Ratio
0.50
0.25
0.00
-0.25
-0.50
0
2
4
6
8
10
12
Age (years)
βAge
βAge*
βW
βB
βB*

Figure 1: Schematic overview of standardised effects of age from best-fitting models. Predicted values of Hp, NAbs, Ca, and HL ratio showing the overall effect of age (β_{Age}) from cross-sectional models (left), and the separate between-individual (β_B) and within-individual (β_W) age effects from within-subject-centred models (right). Fitted values were calculated from β-estimates of respective best-fitting models standardised by two standard deviations (table 1), while all other fixed effects were set to mean values. Ribbons show 95% confidence intervals around age effect β-estimates only. Initial y-axis values were set to 0. Effects labelled with '*' show confidence intervals not containing zero.

Longitudinal within-subject centring analyses

Best-supported age functions in all longitudinal analyses were the same as those in cross-sectional analyses (tables 1, S5). NAbs decreased linearly with age between individuals, suggesting individuals with high NAbs disappear from the sample population with age (β_B(Std) = -0.134, CI = -0.203, -0.065; table 1, figs. 1, 2a), while a weaker decline was observed within individuals (β_W(Std) = -0.040, CI = -0.122, 0.042; table 1, figs. 1, 2a). HL ratio increased linearly with age between individuals, suggesting individuals with low HL ratios disappear from the population with age (β_B(Std) = 0.110, CI = 0.023, 0.197; table 1, figs. 1, 2b), but within individuals a weaker age-related change was observed (β_W(Std) = 0.065, CI = -0.027, 0.155; table 1, figs. 1, 2b). The between-individual effects in both NAbs and HL ratio indicate selective disappearance of individuals with high and low values of these indices respectively, and could indicate heterogeneity in mortality risk. Controlling for post-capture survival (*i.e.* failure to recapture) did not affect the outcomes of any best-fitting longitudinal model (table S8), providing no evidence that a sampling artefact might generate these between-individual effects.

Additionally, we formally tested whether the slopes of within- and between-individual effects in each best-supported longitudinal model for NAbs and HL ratio differed significantly from one another. Despite the evidence for there being stronger between-individual effect sizes than for within-individual effects, the two slopes were not significantly different for either index (table S7). Thus, we cannot conclude definitively that the observed age-related changes in NAbs (decline) and HL ratio (increase) are exclusively due to selective disappearance of individuals, but could partly be attributable to weak age-related changes within individuals.

Finally, for Hp and Ca, longitudinal age models were neither more probable than the respective null models (ER = 0.52 and ER = 1.01, respectively; tables 1, S5), nor showed partitioned β_W and β_B effect sizes that were distinct from zero (table 1). This excluded the possibility that opposing within- and between-individual effects were masked in cross-sectional models. However, the slightly positive within-individual age effects in both indices

had highly asymmetric 95% confidence intervals almost entirely in positive space, suggesting a possible maintenance or even slight enhancement with age, but not immunosenescence. Coefficients from all models compared are reported in table S9.

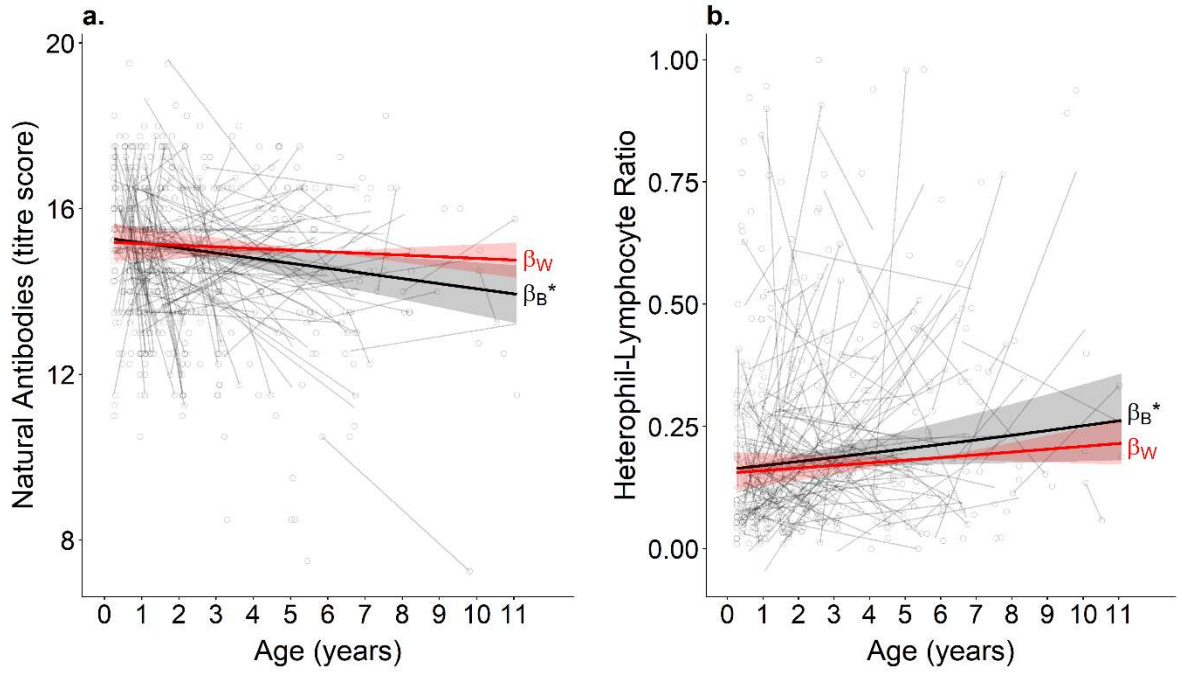

Figure 2: Stronger evidence for between-individual effects (β_B, indicative of selective disappearance) than age-related changes within individuals (β_W, senescence). Fitted lines showing within-individual (β_W, red) and between-individual (β_B, black) age-related changes from **a.** NAbs and **b.** HL ratio non-standardised longitudinal models (table S5), with other variables fixed at mean values. Ribbons indicate 95% confidence intervals, with "*" indicating those not containing a slope of zero. Plot **b.** is back-transformed to original units on the y-axis. Raw data are plotted, with fitted grey lines showing reaction norms for individuals with multiple repeated measures, obtained from a simplified linear regression of immune index ~ linear age, while individuals with single measures have no line.

Discussion

The present study aimed to assess age-related changes in several immune indices in a wild tropical passerine, the purple-crowned fairy-wren, and to distinguish within- and between-individual processes. We found overall very limited evidence for true (within-individual) immunosenescence, with no age-related trends for haptoglobin (Hp) and complement activity (Ca). For natural antibodies (NAbs) and heterophil-lymphocyte ratio (HL ratio), within-subject centring showed that observed differences between age classes could not be clearly attributed to within-individual senescence, and instead, appeared to be driven primarily by differences between individuals in the population.

No decline in immune function: complement and haptoglobin

Although we predicted senescence in Ca, this function was clearly maintained into older age, with a small positive effect size both within and between individuals (though not statistically distinct from zero; table 1, fig. 1). With advancing age, there is the theoretical expectation that self-maintenance increasingly declines as the age-adjusted risk of mortality increases (Kirkwood and Rose, 1991). Consequently, at a cellular level, organisms can accumulate senescent or apoptotic cells (Vicencio et al., 2008). One less well-known – but important – function of the complement system is to remove apoptotic cells from the body (Ricklin et al., 2010). In humans, the age-related increase in apoptotic cells stimulates a chronically attenuated Ca response (fig. 1c in Ricklin et al., 2010). This can explain levels of Ca being maintained (as appears overall common, Peters et al., 2019) or possibly increasing with age, rather than declining, as maintaining these humoral components might mitigate or delay the consequences of not adequately clearing dead cells (Nagata, 2010).

The cumulative effects of damage and cellular debris acquired with age are also thought to result in a chronic systemic increase in the inflammatory system (i.e. inflammaging), a process closely linked to immunosenescence and age-related illness (Bruunsgaard et al., 2001; Franceschi et al., 2017, 2006; Pawelec, 2018). We thus predicted that haptoglobin (Hp) would undergo age-related increase as a part of inflammaging. Although Hp can increase multiple-fold from baseline levels during an inflammatory response at any age, its baseline levels are primarily controlled by the interleukin-6 (IL-6) inflammatory cytokine (Quaye, 2008) which strongly increase with age, at least in humans (Franceschi et al., 2006). Considering Hp production is stimulated by inflammatory cytokines to dampen the effects of inflammation (Quaye, 2008), the absence of age-related increase in Hp that we observed, suggests that either IL-6 does not increase, or does not stimulate Hp, or possibly, there is no inflammaging overall, in aging purple-crowned fairy-wrens. This apparent lack of inflammaging contrasts

with the general inflammaging seen in other wild animals (Peters et al., 2019), though there is some evidence that reduced inflammaging may be beneficial for longevity (Shanley et al., 2009), and potentially adaptive for a long-lived fairy-wren.

Immunosenescence or selective disappearance? Natural antibodies and HL ratio

NAbs showed a clear decline with age overall, in line with our predictions and some published avian studies (Møller and Haussy, 2007; Vermeulen et al., 2017; although a general decline is not found across species, Peters et al., 2019). From the within-subject centred model, there was also an indication of selective disappearance from the sample of older individuals with higher NAbs levels, but limited evidence for within-individual senescence. The minimal within-individual effect here could simply reflect that NAb levels do not change with age, as reported in cross-sectional avian studies elsewhere (Lecomte et al., 2010; Palacios et al., 2007). Nevertheless, a lack of detectable difference between the between- and within-individual effect sizes (table S7), demonstrates that the within-individual effect is placed indistinctly between zero and the between-individual effect, and equally we cannot exclude the possibility of immunosenescence. As NAbs primarily function for frontline immune defence and surveillance, high NAb levels are probably more beneficial for survival (Baumgarth et al., 2005; Ochsenbein et al., 1999); however, the observed between-individual effect suggests that individuals with high NAbs are disappearing from the population. Possibly, higher constitutive levels of NAbs are a consequence of a higher background infection risk (Horrocks et al., 2015), and therefore indirectly related to the increased mortality associated with higher disease risk. Alternatively, disappearance of individuals may covary with high NAbs as a consequence of the role of self-reactive classes of natural (auto)antibodies, which can increase with age as part of a debris clearance system (Grönwall et al., 2012; Nagele et al., 2013; Panda and Ding, 2015). In the context of aging, autoantibodies are produced in response to increasingly dysregulated cellular and molecular components, if there is selective disappearance of the most dysregulated individuals, these individuals would also have the highest NAbs titres, which could explain our observations.

We predicted that HL ratio might increase with age due to faster senescence of cellular adaptive immunity relative to cellular innate immunity, particularly through reduced lymphopoiesis in older individuals (Shanley et al., 2009). In agreement with our prediction, the cross-sectional model showed a clear increase in HL ratio with age. However, the longitudinal analysis showed that this was possibly due to disappearance of individuals with lower HL ratios in old age, with no clear evidence for within-individual age-related change. However, as for NAbs, a lack of significant difference between the between- and within-individual slopes

(table S7) demonstrates that the within-individual effect is not substantially weaker than the between-individual effect, and there may be some age-related change within individuals. As HL ratio is also a well-established indicator of chronic stress (Davis and Maney, 2018), individuals with high HL ratio may exhibit suppressed immune responses and lower survival rates (Krams et al., 2012; Minias et al., 2018). This makes the selective disappearance of individuals with low HL ratio seem somewhat contrary to survival costs of chronic stress.

Longitudinal studies: limitations

Within-subject centred models can be open to alternative interpretations regarding the presence and role of immunosenescence if results are not clear-cut, limiting the conclusions that can be drawn. For example, it seems that when within- and between-individual trends show alignment in the same direction, it becomes challenging to definitively tease them apart. In such a case, between-individual effects can also be a consequence of within-individual effects (van de Pol and Wright, 2009). Stronger between-individual than within-individual effects however, similar to those that we observed, can only be confidently interpreted when effect slopes are significantly different from one another (Vaupel et al., 1979; Verhulst et al., 2014). Furthermore, interpreting these slopes as resulting from heterogeneity in mortality typically assumes that disappearance from the sample signifies death, yet birds in our sample were often not recaptured and lived beyond their final measurement. Despite this imperfect sampling, common among wild studies, a strength of our study is exceptional survival estimate accuracy, which allowed us to assess death *vs.* failed recapture post-measurement. Although we found no evidence of bias from failed recapture (table S8), it remains possible that selective recapture biases the results. For example, individual personality (boldness, tendency to explore) has been linked to immunity (Guenther et al., 2018), including in a closely related fairy-wren (Jacques-Hamilton et al., 2017) such that more exploratory, bolder individuals might be captured more easily using passive methods (Michelangeli et al., 2016) and systematically bias the sampled immune traits.

Despite our large sample size, multiple repeated measures (fig. S3), and representative sampling across all older age classes (fig. S2), it is possible that we had slightly less power to detect within-individual change than between-individual processes. The average sampling period within individuals was ~2y (range 0.5-4.5y) and relatively narrow ‘windows’ of repeated measures of individuals may not fully capture within-individual change across the entire lifetimes of individuals. To our knowledge this has not explicitly been considered previously, and it may be an important consideration in the design of future longitudinal studies.

Conclusion

Our study highlights the importance of partitioning contributions of between- and within-individuals changes in studies of immunosenescence. Had we analysed our dataset without within-subject centring, we would have concluded that there is clear evidence for senescence or age-related changes in circulating levels of NAbs and the HL ratio. Instead we showed a more nuanced breakdown of the results that did not conclusively support within-individual changes, but instead hinted at selective disappearance. The absence of senescence observed here in haptoglobin, lytic complement activity (and arguably natural antibodies) is itself notable, and could be selected for if innate immunity remains critically important throughout life. The overall persistence of innate immunity into old age might compensate for a reduced ability of the adaptive immune system to develop specific antibodies to novel antigens with fewer circulating naïve T-cells. However, innate immune components also have different self-maintenance roles (*e.g.* clearance of cellular debris and apoptotic cells), which become more important later in life as other physiological systems become dysregulated. This alternative functionality has been well documented in humans (Holodick et al., 2017; Quaye, 2008; Ricklin et al., 2010), but are rarely considered in ecological studies. Such dual-function could show a functional shift with age, with a defensive role becoming less relevant in older age. It is therefore essential that alternative functions are explicitly considered and integrated when interpreting age-related patterns of immune parameters, even for such widely measured parameters, which will be a challenge for wild ecoimmunology. Applying the increasingly detailed knowledge of human physiological aging more broadly to other organisms may yield unexpected insight into the evolution of the mechanisms of senescence.

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Supplementary Materials

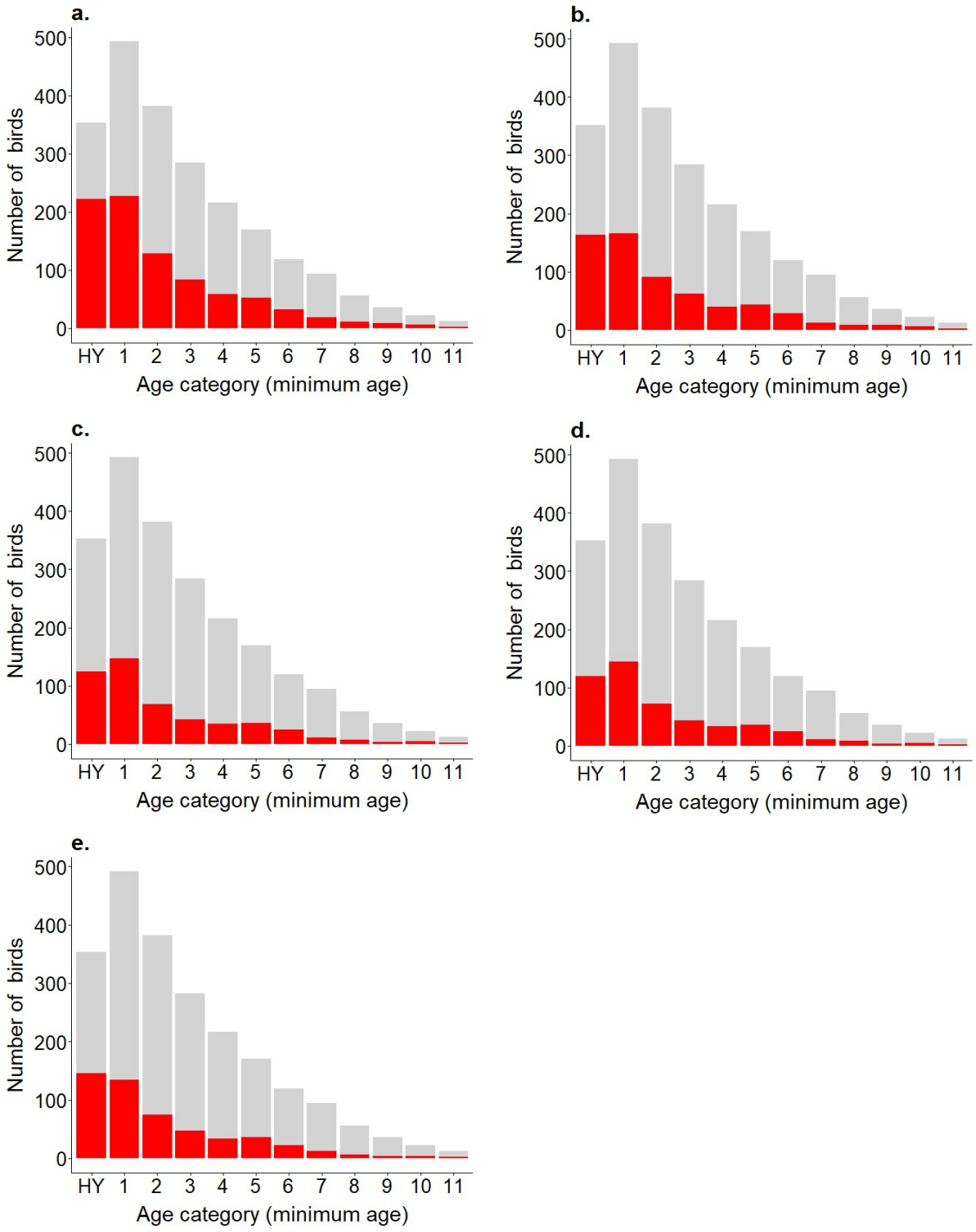

Figure S1: Histograms showing distributions of ages sampled, binned at one-year intervals of minimum age. Hatch year birds, 'HY', are adults sampled at > 90 days old but less than one year old. Grey bars **a-e.** show the cumulative entire study population theoretically available to sample between April 2012 and June 2017. Red bars show individuals sampled with **a.** total captures made (not all indices could be measured for every capture), **b.** haptoglobin, **c.** natural antibodies, **d.** complement activity, and **e.** heterophil-lymphocyte ratio.

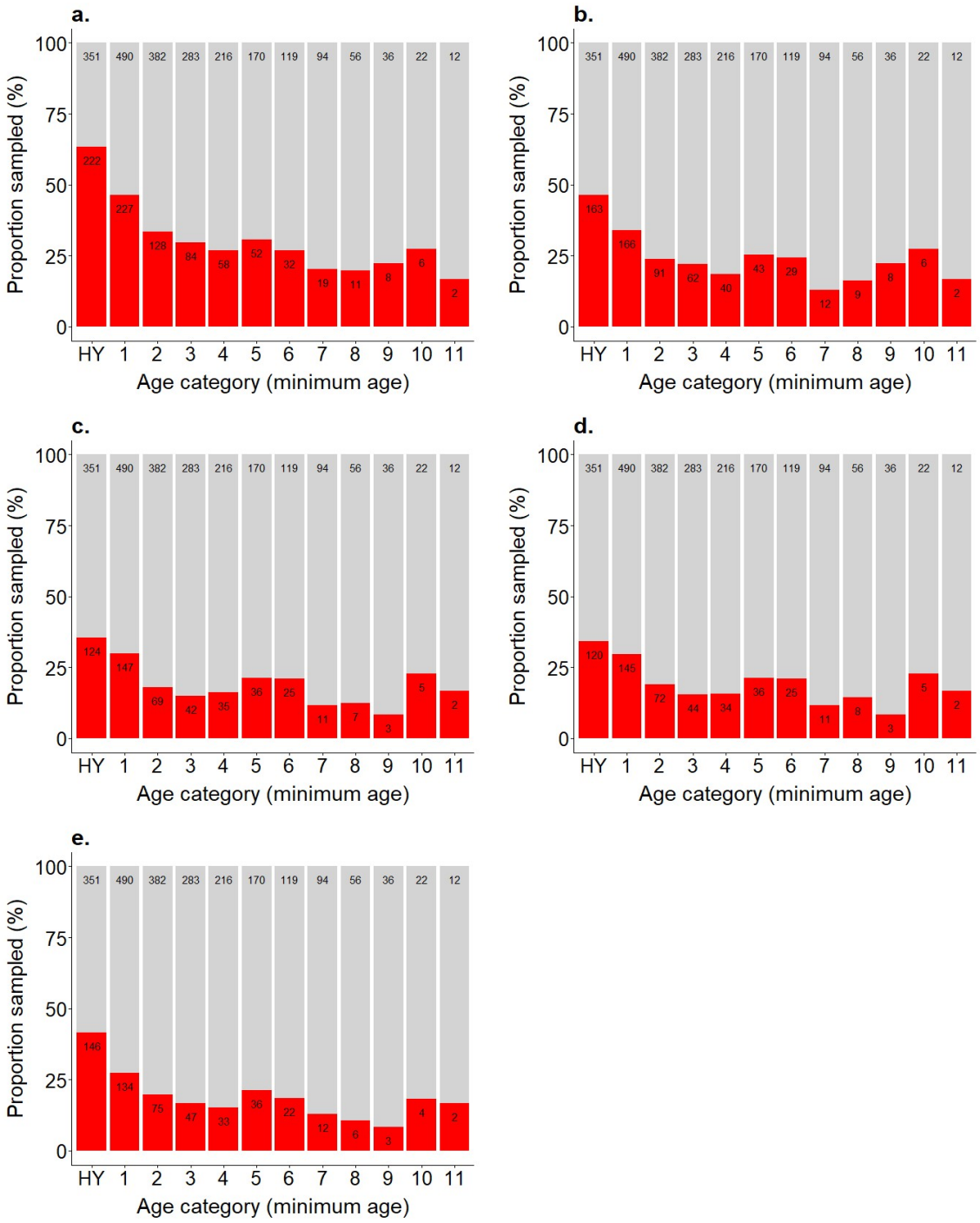

Figure S2: Bar charts showing proportions of ages sampled, binned at one-year intervals of minimum age. Hatch year birds, 'HY', are adults sampled at > 90 days old but less than one year old. Proportionally more HY birds were caught as a consequence of targeted mist-netting to capture unbanded birds that were offspring since the previous sampling season. Grey bars **a-e.** show the cumulative entire study population theoretically available to sample between April 2012 and June 2017 (100%). Red bars show individuals sampled with **a.** total captures made (not all indices could be measured for every capture), **b.** haptoglobin, **c.** natural antibodies, **d.** complement activity, and **e.** heterophil-lymphocyte ratio.

