Causes and fitness consequences of telomere dynamics in a wild social bird

Submitted by

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I certify that all material in this thesis which is not my own work has been identified and that no material has previously been submitted and approved for the award of a degree by this or any other University.

Summary

Telomeres are increasingly used as biomarkers of somatic maintenance and could conceivably play a causal role in life history trade-offs. In this thesis, I use longitudinal telomere measures from a wild population of cooperatively breeding white-browed sparrow weavers (*Plocepasser mahali*) to further our understanding of the causes and fitness consequences of individual variation in somatic maintenance, with particular focus on hitherto unexplored effects of the social environment. In Chapter 2, I start by investigating the key prediction of life-history theory that shortfalls in somatic maintenance in early life entail later-life costs, and find supporting evidence. Nestlings with higher within-individual rates of telomere attrition show reduced survival to the following season, even after controlling for the effects of variation in body mass. In Chapter 3, I then investigate the effects of the social and abiotic environment on nestling telomere length and attrition rates and find the first support, to my knowledge, for the key prediction that helpers in cooperatively breeding societies alleviate telomere attrition rates in growing offspring (consistent with the expectation that helper contributions to nestling feeding relax resource allocation trade-offs in offspring). In addition, I find that rainfall prior to egg-laying has a positive effect on hatchling telomere length; an effect that most likely arises via egg- or incubation-mediated maternal effects. In Chapter 4, I investigate the causes of variation in telomere attrition rates in adults, and while there are no overall differences in telomere length or long-term within-individual telomere dynamics between dominant and subordinate birds, my findings are suggestive of dominance-related differences in the short-term regulation of telomere length. In addition, and in concordance with predictions of life-history theory regarding trade-offs between somatic maintenance and reproduction, I find that annual rainfall (a proxy for reproduction-related activity during the breeding season) negatively predicts the within-individual rate of change in telomere length in adults specifically over the breeding season; there was no such relationship in the non-breeding season. Finally, in Chapter 5, I investigate the extent to which natural variation

in oxidative state predicts variation in within-individual rates of change in telomere length over time. This chapter provides evidence suggestive of associations between oxidative state and telomere dynamics in a natural population, and highlights complexity in the nature of these relationships. Together my findings provide novel support for key predictions of life-history theory regarding the causes and consequences of variation in somatic maintenance, and lend strength to the view that longitudinal field studies of telomere dynamics can offer useful insights in this regard. Furthermore, my findings highlight the potential for diverse effects of the social environment on patterns of somatic maintenance, and specifically hitherto unexplored downstream effects of helping behaviour on later-life performance and ageing trajectories.

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Authors Declaration

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All chapters in this thesis were written by Emma M Wood with comments and editing by Dr. Andrew J Young. Comments were also provided by Dr. Dominic Cram, Pablo Capilla-Lasheras and Madeleine Vierbuchen. Guidance was provided throughout by Dr. Andrew J. Young. The population of white-browed sparrow weavers (*Plocepasser mahali*) used in this thesis are part of a long-term project led by Dr. Andrew J. Young.

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Chapter 1

General Introduction

1.1 Overview

Telomere dynamics are of particular interest in evolutionary biology as they are thought to act as biomarkers of somatic maintenance and could also conceivably play a causal role in life history trade-offs. In this introductory chapter, I give an overview of the structure of telomeres and current understanding of their function regarding cellular senescence, before outlining some determinants of telomere length and dynamics. I then discuss the potential for studies of telomere dynamics to shed light on life-history trade-offs through their role as a biomarker of somatic maintenance. In particular, I highlight our currently limited understanding of the impact of social behaviour on patterns of somatic maintenance in animal societies. Finally, I outline the primary aims of this thesis, which collectively seek to address this shortfall in our understanding, and introduce the model system that was utilised to address these aims.

1.2 Telomere structure and function

Telomere structure

Telomeres are located at the ends of linear chromosomes and comprise a highly conserved repetitive non-coding sequence of nucleotides (in vertebrates TTAGGG; Blackburn & Szostak 1984; Meyne *et al.* 1989) that form a scaffold for telomeric proteins, including a suite of telomere-specific proteins known as shelterin (Blackburn 1991; de Lange 2005). Telomeric repeats and shelterin are both required for telomeres to function (de Lange 2015). Indeed, telomeres are thought to have evolved as a mechanism to stabilise linear chromosomes (de Lange 2015); without them chromosome ends would be recognised as damaged DNA, resulting in cell cycle arrest, inappropriate recombination, or chromosome end-to-end-fusions (Blackburn & Szostak 1984; Mieczkowski *et al.* 2003; Feldser & Greider 2007; de Lange 2009). In order to avoid triggering DNA damage response mechanisms, it is thought that the telomeric sequence doubles back on itself with the assistance of particular subunits of shelterin (in mammals, thought to be TRF1 and TRF2; Bianchi *et al.* 1999; Stansel *et al.* 2001), to form a 't-loop' structure (Griffith *et al.* 1999; Stansel *et al.* 2001). This is made possible

by a 3′ single-strand overhang at the terminus of the telomere (Henderson & Blackburn 1989; Makarov *et al.* 1997; Wright *et al.* 1997), which is sequestered into the duplex region of the telomere by strand invasion (Griffith *et al.* 1999). The resulting 'D-loop' of displaced TTAGGG repeats is single-stranded and thus has the potential to generate a DNA damage response. However, a subunit of shelterin that binds to single-strand telomeric DNA (in mammals; POT1) appears to prevent this (Denchi & de Lange 2007; Barrientos *et al.* 2008). By blocking the DNA repair machinery from acting at the telomere end, these, and potentially other mechanisms, effectively 'cap' the telomere. Telomeres are, however, dynamic structures that can become 'uncapped' either in a regulated or unregulated manner, and can be both shortened (which I refer to throughout this thesis as telomere attrition) and, under some circumstances, extended.

Telomere attrition and cellular senescence

Telomeres protect coding DNA from loss during cell replication, as conventional DNA polymerases are unable to fully replicate the lagging strand (the "end replication problem"; Watson 1972; Olovnikov 1973) and additional erosion is thought to occur during processing of the 3′ single-strand overhang (Makarov *et al.* 1997; Sfeir *et al.* 2005). Thus, in the absence of functional telomere repair mechanisms, telomeres (rather than important regions of coding DNA) become shorter with every cell division (Harley *et al.* 1990). Once enough telomeres in a cell become too short (e.g. such that they can no longer form a t-loop), chromosomal instability ensues or DNA damage response mechanisms trigger cellular senescence (in which cellular division is arrested) or programmed cell death (apoptosis) (Karlseder 1999; Hemann *et al.* 2001a; Herbig *et al.* 2004; Feldser & Greider 2007; Lloyd *et al.* 2009). The process of telomere attrition thereby limits the proliferative potential of cells (Harley *et al.* 1990), and thus too the potential for tissue regeneration (Hao *et al.* 2005; Reichert *et al.* 2014a). In so doing, they contribute to the accumulation of senescent cells in tissues. Senescent cells that do not die have an altered secretory profile and can contribute to inflammatory disease and undermine health (Kipling 2001; Herbig *et al.* 2006; Campisi & d'Adda di Fagagna 2007; Coppé *et al.* 2010). Thus, telomere dynamics are of interest in evolutionary biology as they have the potential to act as a biomarker of age-related declines in cellular-level

somatic redundancy (Boonekamp *et al.* 2013; Simons 2015). Indeed numerous studies carried out on whole organisms both in captivity and now too in the wild, have shown that telomeres on average get shorter with age (Salomons *et al.* 2009; Barrett *et al.* 2013; Young *et al.* 2013; Beirne *et al.* 2014; Asghar *et al.* 2015), and in some cases are a better predictor of remaining lifespan than chronological age is (Bize *et al.* 2009).

It is therefore tempting to think of gradual telomere erosion as a 'mitotic clock', counting down the number of cell divisions remaining before telomeres reach a 'critical length' that heralds the end of cellular replication (Hayflick 1965; Vaziri *et al.* 1994). However, it is increasingly apparent that the true relationship between telomeres and the triggering of cellular senescence is more complex. In addition to the incremental shortening of telomeres during cellular replication, large tracts of telomere may sporadically be lost (Baird *et al.* 2003; Lansdorp 2005). Furthermore, action by telomere repair mechanisms can decelerate telomere loss, or even extend telomeres, thereby invalidating the view that telomeres act as a simple 'mitotic clock', at least in cells where such mechanisms are active (Blackburn 2000). Below I address each in turn and reflect on what these processes mean for our understanding of telomere dynamics at both the cellular and organismal levels.

Additional mechanisms leading to telomere attrition

Processes other than the cell replication-associated mechanisms described above can cause the loss of large tracts of telomere. This was highlighted by Baird *et al* (2003) who measured telomere lengths at individual chromosomes and showed that telomeres of telomerase-negative fibroblasts undergo large-scale stochastic reductions in length. A number of mechanisms by which this may occur have been proposed (Lansdorp 2005), including deletion of the t-loop during homologous recombination events (Wang *et al.* 2004), and failure to correctly process higher order telomeric structures (Ding *et al.* 2004). The most commonly cited cause of accelerated telomere attrition is, however, oxidative stress.

Oxidative stress arises when the rate of production of reactive oxygen species (ROS) overwhelms a complex system of exogenous and endogenous antioxidant protection and causes damage to proteins, lipids, and DNA (Finkel & Holbrook 2000; Halliwell & Gutteridge 2007a). Over 20 years ago, von Zglinicki et al (1995) demonstrated that human fibroblasts cultured under hyperoxic conditions (and therefore under conditions of oxidative stress) had a more than 5-fold increase in their telomere attrition rate and underwent fewer population doublings than fibroblasts cultured under normoxia. Since then, the focus of *in vitro* work has progressed to investigating the mechanisms by which oxidative stress may increase telomere attrition rate. The overrepresentation of guanine is a highly conserved feature of telomeres across taxa (Williamson 1994), and may explain the particular vulnerability of telomeres to oxidative damage, as it has a lower oxidation potential than other bases (Burrows & Muller 1998), and is particularly prone to damage when repeated – as it is in telomeres (Oikawa & Kawanishi 1999; Kawanishi *et al.* 2001). One of the most common forms of damage induced by oxidative stress are 8-oxo-gaunine lesions (8-oxoG; modified guanine bases), which can result in single strand breaks. Treatment of fibroblasts with hydrogen peroxide (H_2O_2; a reactive oxygen species) has shown that this type of damage persists in telomeres, despite being rapidly repaired in other non-transcribed repetitive sequences and in the bulk of the genome (Petersen *et al.* 1998; also see Oikawa & Kawanishi 1999; Kawanishi & Oikawa 2004; Coluzzi *et al.* 2014). This feature of telomeres has been hypothesised to render them 5-10 times more vulnerable to oxidative damage than other parts of the DNA (Henle *et al.* 1999; Kawanishi *et al.* 2001; Kawanishi & Oikawa 2004). Indeed Richter & von Zglinicki (2007) found a strong exponential correlation between intracellular levels of ROS and telomere shortening rate in a number of cell types. That telomeres appear to be both particularly prone to oxidative damage (Kawanishi *et al.* 2001; Kawanishi & Oikawa 2004) and unusually resistant to its repair has led to the proposal that telomeres are 'sentinels' of damage, acting to prevent continued replication of cells that have faced so much damage as to become dangerous (von Zglinicki 2002). The mechanism (or mechanisms) by which damage to the telomere results in accelerated telomere attrition is unclear, though it is thought that cell replication is required for attrition to take place and it has been

suggested that disruption of the replication fork (Sitte *et al.* 1998; von Zglinicki 2002; Coluzzi *et al.* 2014) and reduced binding efficiency of shelterin may play important roles (Opresko *et al.* 2005).

In addition to the negative effects of ROS on telomere length, it has been demonstrated that experimental increases of endogenous and exogenous antioxidant levels both attenuate telomere attrition rate and increase the proliferative potential of cells (Furumoto *et al.* 1998; Liu *et al.* 2002; Kashino *et al.* 2003; Serra *et al.* 2003; Kurz *et al.* 2004). This lends further support to the hypothesis that oxidative stress is an important determinant of telomere attrition rates at least *in vitro*. If oxidative stress also causes the acceleration of telomere attrition in whole organisms, telomere dynamics have the potential to act as a biomarker of accumulated exposure to oxidative damage. In addition, the finding that increased levels of antioxidants mitigate the effects of ROS on telomere attrition suggests that animals may be able to avoid detriments to telomeres by investing in antioxidant protection. However, evidence for a role of oxidative stress in rates of telomere attrition *in vivo* is far from clear cut, particularly in free-living animals, with associations found between telomere dynamics and measures of ROS or of damage in some species (Ballen *et al.* 2012; Geiger *et al.* 2012) but not others (Nettle *et al.* 2015; Giraudeau *et al.* 2016; Boonekamp *et al.* 2017). Similarly, antioxidants have been shown to alleviate telomere attrition in some species (Cattan *et al.* 2008; Badás *et al.* 2015), but to only have effects on some classes of individual in others (Kim & Velando 2015; Noguera *et al.* 2015; Taff & Freeman-Gallant 2017) and in some species to have no effect. For example Boonekamp et al (2017) recently found no association between any of six measures of oxidative status (both damage and protection) and telomere attrition rate in nestling jackdaws, *Corvus monedula*, despite highly variable rates of telomere attrition. This ambiguity in whole organism studies is perhaps to be expected, as oxidative stress is a complex state that comprises multiple components of both damage and defence, and is thus difficult to measure (Monaghan *et al.* 2009; Hõrak & Cohen 2010). In addition, animals, particularly in the wild, may have behavioural and physiological adaptations to limit oxidative damage that obscure the relationship between any single measure of oxidative status and telomere attrition. Improvement in

our understanding of the proximate causes of variation in telomere dynamics *in vivo* is thus called for, particularly in the wild.

Telomere extension

Telomere attrition is not inevitable. Telomeres may be extended by the enzyme telomerase through *de novo* addition of nucleotides (Greider & Blackburn 1985), or by alternative lengthening (ALT) during homologous recombination events (Henson *et al.* 2008; Cesare & Reddel 2013). As extension of telomeres by ALT is repressed in healthy cells and (thus far) has only been found in abnormal tissues, such as cancer (Henson *et al.* 2008; Cesare & Reddel 2013), I will focus here on extension by telomerase. Although conventional DNA replication machinery is able to replicate the majority of the telomere (Wellinger & Zakian 2012), it is unable to replicate it to the end. For this, a specialised enzyme is required, namely telomerase. Telomerase is a ribonucleoprotein reverse transcriptase formed of an RNA component (TERC) and the protein TERT. By copying a short template sequence in the RNA moiety, telomerase is able to synthesise telomeric DNA, running in a 5′ to 3′ direction towards the distal end of the telomere. The number of repeats added by telomerase at any one extension event is independent of the length of the telomere – in other words, the loss of telomeric repeats at cell division is not matched by the action of telomerase (Teixeira *et al.* 2004). In addition, evidence suggests that telomerase acts on only a subset of telomeres at cell division and appears to act preferentially on shorter telomeres (Marcand *et al.* 1999; Teixeira *et al.* 2004). Telomere length homeostasis could, then, be achieved by this process: as short telomeres are extended, the likelihood of further extension decreases until telomerase's action is inhibited, whereupon the telomere once again becomes shortened, and so on (Zhu *et al.* 1998; Hemann *et al.* 2001b; Samper *et al.* 2001; Teixeira *et al.* 2004; Goldman *et al.* 2005).

The regulation of telomere length by telomerase is an intense area of research but the intricacies of the mechanisms involved have not yet been fully elucidated. However, shelterin is thought to play a major role (see Bianchi & Shore 2008 review). Most proposed mechanisms of telomere length regulation involve a protein counting

mechanism in which shelterin proteins exert an additive inhibitory effect on telomerase, blocking it from access to the telomere end (Grunstein 1997; Marcand *et al.* 1999; Teixeira *et al.* 2004). More recently, Greider (2016) proposed a model in which telomerase travels with the replication fork and has to reach the end of the telomere before it can act – as proteins bound along the telomere have the potential to cause the telomerase to dissociate from the replication fork, long telomeres are less likely to be extended. A third mechanism by which telomere length homeostasis may arise is through TPE-OLD (telomere position effect – over long distance), whereby telomeres loop to specific loci where they are instrumental in gene expression (Robin *et al.* 2014). Kim et al (2016) have recently provided evidence consistent with suppression of TERT (the protein component of telomerase) by association of long telomeres with the *TERT* gene: As telomeres shorten they disengage with the region of chromosome near *TERT* and telomerase expression is promoted. In addition to its potential role in regulating telomere length this mechanism raises the intriguing possibility that telomere dynamics may play a role in age-associated changes in gene expression. It may also explain why after artificial shortening *or* lengthening of telomeres, they reset to the length that is characteristic for that cell (Marcand *et al.* 1999; Negrini *et al.* 2007). It is thought that telomerase does not extend the lifespan of healthy cells indefinitely, and gradual telomere attrition occurs even in cells with relatively high expression of telomerase (Vaziri *et al.* 1994; Blasco 2007). Several studies have recently suggested that telomeres may undergo periods of extension at the level of the organism (Ujvari & Madsen 2009; Fairlie *et al.* 2016; Hatakeyama *et al.* 2016; Hoelzl *et al.* 2016a). However, whether increase in mean telomere length at the level of entire tissues is a phenomenon that can occur *in vivo* is hotly debated (Steenstrup *et al.* 2013; Bateson & Nettle 2016).

Patterns of telomerase expression vary both with cell type and ontogeny and, in general, telomerase expression is much higher in tissues that are required to undergo large numbers of divisions, such as stem cells and cells in the germ line (Lansdorp 2008). Following this logic, telomerase is often expressed to a greater degree in early life when growth rate is high (Haussmann *et al.* 2004; Korandová & Frydrychová 2016) and is thought to be entirely absent in most human adult somatic tissues (Collins & Mitchell

2002) – though in some long-lived species telomerase continues to be expressed in adult somatic tissues (Haussmann *et al.* 2004).

Telomere dynamics as a biomarker of somatic maintenance: the role of telomerase

It is possible that extension of telomeres by telomerase reduces the strength of association between telomere dynamics and accumulated exposure to oxidative damage. However, given the hypothesised function of telomeres as sentinels of cellular damage, one would expect selection against the decoupling of this link between telomere dynamics and somatic maintenance, as such decoupling could open the door to the runaway proliferation of damaged cells (Wright & Shay 2005). Indeed, there is growing evidence that telomerase has roles beyond telomere length regulation, suggesting that telomerase expression may not decouple telomere dynamics and somatic maintenance. For example, Ahmed et al (2008) found that the telomeres of fibroblasts cultured under hyperoxia shortened despite being telomerase positive, as TERT was gradually exported out of the nucleus to the mitochondria, where damage to mitochondrial DNA and the production of ROS were subsequently reduced. There is also evidence to suggest that telomerase expression and activity may be sensitive to oxidative stress exposure in a dose dependent manner, such that chronic exposure to oxidative stress may inhibit telomerase-mediated telomere extension (Ahmed *et al.* 2008; Beery *et al.* 2012). In addition, the expression of telomerase has been shown to be associated with improved repair of non-telomeric DNA, increased stress resistance to specific DNA damaging agents, and improved antioxidant defence (Sharma *et al.* 2003; Armstrong *et al.* 2005). Furthermore, there is evidence to suggest that telomerase protects cells against apoptosis independently of its effect on telomere length (Fu *et al.* 2000; Armstrong *et al.* 2005).

1.3 Telomeres as an organismal biomarker of somatic maintenance

The process of somatic maintenance utilises resources that may otherwise be used in (for example) growth or reproduction, in order to protect or repair cellular components. In so doing, somatic maintenance aims to preserve the integrity of the whole organism.

If resources were infinite and somatic maintenance perfect, age-related declines in somatic function would not occur. In a world of finite resources, however, the investment of resources in somatic maintenance is traded off against investment in other fitness-associated traits, and thus age-related declines in function *do* occur (Stearns 1989; Kirkwood & Rose 1991). Telomere length and dynamics clearly have the potential to reflect the biological age of cells, the accumulation of senescent cells and the depletion of stem cell stocks – all of which may contribute to the aging phenotype (Campisi 2005; Herbig *et al.* 2006; Baerlocher *et al.* 2007; Blasco 2007; Boonekamp *et al.* 2013). In addition, telomere length is thought to be determined in part by exposure to oxidative damage, from which telomeres may be protected by investment in antioxidant protection and repair by telomerase (Ballen *et al.* 2012; Badás *et al.* 2015; Hatakeyama *et al.* 2016). It is also possible that telomeres themselves accelerate tissue degeneration and thus compromise organismal survival prospects (Price *et al.* 2002; Trougakos *et al.* 2006; Campisi & d'Adda di Fagagna 2007; Coppé *et al.* 2010). Even if telomeres are not a *causal* agent in organismal senescence their potential as a biomarker of somatic maintenance remains (Simons 2015). Indeed telomere length is generally observed to decrease with organismal age, particularly in early life (Salomons *et al.* 2009; Barrett *et al.* 2013; Young *et al.* 2013; Beirne *et al.* 2014; Asghar *et al.* 2015), and has been shown in some cases to predict lifespan or survival (Geiger *et al.* 2012; Barrett *et al.* 2013; Asghar *et al.* 2015; Watson *et al.* 2015). However, the strength of association between telomere length and survival varies among species. In some species no association has been found (Caprioli *et al.* 2013; Fairlie *et al.* 2016; Ouyang *et al.* 2016), which may be due to large among individual variation in telomere length caused by genetic and epigenetic effects that obscure the relationship between telomere length and somatic maintenance (Slagboom *et al.* 1994; Delany *et al.* 2003; Broer *et al.* 2013). Parental age may even confound this relationship as it has been shown to predict offspring telomere length (Njajou *et al.* 2007; De Meyer *et al.* 2007; Olsson *et al.* 2011; Broer *et al.* 2013), and can also be a determinant of the quality of care received by offspring (Tardif *et al.* 1984; Weladji *et al.* 2006). The utility of telomere *length* as a biomarker of somatic maintenance may additionally be reduced in studies measuring telomere length by

quantitative PCR (qPCR), as this method incorporates into estimates of telomere length the quantity of interstitial telomeric repeats (found *within* chromosomes) that can vary in length among individuals (Delany *et al.* 2003; Criscuolo *et al.* 2009a; Foote *et al.* 2013).

Within-individual changes in telomere length, however, should not be affected by these genetic and epigenetic sources of noise. In addition, telomere dynamics offer better resolution than telomere length when asking questions about the causes of variation in somatic maintenance: Telomere dynamics can be assessed over short time periods, whereas telomere length reflects a lifetime of changes in addition to genetic and epigenetic effects. Telomere dynamics (specifically within-individual rates of change in telomere length over time) are therefore thought to provide a more informative measure of somatic maintenance than among-individual variation in telomere length *per se*, but to date there has been little investigation of the downstream implications of variation in within-individual telomere attrition rates, particularly in the wild and in dependent young (*in dependent young*: Boonekamp *et al.* 2014a; Fairlie *et al.* 2016; *in adults*: Bize *et al.* 2009b; Salomons *et al.* 2009a; Barrett *et al.* 2013; Fairlie *et al.* 2016; Ujvari *et al.* 2016; Taff & Freeman-Gallant 2017). In this thesis, I therefore utilise measures of within-individual telomere dynamics in addition to telomere length wherever possible.

Use of telomere dynamics as an organismal-level biomarker of somatic maintenance opens up previously unexplored avenues of investigation into the long-hypothesised trade-offs between key life-history traits such as growth and reproduction on the one hand and somatic maintenance on the other, as well as the downstream impacts of failure to invest in the latter on later-life performance and ageing. Indeed, there is growing evidence to suggest that telomere dynamics do provide a useful biomarker of somatic maintenance in both regards. For example, the findings of a growing number of studies concord with predictions of life-history theory regarding trade-offs between somatic maintenance and growth (De Block & Stoks 2008; Geiger *et al.* 2012; Tarry-Adkins *et al.* 2013; Boonekamp *et al.* 2014a), and between somatic maintenance and

reproduction (Kotrschal *et al.* 2007; Heidinger *et al.* 2012; Sudyka *et al.* 2014; Reichert *et al.* 2014b) particularly under poor conditions (Stearns 1989).

Despite increasing interest in the causes of variation in telomere dynamics in free-living organisms, a major factor that may modify somatic maintenance schedules has been largely neglected: the social environment. While several studies have investigated the effects on telomere dynamics of competitive interactions among nestling birds (Voillemot *et al.* 2012; Boonekamp *et al.* 2014a; Reichert *et al.* 2014b; Costanzo *et al.* 2016), there has been virtually no research to date on the impacts of cooperative behaviour on telomere dynamics, with the little research that has been conducted being restricted to social insects (Jemielity *et al.* 2007; Korandová & Frydrychová 2016). The overarching goal of this thesis is to address this shortfall in our understanding with a focussed investigation of the causes and consequences of variation in telomere dynamics in a wild population of highly cooperative vertebrates.

Somatic maintenance and senescence in cooperative breeders

In cooperatively breeding societies some individuals, termed 'helpers', routinely provide care for offspring that are not their own (Cockburn 1998; Koenig & Dickinson 2004, 2016). There is great diversity in the form of cooperative breeding, with helpers varying in the extent to which they breed and considerable variation in the extent to which cooperatively breeding groups comprise simple nuclear families (Cockburn 1998; Koenig & Dickinson 2004, 2016). Arguably the simplest form of cooperative breeding occurs when offspring delay dispersal from their parents' territory, forego reproduction while remaining philopatric, and help to rear future generations of their parents' young while awaiting an opportunity to secure a breeding vacancy of their own (Emlen 1991). The model system for the research conducted in this thesis fits this latter description very well (Harrison *et al.* 2013a; b, 2014).

In cooperatively breeding societies there is therefore considerable potential for the social environment to impact patterns of somatic maintenance. First, reductions in mortality risk with increasing group size (Clutton-Brock *et al.* 1999; Courchamp *et al.*

2000, but see Brouwer *et al.* 2006 for an exception) have the potential to favour increased investment in somatic maintenance in individuals living in large groups, given their greater expected longevity if their group remains large (Jemielity *et al.* 2005). Second, competition between dominants and subordinates over rank and reproduction has the potential to entail energetic costs and social stress that may increase exposure to oxidative stress and thereby hasten the accumulation of somatic damage among either subordinates (Abbott *et al.* 2003; Young & Clutton-brock 2006; Sharp & Clutton-Brock 2011) or dominants (Creel *et al.* 1996; Bell *et al.* 2012). Third, cooperative behaviour, in particular the cooperative contributions of helpers to the rearing of breeders' young, has the potential to alleviate resource allocation trade-offs between investment in somatic maintenance and other traits in both the parents and offspring that the helpers assist. The extent to which helper contributions benefit parents and offspring varies across species (Hatchwell 1999; Dickinson & Hatchwell 2004), ranging from scenarios in which (i) parents maintain their own levels of offspring provisioning in the presence of helpers, which leaves helper contributions increasing the net rate of resource provisioning to offspring (so called "additive care"; Hatchwell 1999), to scenarios in which (ii) parents completely compensate for the contributions of helpers by reducing their own offspring provisioning rates, such that parents, rather than offspring, are the beneficiaries of helping via 'workload-lightening' (so called "compensatory care"; Hatchwell 1999). As such, where helping is additive, the cooperative contributions of helpers are expected to increase net resource availability for developing young and may thereby alleviate resource allocation trade-offs within offspring between essential processes such as growth on the one hand and somatic maintenance on the other (Brouwer *et al.* 2006). Likewise, in species with compensatory care, the cooperative contributions of helpers may alleviate such trade-offs in parents, by lightening their workloads and consequently both releasing resources for somatic maintenance and reducing workload-related exposure to oxidative stress (Cram *et al.* 2015a, b).

Whilst the impact of cooperative breeding in vertebrates on patterns of somatic maintenance and its downstream effects remain largely unexplored, a variety of forms of evidence highlight the promise of investigating such effects. First, studies of eusocial

insect societies have revealed that queens may enjoy markedly longer lifespans than their workers; a pattern attributed to selection for increased somatic maintenance given the reduced extrinsic mortality risk frequently experienced by queens and their often-documented rise in fecundity with age as their workforce increases in size (Keller & Genoud 1997; Hartmann & Heinze 2003; Jemielity *et al.* 2005). Indeed, research into the mechanistic underpinnings of the divergent lifespans of queens and workers has implicated a role for pathways involved in somatic maintenance, including antioxidant mechanisms (Jemielity *et al.* 2005; Seehuus *et al.* 2006). While such markedly divergent ageing trajectories of breeders (queens) and helpers (workers) may not be expected of most cooperative vertebrates, recent studies have revealed evidence of differences in lifespan between breeders and helpers in captive populations of two cooperatively breeding mole-rat species (Dammann & Burda 2006; Dammann *et al.* 2011). Second, there is growing evidence to suggest negative downstream effects of social competition for resources or reproduction that could be a product of their expected detrimental effects on somatic maintenance. For example, in cooperatively breeding meerkats subordinate females are frequently subjected to extended periods of chronic stress by their dominants in order to suppress subordinate reproduction (Young *et al.* 2006), and females that have been exposed to more such periods show accelerated reproductive senescence when they ultimately become dominants themselves (Sharp & Clutton-Brock 2011; see also Lemaître *et al.* 2014; Beirne *et al.* 2016 for similar evidence from non-cooperative species). Additionally, there is evidence from one species suggestive of downstream effects of the cooperative care provided by helpers on the longevity and senescence trajectories of the offspring that they help to rear (Sparkman *et al.* 2011). Finally, at least two studies have highlighted the potential for downstream effects of 'workload lightening' by helpers on patterns of somatic maintenance among parents. In the red cockaded woodpecker, *Picoides borealis*, the presence of subordinate helpers at the nest is correlated with increased breeder longevity even when controlling for territory quality (Khan & Walters 2002), and experiments conducted on white-browed sparrow weaver, *Plocepasser mahali*, societies suggest that the impacts of reproduction on oxidative stress are mitigated in groups with more helpers (Cram *et al.* 2015b).

1.4 Study system

In this thesis, I use a natural population of white-browed sparrow weavers (*Plocepasser mahali mahali*) to investigate the causes and consequences of variation in telomere dynamics in the wild, with particular focus on the impacts of sociality. The white-browed sparrow weaver is a cooperatively breeding passerine bird that lives in groups consisting of a dominant breeding pair and up to ten subordinate birds of both sexes, principally offspring that have delayed dispersal from their natal group (Collias & Collias 1978; Lewis 1981, 1982; Wingfield & Lewis 1993; Harrison *et al.* 2013a). This species provides an ideal model system for investigating the effects of sociality on telomere dynamics as the dominant breeding pair completely monopolise reproduction, group size is highly variable, and all group members contribute to varying degrees to a range of cooperative activities (Harrison *et al.* 2013a). Genetic studies of patterns of parentage confirm that the dominant female completely monopolises reproduction within her group, being the sole egg producer and incubator (Harrison *et al.* 2013a). Dominant males completely monopolise within-group paternity, but lose 12-18% of paternity to extra-group males, that are near-exclusively dominant males in other groups (Harrison *et al.* 2013b). Dominant males also sing dawn song throughout the breeding season, while subordinates males do so less frequently and invariably produce shorter performances (York *et al.* 2016b). All group members participate in a range of cooperative behaviours including the provisioning of nestlings and fledglings, weaving roosts and nests, a form of vigilance known as sentinelling, and territory defence (Collias & Collias 1978; Lewis 1981, 1982; Wingfield & Lewis 1993). With regard specifically to cooperative nestling provisioning, the dominant female provisions offspring at the highest rates, with dominant males and subordinate females provisioning at intermediate rates and subordinate males contributing the least (Walker 2016; Young et al. unpublished data). Research to date suggests that white-browed sparrow weavers have a *partially* compensatory care system, showing evidence of both (i) additive care (experiments have confirmed that the net rate of food delivery to offspring is substantially higher in groups with more helpers; Young et al. unpublished

data) and (ii) compensatory care (parents provision offspring at lower rates [i.e. experience 'workload-lightening'] when assisted by more helpers; Young et al. unpublished data; Lewis 1982). As such, helpers in this species have the potential to yield beneficial improvements in somatic maintenance among both offspring *and* parents via the mechanisms outlined above. Indeed, experimental studies to date have revealed that reproduction in this species entails an oxidative stress cost to carers that appears to be mitigated in groups with more helpers (Cram *et al.* 2015b).

1.5 Thesis aims and outline

In this thesis, I investigate the determinants of telomere dynamics and their downstream implications in a free-living social bird. Despite marked potential for the social environment to impact patterns of somatic maintenance in social species, there has been surprisingly little investigation of the effects of group size, dominance status and helping behaviour on telomere dynamics. Here I utilise extensive longitudinal within-individual sampling of telomere length over the course of a continuous long-term field study of 40 social groups of white-browed sparrow weavers to assess the effects of the abiotic (rainfall) and social environment (both group size and social dominance status) on the telomere dynamics of nestlings and adults. In addition, I contrast the utility of telomere length and dynamics as biomarkers of somatic maintenance by assessing their ability to predict downstream survival, and investigate the extent to which natural variation in oxidative state is a determinant of telomere dynamics in the wild. In summary:

Chapter 2 investigates whether individual variation in telomere length and within-individual telomere attrition rates predict the downstream survival of nestlings, and its findings provide support for the view that detriments in somatic maintenance entail future fitness costs.

Chapter 3 investigates the impacts of the social environment (group size and competition from nest-mates) and abiotic environment on hatchling telomere lengths and the within-individual rates of telomere attrition in nestlings in their early and late

developmental periods. This chapter provides the first support to date for the hypothesis that helpers mitigate telomere attrition rates in the offspring that they help to rear.

Chapter 4 utilises a large longitudinal dataset of telomere measures of adult birds to investigate the determinants of both long- and short-term telomere dynamics in adulthood. This chapter focuses on the effects of dominance status and rainfall-related reproductive activity, and reveals support for impacts of each on telomere attrition rates.

Chapter 5 investigates the extent to which natural variation in oxidative state in the wild predicts variation in within-individual rates of change in telomere length over time. Despite strong *in vitro* evidence that oxidative stress can accelerate telomere attrition, there is equivocal evidence that this is the case at the organismal level. While our findings provide evidence suggestive of associations between oxidative state and telomere dynamics in a natural population, they also highlight unexpected complexity in the nature of these relationships.

Finally, ***Chapter 6*** provides a brief overview of the findings of the work.

Chapter 2

Telomere length and attrition rate predict nestling survival in a wild social bird, but not as expected

2.1 Abstract

Life-history theory assumes that detriments in somatic maintenance entail future fitness costs, but mechanistic evidence in support of this view remains scarce. Telomere attrition rates are considered a useful biomarker of levels of somatic maintenance, providing a promising approach for addressing this fundamental question. While a number of studies have revealed that telomere *length* can predict survival in natural populations, effects of genetic and epigenetic factors on variation in telomere length may confound such associations with fitness components. Investigating whether individual variation in telomere attrition rates predicts survival would circumvent these problems, but few such studies have been conducted to date. Here we investigate whether individual variation in both telomere length and within-individual telomere attrition rates predict the survival of nestlings in a wild social bird, the white-browed sparrow weaver (*Plocepasser mahali*). Our analyses of telomere length reveal counterintuitive patterns: while individual variation in telomere length in the mid and late nestling period do not predict survival, telomere length close to hatching (at 4 days of age) negatively predicts survival. This pattern runs contrary to expectation and appears to be driven by differentially high predation of nestlings with long telomeres (potentially due to an environmental confound, such as variation in pre-laying rainfall, which may increase both predation risk and hatchling telomere length). Accordingly, when predated broods were removed from the analysis telomere length no longer predicted survival. Our analyses of telomere attrition rates revealed much clearer results. Individuals that showed a higher rate of within-individual telomere attrition in the early nestling phase were less likely to survive to adulthood, regardless of their telomere length and controlling for effects of body mass. Our findings provide support for the view that detriments in somatic maintenance entail future fitness costs, and highlight the utility of longitudinal assessments of telomere attrition rates when investigating the links between somatic maintenance and fitness.

2.2 Introduction

Life-history theory is underpinned by the concept that in the face of limited resources trade-offs occur between life-history traits, where elevated investment in one trait reduces the resources available for investment in others. Variation in lifespan, reproductive rate and growth are thought to stem from these trade-offs (Stearns 1989). The disposable soma theory of aging states that the optimal level of somatic maintenance and repair is related to the length of time that individuals can reasonably expect to survive in the wild, given external mortality pressures such as predation and disease. Resources invested in somatic maintenance that maintain the body beyond this time could be better invested in other fitness enhancing processes such as reproduction, yet diverting resources away from somatic maintenance is envisaged to ultimately come at the price of exacerbated senescence and reduced longevity (Kirkwood & Holliday 1979). Despite widespread adoption of this approach to understanding life-histories (Lemaître *et al.* 2015), mechanistic evidence that shortfalls in somatic maintenance are indeed associated with detriments in later-life performance remains scarce.

Telomeres are a promising tool for investigating this link (see Kirkwood 2005). Telomeres are located at the ends of eukaryotic chromosomes and comprise of a repetitive non-coding sequence of DNA and associated proteins. They act as protective chromosomal caps that increase chromosome stability (Blackburn & Szostak 1984; Blackburn 1991) and buffer coding DNA from the gradual erosion that occurs during cell replication when the lagging strand cannot be fully replicated (Watson 1972; Olovnikov 1973; de Lange 2009). Additional loss of telomeric repeats is thought to occur during processing of the telomere end (Makarov *et al.* 1997; Sfeir *et al.* 2005), and thus, in the absence of telomere repair mechanisms, telomeres gradually get shorter with every cell division until they cease to function properly. This eventually results in genome instability, cell senescence, or apoptosis (Karlseder 1999; Blackburn 2000; Hemann *et al.* 2001a; O'Sullivan & Karlseder 2010). Telomeres are considered to be useful biomarkers of somatic maintenance as they are thought to get shorter not only due to the mechanisms referred to above, but also in response to oxidative stress, which

can be a major source of cellular damage and may contribute to senescence (Finkel & Holbrook 2000; Monaghan *et al.* 2009; Selman *et al.* 2012). Oxidative stress occurs when reactive oxygen species (ROS) overcome a complex system of antioxidants to cause damage to proteins, lipids, and DNA (Finkel & Holbrook 2000; Halliwell & Gutteridge 2007a) and has been shown to accelerate telomere attrition *in vitro* (von Zglinicki 2002; Richter & von Zglinicki 2007). Indeed, it is thought that telomeres are particularly susceptible to persistent oxidative damage compared to the rest of the genome (Henle *et al.* 1999; Kawanishi *et al.* 2001; Kawanishi & Oikawa 2004). This quality has earned them the reputation of being 'sentinels' of DNA damage, with high levels of telomere damage thought to be indicative of high levels of damage in coding DNA. It has therefore been proposed that a key function of telomeres is to cause cells that have become 'dangerous' to cell function (through unrepaired or incorrectly repaired damage) to cease proliferating (von Zglinicki, 2002; also see Feldser and Greider, 2007). There is growing evidence that acceleration of telomere attrition due to oxidative damage may also be pertinent at the level of the whole organism both in captive and free-living animal populations (Badás et al., 2015; Ballen et al., 2012; Cattan et al., 2008; Geiger et al., 2012; also see Chapter 5). Furthermore, experimental increases in antioxidant levels both *in vitro* and *in vivo*, have shown that the detrimental effects of ROS to cellular components, including telomeres, may be counteracted by the action of antioxidants (Yu 1994; Furumoto *et al.* 1998; Liu *et al.* 2002; Serra *et al.* 2003). Differential investment in antioxidant defences is therefore expected to both reduce levels of somatic damage and reduce telomere attrition rate.

Though telomeres are consequently considered a useful biomarker of somatic maintenance, the strength of the relationship between telomere length and survival varies among species, with strong evidence for a positive effect of telomere length on survival in some species, but no evidence of a relationship in others (Table 1). Indeed, in juvenile Atlantic salmon, telomere length was found to negatively predict survival (McLennan *et al.* 2017). . Even within species, results are variable. This may in part be due to changes in the strength of the association between survival and telomere length at different ages, as has been shown in humans (Boonekamp *et al.* 2013) and zebra

finches (Heidinger *et al.* 2012). Alternatively, studies may fail to find a relationship between telomere length and survival due to sources of variation in telomere length estimates that limit their utility as a biomarker of somatic maintenance. For example, inter-individual variation in the extent of interstitial telomeric sequences (found within chromosomes) could add noise to telomere length estimates obtained via quantitative PCR approaches, which could in turn obscure relationships between survival and true telomere length (Delany *et al.* 2003; Criscuolo *et al.* 2009a; Foote *et al.* 2013). In addition, telomere length can be affected by both genetic and epigenetic effects, such as parental age (Njajou *et al.* 2007; De Meyer *et al.* 2007; Olsson *et al.* 2011; Broer *et al.* 2013), leaving telomere length *per se* a potentially poor biomarker of investment in somatic maintenance. If the variation in telomere length caused by such effects is greater than the variation caused by somatic damage, telomere length may fail to show a relationship between somatic maintenance and survival. Furthermore, if genetic or epigenetic drivers of telomere length are also drivers of survival, telomere length may predict survival but not because of telomere length *per se*. For example, parental age has been shown in some species to predict offspring telomere length (Njajou *et al.* 2007; De Meyer *et al.* 2007; Olsson *et al.* 2011; Broer *et al.* 2013), and can also be a determinant of offspring survival (Tardif *et al.* 1984; Weladji *et al.* 2006). To investigate the link between somatic maintenance and survival, studies that also examine the downstream effects of individual variation in telomere attrition rates would be beneficial. However, such studies are rare, especially in dependent young (Table 1).

Telomere dynamics in early life are of particular interest as organisms are likely to experience particularly acute challenges regarding resource allocation during periods of intense growth, with the potential for marked downstream effects of shortfalls in somatic maintenance at this time. Here we use data from a natural population of white-browed sparrow weavers (*Plocepasser mahali*) to investigate whether telomere length and attrition rate during the nestling stage predict subsequent early life survival. We measured relative telomere length (RTL) in whole blood: Erythrocytes in birds are nucleated (though do not proliferate) and vastly outnumber other cell types (Williams 1972). Thus telomere length of avian whole blood is likely to reflect current telomere

length of the hematopoietic stem cells, of which erythrocytes are descendants (Vaziri *et al.* 1994; Schroeder 2010).

First, we investigate whether the telomere length of nestlings predicts their downstream survival to the following breeding season. As the extent to which telomere length predicts survival can vary with the focal life stage (see above), we repeat this analysis for telomere length measurements taken at three different time points during the nestling period: at 4, 12 and 30 days of age (reflecting post-hatching, middle-aged nestling, and fledgling telomere lengths). If fitness-relevant individual differences in telomere attrition occur principally before 4 days of age (e.g. during development in the egg), then relationships between telomere length and survival may be most apparent at this time, as selective disappearance of individuals with short telomeres may compress the available variance in telomere length as the nestling period advances. Whereas if fitness-relevant individual differences in telomere attrition principally occur over the course of the nestling period, then relationships with survival may be most apparent in the telomere length measurements of middle-aged nestlings or fledglings. Having established that telomere length solely at 4 days of age predicts survival, we then investigate whether telomere length at 4 days of age predicts short-term survival during the nestling period, teasing apart the contributions of mortality arising from predation versus starvation. We control statistically throughout for individual variation in body mass, to ensure that the predictive power of telomere length does not arise from correlated variation in body mass.

Second, we investigate whether variation in within-individual rates of telomere attrition between 4 and 12 days of age predicts survival to the following breeding season, while controlling for variation in nestling body mass at 12 days of age and telomere length at 4 days of age. Viewing telomere attrition rates as a biomarker of accumulated deficits in somatic maintenance that likely reflect the accrual of damage in multiple body tissues, we predict that nestlings that showed higher rates of telomere attrition will have reduced downstream survival prospects even after controlling for variation in body mass.

Table 1 Vertebrate studies investigating whether natural variation in telomere length and/or within-individual attrition rates predicts survival. Studies are grouped first by whether they focussed on dependent young or adults, and then by taxonomic group. Telomere length: 'Yes' where shorter telomeres predict shorter lifespan and/or reduced survival. Telomere attrition: 'Yes' where higher rates of telomere attrition predict shorter lifespan and/or reduced survival. Where survival to more than one age was tested, results for each age are presented on separate lines. Sample sizes (n; number of individuals) are given in parentheses.

*[1] only in nestlings that never contracted malaria; sample size is therefore given for uninfected birds only. Infected birds: n = 32 *[2] only in late-born chicks; sample size is, however, only given for *all* chicks. *[3] Pythons that were recaptured had shorter telomeres than those that were not, *[4] increase in % of short telomeres rather than telomere attrition *per se.* *[5] Middle quartile had lowest survival. *6 A correlation between survival and telomere length was only found in 80-89 year-old women.

Species	survival measure	Captive/ wild	Telomere length (n)	Telomere attrition (n)	Reference
Studies of dependent young					
Great reed warbler (*Acrocephalus arundinaceus*)	lifespan	wild	**Yes*[1]** (49)	-	Asghar *et al.* 2015
Jackdaw (*Corvus monedula*)	survival to post-fledging and to recruitment	wild	**No** (152)	**Yes** (152)	Boonekamp *et al.* 2014
Barn swallow *(Hirundo rustico)*	lifespan	wild	**No** (60)	-	Caprioli *et al.* 2013
King penguin (*Aptenodytes patagonicus)*	growth-period survival	wild	**Yes** (44)	-	Geiger *et al.* 2012
King penguin (*Aptenodytes patagonicus*)	survival to fledging survival to winter	wild	**Yes*[2]** (*66*) **No** (63)	- -	Stier *et al.* 2014
zebra finch (*Taeniopygia guttata*)	Lifespan	captive	**Yes** (99)	**No** (79)	Heidinger *et al.* 2012
European storm petrel (*Hydrobates pelagicus*)	survival to fledging	wild	**Yes** (59)	-	Watson *et al.* 2015
black-legged kittiwake (*Rissa tridactyla*)	survival to fledging	wild	**Yes** (107)	-	Young *et al.* 2017
Soay sheep (*Ovis aries*)	first winter survival: neonates lambs in their 1st August	wild	 **No** (115) **Yes** (115)	**No**	Fairlie *et al.* 2016
Water python (*Liasis fuscus*)	recapture	wild	**No** (20)	-	Ujvari & Madsen 2009
Studies of adults					
American redstart ♂ (*Setophaga ruticilla*)	return rate	wild	**Yes** (63)	-	Angelier *et al.* 2013
Seychelles warbler (*Acrocephalus sechellensis*)	survival to following year post-sampling lifespan	wild	**Yes** (204) **Yes** (204)	- **Yes** (96)	Barrett *et al.* 2013

Species	survival measure	Captive/ wild	Telomere length (n)	Telomere attrition (n)	Reference
Alpine swift (*Apus melba*)	Length of survival over 7 years	wild	**Yes** (98)	**Yes** (22)	Bize *et al.* 2009
Southern giant petrel (*Macronectes giganteus*)	survival over 8 years	wild	**Yes** (47)	-	Foote *et al.* 2010
Zebra finch (at age 1 yr) (*Taeniopygia guttata*)	Lifespan	captive	**Yes** (79)		Heidinger *et al.* 2012
Tree swallow (*Tachycineta bicolor*)	return rate	wild	**Yes** (22)	-	Haussmann *et al.* 2005
Tree swallow (*Tachycineta bicolor*)	return rate over 2 years	wild	**No** (82)	-	Ouyang *et al.* 2016
Sand martin (*Riparia riparia*)	minimum lifespan (age last seen)	wild	**Yes** (23)	-	Pauliny *et al.* 2006
Dunlin (*Calidris alpina*)	minimum lifespan (age last seen)	wild	**No** (30)	-	Pauliny *et al.* 2006
Jackdaw (*Corvus monedula*)	survival to following year	wild	**Yes** (48)	**Yes**	Salomons *et al.* 2009
Yellowthroat ♂ (*Geothlypis trichas*)	survival to the following year	wild	**No** (59)	**No** (47)	Taff & Freeman-Gallant 2017
Water python (*Liasis fuscus*)	recapture	wild	**No***[3] (50)	-	Ujvari & Madsen 2009
Frill-necked lizard (*Chlamydosaurus kingie*)	recapture	wild	**No** (9393)	**No** (40)	Ujvari *et al.* 2016
Soay sheep (*Ovis aries*)	Lifespan and survival of 3+ yr olds Survival over high - mortality winter	wild	**No** (216) **Yes** (86)		Fairlie *et al.* 2016
Mouse (*Mus musculus*) wild type TgTERT	minimum lifespan	captive	-	**Yes***[4] (18) **Yes***[4] (16)	Vera *et al.* 2012
Human 85 -99 yrs old 100 – 104 yrs old 105+ yrs old	Survival over follow up period (max = 10 years)	-	**Yes***[5] (532) **No** (275) **No** (403)	-	Arai *et al.* 2015
Human 63 – 95 yrs old	survival over follow up period (mean = 6.9 years)	-	**Yes** (350)	-	Bakaysa *et al.* 2007
Human 73 – 101 yrs old	survival over follow up period (7 - 8 years)	-	**No***[6] (812)	-	Bischoff *et al.* 2006
Human 60 – 85+ yrs old	survival over follow up period (15 years)	-	**Yes** (143)	-	Cawthon *et al.* 2003
Human ♂ 70 – 79 yrs old	survival over 12 years	-	**Yes** (236)	**Yes** (236)	Epel *et al.* 2009
Human 79 yrs old	survival over follow up period (5 years)	-	**No** (190)	-	Harris *et al.* 2006
Human 65 – 105 yrs old	survival over follow up period (9 - 11 years)		**Yes** (257)	-	Honig *et al.* 2006
Human 73 – 94 yrs old	survival over follow up period (9 - 10 years)	-	**Yes** (548)	-	Kimura *et al.* 2008

Species	survival measure	Captive/ wild	Telomere length (n)	Telomere attrition (n)	Reference
Human 85 – 101 yrs old	survival over follow up period (12 - 15 years)	-	**No** (598)	-	Martin-Ruiz *et al.* 2005
Human post-stroke 75 - 94	survival over follow up period (5 years)	-	**Yes** (195)	-	Martin-Ruiz *et al.* 2006

2.3 Methods

2.3.1 Study species and field methods

This study was conducted on a population of 38 cooperative groups of white-browed sparrow weavers (*Plocepasser mahali*) in the semi-arid Kalahari Desert at Tswalu Kalahari Reserve, South Africa (27°16'S, 22°25'E). Nests were monitored for the laying of new eggs at least every other day and eggs were weighed on the day they were laid. Once clutches were complete (clutch size = 1 – 3 eggs), nests were left undisturbed until 15 days after clutch initiation when nest checks were resumed and continued until all viable eggs had hatched. Once hatched, nestlings were assigned either a left or right 'haircut', in which feathers were trimmed on one side of the head, or no 'haircut', which was used to distinguish nestlings until they were large enough for a uniquely numbered metal ring, which was fitted at approximately 12 days of age (SAFRING licence 1444). Subsequently nests were checked on the 4th, 8th, 12th and 16th day of life of the first-hatched nestling. Henceforth age-group of nestlings is referred to as the day of life of the first hatched nestling (i.e. the 4th day of life of the first hatched nestling is day 4 for all nestlings in that brood). At each nest check, nestling body mass was recorded to the nearest 0.01 g (Durascale 100; MyWeigh, Phoenix, AZ). On day 4 and day 12 a small blood sample (<25uL) was collected from nestlings via brachial venepuncture using a 26G needle and non-heparinized capillary tube and stored in approximately 500µl of 100% ethanol at ambient temperature until extraction. In order to avoid the risk of pre-fledging, nestlings were not disturbed again after the 16th day, but groups were monitored closely to identify whether nestlings survived to fledgling. Fledglings were captured approximately 30 days post-hatching (henceforth 'day 30') by flushing them from their individual roost chambers into a custom capture bag, whereupon they were weighed and a further blood sample taken as described above. Three colour rings were fitted alongside the metal ring to create a unique combination with which to identify birds without the need for capture. Fledglings were then returned to their roost chambers. Sex of birds that survived to adulthood was determined by beak colour,

which is sexually dimorphic in this subspecies (P. m. mahali; Leitner *et al.* 2010), and molecular sexing was used for nestlings that did not survive (Dawson *et al.* 2015). Group composition was assessed for each group at least every other week: social groups were considered to comprise birds seen consistently foraging, and roosting together (Harrison *et al.* 2013b). Adult group sizes (birds over 6 months of age) were calculated from this data and group size at the time of hatching was used in analyses.

2.3.3 Survival assessments

Survival of nestlings was recorded during standard nest checks (described above). Nestlings found dead in the nest were recorded as 'deceased'. Slender mongooses, *Galerella sanguinea*, have been observed to create a round hole at the back of nests to predate nestlings, so any nests found with this damage were noted in the field as 'predated'. Three weeks after the first nestling hatched, groups were thoroughly checked for the presence of fledglings and fledgling identity was confirmed upon capture at day 30. Nestlings were recorded as having not survived to fledging if they were never observed outside the nest.

Survival to the following season was ascertained using data generated through group observations that were carried out at each group at least every other week (usually groups were monitored once a week) and capture records. Birds that were never recorded as present from the 1st of October in the season following hatching were recorded as having not survived. Fledglings have only very rarely been observed to disperse from their natal group within the study site in their first year (5 of 341 fledglings over 8 years). It is therefore highly unlikely that significant numbers of our 'non-survivors' include nestlings that dispersed beyond the bounds of our study population prior to assessment of survival.

2.3.4 DNA extractions

We extracted DNA using Gentra PureGene Genomic DNA Purification Kit (Qiagen) and assessed DNA integrity by gel electrophoresis, discarding samples with poor integrity (see Appendix A). We measured DNA concentration and quality on a NanoVue 4282 Spectrophotometer (v1.7.3) and accepted ratios of between 1.7 and 2.0 for 260/280, and between 1.9 and 2.2 for 260/230. We re-extracted samples with ratios outside these ranges or rejected them if further extractions also fell outside these ranges. DNA samples were diluted in elution buffer to a concentration of 12.5ng/uL and stored at -20˚C until telomere analysis.

2.3.5 Telomere measurement by qPCR

We used quantitative PCR (hereafter qPCR) as described in Cawthon (2002) to quantify whole blood telomere length relative to a non-variable copy-number control gene, thus controlling for variation in DNA concentration (termed RTL for brevity in the methods, but 'telomere length' for clarity in the results and discussion). This method is well-suited to ecological field studies as it requires only a small amount of DNA and is high-throughput. RTL measures quantified by qPCR include interstitial repeats located away from the chromosome ends and are therefore not a measure of 'true' telomere length. However, where the metric of interest is the within-individual change in telomere length over time, this inclusion of interstitial repeats could increase noise in the data set, but should not otherwise influence the results (Delany *et al.* 2003).

A master mix was prepared for each primer set, containing 10uL SybrGreen fluorescent dye with low ROX (Agilent Technologies), and all primers at a concentration of 200nM in a 20uL reaction. For our control gene we used Glyceraldehyde-3-phosphate-dehydrogenase (GAPDH) using primers specific to *P. mahali* (GAPDH-F 5'AAA CCAGCCAAGTATGATGACAT–3'; GAPDH-R 5'-CCATCAGCAGCAGCCTTCA-3', see Appendix A for details of primer optim-isation). Telomere forward and reverse primers were as follows Tel1b (5′-CGGTTTGTTTGGGTTTGGGTTTGGGTTTGGG

TTTGGGTT-3′), Tel2b (5′-GGCTTGCCTTACCCTTACCCTTACCCTTACCCTTACCCT-3′). Each reaction contained a total of 5ng DNA. All samples were run in triplicate and a between-plate calibration sample consisting of pooled DNA from three individuals was included in triplicate on each plate as a calibrator to standardise across plates. A 2x serial dilution of pooled DNA from 10ng to 0.625ng was also included on each plate to generate a standard curve to assess reaction efficiencies (efficiencies between 85% - 115% were deemed acceptable) and to ascertain that sample measurements had been taken within the linear phase of amplification. GAPDH and telomere reactions had different optimal annealing temperatures and so were carried out on separate 96-well plates on a Stratagene Mx3000 instrument. Thermal cycles for telomere reactions were set to 95˚C for 15 minutes, followed by 40 cycles of 95 ˚C for 15 seconds, 57 ˚C annealing for 30 seconds, and 73 ˚C extension for 30 seconds. Thermal cycles for GAPDH were the same with the exception of the annealing temperature which was 60 ˚C. Readings were taken during the extension phase. Disassociation curves for both GAPDH and telomeres were generated to assess specificity of binding, and no template controls were used to check for contamination. Standard curves were examined after every run and all had acceptable efficiencies and R^2 values as calculated by the Stratagene Mx3000 software (GAPDH efficiency: 90.6 -110.4, mean = 97.81, Standard deviation (SD) = 4.92; GAPDH r^2 : 0.99-1.00, mean = 0.999, SD = 0.001; telomere efficiency: 85.8-109.4; mean = 98.04, SD = 5.57, telomere r^2: 0.99 -1, mean = 0.996, SD = 0.003) (see Appendix A for disassociation curves and standard curves).

We exported data for ROX (which measures background fluorescence) and SYBR Green (the reporter dye) to Microsoft Excel, where we manually corrected for background fluorescence by dividing the fluorescence emission intensity of SYBR Green by the fluorescence emission intensity of ROX. We then used the software LinRegPCR (Ruijter *et al.* 2009), to correct baseline fluorescence, to set a window of linearity for each amplicon group, and to set constant fluorescence thresholds within the windows of linearity for GAPDH (0.156) and telomere (0.161). The cycle at which fluorescence crosses this threshold is termed the Cq value. We exported Cq values and well efficiencies for all samples and standards into Excel, where we calculated the mean

and coefficient of variation (%CV) of Cq and efficiency for each triplicate. We calculated mean well efficiency for each plate, excluding well efficiencies outside the 5th and 95th percentiles. Mean reaction efficiencies were between 1.89 and 1.93 for GAPDH plates, and between 1.77 and 1.84 for telomere plates. Triplicate mean efficiencies that were different to the mean plate efficiency for their plate by more than 5% and triplicates for which the Cq %CV was greater than 5% were highlighted and examined individually. Reactions that were clear outliers within their triplicate were excluded from the triplicate and mean Cq and efficiency was recalculated from the remaining reactions. Triplicates for which there was no clear outlier were excluded from further analysis. Samples that fell outside the range of the standard curve were also excluded from further analysis.

RTL was calculated as the ratio of the quantity of telomere to control gene (T/S) according to Pfaffl (2001) using the following equation, where E_{TEL} and E_{GAPDH} are the mean plate well efficiencies for telomeres and GAPDH respectively:

$$RTL = (E_{TEL} \wedge (Cq_{TEL[Calibrator]} - Cq_{TEL[Sample]})) / (E_{GAPDH} \wedge (Cq_{GAPDH[Calibrator]} - Cq_{GAPDH[Sample]}))$$

Samples for further studies were run alongside those used here. The samples used in this chapter were run on 22 plate pairs, and we kept samples that belonged to the same individual on a single plate. We calculated inter-plate repeatability from 141 samples that were run on at least 2 plates, which included samples used in further studies; these were 13 adult and 12 nestling samples on three 'repeatability' plates, 1 adult sample run on 16 plates, and a further 114 samples (109 from adults, and 5 from nestlings) run on 28 plates. Inter-plate co-efficient of variation of RTL was 13.09% (SD = 8.15). Inter-plate co-efficient of variation of just the 17 nestling samples was 13.62% (SD = 8.15). Intra-plate %CVs of all samples across all plates, after removal of samples that were removed due to poor intra-plate repeatability were 0.32% (SD = 0.08) for GAPDH and 0.85% (SD =0.13) for telomere.

2.3.6 Statistical analyses

Statistical analyses were carried out in 'R' (version 3.3.1). For all analyses we adopted an information theoretic (IT) model selection approach, using Aikaike's information criterion correcting for small sample size (AICc) to compare models (Burnham & Anderson 2002). Global models were constructed that included all variables of interest, and variance inflation factors (VIFs) were used to assess multicollinearity in each global model; all VIFs were below 5 ("car" package; Fox & Weisberg 2011). As we have no *a priori* reason to suppose one combination of variables is more likely to describe the data better than another, all combinations of variables were compared and ranked based on AICc using the package MuMIn (Barton 2016). Two-way interactions and quadratic terms were only included in models where the corresponding first order terms were present. We retained all models within Δ6 AICc of the top model (the model with the highest Akaike weight and lowest AICc), which allows confidence that the most parsimonious model is included in the candidate model set, but removes models with only very weak support (Richards 2005). In order to avoid the selection of overly complex models, we disregarded models that had a lower ΔAICc value than simpler nested models ("model nesting rule"; Richards *et al.* 2011); top model sets prior to implementation of this rule are presented in Appendix B. As we used this model nesting rule we plotted results using effect size estimates from the top model (for terms within the top model; and the best model containing the term of interest for any terms not within the top model) rather than using model averaging. All continuous predictors in models were standardised using the 'scale' function in the R Base package (in which variables are centred and scaled by their standard deviations), but for clarity they were back-transformed for plotting. We checked for overdispersion in all global models, and in the top models of each candidate model set. In all cases the point estimate of dispersion was below 1.5.

(i) Does telomere length predict survival?

We conducted three different binomial generalised linear mixed models (GLMM; glmer function in the package "lme4", with the bobyqa optimiser; Bates et al., 2014) to test

whether RTL at days 4, 12 and 30 predicted survival from the point of telomere assessment to the start of the following season. In addition to RTL, we tested for effects of body mass (see details below), sex, age at sampling, adult group size at hatching (the number of birds over 6 months of age), two measures of rainfall (total rainfall over the month prior to egg-laying; 'pre-lay rainfall', and total rainfall over the ~50 day period from egg-laying to day 30; 'post-lay rainfall'), and the age nestlings would be at the start of the following season (whether they survived or not), which was calculated as the number of days between hatching and the 1st October the following season. This latter variable acts as a control for individual variation in the length of time that nestlings had to survive in order to be classed as 'survived' (as nestlings could hatch at any time from the end of September through to mid-April and survival to the next season was assessed the following October as described above). Social group, season and brood identity were included as random factors in all models.

For nestlings measured at day 4 and day 12 hatch date was known to within a day, whilst for those measured at day 30 hatch date was known to within 2 days. Not all chicks were sampled at exactly 4, 12 and 30 days of age (day 4 range = 3–6 days of age, mean = 4.4, SD = 1.02; day 12 range = 10–14 days of age, mean = 11.7, SD = 0.77, day 30 range = 26-35 days of age, mean = 29.3, SD = 1.57). Nestling mass was taken on the day of sampling, but due to fast growth during the nestling period we adjusted mass measures to the same age for all nestlings (4 days of age for the first model, or 12 days for the second) using the slope from a linear model of age on mass for birds between 3 and 6, and 10 and 13 days of age respectively. To calculate this adjustment, we used all relevant measures taken over the course of the sparrow weaver project (day 4 calculations: n = 564 nestlings, slope = 2.81g/day, day 12 calculations: n= 390 nestlings, slope = 1.92g/day). This correction was made possible by the large number of masses in the full database, and the known relationship between age and mass. We did not correct RTL measures in the same manner as our data did not reveal a clear pattern of telomere loss with age. Not all nestlings were sampled at each stage: The number of nestlings included in multiple analyses is indicated in Table 2.

Sparrow weaver broods suffer relatively high levels of in-nest predation (see results). Accordingly, the majority of disappearances of the nestlings in the model of day 4 RTL occurred during the nestling period, when we have some limited information on the cause of disappearance. As the analyses above revealed that RTL at day 4 predicted survival to the following season, we repeated this model with survival to fledging as the response and, having confirmed that the relationship also held for survival to fledging, conducted the following tests with a view to teasing apart the contributions of predation- and starvation-related mortality within the nest to this survival-to-fledging relationship. Nestlings were categorised as having been 'predated' when signs of predation were noted in the field following brood disappearance (see methods), or when all nestlings in broods of more than one nestling disappeared in the same interval between our standard brood survival checks (n = 14 nestlings from 7 broods; all of which were in good condition prior to disappearance). Nestlings found dead within the nest, or nestlings that disappeared and left behind a heavier surviving sibling were categorised as 'expired' (n = 8 nestlings from 8 broods). We then repeated model selection for the survival-to-fledging model twice: first with 'predated' nestlings excluded from the full data set and second with 'expired' nestlings excluded from the data set. The ten nestlings (from 10 broods) that did not survive, but did not fall into either the 'predated' or 'expired' categories, were retained in both models.

(ii) Does rate of change in RTL predict survival to start of the next season?

We used binomial GLMMs (glmer function in the package "lme4", with the bobyqa optimiser; Bates *et al.* 2014) to test whether the within-nestling rate of change in RTL between day 4 and day 12 predicted survival to the start of the following season. We calculated rate of change in telomere length as (day 12 RTL – day 4 RTL) / number of days between sampling. As the rate of change in telomere length could have non-linear effects on downstream survival (e.g. differentially large effects of high rates of telomere loss), we also included the quadratic effect of rate of change in RTL. In addition, we included, as fixed effect predictors, variables with good support in the Δ6 AICc candidate model sets for either of the analyses conducted above regarding the effects of RTL (of day 4 and day 12 nestlings) on survival to the start of the following season.

These were day 4 RTL, age at the start of the following season, pre-lay rain, and day 12 mass. Of the 39 nestlings for which we had RTL measures at both day 4 and day 12, 16 did not survive to the following season, but we had no reason to suspect that any had been predated in the nest (see classification of 'predated' nestlings above). Social group and brood were fitted as random effects.

Table 2. Summary of the nestling sample sizes in the survival models for each age group.

	Age group			
	4	**12**	**30**	**4 & 12**
Nestlings	82 (*40 and 24 also measured at day 12 and day 30 respectively*)	146 (*52 also measured at day 30*)	71	39
Broods	59	100	55	32
Social groups	30	36	31	25
Seasons	2010-11, 2013-14, 2014-15	2010-11, 2011-12, 2012-13, 2013-14, 2014-15	2011-12, 2012-13, 2013-14, 2014-15	2013-14, 2014-15

2.4 Results

(i) Does telomere length predict survival?

The Δ6 AICc top model sets showing adjusted weight for each model after implementation of the model nesting rule (Richards *et al.* 2011) are presented in Table 3. In the analysis of day 4 nestlings, we found strong evidence that telomere length *negatively* predicted survival to the following season, as it was present in both models in the top model set (Table 3). This unusual finding runs counter to predictions: nestlings with longer telomeres at day 4 were *less* likely to survive to the following season. There was no evidence that telomere length at day 12 or day 30 predicted survival to the following season, though it should be noted that only 12 of the 71 fledglings sampled at day 30 actually disappeared (Figure 1). Nestling mass at day 12 was an important predictor of survival to the following season, with heavy chicks more likely to survive, as we would expect, but body mass was not present in either the day 4 or day 30 top model sets (Table 1; Figure 1). There was strong evidence in both the day 4 and day 12 models that the age the focal nestling would be at the start of the following breeding season negatively predicted survival: Nestlings born early in the season were less likely to survive to the following season, which is as expected given that they had to survive for longer in order to class as 'survived'. Additionally, for day 12 nestlings we found strong evidence for a negative effect of rainfall (there was more evidence for an effect of rainfall prior to egg laying, than between egg-lay and day 30). Nestlings that hatched from eggs laid after greater rainfall were less likely to survive. One of the models in the top model set for day 12 nestlings included sex (with poorer survival in males), but the model had weak support, and sex was not present in any other model. Finally, there was no evidence for offspring of any age of effects of group size or the true age at which the sample was taken on survival.

Table 3: Model selection table showing predictors of survival of white-browed sparrow weavers to the start of the following season for (a) day 4 nestlings, (b) day 12 nestlings and (c) day 30 fledglings. Δ6 AICc top model set after implementation of the model nesting rule are presented (Richards *et al.* 2011). Where the null model is not in the top model set it is included and shaded in blue. Effect sizes are given with standard errors in parentheses. Continuous variables were standardised (centred and scaled). For sex, estimates and standard errors are given for males (M) relative to females. Int = intercept, AW = adjusted weight after implementation of the model nesting rule. The same predictors were tested in all global models, with RTL, age at sampling and body mass being specific to the age of measurement. * Predictors that were included in the global models but were not present in any of the models in the top model sets.

Int	RTL	age next season	body mass	pre-lay rain	post-lay rain	Sex (M)	df	logLik	AICc	Δ AICc	AW
(a) *Day 4 nestlings (n = 82 nestlings from 59 broods in 30 groups)*											
-0.073 (0.245)	-1.063 (0.319)	-0.595 (0.274)					6	-48.56	110.2	0.00	0.814
-0.078 (0.238)	-0.843 (0.281)						5	-51.20	113.2	2.95	0.186
-0.027 (0.241)							4	-57.39	123.3	11.25	NA
(b) *Day 12 nestlings (n =146 nestlings from 100 broods in 36 groups)*											
0.604 (0.672)		-2.164 (0.878)	0.695 (0.319)	-1.703 (0.730)	-0.596 (0.375)		8	-84.68	186.4	0.00	0.368
0.931 (0.508)		-1.933 (0.861)	0.931 (0.420)	-1.718 (0.814)		-0.915 (0.683)	8	-84.98	187.0	0.60	0.273
0.597 (0.623)		-1.733 (0.774)	0.642 (0.327)	-1.399 (0.646)			7	-86-14	187.1	0.69	0.260
0.783 (0.752)		-2.141 (0.807)		-1.419 (0.635)			6	-88.63	189.9	3.45	0.065
0.459 (0.543)			0.779 (0.311)				5	-90.36	191.2	4.75	0.034
0.427 (0.564)							4	-95.06	198.4	12.00	NA
(c) *Day 30 fledglings (n = 71 fledglings from 55 broods in 31 groups)*											
1.054 (0.843)							4	-30.10	68.8	0.00	1

* Age at sampling, adult group size

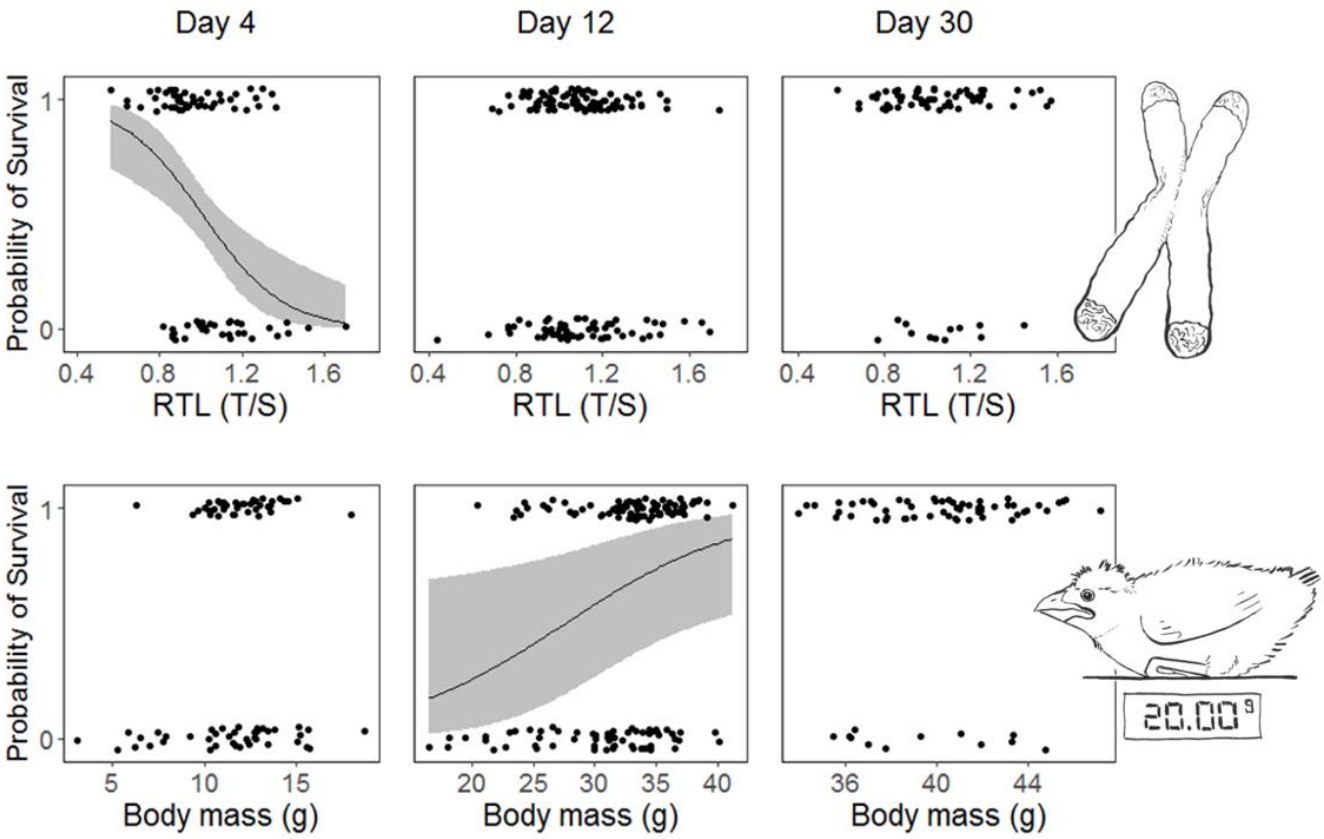


Figure 1. Model predictions for the effect of relative telomere length (RTL; top row) and body mass (bottom row) on survival of white-browed sparrow weaver nestlings to the start of the following season, at **(left)** day 4, **(middle)** day 12 and **(right)** day 30. Nestlings fledge at approximately day 20. Where survived = 0 the nestling was not observed in the following season, where survived = 1, the nestling was observed in the following season. RTL is calculated as the ratio between quantity of telomeric DNA and the reference gene. Points are jittered raw data. Where body mass or telomere length were present in the top model set, the effect is shown by the mean predicted line from the top model when all other variables are held at their mean value. Shaded areas show 95% confidence intervals.

That the telomere length - survival association detected above was apparent for day 4 nestlings but no longer evident in middle-aged nestlings and fledglings (Figure 1), suggests that this association is a product of early life processes. To investigate the origins of this survival relationship, we therefore verified that telomere length at day 4 predicted survival to fledging, before teasing apart the relative contributions of predation and starvation-related mortality to this association. We found strong evidence that telomere length at day 4 *negatively* predicted survival to fledging, with

telomere length again present in all models in the Δ6 AICc top model set (Table 4, section a; Figure 2). In addition to telomere length, there was strong evidence that body mass of day 4 nestlings positively predicted survival to fledging. Our subsequent analyses suggest that the counterintuitive relationship between telomere length at day 4 and survival arose because broods with longer telomeres suffered higher in-nest predation risk (perhaps due to a shared environmental driver of long telomeres and predation risk; see discussion). The removal of 'predated' nestlings from the original data set (14 of the 32 nestlings that disappeared) resulted in a Δ6 AICc top model set (Table 4, section b) that retained only the positive effect of nestling mass, with telomere length attracting no support as a predictor. By contrast, the removal of 'expired' nestlings from the original data set (8 of the 32 nestlings that disappeared) resulted in a Δ6 AICc top model set (Table 4, section c) that retained the negative effect of telomere length in the top model, but did not contain nestling mass. This top model also contained a negative effect of rainfall between egg laying and day 30, but was only 0.08 AIC points above the intercept only model. These results suggest that the counterintuitive association between telomere length and survival is a product principally of predation-related mortality, with mortality following the exclusion of known predation being explained instead by variation in nestling mass.

Table 4: Model selection table showing predictors of survival of day 4 white-browed sparrow weaver nestlings to fledging, for **(a)** all samples, **(b)** when 'predated' nestlings were excluded and **(c)** when 'expired' nestlings were excluded. Δ6 AICc top model sets after implementation of the model nesting rule are presented (Richards *et al.* 2011). Where the null model was not present in the top set it is still included but highlighted in blue. Effect sizes are given followed by standard errors in parentheses. All continuous variables were standardised (centred and scaled). For sex, estimates and standard errors are given for males (M) relative to females. Int = intercept, AW = adjusted weight after implementation of the model nesting rule. The same predictors were tested in all global models, with telomere length (RTL), age at sampling and body mass being specific to the age of measurement. * Predictors that were included in the global models but were not present in any of the models in the top model sets.

Intercept	RTL	Body Mass	Sex (M)	Group size	post-lay rain	df	logLik	AICc	Δ AICc	AW
(a) *All samples (n = 82 nestlings from 59 broods from 30 groups)*										
0.354	-0.825	0.578		-0.424		7	-47.30	110.1	0.00	0.296
(0.260)	(0.316)	(0.295)		(0.259)						
0.381	-0.888	0.686			-0.431	7	-47.42	110.4	0.24	0.262
(0.281)	(0.334)	(0.335)			(0.293)					
0.367	-0.862	0.560				6	-48.66	110.4	0.33	0.251
(0.277)	(0.327)	(0.307)								
-0.055	-0.845		0.760			6	-49.62	112.4	2.24	0.097
(0.342)	(0.302)		(0.502)							
0.329	-0.804					5	-50.81	112.4	2.30	0.094
(0.258)	(0.300)									
0.383						4	-56.15	120.8	9.99	0.002
(0.280)										
(b) *Excluding 'predated' nestlings (leaves n = 68 nestlings from 52 broods from 30 groups)*										
0.859		0.571				5	-39.86	90.7	0.00	0.733
(0.275)		(0.287)								
0.806						4	-42.04	92.7	2.01	0.267
(0.263)										
(b) Excluding 'expired' nestlings (leaves n = 74 nestlings from 55 broods from 30 groups)										
0.941	-1.229				-0.673	6	-40.21	93.7	0.00	0.51
(0.575)	(0.533)				(0.479)					
8.242						4	-42.59	93.8	0.08	0.49
(1.691)										

* Age at sampling, pre-lay rain

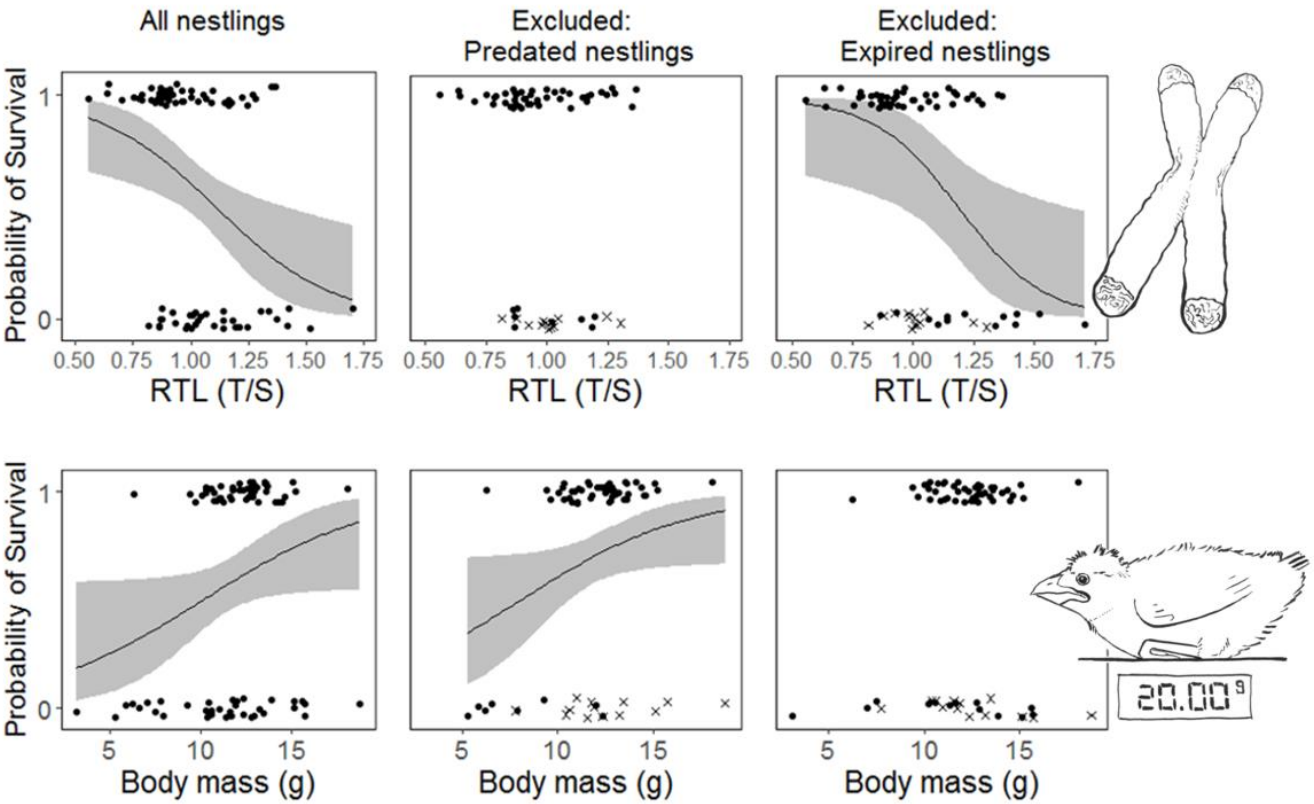


Figure 2. Model predictions for the effect of relative telomere length (RTL; top row) and body mass (bottom row) of white-browed sparrow weavers at day 4 for **(left)** all nestlings, **(middle)** all nestlings except those classified as 'predated' and **(right)** all nestlings except those classified as 'expired'. Where survived = 0 the nestling was never observed as a fledgling, where survived = 1, the nestling was observed to have fledged. RTL is calculated as the ratio between quantity of telomeric DNA and the reference gene. Points are jittered raw data. In the central panel, where 'predated' nestlings are excluded, • are nestlings classified as 'expired', or nestlings that survived; **x** are nestlings that had an unclassified cause of disappearance. In the right-hand panel, where 'expired' nestlings are excluded, • show nestlings classified as 'predated'; **x** are nestlings that had an unclassified cause of disappearance, or that survived. Where the variable was present in the top model set, the effect is shown by the mean predicted line from the top model when all other variables are held at their mean value, and the grey ribbon is 1.96 * se from the model estimate.

(ii) Does the rate of change in telomere length predict survival to the start of the next season?

The Δ6 AICc top model set revealed strong support for an effect of the within-individual rate of change in telomere length between day 4 and day 12 on an individual's

probability of survival to the following season (rate of change in telomere length was present in the top 3 models in the top model set; Table 5). While a positive linear effect of the rate of change in telomere length was present in the top 3 models, our findings suggest that a quadratic relationship best explains the data, as a negative effect of the quadratic rate change in telomere length was also present in the top 2 models (Table 5, Figure 3). This relationship was robust to the exclusion of an outlier in rate of change in telomere length (this point was twice the interquartile range below the 1st quartile), with the linear and quadratic terms still retained within the 2 top models (Table 5).

Given the support above for a quadratic relationship between rate of change in telomere length and survival, we then split the data at the value corresponding to the peak of the quadratic curve (rate of change in telomere length per day = 0.012) and repeated the model comparison process (i) for data with values below the peak (to establish whether there was evidence of a *positive* association between the rate of change in telomere length and survival in these data) and (ii) for data with rate values above the peak (to establish whether there was evidence of a *negative* association between the rate of change in telomere length and survival in these data). For these we constructed a global model that only included terms present in the original top model set (rate of change in telomere length, RTL at day 4 and body mass), and compared all possible nested models. We were unable to control for brood or social group for data that fell below the peak due to convergence errors, so for this we used general linear modles (GLMs) with no random effects. This approach revealed strong support for a positive effect of the rate of change in telomere length on survival to the following season in data below the peak of the quadratic (Table 5). This result cannot be attributed to a failure to control for brood as a random factor in this GLM, as re-running the analysis utilising just one nestling per brood (for the 2 broods that contained more than one nestling) yielded the same result for all possible combinations of retained nestlings. By contrast, in the data above the peak of the quadratic there was no evidence for an effect of the rate of change in telomere length on survival. This is consistent with the negative effect of the quadratic in the full data set reflecting an asymptote in the increase in survival as rate of change in telomere

length increases beyond zero, rather than reductions in survival arising from larger positive values of the rate of change in telomere length.

Table 5. Model selection table showing predictors of survival of day 12 white-browed sparrow weaver nestlings to the start of the following season for **(a)** all data, **(b)** after removal of an outlier, **(c)** for data below the peak of the quadratic, and **(d)** data above the peak of the quadratic. The Δ6 AICc top model set after implementation of the model nesting are presented (Richards *et al.* 2011). Where the null model is not present in the top model set it is highlighted in blue. All models were linear mixed models with the exception of (c) for which random effects were not included (see main body of text). Estimates are given followed by standard errors in parentheses. Continuous variables were centred and scaled. Estimates and standard errors of sex are given for males (M) relative to females. Body mass reflects the nestling's mass at day 12. RTL reflects the nestling's RTL at day 4, prior to the period over which Δ RTL was calculated. Δ RTL reflects the rate of change in RTL from day 4 to day 12. * Predictors that were included in the global model for (a) but were not present in any of the models in the top model set. Predictors for (b) and (c) were those present in the top model set of (a)

Int	Body mass	RTL	Δ RTL	Δ RTL^2	df	logLik	AICc	Δ AICc	AW
a) all data									
1.150 (0.557)	1.007 (0.573)		1.036 (0.605)	-1.180 (0.631)	6	-18.337	51.3	0.00	0.496
1.366 (0.521)			0.747 (0.516)	-1.351 (0.574)	5	-20.256	52.3	1.03	0.296
0.306 (0.381)	1.299 (0.554)		0.862 (0.446)		5	-21.199	54.2	2.92	0.115
0.381 (0.407)	0.981 (0.584)	-0.688 (0.472)			5	-22.099	56.0	4.72	0.047
0.358 (0.353)	0.903 (0.430)				4	-23.451	56.1	4.78	0.045
0.363 (0.326)					3	-26.401	59.5	8.19	NA
b) with outlier removed									
1.150 (0.557)	1.007 (0.573)		1.036 (0.606)	-1.180 (0.631)	6	-18.337	51.4	0.00	0.506
1.366 (0.521)			0.747 (0.516)	-1.351 (0.574)	5	-20.256	52.4	1.00	0.306
0.448 (0.365)	0.998 (0.452)				4	-22.078	53.4	1.98	0.188
0.427 (0.332)					3	-25.491	57.7	6.30	NA
c) data below the peak of the quadratic									
0.786 **(0.529)**			1.598 (0.658)	NA	2	-11.685	27.9	0.00	0.949
0.595 (0.449)	0.877 (0.550)			NA	2	-14.602	33.7	5.84	0.051
0.575				NA	2	-16.335	34.8	6.93	NA
d) data above the peak of the quadratic									
0.1542 (0.556)				NA	3	-8.972	26.6	0.00	1.00

* Day 4 RTL, Age at the start of the following season, pre-lay rainfall, post-lay rainfall

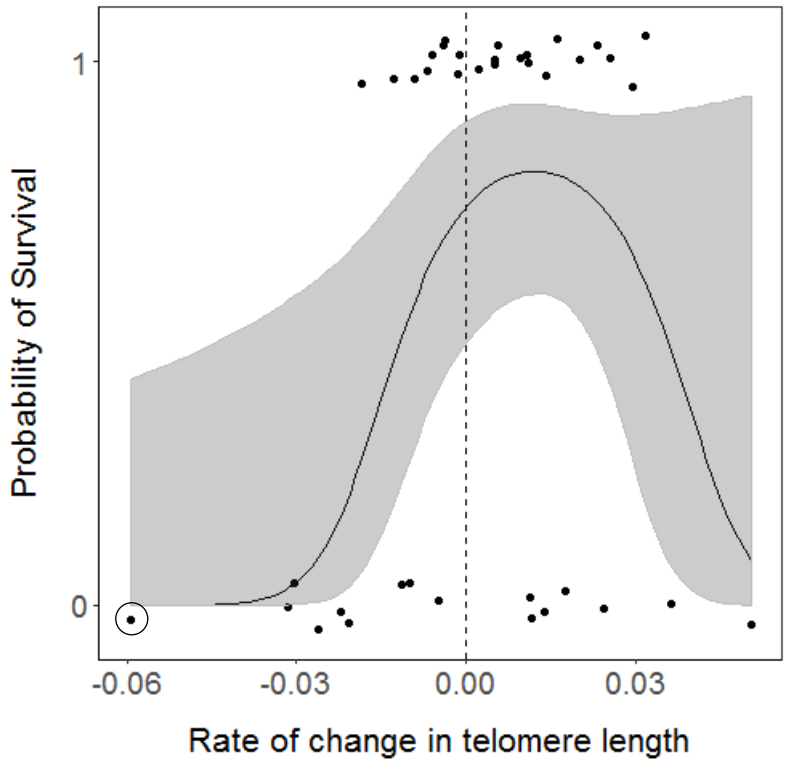


Figure 3: Model predicted line showing the effect of change in RTL per day between day 4 and day 12 on the survival of white browed sparrow weaver nestlings to the following season. Where survived = 0 the nestling was not observed in the following season, where survived = 1, the nestling was observed in the following season. Points are jittered raw data. Solid line is the mean predicted line when body mass is held at its mean value, grey ribbon is 1.96 * se from the mean. This relationship remains in the top model when the outlier (circled) is removed. RTL is calculated as the ratio between quantity of telomeric DNA and the reference gene. As change in RTL was corrected for regression to the mean using Verhulst's D, which renders 0 equal to the mean change in telomere length, we corrected the position of 0 before calculating the rate of change in RTL per day for analysis. Thus, data to the left of the dashed line indicate telomere loss, while points to the right indicate increase.

2.5 Discussion

We investigated whether both telomere length and within-individual rates of change in telomere length predict early life survival in a wild bird. Nestlings and fledglings with shorter mean telomere lengths were *not* less likely to survive than those with longer telomeres. Indeed, contrary to our expectations we found the opposite effect in very young (day 4) nestlings: telomere length just after hatching strongly *negatively* predicted early life survival. Our analyses suggest that this relationship is driven by differentially high in-nest predation of broods with longer telomeres, as the telomere lengths of day 4 nestlings were not predictive of survival when predated nestlings were removed from the analysis, but were predictive of survival following the removal of nestlings that expired for reasons other than predation. In addition, the association between telomere length and survival was no longer apparent in offspring at day 12 or day 30. By contrast, our findings with respect to within-individual rates of change in telomere length were much clearer: individuals that experienced higher rates of telomere *shortening* were subsequently less likely to survive. Our findings provide mechanistic evidence in support of the hypothesis that deficits in somatic maintenance are associated with reduced survival prospects. They also highlight the benefits of utilising a longitudinal approach to assess within-individual telomere dynamics when seeking to relate telomere measures to components of fitness.