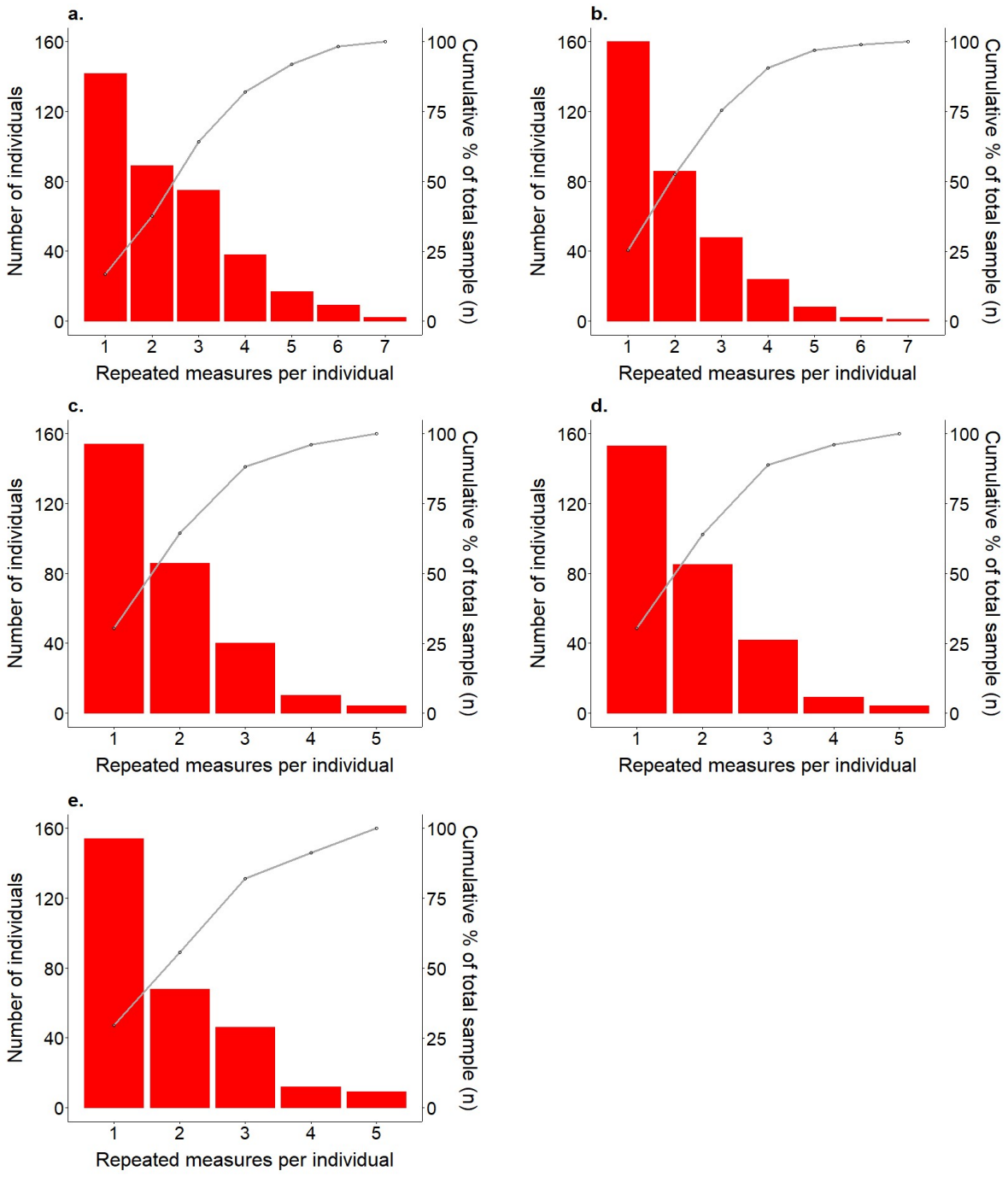

Figure S3: Plots showing the individuals with single and repeated measures and their contribution to the final sample for each index. Red bars show the number individuals that had different series lengths of measures collected for **a.** total captures made (not all indices could be measured for every capture), **b.** haptoglobin, **c.** natural antibodies, **d.** complement activity, and **e.** heterophil-lymphocyte ratio. For each index **b-e.** the number of individuals sampled were n = 329, 294, 293 and 289, respectively. Lines show the cumulative percentage of the total sample size that is derived from individuals with different measures series lengths, for each plot **a-e.**, respectively. The majority of the total sample size derives from individuals with multiple repeated measures (**a.** 83%; **b.** 75%; **c-e.** 70%), even though individuals most frequently have single measures.

Table S1: Binned ages of emigrant dispersers at dates last seen in the core population before dispersal, between 2007-2018 (median = 252d, standard deviation = 342d).

Age (years)	**n**	**%**	**Cumulative %**
0-1	39	73.6	73.6
1-2	7	13.2	86.8
2-3	2	3.8	90.6
3-4	5	9.4	100
4+	0	0	100

Table S2: Model structures of 'null models' for each modelled response variable.

Structure	**Hp**	**NAbs**	**Ca**	**HL ratio**
Fixed effects	Sex	Sex	Sex	Sex
	Time bled	Time bled	Time bled	Time bled
	Time wait	Time wait	Time wait	Time wait
		Plate standard	Plate standard	
Random effects	Plate ID	Field season	Field season	Scorer ID
	Field season	Individual ID	Individual ID	Field season
	Individual ID			Individual ID

Table S3: Testing the influence of founders and immigrant birds. In **a-d)**, fixed effect outputs only from LMMs used to inform inclusion of observations of founders and immigrants in the final sample. β-estimates are from non-standardised models. Reference categories of variables 'Sex' and 'Origin' are 'Female' and 'Known-Age' respectively, with other factor levels indented. Bold typeface indicates fixed effects with p-values < 0.05. Observations from founders were excluded and observations from immigrant birds were retained in all subsequent models.

a)

Response: Haptoglobin (n = 643)					
Fixed effects	***β***	**SE**	**df**	***t***	***p***
Intercept	6.28E-01	7.68E-02	200.6	8.171	<0.001
Age Log	6.09E-03	9.69E-03	626.6	0.629	0.530
Sex					
Male	-1.10E-02	1.81E-02	617.1	-0.611	0.541
Time Bled	9.21E-06	7.49E-05	618.8	0.123	0.902
Time Wait	-2.44E-04	4.68E-04	626.8	-0.521	0.603
Origin					
Founder	-1.54E-03	6.34E-02	613.4	-0.024	0.981
Immigrant	1.29E-03	2.44E-02	622.2	0.053	0.958

b)

Response: Natural Antibodies (n = 516)					
Fixed effects	***β***	**SE**	**df**	***t***	***p***
Intercept	9.93E+00	7.85E-01	77.5	12.662	<0.001
Age Linear	**-3.14E-04**	**8.19E-05**	**511.7**	**-3.833**	**<0.001**
Sex					
Male	8.68E-03	1.39E-01	509.4	0.062	0.950
Time Bled	4.58E-04	5.31E-04	513.8	0.863	0.388
Time Wait	6.62E-03	3.62E-03	515.9	1.830	0.068
Plate Standard	**5.08E-01**	**6.63E-02**	**514.2**	**7.670**	**<0.001**
Origin					
Founder	**1.46E+00**	**5.06E-01**	**509.8**	**2.886**	**0.004**
Immigrant	-9.03E-02	1.87E-01	509.1	-0.483	0.630

c)

Response: Complement Activity (n = 515)					
Fixed effects	***β***	**SE**	**df**	***t***	***p***
Intercept	6.58E-01	3.32E-01	284.8	1.979	0.049
Age Log	5.06E-02	3.12E-02	400.4	1.622	0.106
Sex					
Male	-2.02E-02	5.98E-02	283.6	-0.338	0.736
Time Bled	-1.22E-04	2.16E-04	514.5	-0.566	0.572
Time Wait	-1.07E-03	1.45E-03	488.9	-0.740	0.459
Plate Standard	4.87E-02	6.53E-02	479.0	0.745	0.456
Origin					
Founder	-3.89E-01	2.09E-01	255.7	-1.867	0.063
Immigrant	-2.39E-02	8.04E-02	309.8	-0.298	0.766

d)

Response: Heterophil-lymphocyte Ratio (n = 531)					
Fixed effects	***β***	**SE**	**df**	***t***	***p***
Intercept	3.48E-01	2.86E-02	23.5	12.173	<0.001
Age Linear	**2.72E-05**	**1.05E-05**	**312.9**	**2.604**	**0.010**
Sex					
Male	2.89E-02	1.76E-02	276.1	1.647	0.101
Time Bled	**4.19E-04**	**6.16E-05**	**496.1**	**6.807**	**<0.001**
Time Wait	**-1.44E-03**	**4.04E-04**	**359.7**	**-3.564**	**<0.001**
Origin					
Founder	-4.41E-02	6.41E-02	284.9	-0.687	0.492
Immigrant	4.23E-02	2.34E-02	287.4	1.807	0.072

Table S4: Testing possible interactive effects of social status and age on each immune index **a-d)**. Interaction terms were fitted with best-fitting age models from cross-sectional analyses. Significant model parameters are highlighted in bold typeface. Reference category for 'Status' is 'Dominant', and for 'Sex' is 'Female'.

a)

Response: Haptoglobin (n = 631)					
Fixed effects	***β***	**SE**	**df**	***t***	***p***
Intercept	6.46E-01	1.31E-01	537.2	4.946	<0.001
Age Log	3.25E-03	1.77E-02	612.4	0.183	0.855
Status					
Subordinate	-6.66E-02	1.53E-01	606.8	-0.436	0.663
Sex					
Male	-1.38E-02	1.78E-02	604.3	-0.778	0.437
Time Bled	1.91E-05	7.55E-05	606.0	0.253	0.800
Time Wait	-2.44E-04	4.79E-04	613.9	-0.510	0.610
Age Log x Status					
Subordinate	1.15E-02	2.31E-02	606.2	0.498	0.619

b)

Response: Natural Antibodies (n = 506)					
Fixed effects	***β***	**SE**	**df**	***t***	***p***
Intercept	1.01E+01	7.96E-01	81.9	12.674	<0.001
Age Linear	**-3.48E-04**	**1.06E-04**	**500.0**	**-3.284**	**0.001**
Status					
Subordinate	-8.50E-03	2.29E-01	499.7	-0.037	0.970
Sex					
Male	1.25E-01	1.35E-01	499.4	0.928	0.354
Time Bled	4.10E-04	5.31E-04	503.8	0.772	0.441
Time Wait	6.61E-03	3.68E-03	505.9	1.794	0.073
Plate Standard	**4.99E-01**	**6.65E-02**	**504.3**	**7.508**	**<0.001**
Age Linear x Status					
Subordinate	-4.14E-04	2.59E-04	499.6	-1.596	0.111

c)

Response: Complement Activity (n = 505)					
Fixed effects	***β***	**SE**	**df**	***t***	***p***
Intercept	9.14E-01	4.59E-01	408.7	1.990	0.047
Age Log	1.02E-02	5.47E-02	392.8	0.186	0.853
Status					
Subordinate	-3.97E-02	4.78E-01	482.8	-0.083	0.934
Sex					
Male	1.09E-02	5.88E-02	272.0	0.186	0.853
Time Bled	-1.81E-04	2.16E-04	503.2	-0.838	0.403
Time Wait	-6.36E-04	1.47E-03	476.0	-0.431	0.667
Plate Standard	6.15E-02	6.67E-02	462.3	0.923	0.356
Age Log x Status					
Subordinate	-1.56E-02	7.29E-02	485.6	-0.214	0.831

d)

Response: Heterophil-lymphocyte Ratio (n = 521)					
Fixed effects	***β***	**SE**	**df**	***t***	***p***
Intercept	3.85E-01	3.09E-02	36.4	12.447	<0.001
Age Linear	1.55E-05	1.35E-05	377.7	1.146	0.253
Status					
Subordinate	-3.96E-02	2.66E-02	511.2	-1.491	0.137
Sex					
Male	2.06E-02	1.66E-02	269.2	1.242	0.215
Time Bled	**4.24E-04**	**6.16E-05**	**483.5**	**6.888**	**<0.001**
Time Wait	**-1.32E-03**	**4.10E-04**	**332.8**	**-3.208**	**0.001**
Age Linear x Status					
Subordinate	1.47E-05	3.01E-05	500.3	0.489	0.625

Table S5: Comparing the relative fit of models with different linear and non-linear age effects, in both cross-sectional and longitudinal model sets for each immune index. AIC = Akaike Information Criterion, ΔAIC = difference from best-fitting model in set, ω = model probability within model set, ER = evidence ratio relative to best-fitting model of set, μ/Δ = either mean (between-individual) or delta (within-individual) effects in partitioned longitudinal models, β = standardised effect size, CI = 95% confidence intervals of β standardised effect size, NA = values not applicable and '-' = values not computed as ΔAIC > 2 in the model set. β-estimates and associated CIs are of models with explanatory and response variables standardised with data values divided by two standard deviations (Gelman and Su, 2016; arm::standardize). For quadratic models, quadratic parameter estimates only are reported, linear components are not reported. Bold typeface shows the best-fitting model in each set, and italicised typeface shows models where ΔAIC < 2 and are similarly well-supported.

a) Haptoglobin

Set	Model	AIC	ΔAIC	ω	ER	μ/Δ	β (Std)	CI (Std)
Cross-sectional	Null model	**-106.56**	**0.00**	**0.47**	**1.00**	**NA**	**NA**	**NA**
	Age (linear)	*-104.64*	*1.92*	*0.18*	*2.61*	*NA*	*0.010*	*(-0.056, 0.075)*
	Age (quadratic) + Age (linear)	-103.89	2.67	0.12	3.79	NA	-	-
	Age (log)	*-104.99*	*1.57*	*0.22*	*2.19*	*NA*	*0.022*	*(-0.044, 0.089)*
	Age (factor)	-98.17	8.39	0.01	66.42	NA	-	-
Longitudinal	Null model	**-106.56**	**0.00**	**0.57**	**1.00**	**NA**	**NA**	**NA**
	μ age (linear) + Δ age (linear)	-103.31	3.25	0.11	5.07	μ	-	-
						Δ	-	-
	μ age (quadratic) + Δ age (quadratic) + μ age (linear)*Δ age (linear)	-100.65	5.91	0.03	19.20	μ	-	-
						Δ	-	-
	μ age (log) + Δ age (log)	*-105.23*	*1.33*	*0.29*	*1.94*	*μ*	*-0.003*	*(-0.066, 0.061)*
						Δ	*0.061*	*(-0.012, 0.134)*

b) Natural antibodies

Set	Model	AIC	ΔAIC	ω	ER	μ/Δ	β (Std)	CI (Std)
Cross-sectional	Null model	1865.28	12.44	0.00	503.81	NA	NA	NA
	Age (linear)	**1852.84**	**0.00**	**0.55**	**1.00**	**NA**	**-0.141**	**(-0.213, -0.069)**
	Age (quadratic) + Age (linear)	*1854.83*	*1.99*	*0.20*	*2.71*	*NA*	*0.004*	*(-0.097, 0.104)*
	Age (log)	*1854.74*	*1.90*	*0.21*	*2.59*	*NA*	*-0.133*	*(-0.206, -0.060)*
	Age (factor)	1858.63	5.79	0.03	18.10	NA	-	-
Longitudinal	Null model	1865.28	11.14	0.00	261.89	NA	NA	NA
	μ age (linear) + Δ age (linear)	**1854.14**	**0.00**	**0.67**	**1.00**	**μ**	**-0.134**	**(-0.203, -0.065)**
						Δ	**-0.040**	**(-0.122, 0.042)**
	μ age (quadratic) + Δ age (quadratic) + μ age (linear)*Δ age (linear)	1857.77	3.63	0.11	6.14	μ	-	-
						Δ	-	-

	μ age (log) + Δ age (log)	1856.44	2.30	0.21	3.16	μ	-	-
						Δ	-	-

c) Complement activity

Set	Model	AIC	ΔAIC	ω	ER	μ/Δ	β (Std)	CI (Std)
Cross-sectional	Null model	*935.99*	*0.55*	*0.27*	*1.32*	*NA*	*NA*	*NA*
	Age (linear)	*936.32*	*0.89*	*0.22*	*1.56*	*NA*	*0.061*	*(-0.032, 0.153)*
	Age (quadratic) + Age (linear)	*937.04*	*1.60*	*0.16*	*2.23*	*NA*	*-0.072*	*(-0.195, 0.052)*
	Age (log)	**935.43**	**0.00**	**0.35**	**1.00**	**NA**	**0.075**	**(-0.017, 0.166)**
	Age (factor)	950.14	14.71	0.00	1561.50	NA	-	-
Longitudinal	Null model	*935.99*	*0.02*	*0.35*	*1.01*	*NA*	*NA*	*NA*
	μ age (linear) + Δ age (linear)	*937.65*	*1.68*	*0.15*	*2.32*	*μ*	*0.034*	*(-0.056, 0.124)*
						Δ	*0.067*	*(-0.031, 0.164)*
	μ age (quadratic) + Δ age (quadratic) + μ age (linear)*Δ age (linear)	*937.94*	*1.97*	*0.13*	*2.68*	*μ*	*-0.043*	*(-0.179, 0.092)*
						Δ	*-0.385*	*(-0.717, -0.053)*
	μ age (log) + Δ age (log)	**935.96**	**0.00**	**0.36**	**1.00**	**μ**	**0.040**	**(-0.049, 0.129)**
						Δ	**0.087**	**(-0.006, 0.181)**

d) Heterophil-lymphocyte ratio

Set	Model	AIC	ΔAIC	ω	ER	μ/Δ	β (Std)	CI (Std)
Cross-sectional	Null model	-351.22	5.73	0.03	17.56	NA	NA	NA
	Age (linear)	**-356.95**	**0.00**	**0.47**	**1.00**	**NA**	**0.128**	**(0.038, 0.217)**
	Age (quadratic) + Age (linear)	*-355.01*	*1.94*	*0.18*	*2.64*	*NA*	*-0.015*	*(-0.133, 0.104)*
	Age (log)	*-356.14*	*0.81*	*0.32*	*1.50*	*NA*	*0.117*	*(0.030, 0.204)*
	Age (factor)	-346.89	10.06	0.00	153.05	NA	-	-
Longitudinal	Null model	-351.22	3.73	0.06	6.46	NA	NA	NA
	μ age (linear) + Δ age (linear)	**-354.95**	**0.00**	**0.37**	**1.00**	**μ**	**0.110**	**(0.023. 0.197)**
						Δ	**0.065**	**(-0.027, 0.155)**
	μ age (quadratic) + Δ age (quadratic) + μ age (linear)*Δ age (linear)	*-354.36*	*0.60*	*0.27*	*1.35*	*μ*	*-0.112*	*(-0.247, 0.023)*
						Δ	*0.178*	*(-0.090, 0.446)*
	μ age (log) + Δ age (log)	*-354.55*	*0.40*	*0.30*	*1.22*	*μ*	*0.114*	*(0.029, 0.199)*
						Δ	*0.035*	*(-0.051, 0.120)*

Table S6: General Additive Mixed Models (GAMMs) testing for more complex non-linear age-related change in immune indices. Fitted with *gamm4* package in R, age at capture (Age Linear) was included in each GAMM as a smooth term in respective cross-sectional models for **a)** haptoglobin, **b)** natural antibodies, **c)** complement activity and **d)** heterophil-lymphocyte ratio. For smooth terms, *β*-estimates and associated standard error are reported as coefficients for *f*(x1), with approximate significance of smooth terms; estimated degrees of freedom (edf) indicate increasing linearity as values approach 1. No model supports complex non-linear age-related change in immune indices, however, natural antibodies and heterophil-lymphocyte ratio indicate significant linear age-related change, as found in LMMs. Significant model parameters are highlighted in bold typeface. Reference category of 'Sex' variable is 'Female'.

a)

Response: Haptoglobin (n = 631)					
Fixed effects	***β***	**SE**	***t***	***p***	
Intercept	6.67E-01	4.71E-02	14.144	<0.001	-
Sex					
Male	-1.14E-02	1.71E-02	-0.670	0.503	-
Time Bled	1.21E-05	7.55E-05	0.160	0.873	-
Time Wait	-2.22E-04	4.78E-04	-0.465	0.642	-
Smooth terms	***β* (*f*(x1))**	**SE (*f*(x1))**	***F***	***p***	**edf**
s(Age Linear)	2.49E-03	9.17E-03	0.074	0.781	1.019
Random effects					σ^2
Individual ID	-	-	-	-	0.000
Plate ID	-	-	-	-	0.023
Season	-	-	-	-	0.008
Residual	-	-	-	-	0.043

b)

Response: Natural Antibodies (n = 506)					
Fixed effects	***β***	**SE**	***t***	***p***	
Intercept	9.66E+00	8.08E-01	11.962	<0.001	-
Sex					
Male	5.92E-02	1.33E-01	0.446	0.656	-
Time Bled	4.66E-04	5.36E-04	0.869	0.385	-
Time Wait	5.76E-03	3.70E-03	1.557	0.120	-
Plate Standard	**5.03E-01**	**6.72E-02**	**7.488**	**<0.001**	**-**
Smooth terms	***β* (*f*(x1))**	**SE (*f*(x1))**	***F***	***p***	**edf**
s(Age Linear)	**-2.62E-01**	**6.89E-02**	**14.440**	**<0.001**	**1**
Random effects					σ^2

Individual ID	-	-	-	-	0.000
Season	-	-	-	-	1.329
Residual	-	-	-	-	2.116

c)

Response: Complement Activity (n = 505)					
Fixed effects	***β***	**SE**	***t***	***p***	
Intercept	9.60E-01	2.81E-01	3.414	<0.001	-
Sex					
Male	-1.13E-02	5.72E-02	-0.197	0.844	-
Time Bled	-1.66E-04	2.18E-04	-0.762	0.446	-
Time Wait	-9.41E-04	1.48E-03	-0.636	0.525	-
Plate Standard	5.48E-02	6.76E-02	0.811	0.418	-
Smooth terms	***β* (*f*(x1))**	**SE (*f*(x1))**	***F***	***p***	**edf**
s(Age Linear)	3.85E-02	2.94E-02	1.718	0.191	1
Random effects					σ^2
Individual ID	-	-	-	-	0.039
Season	-	-	-	-	0.078
Residual	-	-	-	-	0.315

d)

Response: Heterophil-lymphocyte Ratio (n = 521)					
Fixed effects	***β***	**SE**	***t***	***p***	
Intercept	3.86E-01	2.69E-02	14.36	<0.001	-
Sex					
Male	1.59E-02	1.65E-02	0.965	0.335	-
Time Bled	**4.27E-04**	**6.20E-05**	**6.892**	**<0.001**	**-**
Time Wait	**-1.40E-03**	**4.11E-04**	**-3.401**	**<0.001**	**-**
Smooth terms	***β* (*f*(x1))**	**SE (*f*(x1))**	***F***	***p***	**edf**
s(Age Linear)	**2.38E-02**	**8.55E-03**	**7.730**	**0.006**	**1**
Random effects					σ^2
Individual ID	-	-	-	-	0.005
Season	-	-	-	-	0.001
Scorer ID	-	-	-	-	0.001
Residual	-	-	-	-	0.024

Table S7: Testing the difference between within- and between-individual effect slopes in longitudinal models. The difference between slopes of within- and between individual effects is estimated by rearrangement of the longitudinal model regression equation, from:

$$y_{ij} = \beta_0 + \beta_W\left(x_{ij} - \bar{x}_j\right) + \beta_B\bar{x}_j + u_{0j} + e_{0ij}$$

to:

$$y_{ij} = \beta_0 + \beta_W x_{ij} + (\beta_B - \beta_W)\bar{x}_j + u_{0j} + e_{0ij}$$

where within-subject centred 'Δ' and 'μ' variables are $\left(x_{ij} - \bar{x}_j\right)$ and $\bar{x}_j$ model parameters respectively. Here, the original age variable (not within-subject centred, x_{ij}) included in the model will produce an estimate identical to the within-individual estimate in the longitudinal model, and the 'μ' variable estimate will be equivalent to the difference between the slopes (Equation 3; van de Pol and Wright, 2009). A significant effect of this fixed effect in the new model indicates that the slopes of Δ and μ components in the longitudinal model are significantly different from one another. Tables **a-b)** correspond to the differences between slopes plotted in fig. 2 a-b.

a)

Response: Natural Antibodies (n = 506)					
Fixed effects	***β***	**SE**	**df**	***t***	***p***
Intercept	1.00E+01	7.89E-01	79.4	12.721	<0.001
Age Linear (β_W)	-1.78E-04	1.85E-04	505.1	-0.959	0.338
μ Age Linear (β_B- β_W)	-1.71E-04	2.05E-04	504.4	-0.832	0.406
Sex					
Male	6.88E-02	1.33E-01	499.7	0.519	0.604
Time Bled	4.89E-04	5.34E-04	503.8	0.917	0.360
Time Wait	5.88E-03	3.68E-03	505.9	1.597	0.111
Plate Standard	**4.98E-01**	**6.69E-02**	**504.4**	**7.451**	**<0.001**

b)

Response: Heterophil-lymphocyte Ratio (n = 521)					
Fixed effects	***β***	**SE**	**df**	***t***	***p***
Intercept	3.61E-01	2.68E-02	23.2	13.466	<0.001
Age Linear (β_W)	3.01E-05	2.12E-05	176.0	1.421	0.157
μ Age Linear (β_B- β_W)	-8.54E-07	2.37E-05	301.0	-0.036	0.971
Sex					
Male	1.61E-02	1.64E-02	252.5	0.981	0.327
Time Bled	**4.26E-04**	**6.17E-05**	**483.3**	**6.910**	**<0.001**
Time Wait	**-1.41E-03**	**4.07E-04**	**324.5**	**-3.467**	**<0.001**

Table S8: Testing whether final repeated measures in the sequence for each individual (either a terminal sample or failure to recapture) had an effect on the model conclusions. Survival greater than 90 days post-capture (i.e. to the next sampling period) was included as a binary variable, 'Survival90' to best-fitting longitudinal models. In **a-d)**, fixed effect outputs only from LMMs. β-estimates are from non-standardised models. Reference category of 'Sex' variable is 'Female'. Bold typeface indicates fixed effects with p-values < 0.05. Model outcomes were not changed, nor was 'Survival90' significant in any model.

a)

Response: Haptoglobin (n = 632)					
Fixed effects	***β***	**SE**	**df**	***t***	***p***
Intercept	6.83E-01	8.32E-02	254.2	8.209	<0.001
Δ Age Log	2.95E-02	2.04E-02	618.6	1.441	0.150
μ Age Log	3.99E-04	1.10E-02	609.9	0.036	0.971
Survival90	-2.18E-02	3.15E-02	612.1	-0.691	0.490
Sex					
Male	-1.05E-02	1.71E-02	604.5	-0.616	0.538
Time Bled	1.42E-05	7.50E-05	607.2	0.189	0.850
Time Wait	-1.99E-04	4.75E-04	616.4	-0.419	0.676

b)

Response: Natural Antibodies (n = 506)					
Fixed effects	***β***	**SE**	**df**	***t***	***p***
Intercept	1.05E+01	8.20E-01	92.4	12.778	<0.001
Δ Age Linear	-2.20E-04	1.86E-04	505.2	-1.182	0.238
μ Age Linear	**-3.21E-04**	**9.21E-05**	**500.0**	**-3.490**	**<0.001**
Survival90	-4.61E-01	2.46E-01	499.6	-1.871	0.062
Sex					
Male	6.54E-02	1.32E-01	499.7	0.495	0.621
Time Bled	4.38E-04	5.32E-04	503.8	0.822	0.411
Time Wait	5.78E-03	3.67E-03	505.8	1.577	0.115
Plate Standard	**4.95E-01**	**6.67E-02**	**504.3**	**7.421**	**<0.001**

c)

Response: Complement Activity (n = 505)					
Fixed effects	***β***	**SE**	**df**	***t***	***p***
Intercept	7.22E-01	3.54E-01	298.7	2.039	0.042
Δ Age Log	1.27E-01	6.53E-02	361.3	1.940	0.053
μ Age Log	2.50E-02	3.59E-02	303.1	0.696	0.487
Survival90	7.74E-02	1.00E-01	497.1	0.773	0.440
Sex					
Male	-7.44E-03	5.75E-02	255.2	-0.129	0.897
Time Bled	-1.36E-04	2.16E-04	503.9	-0.630	0.529
Time Wait	-9.20E-04	1.47E-03	477.0	-0.627	0.531
Plate Standard	5.34E-02	6.69E-02	463.0	0.799	0.425

d)

Response: Heterophil-lymphocyte Ratio (n = 521)					
Fixed effects	***β***	**SE**	**df**	***t***	***p***
Intercept	3.64E-01	3.72E-02	69.8	9.792	<0.001
Δ Age Linear	2.96E-05	2.15E-05	172.4	1.378	0.170
μ Age Linear	**2.94E-05**	**1.18E-05**	**233.6**	**2.489**	**0.014**
Survival90	-3.64E-03	2.87E-02	516.4	-0.127	0.899
Sex					
Male	1.61E-02	1.64E-02	251.7	0.979	0.329
Time Bled	**4.26E-04**	**6.17E-05**	**483.2**	**6.901**	**<0.001**
Time Wait	**-1.41E-03**	**4.07E-04**	**324.3**	**-3.468**	**<0.001**

Table S9: Complete model outputs for each final model assessed cross-sectionally and longitudinally with each immune index. Tables for cross-sectional and longitudinal model sets respectively are **a-b)** for haptoglobin, **c-d)** for natural antibodies, **e-f)** for complement activity, **g-h)** for heterophil-lymphocyte ratio. Models in each set are labelled **i-v)** in cross-sectional sets and **i-iv)** in longitudinal sets as age as a factor was not assessed longitudinally. F/R denote inclusion in model as a fixed or random effect, β-estimates are not standardised. Reference category for 'Sex' is 'Female' and for 'Age Factor' is 'Hatch Year'.

a) i)

Model Set: Cross-sectional; Response: Haptoglobin (n = 632), Null							
F/R	**Effect**	***β***	**SE**	**df**	***t***	***p***	σ^2
	Intercept	6.66E-01	4.60E-02	31.3	14.483	<0.001	-
F	Sex						
	Male	-1.09E-02	1.70E-02	605.1	-0.644	0.520	-
F	Time Bled	1.19E-05	7.51E-05	607.9	0.158	0.874	-
F	Time Wait	-2.25E-04	4.76E-04	616.1	-0.472	0.637	-
R	Individual ID	-	-	-	-	-	0.000
R	Plate ID	-	-	-	-	-	0.023
R	Season	-	-	-	-	-	0.007
	Residual	-	-	-	-	-	0.043

a) ii)

Model Set: Cross-sectional; Response: Haptoglobin (n = 632), Age Linear							
F/R	**Effect**	***β***	**SE**	**df**	***t***	***p***	σ^2
	Intercept	6.64E-01	4.69E-02	34.1	14.144	<0.001	-
F	Age Linear	2.86E-06	1.05E-05	611.4	0.272	0.786	-
F	Sex						
	Male	-1.12E-02	1.70E-02	604.8	-0.660	0.510	-
F	Time Bled	1.25E-05	7.51E-05	607.9	0.166	0.868	-
F	Time Wait	-2.24E-04	4.76E-04	616.2	-0.470	0.638	-
R	Individual ID	-	-	-	-	-	0.000
R	Plate ID	-	-	-	-	-	0.023
R	Season	-	-	-	-	-	0.007
	Residual	-	-	-	-	-	0.043

a) iii)

Model Set: Cross-sectional; Response: Haptoglobin (n = 632), Age Quadratic							
F/R	**Effect**	***β***	**SE**	**df**	***t***	***p***	σ^2
	Intercept	6.60E-01	4.69E-02	34.5	14.067	0.000	-
F	Age Quadratic	-1.07E-08	9.74E-09	605.5	-1.100	0.272	-
F	Age Linear	1.55E-05	1.56E-05	612.5	0.996	0.319	-
F	Sex						
	Male	-1.19E-02	1.70E-02	604.8	-0.702	0.483	-

F	Time Bled	1.44E-05	7.51E-05	607.8	0.191	0.848	-
F	Time Wait	-2.45E-04	4.75E-04	616.0	-0.516	0.606	-
R	Individual ID	-	-	-	-	-	0.000
R	Plate ID	-	-	-	-	-	0.023
R	Season	-	-	-	-	-	0.007
	Residual	-	-	-	-	-	0.043

a) iv)

Model Set: Cross-sectional; Response: Haptoglobin (n = 632), Age Log

F/R	Effect	β	SE	df	t	p	σ^2
	Intercept	6.28E-01	7.65E-02	199.7	8.212	<0.001	-
F	Age Linear	5.98E-03	9.60E-03	614.4	0.623	0.533	-
F	Sex						
	Male	-1.19E-02	1.71E-02	604.7	-0.699	0.485	-
F	Time Bled	1.43E-05	7.52E-05	607.7	0.190	0.849	-
F	Time Wait	-2.28E-04	4.75E-04	616.0	-0.479	0.632	-
R	Individual ID	-	-	-	-	-	0.000
R	Plate ID	-	-	-	-	-	0.023
R	Season	-	-	-	-	-	0.007
	Residual	-	-	-	-	-	0.043

a) v)

Model Set: Cross-sectional; Response: Haptoglobin (n = 632), Age Factor

F/R	Effect	β	SE	df	t	p	σ^2
	Intercept	6.55E-01	4.72E-02	36.2	13.868	<0.001	-
F	Age Factor						
	1+	6.44E-04	2.42E-02	616.5	0.027	0.979	-
	2+	4.31E-02	2.88E-02	612.2	1.496	0.135	-
	3+	1.70E-02	3.29E-02	615.2	0.517	0.605	-
	4+	6.36E-02	3.88E-02	612.8	1.642	0.101	-
	5+	-2.85E-02	3.68E-02	609.6	-0.773	0.440	-
	6+	1.66E-02	4.33E-02	606.8	0.383	0.702	-
	7+	9.64E-02	6.30E-02	604.8	1.530	0.126	-
	8+	-3.51E-02	7.41E-02	608.1	-0.474	0.636	-
	9+	-2.15E-02	5.58E-02	608.1	-0.386	0.700	-
F	Sex						
	Male	-1.09E-02	1.71E-02	604.3	-0.637	0.524	-
F	Time Bled	5.06E-06	7.50E-05	608.2	0.067	0.946	-
F	Time Wait	-1.95E-04	4.74E-04	615.6	-0.412	0.680	-
R	Individual ID	-	-	-	-	-	0.000
R	Plate ID	-	-	-	-	-	0.023
R	Season	-	-	-	-	-	0.007
	Residual	-	-	-	-	-	0.042

b) i)

Model Set: Longitudinal; Response: Haptoglobin (n = 632), Null

F/R	Effect	*β*	SE	df	*t*	*p*	σ^2
	Intercept	6.66E-01	4.60E-02	31.3	14.483	<0.001	-
F	Sex						
	Male	-1.09E-02	1.70E-02	605.1	-0.644	0.520	-
F	Time Bled	1.19E-05	7.51E-05	607.9	0.158	0.874	-
F	Time Wait	-2.25E-04	4.76E-04	616.1	-0.472	0.637	-
R	Individual ID	-	-	-	-	-	0.000
R	Plate ID	-	-	-	-	-	0.023
R	Season	-	-	-	-	-	0.007
	Residual	-	-	-	-	-	0.043

b) ii)

Model Set: Longitudinal; Response: Haptoglobin (n = 632), Age Linear

F/R	Effect	*β*	SE	df	*t*	*p*	σ^2
	Intercept	6.66E-01	4.68E-02	34.7	14.231	<0.001	-
F	Δ Age Linear	2.21E-05	2.58E-05	619.6	0.857	0.392	-
F	μ Age Linear	-8.24E-07	1.14E-05	607.1	-0.072	0.943	-
F	Sex						
	Male	-1.03E-02	1.71E-02	604.9	-0.602	0.547	-
F	Time Bled	1.28E-05	7.51E-05	607.4	0.170	0.865	-
F	Time Wait	-2.00E-04	4.76E-04	616.9	-0.420	0.674	-
R	Individual ID	-	-	-	-	-	0.000
R	Plate ID	-	-	-	-	-	0.023
R	Season	-	-	-	-	-	0.007
	Residual	-	-	-	-	-	0.042

b) iii)

Model Set: Longitudinal; Response: Haptoglobin (n = 632), Age Quadratic

F/R	Effect	*β*	SE	df	*t*	*p*	σ^2
	Intercept	6.61E-01	4.64E-02	35.8	14.265	<0.001	-
F	Δ Age Quadratic	-9.33E-08	5.46E-08	612.1	-1.708	0.088	-
F	μ Age Quadratic	-6.88E-09	1.20E-08	608.7	-0.575	0.566	-
F	Δ Age Linear	-1.24E-04	1.10E-04	614.5	-1.132	0.258	-
F	μ Age Linear	8.02E-06	1.89E-05	612.5	0.424	0.672	-
F	Δ Age Linear x μ Age Linear	1.58E-07	1.05E-07	612.1	1.501	0.134	-
F	Sex						
	Male	-1.07E-02	1.70E-02	605.2	-0.628	0.530	-
F	Time Bled	1.97E-05	7.50E-05	606.1	0.263	0.793	-
F	Time Wait	-2.45E-04	4.76E-04	616.4	-0.515	0.606	-
R	Individual ID	-	-	-	-	-	0.000
R	Plate ID	-	-	-	-	-	0.023

R	Season	-	-	-	-	-	0.006
	Residual	-	-	-	-	-	0.042

b) iv)

Model Set: Longitudinal; Response: Haptoglobin (n = 632), Age Log

F/R	Effect	*β*	SE	df	*t*	*p*	σ^2
	Intercept	6.73E-01	8.19E-02	248.7	8.216	<0.001	-
F	Δ Age Log	3.25E-02	2.00E-02	617.9	1.624	0.105	-
F	μ Age Log	-1.30E-03	1.07E-02	611.4	-0.121	0.904	-
F	Sex						
	Male	-9.81E-03	1.71E-02	604.5	-0.575	0.566	-
F	Time Bled	1.51E-05	7.50E-05	607.1	0.201	0.840	-
F	Time Wait	-1.90E-04	4.75E-04	616.4	-0.399	0.690	-
R	Individual ID	-	-	-	-	-	0.000
R	Plate ID	-	-	-	-	-	0.023
R	Season	-	-	-	-	-	0.007
	Residual	-	-	-	-	-	0.042

c) i)

Model Set: Cross-sectional; Response: Natural Antibodies (n = 506), Null

F/R	Effect	*β*	SE	df	*t*	*p*	σ^2
	Intercept	9.85E+00	8.09E-01	74.1	12.178	<0.001	-
F	Sex						
	Male	2.01E-02	1.35E-01	500.5	0.149	0.882	-
F	Time Bled	3.70E-04	5.44E-04	504.5	0.679	0.497	-
F	Time Wait	5.28E-03	3.76E-03	506.9	1.403	0.161	-
F	**Plate Standard**	**4.91E-01**	**6.83E-02**	**505.2**	**7.182**	**<0.001**	-
R	Individual ID	-	-	-	-	-	0.000
R	Season	-	-	-	-	-	1.245
	Residual	-	-	-	-	-	2.193

c) ii)

Model Set: Cross-sectional; Response: Natural Antibodies (n = 506), Age Linear

F/R	Effect	*β*	SE	df	*t*	*p*	σ^2
	Intercept	9.98E+00	7.95E-01	78.4	12.559	<0.001	-
F	**Age Linear**	**-2.95E-04**	**8.32E-05**	**502.5**	**-3.549**	**<0.001**	-
F	Sex						
	Male	4.43E-02	1.34E-01	500.5	0.332	0.740	-
F	Time Bled	5.08E-04	5.39E-04	504.8	0.943	0.346	-
F	Time Wait	5.45E-03	3.72E-03	506.9	1.465	0.144	-
F	**Plate Standard**	**5.00E-01**	**6.75E-02**	**505.1**	**7.401**	**<0.001**	-
R	Individual ID	-	-	-	-	-	0.000
R	Season	-	-	-	-	-	1.157

	Residual	-	-	-	-	-	2.141

c) iii)

Model Set: Cross-sectional; Response: Natural Antibodies (n = 506), Age Quadratic

F/R	Effect	*β*	SE	df	*t*	*p*	σ^2
	Intercept	9.98E+00	7.95E-01	78.4	12.560	<0.001	-
F	Age Quadratic	-1.12E-08	7.68E-08	500.2	-0.146	0.884	-
F	**Age Linear**	**-2.83E-04**	**1.21E-04**	**501.7**	**-2.341**	**0.020**	-
F	Sex						
	Male	4.34E-02	1.34E-01	500.5	0.325	0.746	-
F	Time Bled	5.08E-04	5.39E-04	504.8	0.943	0.346	-
F	Time Wait	5.43E-03	3.72E-03	506.9	1.459	0.145	-
F	**Plate Standard**	**4.99E-01**	**6.76E-02**	**505.1**	**7.391**	**<0.001**	-
R	Individual ID	-	-	-	-	-	0.000
R	Season	-	-	-	-	-	1.157
	Residual	-	-	-	-	-	2.141

c) iv)

Model Set: Cross-sectional; Response: Natural Antibodies (n = 506), Age Log

F/R	Effect	*β*	SE	df	*t*	*p*	σ^2
	Intercept	1.12E+01	9.11E-01	117.7	12.317	<0.001	-
F	**Age Log**	**-2.31E-01**	**7.46E-02**	**502.5**	**-3.099**	**0.002**	-
F	Sex						
	Male	6.15E-02	1.34E-01	500.5	0.458	0.647	-
F	Time Bled	4.27E-04	5.39E-04	504.7	0.792	0.429	-
F	Time Wait	5.61E-03	3.73E-03	506.9	1.505	0.133	-
F	**Plate Standard**	**4.97E-01**	**6.77E-02**	**505.1**	**7.346**	**<0.001**	-
R	Individual ID	-	-	-	-	-	0.000
R	Season	-	-	-	-	-	1.177
	Residual	-	-	-	-	-	2.153

c) v)

Model Set: Cross-sectional; Response: Natural Antibodies (n = 506), Age Factor

F/R	Effect	*β*	SE	df	*t*	*p*	σ^2
	Intercept	9.85E+00	7.95E-01	77.9	12.385	<0.001	-
F	Age Factor						
	1+	-8.95E-02	1.85E-01	502.0	-0.484	0.629	-
	2+	-1.99E-01	2.28E-01	501.6	-0.872	0.384	-
	3+	-3.02E-01	2.69E-01	502.1	-1.123	0.262	-
	4+	-3.92E-01	2.84E-01	501.4	-1.382	0.168	-
	5+	**-9.72E-01**	**2.81E-01**	**500.6**	**-3.459**	**<0.001**	-
	6+	-4.37E-01	3.30E-01	501.0	-1.323	0.186	-
	7+	-2.87E-01	4.60E-01	500.6	-0.624	0.533	-

	8+	8.99E-02	5.77E-01	501.1	0.156	0.876	-
	9+	**-1.64E+00**	**4.88E-01**	**501.1**	**-3.361**	**<0.001**	-
F	Sex						
	Male	7.39E-02	1.35E-01	500.2	0.546	0.585	-
F	Time Bled	4.31E-04	5.38E-04	505.1	0.801	0.424	-
F	Time Wait	5.20E-03	3.71E-03	506.8	1.402	0.162	-
F	**Plate Standard**	**5.08E-01**	**6.74E-02**	**504.9**	**7.536**	**<0.001**	-
R	Individual ID	-	-	-	-	-	0.000
R	Season	-	-	-	-	-	1.164
	Residual	-	-	-	-	-	2.099

d) i)

Model Set: Longitudinal; Response: Natural Antibodies (n = 506), Null

F/R	Effect	β	SE	df	t	p	σ^2
	Intercept	9.85E+00	8.09E-01	74.1	12.178	<0.001	-
F	Sex						
	Male	2.01E-02	1.35E-01	500.5	0.149	0.882	-
F	Time Bled	3.70E-04	5.44E-04	504.5	0.679	0.497	-
F	Time Wait	5.28E-03	3.76E-03	506.9	1.403	0.161	-
F	**Plate Standard**	**4.91E-01**	**6.83E-02**	**505.2**	**7.182**	**<0.001**	-
R	Individual ID	-	-	-	-	-	0.000
R	Season	-	-	-	-	-	1.245
	Residual	-	-	-	-	-	2.193

d) ii)

Model Set: Longitudinal; Response: Natural Antibodies (n = 506), Age Linear

F/R	Effect	β	SE	df	t	p	σ^2
	Intercept	1.00E+01	7.98E-01	78.7	12.576	<0.001	-
F	Δ Age Linear	-1.51E-04	1.87E-04	506.0	-0.808	0.420	-
F	**μ Age Linear**	**-3.29E-04**	**9.20E-05**	**501.1**	**-3.576**	**<0.001**	-
F	Sex						
	Male	5.46E-02	1.34E-01	500.7	0.407	0.684	-
F	Time Bled	5.31E-04	5.39E-04	504.8	0.984	0.325	-
F	Time Wait	5.58E-03	3.72E-03	506.9	1.500	0.134	-
F	**Plate Standard**	**4.96E-01**	**6.76E-02**	**505.5**	**7.337**	**<0.001**	-
R	Individual ID	-	-	-	-	-	0.000
R	Season	-	-	-	-	-	1.169
	Residual	-	-	-	-	-	2.138

d) iii)

Model Set: Longitudinal; Response: Natural Antibodies (n = 506), Age Quadratic

F/R	Effect	β	SE	df	t	p	σ^2
	Intercept	9.97E+00	8.00E-01	76.4	12.464	<0.001	-

F	Δ Age Quadratic	5.18E-07	4.13E-07	501.5	1.254	0.210	-
F	μ Age Quadratic	-9.04E-08	9.50E-08	500.3	-0.952	0.342	-
F	Δ Age Linear	4.53E-04	7.96E-04	502.0	0.569	0.570	-
F	μ Age Linear	-2.24E-04	1.42E-04	501.0	-1.573	0.116	-
F	Δ Age Linear x μ Age Linear	-7.61E-07	7.83E-07	501.4	-0.973	0.331	-
F	Sex						
	Male	4.35E-02	1.34E-01	500.7	0.325	0.746	-
F	Time Bled	6.20E-04	5.40E-04	504.4	1.148	0.252	-
F	Time Wait	5.66E-03	3.72E-03	506.9	1.520	0.129	-
F	**Plate Standard**	**4.98E-01**	**6.76E-02**	**505.7**	**7.379**	**<0.001**	-
R	Individual ID	-	-	-	-	-	0.000
R	Season	-	-	-	-	-	1.196
	Residual	-	-	-	-	-	2.125

d) iv)

Model Set: Longitudinal; Response: Natural Antibodies (n = 506), Age Log

F/R	Effect	*β*	SE	df	*t*	*p*	σ^2
	Intercept	1.13E+01	9.45E-01	132.6	12.006	<0.001	-
F	Δ Age Log	-1.61E-01	1.67E-01	505.3	-0.967	0.334	-
F	**μ Age Log**	**-2.47E-01**	**8.22E-02**	**501.4**	**-3.010**	**0.003**	-
F	Sex						
	Male	6.91E-02	1.35E-01	500.8	0.511	0.610	-
F	Time Bled	4.36E-04	5.40E-04	504.7	0.808	0.419	-
F	Time Wait	5.69E-03	3.73E-03	506.9	1.525	0.128	-
F	**Plate Standard**	**4.95E-01**	**6.78E-02**	**505.2**	**7.298**	**<0.001**	-
R	Individual ID	-	-	-	-	-	0.000
R	Season	-	-	-	-	-	1.180
	Residual	-	-	-	-	-	2.152

e) i)

Model Set: Cross-sectional; Response: Complement Activity (n = 505), Null

F/R	Effect	*β*	SE	df	*t*	*p*	σ^2
	Intercept	9.29E-01	2.74E-01	167.8	3.387	<0.001	-
F	Sex						
	Male	-9.74E-03	5.69E-02	255.6	-0.171	0.864	-
F	Time Bled	-1.39E-04	2.16E-04	504.8	-0.641	0.522	-
F	Time Wait	-1.07E-03	1.47E-03	470.9	-0.731	0.465	-
F	Plate Standard	5.99E-02	6.71E-02	455.2	0.894	0.372	-
R	Individual ID	-	-	-	-	-	0.039
R	Season	-	-	-	-	-	0.058
	Residual	-	-	-	-	-	0.314

e) ii)