The unexpected negative association in very young (day 4) nestlings between mean telomere length and early life survival may reflect short-term survival costs of investment in somatic maintenance in very early life, which leave offspring with long telomeres with poorer survival prospects. For example, very young nestlings that invested more in somatic maintenance could consequently have had fewer reserves to weather any short-term resource shortages during their subsequent growth. However, that this survival association was no longer apparent once predated nestlings were excluded from the analysis (but remained apparent when nestlings that expired for other reasons were excluded), suggests that 4-day-old nestlings with longer telomeres may have been differentially vulnerable to predation, rather than starvation. Nestlings

with longer telomeres at day 4 could be of higher intrinsic quality, and may therefore beg harder during the nestling period (Kilner 2001, though see Price et al. 1996), leaving them more likely to attract nest predators (Leech & Leonard 1997). Alternatively, the positive association with survival could reflect an environmental confound of long telomere lengths in young nestlings (e.g. high rainfall), which itself influences predation risk. For example, our work has revealed that high rainfall prior to egg laying positively predicts nestling telomere lengths at hatching (Chapter 3) and high rainfall could also increase nest predation risk if predators are rain-dependent breeders and/or track hot-spots of recent rainfall (both being likely in this semi-arid region; Jaksić *et al.* 1993; Yang *et al.* 2008). Indeed, we found some evidence of an otherwise counterintuitive negative effect of rainfall on nestling survival after expired nestlings were excluded from analyses (Table 4).

That we found strong evidence that higher telomere attrition rates during the nestling period negatively predicted survival, but no evidence that nestlings with shorter telomeres were less likely to survive, highlights a potential problem with cross-sectional studies seeking to use among-individual variation in telomere length at a single time point to investigate impacts of somatic maintenance patterns on downstream fates. Among-individual variation in telomere length *per se*, particularly in early life, will be a product not only of individual variation in telomere attrition rates (likely a useful biomarker of somatic maintenance; Boonekamp et al., 2013; Houben et al., 2008), but of genetic and epigenetic sources of variation in 'initial' telomere length (and indeed, in studies that assess telomere length using qPCR, as here, of the extent of interstitial telomeric repeats; Delany *et al.* 2003). Where genetic or epigenetic drivers of variation in telomere length estimates are uncorrelated with offspring survival prospects, they have the potential to obscure relationships that might otherwise have been apparent between telomere length and fitness components. More problematic though is the possibility that such drivers are actually correlated with offspring survival prospects, which could confound relationships detected between telomere length *per se* and fitness components. For example, parental age may independently affect both offspring telomere length (Njajou *et al.* 2007; De Meyer *et al.* 2007; Olsson *et al.* 2011; Broer *et al.*

2013) and offspring survival prospects (Tardif *et al.* 1984; Weladji *et al.* 2006). For both reasons, the use of longitudinal assessments of within-individual telomere attrition rates should be prioritised in future studies seeking to test whether detriments in somatic maintenance predict survival prospects. Indeed, in our study, among-individual noise in telomere length estimates due to interstitial repeats, coupled with potential genetic and epigenetic effects on among-individual variation in actual telomere length, provide plausible explanations for why within-individual change in telomere length negatively predicts survival but having shorter mean telomere lengths does not.

Our key finding, that higher rates of telomere attrition negatively predict survival, concords with expectations regarding the negative fitness consequences of shortfalls in somatic maintenance, and is apparent even after allowing for positive effects on survival of body mass (which might otherwise confound such a relationship). This finding is significant, as investigations of the relationship between within-individual telomere dynamics and survival in natural populations remain rare (Bize *et al.* 2009; Salomons *et al.* 2009; Barrett *et al.* 2013; Boonekamp *et al.* 2014a; Fairlie *et al.* 2016; Ujvari *et al.* 2016; Taff & Freeman-Gallant 2017). These results are in agreement with those of the only other study to date to have investigated relationships between both telomere length and attrition rate in free-living nestlings and their downstream survival, in which jackdaw telomere attrition but not length predicted survival to recruitment (Boonekamp *et al.* 2014). In nestlings, higher telomere attrition rates may be caused by rapid cellular proliferation *per se* (Allsopp *et al.* 1995) or by oxidative stress generated by rapid growth (Alonso-Alvarez *et al.* 2007b) and other stressors (von Zglinicki 2002; Ballen *et al.* 2012; Geiger *et al.* 2012; Asghar *et al.* 2015; Meillère *et al.* 2015). As such, higher telomere attrition rates could predict poorer future survival prospects (as reported here) through either of the following mechanisms acting in isolation or concert. First, faster telomere attrition may hasten the accumulation of senescent cells and the depletion of stem cell stocks (Campisi 2005; Herbig *et al.* 2006; Baerlocher *et al.* 2007; Blasco 2007; Boonekamp *et al.* 2013) thereby accelerating tissue degeneration and compromising organismal survival prospects (Price *et al.* 2002; Zhang *et al.* 2003; Trougakos *et al.* 2006; Campisi & d'Adda di Fagagna 2007; Coppé *et al.* 2010). Second, higher telomere

attrition rates may effectively act as a biomarker of accumulated oxidative damage to other tissues across the body that may itself have causal negative effects on fitness (i.e. negative effects correlated with telomere shortening; Metcalfe & Alonso-Alvarez 2010; Selman *et al.* 2012). Net telomere loss is not, however, inevitable during this period as telomeres can be maintained by the enzyme telomerase (Greider & Blackburn 1985), which is active in the cells of hatchling birds, particularly in the bone marrow (Haussmann et al. 2007). Telomere lengths of the hematopoietic stem cells in the bone marrow are highly likely to be reflected in measures of telomere length taken from avian blood (as in this study), as their nucleated erythrocyte descendants vastly outnumber other cell types in circulating blood, and do not proliferate (and thus do not *themselves* undergo telomere attrition) (Williams 1972; Vaziri *et al.* 1994; Schroeder 2010). The actions of telomerase needn't leave telomere dynamics decoupled from the levels of oxidative damage to other tissues, however, as telomere repair by telomerase may be inhibited by oxidative damage (Ahmed *et al.* 2008), and has also been shown to have restorative impacts on structures other than telomeres (Fu *et al.* 2000; Sharma *et al.* 2003; Ahmed *et al.* 2008).

Uniquely, to our knowledge, our analyses revealed a non-linear association between telomere attrition rate and survival: Faster rates of telomere attrition were associated with reduced survival prospects, but large *increases* in telomere length did not confer greater survival benefits than small increases (Figure 3). A large number of nestlings in this study appeared to show telomere lengthening. We must naturally be cautious interpreting these findings as evidence of increases in mean telomere length, given the potential for measurement error to generate such patterns (Steenstrup et al., 2013; though see Bateson and Nettle, 2016). However, telomerase activity has been found to be high in the bone marrow of hatchlings of a number of species (Haussmann *et al.* 2004, 2007), and thus net increases in mean telomere length between the ages of 4 and 12 days are at least conceivable. There are a number of potential explanations for increases in telomere length, beyond measurement error: Telomere extension may reflect either i) stem cell turnover (if cycling hematopoietic stem cells with short telomeres are replaced by a 'younger' subset of previously quiescent cells with longer

telomeres; Ogawa, 1993; Suda et al., 2011), ii) increased investment in somatic maintenance (regardless of past telomere dynamics), or iii) compensation for past attrition through increases in telomerase activity (Beery *et al.* 2012). That we found a negative effect of telomere attrition but not of telomere *extension* is more consistent with the latter two explanations as we would expect the effect of stem cell dropout to be consistent with that of telomere attrition (as it reflects low somatic maintenance). Telomere extension as a response to past attrition is more consistent with our results as it suggests that extension may in fact reflect no *net* change in telomere length and thus no survival benefit. On the other hand, if telomere extension is indicative of high somatic maintenance regardless of past dynamics we might expect telomere extension to positively predict survival (which we did not find to be the case). However, we cannot rule this out as a possibility as heavy investment in somatic maintenance in early life may have fitness benefits that only appear at older ages, and may also carry shorter term costs (as not all fitness components can be maximised simultaneously; Stearns 1989).

Our results indicate that detriments in somatic maintenance during the nestling period, as reflected by telomere *attrition*, may impact downstream survival prospects. In contrast, contrary to our expectations, nestlings with long telomeres were less likely to survive - an effect that appears to be driven by differentially high rates of predation of nestlings with long telomeres. Even after removal of nestlings suspected to have been predated we did not find a negative effect of telomere length on survival, which may be due to a confound, such as variation in pre-laying rainfall, which may increase both predation risk and hatchling telomere length. Our telomere *length* results therefore highlight potential drawbacks of cross-sectional studies. Whether deficits in somatic maintenance in early life are carried through beyond the first year of life is a subject for further study in our species, as we are currently unable to investigate potential effects on lifespan or other components of fitness due to the large proportion of sampled nestlings that remain alive at the time of writing. To conclude, our findings provide mechanistic support for the view that detriments in somatic maintenance entail future fitness costs, and highlight the value of using longitudinal assessments of telomere

attrition alongside cross-section assessments of mean telomere length, when using telomeres as a proxy for biological age or patterns of somatic maintenance.

Chapter 3

Environmental effects on early-life telomere dynamics in a cooperatively breeding bird

3.1 Abstract

Recent studies have highlighted the potential for the social environment experienced during early life to have marked downstream effects on later-life performance and ageing trajectories, a link that is thought to arise via impacts on somatic maintenance. While research to date has focussed almost exclusively on the deleterious downstream effects of exposure to competitive social interactions, the potential for beneficial downstream effects of cooperative social interactions remains largely unexplored. Here we investigate the impact of cooperative helping behaviour on biomarkers of somatic maintenance in offspring (telomere length and attrition rates) in a wild population of cooperatively breeding white-browed sparrow weavers, *Plocepasser mahali* in a semi-arid region. In this species, parental pairs rear their offspring unassisted or with the assistance of up to ten non-breeding helpers. Our analyses reveal that hatchling telomere lengths are unrelated to the number of helpers in the group but are positively predicted by rainfall during the period prior to egg laying. This pattern could arise via positive effects of rainfall on maternal condition and consequent beneficial effects of egg- or incubation-mediated maternal effects on developing embryos. By contrast, we found evidence suggesting that the telomere attrition rates of nestlings *are* impacted by the presence of cooperative helpers: nestlings reared by groups containing helpers showed lower rates of telomere attrition than nestlings in groups without helpers (pairs). This concords with the expectation that positive effects of helpers on resource delivery rates to offspring relax offspring resource allocation trade-offs between somatic maintenance and investment in other traits, such as growth. Our findings provide the first evidence, to our knowledge, suggestive of beneficial effects of cooperative social interactions on patterns of somatic maintenance, and highlight the possibility of hitherto unexplored downstream effects of helping behaviour on late-life performance and ageing trajectories.

3.2 Introduction

Recent research has highlighted that individual variation in late-life performance and ageing trajectories may frequently be attributable in part to downstream effects of the social environment experienced in early life (Nussey *et al.* 2007; Sharp & Clutton-Brock 2011; Brouwer *et al.* 2012; Beirne *et al.* 2015; Lemaître *et al.* 2015; Bebbington *et al.* 2017). Competitive social interactions experienced in early adulthood, for example, have recently been found to predict late-life performance in wild vertebrate populations (Lemaitre *et al.* 2014, Beirne *et al.* 2015, Nussey *et al.* 2007) and can even account for sex differences in ageing trajectories (Beirne *et al.* 2015). While it has long been hypothesised that such negative downstream effects arise because social competition entails costs to somatic maintenance (Bonduriansky *et al.* 2008), only recently has the advent of viable biomarkers of somatic maintenance (such as telomere attrition rates; see below) allowed this hypothesis to be tested. Consistent with this idea, a number of studies have now highlighted that experimental increases in the intensity of early-life competition can lead to detriments in somatic maintenance that might themselves be expected to have negative downstream effects on performance (though see Voillemot *et al.* 2012; Nettle *et al.* 2013, 2015; Boonekamp *et al.* 2014a; Reichert *et al.* 2014b; Costanzo *et al.* 2016). While most research on downstream effects of the social environment has focussed on the effects of competitive interactions (Komdeur 1994; Sharp & Clutton-Brock 2011; Boonekamp *et al.* 2014a; Lemaître *et al.* 2014; Beirne *et al.* 2015; Bebbington *et al.* 2017), cooperative interactions are also pervasive (Solomon & French 1997; Dickinson & Hatchwell 2004; Koenig & Dickinson 2004) and have the potential to yield *positive* downstream effects on late life performance that have rarely been considered (Russell *et al.* 2007, Sparkman *et al.* 2011). For example, in cooperatively breeding species, helpers assist parents by feeding their offspring (Solomon & French 1997; Dickinson & Hatchwell 2004; Koenig & Dickinson 2004). While there is widespread evidence that helpers can consequently have positive effects on offspring food intake, growth and survival (Clutton-Brock *et al.* 2001; Dickinson & Hatchwell 2004; Hodge *et al.* 2005), there has been remarkably little investigation of helper effects on life-histories

(Russell *et al.* 2007b; Sparkman *et al.* 2011; Brouwer *et al.* 2012; Hammers *et al.* 2013). In particular, whether helpers have life-long downstream effects on components of fitness by promoting somatic maintenance in early life has yet to be investigated.

Trade-offs between somatic maintenance and investment in other traits are widely thought to mediate the link between early-life conditions and later life effects (Kirkwood 1977; Kirkwood & Rose 1991; Cichon *et al.* 2001; Lemaître *et al.* 2015). The rationale is that resources (and the ability to acquire them) are finite and thus not all aspects of fitness can be maximised simultaneously (Stearns 1989). Thus, if resources are preferentially allocated to reproductive- or survival-enhancing activities, shortfalls in somatic maintenance may occur. The resultant accumulation of molecular damage is thought to accelerate senescence and reduce longevity (Kirkwood 1977; Kirkwood & Rose 1991). Poor investment in somatic maintenance and repair may therefore incur a large fitness cost, particularly if it occurs in early life when remaining reproductive value is high (Kirkwood 1977; Kirkwood & Rose 1991; Cichon *et al.* 2001). The consequences of trade-offs between somatic maintenance and other activities are predicted to be most apparent when resource availability is poor. For example, while growth rate and somatic maintenance may be unrelated or even positively correlated when resources are plentiful, under poor conditions (or following poor conditions i.e. during compensatory growth) growth rate has been found to negatively predict both physiological condition (De Block & Stoks 2008; Geiger *et al.* 2012; Tarry-Adkins *et al.* 2013; Boonekamp *et al.* 2014a) and lifespan (Jennings *et al.* 1999; Inness & Metcalfe 2008; Lee *et al.* 2013). Resource availability is impacted by aspects of both the abiotic environment, such as rainfall; a major driver of food abundance, and the social environment. For instance, competition among siblings of the same brood or litter may reduce the availability of resources to any particular individual, and increase the costs of acquiring them (Neuenschwander *et al.* 2003; e.g. through increased begging or aggression between competitors; Hodge *et al.* 2009). However, social environment also has the potential to *increase* the resources available to any particular individual: In cooperatively breeding species helpers may increase overall provisioning rate and thus the total resources available to offspring (Hatchwell 1999). Overall increases in resource availability for

offspring are predicted to alleviate trade-offs between somatic maintenance and other activities. Thus, both abiotic and social environmental conditions in early life may be profound drivers of variation in investment in somatic maintenance with important downstream consequences.

Telomeres – the dynamic caps of linear chromosomes – are considered a biomarker of somatic maintenance (Houben *et al.* 2008; Boonekamp *et al.* 2013; Simons 2015). Telomeres consist of a repetitive nucleotide sequence and associated proteins that act together to prevent the ends of linear chromosomes from being seen by DNA repair mechanisms as damaged DNA (Blackburn & Szostak 1984; Karlseder 1999). In so doing, they preclude inappropriate 'repair' that could result in chromosome end-to-end fusions, apoptosis (programmed cell death) or cellular senescence (when cell division is arrested) (Hemann *et al.* 2001a; Feldser & Greider 2007; Campisi & d'Adda di Fagagna 2007). In addition to this vital function, telomeres protect coding DNA from being eroded during cell replication when standard DNA polymerases are unable to fully replicate the lagging strand (Watson 1972; Olovnikov 1973). Thus, when cells proliferate it is telomeres rather than stretches of important coding DNA that are eroded, and consequently telomeres get shorter as cell populations age (Harley *et al.* 1990). When telomeres become too short they cease to function properly and trigger cellular senescence or apoptosis (Hemann *et al.* 2001a; Herbig *et al.* 2004; Feldser & Greider 2007). Telomere attrition thereby has the capacity to limit the proliferative potential of cells (Vaziri *et al.* 1994) and thus also the potential for tissue regeneration (Hao *et al.* 2005; Reichert *et al.* 2014a).

In addition to the gradual and predictable erosion that occurs at each cell cycle, larger tracts of telomere can stochastically be lost (Baird *et al.* 2003; Lansdorp 2005). A major cause of such losses is thought to be oxidative stress (von Zglinicki 2002; Richter & von Zglinicki 2007): When antioxidant defences are overwhelmed, reactive oxygen species (ROS) cause damage to cellular components (Halliwell & Gutteridge 2007a), and telomeres appear to be both particularly prone to oxidative damage (Kawanishi *et al.* 2001; Kawanishi & Oikawa 2004) and unusually resistant to its repair (Petersen *et al.*

1998; Oikawa & Kawanishi 1999; Coluzzi *et al.* 2014), with unrepaired damage leading to accelerated rates of attrition (Sitte *et al.* 1998; von Zglinicki 2002; Coluzzi *et al.* 2014). Thus telomeres may be thought of as 'sentinels' of the damage experienced by cells, that can act to prevent dangerously damaged cells from replicating (von Zglinicki 2002). Telomere attrition is not, however, unavoidable. Organisms may be able to extend somatic lifespan by investing more in antioxidant defences and thus precluding damage (Serra *et al.* 2003; Cattan *et al.* 2008; Badás *et al.* 2015), or by investing in cellular repair mechanisms such as telomerase (an enzyme that extends telomeres; Greider and Blackburn, 1985). The precise relationship between telomerase and somatic maintenance is not well understood, however it is possible that telomeres are only repaired by telomerase to a level that is reflective of overall cell health (Sharma *et al.* 2003; i.e when other cellular components are also efficiently repaired; see for example Ahmed *et al.* 2008). Thus, even in cells with active telomerase, telomeres are thought to eventually reach a length when they are no longer able to function properly, and cellular senescence or apoptosis is triggered. Both reduced cell renewal capacity and accumulation of senescent cells are associated with the aging phenotype (Kipling 2001; Herbig *et al.* 2006; Campisi & d'Adda di Fagagna 2007; Coppé *et al.* 2010). Indeed, short telomeres and high rates of telomere attrition are associated with age-related diseases (Blackburn *et al.* 2015) and have been shown to predict reduced survival and shorter lifespan in several species (see chapter 2, table 1; Epel *et al.* 2009; Heidinger *et al.* 2012; Vera & Blasco 2012; Barrett *et al.* 2013). Telomeres may therefore potentially be both a biomarker of somatic damage, and an active agent mediating early-late life trade-offs.

Telomere attrition rates are often found to be fastest in early life (potentially because of rapid cellular division and oxidative stress generated by rapid growth; Alonso-Alvarez *et al.* 2007b). Early life exposure to competition or cooperative interactions thus has the capacity to impact telomere attrition rates, with potentially life-long downstream consequences. Indeed, there is compelling evidence that competition in early life can impact telomere length and attrition rate (Boonekamp *et al.* 2014a; Reichert *et al.* 2014b; Costanzo *et al.* 2016; though see Voillemot *et al.* 2012). For example, using brood manipulations in jackdaws, *Corvus monedula*, Boonekamp et al. (2014) demonstrated

that nestlings in enlarged broods had higher rates of telomere attrition than those in broods that were reduced in size, and higher rates of telomere attrition were found to be predictive of reduced early life survival. The extent of any detrimental effects of competition depend not only the number of competitors, but also on relative competitive ability (Nettle *et al.* 2013, 2015). European starling, *Sturnus vulgaris*, nestlings assigned to broods in which they had a competitive disadvantage (where they were smaller than brood-mates) were found to have higher rates of telomere attrition than those with a competitive advantage (larger than brood-mates), despite there being no overall effect of this manipulation on body mass gain (Nettle *et al.* 2015). Social competition can, therefore, have demonstrably negative effects on telomere dynamics, which is likely to be indicative of more widely compromised somatic maintenance (see above). While cooperative interactions, such as helping to feed the offspring of others, are widely appreciated to alleviate food constraints in developing young in those cooperative breeders with additive helping (Hatchwell 1999), how these interactions impact telomere dynamics and ultimately somatic maintenance remains to be investigated.

Here we investigate the effects of both cooperation and competition on hatchling telomere length and the within-individual telomere dynamics of nestlings in societies of the cooperatively breeding white-browed sparrow weaver (*Plocepasser mahali*). In previous work we found that telomere dynamics of sparrow weaver nestlings predict survival (Chapter 2), and thus any impacts of the environment on telomere attrition could have profound consequences for offspring fitness. White-browed sparrow weavers inhabit semi-arid regions of sub-Saharan Africa, and are dependent on sufficient rain in order to lay eggs and successfully raise offspring. These birds occupy year-round territories that are defended by the whole group (Collias & Collias 1978). Groups consist of between 2 and 12 birds, but contain only a single dominant pair who monopolise within-group reproduction (Harrison *et al.* 2013a; b). Cooperation is well developed and subordinate helpers assist in sentinelling, weaving, and territory defence, in addition to the care of young (Collias & Collias 1978; Lewis 1982; Walker *et al.* 2016). The majority of subordinates help provision the broods of between one and three

nestlings, with the result that nestlings raised by more helpers are provisioned at higher rates (Young, unpublished data). Whilst some immigrant subordinates provision at a much lower rate than natal subordinates, these birds actively help in territory defence and sentinelling (Young, unpublished data), potentially enabling a higher rate of provisioning by other birds. The presence or number of helpers could also alleviate resource constraints in laying mothers, for example by easing the trade-off between foraging and sentinelling. In so doing helpers could allow mothers to invest differentially in key resources in their eggs (Paquet *et al.* 2013). Alternatively, mothers with helpers may pre-emptively reduce egg quality, in anticipation that helper investment in offspring provisioning would compensate for the shortfall (Russell *et al.* 2007a).

Specifically, we assess the impact of the social environment (in particular group size; the number of helpers plus two dominant birds) and abiotic environment (rainfall) on (i) telomere length close to hatching (at 4 days of age; hereafter 'hatchling' telomere length), and (ii) within-individual telomere attrition rate in the early developmental phase (between 4 and 12 days of age, during the nestling period) and the late developmental phase (between 12 and 30 days of age; the latter being immediately post-fledging). We test the following predictions. First, we predict positive effects of egg mass and/or pre-laying rainfall on hatching telomere length, as each has the potential to positively influence the resources available to support maternal *in-ovo* development. Group size may either positively or negatively impact hatchling telomere length through effects of maternal investment in egg composition (see above). Second, we predict positive effects of rainfall and group size and negative effects of brood size on within-individual telomere attrition rates in both the early and late developmental contexts, given that existing evidence strongly suggests that both rainfall and helpers enhance resource delivery to the developing brood in this species (Young *et al.* unpublished data), and because the resources delivered are divided among brood members.

3.3 Methods

3.3.1 Study population and field methods

This study was conducted on a colour-ringed population of white-browed sparrow weavers at Tswalu Kalahari Reserve, South Africa (27°16'S, 22°25'E). Nests were monitored at least every other day until eggs were discovered, then every day until clutch completion, after which nests were left undisturbed until 15 days after clutch initiation when daily nest checks were resumed to establish hatch dates. Upon first discovery eggs (typically within two days of laying) and nestlings (typically on their day of hatching, given daily nest checks at this time) were weighed to the closest 0.01g (Durascale 100; MyWeigh, Phoenix, AZ) and nestlings were assigned either a left or right 'haircut', in which feathers were trimmed on one side of the head, or no 'haircut', allowing them to be distinguished until the application of a metal leg ring with a unique number at approximately 12 days of age (SAFRING licence 1444). Nests were subsequently checked on the 4th, 8th, 12th and 16th day of life of the first-hatched nestling. The age of nestlings is henceforth referred to as the day of life of the first hatched nestling (i.e. the 4th day of life of the first hatched nestling is day 4 for all nestlings in that brood). At each nest check nestling body mass was recorded to the nearest 0.01 g (Durascale 100; MyWeigh, Phoenix, AZ) and on day 4 and day 12 a small blood sample (<25µl) was taken via brachial venepuncture with a 26G needle for telomere length determination. Blood was collected in a non-heparinized capillary tube and stored in approximately 500µl of absolute ethanol at ambient temperature until extraction. Fledglings were caught at night from their roost chambers at approximately 30 days of age (day 30) by flushing them from their individual roost chambers at night into a custom capture bag, whereupon a small blood sample, along with a measure of body mass were taken as described above. Fledglings were then returned to their roost chambers. Nestlings that survived to adulthood were sexed by beak colour (a sexually dimorphic trait in adults of this subspecies; *P. m. mahali*; Leitner et al., 2010), or by molecular sexing if not (Dawson *et al.* 2015). In order to assess adult group size (the number of birds over 6 months of age), birds were noted as present or absent during group composition scans that were

conducted for each group at least once every two weeks. Birds were considered to belong to a social group when they were consistently observed to forage with that group and roost in trees in the groups territory (Harrison *et al.* 2013b). Start and end dates for group membership were calculated as the date midway between when they were last known to be absent from the group and first seen as accepted in the group (for the start of their group membership) and between when they were last known to be present and first confidently recorded as absent (for the end of their group membership). Group size for each breeding attempt was calculated as the average number of adult birds (over 6 months of age) present per day between the hatch date and day 30 of each breeding attempt (the number of potential helpers is simply group size minus 2).

3.3.2 DNA extraction and telomere measurement

DNA extraction and qPCR methods are described fully in chapter 2. Briefly, DNA was extracted using the Gentra PureGene Genomic DNA Purification Kit (Qiagen), and DNA quantity, integrity and quality were assessed. Real-time quantitative PCR (qPCR), as described in Cawthon (2002), was used to measure relative telomere length (termed RTL for brevity in the methods, but 'telomere length' for clarity in the results and discussion). This method gives an average telomere length per sample relative to a constant copy number control gene, for which we used Glyceraldehyde-3-phosphate-dehydrogenase (GAPDH) using primers specific to the white-browed sparrow weaver (GAPDH-F 5′AAACCAGCCAAGTATGATGACAT –3′; GAPDH-R 5′-CCATCAGCAGCAGCCTT CA-3′ see Appendix A). Telomere primers were as follows: Tel1b 5′-CGGTTTGTTTGGGTTTGGGTTTGGGTTTGGGTTTGGGTT-3′; Tel2b 5′-GGCTTGCCTTACCCTTACCCTTACCCTTACCCTTACCCT-3′. All primers were used at a concentration of 200nM in 20µl reactions that also included 10uL SybrGreen fluorescent dye with low ROX (Agilent Technologies) and 5ng (total) DNA. All samples were run in triplicate on 96-well plates, and samples from the same individual were run on the same plate to reduce noise in within-individual change in telomere length measures (telomere and GAPDH reactions were carried out on separate plates). Plates also included a between-plate calibration sample consisting of pooled DNA from three

birds. With the exception of the annealing temperature, thermal cycles for telomere and GAPDH were the same: 95˚C for 15 minutes, followed by 40 cycles of 95˚C for 15 seconds, 57˚C annealing for 30 seconds (Telomere) or 60˚C (GAPDH), and 73˚C extension for 30 seconds. LinRegPCR (Ruijter *et al.* 2009) was used to correct baseline fluorescence, set a window of linearity for each amplicon group and to set constant fluorescence thresholds within the windows of linearity for GAPDH (0.156) and telomere (0.161). These data were then used to calculate RTL following Pfaffl (2001).

Samples used in this chapter were run on 22 pairs of plates, some of which also included samples used for further studies. Samples from the same individual were run on the same plate to reduce noise in calculations of change in RTL. The inter-plate coefficient of variation for RTL was 13.09% (Standard deviation; SD = 8.15) across all plates (including those used in further studies), while the intra-plate coefficients of variation for all samples across all plates were 0.32% (SD = 0.08) for GAPDH and 0.85% (SD =0.13) for telomere.

3.3.3 Statistical analyses

All statistical methods were carried out in 'R' (version 3.3.1). We used an information theoretic (IT) model selection approach to compare linear mixed effects models, using Aikaike's information criterion correcting for small sample size (AICc) to compare models (Burnham & Anderson 2002). We constructed global models as outlined below, and as we had no *a priori* reason for excluding certain combinations of terms, all combinations were compared and ranked based on AICc (except in one exceptional case, highlighted in the results, in which group size was competed against, but not included alongside, the presence or absence of helpers) using the package MuMIn (Barton 2016). Interaction terms were only included in models where the corresponding first order terms were present. All models within Δ6 AICc of the top model (with the highest Akaike weight and lowest AICc) were retained in a 'top model set' of the best supported models. This cutoff allows confidence that the most parsimonious model is included in the candidate model set, but removes models with only very weak support

(Richards 2005). In order to avoid the selection of overly complex models, we disregarded models that had a higher AICc value than simpler nested models ("model nesting rule"; Richards *et al.* 2011), however all top model sets prior to implementation of the model nesting rule are presented in Appendix C. As we used this model nesting rule we plotted results using effect size estimates from the top model (for terms within the top model; and the best model containing the term of interest for any terms not within the top model) rather than using model averaging. All continuous predictors in linear mixed models were centred and scaled but were back-transformed for plotting. Prior to model comparison, in order to ascertain independence of predictors, correlations between all predictor variables and variance inflation factors (VIF) of each global model were checked to assess multicolliearity: all VIFs were below 5 ("car" package; Fox & Weisberg 2011). Model residuals of global models were assessed to confirm compliance with model assumptions. Cook's distances were examined to check for points of high influence using the package influence.ME (Nieuwenhuis et al 2012).

(1) Hatchling telomere length

We used linear mixed models (lmer function in the package "lme4"; Bates *et al.* 2014) to test whether social or environmental variables predict RTL of nestlings on day 4 (hatch date was known to within a day; range = 3–6 days of age, SD = 1.03, n = 87 nestlings), which was the earliest we could safely take blood samples. The 87 nestlings sampled were from 59 broods in 32 social groups over 3 breeding seasons. Residuals of the global model were right-skewed so we logged our response term (RTL). Our candidate explanatory variables of interest were mean adult group size between hatching and day 30, rainfall (mm) in the month prior to the onset of egg laying, mean egg mass for the clutch (see below), nestling mass at sampling, clutch size, and nestling sex. We also included the age at which the nestling was sampled as a covariate predictor, given slight variation among nestlings in the age at sampling (see above). We logged adult group size as we expect increases in group size to become less important the larger the group. As not all nestlings were sampled and hence weighed at precisely 4 days of age, nestling mass was adjusted using the slope of the regression of nestling mass on age for all nestlings (measured over the course of the long-term project) between 3 and 6

days of age (n = 564 nestlings, slope = 2.81 g / day). As we could not always tell which nestling hatched from which egg, we instead fitted the mean egg mass for the clutch, excluding eggs that did not hatch. Viable eggs within a clutch tend to be very similar in mass: When unhatched eggs are excluded from all eggs measured over the long-term project, Pearson's rho with pairwise comparisons (as some clutches contained three eggs) revealed that egg mass was repeatable within clutches (n = 268 clutches, r = 0.86). Eggs for 68 of the 87 nestlings were weighed when eggs were less than 3 days old. The remaining were weighed later, or have uncertain lay dates. As eggs become lighter over time this may generate noise but should not result in a type I error. Rainfall prior to egg laying may impact egg content, depending on the number of eggs laid. We therefore included the interaction between clutch size and rainfall prior to egg laying. Social group, brood, breeding season (e.g. 2014-15) and qPCR plate were included as random effects.

(2) Rate of change in telomere length

We examined causes of variation in the rate of change in RTL over two periods: the early developmental period between day 4 and day 12 (day 4 range = 3–5 days of age, SD = 0.48; day 12 range = 11–12 days of age, SD = 0.42), and the late developmental period between day 12 and day 30 (day 12 range = 10–13 days of age, SD = 0.61; day 30 range = 28–34 days of age, SD = 1.41). Hatch date was known to within a day. Nestlings fledge at approximately 20-25 days of age; this is difficult to determine precisely as checking whether nestlings have fledged tends to stimulate them to fledge early from about 17 days of age and once nestlings have fledged naturally they are initially very cryptic. In the early developmental period, we measured change in RTL in 40 birds from 32 broods in 25 social groups. All nestlings hatched within a single calendar year (2014), but over two breeding seasons (2013-14 and 2014-15). In the late developmental period, we measured change in RTL in 48 birds from 38 broods in 25 social groups. Sampled birds hatched over the course of three seasons (2012-13, 2013-14 and 2014-2015). For both developmental periods, we used paired t-tests to test whether there was a significant decrease in telomere length (i) for all nestlings, (ii) for nestlings raised by *social groups*

(parental pairs with helpers; group size > 2) and (iii) for nestlings raised by *unassisted pairs* (parental pairs without helpers).

We used two separate linear mixed effects models to test the factors that affect the rate of change in RTL over the early and late development periods, including whether there was a difference in the rate of change in RTL between broods raised by social groups versus unassisted pairs. For the response term in each model we calculated the change in RTL between the baseline (i.e. day 4 in early development period, or day 12 in the late development period) and follow-up measure (day 12 and day 30 respectively) with correction for regression to the mean, following Verhulst (2013). Briefly, the mean-centred baseline measures were multiplied by the correlation coefficient between the mean-centred baseline and mean-centred follow-up RTL measures, and then subtracted from the mean-centred follow-up RTL to yield a value of 'D'. As this calculation involves mean centring values for both baseline and follow-up measurements, the average D is equal to zero. For clarity, we corrected the position of zero (i.e. where no change in telomere length occurred) by adding the difference between the mean baseline RTL and mean follow-up RTL to all values of D. We then calculated the rate of change in telomere length as D / number of days between the sampling time-points. Both global models included the following fixed effect predictors: start RTL (the baseline measure in each case i.e. day 4 RTL in the early developmental period, and day 12 RTL in the late developmental period), two measures of rainfall: total rainfall (mm) in the month prior to the onset of egg laying and total rainfall (mm) between the onset of egg laying and day 12 in the early developmental period or day 30 in the late developmental period, and a measure of nestling competition ('true' if there was more than one nestling in the brood at any time in the period of interest, and 'false' if not). We used this nestling competition variable rather than brood size as nestlings from broods of three were rare in our sample (2 broods in the early development data set and 3 broods in the late development data set).

In order to control for potential effects of variation in growth on telomere dynamics we also fitted body mass at day 12 as a fixed effect predictor in the early developmental

period model. As not all nestlings were sampled and hence weighed at precisely 12 days of age, nestling mass was adjusted using the slope of the regression of nestling mass on age for all nestlings measured over the course of the long-term project) between 10 and 13 days of age (n= 521 measures from 390 birds, slope = 1.914). Fledgling (day 30) mass was not adjusted for the precise age at weighing as mass gain by this stage is minimal. As the available sample size was larger for the late developmental period model, we were able to allow for more complex growth effects by fitting body mass at day 12, mass gain between days 12 and 30, and the interaction between these two terms. In order to allow for the possibility that helper effects could manifest as either an effect of continuous variation in group size or an effect of whether or not a parental pair has helpers (as the latter could be true in this species as the contributions of individual helpers to offspring provisioning do significantly decrease with increasing group size; Young et al. unpublished data), we also tested the effects of including *either* group size (number of group members over 6 months of age) *or* group type (whether the parental pair had helpers: social group or unassisted pair) as fixed effect predictors; these terms were never fitted in the same model together. Social group, clutch and qPCR plate were included as random factors in both global models. Samples for the early development period were all taken within a single calendar year and so season was not included as a random effect in this model, though it was in the model for the late developmental period.

3.4 Results

3.4.1 Hatchling telomere length

Mixed effects modelling of hatchling telomere length revealed evidence that the total rainfall in the month prior to egg-laying positively predicted hatchling telomere length (Table 1; Figure 1a). Models utilising the full data set did not have full support for this rainfall effect (Table 1a): while pre-laying rainfall was present in the top model, this model was only 0.35 AICc points above the intercept-only model. However, the removal of a single influential outlier (with a high Cook's distance of 1.74, whilst all other points were below 0.05; see circled point in Figure 1a) resulted in a substantial increase in the overall strength of model fit, an increase in the effect size of pre-laying rainfall, and its presence within all models in the top model set (Table 1b). After removal of this influential point the top model additionally included sex, with males having longer telomeres than females (Figure 1b), but support for an effect of sex was limited as it was absent from the second-best supported model, just 0.69 AICc points below the top model (Table 1b). We found no evidence that aspects of the social environment (either group size or clutch size) or egg mass, nestling mass, or post egg-laying rainfall predicted hatchling telomere length. There was also no evidence that the age of nestlings at the time of sampling had an effect.

Table 1. Model selection table showing predictors of telomere length of day 4 white-browed sparrow weaver nestlings, for **(a)** all samples, and **(b)** when a single influential point is excluded (see circled point in Figure 1a). Δ6 AICc top model sets after implementation of the model nesting rule are presented (Richards *et al.* 2011). Where it is not present in the top model set, the null model is shown, highlighted in blue. All continuous variables were centred and scaled. Estimates for each term present in each model are given followed by the standard error in parentheses. For sex, the estimate and standard error are given for males (M) relative to females. *Variables present in the global model but not the top model set

Intercept	pre-lay rain	Sex (M)	df	logLik	AICc	Δ AICc	Adjusted weight
(a) *full data*							
0.007 (0.029)	0.040 (0.023)		7	20.755	-26.1	0.00	0.544
0.008 (0.029)			6	19.395	-25.7	0.35	0.456
(b) *influential point removed*							
-0.014 (0.034)	0.073 (0.019)	0.058 (0.032)	8	32.646	-47.4	0.00	0.581
0.017 (0.030)	0.076 (0.020)		7	31.085	-46.7	0.69	0.415
0.017			6	24.617	-36.2	11.25	NA

* Age, Egg mass, Nestling mass at day 4, log Group size, Clutch size, Clutch size: pre-lay rain

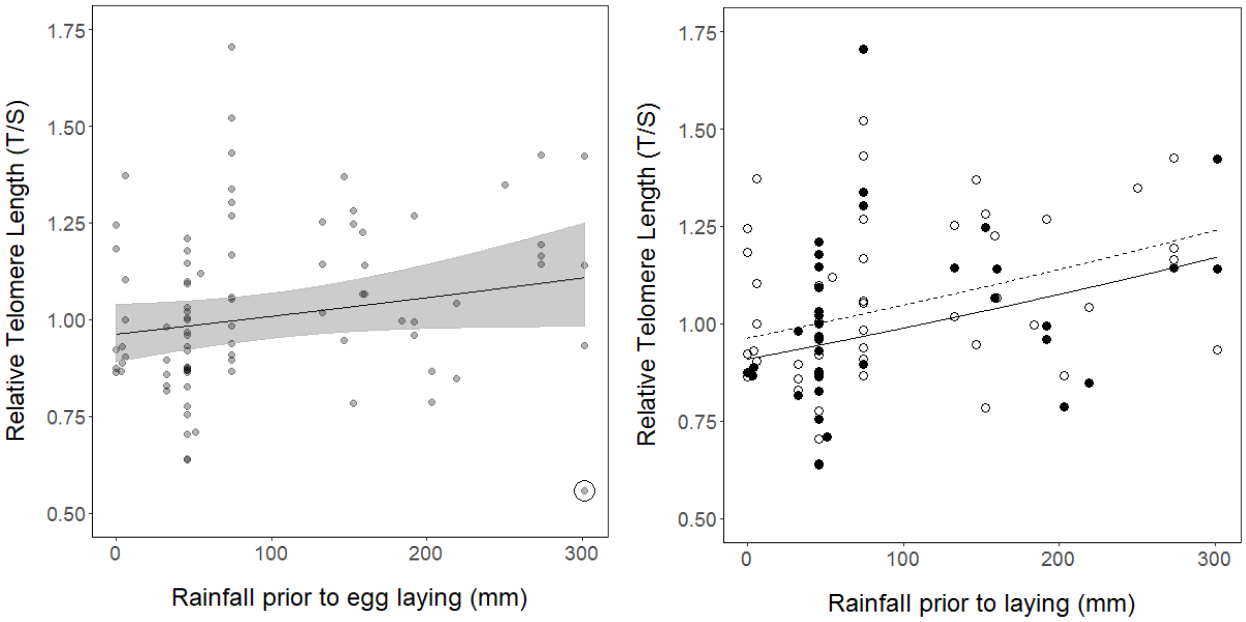


Figure 1: Model predicted slope of the effect of rainfall in the month prior to egg laying on hatchling white-browed sparrow weaver relative telomere length (RTL) for **(a)** all data, with the influential point circled, and **(b)** after removal of the influential point, with the data and regression lines for the sexes now indicated for females (filled circles, solid line) and for males (open circles, dashed line). RTL has been back-transformed from log RTL. Grey ribbon shows 95% confidence intervals (not included in b for ease of viewing, but see Table 1 for standard errors). Points that overlap in figure (a) appear darker.

3.4.2 Within-individual change in telomere length during development

Telomere dynamics during early development (day 4 to day 12)

Paired comparisons revealed that nestlings did not experience a significant within-individual change in mean telomere length between 4 and 12 days of age (n = 40 nestlings sampled at both time points, paired t = 0.33, df = 39, p = 0.74, Figure 3a). Nestlings raised by unassisted pairs showed a non-significant trend towards a within-individual decrease in telomere length over this period (n = 6 nestlings, paired t = -1.73, df = 5, p = 0.14, Figure 3a), whereas nestlings reared by social groups did not (n = 34 nestlings, paired t = 0.97, df = 33, p = 0.34, mean of the differences = 0.029, Figure 3a). Accordingly, mixed modelling revealed no evidence of a difference between the within-individual rate of change in telomere length for broods raised by social groups and broods raised by unassisted pairs (Table 2, Figure 3c).

Mixed modelling revealed that only hatchling telomere length (at day 4) predicted the within-individual rate of change in telomere length between 4 and 12 days of age. Nestlings that had longer telomeres at day 4 showed higher rates of telomere attrition (i.e. rates of change in telomere length that are more negative) than those with shorter telomeres at day 4: while day 4 telomere length was present in the top model, this model was only 1.35 AICc points above the intercept-only model (Figure 2).

None of our measures of either social environment (nestling competition, group size, or the presence of helpers in the group) or abiotic environment (rainfall in the month before egg laying), predicted the within-individual rate of change in telomere length in the early developmental period, and neither did nestling sex or body mass.

Table 2. Model selection table showing predictors of the rate of change in relative telomere length during early development (between day 4 and day 12) of white-browed sparrow weaver nestlings. Δ6 AICc top model set after implementation of the model nesting rule is presented (Richards *et al.* 2011). All continuous variables were centred and scaled. Estimates are presented with standard errors in parentheses. *Variables present in the global model but not the top model set

Intercept	Day 4 RTL	df	logLik	AICc	Δ AICc	Adjusted Weight
0.001 (0.0036)	-0.005 (0.0025)	6	109.416	-204.3	0.00	0.662
0.001 (0.0034)		5	107.353	-202.9	1.35	0.338

* nestlings mass at day 12, sex, nestling competition, group type (unassisted pairs or social group), group size, pre-lay rainfall, post-lay rainfall

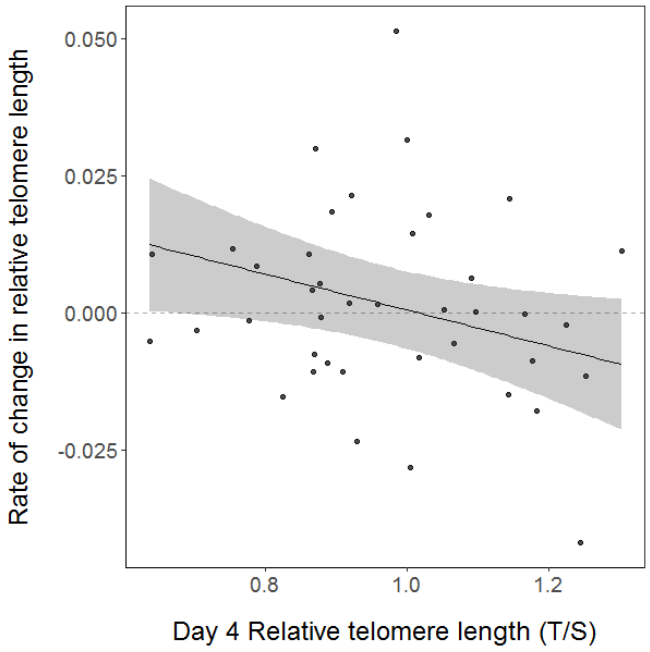


Figure 2. Effect of relative telomere length (RTL) of white-browed sparrow weaver nestlings at day 4 on the within-individual rate of change in RTL between day 4 and day 12. Model predicted slope and 95% confidence intervals are shown. RTL is calculated as the ratio between quantity of telomeric DNA and the reference gene. As change in RTL was corrected for regression to the mean using Verhulst's D, which renders 0 equal to the mean change in telomere length, we corrected the position of 0 before calculating the rate of change in RTL for analysis. Thus, data below the dashed line indicate telomere loss. Points show raw data, and the grey ribbon shows confidence intervals around the estimate.

Telomere dynamics during late development (day 12 to day 30)

Paired comparisons revealed that nestlings experienced a significant within-individual decrease in mean telomere length between day 12 and day 30 (n = 48 birds; paired t = -2.35, df = 47, p = 0.02, Figure 3b). Nestlings raised by unassisted pairs experienced a significant within-individual decrease in mean telomere length over this period (n = 14 nestlings, paired t = -4.798, df = 13, p = 0.0003, Figure 3b), while nestlings raised by social groups did not (n = 34 nestlings, paired t = -0.54, df =33, p = 0.30, Figure 3b).

Results from model comparisons suggest that this contrast arises because the rates of change in telomere length are lower (i.e. telomere attrition is significantly more severe) in offspring reared by unassisted pairs than offspring reared in social groups (Table 3, Figure 3d). Helper effects appeared in the top model regardless of whether they were characterised by fitting group type (unassisted pairs or social groups) as the focal predictor or continuous variation in group size. However, there was stronger support for the model utilising the binomial classification (1.55 AICc points above the intercept only model) than the model utilising continuous variation in group size (just 0.85 AICc points above the intercept-only model); this contrast being suggestive of asymptotic benefits of increasing helper numbers. We found no evidence that nestling competition (brood size), telomere length at day 12, rainfall (either before or after egg laying), nestling mass at day 12, mass gain from day 12 to day 30 (nor the interaction between the two) predicted the within-individual rate of change in telomere length.

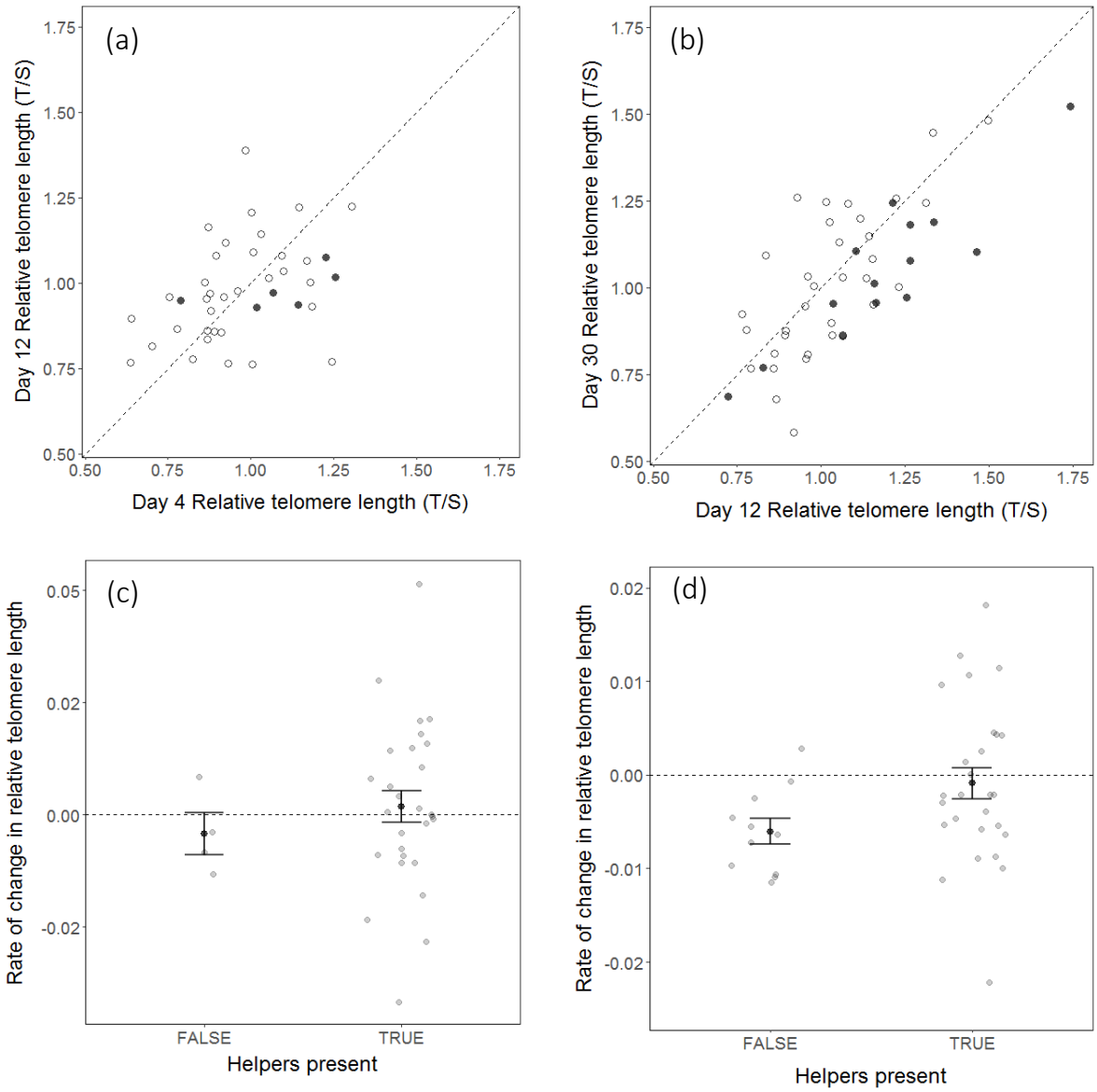


Figure 3. Change in relative telomere length (RTL) of white-browed sparrow weaver nestlings between **(a)** day 4 and day 12, and **(b)** day 12 and 30 and *rate* of change in telomere length between **(c)** day 4 and day 12, and **(d)** day 12 and 30. Nestlings fledge at approximately day 20. In all plots, the dashed line shows the line of no change, with points below the line indicating telomere attrition. In (**a** and **b**) open circles are offspring raised by social groups, while closed points are offspring raised by unassisted pairs. Relative telomere length is calculated as the ratio between quantity of telomeric DNA and the reference gene. In plots (**c** and **d**) grey points show the raw data, while the black point and whiskers show the mean ± one standard error from the mean from the raw data. As change in RTL was corrected for regression to the mean using Verhulst's D, which renders 0 equal to the mean change in telomere length, we corrected the position of 0 before calculating the rate of change in RTL for analysis.

Table 3. Model selection table showing predictors of the rate of change in relative telomere length of white-browed sparrow weaver nestlings during late development (between day 12 and day 30). Nestlings fledge at approximately day 20. Δ6 AICc top model set after implementation of the model nesting rule is shown (Richards *et al.* 2011). Estimates are presented with standard errors in parentheses. Group size was centred and scaled. For group type the estimate is given for unassisted pairs relative to social groups. *Variables present in the global model but not the top model set

(Intercept)	Group type (Unassisted Pairs)	Group size	df	logLik	AICc	Δ AICc	Adjusted Weight
-0.0012 (0.0014)	-0.005 (0.0014)		7	165.21	-313.6	0.00	0.473
-0.0029 (0.0012)		0.0022 (0.0011)	7	164.78	-312.8	0.85	0.310
-0.0029 (0.0012)			6	163.06	-312.1	1.55	0.218

* nestlings mass at day 12, nestlings mass gain, nestling competition, start RTL, pre-lay rainfall, post-lay rainfall, nestling mass at day 12: mass gain

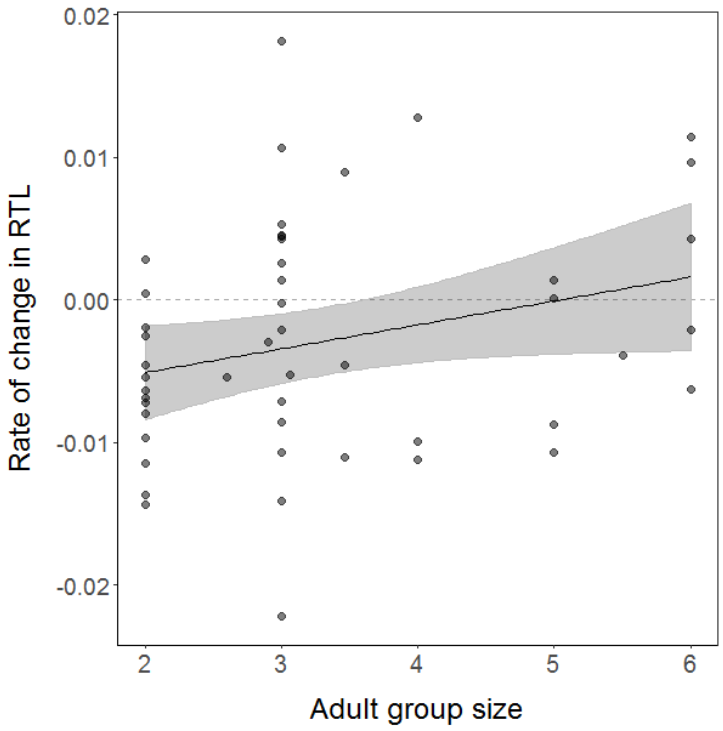


Figure 4. The effect of white-browed sparrow weaver adult group size on the rate of change in nestling relative telomere lengths (RTL) between day 4 and day 12: Rate of change in RTL is calculated as day 12 RTL – day 4 RTL corrected for regression to the mean using Verhulst's D, divided by the number of days between samples. Correcting for regression to the mean involves mean-centering RTL values, but for clarity zero has been readjusted to indicate no change in RTL. Thus, data below the dashed line indicate telomere loss. Points show raw data, and the grey ribbon shows 95% confidence intervals around the model predicted estimate (black line) from the second best supported model. Points show raw data and are darker where they overlap.

3.5 Discussion

Both the social and abiotic environment experienced in early life have the potential to impact the future fitness of offspring through their effects on resource availability and thus somatic maintenance. Here we used telomere dynamics to investigate this link. From previous work we already know that telomere dynamics in early life predict survival (Chapter 2), and so any apparent impacts of the environment on telomere attrition detected here could have profound consequences for offspring fitness. We found that rainfall in the month prior to egg laying had a positive effect on hatchling telomere length at day 4, though we detected no effect of rainfall on subsequent rates of telomere attrition. Whilst telomere attrition rates are often higher in early life than in adulthood (Hall *et al.* 2004; Baerlocher *et al.* 2007; Salomons *et al.* 2009; Monaghan *et al.* 2012; Fairlie *et al.* 2016), we found no evidence of telomere attrition on average during the early developmental period, and evidence of significant but only weak telomere attrition on average during the late developmental period. However, our analyses did reveal evidence consistent with beneficial effects of helpers on nestling telomere dynamics: nestlings reared in groups containing helpers (that increase the overall rate of food delivery to nestlings; Young et al. unpublished data) showed lower rates of telomere attrition than offspring reared by unassisted pairs. While offspring growth rate and the extent of nestling competition have previously been reported to impact early life telomere dynamics in birds (Geiger *et al.* 2012; Boonekamp *et al.* 2014a; Stier *et al.* 2015), we found no support for effects of either process in either the early or late developmental periods. To our knowledge this is the first study to report evidence suggestive of positive effects of social behaviour (in this case cooperative helping behaviour) on within-individual telomere dynamics; a relationship that could have hitherto unexplored downstream effects for offspring health and longevity.

Our analyses revealed that rainfall prior to egg-laying positively predicted hatchling telomere length. White-browed sparrow weavers are rain-dependent breeders in the Kalahari desert, with variation in rainfall being a strong positive predictor of both clutch initiation and annual reproductive success (Young et al. unpublished data).

Surprisingly, we found no support for a relationship between rainfall (either pre- or post-laying) and nestling telomere attrition rates in either the early or late developmental periods. It therefore seems unlikely that the apparent effect of pre-laying rainfall on hatchling telomere length was mediated by variation in food availability in the short period between hatching and day 4 (when the hatchling telomere length measures were taken). Rather, it seems more likely that poor resource availability for the breeding female in drier conditions led to deficits in egg composition and/or lower or more irregular incubation effort, which could each have conceivably impacted embryonic telomere attrition *in ovo* via constraints on the availability of key resource and/or the induction of stress responses (Haussmann *et al.* 2012; Tarry-Adkins & Ozanne 2014; Giordano *et al.* 2015). While variation in environmental conditions has been implicated as a likely cause of variation in offspring telomere length in a number of bird and mammal species (Voillemot *et al.* 2012; Becker *et al.* 2015; Watson *et al.* 2015; Bebbington *et al.* 2016; Fairlie *et al.* 2016), to our knowledge this is the first case where a specific abiotic environmental condition has been shown to predict variation in offspring telomere length, which we propose may arise due to variation in egg composition: Experimental evidence in chickens shows that higher levels of glucocorticoids in eggs leads to an over-representation of short telomeres in hatchlings (Haussmann et al 2011). Whilst direct evidence that natural variation in egg composition impacts telomere length is lacking, the findings of Mizutani et al. (2016) provide some support for this hypothesis: first-hatched black-tailed gull nestlings have longer telomeres than second-hatched chicks (Mizutani *et al.* 2016; see also Noguera *et al.* 2016) and lower maternal investment in eggs laid later in a clutch (e.g. the eggs of second-hatched chicks) is not uncommon (Royle *et al.* 1999; Saino *et al.* 2002). As with egg composition, there has been only limited research to date into the effects of incubation patterns on offspring telomere lengths. However, if drier incubation periods result in females undertaking longer periods of foraging, eggs may be subjected to sub-optimal temperatures for longer periods, which can increase both corticosterone levels (DuRant *et al.* 2010) and metabolic rates (DuRant *et al.* 2012) in hatchlings, both of

which have the potential to increase telomere attrition rates (Haussmann *et al.* 2012; Herborn *et al.* 2014).

While it is conceivable that social effects could have had an effect on hatchling telomere length through either (i) helper effects on maternal condition that may modify hormonal composition or nutritional content of eggs (Russell & Lummaa 2009; Haussmann *et al.* 2012; Paquet *et al.* 2013; Valencia *et al.* 2016) or (ii) pre-emptive maternal modification of egg constituents according to the presence of helpers (Russell *et al.* 2007a), we found no evidence to suggest that this was the case. It should be noted, however, that the data we used for this question were necessarily cross-sectional in nature, which can lead to reduced likelihood that effects will be detected. For example, inter-individual variation in hatchling telomere measures born of uncontrolled variation in genetic and epigenetic processes (Njajou *et al.* 2007; Olsson *et al.* 2011; e.g. parental age effects; Broer *et al.* 2013), or incidence of interstitial telomeric repeats (Delany *et al.* 2003), can obscure effects on true telomere length, particularly if those effects are subtle.

We found evidence that the presence of helpers (and to a lesser extent continuous variation in group size), positively predicted the within-individual rate of change in telomere length during the late developmental period. This finding is consistent with our prediction that helpers should alleviate resource allocation trade-offs in offspring (e.g. between growth and somatic maintenance), given that offspring reared in groups with more helpers are fed at significantly higher rates and with food items of comparable size (Young et al. unpublished data). That there was weaker support for a linear effect of continuous variation in group size than for a simple contrast between groups with and without helpers is suggestive of asymptotic benefits of helper numbers for offspring telomere dynamics. Indeed, such a pattern would be expected on the basis of resource intake rates alone, as the provisioning rates of individual helpers in this population significantly decline with increasing group size, leading to a steadily diminishing effect of increases in helper numbers on the overall rates at which offspring are provisioned (Young et al. unpublished data). Somatic maintenance deficits in offspring reared by unassisted pairs could arise not only from reductions in resource intake rates, but also

from reductions in the *predictability* of resource intake. Such a change in predictability could conceivably elevate nestling corticosterone levels (Reneerkens *et al.* 2002; Ulyan *et al.* 2006) and thereby exacerbate telomere attrition (Haussmann *et al.* 2012; Herborn *et al.* 2014). While we did not find comparable evidence in the early provisioning period for an association between the presence of helpers and offspring telomere attrition rates, our sample size of offspring raised by unassisted pairs in this period was very small, which is likely to have limited our power to detect such an effect (Figure 3). It is also possible that helper contributions become differentially important as development advances, as the resource demands of older, larger nestlings are likely to be higher.

While we found evidence suggesting that cooperative behaviour impacts telomere dynamics, we found no evidence that telomere attrition was affected by whether nestlings were reared as singletons or had within-brood competition from siblings. Experimental manipulations of brood size in both captive and free-living birds tend to show that experimentally increased levels of competition lead to faster rates of telomere attrition (Boonekamp et al., 2014; Costanzo et al., 2016; Reichert et al., 2014; Young et al., 2017; though see Voillemot et al 2012). The absence of an effect of natural variation in brood size in this study may therefore reflect adjustment of brood size by mothers (or indeed by environmentally-driven patterns of nestling mortality) such that larger broods are more likely to occur in relative resource-rich environments, negating such competition effects (Temme & Charnov 1987). Another possible explanation is that all but one of these experimental studies were conducted in species that have relatively large broods, which may result in much higher levels of nestling competition than are seen in white-browed sparrow weavers, which have small broods (modal brood size = 2; Harrison *et al.* 2013a) and low variation in brood size (1 - 3 nestlings). We also found no evidence of a correlation between offspring growth rates and telomere attrition rates. In the presence of natural variation in the availability of resources for investment in *either* trait this is perhaps not surprising, even if individuals do experience the commonly-invoked resource allocation trade-off between growth and somatic maintenance (Stearns 1989). Indeed, many studies have found no association between growth rate (or mass) and telomere length (or attrition), even where manipulations have

increased the costs of growth (Nettle *et al.* 2013; Meillère *et al.* 2015; Salmón *et al.* 2016; Stier *et al.* 2016). Where negative effects of mass or growth on telomeres have been found, it has only been in individuals facing more challenging conditions, for example in enlarged but not reduced broods (Jackdaws; Boonekamp *et al.* 2014a) or in harsh but not benign environmental conditions (Atlantic salmon, Salmo salar; McLennan *et al.* 2016). The absence of a *positive* among-individual correlation between growth rates and telomere maintenance is arguably interesting, as a simplistic resource allocation trade-off model would tend to predict that individuals with greater access to resources should invest more heavily in *both* traits (Bouwhuis). It has recently been suggested that the lack of such a correlation could reflect past selection for the developmental canalisation of one of the traits, given historically stronger associations between that trait and fitness (Bouwhuis). Whether this is the case in our species is currently unclear and it would seem premature to invoke such an explanation given the potential limitations in the power of our analyses arising from sample size constraints and/or extraneous sources of variability in our growth and telomere attrition estimates.

In summary, this study provides for the first time, to our knowledge, evidence suggestive of positive effects of social interactions on the within-individual telomere dynamics of offspring, which may have important downstream implications for their fitness. In addition we show that rainfall prior to egg laying is positively associated with hatchling telomere length, an effect that seems likely to have arisen via egg- or incubation-mediated maternal effects. Future studies might now profitably seek to test the causality of these relationships with social and abiotic environmental manipulations in the field, and, moreover, explore the potential downstream effects of these environmental drivers on later-life performance and ageing trajectories.

Chapter 4

Social dominance and rainfall predict adult telomere dynamics in a cooperatively breeding arid-zone bird

4.1 Abstract

Social dominance often carries the benefit of differentially high reproductive success, but downstream effects of dominance on health and lifespan remain poorly understood. Whilst investment in reproduction may give rise to reduced somatic maintenance and thus have negative downstream effects, other aspects of being dominant, such as improved access to resources, may compensate for this. Telomeres, which generally get shorter with age and exposure to oxidative stress, are increasingly used as biomarkers of ageing, yet the relationships between social dominance and telomere dynamics remain unexplored. Here we use a large data set of repeated measures of telomere length from a cooperatively breeding bird (the white-browed sparrow weaver, *Plocepasser mahali*), to investigate age-related changes in mean telomere length in adulthood and the extent to which these vary with dominance status and rainfall-related reproductive activity. First, we report that while population-level variation in telomere length with age is suggestive of age-related declines in mean telomere length, this pattern appears to have arisen solely from among-individual effects; we found no statistical support for an overall within-individual age-related decline in mean telomere length in adulthood despite extensive longitudinal sampling. Second, our analyses of the causes of variation in within-individual telomere attrition rates revealed more severe telomere attrition during years of high rainfall, but only during the breeding season. As rainfall is the key driver of reproduction-related activity during the breeding season of this arid-zone bird, these findings provide support for the core assumption of life-history theory that reproduction-related activities entail costs to somatic maintenance. Third, our analyses reveal that dominant birds have neither shorter telomeres nor faster telomere attrition rates than subordinates over the long-term, despite monopolising within-group reproduction. Our findings do, however, suggest that dominant birds may regulate telomere length in a manner that subordinates do not, with evidence suggestive of differential telomere elongation in dominants with short telomeres. Together, our findings provide evidence of a link between reproduction-related activities and telomere attrition rates, and the first evidence to our knowledge of associations between dominance status and within-individual telomere dynamics.

4.2 Introduction

In many animal societies, socially dominant individuals enjoy substantially higher reproductive success than subordinates, but the downstream effects of social dominance on organismal health, lifespan and ageing trajectories remain poorly understood. On the one hand, greater investment by dominants in reproduction or the defence of social rank could entail significant costs to their investment in somatic maintenance, manifesting as later-life deficits in health, lifespan and ageing trajectories (Creel *et al.* 1996; Creel 2001; Bell *et al.* 2012; Verhulst *et al.* 2014; Boonekamp *et al.* 2014b). On the other, dominants may enjoy differential access to the resources needed for somatic maintenance (Wrangham 1981; Gilmour 2005; Murray *et al.* 2006) and may be free of the socially-induced stress that subordinates can experience (Abbott *et al.* 2003; Young *et al.* 2006). In addition, where dominant individuals enjoy extreme fecundity and/or reduced extrinsic mortality risks, selection may favour disproportionate investment in somatic maintenance (Keller & Genoud 1997; Jemielity *et al.* 2005; as in some eusocial and cooperatively breeding vertebrates; Dammann & Burda 2006; Dammann *et al.* 2011). While advances in our understanding of the impact of dominance on life-histories therefore require that we now investigate the net effect of social dominance on patterns of somatic maintenance, few such studies have been conducted to date.

In social species, dominance status is typically a key determinant of reproductive rate, with socially dominant individuals of both sexes being the most reproductively active (Deutsch *et al.* 1990; Cowlishaw 1991; Young 2009; Cant *et al.* 2010; Harrison *et al.* 2013a). Trade-offs between investment in reproduction and somatic maintenance are generally expected to result in reproductive effort entailing costs to later-life components of fitness (Williams 1966; Reznick 1985; Nussey *et al.* 2006; Harshman & Zera 2007; Boonekamp *et al.* 2014b). Where socially dominant individuals do invest more in reproduction, theory would therefore predict that, all else being equal (though see below), they should consequently suffer greater deficits in later-life health or lifespan (Kirkwood, 1977; Kirkwood and Holliday, 1979). Specifically, the energetic demands of reproduction can elevate metabolic rate (Bryant 1988; Angilletta & Sears 2000; Nilsson

2002; Cram *et al.* 2015c). Under certain circumstances this can increase the production of reactive oxygen species (ROS), and subject to the extent of an individual's antioxidant defences, to exposure to oxidative damage to proteins, lipids and DNA (oxidative stress; Speakman & Garratt 2014; Cram *et al.* 2015b). While compelling evidence that reproduction elevates levels of oxidative stress (as predicted by life-history theory) remains scarce (Beaulieu *et al.* 2011; Costantini *et al.* 2014; Cram *et al.* 2015b), this may well be due to the difficulties involved in testing the idea, rather than that it does not occur (Cram *et al.* 2015b; Blount *et al.* 2016). In addition to potentially increasing oxidative stress, reproductive investment is thought to direct resources away from the immune system, potentially increasing susceptibility to infection and disease (Deerenberg *et al.* 1997; Cichon *et al.* 2001; Hanssen *et al.* 2005; Neggazi *et al.* 2016) that themselves have the potential to compromise somatic integrity further. That reproductive effort may frequently entail costs to somatic maintenance is further illustrated by research on the endocrine pathways that evolved to regulate such trade-offs. For example, the upregulation of circulating testosterone levels (e.g. in association with reproductive effort in males) is thought to compromise somatic maintenance via effects on metabolism, immunity and oxidative balance (Ling *et al.* 2002; Beehner *et al.* 2005; Alonso-Alvarez *et al.* 2007a; Mougeot *et al.* 2009; Muehlenbein & Watts 2010).

In addition to the costs associated with reproduction, dominant individuals may also incur differentially high levels of somatic damage, through investment in activities that enable them to maintain their social rank. In a number of social species, social dominance is associated with a higher frequency of agonistic encounters, which can elevate circulating levels of stress hormones (glucocorticoids), ultimately leaving dominant individuals exposed to potentially hazardous chronic stress (Creel *et al.* 1996; Creel 2001). This could compromise somatic maintenance via various pathways including elevated levels of oxidative damage (Costantini *et al.* 2011; Haussmann *et al.* 2012). Additionally, high ranking individuals may be at greater risk of parasite infection (Habig & Archie 2015; Smyth & Drea 2016) due to behavioural differences that lead to higher parasite encounter rates, or reduced resistance to parasite infection (Halvorsen 1986; Altizer *et al.* 2003; Li *et al.* 2007; Evans *et al.* 2015). Beyond differential exposure

to stress and parasites, a higher rate of exposure to agonistic encounters may also simply increase the risk of physical injury and any accompanying deficits in somatic maintenance that may arise from the physical damage itself. Such deficits may arise through reductions in foraging efficiency, and costs arising from tissue repair and fighting any associated infection (Martin *et al.* 2006; Archie *et al.* 2014). These non-reproductive costs also have the potential to exacerbate any impacts of reproductive effort via their effects on body condition (Garratt & Brooks 2012).

The effects of social dominance on somatic maintenance need not, however, be negative. The above costs may only be relevant under particular scenarios, such as during peaks of physical aggression in unstable dominance hierarchies, or when environmental conditions are poor (Monaghan & Metcalfe 1985; Sapolsky 2005; Beaulieu *et al.* 2014). The potential for negative downstream effects of dominance status may therefore vary among species, populations, or cohorts. In addition, elevated social status may carry benefits that partially offset, or more than compensate for, the costs that might otherwise be entailed in differential investment in reproductive effort or maintenance of status. High rank may itself offer differential access to resources (Barton & Whiten 1993; Murray *et al.* 2006) potentially alleviating trade-offs that might otherwise arise between investment in somatic maintenance and other fitness-related traits (such as reproductive effort and immunity). Not only may high rank provide better access to resources, it may also result in avoidance of the physiological stress and elevated risk of injury that subordination entails in some species (Abbott et al., 2003; Archie et al., 2012; Young et al., 2006). Finally, in eusocial insects and some highly cooperative vertebrates, dominant individuals enjoy both reduced extrinsic mortality risk and extreme fecundity that may increase substantially with age (Keller & Genoud 1997; Remolina & Hughes 2008; Schmidt *et al.* 2013). Under the disposable soma theory of aging (Kirkwood, 1977; Kirkwood and Holliday, 1979), both these traits could be predicted to favour differential investment in somatic maintenance. This theory provides compelling explanations for observations in some highly social species, of dominant individuals showing stronger resistance to oxidative stress (Haddad *et al.* 2007; Aamodt 2009) and longer captive

lifespans than their non-reproductive subordinates (Keller & Genoud 1997; Carey 2001; Jemielity *et al.* 2005; Dammann & Burda 2006; Dammann *et al.* 2011).

Investigation of telomere dynamics may shed light on the likely downstream effects of social dominance on later-life health and lifespan, as they are thought to provide a useful biomarker of patterns of somatic maintenance. Telomeres are nucleoprotein structures that cap and protect the ends of linear chromosomes (Blackburn & Szostak 1984; Blackburn 2000) and, in the absence of telomere maintenance mechanisms, get shorter with each cell replication (Olovnikov, 1973; Watson, 1972; Makarov et al., 1997; Sfeir et al., 2005). Rates of attrition are accelerated under conditions of oxidative stress (von Zglinicki 2002; Cattan et al., 2008), hastening apoptosis or cell senescence, which are thought to be triggered by short telomeres (Feldser and Greider, 2007; Hemann et al., 2001a; Herbig et al., 2004). The proliferative potential of cells (and thus the capacity for tissue renewal) therefore declines with cell replication and oxidative damage (Harley *et al.* 1990; Hao *et al.* 2005; Reichert *et al.* 2014a). Accumulation of senescent cells is thought to further contribute to senescence at the organismal level (Campisi and d'Adda di Fagagna, 2007; Coppé et al., 2010; Herbig et al., 2006; Kipling, 2001). Telomere dynamics may thus provide not only a useful biomarker of somatic maintenance, but also one plausible proximate mechanism via which deficits in somatic maintenance may ultimately compromise downstream health and lifespan (Kirkwood & Rose 1991; Kirkwood 2005). Indeed, telomere dynamics have been shown to predict health (Cawthon *et al.* 2003; Blackburn *et al.* 2015) and lifespan in a number of species (See Chapter 2).

Telomeres have been observed to get shorter with age in a number of taxa (Salomons *et al.* 2009; Barrett *et al.* 2013; Young *et al.* 2013; Beirne *et al.* 2014; Asghar *et al.* 2015) though in several cases no clear trend of attrition in adulthood has been found (Mizutani *et al.* 2013; Fairlie *et al.* 2016; Ujvari *et al.* 2016). A lack of attrition, and even apparent telomere extension have also been reported in cross-sectional studies (Haussmann *et al.* 2003; Hall *et al.* 2004). While reports of telomere extension in cross-sectional studies may be generated by selective disappearance of individuals with short

telomeres (Haussmann & Mauck 2008), there are also cases of apparent within-individual lengthening with age at particular life stages (Ujvari & Madsen 2009; Fairlie *et al.* 2016; Hatakeyama *et al.* 2016; Hoelzl *et al.* 2016b). In some cases this may be due to increased action by telomerase (Hatakeyama *et al.* 2016); an enzyme that is capable of maintaining telomere length by the *de novo* addition of bases to telomere (Greider & Blackburn 1985). Telomerase may have restorative impacts on cells beyond its effect on telomere length (Fu *et al.* 2000; Haendeler *et al.* 2003; Sharma *et al.* 2003; Ahmed *et al.* 2008), and telomere repair by telomerase may be inhibited by oxidative damage (Ahmed *et al.* 2008). Thus, investment in these mechanisms may extend somatic life span. Telomerase is downregulated in adult somatic cells of some species but not others (Haussmann *et al.* 2004, 2007) and, intriguingly, telomerase activity has been found to vary among classes within a species, for example, it is much more active in long-lived honey bee queens than in short-lived workers (Korandová & Frydrychová 2016).

Given the potential for telomere dynamics to reflect levels of somatic maintenance, there has been remarkably little investigation of the relationships between dominance status and telomere length, and not a single study has investigated relationships between social dominance and within-individual telomere attrition rates in non-human animals. In humans, however, low socio-economic status has been found to predict shorter telomeres and higher rates of telomere attrition (Cherkas et al., 2006; Shiels et al., 2011, though see Woo et al., 2009). With regard to the studies of dominance relationships with telomere length, queens of the black garden ant, *Lasius niger* (the only females to reproduce, and the dominant caste), have vastly longer lifespans than female workers, yet their telomere lengths are remarkably similar (Jemielity *et al.* 2007). However, the samples used to compare queen and worker telomere lengths were taken very early in life. Therefore, any differences in telomere dynamics that may subsequently have arisen due to life-history, behavioural, and consequent resource allocation differences between the castes may not have been captured at this time. Korandová and Frydrychová (2016) also found no statistical difference in telomere length between queens and workers of the eusocial honeybee, *Apis mellifera*, though they found much higher levels of telomerase in queens. In vertebrates, to our knowledge, there has been only one study

of the associations between social dominance and telomere length. Lewin et al (2015) found that telomere length of high-ranking spotted hyenas was greater than that of subordinates. However, like the studies of eusocial insects described above, these data were cross sectional and thus it is not clear whether rank-related differences in telomere length were due to differences in the rates of within-individual changes in telomere length with age, or other sources of among-individual variation, such as selective disappearance effects (van de Pol & Verhulst 2006; Haussmann & Mauck 2008). While few studies have tested effects of dominance *per se,* a number of studies have provided experimental (Kotrschal *et al.* 2007; Heidinger *et al.* 2012; Sudyka *et al.* 2014; Reichert *et al.* 2014b) and correlational evidence (Young *et al.* 2016) that high reproductive effort (which is commonly associated with social dominance; see above) is associated with increases in telomere attrition rate or shorter telomeres (Plot *et al.* 2012). Such relationships are expected, as reproductive effort can generate higher levels of oxidative damage (Beaulieu *et al.* 2011; Costantini *et al.* 2014; Cram *et al.* 2015b; c), which itself has been shown to increase telomere attrition rate (von Zglinicki 2002; Ballen *et al.* 2012; though see Boonekamp *et al.* 2017). However, effects of reproduction on telomere length may be transient (Heidinger *et al.* 2012), or may not exist at all if antioxidant levels are also elevated to combat somatic damage (Beaulieu *et al.* 2011; Sudyka *et al.* 2016).

Here we use extensive longitudinal within-individual sampling of telomere lengths in adulthood to investigate the effects of dominance status and reproductive effort on telomere dynamics in a wild cooperatively breeding bird, the white browed sparrow weaver, *Plocepasser mahali.* These birds live in year-round territorial groups of between 2 and 12 individuals, in which a single dominant pair monopolise reproduction within the group (~15% of offspring are sired by dominant males in other groups; Harrison *et al.* 2013a; b). Non-breeding subordinates assist with offspring care, weaving, sentinelling and territory defence (Lewis 1982; Walker *et al.* 2016). Reproductive activity can occur throughout a long 'breeding season' (the Southern summer; September to April inclusive) and is tightly linked to rainfall: rates of weaving, dawn song production by the dominant male (subordinate males do sing but invariably for

shorter periods than their dominant; York *et al.* 2016b), and clutch initiation by the dominant female all increase markedly in response to rain (Young *et al.* unpublished data). During the breeding season, dominant females can lay up to six clutches in succession, are the sole incubators, provision nestlings at markedly higher rates than all other classes of bird (Harrison *et al.* 2013a; Walker 2016), and experience stronger within-individual declines in antioxidant levels than all other classes (Cram *et al.* 2015c). All group members contribute to provisioning nestlings and there is evidence to suggest that subordinate helpers (i) increase the reproductive success of the dominant pair (Lewis 1981), and (ii) lighten the workloads of dominant females (Lewis 1982; Cram *et al.* 2015b) and allow them to initiate clutches more frequently (Young *et al.* unpublished data). Indeed, experimental work suggests that reproductive effort entails an oxidative stress cost in this species, and that these costs (for any one breeding attempt) are mitigated by workload sharing in larger groups (Cram *et al.* 2015b). While immigrant subordinates contribute far less to provisioning than natal subordinates, they also contribute to territory defence and sentinelling, potentially allowing greater provisioning effort by other group members (Young, unpublished data).

We conduct our analyses in two phases. First, we investigate the effects of dominance status and sex on telomere length and long-term within-individual telomere length dynamics. We utilise a within-individual centring approach (van de Pol & Verhulst 2006) to isolate the effects of within-individual changes in age from the potentially confounding effects of among-individual variation in age, which could, for example, arise from selective disappearance effects (Beirne et al., 2014; Beirne et al., 2016; van de Pol and Verhulst, 2006; Haussmann & Mauck 2008). Specifically, we predict no marked sex difference in telomere attrition rates (and hence length) as this species is principally monogamous (Clutton-Brock & Isvaran 2007; and such species tend to lack clear sex differences in lifespans and ageing trajectoies; Bonduriansky *et al.* 2008), without marked sexual size dimorphism (Barrett & Richardson 2011; Harrison *et al.* 2013a). That said, male biased attrition could be predicted given that males of this species are slightly larger than females (males are 5.5% heavier, and have a wing length 2.9% longer than females; Leitner et al., 2010), while, among dominants, female-biased telomere

attrition could be predicted given the greater investment of breeding females in egg laying, incubation and nestling provisioning (Harrison *et al.* 2013a; Walker *et al.* 2016). Regarding dominance, if the major driver of individual variation in telomere attrition rates is variation in the intensity of metabolic work, we would predict that dominant birds should show higher rates of telomere attrition than subordinates, given their substantially greater investment than subordinates in territorial defence (Young et al. unpublished data) and reproductive activities (Harrison et al. 2013a). The extent to which this manifests in nature, however, may also depend upon the extent to which dominants are of intrinsically higher 'quality' (which could be associated with longer telomeres and lower attrition rates).

Second, we utilise analyses of telomere length dynamics over shorter periods to investigate the extent to which the rates of within-individual change in telomere length in adults are impacted not only by sex and dominance status, but by the breeding season (versus the non-breeding season), rainfall patterns, and social group size. Specifically, we predict that greater telomere attrition may occur in the breeding season, given differential reproductive and oxidative stress costs entailed (Cram *et al.* 2015c), particularly when rainfall is high as rainfall triggers prolific reproduction (Lewis 1982) and/or when many breeding attempts are initiated. Predictions regarding group size are more complex: while our work to date suggests that workload sharing in larger groups may mitigate the oxidative stress costs of a given reproductive attempt (Cram *et al.* 2015b; leading one to predict reduced rates of telomere attrition in larger groups) such benefits may be offset by the fact that dominant females in larger groups lay new clutches more frequently (Young et al. unpublished data).

4.3 Methods

4.3.1 Study population and field methods

Data were collected from a population of 40 social groups of white-browed sparrow weavers in the semi-arid Kalahari desert at Tswalu Kalahari Reserve, South Africa (27°16'S, 22°25'E). All birds in the population were fitted with a unique combination of three colour rings and a numbered metal ring under SAFRING licence 1444. Sex was readily determined by beak colour, which is a sexually dimorphic trait in this subspecies (Leitner *et al.* 2010). Group composition and dominance status were recorded during weekly behavioural monitoring of each social group. The dominant pair in each social group was identified by dominance-related displacement, aggression, and territorial and reproduction-associated behaviours (for more detail see Harrison *et al.* 2013a; York *et al.* 2014). Group size was calculated as the average number of adult birds (over 6 months of age) belonging to the group over each breeding or non-breeding season (see Chapter 3 for detail on calculations of group membership windows); birds were considered to be group members if they frequently foraged and performed territorial displays with that group, and slept in the same trees. Breeding attempts that occurred during the breeding season were recorded (nest checks began between mid- September and mid-October depending on the year, and continued until mid-April), with nest checks carried out in each group at least every other day until eggs were discovered, then every day until clutch completion. Eggs were checked once mid-way through the incubation period but were otherwise left undisturbed. 15 days after clutch initiation daily nest checks were resumed to establish hatch dates. After hatching, nests were subsequently checked on the 4^{th}, 8^{th}, 12^{th} and 16^{th} day of life of the first-hatched nestling. If the clutch failed, standard nest checks were immediately re-established so that breeding attempts were not missed.

Birds were captured at night by flushing them from their individual roost chambers into a custom capture bag. A small blood sample (~25uL) was collected via brachial venepuncture using a 26G needle and a non-heparinized capillary tube, and was

immediately stored in absolute ethanol, where it was kept at ambient temperature until extraction. All samples were taken between September and November (start of the breeding season) and between March and May (end of the breeding season). The mean length of time between each bird's start and end of season samples for analyses of within-individual changes in telomere length over a season was 197 days over the breeding season (range 122 – 237 days, Standard deviation; SD = 33.3) and 198 days over the non-breeding period (range 142 - 248 days, SD = 25.3). We subsequently accounted for variation in this interval in our calculations of the rate of change in telomere length (see statistical analyses).

4.3.2 DNA extraction and measurement of telomere length by qPCR

Full methods are described in Chapter 1. Briefly, DNA was extracted from whole blood (and thus was predominantly from erythrocytes) using Gentra PureGene Genomic DNA Purification Kits (Qiagen). Quantity, quality, and integrity were assessed and any samples that failed were re-extracted, or discarded if further extractions also failed. DNA samples were stored at -20˚C until telomere analysis. We used quantitative PCR (qPCR) as described in Cawthon (2002) to quantify mean relative telomere length of whole blood (referred to here as 'RTL', but as 'telomere length' in the results and discussion). This gives a measure of telomere length relative to a non-variable copy-number control gene that controls for variation in DNA concentration. Control gene (for which we used glyceraldehyde-3-phosphate dehydrogenase; GAPDH) and telomere reactions were carried out on separate 96-well plates on a Stratagene Mx3000 instrument. Thermal cycles were set to 95˚C for 15 minutes, then 40 cycles of 95˚C for 15 seconds, 57˚C (telomere) or 60˚C (GAPDH) annealing for 30 seconds, and 73˚C extension for 30 seconds. GAPDH primers were specific to *P. mahali* (GAPDH-F 5′AAACCAGCCAAGTATGATGACAT–3′; GAPDH-R 5′-CCATCAGCAGCAGCCT TCA-3′; see Appendix A). Telomere primers were Tel1b: 5′-CGGTTTGTTTGGGTTT GGGTTTGGGTTTGGGTTTGGGTT-3′, and Tel2b: 5′-GGCTTGCCTTACCCTTA CCCTTACCCTTACCCTTACCCT-3. Both sets of primers were used at a concentration of 200nM in a 20uL reaction, alongside 10uL SybrGreen fluorescent dye

with low ROX (Agilent Technologies) and 5ng total DNA. Samples were run in triplicate, as was a between-plate calibration sample (pooled from 3 birds) which was included on every plate. Samples from the same individual were kept together wherever possible for the analysis of long-term changes in telomere length, and always on the same plate for the analysis of change in telomere length over the season. Samples were run on 27 plate pairs in total. Background corrected data were exported to LinRegPCR (Ruijter *et al.* 2009) where baseline fluorescence was corrected and a window of linearity set for each amplicon group and constant fluorescence thresholds were set within the windows of linearity for GAPDH (0.156) and telomere (0.161). RTL was calculated using well efficiencies following the equation in Pfaffl (2001), which gives the ratio of the quantity of telomeric DNA to control gene (T/S).

4.3.3 Statistical analyses

Analyses were carried out using an Information-Theoretic (I-T) framework in R 3.0.2 (R Core team 2013). For each of the questions below a global linear mixed effects model (lmer in lme4; Bates et al., 2014) was constructed including all terms of interest. As we have no *a priori* reason to believe any combination of these terms to be less plausible than others, we compared all nested models of each global model, but two-way interactions and quadratic terms were only included in models where the corresponding first order terms were present. Models were ranked by AICc using the dredge and subset functions in the MuMIn package (Barton 2016). Models within 6 AICc points of the top model were retained in the top model set unless they were more complex than nested models with a lower AICc, in which case they were dropped in the interests of parsimony, and adjusted Akaike weights of the remaining models were calculated (Richards *et al.* 2011). Models with a high Akaike weight have more support than those with a low weight. As we used this model nesting rule (Richards *et al.* 2011) we plotted results using effect size estimates from the top model (for terms within the top model; and the best model containing the term of interest for any terms not within the top model) rather than using model averaging. Unabridged top model sets (prior to implementation of the model nesting rule) are presented in Appendix E. All continuous

predictors were centred and scaled using the 'scale' function in the 'R' Base package, but were back-transformed for plotting. Prior to model comparison, in order to ascertain independence of predictors, correlations between all predictor variables were checked and variance inflation factors (VIF) of each global model were checked to assess multicolliearity: all VIFs were below 3 ("car" package; Fox & Weisberg 2011). Model residuals of global models were assessed to confirm compliance with model assumptions. Cook's distances were examined to check for points of high influence using the package influence.ME (Nieuwenhuis et al 2012).

Long-term telomere dynamics

In total, our dataset consisted of 299 RTL measures from 87 adult birds of known age (54 subordinates, 26 dominants and 7 birds with samples for both classes). All birds included were adults (older than 6 months) at the time of sampling. For each bird included in the analysis we had between 2 and 8 RTL measures (median number of samples per bird = 3). The distribution of RTL was heavily right skewed so in order to normalise model residuals we used log RTL as our response for all models.

We analysed long-term telomere dynamics in two phases i) population level patterns (without age partitioning), and ii) partitioning age to isolate within-individual dynamics. These models are described in more detail below, but all models included the following random effects: qPCR plate (to account for between plate variation in telomere measures), social group (to account for heterogeneity in territory quality and genetic variation), period, and bird ID. The 'period' term used in this chapter differed subtly from the season term used in previous chapters in that it included information on (i) which breeding season the samples were collected in (breeding seasons are approximately 7 months long and span two calendar years eg: '2013-14'), and (ii) whether the sample was taken towards the beginning or end of that breeding season (eg '2013-14_start'). We initially included season as a random effect without including within it information on whether it was the start or end of the breeding season in question (the way that season is specified in other chapters), but this approach yielded poor model fit, and model comparisons produced results that qualitatively varied

depending on whether or not data from the end of 2014-15 were included (see Appendix D).

i) Population level patterns (without age partitioning)

We constructed a global model that included dominance, sex, and age as fixed effect predictors alongside the random effects outlined above. As change in telomere length with age has been found in other species to be non-linear (e.g. Heidinger *et al.* 2012), we also included the quadratic effect of age. Age and dominance were strongly associated, but the variance inflation factors (VIF) for these variables were acceptable (highest VIF = 2.04), thus we deemed it appropriate to include both.

ii) Partitioning age to isolate within-individual dynamics

In order to investigate within-individual changes in RTL whilst controlling for among-individual variation we partitioned age into the mean age of all samples for each bird (henceforth 'mean age'), and the age at which a given sample was collected minus the bird's mean age (henceforth 'delta age'; van de Pol & Wright 2009). For the 7 birds with measures taken at both subordinate and dominant life stages, mean and delta age were calculated separately within each life stage.

We constructed a global model that included dominance, sex, mean age and delta age and all two way interactions between these terms as fixed effect predictors, alongside the random effects outlined above. In addition, we included the quadratic effects of delta age and mean age. Although mean age and dominance status were statistically associated, the variance inflation factors (VIF) for all variables were acceptable (highest VIF = 2.21).

Causes of short-term variation in within-individual telomere attrition rates

To investigate the effects on telomere attrition rates of variables that change frequently within an individual's lifetime such as rainfall, group size and whether it is the breeding or non-breeding season, we utilised our collection of samples taken at the start and end of four successive breeding seasons to calculate within-individual changes in RTL over

individual breeding seasons (approximately 7 months in duration) and intervening non-breeding seasons (approximately 5 months in duration). The calculation of the within-individual change in RTL over each season were corrected for regression to the mean using Verhulst's D (Verhulst *et al.* 2013), and, as this method involves mean centring values for the initial and follow-up RTL measures, we adjusted all corrected measures by a single correction offset to ensure that a zero D value reflected no change in telomere length (by adding the difference between the mean baseline RTL and mean follow-up RTL to all values of D). As the length of time elapsed between successive samples varied (range 4.1 to 8.3 months, median = 6.9 months, mean = 6.6 months, SD = 1.02), we used rate of change in RTL by dividing all values of D by the time elapsed between samples (in months).