Model Set: Cross-sectional; Response: Complement Activity (n = 505), Age Linear

F/R	Effect	*β*	SE	df	*t*	*p*	σ^2
	Intercept	9.04E-01	2.76E-01	164.8	3.279	0.001	-
F	Age Linear	4.87E-05	3.50E-05	321.4	1.392	0.165	-
F	Sex						
	Male	-1.39E-02	5.69E-02	256.3	-0.245	0.807	-
F	Time Bled	-1.56E-04	2.16E-04	504.8	-0.721	0.471	-
F	Time Wait	-1.07E-03	1.47E-03	475.7	-0.730	0.466	-
F	Plate Standard	5.68E-02	6.70E-02	459.4	0.847	0.397	-
R	Individual ID	-	-	-	-	-	0.038
R	Season	-	-	-	-	-	0.063
	Residual	-	-	-	-	-	0.313

e) iii)

Model Set: Cross-sectional; Response: Complement Activity (n = 505), Age Quadratic

F/R	Effect	*β*	SE	df	*t*	*p*	σ^2
	Intercept	8.90E-01	2.76E-01	164.1	3.225	0.002	-
F	Age Quadratic	-3.80E-08	3.13E-08	488.9	-1.215	0.225	-
F	Age Linear	9.07E-05	4.91E-05	467.2	1.847	0.065	-
F	Sex						
	Male	-1.76E-02	5.70E-02	258.2	-0.309	0.758	-
F	Time Bled	-1.56E-04	2.16E-04	504.6	-0.723	0.470	-
F	Time Wait	-1.10E-03	1.47E-03	475.9	-0.747	0.456	-
F	Plate Standard	5.79E-02	6.69E-02	460.4	0.865	0.388	-
R	Individual ID	-	-	-	-	-	0.040
R	Season	-	-	-	-	-	0.064
	Residual	-	-	-	-	-	0.311

e) iv)

Model Set: Cross-sectional; Response: Complement Activity (n = 505), Age Log

F/R	Effect	*β*	SE	df	*t*	*p*	σ^2
	Intercept	6.24E-01	3.36E-01	293.9	1.859	0.064	-
F	Age Log	5.01E-02	3.12E-02	392.1	1.605	0.109	-
F	Sex						
	Male	-1.70E-02	5.71E-02	257.2	-0.297	0.767	-
F	Time Bled	-1.51E-04	2.16E-04	504.1	-0.698	0.485	-
F	Time Wait	-1.06E-03	1.47E-03	475.2	-0.720	0.472	-
F	Plate Standard	5.79E-02	6.69E-02	461.3	0.866	0.387	-
R	Individual ID	-	-	-	-	-	0.039
R	Season	-	-	-	-	-	0.063
	Residual	-	-	-	-	-	0.311

e) v)

Model Set: Cross-sectional; Response: Complement Activity (n = 505), Age Factor

F/R	Effect	*β*	SE	df	*t*	*p*	σ^2
	Intercept	9.11E-01	2.78E-01	166.9	3.277	0.001	-
F	Age Factor						
	1+	2.77E-02	7.58E-02	502.6	0.365	0.715	-
	2+	3.35E-02	9.20E-02	497.0	0.365	0.716	-
	3+	1.59E-01	1.08E-01	487.6	1.480	0.140	-
	4+	8.10E-02	1.19E-01	501.7	0.682	0.496	-
	5+	3.31E-02	1.14E-01	501.3	0.290	0.772	-
	6+	1.19E-01	1.35E-01	497.6	0.879	0.380	-
	7+	1.75E-01	1.89E-01	497.3	0.929	0.353	-
	8+	2.85E-01	2.21E-01	502.0	1.290	0.198	-
	9+	4.86E-02	2.07E-01	370.0	0.235	0.814	-
F	Sex						
	Male	-1.13E-02	5.81E-02	262.0	-0.194	0.847	-
F	Time Bled	-1.73E-04	2.18E-04	504.5	-0.797	0.426	-
F	Time Wait	-1.19E-03	1.48E-03	473.4	-0.804	0.422	-
F	Plate Standard	5.58E-02	6.73E-02	461.8	0.830	0.407	-
R	Individual ID	-	-	-	-	-	0.038
R	Season	-	-	-	-	-	0.063
	Residual	-	-	-	-	-	0.312

f) i)

Model Set: Longitudinal; Response: Complement Activity (n = 505), Null

F/R	Effect	*β*	SE	df	*t*	*p*	σ^2
	Intercept	9.29E-01	2.74E-01	167.8	3.387	<0.001	-
F	Sex						
	Male	-9.74E-03	5.69E-02	255.6	-0.171	0.864	-
F	Time Bled	-1.39E-04	2.16E-04	504.8	-0.641	0.522	-
F	Time Wait	-1.07E-03	1.47E-03	470.9	-0.731	0.465	-
F	Plate Standard	5.99E-02	6.71E-02	455.2	0.894	0.372	-
R	Individual ID	-	-	-	-	-	0.039
R	Season	-	-	-	-	-	0.058
	Residual	-	-	-	-	-	0.314

f) ii)

Model Set: Longitudinal; Response: Complement Activity (n = 505), Age Linear

F/R	Effect	*β*	SE	df	*t*	*p*	σ^2
	Intercept	9.15E-01	2.76E-01	160.7	3.310	0.001	-
F	Δ Age Linear	1.01E-04	7.22E-05	397.0	1.403	0.161	-
F	μ Age Linear	3.31E-05	3.97E-05	228.6	0.834	0.405	-
F	Sex						

	Male	-9.87E-03	5.70E-02	255.9	-0.173	0.863	-
F	Time Bled	-1.49E-04	2.16E-04	505.0	-0.686	0.493	-
F	Time Wait	-1.01E-03	1.47E-03	476.0	-0.691	0.490	-
F	Plate Standard	5.68E-02	6.70E-02	462.4	0.847	0.397	-
R	Individual ID	-	-	-	-	-	0.039
R	Season	-	-	-	-	-	0.065
	Residual	-	-	-	-	-	0.312

f) iii)

Model Set: Longitudinal; Response: Complement Activity (n = 505), Age Quadratic

F/R	Effect	*β*	SE	df	*t*	*p*	σ^2
	Intercept	9.30E-01	2.77E-01	163.2	3.362	<0.001	-
F	**Δ Age Quadratic**	**-3.56E-07**	**1.57E-07**	**263.0**	**-2.264**	**0.024**	-
F	μ Age Quadratic	-2.98E-08	4.05E-08	278.7	-0.736	0.463	-
F	Δ Age Linear	-4.50E-04	3.08E-04	269.6	-1.460	0.145	-
F	μ Age Linear	6.48E-05	5.97E-05	322.8	1.086	0.278	-
F	**Δ Age Linear x μ Age Linear**	**6.15E-07**	**2.99E-07**	**261.9**	**2.054**	**0.041**	-
F	Sex						
	Male	-1.14E-02	5.72E-02	256.5	-0.199	0.843	-
F	Time Bled	-1.66E-04	2.16E-04	504.6	-0.769	0.442	-
F	Time Wait	-1.23E-03	1.46E-03	474.6	-0.838	0.402	-
F	Plate Standard	5.22E-02	6.68E-02	462.6	0.781	0.435	-
R	Individual ID	-	-	-	-	-	0.042
R	Season	-	-	-	-	-	0.064
	Residual	-	-	-	-	-	0.305

f) iv)

Model Set: Longitudinal; Response: Complement Activity (n = 505), Age Log

F/R	Effect	*β*	SE	df	*t*	*p*	σ^2
	Intercept	7.18E-01	3.51E-01	293.9	2.046	0.042	-
F	Δ Age Log	1.22E-01	6.46E-02	358.4	1.889	0.060	-
F	μ Age Log	3.63E-02	3.47E-02	300.7	1.046	0.296	-
F	Sex						
	Male	-1.23E-02	5.74E-02	256.5	-0.215	0.830	-
F	Time Bled	-1.36E-04	2.16E-04	505.1	-0.630	0.529	-
F	Time Wait	-1.01E-03	1.47E-03	477.3	-0.686	0.493	-
F	Plate Standard	5.48E-02	6.69E-02	462.8	0.819	0.413	-
R	Individual ID	-	-	-	-	-	0.040
R	Season	-	-	-	-	-	0.066
	Residual	-	-	-	-	-	0.310

g) i)

Model Set: Cross-sectional; Response: Heterophil-lymphocyte Ratio (n = 521), Null							
F/R	**Effect**	***β***	**SE**	**df**	***t***	***p***	σ^2
	Intercept	3.86E-01	2.56E-02	17.7	15.061	<0.001	-
F	Sex						
	Male	1.79E-02	1.66E-02	255.0	1.082	0.280	-
F	**Time Bled**	**4.35E-04**	**6.22E-05**	**494.3**	**6.989**	**<0.001**	-
F	**Time Wait**	**-1.38E-03**	**4.12E-04**	**339.4**	**-3.342**	**<0.001**	-
R	Individual ID	-	-	-	-	-	0.005
R	Season	-	-	-	-	-	0.001
R	Scorer ID	-	-	-	-	-	0.000
	Residual	-	-	-	-	-	0.024

g) ii)

Model Set: Cross-sectional; Response: Heterophil-lymphocyte Ratio (n = 521), Age Linear							
F/R	**Effect**	***β***	**SE**	**df**	***t***	***p***	σ^2
	Intercept	3.61E-01	2.66E-02	20.9	13.580	<0.001	-
F	**Age Linear**	**2.94E-05**	**1.05E-05**	**302.2**	**2.811**	**0.005**	-
F	Sex						
	Male	1.61E-02	1.64E-02	253.0	0.981	0.328	-
F	**Time Bled**	**4.26E-04**	**6.17E-05**	**483.8**	**6.913**	**<0.001**	-
F	**Time Wait**	**-1.41E-03**	**4.07E-04**	**325.1**	**-3.466**	**<0.001**	-
R	Individual ID	-	-	-	-	-	0.005
R	Season	-	-	-	-	-	0.001
R	Scorer ID	-	-	-	-	-	0.000
	Residual	-	-	-	-	-	0.024

g) iii)

Model Set: Cross-sectional; Response: Heterophil-lymphocyte Ratio (n = 521), Age Quadratic							
F/R	**Effect**	***β***	**SE**	**df**	***t***	***p***	σ^2
	Intercept	3.60E-01	2.67E-02	21.3	13.474	<0.001	-
F	Age Quadratic	-2.26E-09	9.15E-09	493.6	-0.247	0.805	-
F	**Age Linear**	**3.18E-05**	**1.43E-05**	**460.9**	**2.225**	**0.027**	-
F	Sex						
	Male	1.59E-02	1.64E-02	249.9	0.972	0.332	-
F	**Time Bled**	**4.27E-04**	**6.17E-05**	**483.2**	**6.919**	**<0.001**	-
F	**Time Wait**	**-1.41E-03**	**4.07E-04**	**325.0**	**-3.460**	**<0.001**	-
R	Individual ID	-	-	-	-	-	0.005
R	Season	-	-	-	-	-	0.001
R	Scorer ID	-	-	-	-	-	0.000
	Residual	-	-	-	-	-	0.024

g) iv)

Model Set: Cross-sectional; Response: Heterophil-lymphocyte Ratio (n = 521), Age Log

F/R	Effect	*β*	SE	df	*t*	*p*	σ^2
	Intercept	2.37E-01	6.11E-02	159.1	3.876	<0.001	-
F	**Age Log**	**2.33E-02**	**8.76E-03**	**394.8**	**2.661**	**0.008**	-
F	Sex						
	Male	1.45E-02	1.64E-02	250.8	0.888	0.375	-
F	**Time Bled**	**4.36E-04**	**6.17E-05**	**490.0**	**7.066**	**<0.001**	-
F	**Time Wait**	**-1.38E-03**	**4.09E-04**	**334.7**	**-3.389**	**<0.001**	-
R	Individual ID	-	-	-	-	-	0.004
R	Season	-	-	-	-	-	0.001
R	Scorer ID	-	-	-	-	-	0.000
	Residual	-	-	-	-	-	0.024

g) v)

Model Set: Cross-sectional; Response: Heterophil-lymphocyte Ratio (n = 521), Age Factor

F/R	Effect	*β*	SE	df	*t*	*p*	σ^2
	Intercept	3.64E-01	2.74E-02	22.1	13.284	<0.001	-
F	Age Factor						
	1+	1.20E-02	2.06E-02	509.0	0.584	0.560	-
	2+	3.74E-02	2.41E-02	506.8	1.553	0.121	-
	3+	4.78E-02	2.82E-02	508.3	1.696	0.091	-
	4+	-3.20E-03	3.34E-02	512.2	-0.096	0.924	-
	5+	**7.70E-02**	**3.18E-02**	**511.8**	**2.422**	**0.016**	-
	6+	3.57E-02	3.98E-02	501.7	0.897	0.370	-
	7+	5.60E-02	5.09E-02	518.7	1.101	0.271	-
	8+	5.00E-02	7.02E-02	509.8	0.712	0.477	-
	9+	**1.63E-01**	**6.29E-02**	**376.2**	**2.591**	**0.010**	-
F	Sex						
	Male	1.42E-02	1.66E-02	251.9	0.857	0.392	-
F	**Time Bled**	**4.33E-04**	**6.16E-05**	**473.7**	**7.027**	**<0.001**	-
F	**Time Wait**	**-1.44E-03**	**4.06E-04**	**307.7**	**-3.538**	**<0.001**	-
R	Individual ID	-	-	-	-	-	0.005
R	Season	-	-	-	-	-	0.001
R	Scorer ID	-	-	-	-	-	0.000
	Residual	-	-	-	-	-	0.023

h) i)

Model Set: Longitudinal; Response: Heterophil-lymphocyte Ratio (n = 521), Null

F/R	Effect	*β*	SE	df	*t*	*p*	σ^2
	Intercept	3.86E-01	2.56E-02	17.7	15.061	<0.001	-
F	Sex						
	Male	1.79E-02	1.66E-02	255.0	1.082	0.280	-

F	**Time Bled**	**4.35E-04**	**6.22E-05**	**494.3**	**6.989**	**<0.001**	-
F	**Time Wait**	**-1.38E-03**	**4.12E-04**	**339.4**	**-3.342**	**<0.001**	-
R	Individual ID	-	-	-	-	-	0.005
R	Season	-	-	-	-	-	0.001
R	Scorer ID	-	-	-	-	-	0.000
	Residual	-	-	-	-	-	0.024

h) ii)

Model Set: Longitudinal; Response: Heterophil-lymphocyte Ratio (n = 521), Age Linear

F/R	Effect	*β*	SE	df	*t*	*p*	σ^2
	Intercept	3.61E-01	2.68E-02	23.2	13.466	<0.001	-
F	Δ Age Linear	3.01E-05	2.12E-05	176.0	1.421	0.157	-
F	**μ Age Linear**	**2.92E-05**	**1.17E-05**	**231.9**	**2.493**	**0.013**	-
F	Sex						
	Male	1.61E-02	1.64E-02	252.5	0.981	0.327	-
F	**Time Bled**	**4.26E-04**	**6.17E-05**	**483.3**	**6.910**	**<0.001**	-
F	**Time Wait**	**-1.41E-03**	**4.07E-04**	**324.5**	**-3.467**	**<0.001**	-
R	Individual ID	-	-	-	-	-	0.005
R	Season	-	-	-	-	-	0.001
R	Scorer ID	-	-	-	-	-	0.000
	Residual	-	-	-	-	-	0.024

h) iii)

Model Set: Longitudinal; Response: Heterophil-lymphocyte Ratio (n = 521), Age Quadratic

F/R	Effect	*β*	SE	df	*t*	*p*	σ^2
	Intercept	3.55E-01	2.63E-02	25.3	13.465	<0.001	-
F	Δ Age Quadratic	5.38E-08	4.12E-08	272.9	1.306	0.193	-
F	μ Age Quadratic	-2.00E-08	1.22E-08	236.3	-1.637	0.103	-
F	Δ Age Linear	7.67E-05	8.05E-05	272.4	0.953	0.341	-
F	**μ Age Linear**	**5.14E-05**	**1.78E-05**	**317.1**	**2.893**	**0.004**	-
F	Δ Age Linear x μ Age Linear	-6.94E-08	8.09E-08	273.4	-0.858	0.392	-
F	Sex						
	Male	1.47E-02	1.63E-02	250.1	0.902	0.368	-
F	**Time Bled**	**4.41E-04**	**6.19E-05**	**482.8**	**7.132**	**<0.001**	-
F	**Time Wait**	**-1.44E-03**	**4.04E-04**	**304.1**	**-3.559**	**<0.001**	-
R	Individual ID	-	-	-	-	-	0.004
R	Season	-	-	-	-	-	0.001
R	Scorer ID	-	-	-	-	-	0.000
	Residual	-	-	-	-	-	0.024

h) iv)

Model Set: Longitudinal; Response: Heterophil-lymphocyte Ratio (n = 521), Age Log							
F/R	**Effect**	***β***	**SE**	**df**	***t***	***p***	σ^2
	Intercept	2.19E-01	6.74E-02	243.7	3.249	0.001	-
F	Δ Age Log	1.38E-02	1.71E-02	256.9	0.811	0.418	-
F	**μ Age Log**	**2.63E-02**	**9.93E-03**	**304.3**	**2.650**	**0.008**	-
F	Sex						
	Male	1.38E-02	1.64E-02	249.3	0.842	0.401	-
F	**Time Bled**	**4.37E-04**	**6.17E-05**	**493.1**	**7.084**	**<0.001**	-
F	**Time Wait**	**-1.39E-03**	**4.09E-04**	**335.6**	**-3.399**	**<0.001**	-
R	Individual ID	-	-	-	-	-	0.004
R	Season	-	-	-	-	-	0.001
R	Scorer ID	-	-	-	-	-	0.000
	Residual	-	-	-	-	-	0.024

Chapter 4: Fitness outcomes in relation to individual variation in constitutive innate immune function

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Abstract

Maintenance of immune functions is crucial for the survival of organisms in the face of parasite and pathogen pressure. However, costs of immune function may result in trade-offs with competing physiological processes that translate into costs for (other) fitness components. Optimising, not maximising, immune function is therefore assumed to be the best solution for hosts to maximise overall fitness. The maintenance costs of immune function are considered relatively low compared to immune activation and have seldom been investigated in relation to fitness in the wild. Here, we assess how measures of constitutive immune function (haptoglobin, natural antibodies, complement activity) are related to subsequent fitness outcomes (survival, reproductive success, dominance acquisition) in a wild passerine, the purple-crowned fairy-wren (*Malurus coronatus*). We also investigate whether chronic stress (heterophil-lymphocyte ratio) and body condition (size-adjusted body mass as a proxy for energetic reserves) relate to fitness outcomes to assess whether environmental stressors or resource availability might mediate any trade-off. We found that increased survival is not related to high (maximal) or intermediate (optimal) levels of immune indices. Rather, both low and high values of complement activity (quadratic) were associated with increased survival, suggesting immune investment is context-dependent and the optimal strategy might relate to the immediate disease environment. Positive relationships between immune indices – significantly natural antibodies – and reproductive success suggest that variation in individual quality prevails over potential resource reallocation trade-offs within individuals. Body condition did not relate to any fitness-related outcome. Lastly, dominance acquisition was positively related only to chronic stress (HL ratio), which we interpret as linked to intra-specific competition. Overall, our results do not provide evidence that maintaining baseline levels of these constitutive immune components comes at a net cost or benefit for fitness, suggesting that they might be quite tightly regulated according to individual quality.

Keywords: maintenance cost, fitness, trade-off, resource reallocation, constitutive, induced

Introduction

Immune defences are crucial for the health and survival of host organisms among a diversity of parasites, pathogens and diseases. Considering this importance, any individual variation in host immune function is likely to be consequential for defence against disease-causing agents and host survival and longevity (Schmid-Hempel, 2003). Additionally, many parasites and pathogens have sub-lethal effects (Alizon et al., 2009) that either directly or indirectly influence host fitness (lifetime reproductive success). Infections and diseases can disrupt the host's reproductive pathways (Graham et al., 2011; Hall et al., 2007), the ability to acquire resources (Binning et al., 2017; Gegear et al., 2006), ornamentation and the signalling of attractiveness to a mate (Brawner et al., 2000; Shawkey et al., 2009), or even social dominance or prestige (Buck et al., 2018; Poirotte et al., 2017; Rau, 1984, 1983). Parasites can therefore affect many 'fitness-related traits' that will ultimately have consequences for overall fitness of the host. For hosts, the level of investment into diverse and multi-faceted immune systems is expected to reflect parasite pressure (Schulenburg et al., 2009), and should therefore relate to overall fitness.

Operating an immune system is physiologically and energetically costly, incurring development, maintenance, and deployment costs, in addition to any collateral damage sustained through immune-associated inflammation (Ashley et al., 2012; Klasing, 2004). Consequently, when resources are a limiting factor, trade-offs are expected to occur between immune function and other key physiologically demanding processes (Lochmiller and Deerenberg, 2000; Norris and Evans, 2000). Optimising, rather than maximising, immune function, might therefore be the best solution for hosts to maximise overall fitness (Sheldon and Verhulst, 1996; Viney et al., 2005), and as a result tolerance to parasites can emerge (Medzhitov et al., 2012; Råberg et al., 2007). Although there is some uncertainty over when and how the costs of immune function are paid physiologically (Hasselquist and Nilsson, 2012; Lee, 2006; Lochmiller and Deerenberg, 2000), there is good evidence that trade-offs do exist e.g. between immune function and reproduction (Ardia, 2005a; Kulaszewicz et al., 2017; Stahlschmidt et al., 2013), growth (van der Most et al., 2011), and moulting (Moreno-Rueda, 2010; Sanz et al., 2004). Experimentally manipulated resource availability has also demonstrated that different physiological processes compete for resources on some level (Sanz et al., 2004; Stahlschmidt et al., 2013) and it remains possible that energy is not the

only ‘currency’ of these trade-offs (Hasselquist and Nilsson, 2012). Despite attempts to quantify the absolute and relative physiological costs of immune function (Klasing, 2004; Lee, 2006; Lochmiller and Deerenberg, 2000), relatively little is known about how variation in individual immune investment relates to important fitness-related traits, and ultimately, fitness.

Linking the proximate physiological costs to the ultimate fitness costs of immune function has been a core pursuit of ecoimmunological studies (Schoenle et al., 2018), where it is particularly important to examine fitness in the wild, under natural environmental constraints and associated trade-offs (Maizels and Nussey, 2013; Pedersen and Babayan, 2011). Moreover, because immune systems have evolved in an ecological context with diverse parasite pressures that controlled conditions often remove, immune function can be very different under wild *vs.* captive conditions (Abolins et al., 2011). Furthermore, exposure to environmental stressors is known to elicit stress responses which can result in immunosuppression (Martin, 2009), which can mediate trade-offs and outcomes for fitness.

The costs associated with mounting immune responses have been investigated both in the laboratory and in the wild, as immune activation can be relatively easily studied using immune challenge experiments (Brock et al., 2014; Demas et al., 2011). As a result, there is a substantial body of evidence that immune activation is physiologically costly, resulting in trade-offs with fitness-related traits, particularly reproductive effort and success (reviewed for avian species by Hasselquist and Nilsson, 2012). However, because meaningful experimental manipulation of standing (constitutive) immunity is considerably more difficult if not impossible (Lochmiller and Deerenberg, 2000), the costs of standing immune function have received much less attention (but see McKean et al., 2008; Valtonen et al., 2010). Although the costs of immune maintenance are presumably lower than induced immune costs (Klasing, 2004; Lee, 2006), any costs do accumulate continuously, and potentially require more long-term testing. Specifically, how standing immune variation relates to fitness consequences in the wild has not been rigorously tested (but see Derting and Compton, 2003; Kulaszewicz et al., 2017; Møller and Haussy, 2007; Nussey et al., 2014; Schneeberger et al., 2014).

In this study, we assess how individual variation in constitutive immune function is related to subsequent fitness outcomes (survival, reproductive success, dominance acquisition) in a wild, cooperatively breeding, tropical passerine, the purple-crowned fairy-wren (*Malurus coronatus*). We measure three immune indices known to be important for frontline defences (haptoglobin, natural antibodies, complement activity; Baumgarth et al., 2005; Quaye, 2008; Trouw and Daha, 2011). Additionally, we assess how an index of chronic stress and body condition relate to fitness outcomes, to evaluate whether environmental

stressors or resource availability might mediate any covariation between immune indices and fitness outcomes. Using this observational approach, we implicitly interpret fitness outcomes as a subsequent cost of individual variation in maintenance of immune function. Given the importance of immune defences, we predict that individuals with higher immune function, lower chronic stress, and better body condition, will have higher survival probability. Congruent with evidence of resource reallocation trade-offs between reproduction and particularly immune activation (Harshman and Zera, 2007; Hasselquist and Nilsson, 2012), we predict that a higher maintenance of immune function will be associated with reduced subsequent reproductive success. By extension, we expect that higher chronic stress and poorer body condition will also be related to lower reproductive success. Lastly, we hypothesise that acquisition of a dominant breeding position is a function of individual quality and an ability to sequester and efficiently utilise resources. Therefore, we expect that higher immune function, lower chronic stress, and better body condition will predict acquisition of a dominant breeding position.

Methods

Study species

Purple-crowned fairy-wrens are cooperatively breeding sedentary residents of small rivers in north-western Australia. As riparian specialists, the western subspecies *Malurus coronatus coronatus* is highly dependent on *Pandanus aquaticus* vegetation for nest sites, foraging and predator evasion, defending stable year-round territories (Kingma et al., 2011a). Social groups of up to eleven individuals occupy each territory, consisting of a dominant male and female, often with one or more subordinate adults (settled dispersers or previous offspring). The dominant pair are the only birds in each group to breed, while subordinates usually contribute to offspring provisioning (Hall and Peters, 2008; Kingma et al., 2010). The dominant pair are socially and genetically monogamous, with extra-pair paternity of ~4% and used primarily as a means of incest avoidance (Kingma et al., 2013). Breeding occurs in response to recent heavy rainfall, peaking annually during the wet season (December-March; Hidalgo Aranzamendi et al., 2019). The core study population is located at Australian Wildlife Conservancy's (AWC) Mornington Wildlife Sanctuary (126.1°E, -17.5°N), where every individual along a contiguous 15km stretch of Annie Creek and Adcock River has been uniquely colour-banded since 2005. Territory boundaries, social group composition, individual movements, dispersal within the population and survival are monitored through regular censuses. Capture and sampling were done on a biannual basis, during two fieldwork periods

from mid-April to mid-June, and from mid-October to late November, at the start and end of each dry season, respectively.

Capture and sampling

For this study, captures of adult (> 90d old) purple-crowned fairy-wrens were made between April 2012 and June 2017. Passive mist-netting in combination with active audio playback was used to catch fairy-wrens. Once extracted from nets, birds were kept in holding bags before blood sampling (median = 23min, s.d. = 19.6min). Up to 100µl of blood was collected by brachial venepuncture into heparinised capillary tubes, which were sealed and stored on ice in the field, before centrifuging at 13,000rpm for 5min later that day. Red blood cell fractions were stored in ethanol at 4°C for DNA analyses and parental assignment, while plasma fractions were frozen at -20°C for immunological assays. At the end of each field work period, plasma samples were moved to -80 °C. At capture, using a drop of whole blood, a blood smear was created using the wedge-pull method (Campbell, 2015a). Air-dried blood smears were fixed in absolute methanol for no less than 15min.

Immune and stress indices

We assayed natural antibodies (NAbs), complement activity (Ca), and haptoglobin (Hp), which comprise part of the front-line immune defences by identifying, eliminating and mitigating the threats posed by parasites and pathogens (Baumgarth et al., 2005; Quaye, 2008; Trouw and Daha, 2011). NAbs are present in animals prior to any antigenic exposure and non-specifically identify a broad range of bacterial, viral and fungal antigens, providing broad spectrum surveillance (Holodick et al., 2017). NAbs bind to foreign antigenic components, opsonising them for phagocytosis, and initiate the complement system via the classic pathway (Nesargikar et al., 2012). Ca then helps to eliminate the infection through a suite of activated proteins that lyse and break down the pathogen (Ricklin et al., 2010; Trouw and Daha, 2011). Where erythrocyte cell damage is sustained as a consequence of infection, haptoglobin-like haem-binding scavengers (Hp or PIT54, an avian analogue) bind to reactive oxidative haem groups released to mitigate further damaged done by infection (Andersen et al., 2017; Quaye, 2008). These scavengers are tightly linked to the acute phase response and increase multiple-fold during infection and inflammation, however constitutive levels can predict the strength of an immune response (Matson et al., 2012). Lastly, chronic stress causes circulating heterophils to increase while lymphocytes decline in number (Davis and Maney, 2018). The heterophil-lymphocyte ratio (HL ratio) is therefore commonly used by ecologists as an index for chronic stress (Davis et al., 2008), which is known to have immunosuppressive consequences and be predictive of immune responsiveness (Krams et al., 2012), and could

moderate baseline immune function. Detailed methods of each immunological assay used in this study are described in Chapter 2 (Roast et al., 2019) and are only summarised here.

NAbs and Ca were quantified using the same modified haemolysis-haemagglutination assay (Matson et al., 2005; Chapter 2) with minor modifications. Plasma was added along a continuous row of two adjacent two clear 96-well plates, 15µl of 100% plasma in the first column, and 15µl serially diluted by 50% with Dulbecco's phosphate buffered saline (PBS) thereafter (100%, 50%, 25%, 12.5%, and so on). Exogenous rabbit red blood cells (Equicell) were introduced to plasma samples and plates were incubated for 90min at 37.5°C, then at room temperature for 20min tilted 45°, then 70min flat. Scanned images of the plates were taken at 110min and 180min for titre scores of agglutination (NAbs), and lysis (Ca) respectively. Inter-plate chicken plasma standards were scored for both agglutination (mean = 10.1, n = 247) and lysis (mean = 3.55, n = 265) titres respectively, resulting in CV = 0.13 and CV = 0.11.

Hp was assayed using a commercial kit (Phase^{TM} Range, TP801; Tri-Delta Development Ltd.) with a modified protocol (Chapter 2), and a VersamaxPLUS ROM v1.21 microplate reader. All samples were run in duplicate and where plasma volume was limiting, a 50% dilution was prepared with kit 'Diluent' and values obtained were doubled prior to analysis (n = 168). Rabbit plasma (Monash University Animal Research Platform) in triplicate was used to assess inter-plate variation (CV = 0.24, n = 25 plates). Of 732 samples, 82 fell above the 1.25mg/ml optical saturation threshold of the assay and were excluded from further analyses.

Blood smears were immersed for 15 min in 50% diluted May-Grünwald followed by 10% diluted Giemsa stains, then 5 min in distilled water before air-drying. At 1000x magnification, heterophils, lymphocytes, basophils, eosinophils and monocytes were counted for the first 100 leukocytes observed, and HL ratio was derived from the resulting leukocyte profiles. Scoring was undertaken by 4 individual scorers following (Campbell, 2015b). Scorer ID was included in all statistical models to account for variation between individuals.

Survival

Survival was estimated as a binary variable for survival (1) or mortality (0) if a sampled individual survived until the next biannual census and sampling period or not (> 90d post-capture). This estimate of survival is considered robust and reliable, close to the 'true' survival outcomes of individuals, for several reasons. In the core area, individuals that disappeared were conservatively declared dead only after all other group members had been observed 3 times during censuses. Birds initially declared dead but then rediscovered within the core

study population were relatively uncommon and overall this resulted in a 98% detection rate in the core population. Furthermore, this species has limited dispersal abilities, known only to disperse along waterways in the region (Rowley and Russell, 1993; Skroblin and Legge, 2010). To find dispersers outside of the core area, 71% of all suitable habitat in a 40km radius of the core population (Skroblin and Legge, 2013; fig. S1) was surveyed annually using an audio playback method (90% detection rate; Hidalgo Aranzamendi et al., 2016). These approaches allow us to distinguish dispersal from death with high accuracy.

Reproductive Success

Reproductive success was determined from observations of free-flying juveniles that were confirmed as a match to their putative parents using genetic data. DNA was extracted as per Eastwood et al. (2018), followed by microsatellite genotyping at 9 loci (Hidalgo Aranzamendi et al., 2016). With *Cervus 3.0.3* software, parents of each individual were assigned (Kalinowski et al., 2007). All juveniles that survived > 90d post-hatching, the approximate age of independence from parents (Hidalgo Aranzamendi et al., 2019), were deemed recruits, and the genetic parents of these individual were considered to have successfully reproduced. Hatch dates were estimated for all individuals from direct nest observations or based on begging behaviour, morphological and plumage traits of known age of acquisition. To assess the cost of immunity on subsequent reproductive success, for each individual measured in a given sampling season, all recruited offspring whose hatch dates fell during or after that sampling season were counted towards the individual's reproductive success (range 0-4 recruits). As only socially dominant individuals breed (Kingma et al., 2010), all adults that were dominant at the time of sampling that did not raise offspring to 90d were assigned a 0 score for reproductive success, while subordinates were excluded from reproductive success analyses. There were 7 exceptions, where birds sampled as subordinates quickly acquired dominant positions and successfully bred before the next sampling period ~5 months later. Due to low overall nesting success rate (12.7%; fig. S2), reproductive success data were highly zero-inflated and transformed to a binary variable of success (1) or failure (0). While this estimate does not represent overall lifetime reproductive success, it provides a short-term estimate of reproductive success which is most likely to be affected by the immediate physiological and immune status that we measure.

Dominance Acquisition

The social structure in purple-crowned fairy-wrens and the low frequency of extra-pair paternity mean that acquisition of a dominant breeding position is crucial to reproductive success, and competition is high with many subordinate individuals dying before acquiring

dominance (46%). Subordinates can form stable queues within groups to inherit a dominant position (Kingma et al., 2011b), and in males at least, ornaments are associated with the probability to acquire a breeding position elsewhere (Fan et al., 2018), which could indicate a role for individual quality and immune function. Dominance acquisition was recorded as a binary variable of success (1) or failure (0) in acquiring a dominant breeding position prior to the subsequent sampling period. Birds already holding a dominant position were excluded from these analyses.

Statistical Analysis

All analyses were carried out in the R statistical environment (v3.6.0; R Core Team, 2019). To address the key questions of this study, immune indices were treated as explanatory variables to all fitness-related response variables – survival, reproductive success, and dominance acquisition. This model arrangement, in addition to the temporal lag in quantification of response variables (outcome after immune measurements were taken) imply a consequential cost of immune function, though causality cannot explicitly be drawn from this observational study. In order to use immune and stress indices as explanatory variables in models however, raw values were corrected for sources of measurement error with linear mixed-models (LMMs) using *lme4* (Bates et al., 2015). With each immune or stress index as a response variable in separate LMMs (Hp and NAbs were normally distributed, Ca and HL were natural log and square-root transformed, respectively, to comply with assumptions with normality), the following potential sources of measurement error were included as fixed or random effects in each model: time bled – the time of day relative to sunrise (Zylberberg, 2015); time wait – the delay between capture and sampling representing handling stress (Davis, 2005; Zylberberg, 2015); fieldwork season – to account for storage differences between batches of samples; plate ID – to control for assay inter-plate variation; and scorer ID (see table S1 for exact model configurations). Residual values from these models were considered 'corrected' immune and stress index values, and these values were used as explanatory variables in all further analyses. In addition, to avoid conflation of the reproductive success and dominance acquisition responses with survival, only birds that survived to the next fieldwork period were retained in analyses involving reproductive success and dominance acquisition.

Generalised linear mixed effects models (GLMMs) were used to assess the relationship between immune and stress indices and the fitness-related variables using the *lme4* package (Bates et al., 2015). All response variables were binary, so logistic regression mixed models with binomial error structures were applied, with individual ID as a random effect to account for non-independence of repeated measures. Because not all individuals had the

complete panel of indices available, for each fitness-related response, separate GLMM models were constructed using all available samples for each immune or stress index. Age at capture and sex were also included in all models as covariates as these may influence short term fitness outcomes. Additionally, the time of year birds were sampled (May or November) was included in models with reproductive success as response, since this was consistently higher after November sampling as birds entered the wet season breeding peak. To each of these models, a quadratic term of each corrected immune or stress index was also included to test for a non-linear effect that may result from an optimal immune level, but removed if not significant ($p > 0.05$) using *lmerTest* (Kuznetsova et al., 2017). To assess the possibility of condition-dependence of fitness-related responses, a fifth GLMM was constructed for each response variable in which 'body condition' was an explanatory variable similar to the previous four models. Condition was calculated as the residuals of a linear regression model of body mass at capture, corrected for individual tarsus length and time of day.

Finally, for each fitness-related response, one additional model containing all immune and stress indices and body condition as explanatory variables combined was constructed to validate the results obtained in independent models; these models had substantially reduced sample sizes compared to any of the individual models (see table 1 for details). Quadratic terms were also included in combined models, then removed if not significant in the same manner as independent models. A principal component (PC) analysis was conducted across all immune and stress indexes to explore the possibility of using PCs in combined models; however, PCs did not substantially better explain the data or reduce dimensionality among explanatory variables, so were not used further (table S2, fig. S3). In all survival GLMMs, and the reproductive success combined GLMM, models experienced convergence and fitting issues, which were resolved by removing the individual ID random effect. In such cases the equivalent generalised linear model (GLM) without the individual ID random effect was fitted to obtain estimates reported here, but as GLMs do not fully control for non-independence of data points results of these models need to be interpreted with a degree of caution.

Using the combined model approach largely validates and does not contradict independent models. Independently fitted models and combined models showed generally similar magnitude and direction of effects (table S3; fig. S4). In most cases, the effect direction (+/-) was identical between independent and combined models with two exceptions: the effect of Ca on reproductive success, and HL ratio on survival. In both cases (where signs were opposed), the effect size in independent models was essentially zero with large 95% confidence intervals, and only a |1|% change in response probability (tables 2, S3), suggesting

these models were not directly contradictory, but rather different in effect size, with some stochasticity around zero. Across all models, three significant effects were observed in independent models, while only one of these was observed as significant in combined models. It was expected that independent models are more likely to contain significant explanatory effects because of a larger sample size, and therefore greater statistical power. Additionally, 95% confidence intervals are wider in combined models, as smaller sample size results in a typically larger error associated with estimates and predicted values (fig. 1), and are also therefore more likely to contain zeroes. Consequently, only independent models are reported and interpreted further, while complete combined model outputs and comparisons are reported in the supplementary material.

Table 1: Matrix of sample sizes (*n*) for model configurations. All analyses include only adult birds (> 90d), reproductive success analyses include only dominant individuals, and dominance acquisition analyses include only subordinate individuals. The combined model includes only samples for which all five explanatory variables were available.

		Explanatory					
		Hp	*NAbs*	*Ca*	*HL ratio*	*Condition*	*Combined*
Response	*Survival*	648	520	519	536	836	337
	Reproductive Success	305	248	250	253	406	156
	Dominance Acquisition	290	235	231	247	373	158

Results

Survival

Ca was the only index to significantly predict survival (table 2; fig. 1c). Interestingly, it showed a quadratic effect, with intermediate values of Ca having the lowest predicted probability of survival, while individuals that had either higher or lower values were more likely to survive to the next field season (table 2; β = 0.525, 95%CI = 0.098, 1.027). This effect did not appear to be condition-dependent, though there was a general trend that individuals in poorer body condition experienced a slightly lower probability of survival, as was expected (table 2; fig. 1e). Furthermore, individuals with higher NAbs were less likely to survive, and although the effect was fairly large, with -24% change of survival with an increase of 1 s.d. in NAbs, this result was not quite significant (table 2; β = -0.276, 95%CI = -0.621, 0.063).

Reproductive Success

For reproductive success, NAbs appear to be the most important explanatory variable (table 2; β = 0.563, 95%CI = 0.219, 0.962), with a 76% increase in the probability to successfully produce at least one recruit, per standard deviation increase in NAbs (table 2; fig. 1g). There was also a substantial, but not significant, increase in the probability of reproductive success with increased Hp (table 2; β = 0.254, 95%CI = -0.040, 0.574), while all other indices had only negligible effects.

Dominance Acquisition

The probability of successfully acquiring a dominant breeding position was positively related to HL ratio, i.e. level of chronic stress (table 2; β = 0.364, 95%CI = 0.006, 0.722). In addition, body condition to a lesser extent appeared to be important, as individuals with better body condition were also more likely to acquire dominant breeding positions, with a similar effect size to HL ratio (although not significant; table 2; β = 0.301, 95%CI = -0.014, 0.676). Finally, individuals with higher baseline Hp concentrations were 31% more likely to acquire a breeding position in the following months (although not significant; table 2; β = 0.267, 95%CI = -0.046, 0.580).

Table 2: The effects of immune, stress and body condition indices on fitness-related traits. The β-estimates and 95% confidence intervals (CI) are derived from independently modelled variables, and are presented on the logit link function scale. Odds ratios (OR), and the percentage change in the probability of the respective response (Δ% p(Response)) are per standard deviation in the corrected immune, stress and condition indices. Bold typeface shows variables that were significant in independent models, determined by 95%CI that do not contain zero and $p < 0.05$ for z-tests (table S4).

Response	Explanatory	C / I	β	(95% CI)	OR	Δ% p(Response)	Model
Survival	Haptoglobin	I	-0.150	(-0.412, 0.120)	0.86	-14%	GLM
	Natural Antibodies	I	-0.276	(-0.621, 0.063)	0.76	-24%	GLM
	Complement Activity	I	0.252	(-0.136, 0.673)	1.29	29%	GLM
	(Complement Activity)^2	**I**	**0.525**	**(0.098, 1.027)**	**1.69**	**69%**	**GLM**
	HL Ratio	I	-0.011	(-0.325, 0.327)	0.99	-1%	GLM
	Body Condition	I	0.118	(-0.140, 0.375)	1.12	12%	GLM
Reproductive Success	Haptoglobin	I	0.254	(-0.040, 0.574)	1.29	29%	GLMM
	Natural Antibodies	**I**	**0.563**	**(0.219, 0.962)**	**1.76**	**76%**	**GLMM**
	Complement Activity	I	-0.013	(-0.316, 0.298)	0.99	-1%	GLMM
	HL Ratio	I	0.088	(-0.239, 0.418)	1.09	9%	GLMM
	Body Condition	I	-0.026	(-0.266, 0.222)	0.97	-3%	GLMM
Dominance Acquisition	Haptoglobin	I	0.267	(-0.046, 0.580)	1.31	31%	GLMM
	Natural Antibodies	I	-0.014	(-0.382, 0.354)	0.99	-1%	GLMM
	Complement Activity	I	-0.203	(-0.589, 0.182)	0.82	-18%	GLMM
	HL Ratio	**I**	**0.364**	**(0.006, 0.722)**	**1.44**	**44%**	**GLMM**
	Body Condition	I	0.301	(-0.014, 0.676)	1.39	39%	GLMM

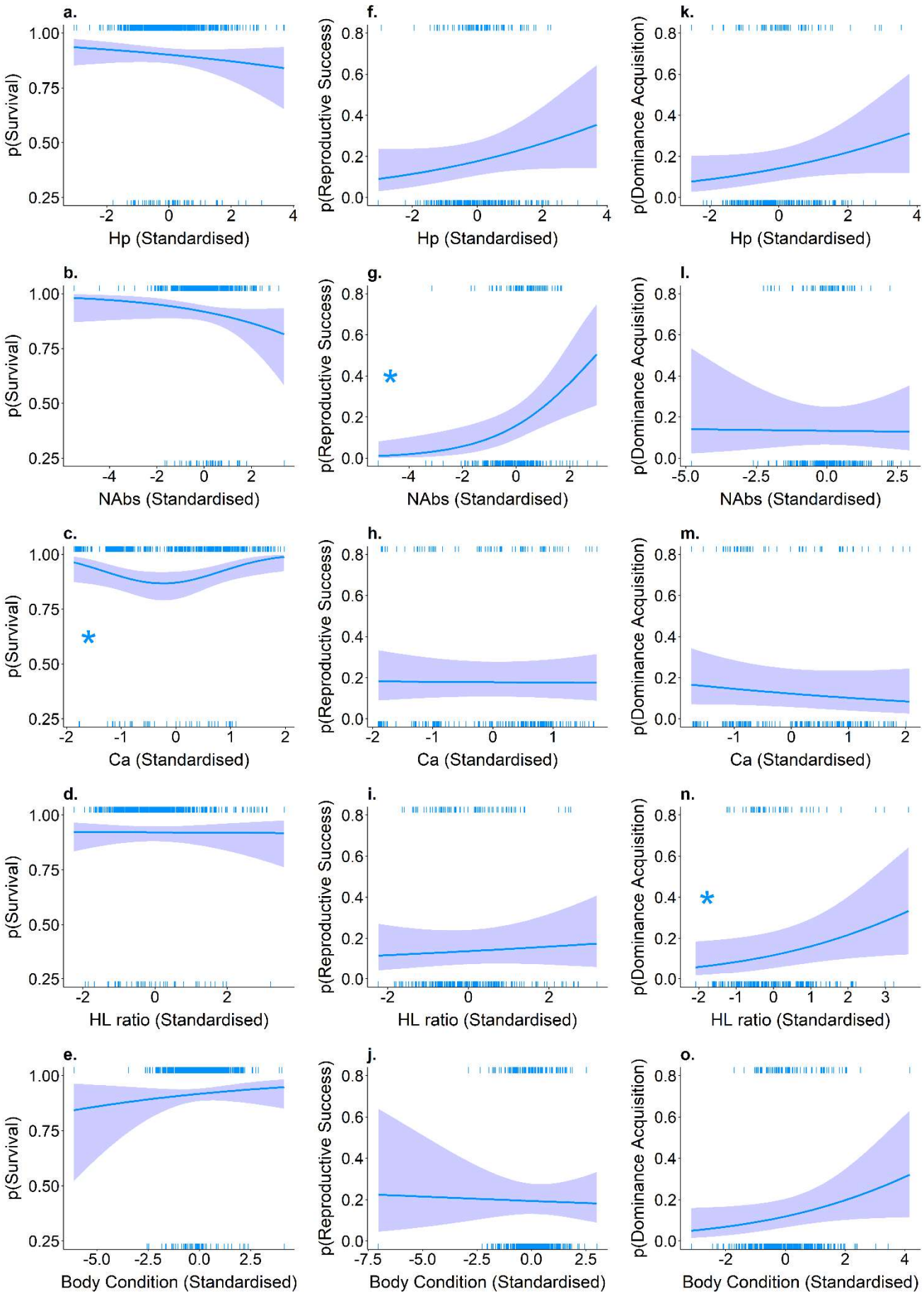
a.
p(Survival)
Hp (Standardised)
b.
p(Survival)
NAbs (Standardised)
c.
p(Survival)
Ca (Standardised)
d.
p(Survival)
HL ratio (Standardised)
e.
p(Survival)
Body Condition (Standardised)
f.
p(Reproductive Success)
Hp (Standardised)
g.
p(Reproductive Success)
NAbs (Standardised)
h.
p(Reproductive Success)
Ca (Standardised)
i.
p(Reproductive Success)
HL ratio (Standardised)
j.
p(Reproductive Success)
Body Condition (Standardised)
k.
p(Dominance Acquisition)
Hp (Standardised)
l.
p(Dominance Acquisition)
NAbs (Standardised)
m.
p(Dominance Acquisition)
Ca (Standardised)
n.
p(Dominance Acquisition)
HL ratio (Standardised)
o.
p(Dominance Acquisition)
Body Condition (Standardised)

Figure 1: The effect of individual variation in immune function, chronic stress and body condition indices on subsequent fitness-related outcomes in purple-crowned fairy-wrens. Fitted lines show the predicted probability of survival (left, **a-e.**), successful reproduction of at least one recruited offspring (centre, **f-j.**) and acquisition of a dominant breeding position (right, **k-o.**), as responses to (from top to bottom) haptoglobin (Hp), natural antibodies (NAbs), complement activity (Ca), heterophil-lymphocyte ratio (HL ratio), and body condition. Ribbons show confidence intervals based upon error of fixed effects only, while all continuous variables are held at median values. Lines are shown for males in May only, as reference categories for 'Sex' (all responses) and 'Time of year' (reproductive success response only). Lines and ribbons are derived from models fitted independently with a single index and maximum sample. Data ticks above and below each plot show raw data values at binary 1 and 0 outcomes. Plots labelled with "*" denote significant effects of indices in final models.