To test for effects of the social environment on rates of change in RTL we included dominance status ('dominant' for either the dominant male of female or 'subordinate' for all other birds), log adult group size and the interaction between the two as fixed effect predictors. As the effect of dominance may vary by sex, or with season (whether it was the breeding or non-breeding season) we also included these interactions (fitting season as a two-level factor). As rainfall (which is positively associated both with food availability and stimulates reproductive activity of both dominant and subordinate birds over the breeding period; see intro) tends to be much higher in the breeding season (the birds are rain-dependent breeders), in order to avoid confounding the season variable with the rainfall varaible we fitted the term 'annual rainfall', calculated as the total rainfall over the year preceding the date on which the second RTL sample (i.e. from the end of the window of interest) was collected, rather than total rainfall during the period between the two samples. We allowed for season-dependent effects of annual rainfall (season : annual rainfall interaction) as high-rainfall breeding seasons may entail greater costs to somatic maintenance due to higher reproduction-related work rates, while high-rainfall non-breeding seasons may not. In addition we included the interaction between dominance status and annual rainfall as the costs of high-rainfall may depend on dominance status. In a number of studies the rate of change in RTL over a given period has been found to be higher in individuals with initially longer telomeres

(Verhulst *et al.* 2013). We therefore included RTL of the first sample of each pair as an additional fixed effect predictor along with its interaction with social status, as dominant and subordinate birds may differ in their regulation of telomere length. Finally, in order to control for potential effects of variation in body condition, we included mass at the start of the focal season and its interaction with the focal bird's within-individual change in mass over the season. We controlled for qPCR plate, bird identity, social group and season (eg '2013-14' refers to the breeding season that fell over the end of 2013 and start of 2014, and also the non-breeding season that followed in 2014) in the random effects of all models.

As we did not know the age of all birds in this study we initially used a reduced dataset containing only the subset of samples for which age was known to within 3 months, which consisted of 166 rate estimates for 82 birds (69 rate estimates from 28 birds as dominants, and 97 rate estimates from 60 birds as subordinates). However, as age was not present in the Δ6 AICc top model set using this approach (see Appendix E) we then expanded the data set to birds of unknown age and removed the fixed effect of age from the model, thereby increasing our sample size to 187 rate estimates for 101 birds (87 rate estimates for 45 birds as dominants, and 100 rate estimates from 63 birds as subordinates). 17 of these unknown-age birds were dominant and 3 were subordinate birds first ringed as adults. We did not know ages of these birds as they were already present as adults when the project began in 2007 (n = 14 birds), or arrived as adult immigrants from outside the study site (n = 6 birds). These birds were only included in analyses once they had definitely reached adulthood: fledgling characteristics become less evident at the age of three months, so unknown age subordinates were only termed 'adult' for this analysis after being in the population for three months. The full data contained six outliers (points that were greater than 1.5 times the interquartile range beyond the upper and lower quartiles). We checked whether the removal of these points changed the results, and found that the results remained qualitatively similar so retained all outliers in the final model comparison.

Finally, as we only collect information on breeding attempts during the breeding season (when the vast majority of breeding occurs), in order to test the effect of the number of breeding attempts that each bird was present for on its rate of change in RTL during that breeding season we conducted additional model comparisons restricting our data set only to that for breeding seasons. This dropped the sample size to 60 rate estimates from 43 birds as dominants and 60 rate estimates from 53 birds as subordinates. For birds that moved between groups during the season, we calculated the number of breeding attempts that they were present for in each group and only included breeding attempts that at least partially occurred between sampling dates. In addition to the number of breeding attempts that each bird was present for over its sampling period, we included the 3-way interaction with this variable and both dominance status and sex, as helper effort varies with sex (subordinate females provisioning young at significantly higher rates than males; Young et al. unpublished data), and the telomeres of dominant females are expected to be differentially impacted by breeding attempts given their differential reproductive investment (see above). We also included the interaction between dominance status and annual rainfall as this was present in the Δ6 AICc top model set from the full model including both breeding and non-breeding seasons. Random terms were as described above.

4.4 Results

4.4.1. Long-term telomere dynamics

Population level patterns (without age partitioning)

Analyses at the population level (prior to age partitioning and therefore semi-cross sectional in nature) revealed evidence of a negative effect of variation in age (Table 1; Figure 1a) on RTL. However, the evidence for this effect was not strong, as the age effect was not present in the second-best-supported model, just 0.32 AICc points below the top model. In addition we found (stronger) evidence that males had shorter telomeres than females (Table 1). There was no evidence of an effect of dominance status on mean RTL nor for any interactions between mean or delta age and either sex or dominance status.

Table 1. Model selection table of predictors of relative telomere length of adult white-browed sparrow weavers. Δ6 AICc top model set after implementation of the model nesting rule is presented (Richards *et al.* 2011). Age was scaled and centred. Estimates are given for each variable; for sex, the estimate is given for males relative to females, and standard errors are reported in parentheses. AW = adjusted Akaike weight. * Terms that appeared in the global model but are not present in the top model set.

Intercept	Sex (M)	Age	df	logLik	AICc	Δ AICc	AW
-0.0228 (0.051)	-0.090 (0.034)	-0.0413 (0.021)	8	104.088	-191.7	0.00	0.477
-0.0319 (0.046)	-0.077 (0.035)		7	102.873	-191.4	0.32	0.407
-0.0726 (0.043)			6	100.568	-188.8	2.83	0.116

* Dominance status, Age^2

Partitioning age to isolate within-individual dynamics

Following age partitioning, there was no evidence of an effect of within-individual change in age (Δ age) on telomere length in birds of known age (Table 2; Figure 1b). However, we did find evidence of a negative effect of mean age (Table 2; Figure 1c), suggestive of the selective disappearance from our data set of birds with long telomeres, selective appearance of birds with short telomeres or some form of cohort effect. The evidence for an effect of mean age was not strong, however, as the mean age effect was not present in the second-best-supported model, just 0.30 AICc points below the top model.

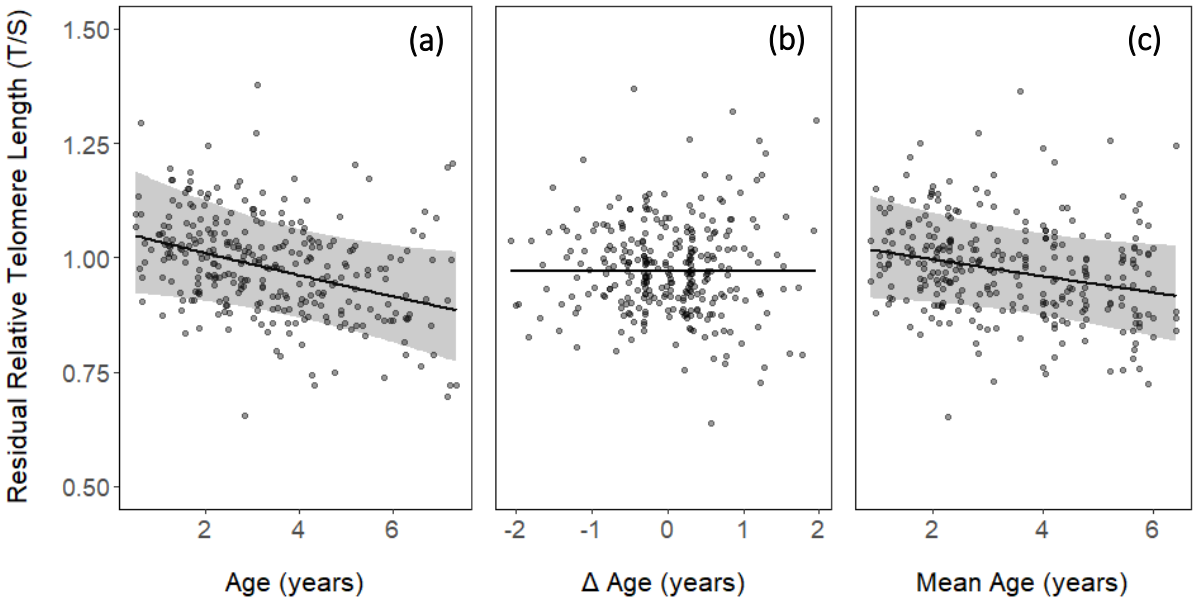


Figure 1: Changes in white-browed sparrow weaver adult relative telomere length with (**a**) increasing age for the entire population (including selective appearance and disappearance effects), (**b**) within-individual changes in age, and (**c**) among-individual changes in the mean age at which individuals were sampled, suggestive of a selective disappearance or selective appearance effect. Solid line shows the model estimate from the best supported model in each case and is plotted for females as sex was also present in the top model. Points are adjusted to control for the random effects present in the model (season, social group, qPCR plate and bird ID), and for sex. Shaded areas show 95% confidence intervals where the focal variable was present in the best supported model. The y axis has been back-transformed from log (RTL). Relative telomere length is calculated as a ratio of the quantity of telomeric DNA (T) to reference gene (S).

Table 2. Model selection table of predictors of log RTL, using age partitioning. Δ6 AICc top model set after implementation of the model nesting rule is presented (Richards *et al.* 2011). All continuous variables were centred and scaled. Effect size estimates are given for each term; for sex, the estimate is given for males relative to females. Standard errors are given in parentheses. * Terms that appeared in the global model but are not present in the top model set.

Intercept	Sex (M)	mean age	df	logLik	AICc	Δ AICc	Adjusted weight
-0.029 (0.047	-0.086 (0.034)	-0.028 (0.017)	8	104.079	-191.7	0.00	0.475
-0.032 (0.046)	-0.077 (0.035)		7	102.873	-191.4	0.30	0.409
-0.073 (0.043)			6	100.568	-188.8	2.81	0.116

* Dominance status, Δ age, Δ age: Dominance status, mean age: Dominance status, Sex: Dominance status, Δ age: mean age, Δ age: Sex, Δ age^2

4.4.2. Causes of short-term variation in within-individual telomere attrition rates

Modelling the causes of variation in the within-individual rates of change in telomere length over the breeding and non-breeding seasons revealed strong support for an interaction between annual rainfall and whether the focal season was the breeding or non-breeding season (Table 3; Figure 2). Inspection of the effect sizes and confidence intervals (Table 3) reveals that during the breeding season there was strong evidence of a negative effect of annual rainfall on the rate of within-individual change in telomere length (Figures 3a and 3b), while the same was not true for the non-breeding season (Figures 3c and 3d). There was also evidence of an interaction between annual rainfall and dominance status, in which annual rainfall had a more negative effect in subordinates (Figures 3b and 3d) than dominants (Figures 3a and 3c), though this

interaction was only present in the top model (Table 3; Figure 2) and not the second-best-supported model (1.14 AICc points below).

We also found strong evidence of an interaction between telomere length at the start of the season and dominance status; while dominant birds with longer telomeres at the start of the season lost more telomere length over the course of the season, no such relationship was apparent among subordinates (Table 3; Figure 3). We found no evidence that body mass, change in body mass over the season, social group size, or sex, had any effect on the within-individual rate of change in telomere length. Removal of outliers (see methods for details) did not alter the term composition of the top model.

Table 3: Model selection table showing predictors of within-individual change in relative telomere length over the breeding and non-breeding seasons for adult white-browed sparrow weavers. Δ6 AICc top model set after implementation of the model nesting rule is presented (Richards *et al.* 2011), along with the null model (not present in the top model set) highlighted in blue. All continuous variables were scaled and centred. Effect size estimates are given for each term, with standard errors in parentheses. For dominance status and season, and interactions containing these terms, estimates are given for subordinate birds (Sub) relative to dominants and for the non-breeding season (N-B) relative to the breeding season. Int: intercept, AW: adjusted Akaike weight. * Variables that were in the global model but do not appear in the top model set

Int	Dominance Status (Sub)	Season (N-B)	start RTL	Annual rain	Status (Sub): start RTL	Status (Sub): annual rain	Season (N-B): annual rain	df	logLik	AICc	Δ AICc	AW
0.006 (0.003)	-0.006 (0.003)	0.000 (0.004)	-0.008 (0.002)	-0.011 (0.004)	0.011 (0.003)	-0.006 (0.003)	0.015 (0.005)	13	451.06	-874.01	0.00	0.606
0.006 (0.004)	-0.006 (0.004)	0.002 (0.004)	-0.008 (0.002)	-0.015 (0.003)	0.010 (0.003)		0.017 (0.005)	12	449.33	-872.86	1.14	0.342
0.003								6	431.80	-851.1	22.87	NA

* Sex, log Group size, Mass, Δ Mass, Status: Sex, Status: Group size, Mass: Δ Mass

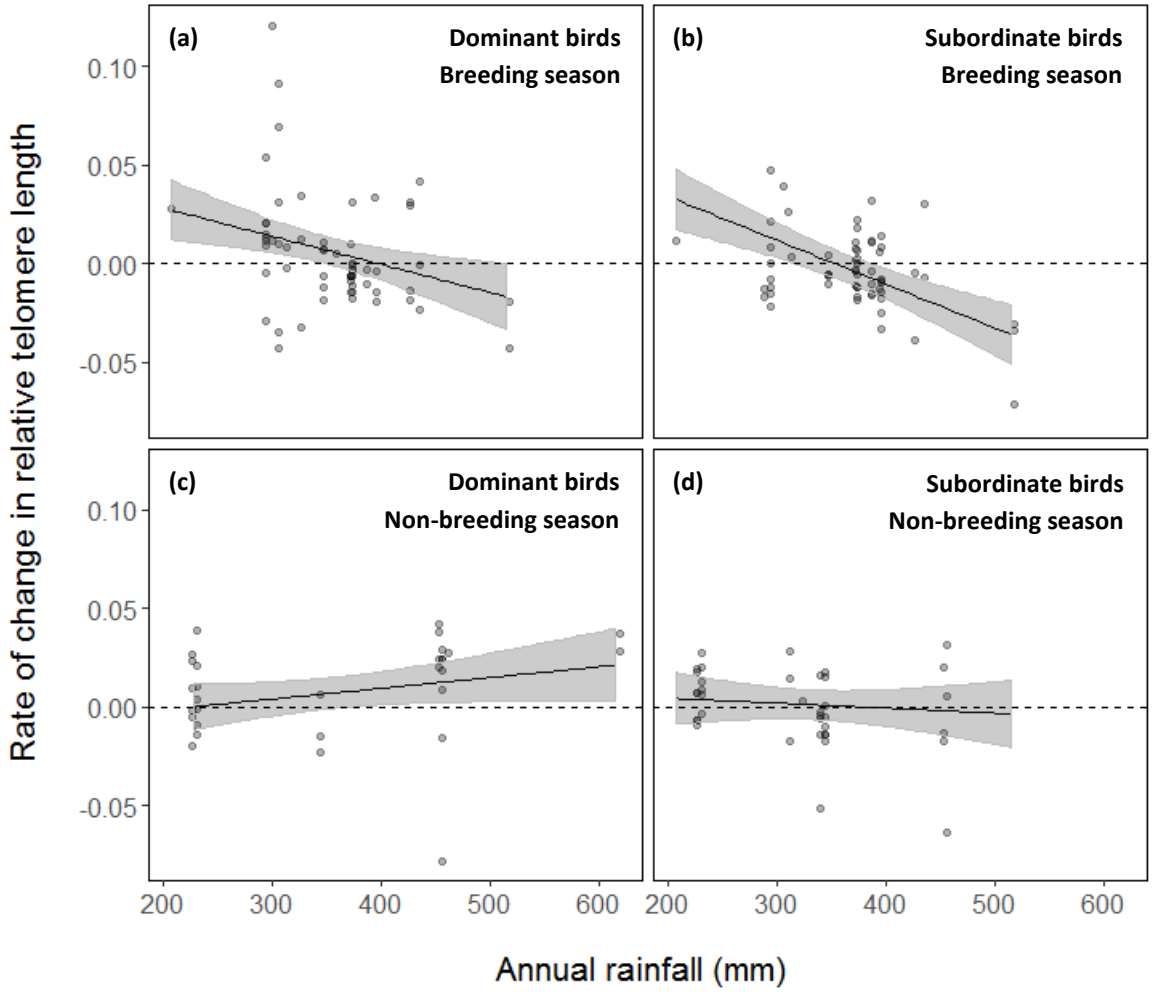


Figure 2. The effect of annual rainfall (mm) on the within-individual rate of change in RTL per month in adult white-browed sparrow weavers for **(a)** dominant birds in the breeding season, **(b)** subordinate birds in the breeding season, **(c)** dominant birds in the non-breeding season, and **(d)** subordinate birds in the non-breeding season. Plots reflect the interactions between dominance status and annual rainfall, and between season and annual rainfall, both present in the top model in Table 3. Solid lines show the estimates from the best supported model for the mean value of initial RTL. Grey ribbons show 95% confidence intervals around the estimate. Points are raw data and are partially opaque to show overlapping points. As change in RTL was corrected for regression to the mean using Verhulst's D, which renders 0 equal to the mean change in telomere length, we corrected the position of 0 before calculating the rate of change in RTL for analysis. As such, the dashed line in all panels shows the point of no change in telomere length over time; points below the line show decreases in telomere length estimates over time and points above the line show increases in telomere length estimates over time.

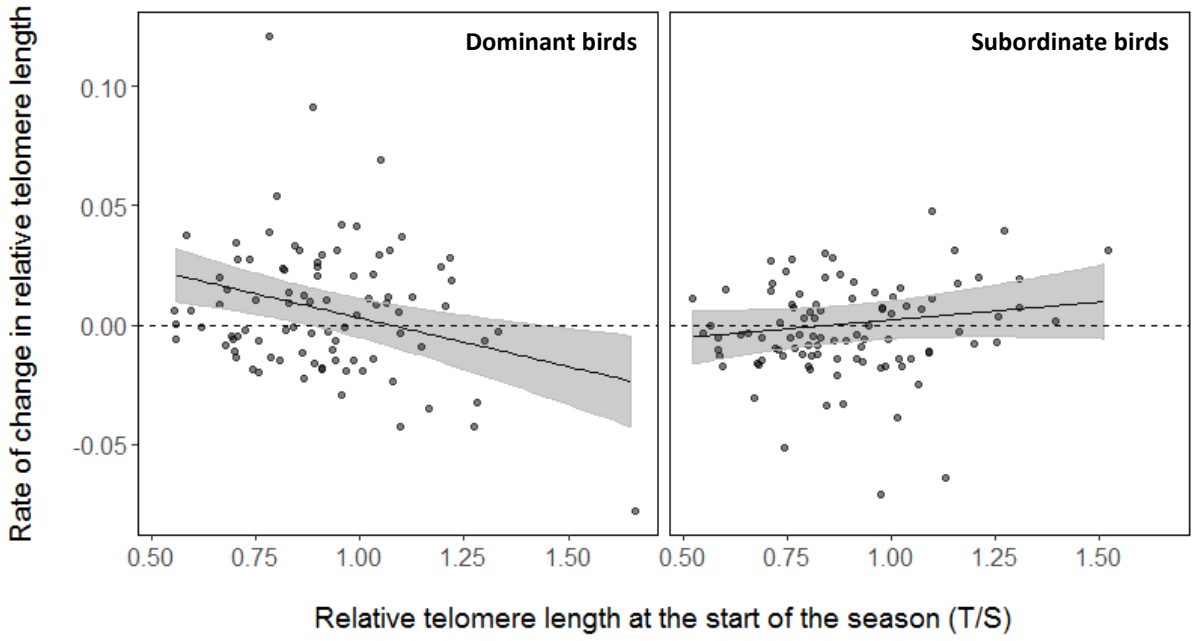


Figure 3. Model predicted estimates of the effect of telomere length (RTL) at the start of the season on the rate of change in RTL during that season, for dominant and subordinate adult white-browed sparrow weavers. Solid line shows the estimate from the top model for the non-breeding period and for the mean value of annual rainfall. Grey ribbons show 95% confidence intervals around the estimate. Points are raw data and are partially opaque to show overlap. RTL was calculated as a ratio of the quantity of Telomeric DNA (T) to reference gene (S). As change in RTL was corrected for regression to the mean using Verhulst's D, which renders 0 equal to the mean change in telomere length, we corrected the position of 0 before calculating the rate of change in RTL for analysis. As such, the dashed line in all panels shows the point of no change in telomere length over time; points below the line show decreases in telomere length estimates over time and points above the line show increases in telomere length estimates over time.

When we restricted the data set to include only the breeding season in order to test for effects of breeding attempt number, the top model set remained qualitatively similar, with the exception that the interaction between dominance status and rainfall dropped out. There remained strong support for a negative effect of annual rainfall on the rate of change in telomere length (Table 4). The number of breeding attempts the focal individual was present for during the breeding season in question did not predict rate of change in telomere length, either on its own, or in interactions with dominance status, sex, or group size.

Table 4: Model selection table showing predictors of change in relative telomere length of adult white-browed sparrow weavers over the breeding period. Δ6 AICc top model set after implementation of the model nesting rule is presented (Richards *et al.* 2011), along with the null model (not present in the top model set), highlighted in blue. All continuous variables were scaled and centred. Effect size estimates are given for each term, with standard errors in parentheses. For dominance status, and the interaction containing this term, estimates and standard errors are given for subordinates (Sub) relative to dominants. Int = intercept, AW = adjusted Akaike weight. * Terms that were present in the global model but are not present in the top model set

Int	Status (Sub)	start RTL	Annual rainfall	Status (Sub): start RTL	df	logLik	AICc	Δ AICc	AW
0.0046 (0.0037)	-0.0059 (0.0041)	-0.0059 (0.0031)	-0.0098 (0.0025)	0.0105 (0.0041)	10	285.85	-549.7	0.00	0.476
0.0043 (0.0030)	-0.0068 (0.0042)		-0.0093 (0.0021)		8	282.88	-548.5	1.22	0.259
0.0014 (0.0029)			-0.0100 (0.0025)		7	281.72	-548.4	1.23	0.257
0.003					6	275.61	-538.5	11.21	NA

* Number of breeding attempts (BA), Sex, log Group size, Status : Rain, Status : Sex, Status : BA, Sex : BA, BA : log Group size, Status : Sex : BA

4.5 Discussion

While dominance status is known to markedly impact reproductive success in many animal societies, the possibility that it also has implications for patterns of somatic maintenance, due in part to trade-offs with reproductive effort, remains largely unexplored. Here we investigate associations between dominance status and patterns of somatic maintenance using the telomere dynamics of white-browed sparrow weavers, a species of cooperatively breeding bird in which the dominant pair in each group reproduce and their subordinate non-breeders help. First, despite a large data set containing extensive within-individual longitudinal sampling of telomere length over time, we found no evidence of net within-individual telomere attrition with increasing age in adult birds, and no evidence of a difference in mean telomere length between the dominance classes. However, our findings do reveal evidence of a negative impact of elevated reproduction-related effort on the within-individual telomere dynamics of birds of both dominance classes: rainfall (which is the major environmental driver of reproductive activity in the breeding season for this arid-zone bird) negatively predicted the rate of change in telomere length over the course of the breeding season, but not the non-breeding season. Our results also suggest that despite monopolising reproduction, dominant birds may better maintain their telomeres than subordinates, as (i) elevated annual rainfall had a more negative effect on the within-individual rate of change in telomere length in subordinate birds than dominants, and (ii) while telomere attrition was unrelated to telomere length in subordinates, our findings suggest that dominant birds with short telomeres may experience increases in mean telomere length. Our findings therefore provide evidence that both reproductive effort and dominance status predict within-individual telomere dynamics in the wild.

A number of longitudinal studies have found within-individual declines in telomere length with age in adults (Barrett *et al.* 2013; Young *et al.* 2013; Beirne *et al.* 2014; Asghar *et al.* 2015). However, though our population-level data suggested that older individuals had shorter telomeres, we found no evidence of net within-individual decreases in telomere length over time (Figures 1a and 1b). Whilst a lack of age-related decline in

telomere length is not uncommon in cross-sectional studies, particularly in long-lived species (Haussmann *et al.* 2003; Hall *et al.* 2004; Foote *et al.* 2011; Le Vaillant *et al.* 2015; Lewin *et al.* 2015), it is possible in this type of study that within-individual declines are obscured by the selective disappearance of individuals with short telomeres with advancing age and/or by considerable among-individual variation. Evidence of a lack of *within-individual* declines in telomere length over time is decidedly rarer, yet there are a few cases that call into question the universality of progressive systematic declines in telomere length with age. Fairlie et al (2016) found periods of within-individual increase in Soay sheep leukocyte telomere length in addition to periods of attrition, and Hoelzl et al (2016b) report telomere length increases in buccal mucosa cells of older dormice, following telomere length declines in early life. Finally, Ujvari & Madsen (2009) found within-individual increases in telomere length in all hatchling water pythons for which they had these measures (n = 8 snakes), though they consistently found within-individual declines in telomere length in adulthood. Our analyses on a finer-temporal scale, focussing on within-individual telomere dynamics across breeding or non-breeding seasons provided a potential explanation for these findings, with dynamics being characterised by attrition in some contexts and apparent increases in others. We did not, however, find any evidence of the specific early- or late-life increases found in water pythons (Ujvari & Madsen 2009) and dormice respectively (Hoelzl *et al.* 2016b), as we found no evidence of an effect of age on the within-individual rate of change in telomere length in adults (though it is certainly possible that sample size limitations in the oldest age classes precluded the detection of terminal declines, or indeed increases, in telomere length [Salomons *et al.* 2009a]). We discuss the plausibility and implications of apparent increases in telomere length below.

Though we found no net within-individual decline in telomere length with age, our results do suggest that birds whose samples were, on average, taken at an older age (i.e. those with a higher mean age value), had shorter telomeres whenever they were sampled (Figure 1c). Given that higher within-individual telomere attrition rates predict reduced survival during the first year of life in this species (Chapter 2), it seems unlikely that the negative relationship apparent here in adulthood between mean age and telomere

length is due to a *causal* negative effect of telomere length on survival. However, other mechanisms could conceivably have led to the selective disappearance of individuals with longer telomeres from our population. For example, if adults of higher quality differentially engage in high risk / high return activities such as attempting to disperse, challenging for social dominance or investing heavily in reproduction, such a mechanism could leave birds with longer telomeres exposed to an increased mortality risk. Alternatively, while dispersal beyond the bounds of our study population is probably uncommon (Harrison et al 2014) if individuals with longer telomeres were more likely to engage in long distance dispersal this too could explain the apparent selective disappearance of individuals with long telomeres in the absence of an association between telomere length and mortality in adulthood. The evidence, from our study, that an apparent population-level decline in mean telomere length with age can arise in the absence of within-individual declines in telomere length with age highlights the need for extreme caution when drawing inference from purely cross-sectional studies.

Unlike mammals, where males have generally been found to have shorter telomeres than females, there is no clear pattern of sex differences in telomere length in birds (Barrett & Richardson 2011). Here, we found evidence that adult females had longer mean telomere lengths than adult males, in contrast to our previous findings in nestlings where, if anything, the opposite was true (Chapter 3). As we found no evidence of a sex difference in the within-individual rate of change in telomere length in the nestling phase (Chapter 3) or in adulthood (here), it seems likely that the sex difference in mean telomere length in adulthood arises during maturation (i.e. between the nestling-stage analyses conducted in Chapter 3 and the adulthood analyses conducted here). For example, if the slight sexual size dimorphism in this species, with males being slightly larger than females (males are 5.5% heavier, and have a wing length 2.9% longer than females; Leitner et al., 2010), arises in part following the nestling stage it is possible that the associated greater incidence of cellular replication in males than females (in order to generate their larger size) could leave mean telomere length in adulthood slightly shorter in males than females. It is also possible that the sex difference arises in

association with reproductive maturation, from transient impacts of maturation-associated increases in circulating sex steroid concentrations on sex-specific patterns of somatic maintenance (e.g. negative effects of androgens and positive effects of oestrogens; Sugioka *et al.* 1987; Kyo *et al.* 1999; Ling *et al.* 2002; Alonso-Alvarez *et al.* 2007a)

We found that sparrow weavers experienced greater telomere attrition during years with higher rainfall, but only during the breeding season (Figure 2). Rainfall is the major environmental driver of reproduction in this arid-zone bird (Young, unpublished data) due to positive effects on food availability through marked impacts on vegetation and associated insect communities. As such, marked behavioural changes are noted in birds of all classes following high rainfall during the breeding season, including an increased probability of clutch initiation by the dominant female (and subsequent contributions to chick-rearing by all classes), increases in dawn song production by the dominant male and to a lesser extent subordinate males, increases in investment by subordinates of both sexes in extra-territorial prospecting forays for breeding and dispersal opportunities, and increases in weaving activity by birds of all classes (Young et al. unpublished data). Our finding that increased telomere attrition is associated with high rainfall is therefore consistent with the expectation that investment in such 'reproduction-related' activities entails a cost to somatic maintenance. To our knowledge, just one other study to date has found evidence that natural (un-manipulated) reproductive effort appears to entail negative effects on within-individual telomere attrition rates in the wild: Common tern (*Sterna hirundo*) parents that lost their brood at an early stage had less telomere attrition over the following year than those that raised an intermediate number of chicks, though birds that raised the largest number of offspring (and might therefore have been of higher phenotypic quality) also showed reduced telomere attrition (Bauch *et al.* 2013). Associations between phenotypic quality and breeding effort may partially explain why we found no effect specifically of the number of breeding attempts on telomere attrition. In addition, rainfall is likely to be a stronger aggregate predictor of reproductive activity in sparrow weavers than the number of breeding attempts *per se*, in part because groups conducting

large numbers of breeding attempts are likely to have done so because many quickly failed (also see Sudyka et al 2016). There have been few studies of the effect of reproductive investment on within-individual telomere dynamics in adults, particularly in free-living animals, but our rainfall results concur with the general finding that higher reproductive effort is associated with greater telomere attrition (Bauch et al., 2013; Heidinger et al., 2012; Kotrschal et al., 2007; Reichert et al., 2014; Sudyka et al., 2014, though see Beaulieu et al., 2011; Sudyka et al., 2016).

Despite their markedly higher rates of reproduction, dominant birds appear to suffer no net cost to somatic maintenance in association with their dominance status, as they did not, on average, have either shorter telomeres than subordinates or show evidence of higher overall telomere attrition rates (Figure 2). The only other study to our knowledge that has investigated the effect of social dominance on telomere length in a vertebrate reported that socially dominant hyenas (*Crocuta crocuta*) had *longer* telomeres than less dominant individuals (Lewin *et al.* 2015). However, when and how these differences arose is not known as the study was cross-sectional. Cross-sectional studies have also been conducted on social insects, revealing no rank-related differences in telomere lengths of adult queens and female workers in either black garden ants (*Lasius niger,* Jemielity *et al.* 2007) or honey bees (*Apis melifera, Korandová* & Frydrychová 2016), suggesting that the extremely high rates of reproduction in queens (and the associated challenges that might be predicted regarding concurrent investment in somatic maintenance) are not pre-empted by starting with longer telomeres. Because cross-sectional studies do not give insight into the means by which differences between classes may arise (or not), studies of within-individual rates of change in telomere length are likely to be much more informative. When investigating the impacts of dominance status on long-term telomere dynamics (Tables 1 and 2), we found that the lack of an apparent dominance-related difference in mean telomere length can be attributed to a lack of an apparent dominance-related difference in the within-individual rate of telomere attrition *over the long term* (Tables 1 and 2). However, two lines of evidence suggest that dominant and subordinate birds do indeed differ in their telomere dynamics *over the short-term* (Table 3), which we will address in turn.

First, we found some limited evidence of an interaction between dominance status and annual rainfall, which suggests that the relationship between rainfall and telomere attrition (i.e. greater telomere attrition in higher rainfall years) is steeper among subordinates than dominants despite the latter monopolising reproduction (Figure 2). One potential explanation is that competitive advantages associated with social dominance afford dominant birds differential access to resources which may in turn alleviate resource allocation trade-offs between investment in rainfall-related activities (see above) and somatic maintenance. It is also possible that while the rain-related reproductive activities of dominants are conspicuous (e.g. egg laying, incubation and dawn song production), the more cryptic rain-related activities of subordinates (including conducting extra-territory forays for reproduction and dispersal opportunities; Young et al 2005; 2007) could conceivably generate more severe trade-offs with investment in somatic maintenance (e.g. prospecting can entail reduced body condition and elevated physiological stress; Young *et al.* 2005; Young & Monfort 2009)

Second, we also found strong support for the within-individual rate of change in telomere length in a given season being predicted by an interaction between dominance status and the bird's telomere length at the start of the season (Table 3). Visualising this interaction suggests that dominant birds may actively regulate their telomere length (i.e. those with shorter telomeres appear likely to experience *increases* in mean telomere length, while those with longer telomeres appear more likely to experience attrition or no change in mean telomere length) in a manner that subordinates do not (see Figure 3). Indeed, the pattern for dominant birds suggests as much evidence, if not more, for telomere length *increases* in birds with short telomeres, as telomere length decreases in birds with long telomeres. As short telomeres are thought to be indicative of declines in somatic integrity that may threaten health, performance and survival (see Introduction), the strategic maintenance of short telomeres (via mechanisms that may also repair other biomolecules; see below) could well explain this pattern. To the extent that this is true, our findings lend strength to the view that selection may favour disproportionate investment in somatic maintenance among dominants, given their markedly higher expected future reproduction than subordinates (as few subordinates

ever become dominant) and conceivably lower exposure to extrinsic mortality (having already achieved potentially risky dispersal); an argument that has previously been made to explain the extraordinary longevity of social insect queens (Keller & Genoud 1997; Carey 2001). It is also possible that this interaction in part reflects dominant birds strategically adjusting their reproductive effort (and hence any consequent trade-off with somatic maintenance) according to their levels of accumulated somatic damage, and indeed effects of shortening telomeres on patterns of gene expression provide a candidate proximate mechanism for making such adjustments (see General Introduction).

The apparent within-individual increases in telomere length detected in this study (and other chapters) may be explained in at least three ways: methodological problems (including measurement error), turnover of hematopoietic stem cell populations, or true telomere extension (most likely through upregulation of the enzyme telomerase; Greider & Blackburn 1985; Haussmann *et al.* 2007; Hatakeyama *et al.* 2016; Korandová & Frydrychová 2016). Apparent increases in telomere length must be interpreted with caution, as they can be generated purely by measurement error (Steenstrup et al., 2013; though see Bateson and Nettle, 2016). However, our data suggest that dominant birds with short telomeres tend to experience within-individual increases in telomere length even after correction for regression to the mean effects (Verhulst *et al.* 2013). Furthermore, if measurement error and regression to the mean effects were the cause of this pattern, we would expect such a pattern to be apparent in subordinates too, but our analyses strongly suggest that this was not the case. Turnover in the active haematopoietic stem cell populations that produced the erythrocytes, from which our whole blood telomere length estimates principally derive, could also have played a role in generating these apparent increases in mean telomere length. In studies of *leukocyte* telomere length in humans and other mammals, apparent within-individual increases in telomere length could be attributable to changes in the relative proportions of the different cell types that make up the leukocyte population (Beirne *et al.* 2014). While such changes in cell type composition are unlikely to cause similar issues in whole blood telomere length assessments (as erythrocytes numerically dominate the cell population

under assessment), it is possible that senescence of previously active haematopoietic stem cells results in an apparent increase in erythrocyte telomere length as previously quiescent stem cells (with longer telomeres) take over the production of erythrocytes (Ogawa 1993; Suda *et al.* 2011). However, whether this process actually would result in an increase in mean telomere length is as yet unclear, as quiescent stem cells too have been shown to accrue damage over time (Beerman *et al.* 2014; Wang *et al.* 2014). Finally, increases in mean telomere lengths in whole blood could be attributable to the upregulation of telomerase in the hematopoietic stem cells. Indeed, in some long-lived birds telomerase is expressed in cells of the bone marrow (which produces hematopoietic stem cells), gonad and intestine (all highly proliferative tissues) throughout life (Haussmann *et al.* 2004, 2007).

In this chapter so far, we have assumed that any changes in mean telomere length are indicative of patterns of somatic maintenance. However, while it is widely appreciated that telomere attrition rates may provide a useful biomarker of somatic maintenance (as telomere attrition may reflect systemic exposure to oxidative stress that damages other biomolecules too; von Zglinicki 2002; Halliwell & Gutteridge 2007a), the possibility that telomeres are elongated by telomerase during adulthood could complicate this view, by decoupling telomere length from the accumulation of oxidative damage to other tissues. Telomerase-induced increases in telomere length may not be as problematic for the utility of telomere attrition as a biomarker as one might think, however, for at least two reasons. First, telomere length itself could have mechanistically causal effects on organismal performance; such that telomerase-induced increases in telomere length alone could indeed constitute one aspect of 'somatic maintenance' (see Simons 2015 and references therein). Second, limited research to date suggests that telomere elongation may be coupled with the upregulation of the wider somatic maintenance machinery (Fu *et al.* 2000; Sharma *et al.* 2003; Armstrong *et al.* 2005; Ahmed *et al.* 2008), which could thereby maintain the link between telomere attrition rates and wider declines in somatic integrity across tissues even in the presence of active telomerase. Nevertheless, it must be acknowledged that if the apparent increases in mean telomere length observed here are real (see Bateson & Nettle 2016 but also Steenstrup *et al.* 2013),

the link between telomere attrition rates and wider patterns of somatic maintenance could be weaker than is generally appreciated in the absence of mechanisms of this kind. Further investigation into the causes of the apparent increases in mean telomere length in this and other species (Ujvari & Madsen 2009; Fairlie *et al.* 2016; Hatakeyama *et al.* 2016; Hoelzl *et al.* 2016a; b), and more specifically the patterns of telomerase expression *in vivo*, are therefore urgently needed.

Together, our findings provide evidence of a link between reproduction-related activities and telomere attrition rates, and, to our knowledge, the first evidence of associations between dominance status and within-individual telomere dynamics. The patterns of telomere attrition in this species suggest that dominant individuals do not suffer higher telomere attrition rates than their subordinates, despite completely monopolising within-group reproduction (Harrison *et al.* 2013a). Whether this is the case in other social species will only become clearer with time, but appreciable inter-specific variation can be expected in this regard. For example, we have no evidence to suggest that white-browed sparrow weavers ever attempt to breed while subordinate, perhaps because selection has favoured complete reproductive restraint in what are principally family groups (Harrison *et al.* 2013a). However, overt rank-related reproductive conflict is more apparent in other societies with more complex kin structures and frequent subordinate reproduction, yielding the potential for differential costs to somatic maintenance among subordinates where they are subjected to chronic harasssment (e.g. Abbott *et al.* 2003; Young *et al.* 2006) or among dominants where the harasser suffers costs of their own (e.g. Creel *et al.* 1996; Creel 2001; Bell *et al.* 2012). Further research is now required to probe this potential complexity, and our findings highlight the critical importance of conducting longitudinal studies of within-individual changes in telomere length over time when seeking to address this goal.

Chapter 5

Oxidative state predicts telomere attrition rate in a wild bird in a sex-dependent manner

5.1 Abstract

Recent years have seen a surge of evolutionary and biomedical interest in the causes of variation in telomere length and attrition rates, given their capacity to predict life-history outcomes and the incidence of age-related disease. Hypothesised links between telomere dynamics and both life-histories and disease frequently assume that telomere attrition arises in part from exposure to oxidative stress during the lifetime, which accelerates telomere shortening that occurs during cellular replication. However, few studies to date have investigated whether oxidative state predicts telomere attrition rates in free-living organisms. Here we investigate, using samples from a wild population of social birds (the white-browed sparrow weaver; *Plocepasser mahali*), whether an individual's oxidative state at the start of a lengthy breeding season, and the within-individual change in oxidative state that it experiences during that season, predict its rate of change in telomere length during that season. Our analyses reveal that aspects of oxidative state at the start of the season (but not the change in oxidative state during the season) do predict telomere attrition rates, but in complex ways. While females with higher erythrocyte concentrations of the intracellular antioxidant enzyme superoxide dismutase (SOD) showed reduced levels of telomere attrition, if anything the reverse was true for males (reflected as evidence for an interaction between sex and SOD activity). We also found weak evidence that levels of plasma antioxidant protection predicted telomere attrition in a manner that depended upon the bird's dominance status. By contrast, circulating levels of a biomarker of oxidative damage (the lipid oxidative damage product malondialdehyde) did not predict telomere dynamics. Our findings provide evidence from natural populations of associations between oxidative state and telomere dynamics, and highlight unexpected complexity in the nature of these relationships.

5.2 Introduction

The past decade has seen a continued rise in interest in telomeres (nucleoprotein structures that cap and protect linear chromosomes) in both evolutionary ecology and biomedicine. Telomere length and dynamics are associated with age-related declines in health (Blackburn *et al.* 2015), can be predictive of remaining lifespan (see chapter 2, Table 1), and may play an important role in life-history trade-offs (Monaghan 2010). The most commonly cited cause of variation in telomere attrition rates is oxidative stress, which arises when the rate of production of so-called reactive oxygen species (ROS) overwhelms a complex system of exogenous and endogenous antioxidant protection (Finkel & Holbrook 2000; Halliwell & Gutteridge 2007a). However, whilst *in vitro* studies have demonstrated that oxidative damage increases telomere attrition and that antioxidants alleviate it (Furumoto *et al.* 1998; von Zglinicki 2002; Kashino *et al.* 2003; Serra *et al.* 2003; Kurz *et al.* 2004), much remains to be investigated about the relationship between oxidative state and telomere dynamics at the organismal level. Animals (particularly in the wild) may be able to avoid the telomere-associated consequences of poor oxidative state through behavioural (e.g. dietary changes, or reduction in energetically expensive activities) or physiological mechanisms (e.g. upregulation of antioxidant defences or telomerase, or reduction in metabolic rate). Whilst experimental induction of oxidative stress in the laboratory has shown that oxidative stress *can* impact telomere dynamics under artificial conditions, it is important that we also conduct investigations under natural conditions to understand whether natural variation in oxidative states is a significant driver of variation in telomere attrition rates in the wild.

Telomeres are highly conserved repetitive sequences of non-coding DNA (in vertebrates: TTAGGG) and associated shelterin proteins that cap the ends of linear chromosomes. They increase chromosomal stability by making chromosome ends distinguishable from double-stranded breaks, thereby preventing initiation of the DNA Damage Response pathway and end-to-end fusions (Blackburn & Szostak 1984; Mieczkowski *et al.* 2003; Feldser & Greider 2007). Furthermore, during cell replication

the lagging strand of the DNA cannot be fully replicated by conventional DNA polymerases (the 'end replication problem'; Watson 1972; Olovnikov 1973), thus gradual erosion occurs with every cell cycle, and the presence of telomeres ensures that coding DNA is not lost in the process. Additional erosion occurs with every cell replication due to processing of the single strand overhang that forms a 'T-loop' structure at the terminus of the telomere (Makarov *et al.* 1997; Sfeir *et al.* 2005). In the absence of telomere repair mechanisms (e.g. the addition of nucleotides by the enzyme telomerase), these processes result in gradual telomere shortening with every cell division. Short telomeres are thought to become functionally compromised and to trigger replicative senescence (a process in which cellular division is arrested), or programmed cell death (Hemann *et al.* 2001a; Herbig *et al.* 2004; Feldser & Greider 2007). Telomere attrition thereby has the capacity to limit the proliferative potential of cells, and thus too the potential for tissue regeneration (Hao *et al.* 2005; Reichert *et al.* 2014a). In addition, senescent cells have altered secretory profiles that may contribute to inflammatory disease, and accumulation of these cells, combined with reduced cell renewal capacity, has been linked to aging phenotypes (Kipling 2001; Herbig *et al.* 2006; Campisi & d'Adda di Fagagna 2007; Coppé *et al.* 2010).

Research strongly suggests that variation in telomere attrition rates also arises via processes other than cellular replication. Using a method that measures telomere lengths at individual chromosomes, Baird et al (2003), showed that in addition to the gradual erosion consistent with replication-associated mechanisms, telomeres of telomerase-negative fibroblasts undergo large-scale stochastic reductions in length. A number of mechanisms by which large tracts of telomere may be intermittently lost have been proposed (Lansdorp 2005), of which the most commonly cited is oxidative stress. Early work on the association between oxidative stress and telomere attrition showed that the telomeres of human fibroblasts cultured under normoxia shortened at a slower rate than those cultured under hyperoxia (von Zglinicki *et al.* 1995). More recently, the focus of in vitro work has progressed to investigating the mechanisms by which oxidative stress increases telomere attrition rate. Telomeres are relatively rich in guanine, which has a lower oxidation potential than other bases, and this has been

hypothesised to render telomeres particularly vulnerable to oxidative damage (Kawanishi *et al.* 2001; Kawanishi & Oikawa 2004). In addition, treatment of fibroblasts with hydrogen peroxide (H_2O_2) causes single strand breaks that persist in telomeres, despite being rapidly repaired in minisatellites (non-transcribed repetitive sequences) in other parts of the genome (Petersen *et al.* 1998; also see Oikawa & Kawanishi 1999; Kawanishi & Oikawa 2004; Coluzzi *et al.* 2014). Unrepaired single strand breaks are thought to translate to increased rate of telomere shortening during the process of cell proliferation, potentially due to disruption of the replication fork (Sitte *et al.* 1998; von Zglinicki 2002; Lansdorp 2005; Coluzzi *et al.* 2014). Further research has suggested that oxidative damage may also exacerbate telomere loss due to reduced binding efficiency of proteins associated with telomere maintenance (Opresko *et al.* 2005). Indeed Richter & von Zglinicki (2007) found a strong exponential correlation between intracellular levels of ROS and telomere shortening rate in a number of cell types. In addition to the apparent negative effects of ROS on telomere length, it has been demonstrated that antioxidant supplementation can reduce telomere attrition rate (Furumoto *et al.* 1998; Liu *et al.* 2002; Kashino *et al.* 2003; Serra *et al.* 2003; Kurz *et al.* 2004), and that action by telomerase to maintain or extend telomeres may be inhibited by oxidative damage (Ahmed *et al.* 2008; Houben *et al.* 2008).