Discussion

High baseline immune function does not predict survival

We predicted that as a consequence of parasite pressure, higher immune function would be directly related to a higher probability of survival. In relation to our three constitutive immune indices, this was not the case, despite their important roles for frontline defences. Haptoglobin and natural antibodies were unrelated to survival probability, while we found a significant quadratic effect of complement activity. We tested for quadratic effects in these models expecting a possible optimal level of immune function; however, instead of a peak for optimal immunity, we unexpectedly observed a trough, showing that intermediate values had the lowest probability of survival. This could suggest a bimodal (high and low) level of optimal Ca, potentially determined by fluctuating environmental variation (Lazzaro and Little, 2009; Nwaogu et al., 2019; Tieleman et al., 2019). Disease risk in the immediate environment may vary periodically or seasonally through fluctuating vector or microbial abundance (Bolling et al., 2005; Horrocks et al., 2015, 2012b, 2012a; Sehgal, 2015), or even spatially through localised disease outbreak or transmission between neighbouring territories (White et al., 2018), shaping which strategy for Ca is optimal in a given time or space. As Ca is a particularly aggressive component of immune function, it can generate greater immunopathological costs (Ricklin et al., 2010), and in the absence of strong selective pressure from parasites and pathogens at certain times or locations, lower Ca could be more advantageous. Survival of individuals with high and low immune function could emerge from plastic levels of immune function within-individuals, or maintained immune variation between individuals in the population. Given that there is very low within-individual repeatability of immune

measurements in this species (Chapter 2), this result is highly suggestive that immune function is plastic and strongly environmentally determined, which is consistent with the quadratic pattern associated with survival.

No evidence of a trade-off between immune function and reproduction

We hypothesised that investment in immune function could result in a trade-off with reproduction as a competing physiological process. Therefore, we expected immune function to be inversely related to subsequent reproductive success, yet this was unsupported for all of our immune indices. Higher levels of NAbs were strongly related to greater likelihood of reproductive success, and the same was true for Hp, albeit to a slightly lesser extent. These results indicate that both reproduction and immunity could be an expression of a third unmeasured variable, an individual or environmental variable ('quality') that overrides a resource reallocation trade-off. Better quality individuals might simultaneously be able to invest in immunity and reproduction (Ardia, 2005b). High quality individuals could be more efficient foragers of resources (Lescroël et al., 2010), or inhabit superior territories providing better access to resources (Arcese, 1989), which could relax energetic trade-off constraints or directly improve offspring provisioning and survival. A genetic basis for differences in individual quality might exist in this species, given that purple-crowned fairy-wrens are particularly vulnerable to inbreeding. In this species, incestuous dominant pairings are highly costly to reproductive success (Hidalgo Aranzamendi et al., 2016; Kingma et al., 2013), while severe habitat fragmentation and limited dispersal ability has reduced genetic diversity in isolated subpopulations (Skroblin et al., 2014). Consequently, as inbreeding has been shown to cause immunosuppression (Reid et al., 2007, 2003), this could result in positive correlations between individual genetic diversity, reproductive success, and immune function.

Our hypothesis that dominance acquisition would be a function of individual quality, and therefore related to individuals with higher immune function was not supported. No immune index significantly related to the probability of dominance acquisition (table 2). Purple-crowned fairy-wrens exhibit sexually dichromatic seasonal plumage ornamentation, which is either structural (males) or melanin-based (males and females) in colour (Fan et al. 2019). This type of colouration can signal individual quality (Jawor and Breitwisch, 2003; Keyser and Hill, 2000; Siefferman and Hill, 2003), and possibly immunocompetence to potential mates (Folstad and Karter, 1992; Hamilton and Zuk, 1982). Male ornamentation is important for male-male competition with 'brighter' ornaments enhancing the probability of acquiring a dominant breeding position (Fan et al. 2018); however, we do not fully understand how this relates to individual quality in female purple-crowned fairy-wrens. Based on what is known about males,

these results from males and females suggest that the mechanisms which convey individual quality through plumage ornamentation to acquire dominance may not be the same as those which convey quality in immune function.

Limited evidence for condition-dependent fitness

We predicted that higher body condition, a rudimentary proxy for energetic resources, would have a positive effect on each of our fitness responses, which to a limited extent was the case. Though not significant, when body condition was higher there were small to moderate increases in the probability of survival (congruent with other bird studies; Morrison et al., 2007; Newton, 1993) and dominance acquisition (as in e.g. Gosler and Carruthers, 1999). As a tropical resident species without a critical migratory or overwintering annual phase where condition is important, any condition-dependence of survival particularly might not be so pronounced (Milenkaya et al., 2015). Additionally, we found no positive relationship between body condition and reproductive success, i.e. no condition-dependence. Together with no evidence of a trade-off between immune function and reproductive success, this suggests there is unlikely to be an energetic constraint that prohibits both maintenance of baseline immune function and reproductive activity. Immune-reproductive trade-offs are consistently tested and found, often in the presence of energetic constraints (Hasselquist and Nilsson, 2012), and so our results are somewhat contrary to those in the literature. However, these results stemmed from immune challenges invoking an acute immune response rather than baseline constitutive immune function and therefore trade-offs may only occur with specific costly immune components (Hasselquist and Nilsson, 2012; Palacios and Bronikowski, 2017). The costs of constitutively maintaining immune components are considered to be lower than those involved in an acute phase response (Hasselquist and Nilsson, 2012; Lee, 2006), and less likely to invoke a (strong) trade-off. Alternatively, when experiencing a pathogenic immune challenge, sickness behaviours can also restrict resource acquisition, perhaps creating energetic bottlenecks at the moment when resources are most needed (Adelman and Martin, 2009; Bashir-Tanoli and Tinsley, 2014; Kyriazakis et al., 1998), increasing the likelihood of trade-offs occurring with induced rather than constitutive immune components. As purple-crowned fairy-wrens reproduce only when conditions are most favourable and there is an abundance of invertebrate prey (Hidalgo Aranzamendi et al. 2019), energetic constraints might be relaxed when physiological demands are high, and baseline immune function can be maintained throughout the annual cycle (Chapter 2), irrespective of body condition or reproductive state.

Elevated stress and dominance acquisition

We hypothesised that, because chronic stress generally depresses constitutive and other immune functions, chronic stress should also relate negatively to fitness-related traits, assuming immune indices relate positively to fitness-related traits. From our results, no clear pattern emerged to support this hypothesis. For reproduction and survival, heterophil-lymphocyte ratio was uninformative, and there was a clear, and significant, positive effect on dominance acquisition. Acute stress responses and elevated corticosterone are hypothesised to be adaptively beneficial to organisms (Cort-Activity/Adaptation Hypotheses; Bonier et al., 2009; Rivers et al., 2012), stimulating activity, energy expenditure, food intake and behavioural responses that help cope with immediate environmental stressors and increase survival (Cote et al., 2006; Rivers et al., 2012). While the HL ratio becomes elevated in response to corticosterone, elevated HL ratio represents more chronic stress (Davis and Maney, 2018; Goessling et al., 2015), that can remain stable within individuals (Minias, 2019) and that is presumed not to be beneficial, and even inflammatory (Cohen et al., 2012). Superficially, our results suggest an apparent benefit of higher levels of chronic stress on acquisition of a dominance position. Alternatively, this could be anticipatory stress – birds poised to take over a dominance position are stressed possibly in response to learned cues of social instability or anticipated dispersal (Boonstra, 2013), which may be further modified by specific group social dynamics and history (Creel et al., 2013). Such an explanation is consistent with high HL ratios that have been related to social stressors (Frigerio et al., 2017), and elevated Hp – an inflammatory marker – in these individuals. High stress reactivity is also commonly observed in more aggressive or bolder individuals (Pusch et al., 2018; van der Meer and van Oers, 2015). Such personality traits can be advantageous in outcompeting conspecifics for a dominant breeding position (Pellegrini, 2008), possibly explaining the positive relationship between HL ratio and the probability of dominance acquisition. In this case, chronic stress may not be directly related to overall fitness, but rather closely linked to personality types that prevail in intra-specific competition.

Conclusion

Unrelenting parasitic and pathogenic selective pressures ensure that immune function remains vital for survival, and constitutive immune defences form an integral part of the immune system. However, we found that survival was not related to high levels of constitutive immune indices. In addition, positive relationships between reproductive success and immune indices – significantly NAbs – suggest that some unmeasured aspect of individual quality is more important than resource allocation trade-offs within individuals. Without evidence of an immune-reproductive trade-off, our results suggest the fitness costs of maintenance of these

constitutive humoral immune components may not be as substantial as the costs of induced or cellular immune responses that invoke trade-offs in other systems. Immune component-specific costs are not a new concept (Lee 2006), yet quantifying the physiological costs of constitutive immune components, that are not amenable to experimental manipulation, has been a difficult task for ecoimmunologists and remains a somewhat unresolved challenge. By developing a more detailed understanding of precise energetic, nutritional, metabolic and oxidative patterns of covariation of specific immune components, we will be able to hypothesise more precisely about how individual variation in immune function relates to fitness outcomes and evolutionary host-parasite dynamics.

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Supplementary Materials

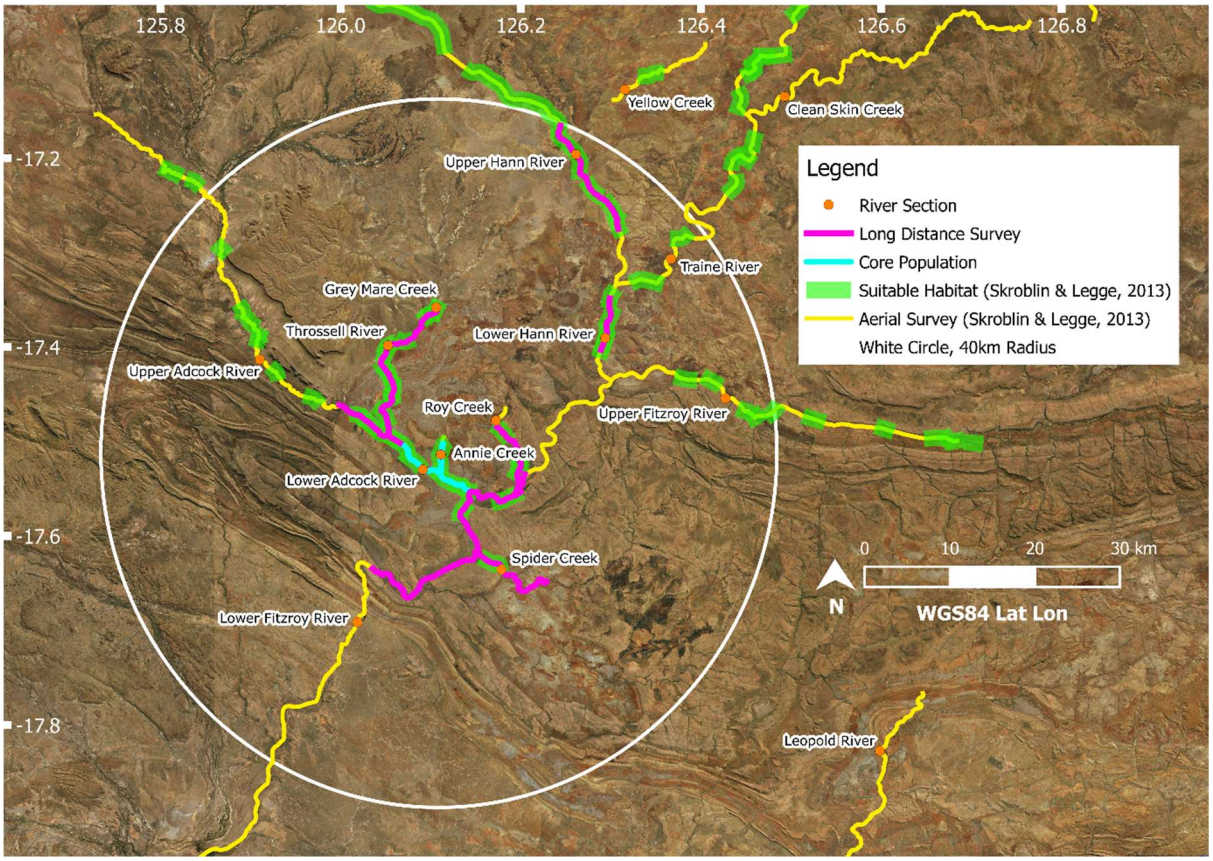


Figure S1: Main rivers and small creeks surveyed for purple-crowned fairy-wrens in the Fitzroy River catchment. Regular census (approximately weekly visits to each territory) of the entire core population was biannual during dry season periods of April-June and October-November, through all years of the present study 2012-2017. Additional census of the core population was carried out on Annie Creek in 2016-2017 during the wet season during January-April. Annually, 'long distance' surveys in a wider area of the Fitzroy catchment were conducted as part of the present study to determine any dispersal outside of the core population into neighbouring rivers with suitable habitat. From 2007-2009, Skroblin and Legge (2013) aerially surveyed many rivers in the catchment for suitable purple-crowned fairy-wren habitat; 44km of river with habitat suitability index > 0.54 were identified. All core and long-distance areas of the present study were previously surveyed by Skroblin and Legge (2013), and locations of suitable habitat have remained relatively unchanged in these areas, though quality can vary with time through floods, fires, regrowth, and grazing management.

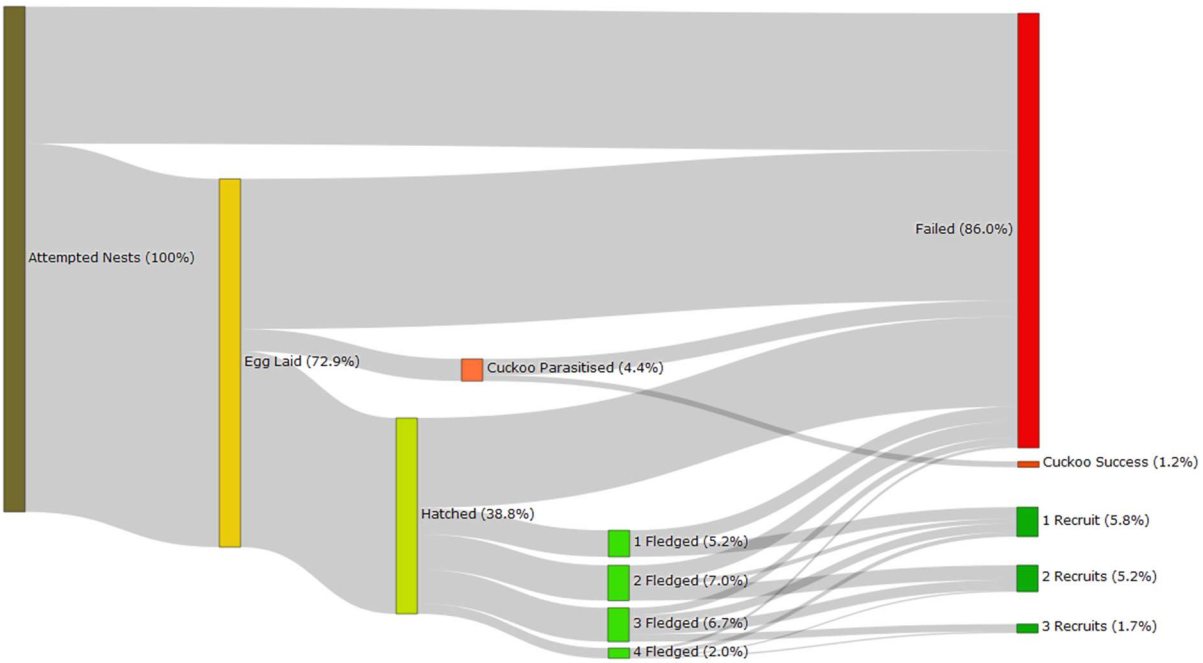


Figure S2: Purple-crowned fairy-wren nest attempt outcomes, Annie Creek 2016-2018. For initiated nest attempts (n = 343), the final outcome was most often failure, failing at all stages of reproductive attempts. Approximately 21% of nests fledged, but only approximately 12.8% resulted in recruits, most commonly only a single recruit. All percentages refer to the initial attempted nests; bars are proportional in size.

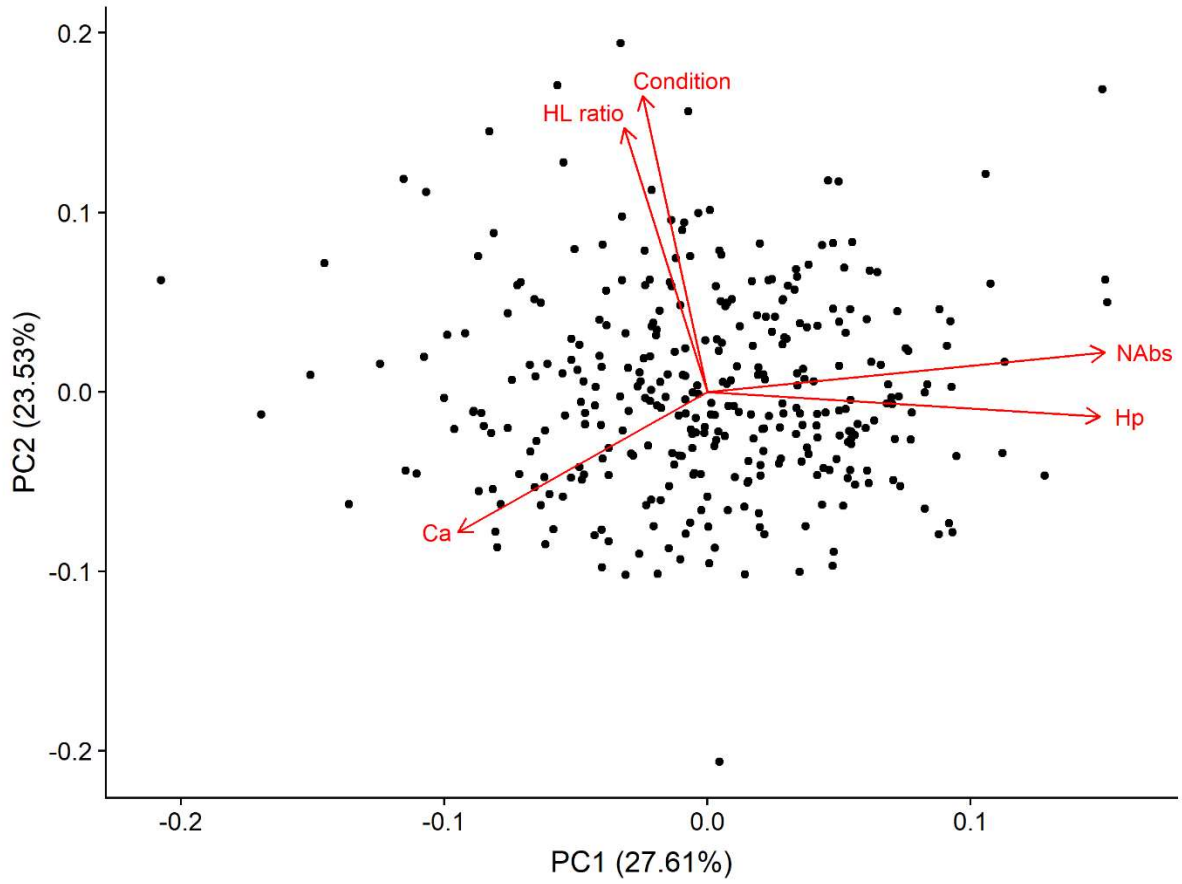
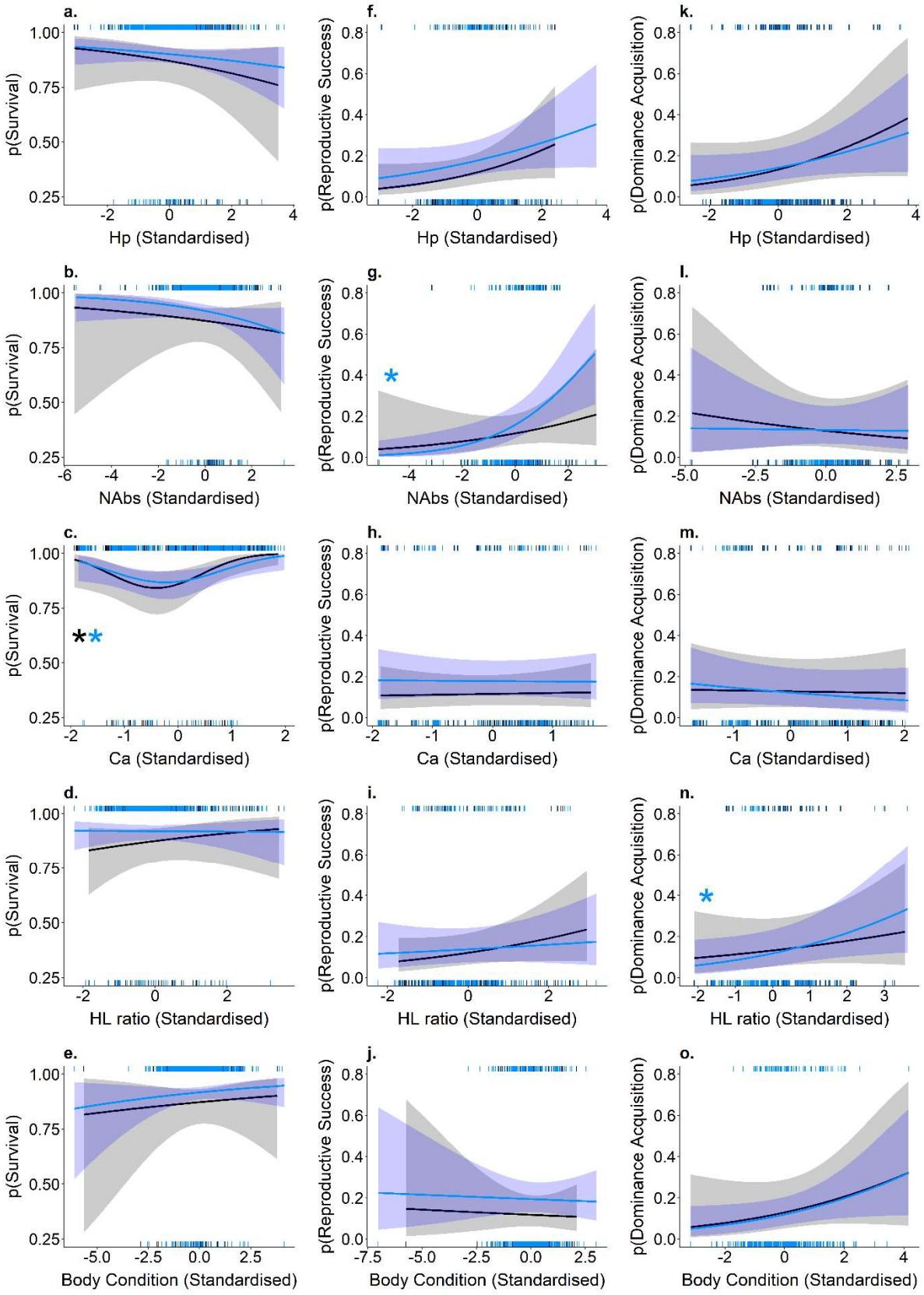


Figure S3: Principal Component Analysis (PCA) of explanatory variables used in combined models. Among 5 axes of variation, the principal components explained between 27.6-14.0% of the variance explained by the dataset. This did not substantially reduce the dimensionality in the dataset and all standardised original variables (haptoglobin, Hp; natural antibodies, NAbs; complement activity, Ca; heterophil-lymphocyte ratio, HL ratio; condition) were retained in combined models. With these exact data points plotted 3-dimensionally with a PC3 axis (18.4%), HL ratio and Condition are also separated in depth, despite the apparent similarity in 2-dimensions, as are NAbs and Hp.


a.
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l.
m.
n.
o.
p(Survival)
p(Reproductive Success)
p(Dominance Acquisition)
Hp (Standardised)
NAbs (Standardised)
Ca (Standardised)
HL ratio (Standardised)
Body Condition (Standardised)
*
**

Figure S4: The effects of immune, stress and condition indices on fitness-related traits in combined (C) *versus* independent (I) models. Fitted lines show the predicted probability of survival (left, **a-e.**), successful reproduction of at least one recruited offspring (centre, **f-j.**) and acquisition of a dominant breeding position (right, **k-o.**), as responses to (from top to bottom) haptoglobin (Hp), natural antibodies (Nabs), complement activity (Ca), heterophil-lymphocyte ratio (HL ratio), and body condition. Black depicts models containing all explanatory variables (restricted sample), while blue depicts models with explanatory variables fitted independently (maximal sample). Ribbons show confidence intervals based upon error of fixed effects only, while all continuous variables are held at median values. Lines are shown for males in May only, as reference categories for 'Sex' (all responses) and 'Time of year' (reproductive success response only). Data ticks above and below each plot show raw data values at binary 1 and 0 outcomes. Plots labelled with "*" denote significant effects of indices in final models.

Table S1: Configurations of Linear Mixed Models (LMMs) used to correct for measurement error in immune and stress indices. Immune and stress indices were fitted as response variables in separate models with the appropriate fixed and random effects applied to calculate residual values. Residual values were then standardised (scaled, centred) and included in the main analyses as explanatory variables.

Structure	**Hp**	**NAbs**	**Ca**	**HL ratio**
Fixed effects	Time bled	Time bled	Time bled	Time bled
	Time wait	Time wait	Time wait	Time wait
	Field Season	Field Season	Field Season	Field Season
Random effects	Plate ID	Plate ID	Plate ID	Scorer ID

Table S2: Principal Component Analysis (PCA) over 5 axes of variation. Data were included in the PCA from the 5 variables included in combined models (haptoglobin, natural antibodies, complement activity, heterophil-lymphocyte ratio, condition). Variance explained by each principal component was not substantially different from 20%, expected from 5 independent and uncorrelated variables. Inclusion of < 5 principal components would leave at least ≈14% of variance unexplained, and to include all 5 would not reduce dimensionality or improve interpretation. Consequently, principal components were not used further in the main analyses.

Principal Component	**Eigenvalue**	**% of Variance Explained**	**Cumulative % of Variance Explained**
PC1	1.38	27.61	27.61
PC2	1.17	23.53	51.13
PC3	0.91	18.35	69.48
PC4	0.82	16.53	86.02
PC5	0.70	13.98	100.00

Table S3: The effects of immune, stress and condition indices on fitness-related traits in combined (C) *versus* independent (I) models. The β-estimates and 95% confidence intervals (CI) are presented on the logit link function scale. Odds ratios (OR), and the percentage change in the probability of the respective response (Δ% p(Response)) are standardised to the change per one standard deviation in the corrected residuals of explanatory variables. Models with convergence issues were fitted as GLMs, and other models with GLMMs. Bold typeface shows variables that were significant in each model, determined by with 95%CI that do not contain zero and $p < 0.05$ for and z-tests (table S4).

Response	Explanatory	C / I	β	(95% CI)	OR	Δ% p(Response)	Model
Survival	Haptoglobin	C	-0.214	(-0.629, 0.203)	0.81	-19%	GLM
		I	-0.150	(-0.412, 0.120)	0.86	-14%	GLM
	Natural Antibodies	C	-0.128	(-0.628, 0.344)	0.88	-12%	GLM
		I	-0.276	(-0.621, 0.063)	0.76	-24%	GLM
	Complement Activity	**C**	**0.667**	**(0.073, 1.38)**	**1.95**	**95%**	**GLM**
		I	0.252	(-0.136, 0.673)	1.29	29%	GLM
	(Complement Activity)^2	**C**	**0.810**	**(0.197, 1.578)**	**2.25**	**125%**	**GLM**
		I	**0.525**	**(0.098, 1.027)**	**1.69**	**69%**	**GLM**
	HL Ratio	C	0.187	(-0.247, 0.684)	1.21	21%	GLM
		I	-0.011	(-0.325, 0.327)	0.99	-1%	GLM
	Body Condition	C	0.077	(-0.345, 0.489)	1.08	8%	GLM
		I	0.118	(-0.140, 0.375)	1.12	12%	GLM
Reproductive Success	Haptoglobin	C	0.389	(-0.034, 0.835)	1.48	48%	GLM
		I	0.254	(-0.040, 0.574)	1.29	29%	GLMM
	Natural Antibodies	C	0.227	(-0.202, 0.694)	1.25	25%	GLM
		I	**0.563**	**(0.219, 0.962)**	**1.76**	**76%**	**GLMM**
	Complement Activity	C	0.044	(-0.352, 0.452)	1.31	31%	GLM
		I	-0.013	(-0.316, 0.298)	0.99	-1%	GLMM
	HL Ratio	C	0.270	(-0.121, 0.666)	1.05	5%	GLM
		I	0.088	(-0.239, 0.418)	1.09	9%	GLMM
	Body Condition	C	-0.045	(-0.446, 0.390)	0.96	-4%	GLM
		I	-0.026	(-0.266, 0.222)	0.97	-3%	GLMM
Dominance Acquisition	Haptoglobin	C	0.372	(-0.107, 0.841)	1.44	44%	GLMM
		I	0.267	(-0.046, 0.580)	1.31	31%	GLMM
	Natural Antibodies	C	-0.130	(-0.604, 0.342)	0.88	-12%	GLMM
		I	-0.014	(-0.382, 0.354)	0.99	-1%	GLMM
	Complement Activity	C	-0.038	(-0.479, 0.414)	0.97	-3%	GLMM
		I	-0.203	(-0.589, 0.182)	0.82	-18%	GLMM
	HL Ratio	C	0.178	(-0.248, 0.632)	1.21	21%	GLMM
		I	**0.364**	**(0.006, 0.722)**	**1.44**	**44%**	**GLMM**
	Body Condition	C	0.280	(-0.216, 0.695)	1.27	27%	GLMM
		I	0.301	(-0.014, 0.676)	1.39	39%	GLMM

Table S4: Complete model outputs for all main analyses reported in the main manuscript. Tables **i-v)** correspond to models fitted with independent indices (haptoglobin, natural antibodies, complement activity, heterophil-lymphocyte ratio, and body condition), and **vi)** fitted with all indices in a combined model. Table sets **a-c)** correspond to each response variable modelled, for survival, reproductive success and dominance acquisition, respectively. Whether fitted as a GLM or GLMM, and sample size of each model is included in each table subheading.

a)i) Survival, Independent, GLM, Haptoglobin (n = 648)

Effect	*β*	SE	*z*	*p*
Intercept	2.10E+00	2.27E-01	9.253	<0.001
Sex				
Female	9.43E-02	2.87E-01	0.329	0.742
Age	1.10E-01	6.65E-02	1.655	0.098
Haptoglobin	-1.50E-01	1.35E-01	-1.109	0.267

a)ii) Survival, Independent, GLM, Natural antibodies (n = 520)

Effect	*β*	SE	*z*	*p*
Intercept	2.31E+00	2.73E-01	8.449	<0.001
Sex				
Female	-1.73E-02	3.36E-01	-0.051	0.959
Age	1.06E-01	7.96E-02	1.331	0.183
Natural antibodies	-2.76E-01	1.75E-01	-1.578	0.115

a)iii) Survival, Independent, GLM, Complement activity (n = 519)

Effect	*β*	SE	*z*	*p*
Intercept	1.81E+00	3.08E-01	5.865	<0.001
Sex				
Female	-5.00E-02	3.27E-01	-0.153	0.879
Age	1.06E-01	7.84E-02	1.353	0.176
Complement activity	2.52E-01	2.04E-01	1.237	0.216
I(Complement activity)^2	**5.25E-01**	**2.35E-01**	**2.230**	**0.026**

a)iv) Survival, Independent, GLM, Heterophil-lymphocyte ratio (n = 536)

Effect	*β*	SE	*z*	*p*
Intercept	2.39E+00	2.66E-01	9.001	<0.001
Sex				
Female	1.47E-02	3.34E-01	0.044	0.965
Age	5.74E-02	7.44E-02	0.771	0.441
Heterophil-lymphocyte ratio	-1.11E-02	1.66E-01	-0.067	0.947

a)v) Survival, Independent, GLM, Body condition (n = 836)

Effect	*β*	SE	*z*	*p*
Intercept	2.38E+00	2.15E-01	11.052	<0.001
Sex				
Female	1.02E-01	2.67E-01	0.382	0.702
Age	2.80E-02	5.66E-02	0.495	0.621
Body condition	1.18E-01	1.32E-01	0.900	0.368

a)vi) Survival, Combined, GLM, All indices (n = 337)

Effect	*β*	SE	*z*	*p*
Intercept	1.81E+00	3.90E-01	4.648	<0.001
Sex				
Female	4.00E-01	4.50E-01	0.889	0.374
Age	2.54E-02	8.74E-02	0.290	0.772
Haptoglobin	-2.14E-01	2.11E-01	-1.013	0.311
Natural antibodies	-1.28E-01	2.49E-01	-0.512	0.608
Complement activity	**6.67E-01**	**3.29E-01**	**2.024**	**0.043**
I(Complement activity)^2	**8.10E-01**	**3.49E-01**	**2.319**	**0.020**
Heterophil-lymphocyte ratio	1.87E-01	2.36E-01	0.794	0.427
Body condition	7.69E-02	2.13E-01	0.361	0.718

b)i) Reproductive Success, Independent, GLMM, Haptoglobin (n = 305)

F/R	Effect	*β*	SE	*z*	*p*	σ^2
	Intercept	-1.60E+00	3.96E-01	-4.045	<0.001	-
F	Sex					
	Female	4.75E-01	3.34E-01	1.421	0.155	-
F	Age	2.04E-02	6.20E-02	0.329	0.742	-
F	Time of year					
	November	4.19E-01	3.34E-01	1.256	0.209	-
F	Haptoglobin	2.54E-01	1.54E-01	1.650	0.099	-
R	Individual ID	-	-	-	-	0.805

b)ii) Reproductive Success, Independent, GLMM, Natural antibodies (n = 248)

F/R	Effect	*β*	SE	*z*	*p*	σ^2
	Intercept	-1.76E+00	4.01E-01	-4.401	<0.001	-
F	**Sex**					
	Female	**8.09E-01**	**3.35E-01**	**2.414**	**0.016**	-
F	Age	2.91E-02	6.28E-02	0.463	0.644	-
F	**Time of year**					
	November	**7.59E-01**	**3.73E-01**	**2.035**	**0.042**	-
F	**Natural antibodies**	**5.63E-01**	**1.87E-01**	**3.015**	**0.003**	-
R	Individual ID	-	-	-	-	0.356

b)iii) Reproductive Success, Independent, GLMM, Complement activity (n = 250)

F/R	Effect	*β*	SE	*z*	*p*	σ^2
	Intercept	-1.62E+00	3.84E-01	-4.215	<0.001	-
F	**Sex**					
	Female	**8.11E-01**	**3.24E-01**	**2.503**	**0.012**	-
F	Age	2.84E-02	5.97E-02	0.475	0.635	-
F	Time of year					
	November	6.77E-01	3.67E-01	1.845	0.065	-
F	Complement activity	-1.33E-02	1.54E-01	-0.087	0.931	-
R	Individual ID	-	-	-	-	0.366

b)iv) Reproductive Success, Independent, GLMM, Heterophil-lymphocyte ratio (n = 253)

F/R	Effect	*β*	SE	*z*	*p*	σ^2
	Intercept	-1.93E+00	4.46E-01	-4.332	<0.001	-
F	Sex					
	Female	5.28E-01	3.55E-01	1.487	0.137	-
F	Age	3.31E-02	6.73E-02	0.491	0.623	-
F	**Time of year**					
	November	**1.40E+00**	**3.96E-01**	**3.525**	**<0.001**	-
F	Heterophil-lymphocyte ratio	8.76E-02	1.65E-01	0.532	0.595	-
R	Individual ID	-	-	-	-	0.678

b)v) Reproductive Success, Independent, GLMM, Body condition (n = 406)

F/R	Effect	*β*	SE	*z*	*p*	σ^2
	Intercept	-1.48E+00	3.24E-01	-4.574	<0.001	-
F	Sex					
	Female	2.43E-01	2.68E-01	0.907	0.364	-
F	Age	1.84E-02	5.25E-02	0.350	0.726	-
F	**Time of year**					
	November	**7.29E-01**	**2.84E-01**	**2.565**	**0.010**	-
F	Body condition	-2.63E-02	1.23E-01	-0.213	0.831	-
R	Individual ID	-	-	-	-	0.392

b)vi) Reproductive Success, Combined, GLM, All indices (n = 156)

Effect	*β*	SE	*z*	*p*
Intercept	-2.12E+00	5.06E-01	-4.181	<0.001
Sex				
Female	**9.48E-01**	**4.28E-01**	**2.217**	**0.027**
Age	3.26E-02	7.55E-02	0.431	0.666
Time of year				

November	**1.04E+00**	**4.25E-01**	**2.448**	**0.014**
Haptoglobin	3.89E-01	2.20E-01	1.764	0.078
Natural antibodies	2.27E-01	2.26E-01	1.003	0.316
Complement activity	4.44E-02	2.04E-01	0.218	0.828
Heterophil-lymphocyte ratio	2.70E-01	1.99E-01	1.360	0.174
Body condition	-4.48E-02	2.11E-01	-0.213	0.832

c)i) Dominance Acquisition, Independent, GLMM, Haptoglobin (n = 290)

F/R	Effect	*β*	SE	*z*	*p*	σ^2
	Intercept	-2.13E+00	4.52E-01	-4.711	<0.001	-
F	**Sex**					
	Female	**1.11E+00**	**3.71E-01**	**3.003**	**0.003**	-
F	Age	3.29E-01	1.97E-01	1.668	0.095	-
F	Haptoglobin	2.67E-01	1.60E-01	1.674	0.094	-
R	Individual ID	-	-	-	-	0.631

c)ii) Dominance Acquisition, Independent, GLMM, Natural antibodies (n = 235)

F/R	Effect	*β*	SE	*z*	*p*	σ^2
	Intercept	-2.25E+00	5.76E-01	-3.897	<0.001	-
F	**Sex**					
	Female	**1.27E+00**	**4.64E-01**	**2.731**	**0.006**	-
F	Age	3.69E-01	2.59E-01	1.426	0.154	-
F	Natural antibodies	-1.41E-02	1.88E-01	-0.075	0.940	-
R	Individual ID	-	-	-	-	1.076

c)iii) Dominance Acquisition, Independent, GLMM, Complement activity (n = 231)

F/R	Effect	*β*	SE	*z*	*p*	σ^2
	Intercept	-2.35E+00	6.08E-01	-3.870	<0.001	-
F	**Sex**					
	Female	**1.44E+00**	**4.90E-01**	**2.949**	**0.003**	-
F	Age	3.82E-01	2.57E-01	1.486	0.137	-
F	Complement activity	-2.03E-01	1.97E-01	-1.033	0.302	-
R	Individual ID	-	-	-	-	1.085

c)iv) Dominance Acquisition, Independent, GLMM, Heterophil-lymphocyte ratio (n = 247)

F/R	Effect	*β*	SE	*z*	*p*	σ^2
	Intercept	-2.41E+00	6.35E-01	-3.799	<0.001	-
F	**Sex**					
	Female	**1.20E+00**	**4.33E-01**	**2.768**	**0.006**	-
F	Age	4.00E-01	2.74E-01	1.458	0.145	-
F	**Heterophil-lymphocyte ratio**	**3.64E-01**	**1.83E-01**	**1.993**	**0.046**	-

R	Individual ID	-	-	-	-	0.570

c)v) Dominance Acquisition, Independent, GLMM, Body condition (n = 373)

F/R	Effect	*β*	SE	*z*	*p*	σ^2
	Intercept	-2.47E+00	5.08E-01	-4.860	<0.001	-
F	**Sex**					
	Female	**1.27E+00**	**3.72E-01**	**3.405**	**<0.001**	-
F	Age	4.62E-01	2.40E-01	1.923	0.054	-
F	Body condition	3.01E-01	1.57E-01	1.917	0.055	-
R	Individual ID	-	-	-	-	1.014

c)vi) Dominance Acquisition, Combined, GLMM, All indices (n = 158)

F/R	Effect	*β*	SE	*z*	*p*	σ^2
	Intercept	-1.91E+00	6.27E-01	-3.048	0.002	-
F	Sex					
	Female	8.85E-01	5.15E-01	1.720	0.085	-
F	Age	9.17E-02	2.46E-01	0.374	0.709	-
F	Haptoglobin	3.72E-01	2.42E-01	1.540	0.124	-
F	Natural antibodies	-1.30E-01	2.38E-01	-0.547	0.584	-
F	Complement activity	-3.84E-02	2.24E-01	-0.171	0.864	-
F	Heterophil-lymphocyte ratio	1.78E-01	2.13E-01	0.835	0.404	-
F	Body condition	2.80E-01	2.40E-01	1.168	0.243	-
R	Individual ID	-	-	-	-	0.548

Chapter 5: General Discussion

Overview

The immune system forms an integral physiological aspect of the whole organism that has evolved to optimally function and reproduce in a wild ecosystem. Individuals vary greatly in their immune function and understanding why this variation exists is essential to know how whole organisms are subject to trade-offs that influence life-histories and evolutionary processes. It is therefore particularly important to address these questions in the wild ecosystems that the organism and its immune system evolved in. My thesis investigates the possible causes and consequences of individual variation in constitutive innate immunity in a wild passerine model system. Through a series of observational studies, I aimed to show how environmental variation relates to variation in constitutive innate immunity (chapter 2); how immune function varies with age at both the individual and population levels (chapter 3); and how individual variation in constitutive innate immunity is linked to fitness-related outcomes (chapter 4). Here I discuss the key findings of these chapters (fig. 1) and their significance from ecoimmunological perspectives, providing suggestions for future research.

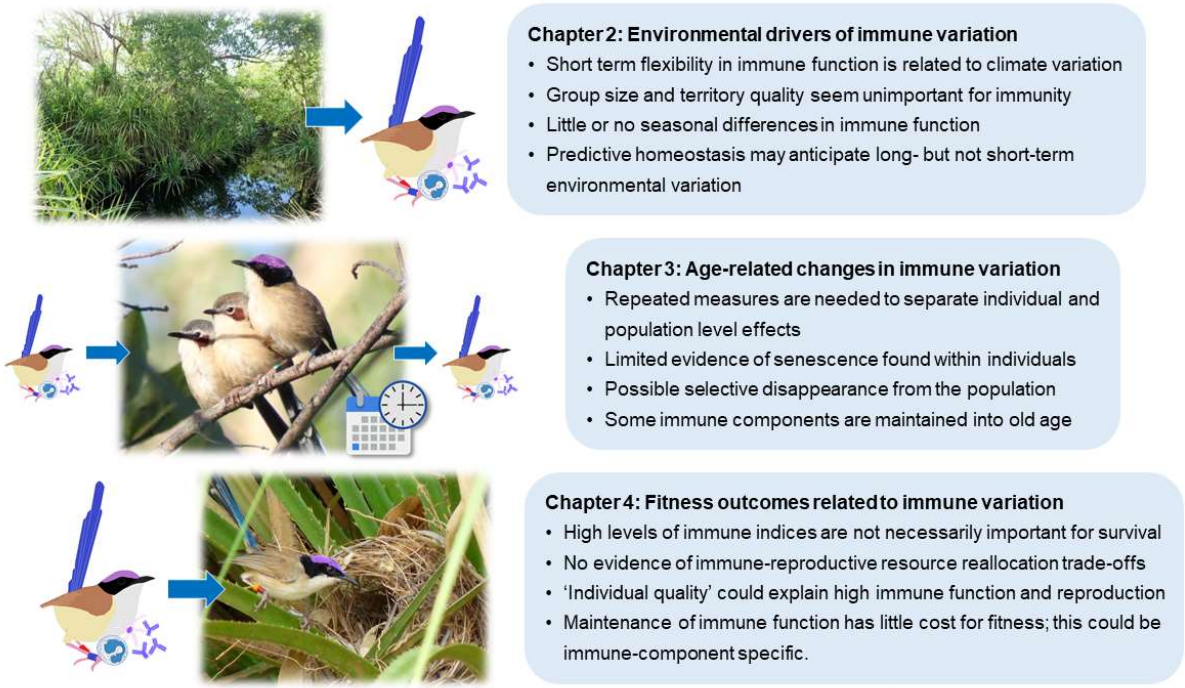

Figure 1: An overview of thesis chapter conclusions.

Further Discussion

Extensive individual variation in immune function

Individual immune variation could be due to genetic or environmental variation, or a combination of both (e.g. genetically controlled mechanisms that respond to environmental cues such as photoperiod; see *winter immune-enhancement hypothesis,* Nelson et al., 1995; Walton et al., 2011). In chapter 2, I focused on testing the strength of relationships between measures of constitutive innate immune function and several environmental (climatic, social and ecological) variables hypothesised to influence immunity. I showed that short-term climatic variation was relatively more important for predicting immune variation than group size and territory quality, which are temporally more stable. Despite the relationships to short-term climatic variables, immune indices remained at relatively stable levels throughout the year, with only limited seasonal differences in natural antibodies. From a reactive scope perspective (Romero et al., 2009), these data suggest that predictive homeostasis may maintain stability in immune function in anticipation of longer-term environmental variation, but unpredictable short-term environmental variation may induce compensatory variation in immune function. These results are consistent with observations that in the tropics, environmental fluctuation is more important than the annual cycle for immune variation (Nwaogu et al., 2019). Furthermore, seasonality for tropical species is less important for immune variation than in temperate species which experience more pronounced environmental variation as part of the annual cycle (Martin et al., 2008). While the ability to make predictive adjustments to immune function could have a genetic component (Versteegh et al., 2014), the immune variation I observed appeared to be highly flexible and had very low within-individual repeatability (Chapter 2, table S5), suggesting that individual differences in immune variation are unlikely to be heritable, similar to humans where non-heritable influences are most important to immune variation (Brodin et al., 2015).

Low within-individual repeatability in immune indices could result from changes in immune function within individuals over time. In chapter 3, I adopted a within-subject centring approach to assess how immune function varied with age at both the individual and population levels (van de Pol and Wright, 2009). Although I found indications that circulating levels of natural antibodies decline within individuals (senescence), and that adaptive cellular immunity senesces at a faster rate than innate cellular immunity, these effects were not significant. These results are consistent with general trends that constitutive innate immune function is maintained into old age (Peters et al., 2019), perhaps because it becomes more important for additional self-maintenance roles beyond immune defence, e.g. to mitigate the increase in inflammation with age (Chapter 3; Franceschi et al., 2017; Holodick et al., 2017; Quaye, 2008;

Ricklin et al., 2010). Nevertheless, there was substantial variability between individuals in repeated measures of immune function as was evident from the diverse individual reaction norms (Chapter 3, figs. 2a, 2b, grey lines). Combined together, the findings from Chapter 2 and 3 indicate that overall levels of constitutive innate immune function may remain stable during the year and with advancing age in purple-crowned fairy-wrens. However, there is still a high degree of immune variation within individuals, explained by 'immunoplasticity' that is related to short-term environmental variation.