Oxidative stress may therefore play a major role in generating variation in rates of telomere attrition, but there has thus far been limited investigation of this link *in vivo*, particularly in free-living organisms. A number of studies have reported concordant impacts of experimental manipulations (e.g. changes in nutrition, growth, or reproduction) on measures of both oxidative state and telomere attrition that are consistent with the hypothesis that oxidative stress impacts telomere attrition rates (Tarry-Adkins *et al.* 2013; Stier *et al.* 2014, 2015; Sudyka *et al.* 2016; Yip *et al.* 2017; Young *et al.* 2017). However, these studies did not explicitly investigate the relationship between oxidative state and telomere dynamics *per se*. We therefore concentrate here on studies that have directly investigated this link.

Experimental manipulations of the oxidative states of organisms, that either increase or depress antioxidant levels or activity, have shown that antioxidants can reduce telomere attrition rates in captive and free-living animals respectively (Cattan *et al.* 2008; Badás *et al.* 2015). Correlative evidence that oxidative damage may increase telomere attrition has also been found in free-living animals: king penguin chicks (*Aptenodytes patagonicus*) that had experienced greater telomere loss by the end of the growth period had higher oxidative damage levels (Geiger *et al.* 2012), and levels of superoxide (an important ROS) in painted dragon lizard hatchlings (*Ctenophorus pictus*) negatively predicted telomere length (Ballen *et al.* 2012). However, other correlative studies of free-living organisms have found no relationship between telomere dynamics and measures of either oxidative damage or protection (Beaulieu *et al.* 2011; Nettle *et al.* 2015; Giraudeau *et al.* 2016), including a recent study by Boonekamp et al (2017) that showed a lack of correlation between any of six different oxidative stress markers and telomere attrition in jackdaw chicks. However, variation among individuals, or among classes of individual, in behavioural and physiological traits and optimal resource allocation strategies, provides potential for status-dependent effects of oxidative state on telomere attrition. Failure to find relationships between oxidative state and telomere attrition rates may arise in part if such effects exist but are not tested. Investigation of status-dependent effects are rare, but they have previously been reported: Experimental increases in antioxidant levels reduced telomere attrition in captive female, but not male, zebra finches (*Taenopygia guttata*, Noguera *et al.* 2015), and in 'bold' but not 'fearful' gull chicks (*Larus michahellis*, Kim & Velando 2015). Furthermore, higher natural levels of antioxidants predicted reduced attrition in old but not young male common yellowthroats (*Geothlypis trichas*, Taff & Freeman-Gallant 2017).

The majority of studies at the organismal level have used measures of plasma total antioxidant capacity as their only measure of protection against oxidative damage. However, antioxidant levels in the plasma may poorly reflect levels of intracellular antioxidant protection, which are an important line of defence against oxidative damage (Parkes *et al.* 1998; Chaudière & Ferrari-Iliou 1999). Superoxide dismutase enzymes (SODs) are a major component of the intracellular antioxidant armoury (Halliwell &

Gutteridge 2007b), yet to our knowledge, there has been very little investigation of whether intra-cellular SOD activity predicts rates of telomere attrition *in vivo* (Houben *et al.* 2009; Tarry-Adkins *et al.* 2013). This is surprising given that *in vitro* studies suggest that action by this enzyme significantly reduces telomere attrition. Indeed of 234 genes involved in the stress response, expression of so-called 'extracellular SOD' (EC-SOD; which contributes to intra-cellular SOD activity too) was found to be the best candidate for maintaining low rates of telomere attrition, and higher expression of Mn-SOD (a SOD form found in the mitochondrion) was also associated with lower rates of telomere attrition (Serra *et al.* 2003). Furthermore, Serra *et al.* (2003) demonstrated that experimental increase of EC-SOD expression resulted in reduced telomere attrition rates in fibroblasts and the elongation of cellular lifespan.

Here we investigate whether oxidative state predicts telomere attrition rate in a wild social bird, the white-browed sparrow weaver (*Plocepasser mahali*). Specifically, we test whether an individual's oxidative state at the start of a lengthy breeding season and its within-individual change in oxidative state during that breeding season, predict its rate of telomere attrition during that breeding season. White-browed sparrow weavers form cooperatively breeding groups that comprise a dominant pair who monopolise within-group reproduction (Harrison *et al.* 2013a; approximately 15% of offspring are sired by extra-group, usually dominant, males), and up to ten subordinate birds who assist with provisioning young, weaving, sentinelling, and territory defence (Lewis 1982; Walker 2016; Walker *et al.* 2016). Dominant males, and to a much lesser degree, subordinate males, participate in solo dawn song, which is likely to be energetically expensive (York *et al.* 2016a), and only dominant females produce and incubate eggs (Harrison *et al.* 2013a; Walker 2016). In addition, dominant females provision offspring at the highest rates, and females of both classes provision offspring at higher rates than males (Young, unpublished data). Given the different behavioural profiles of birds of differing sex and dominance status (and the consequent potential for them to resolve or respond to resource allocation trade-offs in differing ways), we also allow in our analyses for the possibility of sex- or dominance-dependent relationships between oxidative state and telomere dynamics. For example, evidence that sex steroids and sex steroid receptors

impact the expression of DNA repair mechanisms (Kyo *et al.* 1999; Polkinghorn *et al.* 2013; Yip *et al.* 2017) highlights the potential for divergent relationships between oxidative stress and telomere dynamics for the sexes.

Characterising oxidative state requires measures of both oxidative damage and antioxidant protection, and we therefore employ measures of both. Specifically, we assessed (i) plasma concentrations of the lipid oxidative damage product malondialdehyde (MDA), (ii) the activity of the antioxidant enzyme superoxide dismutase (SOD: this measure incorporates all forms of SOD) in cellular (erythrocyte) lysates, and (iii) plasma total antioxidant capacity controlling for the confounding effects of uric acid (here termed 'residual TAC'; which measures the action of both endogenous and exogenous antioxidants in plasma). Previous work on our study population has revealed meaningful associations between all three of these oxidative state variables and expected modulators of oxidative state. For example, consistent with the expectation that elevated reproductive effort entails an oxidative stress cost (Costantini 2008; Metcalfe & Monaghan 2013), our previous work has found that experimental manipulation of reproductive effort has associated impacts on plasma MDA levels (Cram *et al.* 2015b), with birds showing elevated plasma MDA levels during reproductive episodes but only in smaller social groups (in which individual provisioning workloads are higher). Similarly, individuals that provision offspring at higher rates (which is expected to increase ROS production via increases in metabolic work) show significantly lower levels of plasma residual TAC during the peak provisioning period (Cram *et al.* 2015b), and dominant females (that provision offspring at significantly higher rates than subordinate females) experience significantly greater within-individual declines in residual TAC over the course of the breeding season (Cram *et al.* 2015c). Finally, individual variation in levels of erythrocyte SOD activity immediately prior to an immune challenge also predicted the strength of the resulting immune response (Cram *et al.* 2015a).

Specifically, we investigate (i) whether an individual's oxidative state at the start of a long breeding season predicts its within-individual rate of telomere attrition over the

course of the breeding season (allowing for interactions between the oxidative state variables and both sex and dominance status) and (ii) whether an individual's *change* in oxidative state over the course of the season predicts its rate of telomere attrition over the same period. We investigated change in oxidative state in addition to initial state as telomere dynamics are likely to be impacted by state *during* the period over which their change is measured. We predicted that individuals with higher levels of, or greater increases in, oxidative damage (MDA) should show higher rates of telomere attrition, and that individuals with higher levels of, or greater increases in, enzymatic antioxidant levels (SOD) or extracellular antioxidative levels (residual TAC) should show lower rates of telomere attrition. These predictions stem directly from the hypothesis that greater exposure to oxidative stress increases telomere attrition rates. We made no specific predictions about potential interactions between oxidative measures and sex or dominance, seeking only to allow for the possibility that they differed.

5.3 Methods

5.3.1 Study population, field methods and blood sampling

This study was conducted on a population of 40 social groups of white-browed sparrow weavers in the semi-arid Kalahari desert at Tswalu Kalahari Reserve, South Africa (27°16'S, 22°25'E). All birds in the population are fitted with a metal ring (SAFRING licence 1444) and a unique combination of three colour rings for identification. Sex was determined by beak colour (black in males and horn-coloured in females; Leitner *et al.* 2010). Dominant and subordinate birds of each sex (see below for sample size information) were caught for blood sampling during the non-breeding periods immediately before (September 2011) and again after (March and April 2012) the 2011/2012 breeding season. In each case the birds were captured at night for blood sampling by flushing them from their individual roost chambers into a custom capture bag. Two blood samples were immediately collected from each bird at each capture via brachial venepuncture using a 26G needle and heparinized and non-heparinized capillary tubes respectively; one sample for assessment of oxidative state (~300μL) and the other for a matched assessment of whole blood telomere length (~25uL). The length of time between each bird's pre- and post-season samples averaged 222 days (range 178 - 237 days), and we accounted for variation in this interval in our calculations of the rate of change in telomere length (see below). The non-breeding status of groups at sampling was confirmed by nest checks carried out every other day. The pre-season samples were collected before any eggs were laid, and the post-season samples were collected at least two months after the end of egg laying in the population, so birds were not provisioning chicks or young fledglings at either sampling time point. Oxidative stress samples used here are a subset of the those in Cram (2015c), for which we were able to take matched telomere measures. Hence while the analyses presented here are unique (as that paper does not consider telomere dynamics), there are methodological details in common.

5.3.2 Oxidative state metrics

The laboratory methods for the determination of each of the oxidative state metrics are described fully in Cram *et al.* (2015c). Briefly, blood was separated by centrifugation immediately after collection (i.e. in the field) and the erythrocytes from the cellular phase were lysed in four times their volume of ice-cold distilled water and placed on ice for 5 minutes. This solution was then centrifuged for 3 minutes and the erythrocyte lysate drawn off. Plasma from the separated whole blood (for the determination of MDA, TAC and uric acid levels), and the erythrocyte lysate (for the determination of SOD activity), were then both stored on ice until return to base camp where they were transferred to liquid nitrogen (mean ± SD time lag from processing to storage on liquid nitrogen: 131 ± 60 min). All samples were analysed within 7 months of the end of the sampling period.

Oxidative damage to phospholipids (MDA).

Plasma concentrations of circulating malondialdehyde (MDA) were measured using high performance liquid chroma-tography following methods in Nussey et al (2009). A subset of samples run in duplicate showed high repeatability ($F_{66,67} = 15.92$, $r = 0.88$, $P < 0.001$).

Enzymatic antioxidant protection (SOD).

The SOD activity in erythrocyte lysate was measured using a colorimetric assay (Cayman Chemicals, USA) and spectro-photometer (Spectramax M2; Molecular Devices, USA). One unit is defined as the amount of enzyme required to exhibit 50% dismutation of the superoxide radical; enzyme activities are reported as units/ml. A subset of samples run in duplicate on separate plates showed high between-plate repeatability ($F_{37,38} = 6.07$, $r = 0.72$, $p < 0.001$).

Residual Total Antioxidant Capacity (residual TAC).

Plasma 'Total Antioxidant Capacity' (TAC) was estimated using a colorimetric assay kit (Cayman Chemicals, USA) and spectrophotometer (Spectramax M2; Molecular Devices, USA) to measure the capacity of a plasma sample to quench a standardised free radical challenge. Plasma TAC values are expressed as Trolox-equivalent antioxidant concentrations. A subset of plasma samples run in duplicate on separate plates showed high between-plate repeatability ($F_{41,42} = 8.20$, $r = 0.78$, $p < 0.001$).

Uric acid is a nitrogen waste product in birds that may cause up to 90% of the variation in avian plasma TAC (Cohen *et al.* 2007). Its importance as an antioxidant *in vivo* is unclear and as uric acid can itself generate ROS (Dröge 2002), avian plasma TAC values are potentially confounded. We therefore statistically controlled for the effects of uric acid levels on TAC following Cohen et al (2007). Plasma uric acid concentrations significantly predicted TAC (linear mixed model with bird identity as the random factor, $\chi^2_1 = 93.30$, $p < 0.001$, $n = 106$ samples). A separate linear model with TAC as the response and uric acid concentration as the sole predictor was used to generate residuals (hereafter 'residual TAC'). Residual TAC is therefore a measure of plasma antioxidant capacity excluding that arising from uric acid, but retaining any effects of individual identity.

Uric acid.

Plasma concentrations of uric acid were measured using a fluorescence assay kit (Cayman Chemical, USA) and spectrophotometer (Spectramax M2; Molecular Devices, USA). A subset of plasma samples run in duplicate on separate plates showed high between-plate repeatability ($F_{39,40} = 8.35$, $r = 0.79$, $P < 0.001$).

5.3.3 DNA extraction and telomere measurement

DNA extraction and telomere measurement methods are described fully in Chapter 1. Briefly, DNA was extracted from whole blood using the Gentra PureGene Genomic

DNA Purification Kit (Qiagen) and DNA integrity, quantity and quality were assessed. Samples that failed these assessments were re-extracted or rejected if further extractions also failed. DNA samples were diluted in elution buffer and stored at -20˚C until telomere analysis.

We used quantitative PCR (qPCR) as described in Cawthon (2002) to quantify relative telomere length (RTL) of whole blood, which will mostly reflect erythrocyte telomere length as avian erythrocytes are nucleated and comprise the majority of blood cells (Williams 1972). qPCR gives a measure of telomere length relative to a non-variable copy-number control gene (thus controlling for variation in DNA concentration), for which we used glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Estimates of telomere length determined by qPCR include interstitial telomeric repeats located away from the chromosome ends and are therefore not a measure of 'true' telomere length. However, while this issue is expected to generate some *among-individual* variation in RTL independent of variation in true mean telomere length (as individuals may differ in the extent to which they carry interstitial telomeric repeats), it is not anticipated to impact the assessments of *within-individual* changes in mean telomere length that are the focus of this study (as interstitial repeats are not thought to be subject to erosion within the lifetime; Delany *et al.* 2003).

Control gene and telomere reactions were carried out on separate 96-well plates, due to differing optimal annealing temperatures, on a Stratagene Mx3000 instrument. Thermal cycles were: 95˚C for 15 minutes, then 40 cycles of 95˚C for 15 seconds, 57˚C (telomere) or 60˚C (GAPDH) annealing for 30 seconds, and 73˚C extension for 30 seconds. GAPDH primers were specific to *P. mahali* (GAPDH-F 5′AAACCAG CCAAGTATGATGACAT–3′; GAPDH-R 5′-CCATCAGCAGCAGCCTTCA-3′). Telomere forward and reverse primers were as follows Tel1b (5′-CGGTTTGTTT GGGTTTGGGTTTGGGTTTGGGTTTGGGTT-3′), and Tel2b (5′-GGCTTGCCTT ACCCTTACCCTTACCCTTACCCTTACCCT-3′). The master mix contained 10uL SybrGreen fluorescent dye with low Rox (Agilent Technologies) and primers at a concentration of 200nM in a 20uL reaction. Each reaction contained 5ng DNA. Samples

were run in triplicate, as were a no-template control and a between-plate calibration sample which were included on every plate. LinRegPCR (Ruijter *et al.* 2009), was used to correct the baseline fluorescence and set a window of linearity for each amplicon group and to set constant fluorescence thresholds within the windows of linearity for GAPDH (0.156) and telomere (0.161). RTL was calculated using well efficiencies following Pfaffl (2001), which gives the ratio of the quantity of telomeric DNA to reference gene (T/S).

5.3.4 Statistical methods

Statistical analyses were carried out in R3.3.1 (R Core team 2016), using an Information-Theoretic framework to compare models using Akaike's information criterion corrected for small sample sizes (AICc) (Burnham & Anderson 2002). For each of question below we constructed a global model that contained all terms of interest (described below). Given that we had no reason to discount any combination of these terms we competed all possible combinations of the global model (interactions were only included in the presence of their corresponding first order terms), which were ranked by AICc weights using the MuMIn package. The model with the highest Akaike weight and the lowest AICc havs the most support and is referred to as the top model. Models with a Δ AICc > 6 were retained in the top model set unless they were more complex than nested models with a lower AICc, in which case they were dropped in the interests of parsimony and adjusted weights of the remaining models were calculated following the recommendations of Richards (2011). Full top model sets are presented in Appendix F. As we used this rule we plotted results using effect size estimates from the top model (for terms within the top model; and the best model containing the term of interest for any terms not within the top model) rather than using model averaging. All continuous predictors were centred and scaled using the 'scale' function but were back-transformed for plotting.

We assessed the effect of (i) pre-season oxidative state metrics, and (ii) within-individual change in these oxidative state metrics over the course of the breeding season,

on the rate of change in telomere length over the course of the breeding season. In both analyses we corrected 'change in telomere length' for regression to the mean using Verhulst's D (Verhulst *et al.* 2013), in which the difference between mean-centred baseline and mean-centred follow-up RTL measurements is calculated after the baseline RTL value is multiplied by the correlation coefficient between the two (see Verhulst *et al.* 2013 for rationale and details). This gives us a value of D. As this calculation involves mean centring values for both baseline and follow-up measurements, the average D is equal to zero. For clarity, we corrected the position of zero (i.e. where no change in telomere length occurred) by adding the difference between the mean baseline RTL and mean follow-up RTL to all values of D. We then calculated (from this value and the time elapsed between the two estimates of telomere length) the rate of change in D per month, henceforth referred to as rate of change in telomere lenght. Two random intercept terms were fitted in each model: qPCR plate (to account for among plate variation in telomere measures), and social group (to account for heterogeneity in territory quality and genetic variation). Prior to model comparison, in order to ascertain independence of predictors, correlations between all predictor variables were checked and variance inflation factors (VIF) of each global model was checked to assess multicolliearity: all VIFs were below 5 ("car" package; Fox & Weisberg 2011). Model residuals of global models were assessed to confirm compliance with model assumptions and the influence of individual points were tested using Cooks Distance in the influence.ME package (Nieuwenhuis et al 2012).

Does pre-season oxidative state predict the rate of change in telomere length?

All oxidative state measures (MDA, SOD and residual TAC) were fitted in two-way interactions with dominance status and sex to allow for status-dependent effects of oxidative measures on telomere dynamics. In addition, we included the interaction between pre-season (baseline) RTL and dominance status, as we have previously found this to be an important predictor of rate of change in RTL (Chapter 4). The model comparison process is explained above. The data set consisted of measures from 65 individuals from 24 social groups (12 dominant females, 13 dominant males, 16 subordinate females and 24 subordinate males), for whom measures were available for

all three oxidative state metrics at the start of the season and rate of change in RTL over the course of the season.

The two interactions that appeared in the top model set were further investigated by splitting the data by the factor of interest (i.e. sex or dominance status) and conducting model comparisons for each level of that factor (e.g for males and females separately). In each case for these post-hoc analyses, the global model was taken as the model in which the interaction had occurred, and all possible combinations of terms within the global model were compared and ranked.

Does change in oxidative state predict the rate of change in telomere length?

All terms present in the top model from the modelling exercise above (the effect of oxidative state at the start of the season on rate of change in RTL) were included in this model, alongside Δ MDA, Δ SOD and Δ residual TAC (each calculated as the value of the focal oxidative state metric for that individual at the end of the season minus its value at the start). No further interactions were included due to restricted sample size. The sample size for this modelling process was smaller than that for the previous one, as individuals had be excluded if one or more of the oxidative state metrics were not available from them at the end of the season (leaving a sample size of n = 41 individuals from 20 social groups; 7 dominant females, 7 dominant males, 12 subordinate females, 15 subordinate males).

5.4 Results

Does pre-season oxidative state predict the rate of change in telomere length?

We found evidence that an individual's levels of erythrocyte SOD activity at the start of the season predicted their subsequent rate of change in telomere length over the course of the season, but did so in a sex-dependent manner (the best-supported model contained an interaction between sex and SOD; Table 1). While this interaction was not present in all models within the top model set (and so has limited support), inspection of the model parameters suggests that SOD negatively predicted telomere attrition rates (contrary to our predictions) in females, while the effect in males was more-weakly positive. Splitting the data set by sex and comparing for each sex the explanatory power of a model containing an effect of SOD with that of an intercept-only model confirmed these patterns (Table 2): a negative effect of SOD was the top model in females, while a positive effect of SOD was the top model in males after the removal of a single point of high influence (with a Cook's Distance substantially larger than any other point; 0.65).

Table 1. Model selection table showing pre-season oxidative state predictors of rate of change in telomere length of adult white-browed sparrow weavers over a breeding season. Samples were from 65 birds from 24 social groups (12 dominant females, 13 dominant males, 16 subordinate females and 24 subordinate males). Competing models in the Δ6 AICc set after implementation of the model nesting rule are presented (Richards *et al.* 2011). Continuous variables were centred and scaled. Effect sizes are given followed by standard errors in parentheses. For dominance status, the estimate is given for subordinates (Sub) relative to dominants. For sex, the estimate is given for males relative to females. Int = intercept, AW = adjusted weight. *Terms that do not appear in the top model set but were present in the global model.

Int	Status (Sub)	Sex (M)	SOD	residual TAC	Status (Sub): residual TAC	Sex (M): SOD	df	logLik	AICc	Δ AICc	AW
0.0020 (0.0036)		-0.0052 (0.0035)	-0.0052 (0.0024)			0.0096 (0.0036)	7	182.0	-348.0	0.00	0.40
-0.0011 (0.0042)	0.0064 (0.0036)	-0.0066 (0.0033)		-0.0063 (0.0032)	0.0099 (0.0039)		8	182.7	-346.9	1.10	0.23
-0.0048 (0.0037)	0.0065 (0.0038)						5	178.8	-346.6	1.38	0.20
-0.0014 (0.0031)							4	177.5	-346.2	1.74	0.17

* MDA, start RTL, MDA : Sex, MDA : Status, residual TAC : Sex, SOD : Status, start RTL : Status

Table 2. The effect of pre-season SOD on the rate of change in relative telomere length of adult white-browed sparrow weavers. Competing models in the Δ6 AICc set after implementation of the model nesting rule are presented (Richards *et al.* 2011), for **(a)** males (n = 37 birds from 22 social groups), **(b)** males once a point of high influence was removed (see Figure 1), and **(c)** females (n = 28 birds from 18 social groups). SOD was scaled and centred. Effect sizes are given, followed by standard errors in parentheses. All terms present in the global model (only SOD and the intercept) are present in the table.

Intercept	SOD	df	logLik	AICc	Δ AICc	AW
(a) *Males (n = 37 birds)*						
0.0040 (0.0035)		4	103.54	-197.8	0.00	1.000
(b) *Males without point of high influence (n = 36 birds)*						
-0.0047 (0.0023)	0.0058 (0.0022)	5	106.08	-200.2	0.00	0.825
-0.0047 (0.0031)		4	103.18	-197.1	3.10	0.175
(c) *Females (n = 28 birds)*						
0.0010 (0.0029)	-0.0064 (0.0030)	5	76.77	-140.8	0.00	0.644
0.0010 (0.0032)		4	74.68	-139.6	1.18	0.356

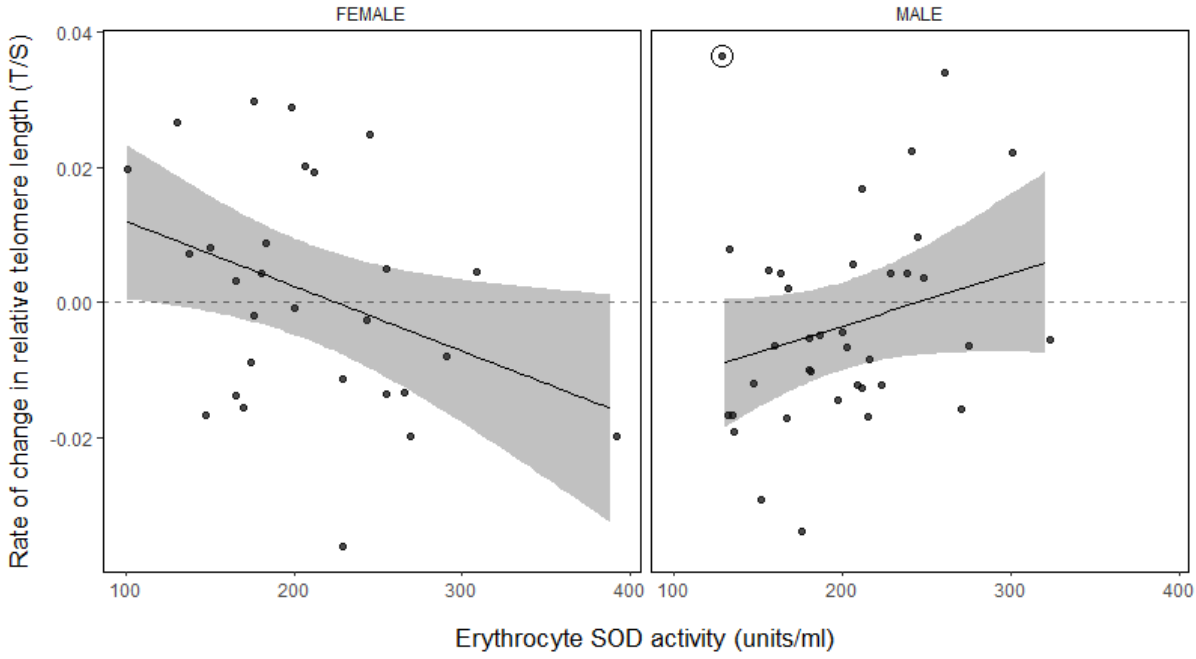


Figure 1. The effect of the interaction between SOD and sex on rate of change in relative telomere length (RTL) of adult white-browed sparrow weavers over a breeding season. Solid lines show the model estimates for females and males, shaded areas show 95 % confidence intervals around the estimate. Points are raw data. Circled point had a substantially higher Cook's distance than any other point (0.65) when data for males and females were separated. RTL is calculated as a ratio of the quantity of Telomeric DNA (T) to reference gene (S). As change in RTL was corrected for regression to the mean using Verhulst's D, which renders 0 equal to the mean change in telomere length, we corrected the position of 0 before calculating the rate of change in RTL for analysis. As such, the dashed line in all panels shows the point of no change in telomere length over time; points below the line show decreases in telomere length estimates over time and points above the line show increases in telomere length estimates over time.

There was competing but very limited evidence for an interaction between residual TAC at the start of the season and dominance status, in which the slope of the relationship between residual TAC and the rate of change in telomere length was weakly negative for dominant birds but very weakly positive for subordinate birds (Table 1). This model additionally contained sex, with males showing higher rates of attrition than females and was only weakly supported, being only 0.6 AICc points different to the intercept only model (Table 1). Furthermore, splitting the data set by dominance status and

comparing for each status the explanatory power of a model containing effects of residual TAC + sex (the two terms other than dominance status within the model containing the interaction with TAC) with simpler forms of this model, revealed no support for effects of TAC in either dominants or subordinates (the top model was the intercept-only model in both cases).

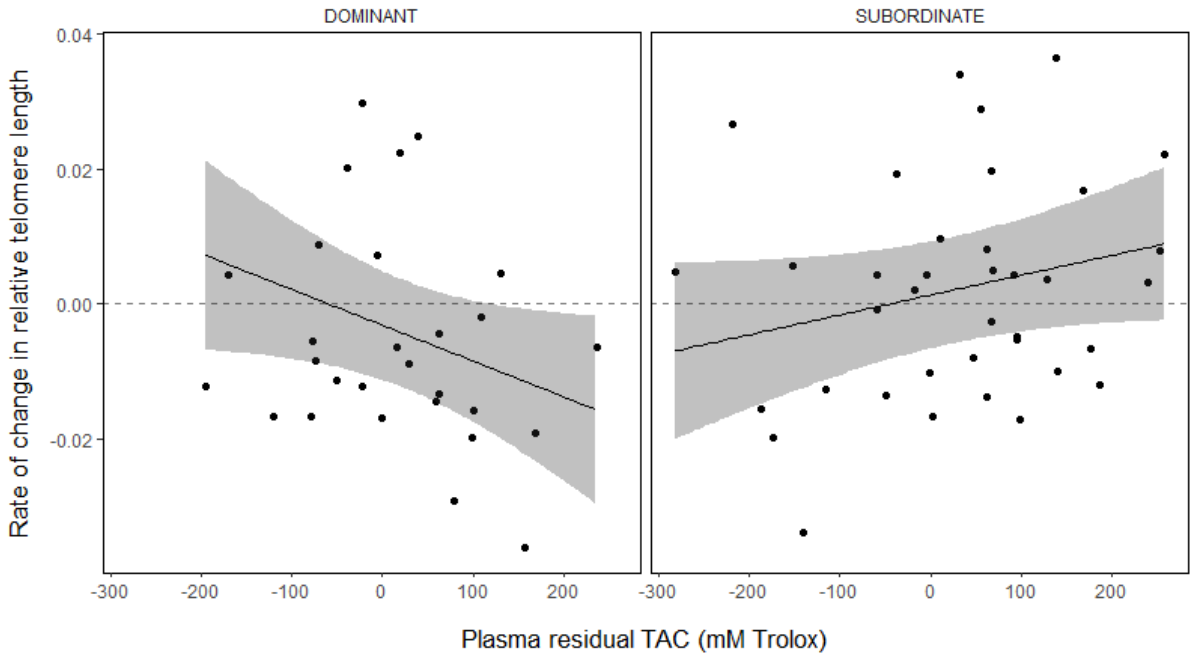


Figure 2. The effect of the interaction between plasma residual total antioxidant capacity (TAC) and dominance status on the rate of change in relative telomere length (RTL) of adult white-browed sparrow weavers over a breeding season. Estimates are taken from the second best supported model, which also included sex. The solid lines show the estimate for the focal dominance status (dominant or subordinate), for the average intercept estimate across males and females. Shaded area shows 95% confidence intervals around the estimate. Points are raw data. RTL is calculated as a ratio of the quantity of Telomeric DNA (T) to reference gene (S). As change in RTL was corrected for regression to the mean using Verhulst's D, which renders 0 equal to the mean change in telomere length, we corrected the position of 0 before calculating the rate of change in RTL for analysis. As such, the dashed line in all panels shows the point of no change in telomere length over time; points below the line show decreases in telomere length estimates over time and points above the line show increases in telomere length estimates over time.

Does change in oxidative state predict the rate of change in telomere length?

We found no evidence that the within-individual change in oxidative status over the course of the season predicted the rate of change in telomere length over the same period (Table 3). As the sample size was small we were unable to test for any status or sex-dependent effects of these within-individual changes in oxidative metrics. Despite the reduced sample size, the interaction between SOD and Sex detected above was retained in the top model.

Table 3. Model selection table showing change in oxidative state predictors of the rate of change in relative telomere length of adult white-browed sparrow weavers over a breeding season. Competing models in the Δ6 AICc set after implementation of the model nesting rule are presented (Richards *et al.* 2011). Continuous variables were scaled and centred. Effect sizes are given followed by standard errors in parentheses. For sex, the estimate is given for males (M) relative to females. AW = adjusted weight after implementation of the model nesting rule. *Terms that do not appear in the top model set but were present in the global model.

Intercept	Sex (M)	SOD	Sex (M): SOD	df	logLik	AICc	Δ AICc	AW
0.0045 (0.0042)	-0.0037 (0.0040)	-0.0073 (0.0025)	0.0138 (0.0042)	7	118.936	-220.5	0.00	0.813
0.0009 (0.0038)				4	113.324	-217.5	2.94	0.187

* Δ SOD, Δ residual TAC, Δ MDA

5.5 Discussion

Oxidative state predicts telomere attrition *in vitro*, yet there has been limited investigation of this relationship at the organismal level, particularly in free-living animals. We found no straightforward relationships between oxidative state metrics and the within-individual rate of telomere attrition in wild white-browed sparrow weavers. In addition, changes in these oxidative state metrics over the course of the season did not predict rates of telomere attrition. Our analyses did, however, provide evidence of sex-specific associations between erythrocyte SOD activity and subsequent telomere dynamics. We also found evidence (though very limited) for a dominance-status-dependent relationship between plasma antioxidant capacity (reflected as residual TAC) and rates of telomere attrition. As we made no predictions about the nature of any sex- or dominance-dependent effects of oxidative state metrics on telomere dynamics (and given the limited statistical support for the TAC interaction in particular), these interactions need to be interpreted with caution. Nevertheless, our results do lend support from a natural population of whole organisms for the hypothesis that aspects of oxidative state impact telomere dynamics, and, given the state-dependent complexity of these associations, may help explain why such associations have not been detected in other studies.

MDA is produced during lipid peroxidation (as triggered by ROS) and is mutagenic primarily at GC base pairs (Niedernhofer *et al.* 2003), which are common in the telomere sequence. MDA therefore has the potential both to directly hasten the erosion of telomeres by causing telomeric lesions and to reflect the wider levels of other ROS molecules within the body that could themselves have similar effects. However, we found no evidence that plasma MDA concentrations at the start of the season, or the within-individual change in MDA concentrations over the course of the season, predict an individual's rate of change in mean telomere length. Several other studies have also reported no apparent relationship between MDA levels and telomere attrition rates (e.g. in free-living animals [Nettle *et al.* 2015] and humans [Boxall *et al.* 2006]), but inverse relationships between MDA and telomere *length* have been reported in humans with

various diseases (Adaikalakoteswari *et al.* 2005; Palmieri *et al.* 2014). To our knowledge no studies have found associations between MDA and telomere attrition *per se*. The lack of any apparent relationship between MDA and telomere attrition may be because MDA in healthy organisms does not constitute a useful indicator of accumulated oxidative stress exposure over the time periods relevant to telomere attrition, but rather reflects only very recent exposure to damage (Boxall *et al.* 2006; Cram *et al.* 2015b). In addition, individuals with good antioxidant protection may not be impacted by the potentially damaging effects of MDA itself, as it may be removed by antioxidant defences prior to causing DNA damage. Unfortunately, our sample sizes did not allow for the fitting of interactions between MDA and measures of antioxidant protection, which could conceivably have provided a better overall assessment of oxidative state. Other measures of ROS have also met with mixed success in predicting telomere dynamics, with negative associations being found between concentrations of hydrogen peroxide and change in telomere length in king penguin chicks (Geiger *et al.* 2012), but not in adult Adelie penguins (Beaulieu *et al.* 2011). The negative association that one might predict between concentrations of the superoxide radical and telomere *length* was apparent in painted dragon lizard hatchlings (Ballen *et al.* 2012), but oxidative damage to DNA (as measured by the Comet assay) did not predict telomere attrition rates in male common yellowthroats (Taff & Freeman-Gallant 2017), and neither did any of three measures of ROS (lipid peroxidation products, hydrogen peroxide and oxidised glutathione) predict telomere attrition in jackdaw nestlings (Boonekamp *et al.* 2017). Whether levels of ROS impact telomere attrition remains equivocal but the evidence thus far (including our findings) suggests that this is not the case. However, investigating the impact of ROS in individuals with greater or lesser antioxidant protection may be necessary to uncover the relationship between ROS and telomere attrition *in vivo*.

Levels of expression of EC-SOD have been shown to strongly positively predict rates of change in telomere length *in vitro* (Serra *et al.* 2003). Our analyses suggest that erythrocyte SOD activity positively predicts rates of change in telomere length in males (as one would predict on the basis of these *in vitro* results), but that the reverse

relationship is apparent in females (Figure 1). To our knowledge this is the first time that a relationship between SOD and telomere dynamics has been investigated at the organismal level. This interaction between sex and SOD activity (and the unexpected negative relationship in females) could be explained by either physiological or behavioural differences between the sexes. First, it is possible that high levels of SOD activity in females reflect an upregulation of antioxidant defences in response to (current or anticipated) exposure to elevated levels of ROS (Shull *et al.* 1991; Serra *et al.* 2003), and could thereby actually be indicative of challenging (current or impending) oxidative loads among females. There could be sex differences in the extent to which this is the case if, for example, females on average experience higher peak levels of ROS production during the breeding season (which is plausible given their differential pre- and post-natal investment in offspring in this species; Walker 2015 [thesis]). Second, if females are indeed exposed to higher peak levels of ROS production than males, higher quality females may simply have both higher start-of-season SOD activity and greater subsequent investment in ROS-inducing activities (e.g. reproductive effort) than lower quality females. Indeed, congruent with this logic, white-browed sparrow weavers that have higher natural SOD activities mount stronger immune responses when subsequently challenged (Cram *et al.* 2015a). Third, in dominant females it is possible that those with high levels of SOD at the start of the season invest higher levels of antioxidants in eggs to the detriment of their own somatic maintenance (see Bize *et al.* 2008). It may clarify the mechanism driving this interaction to investigate the cause of variation in SOD activity at other points in the breeding season, to see if females do indeed upregulate SOD activity prior to heavy workloads. Again, it would also be instructive to look at the interactions between measures of oxidative damage and protection to better understand the origins of the interaction between sex and SOD.

We also found some, but very limited, evidence that the rates of change in telomere length were predicted by an interaction between residual TAC and dominance status (Figure 2). While any conclusions from such a poorly supported result must necessarily be very tentative, there is evidence from previous studies of trait-dependent relationships between TAC (or dietary antioxidants, which TAC should in part reflect)

and telomere attrition. For example, older common yellowthroats (Taff & Freeman-Gallant 2017), female zebra finches (Noguera *et al.* 2015) and 'bold' gull chicks (Kim & Velando 2015) all show reduced telomere attrition when antioxidant levels are higher, whilst this association is weaker (or not apparent) in younger common yellowthroats, male zebra finches and 'fearful' gull chicks respectively. The suggested explanations for the findings in the first two species centred on trade-off decisions. For example, Noguera *et al.* (2015) suggest that the optimal trait in which to invest extra dietary antioxidants for male zebra finches could be plumage quality (a secondary sexual characteristic), whilst for females it could be somatic maintenance. The interaction in our study (to the extent that it reflects biological reality) could reflect a similar dominance-related difference in trade-off resolution in sparrow weavers. For example, it could be beneficial for dominant birds with high residual TAC to invest more of the associated antioxidant resources in to reproduction and/or maintaining their status (rather than somatic maintenance and the consequent retardation of telomere erosion), whilst subordinates with high residual TAC may be free to invest these resources more exclusively in somatic maintenance.

The potential for change in oxidative status to influence telomere dynamics has been neglected in the literature, which is perhaps surprising given that such measures may give a more integrative view of oxidative status over a period of time than measures taken at a single point. However, we did not detect any such associations. This may partially be due to a relatively small sample size, but may also be because oxidative status is likely to undergo changes during the breeding season that are not represented by either start measures or measures of change between the start and end of the season. In addition, the small sample size did not allow us to investigate interactions either among measures of damage and protection, or among these measures and sex or dominance. Given our findings for start-of-season oxidative status this may have hidden any effects of change in oxidative status.

The lack of clear simple effects of markers of oxidative damage or protection on telomere attrition rates highlights the potential complexity of relationships between

oxidative state and telomere dynamics, perhaps due to the strategic regulation of both the complex antioxidant defence systems as well as systems for the repair of DNA damage (for example telomerase). However, we cannot rule out that other markers of oxidative stress would predict telomere attrition in a more straightforward manner. It is also possible that the measures we took were poorly correlated with overall oxidative exposure over the time period relevant to telomere attrition, and more frequent measures of oxidative state may have shed more light on the matter. Finally, we cannot rule out that oxidative state may *not* be a major driver of individual variation in telomere attrition rates in natural populations (see Boonekamp *et al.* 2017). However, given the strong *in vitro*, and growing *in vivo* evidence that oxidative status *can* influence telomere attrition at the organismal level this seems unlikely. Rather, our results support the view that variation in behaviour or physiology may result in differing effects of oxidative measures on telomere attrition.

Chapter 6

General Discussion

6.1 Overview

Telomere dynamics are increasingly used as a biomarker of somatic maintenance to investigate how organisms resolve life-history trade-offs. However, despite the key prediction of life-history theory that early-life investment in somatic maintenance has downstream implications for later life health and longevity (Kirkwood & Holliday 1979; Kirkwood & Rose 1991), this prediction has only twice been tested in free-living animals using within-individual telomere attrition rates as a biomarker of somatic maintenance (Boonekamp *et al.* 2014a; Fairlie *et al.* 2016). Furthermore, the impact of the social environment on the within-individual telomere dynamics of highly social species has been largely neglected, despite the potential for marked impacts of social behaviour on patterns of somatic maintenance (Jemielity *et al.* 2005; Dammann & Burda 2006; Sharp & Clutton-Brock 2011; Beirne *et al.* 2015; Cram *et al.* 2015b). Finally, whilst there is good evidence that oxidative damage can accelerate telomere shortening and that antioxidants can alleviate this effect *in vitro* (von Zglinicki 2002; Serra *et al.* 2003), evidence that variation in oxidative states predicts individual variation in telomere attrition rates at the organismal level remains rare and, to date, equivocal (Beaulieu *et al.* 2011; Ballen *et al.* 2012; Badás *et al.* 2015; Boonekamp *et al.* 2017; Taff & Freeman-Gallant 2017). In this thesis I aimed to address these key shortfalls in our understanding by using extensive longitudinal sampling of telomere length in a population of cooperatively breeding white-browed sparrow weavers, *Plocepasser mahali*, in the semi-arid Kalahari Desert. Specifically, I investigated the effects of rainfall and the social environment on nestling telomere length and dynamics (Chapter 3), and the downstream implications of these telomere measures for nestling survival over their first year of life (Chapter 2). I then moved on to assess the determinants of both long- and short-term telomere dynamics in adulthood, in particular the effects of dominance status and rainfall-related reproductive activity (Chapter 4). Finally, I investigated the extent to which natural variation in oxidative state in the wild predicts variation in within-individual rates of change in telomere length (Chapter 5). In this chapter I discuss wider implications of the work that bridge across the findings of the different chapters and suggest potentially fruitful areas of further research.

6.2 Telomere measures as biomarkers of somatic maintenance

Throughout this thesis I used measures of both telomere length and telomere dynamics (within-individual rates of change in telomere length over time) as candidate biomarkers of somatic maintenance. Investigations of telomere *dynamics* typically yielded results that concorded with predictions, lending support to view that it constituted a useful biomarker of patterns of somatic maintenance. In Chapter 3 I found that, as predicted, nestlings raised by parental pairs with few or no helpers have faster rates of telomere attrition. The downstream implications of being reared by a small group may be severe, as in Chapter 2 I found evidence that nestlings that experience higher rates of telomere attrition are less likely to survive to the following season, even after controlling for the effects of variation in body mass. In addition, I found evidence in Chapter 4 suggestive of a negative impact of reproduction-related activity on within-individual rates of change in telomere length, consistent with the often-predicted trade-off between reproduction and somatic maintenance that is central to life-history theory. Finally, in Chapter 5 I found that natural variation in oxidative state predicts telomere dynamics in these free-living birds, though unexpected complexities were revealed in the nature of the relationships between oxidative state and telomere dynamics. My results suggest that behavioural or physiological variation among individuals or classes (or indeed variation in optimal resource allocation strategies) may lead to class-dependent effects of oxidative state on telomere attrition.

While the above results suggest that telomere dynamics do reflect levels of somatic maintenance, my analyses also revealed an unexpected frequency of apparent within-individual *increases* in telomere length over time, which have the potential to complicate the association between telomere dynamics and somatic maintenance. Telomere attrition is often found to be fastest in early life (Salomons *et al.* 2009; Heidinger *et al.* 2012; Asghar *et al.* 2015), yet in Chapter 3 I report that the mean within-individual change in telomere length of nestlings over the course of the early developmental period (roughly 4 to 12 days of age) was statistically no different to zero, whilst in the late developmental period (roughly 12 to 30 days of age) the mean within-individual change

in telomere length was only significantly less than zero in nestlings raised by unassisted pairs. Furthermore, I also found no net within-individual change in telomere length with age in adults (Chapter 4). These results add to a growing number of studies that have reported both increases and decreases in telomere length (Fairlie *et al.* 2016; Hatakeyama *et al.* 2016; Hoelzl *et al.* 2016b; Ujvari *et al.* 2016), and call into question the broadly held assumption that the mean telomere lengths of whole organisms can only decrease with increasing age (Steenstrup *et al.* 2013). Further investigation is therefore needed into (i) whether these increases are reflective of true extension of telomeres or driven by other processes such as stem-cell dropout (see Chapter 5 discussion) and (ii) if the former is true, the extent to which telomere elongation by telomerase is associated with the maintenance of other cellular components that might thereby maintain the coupling of telomere dynamics to patterns of somatic maintenance regardless (again, see Chapter 5 discussion).

Aside from the potential complexity regarding the implications of telomere lengthening, my results suggest that telomere dynamics are a better marker of somatic maintenance than telomere *length*, as I found only one result utilising telomere length that concurred with my predictions regarding patterns of somatic maintenance (that rainfall prior to egg laying positively predicted nestling telomere length; Chapter 3). In every other case we either found no relationship between the focal predictors and variation in telomere length, or effects that ran directly counter to my expectations. Noise generated by genetic and epigenetic sources of among-individual variation in telomere length could explain the absence of the predicted relationships with telomere length in Chapters 2 and 4. In Chapter 2 I found no apparent downstream survival cost to older nestlings of having short telomeres, despite apparent costs of having suffering accelerated telomere attrition. In Chapter 4 I found no overall difference between the telomere lengths of dominant and subordinate birds in adulthood.

However, noise alone cannot readily account for the two unexpected relationships that I did find, which ran counter to both my predictions regarding patterns of somaitc maintenance *and* my findings regarding within-individual telomere dynamics results.