Parasite pressure to maintain immune defences?

In chapter 4, I aimed to address the ultimate fitness consequences of variation in immune function, where I explored the links between variation in immune function and several fitness-related traits. Although immune-reproductive trade-offs have been extensively tested and found with induced immune responses (Hasselquist and Nilsson, 2012), I found no such trade-offs with constitutive immune indices. Moreover, I found no indication of condition-dependence, suggesting that the maintenance of these immune components requires relatively few resources (or individuals were not subjected to restricted resource availability). In combination with the evidence of overall maintenance of immune function (between seasons and within individual lifetimes; chapters 2 and 3), this could suggest constitutive immune defences are important and relatively uncostly to maintain. If these defences are relatively uncostly and maintained because they are important for baseline defence, then they may not be related to survival differences if all individuals can equally afford these particular defences. Following this logic, parasite pressure in the environment is then likely to be one reason why immune defences are overall maintained.

An unfortunate limitation to my thesis is that I was unable to relate any measure of parasitism to immune status or fitness, so an important aspect of the environmental context is missing (despite an extensive search for ectoparasites, and gut and blood endoparasites; see Appendix). As chapters 2, 3 and 4 are observational studies, any additional relationships uncovered between parasites, immunity and fitness and could have strengthened any inferences made (Graham et al., 2011). Without parasite information, it is also difficult to assess the possibility of parasite tolerance in the balance of any trade-offs. The lack of parasite information was partly due to unusually low levels of evident parasites and disease. Firstly, I found little evidence of ectoparasites (mites, lice, ticks) that can be common on birds (Clayton et al., 2010; Appendix). Additionally, there is a persistently and surprisingly low prevalence (5%) of avian malaria (*Plasmodium, Haemoproteus, Leucocytozoon*) in purple-crowned fairy-wrens despite high prevalence (25-75%) of partially compatible malaria genotypes in closely

sympatric riparian community species, and a closely related fairy-wren of adjacent savannah grasslands (Eastwood et al., 2019). At the same time, purple-crowned fairy-wrens circulate very high levels of natural antibodies at a mean titre score of ~15, compared to other species with mean titres of < 9 (Matson et al., 2005; Chapter 1; *cf.* Matson, 2006, table 1 for comparison to other species). Speculatively, these high levels of natural antibodies may confer high resistance to malaria and explain the low prevalence similar to observations in the Hawaiian Amakihi (Atkinson and Paxton, 2013). Whether these specific immune components are responsible for keeping malaria low may depend on specific parasite detection and host evasion interactions (Gowda and Wu, 2018; Silver et al., 2010), but is not possible to ascertain from my thesis results. Still, it seems reasonable to assume that the general maintenance of immune function in purple-crowned fairy-wrens may be driven by parasite pressure (Cooper and Herrin, 2010; Schulenburg et al., 2009) and will improve longevity and survival.

Immunoplasticity: optimal immune function in variable contexts

Optimal immune function varies because demands on the immune system vary (French et al., 2009; Pedersen and Babayan, 2011; Tieleman, 2018; Viney et al., 2005). Environmental and physiological contexts (i.e. the intrinsic physiological state or condition of an organism) are dynamic and therefore different levels of immune function are likely to be optimal at different times. Between-individual immune variation is perhaps evolutionarily advantageous (French et al., 2009) and this could be the case when polymorphisms of immune genes or phenotypes are selectively maintained (Kubinak et al., 2012; Maizels, 2009). Further still, the ability of an individual to vary immune function plastically could be a trait that confers fitness benefits when the contexts that shape optimal immune investment are dynamic. In chapter 4, the only significant correlation between higher survival probability and any immune index was either a high or low level of (quadratic) complement activity, showing different immune phenotypes can be advantageous, probably in different individual-specific contexts. This could be a consequence of individuals with high or low complement levels being selected for at different times, resulting in selective maintenance of complement activity variation in the population. Alternatively, individuals that can vary between high and/or low complement activity – with higher immunoplasticity – may have higher fitness than less plastic individuals that are limited to intermediate levels of complement activity. More broadly, given that there is low repeatability among individual repeated measures (Chapter 2), which are unstructured with respect to age (Chapter 3), immunoplasticity is a credible explanation for immune variation in purple-crowned fairy-wrens.

Physiological trade-offs between costly immune functionality and reproduction are presumed to function to maximise fitness (Sheldon and Verhulst, 1996). These trade-offs are also shaped by the costs and benefits of current *vs.* future reproduction. In iteroparous species, the optimal trade-off could change with each subsequent reproductive attempt, under the influence of inter-annual variation in ecological, climatic, individual or social conditions. Immunoplasticity in either the magnitude or type of immune response could result in dynamic trade-offs that are selectively advantageous. By fully considering variation in plasticity of immune function as a labile trait, it is possible to gain insights into the adaptive nature of immune variation, and such comparable insights have been gained from the study of plasticity in animal behaviour and life-history traits (Dingemanse et al., 2012; Dingemanse and Wolf, 2013; Nussey et al., 2007). This important aspect of variation has received very little attention to date in relation to immune function; a literature search yielded only one study that expressly examined between-individual differences in within-individual variation/repeatability in immune function, *i.e.* variation in immunoplasticity (Love et al., 2008; Web of Science; 182 results found and assessed; search terms "immun*" and "plastic*" and "fitness"). Incorporating analyses of variation in immunoplasticity across different contexts might better explain how immune variation relates to lifetime reproductive success and could substantially enhance evolutionary perspectives on immune variation.

Beyond optimal immune function in a single context or point in time, I would hypothesise that immunoplasticity is more important for lifetime reproductive success across multiple different contexts and life-stages experienced during an individual's lifetime. From this hypothesis, it can be predicted that individuals that are better able to plastically change their investment in immune function should have a greater fitness, translating to a greater survival probability especially in long-lived species. This could be tested experimentally by fluctuating a component of environmental context, *e.g.* parasite pressure, over successive breeding attempts within the same individuals, while quantifying how variation in plasticity relates to individual reproductive success. Observational approaches might augment experimental work through repeated measurements of immune function from free-living individuals scheduled at different critical phases in the annual cycle. For example, taking measurements when the expectation of immune function is both low such as during a moult phase or during reproductive attempts (Moreno, 2004; Sanz et al., 2004), and high during an overwintering phase when all resources are likely to be allocated to survival and self-maintenance (Martin et al., 2008) would allow individual plasticity to be quantified and related to individual cumulative reproductive success.

Immunity and the pace-of-life

Purple-crowned fairy-wrens are relatively long-lived small passerines, adopting a slow pace-of-life (Montiglio et al., 2018). Needing to wait for ideal breeding conditions in unpredictable environmental conditions (Hidalgo Aranzamendi et al., 2019) may have led to the evolution of a 'sit-and-wait' life-history strategy where immune function is crucial for longevity and survival. Different immune function and life-history strategies among species could contribute to fast- or slow-living syndromes on a single pace-of-life axis (Pap et al., 2015; Tieleman et al., 2005). Immune variation at lower organisational levels, between populations or individuals (Ardia, 2005; Martin et al., 2006) can provide the variation on which selection can drive evolutionary change. Although predictions can be made that lower constitutive innate immune function and greater adaptive immunological memory should relate to a slower pace-of-life (Lee, 2006), a relatively unexplored way to test these predictions may be to focus on telomeres. The length of telomeres – the DNA segments that protectively cap chromosomes – declines with age (Monaghan and Haussmann, 2006), as is the case in purple-crowned fairy-wrens (fig. 2). The rate of telomere shortening is slower in slow-living species and is linked to the maximum lifespans of birds, showing clear links to a pace-of-life (Dantzer and Fletcher, 2015; Tricola et al., 2018). For species where immune function is important for longevity, it might be expected that individual immune variation is related to the rate of telomere shortening. Importantly, relationships between immune function and telomere shortening may be distinct from chronological aging as telomere length also contains information about individual quality (Angelier et al., 2019; Le Vaillant et al., 2015; including purple-crowned fairy-wrens, Eastwood et al., 2019) and accumulated past physiological stress (Monaghan, 2014; Nussey et al., 2014). Although purple-crowned fairy-wrens did not show clear immunosenescence (Chapter 3), there is evidence to suggest telomere shortening as a biomarker of aging is more closely linked to immune function (Kaszubowska, 2008; Katepalli et al., 2008) and may have a proximate mechanistic role in immunosenescence and cellular senescence (Young, 2018). Exploring the relationships between telomere shortening and immune function could provide valuable insight into our understanding of the evolution of pace-of-life syndromes, and the purple-crowned fairy-wren would make an excellent study system in which to test within-species predictions.

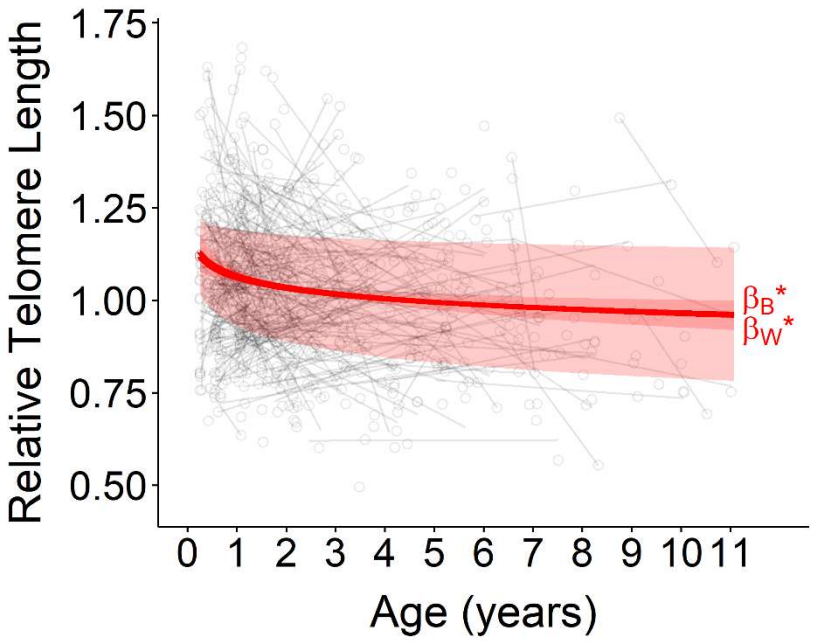

Figure 2: Relative telomere length (rTL) shortens with age in purple-crowned fairy-wrens. Fitted lines (almost perfectly overlaid) show predicted rTL across within-individual (β_W) and between-individual (β_B) changes chronological age. Ribbons indicate 95% confidence intervals, with "*" indicating those that do not contain slopes of zero. Raw data points are plotted, with fitted lines of individual reaction norms of repeated measures, from a simplified linear regression model of rTL ~ age per individual.

Conclusions

My PhD thesis contributes to the current understanding of the causes and consequences of individual variation in immune function by using an exceptionally well-studied model species in a wild and free-living context. I found that immune function in purple-crowned fairy-wrens is most sensitive to short-term changes in temperature and rainfall compared to longer-term environmental variation (chapter 2). Otherwise, immune function is relatively stable during the annual cycle (chapter 2) and is maintained with advancing age, with only limited evidence of immunosenescence (chapter 3). As a species that is already vulnerable to climate change (Reside et al., 2016), interactions with emergent diseases will likely be increasingly common in the future (Brooks and Boeger, 2019; Staley and Bonneaud, 2015), and could pose threats that are exacerbated by immune sensitivity to climatic variation, as will be the case for many other species. Although there was no evidence of resource allocation trade-offs between

constitutive immune function and reproduction (chapter 4), this contrasts with previous work on induced immune components and could indicate immune component-specific trade-offs, or relatively little costs of maintaining immune function. High levels of immune function did not relate to survival, though there was partial evidence in relation to complement activity (chapter 4) and a generally low individual repeatability of immune measures (chapter 2 and 3). This suggests immunoplasticity exists in purple-crowned fairy-wrens, and could be important for survival. Immunoplasticity could have an important role for resilience to predicted increases in extreme and unpredictable weather events in the near future (Stott, 2016). As an aspect of immune variation that has received little attention, this will be an important direction for further research.

Appendix: Notes on the parasitism in purple-crowned fairy-wrens

In attempting to understand the significance of individual variation in immune function, parasitism in the focal species has a crucial role to play. Parasitism is the key evolutionary pressure that has led to the development of complex immune systems in spite of the costs of immunity (Cooper and Herrin, 2010). Changes in parasite pressure can directly lead to changes in individual immune function and can shape the investment trade-offs between immune function and other physiological processes (Tschirren and Richner, 2006). Consequently, it has been recommended that ecoimmunologists aim to quantify levels of parasitism within hosts in conjunction with host immune function and estimates of host fitness to have the best chance of interpreting individual variation in immunity (Graham et al., 2011). During my PhD, I made several attempts to quantify levels of parasitism within individual purple-crowned fairy-wrens and link parasitism to the individual's immune status at capture. I focused on three common general classes of parasites – ectoparasites, gut endoparasites and blood endoparasites – that through different mechanisms could each stimulate changes in immune status.

Different ectoparasites can incur different costs to birds. For example, damage to feathers caused by feather parasites can affect thermal insulation and locomotory efficiency (Barbosa et al., 2002), while ticks and biting insects are likely to be more important for immune function as they can introduce other pathogenic infections through bite wounds (Brinkerhoff et al., 2019). From 2014 to 2017 we screened for ectoparasites during each capture by blowing up the feathers all over the entire head, body and wings of the bird to visually inspect to the base of the feathers and the skin (fig. A1a). During this period, < 6% of captured individuals had any detectable ectoparasites (638 captures of free-flying birds, 37 with parasites, range = 0-7 parasites). Interestingly, no single individual was found that harboured any ticks, and almost all parasites were the same louse species (except a single *Hippoboscidae sp.* fly). Louse parasite specimens were collected and identified in the laboratory as a feather louse, *Myrsidea sp.* (Clay, 1965; fig. A1b), likely an undescribed host-specific species. This genus of feather-chewing lice feeds on lacrimal secretions and feathers of their hosts, which degrades the quality of feathers (Barbosa et al., 2002; Mey, 2013). Given the very low detection rate and unlikely direct influence these lice would have on immune function as they do not bite, it was not useful to investigate this association further. This visual screening method for ectoparasites is likely to have had an imperfect detection rate but should still predict the total parasitism (Koop and Clayton, 2013), however it is surprising that no ticks were found over such a large sample as purple-crowned fairy-wrens occupy an understorey habitat niche where ticks are commonly encountered (Parker et al., 2017). Many bird species have

anatomical adaptations for grooming and adopt self-maintenance behaviours that combat the threat of parasitism (Clayton et al., 2010); close social bonds and frequent allopreening behaviours common among purple-crowned fairy-wrens may provide an effective form of defence against such costly ectoparasites.

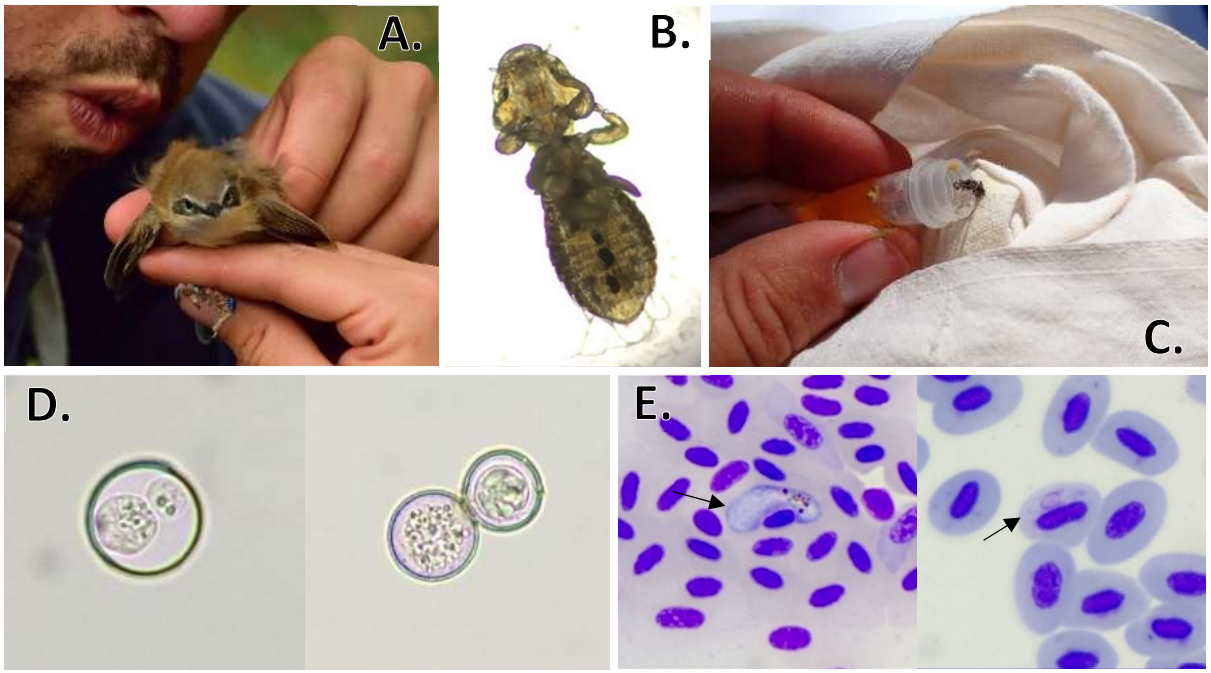

Figure A1: Assessing parasitism in purple-crowned fairy-wrens. **A.** Visual inspection method for counting ectoparasites at capture. **B.** A *Myrsidea sp.* ectoparasite found on a purple-crowned fairy-wren. **C.** Faecal sample collection from bird-holding bags. **D.** Oocysts of coccidian gut endoparasites, putatively *Isospora sp.*. **E.** Intra-cellular malaria parasites at different developmental stages detected in (nucleated) red blood cells from blood smears; *Haemoproteus sp.* and *Plasmodium sp.* lineages were confirmed from through PCR (Eastwood et al., 2019).

Gut endoparasites are another ubiquitous group of parasites that I assessed in purple-crowned fairy-wrens. Continuous exposure to a diverse microbial community in the digestive tract is purported to drive the maintenance of constitutive immunity in order to defend against pathogens or opportunistic commensals that cross the intestinal epithelium (Rakoff-Nahoum and Medzhitov, 2006). Parasites of the subclass *Coccidia*, phylum *Apicomplexa*, are commonly found in passerines (Page and Haddad, 1995) and made a good candidate for study as part of my thesis. Infections are often chronic, and constitutive innate immunity is important in their control; *Coccidia* specifically are directly related to complement activity and

natural antibodies (Pap et al., 2011; Saks et al., 2006). For this reason, from 2015 to 2017 faecal samples were incidentally collected from individuals at capture (fig. A1c), and from these samples *Coccidia* oocysts were extracted by a modified flotation method in a saturated saline solution that consumed the sample; oocysts were then microscopically counted (Dolnik, 2006). As bird capture was most efficient at dawn, a period of high fairy-wren activity and low mist-net visibility, sampling times were substantially biased towards sunrise. Unfortunately, this had unintended consequences as there is a diurnal cycle in the peak times of oocyst shedding (López et al., 2007). This was expected to be statistically controllable, but the phenomenon was so pronounced in purple-crowned fairy-wrens that after n = 154 samples were analysed, not a single sample collected in the first ~4.5 hours of daylight was found to have detectable *Coccidia* present (fig. A2). This contrasted with 45% of samples collected after the first ~4.5 hours of sunlight testing positive (fig. A1d; fig. A2), suggesting that many of the samples collected in the morning were false negatives and the data from the faecal samples could not be meaningfully interpreted. With greater resources, a more sensitive PCR-based approach would be able to detect presence of *Coccidia* from DNA in the sample despite a negative microscopic result (Nakamura et al., 2009), however it was unfeasible in this study.

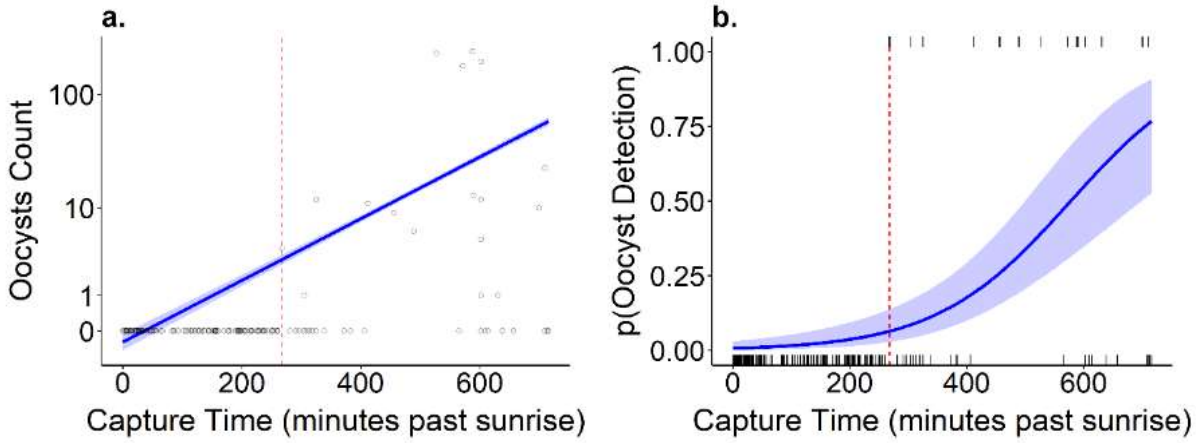

Figure A2: Time of day strongly affects *Coccidia* oocyst shedding in purple-crowned fairy-wrens. **a.** Individual oocyst counts plotted against the time of day faecal samples were collected. Blue line shows Poisson-regression (blue line) of individual oocysts counts and the time of day faecal samples were collected with confidence interval ribbon. Y-axis is base 10 log-transformed. The earliest positive faecal sample collected at 268 minutes (red line); 75% of faecal samples were collected before this time. **b.** Logistic-regression (blue line) of positive oocysts samples and the time of day faecal samples were collected with confidence interval ribbon. Probability of detection is 0.06 at 268 minutes past sunrise (red line).

Lastly, blood endoparasites were assessed using blood samples taken at capture as another an alternative source of parasite information to link to immune status. Specifically, avian malaria causing parasites of *Plasmodium sp.*, *Haemoproteus sp.* and *Leucocytozoon sp.* in purple-crowned fairy-wrens were screened for using nested-PCR approaches as part of another project in the research group (Eastwood et al., 2019), while malarial and other parasites were incidentally observed during white blood cell counts of blood smears (1 sample with *Trypanosoma sp*.; 6 with microfilarial nematodes; 11 with intracellular malarial stages, fig. A1e). All individuals found positive for malaria in blood smears were also positive with the PCR method, but despite the more sensitive PCR screening, only ~5% of purple-crowned fairy-wrens were infected with avian malarial parasites. This low prevalence in purple-crowned fairy-wrens was persistent across years, despite their susceptibility to different malarial lineages, high population density in riparian habitat, and a high malarial prevalence among other bird species in the community (25-75%; buff-sided robin – *Poecilodryas cerviniventris*, white-gaped honeyeater – *Lichenostomus unicolor*, red-backed fairy-wren – *Malurus melanocephalus*; Eastwood et al., 2019). Consequently, there were too few individuals with malaria and associated immune data to assess how immune function is related to malarial infection.

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