In Chapter 2 I found that hatchlings with long telomeres had *poorer* survival prospects than those with shorter telomeres, while in Chapter 4 my results were suggestive of the selective disappearance of birds with *long* telomeres (either through mortality or dispersal). If these counterintuitive relationships reflect genuine relationships in nature, they could reflect (i) novel biological phenomena relating to somatic maintenance that have yet to be widely recognised and/or (ii) relationships that arise from associations between the variables of interest and other confounding factors. First, it is conceivable, for example, that heavier investment in somatic maintenance during early development could leave some hatchlings with longer telomeres but consequently *poorer* body reserves with which to weather subsequent resource shortages during growth, leaving them *more* likely to starve. While in adults, long telomeres could be indicative of higher quality birds that might consequently engage in a higher risk life-history given the potential for higher rewards (e.g. increased prospecting for dominance acquisition, or increased likelihood to make long distance dispersals). On the other hand, both counterintuitive results could conceivably be a product of associations with confounding variables. For example, in Chapter 3 I found that rainfall prior to egg laying positively predicted hatchling telomere length, and so if such pre-laying rainfall also increases predator activity at this time (e.g. by stimulating predator reproduction in this arid environment), this could conceivably leave hatchlings with long telomeres being more likely to suffer predation. In addition, parental age effects on offspring telomere length could also conceivably have generated these counterintuitive relationships in both nestlings and adults, as parental age has been shown to predict offspring telomere length (Njajou *et al.* 2007; De Meyer *et al.* 2007; Olsson *et al.* 2011), and can also be a key determinant of the quality of care received by offspring (Tardif *et al.* 1984; Weladji *et al.* 2006) which could impact survival prospects independent of telomere length. Investigation of parental age effects on offspring telomere length in white-browed sparrow weavers would certainly constitute one fruitful future avenue of research in this species.

As such, my findings lend strong support to previous calls for caution when interpreting cross-sectional studies of telomere length (Monaghan & Haussmann 2006; Nussey *et al.*

2013), and highlight the importance instead of conducting longitudinal studies of within-individual changes in telomere length over time when seeking to understand the causes (Chapter 3 and 4) and fitness consequences (Chapter 2) of variation in somatic maintenance. Previous studies have found that the selective disappearance of individuals with short telomeres has the potential to leave cross-sectional studies of age-related changes in telomere length reaching erroneous conclusions that telomeres do not get shorter as individuals age (Haussmann & Mauck 2008; Salomons *et al.* 2009; Beirne *et al.* 2014). My findings, uniquely to our knowledge, suggest that the opposite problem can also arise, as my analyses of population level variation in telomere length in adulthood (Chapter 5) suggest declines in telomere length with age, yet I found no evidence of such an effect *within* individuals. This gives particular cause for concern given the pervasive use of cross sectional studies of age-related changes in telomere length (particularly in biomedical literature; Valdes *et al.* 2005; van der Harst *et al.* 2007; Zhu *et al.* 2011)van der Harst, valdes et al, zhu et al, which may therefore erroneously attribute population level declines to within individual effects. Solely cross-sectional studies of telomere length should therefore be avoided wherever possible, and interpreted with extreme caution.

6.3 Social environment effects on telomere dynamics

Aspects of social behaviour, particularly in highly social species, have the potential for marked impacts on patterns of somatic maintenance. For example, in cooperatively breeding species, subordinate helpers may both lighten the workloads of dominant breeders and / or increase the total care provided to offspring (Hatchwell 1999). In so doing, subordinate helpers may free up resources for dominant birds and nestlings to invest to a greater extent in somatic maintenance (see Chapter 1). Indeed, in Chapter 3 I provide evidence for the first time, to our knowledge, that the telomere attrition rates of nestlings may indeed be attenuated by the actions of subordinate helpers. Future studies should now seek to experimentally test the causality of this relationship, and investigate the extent to which such effects arise via the mitigation of oxidative or endocrine stress in nestlings. With regard to the effect of workload-lightening in adults,

there is already evidence in white-browed sparrow weavers to suggest that workload-lightening by helpers reduces exposure to oxidative stress during reproductive periods (Cram *et al.* 2015a, b). However, I found no apparent effects of group size on adult telomere length or dynamics (Chapter 4). My findings in Chapter 5 highlight the possibility that the effects of oxidative state on telomere dynamics may depend on the sex or dominance status of the focal individual in question. It is therefore possible that the oxidative benefits of raising offspring in larger groups (Cram *et al.* 2015b) only result in reduced telomere attrition rate in some classes of individual. Alternatively, any beneficial effects of group size on oxidative state during reproductive episodes may be too short-lived to impact telomere dynamics over the timescales monitored here. Investigation of group size effects over shorter periods of time (e.g. over the course of a breeding attempt) may clarify whether this is the case, though such an approach would likely require the employment of higher resolution methods for assessing changes in an individual's distribution of telomere lengths.

Competition between dominant and subordinate individuals over rank and reproduction has the potential to entail energetic costs and social stress that may increase exposure to oxidative stress and thereby hasten the accumulation of somatic damage among either subordinates (Abbott *et al.* 2003; Young *et al.* 2006; Sharp & Clutton-Brock 2011) or dominants (Creel *et al.* 1996; Bell *et al.* 2012). However, I found no evidence of a difference between dominants and subordinates either in mean telomere length or in long-term telomere dynamics (Chapter 4), despite dominants completely monopolising reproduction (Harrison *et al.* 2013a) and prior evidence of differential declines in antioxidant protection levels in dominant females over the course of the breeding season (Cram *et al.* 2015c). Rainfall, which reflects the levels of reproduction-related activity by all classes of individual in this arid-zone bird (see Chapter 5), negatively predicted the within-individual rate of change in telomere length in both dominant and subordinate birds, but the relationship was more negative in subordinates. One potential explanation is that competitive advantages associated with social dominance afford dominant birds differential access to resources which may in turn alleviate resource allocation trade-offs between investment in rainfall-related

activities and somatic maintenance. The higher rates of telomere attrition in subordinates in wet years may potentially be due to costs arising from rain-related increases in the conduct of extra-territorial prospecting forays by subordinates (Young *et al.* 2005, 2007; Young & Monfort 2009). The lack of any clear divergence in the telomere dynamics of dominants and subordinates could also be attributable in part to subordinates in this species apparently exercising complete reproductive restraint (due in part to their typically close relatedness to their dominants; Harrison *et al.* 2013a), such that overt reproductive conflict between dominants and subordinates, of the type that could yield somatic maintenance costs to one or both parties (see above), appears to be rare. Other social species with more complex kin structures, more frequent subordinate reproduction and clearer evidence of overt conflict between dominants and subordinates (Young et al 2006; Creel et al. 1996; Bell et al 2012) might therefore ultimately be found to show strikingly different rank-related patterns of telomere dynamics to those detected here.

Despite a lack of differences in telomere dynamics between dominants and subordinates over the *long*-term, I did find evidence suggestive of rank-related differences in telomere dynamics over shorter periods, with dominant birds appearing to better maintain their telomeres. To our knowledge, this is the first time that associations between dominance status and within-individual telomere dynamics have been found. These findings (that telomere length regulation but not telomere length *per se* differs between classes) concord with results from eusocial insects where queens do not have longer telomeres than workers (Jemielity *et al.* 2007; Korandová & Frydrychová 2016), but honey bee queens do have much higher telomerase activity than workers (Korandová & Frydrychová 2016). Future research into the effects of telomerase on telomere dynamics and other aspects of somatic maintenance, and the regulation of telomerase expression in different classes of individual may therefore shed light on these intriguing results.

References

Aamodt RM (2009) Age-and caste-dependent decrease in expression of genes maintaining DNA and RNA quality and mitochondrial integrity in the honeybee wing muscle. *Experimental Gerontology*, **44**, 586–593.

Abbott D., Keverne E., Bercovitch F. *et al.* (2003) Are subordinates always stressed? a comparative analysis of rank differences in cortisol levels among primates. *Hormones and Behavior*, **43**, 67–82.

Adaikalakoteswari A, Balasubramanyam M, Mohan V (2005) Telomere shortening occurs in Asian Indian Type 2 diabetic patients. *Diabetic Medicine*, **22**, 1151–1156.

Ahmed S, Passos JF, Birket MJ *et al.* (2008) Telomerase does not counteract telomere shortening but protects mitochondrial function under oxidative stress. *Journal of Cell Science*, **121**, 1046–1053.

Allsopp RC, Chang E, Kashefi-Aazam M *et al.* (1995) Telomere shortening is associated with cell division in vitro and in vivo. *Experimental cell research*, **220**, 194–200.

Alonso-Alvarez C, Bertrand S, Faivre B, Chastel O, Sorci G (2007a) Testosterone and oxidative stress: the oxidation handicap hypothesis. *Proceedings. Biological sciences / The Royal Society*, **274**, 819–825.

Alonso-Alvarez C, Bertrand S, Faivre B, Sorci G (2007b) Increased susceptibility to oxidative damage as a cost of accelerated somatic growth in zebra finches. *Functional Ecology*, **21**, 873–879.

Altizer S, Nunn CL, Thrall PH *et al.* (2003) Social Organization and Parasite Risk in Mammals: Integrating Theory and Empirical Studies. *Annual Review of Ecology, Evolution, and Systematics*, **34**, 517–547.

Angelier F, Vleck CM, Holberton RL, Marra PP (2013) Telomere length, non-breeding habitat and return rate in male American redstarts. *Functional Ecology*, **27**, 342–350.

Angilletta MJ, Sears MW (2000) The metabolic cost of reproduction in an oviparous lizard. *Functional Ecology*, **14**, 39–45.

Arai Y, Martin-Ruiz CM, Takayama M *et al.* (2015) Inflammation, but not telomere length, predicts successful ageing at extreme old age: A longitudinal study of semi-supercentenarians. *EBioMedicine*, **2**, 1549–1558.

Archie E a, Altmann J, Alberts SC (2012) Social status predicts wound healing in wild baboons. *Proceedings of the National Academy of Sciences of the United States of America*, **109**, 9017–22.

Archie EA, Altmann J, Alberts SC (2014) Costs of reproduction in a long-lived female primate: Injury risk and wound healing. *Behavioral Ecology and Sociobiology*, **68**, 1183–1193.

Armanios M, Alder JK, Parry EM *et al.* (2009) Short Telomeres are Sufficient to Cause the Degenerative Defects Associated with Aging. *American Journal of Human Genetics*, **85**, 823–832.

Armstrong L, Saretzki G, Peters H *et al.* (2005) Overexpression of telomerase confers growth advantage, stress resistance, and enhanced differentiation of ESCs toward the hematopoietic lineage. *Stem Cells*, **23**, 516–529.

Asghar M, Hasselquist D, Hansson B *et al.* (2015) Hidden costs of infection: Chronic malaria accelerates telomere degradation and senescence in wild birds. *Science*, **347**, 436–438.

Badás EP, Martínez J, Rivero de Aguilar Cachafeiro J *et al.* (2015) Ageing and reproduction: antioxidant supplementation alleviates telomere loss in wild birds. *Journal of evolutionary biology*, **28**, 896–905.

Baerlocher GM, Rice K, Vulto I, Lansdorp PM (2007) Longitudinal data on telomere length in leukocytes from newborn baboons support a marked drop in stem cell turnover around 1 year of age. *Aging cell*, **6**, 121–3.

Baird DM, Rowson J, Wynford-Thomas D, Kipling D (2003) Extensive allelic variation and ultrashort telomeres in senescent human cells. *Nature genetics*, **33**, 203–7.

Bakaysa SL, Mucci LA, Slagboom PE *et al.* (2007) Telomere length predicts survival independent of genetic influences. *Aging cell*, **6**, 769–74.

Ballen C, Healey M, Wilson M (2012) Predictors of telomere content in dragon lizards. , 661–664.

Barrett ELB, Boner W, Mulder E *et al.* (2012) Absolute standards as a useful addition to the avian quantitative PCR telomere assay. *Journal of Avian Biology*, **43**, 571–576.

Barrett ELB, Burke TA, Hammers M, Komdeur J, Richardson DS (2013) Telomere length and dynamics predict mortality in a wild longitudinal study. *Molecular ecology*, **22**, 249–59.

Barrett ELB, Richardson DS (2011) Sex differences in telomeres and lifespan. *Aging cell*, **10**, 913–21.

Barrientos KS, Kendellen MF, Freibaum BD *et al.* (2008) Distinct Functions of POT1 at Telomeres. *Molecular and Cellular Biology*, **28**, 5251–5264.

Barton K (2016) MuMIn: Multi-Model Inference. *R package version 1.15.6*.

Barton RA, Whiten A (1993) Feeding competition among female Olive baboons, Papio anubis. *Animal Behaviour*, **46**, 777–789.

Bates D, Mächler M, Bolker B, Walker S (2014) Fitting Linear Mixed-Effects Models using lme4. *eprint arXiv:1406.5823*, 51.

Bateson M, Nettle D (2016) The telomere lengthening conundrum - it could be biology. *Aging Cell*, **2016**, 1–8.

Bauch C, Becker PH, Verhulst S (2013) Telomere length reflects phenotypic quality and costs of reproduction in a long-lived seabird. *Proceedings. Biological sciences / The Royal Society*, **280**, 20122540.

Beaulieu M, Mboumba S, Willaume E, Kappeler PM, Charpentier MJE (2014) The oxidative cost of

unstable social dominance. *The Journal of experimental biology*, **217**, 2629–32.

Beaulieu M, Reichert S, Le Maho Y, Ancel A, Criscuolo F (2011) Oxidative status and telomere length in a long-lived bird facing a costly reproductive event. *Functional Ecology*, **25**, 577–585.

Bebbington K, Kingma SA, Fairfield EA *et al.* (2017) Consequences of sibling rivalry vary across life in a passerine bird. *Behavioral Ecology*, **28**, 407–418.

Bebbington K, Spurgin LG, Fairfield EA *et al.* (2016) Telomere length reveals cumulative individual and transgenerational inbreeding effects in a passerine bird. *Molecular Ecology*, **25**, 2949–2960.

Becker PJJ, Reichert S, Zahn S *et al.* (2015) Mother-offspring and nest-mate resemblance but no heritability in early-life telomere length in white-throated dippers. *Proceedings of the Royal Society B: Biological Sciences*, Early view.

Beehner JC, Phillips-Conroy JE, Whitten PL (2005) Female testosterone, dominance rank, and aggression in an Ethiopian population of hybrid baboons. *American Journal of Primatology*, **67**, 101–119.

Beerman I, Seita J, Inlay MA, Weissman IL, Rossi DJ (2014) Quiescent hematopoietic stem cells accumulate DNA damage during aging that is repaired upon entry into cell cycle. *Cell Stem Cell*, **15**, 37–50.

Beery AK, Lin J, Biddle JS *et al.* (2012) Chronic stress elevates telomerase activity in rats. *Biology letters*, 1063–1066.

Beirne C, Delahay R, Hares M, Young A (2014) Age-related declines and disease-associated variation in immune cell telomere length in a wild mammal. *PloS one*, **9**, e108964.

Beirne C, Delahay R, Young A (2015) Sex differences in senescence : the role of intra-sexual competition in early adulthood.

Beirne C, Waring L, Mcdonald RA, Delahay R, Young A (2016) Age-related declines in immune response in a wild mammal are unrelated to immune cell telomere length. *Proceedings of the Royal Society B*, **283**, 20152949.

Bell MB V, Nichols HJ, Gilchrist JS, Cant MA, Hodge SJ (2012) The cost of dominance : suppressing subordinate reproduction affects the reproductive success of dominant female banded mongooses. , 619–624.

Bianchi A, Stansel RM, Fairall L *et al.* (1999) TRF1 binds a bipartite telomeric site with extreme spatial flexibility. *EMBO Journal*, **18**, 5735–5744.

Bischoff C, Petersen HC, Graakjaer J *et al.* (2006) No Association Between Telomere Length and Survival Among the Elderly and Oldest Old. *Epidemiology*, **17**, 190–194.

Bize P, Criscuolo F, Metcalfe NB, Nasir L, Monaghan P (2009) Telomere dynamics rather than age predict life expectancy in the wild. *Proceedings. Biological sciences / The Royal Society*, **276**, 1679–83.

Bize P, Devevey G, Monaghan P, Doligez B, Christe P (2008) Fecundity and Survival in Relation to

Resistance to Oxidative Stress in a Free-Living Bird. *Ecology*, **89**, 2584–2593.

Blackburn EH (1991) Structure and function of telomeres. *Nature*, **350**, 569–73.

Blackburn EH (2000) Telomere states and cell fates. *Nature*, **408**, 53–56.

Blackburn EH, Epel ES, Lin J (2015) Human telomere biology: A contributory and interactive factor in aging, disease risks, and protection. *Science*, **350**, 1193–1198.

Blackburn EH, Szostak JW (1984) The Molecular Structure of Centromeres and Telomeres. *Annual Review of Biochemistry*, **53**, 163–194.

Blasco M a (2007) Telomere length, stem cells and aging. *Nature chemical biology*, **3**, 640–9.

De Block M, Stoks R (2008) Compensatory growth and oxidative stress in a damselfly. *Proceedings. Biological sciences / The Royal Society*, **275**, 781–5.

Blount JD, Vitikainen EIK, Stott I, Cant MA (2016) Oxidative shielding and the cost of reproduction. *Biological Reviews*, **91**, 483–497.

Bonduriansky R, Maklakov a., Zajitschek F, Brooks R (2008) Sexual selection, sexual conflict and the evolution of ageing and life span. *Functional Ecology*, **22**, 443–453.

Boonekamp JJ, Bauch C, Mulder E, Verhulst S (2017) Does oxidative stress shorten telomeres? *Biology Letters*, **13**, 20170164.

Boonekamp JJ, Mulder GA, Salomons M, Dijkstra C, Verhulst S (2014a) Nestling telomere shortening , but not telomere length, reflects developmental stress and predicts survival in wild birds. *Proceedings of the Royal Society B*, **281**.

Boonekamp JJ, Salomons M, Bouwhuis S, Dijkstra C, Verhulst S (2014b) Reproductive effort accelerates actuarial senescence in wild birds: an experimental study (G Sorci, Ed,). *Ecology Letters*, n/a-n/a.

Boonekamp JJ, Simons MJP, Hemerik L, Verhulst S (2013) Telomere length behaves as biomarker of somatic redundancy rather than biological age. *Aging cell*, **12**, 330–2.

Bouwhuis S Telomere attrition and growth: a life-history framework and case study in common terns. *Journal of evolutionary biology*.

Boxall MC, Goodship THJ, Brown AL, Ward MC, Von Zglinicki T (2006) Telomere shortening and haemodialysis. *Blood Purification*, **24**, 185–189.

Broer L, Codd V, Nyholt DR *et al.* (2013) Meta-analysis of telomere length in 19 713 subjects reveals high heritability, stronger maternal inheritance and a paternal age effect. *European Journal of Human Genetics*, **21**, 1163–1168.

Brouwer L, Richardson DS, Eikenaar C, Komdeur J (2006) The role of group size and environmental factors on survival in a cooperatively breeding tropical passerine. *The Journal of animal ecology*, **75**, 1321–9.

Brouwer L, Richardson DS, Komdeur J (2012) Helpers at the nest improve late-life offspring performance: Evidence from a long-term study and a cross-foster experiment. *PLoS ONE*, **7**, 16–20.

Bryant DM (1988) Energy Expenditure and Body Mass Changes as Measures of Reproductive Costs in Birds. *Functional Ecology*, **2**, 23–34.

Burnham KP, Anderson DR (2002) *Model selection and multimodel inference: A practical information-theoretic approach.*

Burrows CJ, Muller JG (1998) Oxidative Nucleobase Modifications Leading to Strand Scission. *Chemical Reviews*, **98**, 1109–1152.

Campisi J (2005) Senescent cells, tumor suppression, and organismal aging: Good citizens, bad neighbors. *Cell*, **120**, 513–522.

Campisi J, d'Adda di Fagagna F (2007) Cellular senescence: when bad things happen to good cells. *Nature reviews. Molecular cell biology*, **8**, 729–40.

Cant M a, Hodge SJ, Bell MB V, Gilchrist JS, Nichols HJ (2010) Reproductive control via eviction (but not the threat of eviction) in banded mongooses. *Proceedings. Biological sciences / The Royal Society*, **277**, 2219–26.

Caprioli M, Romano M, Romano a. *et al.* (2013) Nestling telomere length does not predict longevity, but covaries with adult body size in wild barn swallows. *Biology Letters*, **9**, 20130340–20130340.

Carey JR (2001) Demographic mechanisms for the evolution of long life in social insects. *Experimental gerontology*, **36**, 713–22.

Cattan V, Mercier N, Gardner JP *et al.* (2008) Chronic oxidative stress induces a tissue-specific reduction in telomere length in CAST/Ei mice. *Free radical biology & medicine*, **44**, 1592–8.

Cawthon RM (2002) Telomere measurement by quantitative PCR. , **30**, 1–6.

Cawthon RM, Smith KR, O'Brien E, Sivatchenko A, Kerber R a (2003) Association between telomere length in blood and mortality in people aged 60 years or older. *Lancet*, **361**, 393–5.

Cesare AJ, Reddel RR (2013) Alternative Lengthening of Telomeres in Mammalian Cells. , 598–610.

Chaudière J, Ferrari-Iliou R (1999) Intracellular antioxidants: From chemical to biochemical mechanisms. *Food and Chemical Toxicology*, **37**, 949–962.

Cherkas LF, Aviv a, Valdes a M *et al.* (2006) The effects of social status on biological aging as measured by white-blood-cell telomere length. *Aging cell*, **5**, 361–5.

Cichon M, Dubiec A, Chadzinska M (2001) The effect of elevated reproductive effort on humoral immune function in collared flycatcher females. *Acta Oecologica-International Journal of Ecology*, **22**, 71–76.

Clutton-Brock TH, Gaynor D, Mcilrath GM *et al.* (1999) Predation, Group Size and Mortality in a Cooperative Mongoose, Suricata suricata. *Journal of Animal Ecology*, **68**, 672–683.

Clutton-Brock TH, Isvaran K (2007) Sex differences in ageing in natural populations of vertebrates. *Proceedings. Biological sciences / The Royal Society*, **274**, 3097–104.

Clutton-Brock TH, Russell AF, Sharpe LL *et al.* (2001) Effects of Helpers on Juvenile Development and Survival in Meerkats. *Science*, **293**, 2446–2449.

Cockburn A (1998) Evolution of helping in cooperatively breeding birds. *Annual review pf ecology and systematics*, **29**, 141–177Cockburn, Andrew.

Cohen A, Klasing K, Ricklefs R (2007) Measuring circulating antioxidants in wild birds. *Comparative Biochemistry and Physiology - B Biochemistry and Molecular Biology*, **147**, 110–121.

Collias NE, Collias EC (1978) Cooperative Breeding Behavior in the White-Browed Sparrow Weaver. *The Auk*, **95**, 472–484.

Collins K, Mitchell JR (2002) Telomerase in the human organism. *Oncogene*, **21**, 564–579.

Coluzzi E, Colamartino M, Cozzi R *et al.* (2014) Oxidative stress induces persistent telomeric DNA damage responsible for nuclear morphology change in mammalian cells. *PLoS ONE*, **9**.

Coppé J-P, Desprez P-Y, Krtolica A, Campisi J (2010) The senescence-associated secretory phenotype: the dark side of tumor suppression. *Annual review of pathology*, **5**, 99–118.

Costantini D (2008) Oxidative stress in ecology and evolution: Lessons from avian studies. *Ecology Letters*, **11**, 1238–1251.

Costantini D, Bonisoli-Alquati A, Rubolini D *et al.* (2014) Nestling rearing is antioxidant demanding in female barn swallows (Hirundo rustica). *Naturwissenschaften*, **101**, 541–548.

Costantini D, Marasco V, Moller AP (2011) A meta-analysis of glucocorticoids as modulators of oxidative stress in vertebrates. *Journal of comparative phy*, **181**, 447–456.

Costanzo A, Parolini M, Bazzi G *et al.* (2016) Brood size, telomere length, and parent-offspring color signaling in barn swallows. *Behavioral Ecology*, **0**, arw147.

Courchamp F, Grenfell BT, Clutton-Brock TH (2000) Impact of natural enemies on obligately cooperative breeders. *Oikos*, **91**, 311–322.

Cowlishaw G (1991) Dominance rank and mating success in male primates. *Animal Behaviour*, 1045–1056.

Cram DL, Blount JD, York JE, Young AJ (2015a) Immune Response in a Wild Bird Is Predicted by Oxidative Status , but Does Not Cause Oxidative Stress. *PloS one*, **10**, 1–14.

Cram DL, Blount JD, Young AJ (2015b) The oxidative costs of reproduction are group-size dependent in a wild cooperative breeder. *Proceedings of the Royal Society B*, **282**.

Cram DL, Blount JD, Young AJ (2015c) Oxidative status and social dominance in a wild cooperative breeder (J Grindstaff, Ed,). *Functional Ecology*, **29**, 229–238.

Creel S (2001) Social dominance and stress hormones. *Trends in Ecology & Evolution*, **16**, 491–497.

Creel S, Marusha N, Monfort SL (1996) Social stress and dominance. *Nature*, 212.

Criscuolo F, Bize P, Nasir L *et al.* (2009a) Real-time quantitative PCR assay for measurement of avian telomeres. *Journal of Avian Biology*, **40**, 342–347.

Criscuolo F, Bize P, Nasir L *et al.* (2009b) Real-time quantitative PCR assay for measurement of avian telomeres. *Journal of Avian Biology*, **40**, 342–347.

Dammann P, Burda H (2006) Sexual activity and reproduction delay ageing in a mammal. *Current*

Biology, **16**, 117–118.

Dammann P, Šumbera R, Massmann C, Scherag A, Burda H (2011) Extended longevity of reproductives appears to be common in Fukomys mole-rats (Rodentia, Bathyergidae). *PloS one*, **6**, e18757.

Dawson DA, Brekke P, Dos Remedios N, Horsburgh GJ (2015) A marker suitable for sex-typing birds from degraded samples. *Conservation Genetics Resources*, **7**, 337–343.

Deerenberg C, Arpanius V, Daan S, Bos N (1997) Reproductive effort decreases antibody responsiveness. *Proceedings of the Royal Society B-Biological Sciences*, **264**, 1021–1029.

Delany ME, Daniels LM, Swanberg SE, Taylor HA (2003) Telomeres in the Chicken : Genome Stability and Chromosome Ends. *Poultry science*, **82**, 917–926.

Denchi EL, de Lange T (2007) Protection of telomeres through independent control of ATM and ATR by TRF2 and POT1. *Nature*, **448**, 1068–1071.

Deutsch CJ, Haley MP, Le Boeuf BJ (1990) Reproductive effort of male northern elephant seals: estimates from mass loss. *Canadian Journal of Zoology*, **68**, 2580–2593.

Dickinson JL, Hatchwell BJ (2004) Fitness Consequences of Helping. In: *Ecology and Evolution of Cooperative Breeding in Birds* (eds Koenig W, Dickinson J), p. . Cambridge University Press, Cambridge.

Ding H, Schertzer M, Wu X *et al.* (2004) Regulation of murine telomere length by Rtel: An essential gene encoding a helicase-like protein. *Cell*, **117**, 873–886.

DuRant SE, Hepp GR, Moore IT, Hopkins BC, Hopkins WA (2010) Slight differences in incubation temperature affect early growth and stress endocrinology of wood duck (Aix sponsa) ducklings. *The Journal of Experimental Biology*, **213**, 45–51.

DuRant SE, Hopkins WA, Hawley DM, Hepp GR (2012) Incubation temperature affects multiple measures of immunocompetence in young wood ducks (Aix Sponsa). *Biology letters*, **8**, 108–11.

Emlen ST (1991) Evolution of cooperative breeding in birds and mammals J.R. Krebs. In: *Behavioural Ecology: an Evolutionary Approach* (eds Krebs JR, Davies NB), pp. 301–337. Blackwell Science, Oxford, UK.

Epel ES, Merkin SS, Cawthon R *et al.* (2009) The rate of leukocyte telomere shortening predicts mortality from cardiovascular disease in elderly men. *Aging (Albany.NY)*, **1**, 81–88.

Evans MR, Goldsmith AR, Norris SR (2015) The effects of testosterone on antibody production and plumage coloration in male house sparrows (Passer domesticus). *Behavioral Ecology and Sociobiology*, **47**, 156–163.

Fairlie J, Holland R, Pilkington JG *et al.* (2016) Lifelong leukocyte telomere dynamics and survival in a free-living mammal. *Aging Cell*, **15**, 140–148.

Feldser DM, Greider CW (2007) Short Telomeres Limit Tumor Progression In Vivo by Inducing Senescence. *Cancer Cell*, **11**, 461–469.

Finkel T, Holbrook NJ (2000) Oxidants, oxidative stress and the biology of ageing. *Nature*, **408**, 239–

247.

Foote CG, Daunt F, Gonzalez-Solis J *et al.* (2010) Individual state and survival prospects: age, sex, and telomere length in a long-lived seabird. *Behavioral Ecology*, **22**, 156–161.

Foote CG, Gault E a., Nasir L, Monaghan P (2011) Telomere dynamics in relation to early growth conditions in the wild in the lesser black-backed gull. *Journal of Zoology*, **283**, 203–209.

Foote CG, Vleck D, Vleck CM (2013) Extent and variability of interstitial telomeric sequences and their effects on estimates of telomere length. *Molecular ecology resources*.

Fox J, Weisberg S (2011) *An {R} companion to applied regression*. Sage, Thousand Oaks CA.

Fu W, Killen M, Culmsee C *et al.* (2000) The catalytic subunit of telomerase is expressed in developing brain neurons and serves a cell survival-promoting function. *Journal of molecular neuroscience : MN*, **14**, 3–15.

Furumoto K, Inoue E, Nagao N, Hiyama E, Miwa N (1998) Age-dependent telomere shortening is slowed down by enrichment of intracellular vitamin C via suppression of oxidative stress. *Life Sciences*, **63**, 935–948.

Garratt M, Brooks RC (2012) Oxidative stress and condition-dependent sexual signals: more than just seeing red. *Proceedings of the Royal Society B: Biological Sciences*, **279**, 3121–3130.

Geiger S, Le Vaillant M, Lebard T *et al.* (2012) Catching-up but telomere loss: half-opening the black box of growth and ageing trade-off in wild king penguin chicks. *Molecular ecology*, **21**, 1500–10.

Gilmour KM (2005) Physiological Causes and Consequences of Social Status in Salmonid Fish. *Integrative and Comparative Biology*, **45**, 263–273.

Giordano M, Costantini D, Pick JL, Tschirren B (2015) Female oxidative status, egg antioxidant protection and eggshell pigmentation: a supplemental feeding experiment in great tits. *Behavioral Ecology and Sociobiology*, **69**, 777–785.

Giraudeau M, Friesen CR, Sudyka J *et al.* (2016) Ageing and the cost of maintaining coloration in the Australian painted dragon. *Biology letters*, **12**, 734–738.

Goldman F, Bouarich R, Kulkarni S *et al.* (2005) The effect of TERC haploinsufficiency on the inheritance of telomere length. *Proceedings of the National Academy of Sciences of the United States of America*, **102**, 17119–24.

Greider CW (2016) Regulating telomere length from the inside out: The replication fork model. *Genes and Development*, **30**, 1483–1491.

Greider CW, Blackburn EH (1985) Identification of a specific telomere terminal transferase activity in tetrahymena extracts. *Cell*, **43**, 405–413.

Griffith JD, Comeau L, Rosenfield S *et al.* (1999) Mammalian telomeres end in a large duplex loop. *Cell*, **97**, 503–514.

Grunstein M (1997) Molecular model for telomeric heterochromatin in yeast. *Current Opinion in Cell Biology*, **9**, 383–387.

Habig B, Archie EA (2015) Social status, immune response and parasitism in males: a meta-analysis. *Philosophical Transactions of the Royal Society B*, **370**, 20140109.

Haddad LS, Kelbert L, Hulbert AJ (2007) Extended longevity of queen honey bees compared to workers is associated with peroxidation-resistant membranes. *Experimental Gerontology*, **42**, 601–609.

Haendeler J, Hoffmann J, Brandes RP *et al.* (2003) Hydrogen Peroxide Triggers Nuclear Export of Telomerase Reverse Transcriptase via Src Kinase Family-Dependent Phosphorylation of Tyrosine 707 Hydrogen Peroxide Triggers Nuclear Export of Telomerase Reverse Transcriptase via Src Kinase Family-Dependent Pho. , **23**, 4598–4610.

Hall ME, Nasir L, Daunt F *et al.* (2004) Telomere loss in relation to age and early environment in long-lived birds. *Proceedings. Biological sciences / The Royal Society*, **271**, 1571–6.

Halliwell B, Gutteridge JMC (2007a) *Free radicals in biology and medecine*. Oxford University Press, Oxford, UK.

Halliwell B, Gutteridge JMC (2007b) Antioxidant Defences Synthesized in vivo. In: *Free Radical Biology and Medicine*, pp. 77–151. Oxford University Press, Oxford, UK.

Halvorsen O (1986) On the Relationship between Social Status of Host and Risk of Parasitic Infection. *Oikos*, **47**, 71–74.

Hammers M, Richardson DS, Burke T, Komdeur J (2013) The impact of reproductive investment and early-life environmental conditions on senescence: Support for the disposable soma hypothesis. *Journal of Evolutionary Biology*, **26**, 1999–2007.

Hanssen SA, Hasselquist D, Folstad I, Erikstad KE (2005) Cost of reproduction in a long-lived bird: incubation effort reduces immune function and future reproduction. *Proceedings of the Royal Society B: Biological Sciences*, **272**, 1039–1046.

Hao LY, Armanios M, Strong MA *et al.* (2005) Short telomeres, even in the presence of telomerase, limit tissue renewal capacity. *Cell*, **123**, 1121–1131.

Harley CB, Futcher AB, Greider CW (1990) Telomeres shorten during ageing of human fibroblasts. *Nature*, **345**, 458–460.

Harris SE, Deary IJ, MacIntyre A *et al.* (2006) The association between telomere length, physical health, cognitive ageing, and mortality in non-demented older people. *Neuroscience Letters*, **406**, 260–264.

Harrison X a., York JE, Cram DL, Hares MC, Young AJ (2013a) Complete reproductive skew within white-browed sparrow weaver groups despite outbreeding opportunities for subordinates of both sexes. *Behavioral Ecology and Sociobiology*, **67**, 1915–1929.

Harrison X a, York JE, Cram DL, Young a J (2013b) Extra-group mating increases inbreeding risk in a cooperatively breeding bird. *Molecular ecology*.

Harrison XA, York JE, Young AJ (2014) Population genetic structure and direct observations reveal sex-reversed patterns of dispersal in a cooperative. *Molecular Ecology*, **23**, 5740–5755.

Harshman LG, Zera AJ (2007) The cost of reproduction: the devil in the details. *Trends in ecology & evolution*, **22**, 80–6.

van der Harst P, van der Steege G, de Boer RA *et al.* (2007) Telomere Length of Circulating Leukocytes Is Decreased in Patients With Chronic Heart Failure. *Journal of the American College of Cardiology*, **49**, 1459–1464.

Hartmann A, Heinze J (2003) Lay eggs, live longer: Division of labour and life span in a clonal ant species. *Evolution*, **57**, 2424–2429.

Hartmann N, Reichwald K, Lechel A *et al.* (2009) Telomeres shorten while Tert expression increases during ageing of the short-lived fish Nothobranchius furzeri. *Mechanisms of Ageing and Development*, **130**, 290–296.

Hatakeyama H, Yamazaki H, Nakamura KI *et al.* (2016) Telomere attrition and restoration in the normal teleost Oryzias latipes are linked to growth rate and telomerase activity at each life stage. *Aging*, **8**, 62–76.

Hatchwell BJ (1999) Investment Strategies of Breeders in Avian Cooperative Breeding Systems. *The American Naturalist*, **154**, 205–219.

Haussmann MF, Longenecker AS, Marchetto NM, Juliano S a, Bowden RM (2012) Embryonic exposure to corticosterone modifies the juvenile stress response, oxidative stress and telomere length. *Proceedings. Biological sciences / The Royal Society*, **279**, 1447–56.

Haussmann MF, Mauck R a (2008) Telomeres and longevity: testing an evolutionary hypothesis. *Molecular biology and evolution*, **25**, 220–8.

Haussmann MF, Winkler DW, Huntington CE, Nisbet ICT, Vleck CM (2004) Telomerase expression is differentially regulated in birds of differing life span. *Ann. N. Y. Acad. Sci.*, **1019**, 186–190.

Haussmann MF, Winkler DW, Huntington CE, Nisbet ICT, Vleck CM (2007) Telomerase activity is maintained throughout the lifespan of long-lived birds. *Experimental gerontology*, **42**, 610–8.

Haussmann MF, Winkler DW, O'Reilly KM *et al.* (2003) Telomeres shorten more slowly in long-lived birds and mammals than in short-lived ones. *Proceedings. Biological sciences / The Royal Society*, **270**, 1387–92.

Haussmann MF, Winkler DW, Vleck CM (2005) Longer telomeres associated with higher survival in birds. *Biology letters*, **1**, 212–4.

Hayflick L (1965) The limited in vitro lifetime of human diploid cell strains. *Experimental Cell Research*, **37**, 614–636.

Heidinger BJ, Blount JD, Boner W *et al.* (2012) Telomere length in early life predicts lifespan. *Proceedings of the National Academy of Sciences of the United States of America*, **109**, 1743–8.

Hemann MT, Greider CW (2000) Wild-derived inbred mouse strains have short telomeres. *Nucleic acids research*, **28**, 4474–8.

Hemann MT, Rudolph KL, Strong MA *et al.* (2001a) Telomere dysfunction triggers developmentally

regulated germ cell apoptosis. *Molecular Biology of the Cell*, **12**, 2023–2030.

Hemann MT, Strong MA, Hao LY, Greider CW (2001b) The shortest telomere, not average telomere length, is critical for cell viability and chromosome stability. *Cell*, **107**, 67–77.

Henderson ER, Blackburn EH (1989) An overhanging 3' terminus is a conserved feature of telomeres. *Molecular and cellular biology*, **9**, 345–8.

Henle ES, Han Z, Tang N *et al.* (1999) Sequence-specific DNA Cleavage by Fe2+-mediated Fenton Reactions Has Possible Biological Implications. *Journal of Biological Chemistry*, **274**, 962–971.

Henson JD, Neumann AA, Yeager TR, Reddel RR (2008) Alternative lengthening of telomeres in mammalian cells. In: *Origin and Evolution of Telomeres* (eds Nosek J, Tomaska L), pp. 45–57. Landes Bioscience.

Herbig U, Ferreira M, Condel L, Carey D, Sedivy JM (2006) Cellular Senescence in Aging Primates. *Science*, **311**, 1257.

Herbig U, Jobling WA, Chen BPC, Chen DJ, Sedivy JM (2004) Telomere shortening triggers senescence of human cells through a pathway involving ATM, p53, and p21CIP1, but not p16INK4a. *Molecular Cell*, **14**, 501–513.

Herborn KA, Heidinger BJ, Boner W *et al.* (2014) Stress exposure in early post-natal life reduces telomere length : an experimental demonstration in a long-lived seabird. *Proceedings of the Royal Society B*, **281**.

Hodge SJ, Bell MB V, Mwanguhya F *et al.* (2009) Maternal weight, offspring competitive ability, and the evolution of communal breeding. *Behavioral Ecology*, **20**, 729–735.

Hodge SJ, Brown JL, Cant MA *et al.* (2005) Helpers benefit offspring in both the short and long-term in the cooperatively breeding banded mongoose. *Proceedings. Biological sciences / The Royal Society*, **272**, 2479–84.

Hoelzl F, Cornils JS, Smith S, Moodley Y, Ruf T (2016a) Telomere dynamics in free-living edible dormice (Glis glis): the impact of hibernation and food supply. *The Journal of Experimental Biology*, **219**, 2469–2474.

Hoelzl F, Smith S, Cornils JS *et al.* (2016b) Telomeres are elongated in older individuals in a hibernating rodent, the edible dormouse (Glis glis). *Scientific Reports*, **6**, 36856.

Honig LS, Schupf N, Lee JH, Tang MX, Mayeux R (2006) Shorter telomeres are associated with mortality in those with APOE ??4 and dementia. *Annals of Neurology*, **60**, 181–187.

Hõrak P, Cohen A (2010) How to measure oxidative stress in an ecological context: Methodological and statistical issues. *Functional Ecology*, **24**, 960–970.

Houben JMJ, Mercken EM, Ketelslegers HB *et al.* (2009) Telomere shortening in chronic obstructive pulmonary disease. *Respiratory Medicine*, **103**, 230–236.

Houben JMJ, Moonen HJJ, van Schooten FJ, Hageman GJ (2008) Telomere length assessment: biomarker of chronic oxidative stress? *Free radical biology & medicine*, **44**, 235–46.

Inness CLW, Metcalfe NB (2008) The impact of dietary restriction, intermittent feeding and compensatory growth on reproductive investment and lifespan in a short-lived fish. *Proceedings. Biological sciences / The Royal Society*, **275**, 1703–8.

Jaksić FM, Feinsinger P, Jiménez JE *et al.* (1993) A Long-Term Study on the Dynamics of Guild Structure among Predatory Vertebrates at a Semi-Arid Neotropical Site. *Oikos*, **67**, 87–96.

Jemielity S, Chapuisat M, Parker JD, Keller L (2005) Long live the queen: studying aging in social insects. *Age (Dordrecht, Netherlands)*, **27**, 241–8.

Jemielity S, Kimura M, Parker KM *et al.* (2007) Short telomeres in short-lived males: what are the molecular and evolutionary causes? *Aging cell*, **6**, 225–33.

Jennings BJ, Ozanne SE, Dorling MW, Hales CN (1999) Early growth determines longevity in male rats and may be related to telomere shortening in the kidney. *FEBS letters*, **448**, 4–8.

Karlseder J (1999) p53- and ATM-Dependent Apoptosis Induced by Telomeres Lacking TRF2. *Science*, **283**, 1321–1325.

Kashino G, Kodama S, Nakayama Y *et al.* (2003) Relief of oxidative stress by ascorbic acid delays cellular senescence of normal human and Werner syndrome fibroblast cells. *Free Radical Biology and Medicine*, **35**, 438–443.

Kawanishi S, Hiraku Y, Oikawa S (2001) Mechanism of guanine-specific DNA damage by oxidative stress and its role in carcinogenesis and aging. *Mutation Research - Reviews in Mutation Research*, **488**, 65–76.

Kawanishi S, Oikawa S (2004) Mechanism of telomere shortening by oxidative stress. *Annals of the New York Academy of Sciences*, **1019**, 278–284.

Keller L, Genoud M (1997) Extraordinary lifespans in ants : a test of evolutionary theories of ageing. *Nature*, **389**, 3–5.

Khan M, Walters J (2002) Effects of helpers on breeder survival in the red-cockaded woodpecker (Picoides borealis). *Behavioral Ecology and Sociobiology*, **51**, 336–344.

Kilner RM (2001) A growth cost of begging in captive canary chicks. *Proceedings of the National Academy of Sciences of the United States of America*, **98**, 11394–8.

Kim W, Ludlow AT, Min J *et al.* (2016) Regulation of the Human Telomerase Gene TERT by Telomere Position Effect???Over Long Distances (TPE-OLD): Implications for Aging and Cancer. *PLoS Biology*, **14**, 1–25.

Kim S-Y, Velando A (2015) Antioxidants safeguard telomeres in bold chicks. *Biology letters*, **11**, 20150211.

Kimura M, Hjelmborg JVB, Gardner JP *et al.* (2008) Telomere length and mortality: A study of leukocytes in elderly danish twins. *American Journal of Epidemiology*, **167**, 799–806.

Kipling D (2001) Telomeres, replicative senescence and human ageing. *Maturitas*, **38**, 25–38.

Kirkwood TB (1977) Evolution of ageing. *Nature*, **270**, 301–304.

Kirkwood TBL (2005) Understanding the odd science of aging. *Cell*, **120**, 437–447.

Kirkwood TBL, Holliday R (1979) The evolution of ageing and longevity. *Proceedings of the Royal Society B*, **205**, 531–546.

Kirkwood TBL, Rose MR (1991) Evolution of senescence: Late survival sacrificed for reproduction. *Philosophical Transactions of the Royal Society B*, **332**, 15–24.

Koenig WD, Dickinson JL (Eds.) (2004) *Ecology and Evolution of Cooperative Breeding in Birds*. Cambridge University Press, Cambridge.

Koenig WD, Dickinson JL (Eds.) (2016) *Cooperative breeding in vertebrates: studies of ecology, evolution and behavior*. Cambridge University Press, Cambridge.

Komdeur J (1994) Experimental evidence for helping and hindering by previous offspring in the cooperative-breeding Seychelles warbler AcrocephMus sechellensis. , 175–186.

Korandová M, Frydrychová RČ (2016) Activity of telomerase and telomeric length in Apis mellifera. *Chromosoma*, **125**, 405–411.

Kotrschal A, Ilmonen P, Penn DJ (2007) Stress impacts telomere dynamics. *Biology letters*, **3**, 128–30.

Kurz DJ, Decary S, Hong Y *et al.* (2004) Chronic oxidative stress compromises telomere integrity and accelerates the onset of senescence in human endothelial cells. *Journal of cell science*, **117**, 2417–26.

Kyo S, Takakura M, Kanaya T *et al.* (1999) Estrogen activates telomerase. *Cancer Research*, **59**, 5917–5921.

de Lange T (2005) Shelterin: The protein complex that shapes and safeguards human telomeres. *Genes and Development*, **19**, 2100–2110.

de Lange T (2009) How telomeres solve the end-protection problem. *Science*, **326**, 948–52.

de Lange T (2015) A loopy view of telomere evolution. *Frontiers in Genetics*, **6**, 1–5.

Lansdorp PM (2005) Major cutbacks at chromosome ends. *Trends in Biochemical Sciences*, **30**, 388–395.

Lansdorp PM (2008) Telomeres , stem cells , and hematology. *Blood*, **111**, 1759–1766.

Lee W-S, Monaghan P, Metcalfe NB (2013) Experimental demonstration of the growth rate--lifespan trade-off. *Proceedings. Biological sciences / The Royal Society*, **280**, 20122370.

Leech SM, Leonard ML (1997) Begging and the risk of predation in netling birds. *Behav. Ecol.*, **8**, 644–646.

Leitner S, Mundy P, Voigt C (2010) Morphometrics of White-browed Sparrow-Weavers Plocepasser mahali in south-western Zimbabwe. *Ostrich*, **80**, 99–102.

Lemaître J-F, Berger V, Bonenfant C *et al.* (2015) Early-late life trade-offs and the evolution of ageing in the wild. *Proceedings. Biological sciences / The Royal Society*, **282**, 20150209.

Lemaître J-F, Gaillard J, Pemberton JM, Clutton-brock TH, Nussey DH (2014) Early life expenditure in sexual competition is associated with increased reproductive senescence in male red deer. *Proceedings of the Royal Society B*, **281**, 20140792.

Lewin N, Treidel LA, Holekamp KE *et al.* (2015) Socioecological variables predict telomere length in wild spotted hyenas. *Biology letters*, **11**, 20140991.

Lewis DM (1981) Determinants of Reproductive Success of the White-Browed Sparrow Weaver , Plocepasser mahali. *Behavioral Ecology and Sociobiology*, **9**, 83–93.

Lewis DM (1982) Cooperative breeding in a population of white-browed sparrow weavers Plocepasser mahali. *Ibis*, **124**, 511–522.

Li FH, Zhong WQ, Wang Z, Wang DH (2007) Rank in a food competition test and humoral immune functions in male Brandt's voles (Lasiopodomys brandtii). *Physiology and Behavior*, **90**, 490–495.

Ling S, Aozhi D, Williams MRI *et al.* (2002) Testosterone (T) enhances apoptosis-related damage in human vascular endothelial cells. *Endocrinology*, **143**, 1119–1125.

Liu L, Trimarchi JR, Smith PJS, Keefe DL (2002) Mitochondrial dysfunction leads to telomere attrition and genomic instability. *Aging Cell*, **1**, 40–46.

Lloyd P, Andrew Taylor W, Du Plessis MA, Martin TE (2009) Females increase reproductive investment in response to helper-mediated improvements in allo-feeding, nest survival, nestling provisioning and post-fledging survival in the Karoo scrub-robin Cercotrichas coryphaeus. *Journal of Avian Biology*, **40**, 400–411.

Makarov VL, Hirose Y, Langmore JP (1997) Long G tails at both ends of human chromosomes suggest a C strand degradation mechanism for telomere shortening. *Cell*, **88**, 657–666.

Marcand S, Brevet V, Gilson E (1999) Progressive cis-inhibition of telomerase upon telomere elongation. *EMBO Journal*, **18**, 3509–3519.

Martin-Ruiz C, Dickinson HO, Keys B *et al.* (2006) Telomere length predicts poststroke mortality, dementia, and cognitive decline. *Annals of Neurology*, **60**, 174–180.

Martin-Ruiz CM, Gussekloo J, van Heemst D, von Zglinicki T, Westendorp RGJ (2005) Telomere length in white blood cells is not associated with morbidity or mortality in the oldest old: A population-based study. *Aging Cell*, **4**, 287–290.

Martin LB, Weil ZM, Kuhlman JR, Nelson RJ (2006) Trade-offs within the immune systems of female White-footed Mice, Peromyscus leucopus. *Functional Ecology*, **20**, 630–636.

McLennan D, Armstrong JD, Stewart DC *et al.* (2016) Interactions between parental traits, environmental harshness and growth rate in determining telomere length in wild juvenile salmon. *Molecular Ecology*, **25**, 5425–5438.

McLennan D, Armstrong JD, Stewart DC *et al.* (2017) Shorter juvenile telomere length is associated with higher survival to spawning in migratory Atlantic salmon. *Functional Ecology*, **38**, 42–49.

Meillère A, Brischoux F, Ribout C, Ange (2015) Traffic noise exposure affects telomere length in nestling house sparrows. *Biology Letters*, **11**, 2–5.

Metcalfe NB, Alonso-Alvarez C (2010) Oxidative stress as a life-history constraint: the role of reactive oxygen species in shaping phenotypes from conception to death. *Functional Ecology*, **24**, 984–996.

Metcalfe NB, Monaghan P (2013) Does reproduction cause oxidative stress? An open question. *Trends in ecology & evolution*, **28**, 347–350.

De Meyer T, Rietzschel ER, De Buyzere ML *et al.* (2007) Paternal age at birth is an important determinant of offspring telomere length. *Human molecular genetics*, **16**, 3097–102.

Meyne J, Ratliff RL, Moyzis RK (1989) Conservation of the human telomere sequence (TTAGGG)n among vertebrates. *Proceedings of the National Academy of Sciences of the United States of America*, **86**, 7049–53.

Mieczkowski PA, Mieczkowska JO, Dominska M, Petes TD (2003) Genetic regulation of telomere-telomere fusions in the yeast Saccharomyces cerevisae. *Proceedings of the National Academy of Sciences*, **100**, 10854–10859.

Mizutani Y, Niizuma Y, Yoda K (2016) How do growth and sibling competition affect telomere dynamics in the first month of life of long-lived seabird? *PLoS ONE*, **11**, 1–14.

Mizutani Y, Tomita N, Niizuma Y, Yoda K (2013) Environmental perturbations influence telomere dynamics in long-lived birds in their natural habitat. *Biology letters*, **9**, 20130511.

Monaghan P (2010) Telomeres and life histories: the long and the short of it. *Annals of the New York Academy of Sciences*, **1206**, 130–42.

Monaghan P, Haussmann MF (2006) Do telomere dynamics link lifestyle and lifespan? *Trends in ecology & evolution*, **21**, 47–53.

Monaghan P, Heidinger BJ, D'Alba L, Evans NP, Spencer K a (2012) For better or worse: reduced adult lifespan following early-life stress is transmitted to breeding partners. *Proceedings. Biological sciences / The Royal Society*, **279**, 709–14.

Monaghan P, Metcalfe NB (1985) Group foraging in wild brown hares: effects of resource distribution and social status. *Animal Behaviour*, **33**, 993–999.

Monaghan P, Metcalfe NB, Torres R (2009) Oxidative stress as a mediator of life history trade-offs: mechanisms, measurements and interpretation. *Ecology letters*, **12**, 75–92.

Mougeot F, Martínez-Padilla J, Webster LMI *et al.* (2009) Honest sexual signalling mediated by parasite and testosterone effects on oxidative balance. *Proceedings of the Royal Society B: Biological Sciences*, **276**, 1093–1100.

Muehlenbein MP, Watts DP (2010) The costs of dominance: testosterone, cortisol and intestinal parasites in wild male chimpanzees. *BioPsychoSocial medicine*, **4**, 21.

Murray CM, Eberly LE, Pusey AE (2006) Foraging strategies as a function of season and rank among wild female chimpanzees (Pan troglodytes). *Behavioral Ecology*, **17**, 1020–1028.

Neggazi SA, Noreikiene K, ??st M, Jaatinen K (2016) Reproductive investment is connected to innate immunity in a long-lived animal. *Oecologia*, **182**, 347–356.

Negrini S, Ribaud V, Bianchi A, Shore D (2007) DNA breaks are masked by multiple Rap1 binding in yeast: Implications for telomere capping and telomerase regulation. *Genes and Development*, **21**,

292–302.

Nettle D, Monaghan P, Boner W, Gillespie R, Bateson M (2013) Bottom of the heap: Having heavier competitors accelerates early-life telomere loss in the European starling, Sturnus vulgaris. *PLoS ONE*, **8**.

Nettle D, Monaghan P, Gillespie R *et al.* (2015) An experimental demonstration that early-life competitive disadvantage accelerates telomere loss. *Proc. R. Soc. B*, **282**, 20141610.

Neuenschwander S, Brinkhof MW, Kolliker M, Richner H (2003) Brood size, sibling competition, and the cost of begging in great tits (Parus major). *Behavioral Ecology*, **14**, 457–462.

Niedernhofer LJ, Daniels JS, Rouzer CA, Greene RE, Marnett LJ (2003) Malondialdehyde, a product of lipid peroxidation, is mutagenic in human cells. *Journal of Biological Chemistry*, **278**, 31426–31433.

Nilsson J-A (2002) Metabolic consequences of hard work. *Proceedings of the Royal Society B*, **269**, 1735–1739.

Njajou OT, Cawthon RM, Damcott CM *et al.* (2007) Telomere length is paternally inherited and is associated with parental lifespan. *Proceedings of the National Academy of Sciences of the United States of America*, **104**, 12135–9.

Noguera JC, Metcalfe NB, Boner W, Monaghan P (2015) Sex-dependent effects of nutrition on telomere dynamics in zebra finches (Taeniopygia guttata). *Biology letters*, **11**, 20140938-.

Noguera JC, Metcalfe NB, Reichert S, Monaghan P (2016) Embryonic and postnatal telomere length decrease with ovulation order within clutches. *Scientific reports*, **6**, 25915.

Nussey DH, Froy H, Lemaitre JF, Gaillard JM, Austad SN (2013) Senescence in natural populations of animals: Widespread evidence and its implications for bio-gerontology. *Ageing Research Reviews*, **12**, 214–225.

Nussey DH, Kruuk LEB, Donald A, Fowlie M, Clutton-Brock TH (2006) The rate of senescence in maternal performance increases with early-life fecundity in red deer. *Ecology letters*, **9**, 1342–50.

Nussey DH, Kruuk LEB, Morris A, Clutton-Brock TH (2007) Environmental conditions in early life influence ageing rates in a wild population of red deer. *Current biology : CB*, **17**, R1000-1.

Nussey DH, Pemberton JM, Pilkington JG, Blount JD (2009) Life history correlates of oxidative damage in a free-living mammal population. , 809–817.

O'Sullivan RJ, Karlseder J (2010) Telomeres: protecting chromosomes against genome instability. *Nature Reviews Molecular Cell Biology*, **11**, 171–181.

Ogawa M (1993) Differentiation and proliferation of hematopoietic stem cells. *Blood*, **81**, 2844–2853.

Oikawa S, Kawanishi S (1999) Site-specific DNA damage at GGG sequence by oxidative stress may accelerate telomere shortening. *FEBS Letters*, **453**, 365–368.

Olovnikov AM (1973) A theory of marginotomy. The incomplete copying of template margin in enzymic synthesis of polynucleotides and biological significance of the phenomenon. *Journal of*

Theoretical Biology, **41**, 181–190.

Olsson M, Pauliny A, Wapstra E *et al.* (2011) Sex differences in sand lizard telomere inheritance: paternal epigenetic effects increases telomere heritability and offspring survival. *PloS one*, **6**, e17473.

Opresko PL, Fan J, Danzy S, Wilson DM, Bohr VA (2005) Oxidative damage in telomeric DNA disrupts recognition by TRF1 and TRF2. *Nucleic Acids Research*, **33**, 1230–1239.

Ouyang JQ, Lendvai Z, Moore IT, Bonier F, Haussmann MF (2016) Do Hormones, Telomere Lengths, and Oxidative Stress form an Integrated Phenotype? A Case Study in Free-Living Tree Swallows. *Integrative and Comparative Biology*, **56**, 138–145.

Palmieri D, Cafueri G, Mongelli F *et al.* (2014) Telomere shortening and increased oxidative stress are restricted to venous tissue in patients with varicose veins: A merely local disease? *Vascular medicine (London, England)*, **19**, 125–130.

Paquet M, Covas R, Chastel O, Parenteau C, Doutrelant C (2013) Maternal Effects in Relation to Helper Presence in the Cooperatively Breeding Sociable Weaver. *PLoS ONE*, **8**, e59336.

Parkes TL, Elia a J, Dickinson D *et al.* (1998) Extension of Drosophila lifespan by overexpression of human SOD1 in motorneurons. *Nature genetics*, **19**, 171–174.

Pauliny A, Wagner RH, Augustin J, Szép T, Blomqvist D (2006) Age-independent telomere length predicts fitness in two bird species. *Molecular ecology*, **15**, 1681–7.

Petersen S, Saretzki G, Zglinicki T Von (1998) Preferential Accumulation of Single-Stranded Regions in Telomeres of Human Fibroblasts. , **160**, 152–160.

Pfaffl MW (2001) A new mathematical model for relative quantification in real-time RT – PCR. , **29**, 16–21.

Plot V, Criscuolo F, Zahn S, Georges J-Y (2012) Telomeres, age and reproduction in a long-lived reptile. *PloS one*, **7**, e40855.

van de Pol M, Verhulst S (2006) Age-Dependent Traits : A New Statistical Model to Separate Within - and Between - Individual Effects. *The American naturalist*, **May**, 766–773.

van de Pol M, Wright J (2009) A simple method for distinguishing within- versus between-subject effects using mixed models. , **77**, 753–758.

Polkinghorn WR, Parker JS, Lee MX *et al.* (2013) Androgen receptor signaling regulates DNA repair in prostate cancers. *Cancer Discovery*, **3**, 1245–1253.

Price K, Harvey H, Ydenberg R (1996) Begging tactics of nestling yellow-headed blackbirds, *Xanthocephalus xanthocephalus*, in relation to need. *Animal Behaviour*, **51**, 421–435.

Price JS, Waters JG, Darrah C *et al.* (2002) The role of chondrocyte senescence in osteoarthritis. *Aging Cell*, **1**, 57–65.

Reichert S, Bize P, Arrivé M *et al.* (2014a) Experimental increase in telomere length leads to faster feather regeneration. *Experimental gerontology*, **52**, 36–8.

Reichert S, Stier A, Zahn S *et al.* (2014b) Increased brood size leads to persistent eroded telomeres. *Frontiers in Ecology and Evolution*, **2**, 1–11.

Remolina SC, Hughes K a (2008) Evolution and mechanisms of long life and high fertility in queen honey bees. *Age (Dordrecht, Netherlands)*, **30**, 177–85.

Reneerkens J, Piersma T, Ramenofsky M (2002) An experimental test of the relationship between temporal variability of feeding opportunities and baseline levels of corticosterone in a shorebird. *Journal of Experimental Zoology*, **293**, 81–88.

Reznick D (1985) Costs of Reproduction : An Evaluation of the Empirical Evidence. , **44**, 257–267.

Richards SA (2005) Testing Ecological Theory Using the Information-Theoretic Approach : Examples and Cautionary Results. *Ecology*, **86**, 2805–2814.

Richards SA, Whittingham MJ, Stephens PA (2011) Model selection and model averaging in behavioural ecology : the utility of the IT-AIC framework. *Behavioral Ecology and Sociobiology*, **65**, 77–89.

Richter T, von Zglinicki T (2007) A continuous correlation between oxidative stress and telomere shortening in fibroblasts. *Experimental gerontology*, **42**, 1039–42.

Robin JD, Ludlow AT, Batten K *et al.* (2014) Telomere position effect: Regulation of gene expression with progressive telomere shortening over long distances. *Genes and Development*, **28**, 2464–2476.

Royle NJ, Surai PF, McCartney RJ, Speake BK (1999) Parental investiment and egg yolk lipid composition in gulls. *Functional Ecology*, **13**, 298–306.

Ruijter JM, Ramakers C, Hoogaars WMH *et al.* (2009) Amplification efficiency : linking baseline and bias in the analysis of quantitative PCR data. *Nucleic acids research*, **37**, e45.

Russell AF, Langmore NE, Cockburn A, Astheimer LB, Kilner RM (2007a) Reduced Egg Investment Can Conceal Helper Effects in Cooperatively Breeding Birds. *Science*, **317**, 941–944.

Russell AF, Lummaa V (2009) Maternal effects in cooperative breeders: from hymenopterans to humans. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences*, **364**, 1143–67.

Russell a F, Young a J, Spong G, Jordan NR, Clutton-Brock TH (2007b) Helpers increase the reproductive potential of offspring in cooperative meerkats. *Proceedings. Biological sciences / The Royal Society*, **274**, 513–520.

Saino N, Bertacche V, Ferrari RP *et al.* (2002) Carotenoid concentration in barn swallow eggs is influenced by laying order, maternal infection and paternal ornamentation. *Proceedings of the Royal Society B-Biological Sciences*, **269**, 1729–1733.

Salmón P, Nilsson JF, Nord A *et al.* (2016) Urban environment shortens telomere length in nestling great tits, Parus major. *Biology letters*, **12**, 254–260.

Salomons HM, Mulder G a, van de Zande L *et al.* (2009) Telomere shortening and survival in free-living corvids. *Proceedings. Biological sciences / The Royal Society*, **276**, 3157–65.

Samper E, Flores JM, Blasco MA (2001) Restoration of telomerase activity rescues chromosomal instability and premature aging in Terc-/- mice with short telomeres. *EMBO Reports*, **2**, 800–807.

Sapolsky RM (2005) The influence of social hierarchy on primate health. *Science (New York, N.Y.)*, **308**, 648–52.

Schmidt CM, Jarvis JUM, Bennett NC (2013) The long-lived queen : reproduction and longevity in female eusocial Damaraland mole-rats (Fukomys damarensis). *African zoology*, **48**, 193–196.

Schroeder T (2010) Hematopoietic Stem Cell Heterogeneity: Subtypes, Not Unpredictable Behavior. *Cell Stem Cell*, **6**, 203–207.

Seehuus S-C, Norberg K, Gimsa U, Krekling T, Amdam G V. (2006) Reproductive protein protects functionally sterile honey bee workers from oxidative stress. *Proceedings of the National Academy of Sciences*, **103**, 962–967.

Selman C, Blount JD, Nussey DH, Speakman JR (2012) Oxidative damage, ageing, and life-history evolution: where now? *Trends in ecology & evolution*, **27**, 570–7.

Serra V, von Zglinicki T, Lorenz M, Saretzki G (2003) Extracellular superoxide dismutase is a major antioxidant in human fibroblasts and slows telomere shortening. *The Journal of biological chemistry*, **278**, 6824–30.

Sfeir AJ, Chai W, Shay JW, Wright WE (2005) Telomere-end processing: The terminal nucleotidesof human chromosomes. *Molecular Cell*, **18**, 131–138.

Sharma GG, Gupta A, Wang H *et al.* (2003) hTERT associates with human telomeres and enhances genomic stability and DNA repair. *Oncogene*, **22**, 131–46.

Sharp SP, Clutton-Brock TH (2011) Competition, breeding success and ageing rates in female meerkats. *Journal of evolutionary biology*, **24**, 1756–62.

Shiels PG, McGlynn LM, MacIntyre A *et al.* (2011) Accelerated telomere attrition is associated with relative household income, diet and inflammation in the pSoBid Cohort. *PLoS ONE*, **6**, 1–7.

Shull S, Heintz NH, Periasamy M *et al.* (1991) Differential regulation of antioxidant enzymes in response to oxidants. *Journal of Biological Chemistry*, **266**, 24398–24403.

Simons MJP (2015) Questioning causal involvement of telomeres in aging. *Ageing Research Reviews*, **24**, 191–196.

Sitte N, Saretzki G, Von Zglinicki T (1998) Accelerated telomere shortening in fibroblasts after extended periods of confluency. *Free Radical Biology and Medicine*, **24**, 885–893.

Slagboom PE, Droog S, Boomsma DI (1994) Genetic determination of telomere size in humans: a twin study of three age groups. *American journal of human genetics*, **55**, 876–82.

Smyth KN, Drea CM (2016) Patterns of parasitism in the cooperatively breeding meerkat: A cost of dominance for females. *Behavioral Ecology*, **27**, 148–157.

Solomon NG, French JA (Eds.) (1997) *Cooperative Breeding in Mammals.* Cambridge University Press, Cambridge.

Sparkman AM, Adams J, Beyer A *et al.* (2011) Helper effects on pup lifetime fitness in the cooperatively breeding red wolf (Canis rufus). *Proceedings. Biological sciences / The Royal Society*, **278**, 1381–9.

Speakman JR, Garratt M (2014) Oxidative stress as a cost of reproduction: beyond the simplistic trade-off model. *BioEssays : news and reviews in molecular, cellular and developmental biology*, **36**, 93–106.

Stansel RM, De Lange T, Griffith JD (2001) T-loop assembly in vitro involves binding of TRF2 near the 3′ telomeric overhang. *EMBO Journal*, **20**, 5532–5540.

Stearns ASC (1989) Trade-Offs in Life-History Evolution. *Functional Ecology*, **3**, 259–268.

Steenstrup T, Hjelmborg JVB, Kark JD, Christensen K, Aviv A (2013) The telomere lengthening conundrum - Artifact or biology? *Nucleic Acids Research*, **41**, 1–7.

Stier A, Delestrade A, Bize P *et al.* (2016) Investigating how telomere dynamics, growth and life history covary along an elevation gradient in two passerine species. *Journal of Avian Biology*, **47**, 134–140.

Stier A, Massemin S, Zahn S, Tissier ML, Criscuolo F (2015) Starting with a handicap: effects of asynchronous hatching on growth rate, oxidative stress and telomere dynamics in free-living great tits. *Oecologia*, **179**, 999–1010.

Stier A, Viblanc VA, Massemin-Challet S *et al.* (2014) Starting with a handicap: Phenotypic differences between early- and late-born king penguin chicks and their survival correlates. *Functional Ecology*, **28**, 601–611.

Suda T, Takubo K, Semenza GL (2011) Metabolic regulation of hematopoietic stem cells in the hypoxic niche. *Cell Stem Cell*, **9**, 298–310.

Sudyka J, Arct A, Drobniak S *et al.* (2014) Experimentally increased reproductive effort alters telomere length in the blue tit (Cyanistes caeruleus). *Journal of Evolutionary Biology*, **27**, 2258–2264.

Sudyka J, Casasole G, Rutkowska J, Cichon M (2016) Elevated reproduction does not affect telomere dynamics and oxidative stress. *Behavioral Ecology and Sociobiology*, **70**, 2223–2233.

Sugioka K, Shimosegawa Y, Nakano M (1987) Estrogens as natural antioxidants of membrane phospholipid peroxidation. *FEBS Letters*, **210**, 37–39.

Taff CC, Freeman-Gallant CR (2017) Sexual signals reflect telomere dynamics in a wild bird. *Ecology and Evolution*, 1–7.

Tardif SD, Richter CB, Carson RL (1984) Effects of sibling-rearing experience on future reproductive success in two species of callitrichidae. *American Journal of Primatology*, **6**, 377–380.

Tarry-Adkins JL, Martin-Gronert MS, Fernandez-Twinn DS *et al.* (2013) Poor maternal nutrition followed by accelerated postnatal growth leads to alterations in DNA damage and repair, oxidative and nitrosative stress, and oxidative defense capacity in rat heart. *FASEB Journal*, **27**, 379–390.

Tarry-Adkins JL, Ozanne SE (2014) The impact of early nutrition on the ageing trajectory. *The Proceedings of the Nutrition Society*, **73**, 289–301.

Teixeira MT, Arneric M, Sperisen P, Lingner J (2004) Telomere length homeostasis is achieved via a

switch between telomerase-extendible and-nonextendible states. *Cell*, **117**, 323–335.

Temme DH, Charnov EL (1987) Brood size adjustment in birds: Economical tracking in a temporally varying environment. *Journal of Theoretical Biology*, **126**, 137–147.

Trougakos IP, Saridaki A, Panayotou G, Gonos ES (2006) Identification of differentially expressed proteins in senescent human embryonic fibroblasts. *Mechanisms of Ageing and Development*, **127**, 88–92.

Ujvari B, Biro PA, Charters JE *et al.* (2016) Curvilinear telomere length dynamics in a squamate reptile. *Functional Ecology*, **31**, 753–759.

Ujvari B, Madsen T (2009) Short telomeres in hatchling snakes: Erythrocyte telomere dynamics and longevity in tropical pythons. *PLoS ONE*, **4**, 2–6.

Ulyan MJ, Burrows AE, Buzzell CA *et al.* (2006) The effects of predictable and unpredictable feeding schedules on the behavior and physiology of captive brown capuchins (Cebus apella). *Applied Animal Behaviour Science*, **101**, 154–160.

Le Vaillant M, Viblanc VA, Saraux C *et al.* (2015) Telomere length reflects individual quality in free-living adult king penguins. *Polar Biology*, **38**, 2059–2067.

Valdes AM, Andrew T, Gardner JP *et al.* (2005) Obesity, cigarette smoking, and telomere length in women. *Lancet*, **366**, 662–664.

Valencia J, Mateos C, de la Cruz C, Carranza J (2016) Maternal allocation in eggs when counting on helpers in a cooperatively breeding bird. *Journal of Avian Biology*, 1–8.

Vaziri H, Dragowska W, Allsopp RC *et al.* (1994) Evidence for a mitotic clock in human hematopoietic stem cells: loss of telomeric DNA with age. *Proc Natl Acad Sci U S A*, **91**, 9857–9860.

Vera E, Bernardes de Jesus B, Foronda M, Flores JM, Blasco M a (2012) The rate of increase of short telomeres predicts longevity in mammals. *Cell reports*, **2**, 732–7.

Vera E, Blasco M a (2012) Beyond average: potential for measurement of short telomeres. *Aging*, **4**, 379–92.

Verhulst S, Aviv A, Benetos A, Berenson GS, Kark JD (2013) Do leukocyte telomere length dynamics depend on baseline telomere length? An analysis that corrects for "regression to the mean". *European journal of epidemiology*, **28**, 859–66.

Verhulst S, Geerdink M, Salomons HM, Boonekamp JJ (2014) Social life histories: jackdaw dominance increases with age , terminally declines and shortens lifespan. *Proceedings of the Royal Society B*, **281**, 20141045.

Voillemot M, Hine K, Zahn S *et al.* (2012) Effects of brood size manipulation and common origin on phenotype and telomere length in nestling collared flycatchers. *BMC ecology*, **12**, 17.

Walker LA (2016) The evolution and regulation of cooperation in the wild. University of Exeter.

Walker LA, York JE, Young AJ (2016) Sexually selected sentinels? Evidence of a role for intrasexual competition in sentinel behavior. *Behavioral Ecology*, **27**, 1461–1470.

Wang J, Lu X, Sakk V, Klein CA, Rudolph KL (2014) Senescence and apoptosis block hematopoietic activation of quiescent hematopoietic stem cells with short telomeres. *Blood*, **124**, 3237–3240.

Wang RC, Smogorzewska A, De Lange T (2004) Homologous recombination generates t-loop-sized deletions at human telomeres. *Cell*, **119**, 355–368.

Watson JD (1972) Origin of concatemeric T7 DNA. *Nature: New biology*, **239**, 197–201.

Watson H, Bolton M, Monaghan P (2015) Variation in early-life telomere dynamics in a long-lived bird: links to environmental conditions and survival. *The Journal of experimental biology*, **218**, 668–74.

Weladji RB, Gaillard J-M, Yoccoz NG *et al.* (2006) Good reindeer mothers live longer and become better in raising offspring. *Proceedings of the Royal Society B: Biological Sciences*, **273**, 1239–1244.

Wellinger RJ, Zakian VA (2012) Everything you ever wanted to know about Saccharomyces cerevisiae telomeres: Beginning to end. *Genetics*, **191**, 1073–1105.

Williams GC (1966) Natural Selection , the Costs of Reproduction , and a Refinement of Lack â€™ s Principle. *The American Naturalist*, **100**, 687–690.

Williams A. F (1972) DNA Synthesis in Purified Populations of Avian Erythroid Cells. *Journal of Cell Science*, **10**, 27–46.

Williamson JR (1994) G-Quartet Structures I N Telomeric Dna. *Annu.Rev.Biophys.Biomol.Sruct*, **23**, 703–730.

Wingfield JC, Lewis DM (1993) Hormonal and behavioural responses to simulated territorial intrusion in the cooperatively breeding white-browed sparrow weaver, Plocepasser mahali. *Animal Behaviour*, **45**, 1–11.

Woo J, Suen EWC, Leung JCS, Tang NLS, Ebrahim S (2009) Older men with higher self-rated socioeconomic status have shorter telomeres. *Age and Ageing*, **38**, 553–558.

Wrangham RW (1981) Drinking competition in vervet monkeys. *Animal Behaviour*, **29**, 904–910.

Wright WE, Shay JW (2005) Telomere biology in aging and cancer. *Journal of the American Geriatrics Society*, **53**, 292–294.

Wright WE, Tesmer VM, Huffman KE, Levene SD, Shay JW (1997) Normal human chromosomes have long G-rich telomeric overhangs at one end. *Genes and Development*, **11**, 2801–2809.

Yang LH, Bastow JL, Spence KO, Wright AN (2008) What Can We Learn from Resource Pulses. *Ecology*, **89**, 251–256.

Yip BW, Mok HO, Peterson DR *et al.* (2017) Sex-dependent telomere shortening, telomerase activity and oxidative damage in marine medaka Oryzias melastigma during aging. *Marine Pollution Bulletin*, 1–9.

York JE, Radford AN, Groothuis TG, Young AJ (2016a) Dominant male song performance reflects current immune state in a cooperatively breeding songbird. *Ecology and Evolution*, **6**, 1008–1015.

York JE, Radford AN, de Vries B, Groothuis TG, Young AJ (2016b) Dominance-related seasonal song production is unrelated to circulating testosterone in a subtropical songbird. *General and*

Comparative Endocrinology, **233**, 43–52.

York JE, Young AJ, Radford AN (2014) Singing in the moonlight : dawn song performance of a diurnal bird varies with lunar phase. *Biology Letters*, **10**.

Young AJ (2009) *The causes of physiological suppression in vertebrate societies: a synthesis* (H R, JC B, Eds,). Cambridge University Press, Cambridge.

Young AJ, Carlson A a., Clutton-Brock T (2005) Trade-offs between extraterritorial prospecting and helping in a cooperative mammal. *Animal Behaviour*, **70**, 829–837.

Young AJ, Carlson AA, Monfort SL *et al.* (2006) Stress and the suppression of subordinate reproduction in cooperatively breeding meerkats. *PNAS*, **103**, 12005–12010.

Young AJ, Clutton-brock T (2006) Infanticide by subordinates influences reproductive sharing in cooperatively breeding meerkats. , 385–387.

Young RC, Kitaysky AAS, Barger CP *et al.* (2016) Long telomeres: too much of a good thing. *Biology letters*, **3**, 1–10.

Young RC, Kitaysky AS, Haussmann MF *et al.* (2013) Age, sex, and telomere dynamics in a long-lived seabird with male-biased parental care. *PloS one*, **8**, e74931.

Young AJ, Monfort SL (2009) Stress and the costs of extra-territorial movement in a social carnivore. *Biology letters*, **5**, 439–441.

Young AJ, Spong G, Clutton-brock T (2007) Subordinate male meerkats prospect for extra-group paternity : alternative reproductive tactics in a cooperative mammal. , 1603–1609.

Young RC, Welcker J, Barger CP *et al.* (2017) Effects of developmental conditions on growth, stress, and telomeres in black-legged kittiwake chicks. *Molecular Ecology*.

Yu BP (1994) Cellular defenses against damage from reactive oxygen species. *Physiological reviews*, **74**, 139–162.

von Zglinicki T (2002) Oxidative stress shortens telomeres. *Trends in biochemical sciences*, **27**, 339–44.

von Zglinicki T, Saretzki G, Wolf D, Lotze C (1995) Mild hyperoxia shortens telomeres and inhibits proliferation of fibroblasts: A model for senescence? *Experimental Cell Research*, **220**, 186–193.

Zhang X, Lin S, Funk WE, Hou L (2013) Environmental and occupational exposure to chemicals and telomere length in human studies. *Occupational and environmental medicine*, **70**, 743–9.

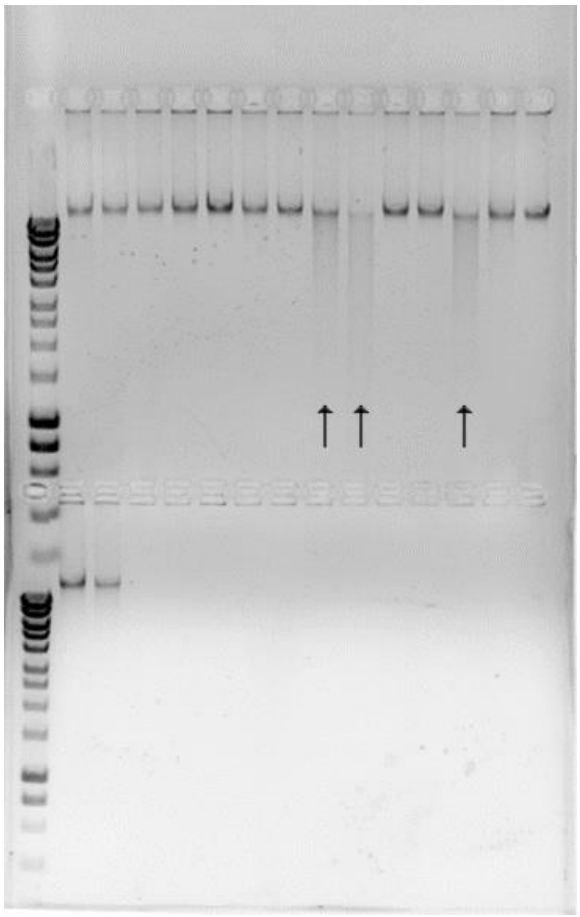
Zhang H, Pan K-H, Cohen SN (2003) Senescence-specific gene expression fingerprints reveal cell-type-dependent physical clustering of up-regulated chromosomal loci. *Proc Natl Acad Sci*, **100**, 3251–3256.

Zhu H, Belcher M, van der Harst P (2011) Healthy aging and disease: role for telomere biology? *Clinical Science*, **120**, 427–440.

Zhu L, Hathcock KS, Hande P *et al.* (1998) Telomere length regulation in mice is linked to a novel chromosome locus. *Proceedings of the National Academy of Sciences of the United States of America*, **95**, 8648–8653.

Sample integrity

All DNA extractions were run on a 1% gel to assess integrity. A single high-weight band indicates good integrity. Extractions that did not show a defined band (i.e that were 'smeared') were re-extracted and re-tested. If the second extraction also showed poor integrity the sample was excluded from further analysis. The figure below shows an example of typical samples alongside three samples that were excluded (indicated by arrows)



GAPDH primers for Plocepasser mahali

To measure telomeres using qPCR it is necessary to amplify both telomere sequence and a non-variable copy number gene. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) has been used as the control gene for a number of passerine species, including zebra finch, alpine swift, American redstart, common yellowthroat and Seychelles warbler (Criscuolo *et al.* 2009b; Bize et al 2009; Heidinger *et al.* 2012; Barrett *et al.* 2012; Angelier *et al.* 2013; Taff & Freeman-Gallant 2017). I designed and tested three primer sets specific to *P. mahali* to amplify part of the GAPDH gene, which is both autosomal and single copy in birds for which the genome is known (NCBI accession no.s: *T. guttata* AF255390, *G. gallus* NW_003763490.1). Bize *et al* (2009), Criscuolo *et al* (2009), Heidinger *et al* (2012), and Barrett *et al* (2012) used the following primers specific to the zebra finch (*Taenopygia guttata*): GAPDH-F (5′-AACCAGCCAAGTACGATGACAT-3′), GAPDH-R (5′-CCATCAGCAGCAGCCTT CA-3′). In order to obtain the *P. mahali* sequence for this region of GAPDH, I used BLAST to align partial cds of house sparrow (*Passer domesticus*), zebra finch (*Taenopygia guttata*), rock dove (*Columbia livia*), chicken (*Gallus gallus*) and Japanese quail (*Coturnix japonica*) against the genomic sequence of zebra finch (NCBI accession no. NC_011462) and designed two sets of primers in highly conserved regions either side of the region of interest (fwd: 5' – ATGGCTTTCCGTGTGCCAACC-3' 5'-GTTGTKGACCTGACCTGCCG-5' rev: 5'-ARTGGTCGTTCAGCGCAATG-3' 5'-CACGGTTGCTGTATCCAWAYTC-3'; Eurofins MWG Operon). Two samples from different individuals were amplified, using the two sets of primers in all possible combinations. DNA was extracted from the gel using Wizard SV Gel and PCR Clean-up System (Promega), tested for quality on a NanoDrop-1000 Specrophotometer (Labtech) and the product verified by electrophoresis on a 1% agarose gel. The four best products were sent to Beckmann Coulter for Sanger sequencing. Three sets of qPCR primers were then designed from the resulting consensus sequence using the online Primer 3 Plus software with qPCR settings. The resulting primer sets were tested by amplifying DNA samples and running the PCR product on a 1% agarose gel with a 100bp ladder (Promega G210A). Primers were excluded if they did not produce a single band of the expected length.

The region of GAPDH that is most commonly used for qPCR primers in telomere studies is highly conserved (figure 1). The primers specific to *P.mahali* are extremely similar to those for *T. guttata* used in previous studies (Bize et al 2009, Criscuolo et al 2009, Heidinger et al 2012, Barrett et al 2012), with only one base difference in the forward primer, and no differences in the reverse primer (GAPDH_F: 5′-AACC AGCCAAGTAT**T**GATGACAT-3′, GAPDH_R: 5′-CCATCAGCAGCAGCCTTCA-3'; henceforth referred to as GAPDH set 1). However, on aligning cd sequences with the genomic sequence for *T. guttata* it was found that the forward primer begins on one exon, and ends on the next, with no sequence aligning to the intron (figure 1). Two further sets of primers were therefore designed: GAPDH set 2: GAPDH_4 (fwd) 5'-GAGGGTAGTGAAGGCTGCTG-3', GAPDH_5 (rev) 5'- GTCCTCTGTGTATGCC AGGA-3' and GAPDH set 3: GAPDH_7 (fwd) 5'- TAGCCATTCCTCCACCTTTG-3', GAPDH_8 (rev) 5'- ACCAGGAAACCAGCTT GACA-3' (figure 1, table 4).

After running PCR products from each primer set, GAPDH set 2 primers were excluded due to production of multiple bands. The other two primer sets produced a single band of the expected size, and produced single-peak melt curves, both of which suggest the product is target sequence (Figures 2 and 3). We therefore were confident in using primer set 1 in further analyses.

```
  2610      2620      2630      2640      2650      2660      2670      2680      2690      2700
                ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
GenomNC_011462  AACTTACTGGAATGGCTTTCCGTGTGCCAACCCCTAACGTGTCTGTTGTGGACCTGACCTGCCGTCTGGAAAAACCAGTAAGTGTTGTGGACAAGGTCCT
Pmahali         -----------------------------------------------GTGGNCNTGACCTNCCGTNTGGAAAAACCAGTAAGTGTTGTGGRTGAGATCCT
SparrAF416452   AACTTACTGGAATGGCTTTCCGTGTGCCAACCCCTAACGTGTCTGTTGTGGACCTGACCTGCCGTCTGGAAAAGCCA~~~~~~~~~~~~~~~~~~~~~~~
FinchAF255390   AACTTACTGGAATGGCTTTCCGTGTGCCAACCCCTAACGTGTCTGTTGTGGACCTGACCTGCCGTCTGGAAAAACCA~~~~~~~~~~~~~~~~~~~~~~~
RdoveAF036934   AACTTACTGGAATGGCTTTCCGTGTGCCAACCCCCAATGTCTCTGTTGTGGACCTGACCTGCCGTCTGGAAAAACCA~~~~~~~~~~~~~~~~~~~~~~~
ChickNM_204305  AGCTTACTGGAATGGCTTTCCGTGTGCCAACCCCCAATGTCTCTGTTGTTGACCTGACCTGCCGTCTGGAGAAACCA~~~~~~~~~~~~~~~~~~~~~~~
QuailEU035555   AGCTTACTGGAATGGCTTTCCGTGTGCCAACCCCCAATGTCTCTGTTGTTGACCTGACCTGCCGTCTGGAGAAACCA~~~~~~~~~~~~~~~~~~~~~~~

                        2710      2720      2730      2740      2750      2760      2770      2780      2790      2800
                ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
GenomNC_011462  AACAACTTGCAGATGTGCCCCT---TCCTCTAAGCTCTGCAT--TGCACCTGGAATTGCC---CTCTCTGCATCCATCTTGTCC-TGAAGAT---ATCTT
Pmahali         CACAACTCCCAGATGTGCCCCTGCTTCCTCTAAGCTCTGTAT--TGCACCTGGAATTGCC---CTCTCTGCCTCCATCTTGTCC-TGAAGATGTGATCTT
SparrAF416452   ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
FinchAF255390   ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
RdoveAF036934   ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
ChickNM_204305  ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
QuailEU035555   ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

                        2810      2820      2830      2840      2850      2860      2870      2880      2890      2900
                ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
GenomNC_011462  TGTGAAAAGAGCACCTGCTGGGGTGTTCCCATGTGTGTCTTCTCCCTCCCATGTCCCCAGTGGACATTTTGTGTGAGTTTGGATCTGATCATCTTGGATC
Pmahali         TGTGAAAAGAGCACCTGCTGGGGTGTTGCCATGTGT--CTCCCCCCTCCCCTGTACCCAGTGGACATTTTGTGTGAGTTGGGATCTCATCATCTTGGATC
SparrAF416452   ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
FinchAF255390   ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
RdoveAF036934   ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
ChickNM_204305  ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
QuailEU035555   ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~


                        2910      2920      2930      2940      2950      2960      2970      2980      2990      3000
                ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
GenomNC_011462  TGTCTCTCCTCAGGCCAAGTACGATGACATCAAGAGGGTAGTGAAGGCTGCTGCTGATGGGCCCCTGAAGGGCATCCTGGCATACACAGAGGACCAGGTG
Pmahali         TGTCTTTCCTCAGGCCAAGTATGATGACATCAAGAGGGTAGTGAAGGCTGCTGCTGATGGGCCCCTGAAGGGCATCCTGGCATACACAGAGGACCAGGTA
Pmahali         TGTCTTTCCTCAGGCCAAGTATGATGACATCAAGAGGGTAGTGAAGGCTGCTGCTGATGGGCCCCTGAAGGGCATCCTGGCATACACAGAGGACCAGGTA
SparrAF416452   ~~~~~~~~~~~~~GCCAAGTACGATGACATCAAGAGGGTAGTGAAGGCTGCTGCTGATGGGCCCCTGAAGGGCATCCTGGCATACACAGAGGACCAGGT~
FinchAF255390   ~~~~~~~~~~~~~GCCAAGTACGATGACATCAAGAGGGTAGTGAAGGCTGCTGCTGATGGGCCCCTGAAGGGCATCTTGGCGTACACAGAGGACCAGGT~
RdoveAF036934   ~~~~~~~~~~~~~GCCAAGTATGATGACATTAAGAGGGTAGTGAAGGCTGCTGCTGATGGGCCCCTGAAGGGCATCCTGGCATACACAGAGGACCAGGT~
ChickNM_204305  ~~~~~~~~~~~~~GCCAAGTATGATGATATCAAGAGGGTAGTGAAGGCTGCTGCTGATGGGCCCCTGAAGGGCATCCTAGGATACACAGAGGACCAGGT~
QuailEU035555   ~~~~~~~~~~~~~GCCAAATATGATGACATCAAGAGGGTAGTGAAGGCTGCTGCTGAGGGGCCCTTGAAGGGCATCCTAGGATACACAGAGGACCAGGT~

                        3010      3020      3030      3040      3050      3060      3070      3080      3090      3100
                ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
GenomNC_011462  AG-GAATGCCTGCTCTC--AGCCTGCAGTTATTGGCTCTTGGCCTCATTTTTGTAAAAGGCTGTTGTGTCAGCAGCTTTGCCAGAATCCTC-TTTGGGCA
Pmahali         GG-GAACGCCTGCTCTCTCAGCCTGCGGTTATTGGCTCTTG-CCTCATTTTTCTAAAAGGCTGTTGT--CAGCAGCTTTGCCAGGATCCTGGTTTGGGCA
SparrAF416452   ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
FinchAF255390   ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
RdoveAF036934   ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
ChickNM_204305  ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
QuailEU035555   ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

                        3110      3120      3130      3140      3150      3160      3170      3180      3190      3200
                ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
GenomNC_011462  CTTGGACTAGAGACTCTGGGCAACCATATGCATTATAATTTCTTGTGTTTCAGGTTGTCTCCTGTGACTTCAATGGTGATAGCCATTCCTCCACCTTTGA
Pmahali         CTTGGAGTCGAGACTGTGGGCAGCCATATGCATTACAATTTCTTGTGTGTCAGGTTGTCTCCTGTGACTTCAATGGTGATAGCCATTCCYCCACCTTTGA
SparrAF416452   ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~TGTCTCCTGTGACTTCAATGGTGATAGCCATTCCTCCACCTTTGA
FinchAF255390   ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~TGTCTCCTGTGACTTCAATGGTGATAGCCATTCCTCCACCTTTGA
RdoveAF036934   ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~TGTCTCATGTGACTTCAACGGTGACAGCCATTCCTCCACCTTTGA
ChickNM_204305  ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~TGTCTCCTGTGACTTCAATGGTGACAGCCATTCCTCCACCTTTGA
QuailEU035555   ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~TGTCTCCTGTGACTTCAATGGTGACAGCCATTCTTCCACCTTTGA

                        3210      3220      3230      3240      3250      3260      3270      3280      3290      3300
                ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
GenomNC_011462  TGCGGGTGCTGGCATTGCACTGAATGACCATTTTGTCAAGCTGGTTTCCTGGTAGGTGCAGCCCTGGGC-ATGTGGGGGTTGGCACAGACAGGTTTCAAA
Pmahali         TGCGGGTGCCGGCATTGCRCTGAACGACCACTTTGTCAAGCTGGTTTCCTGGTNGGTGCAGCCCTGGGAGATGTGGGGGTTGGCACAGACAGGTTACAGA
SparrAF416452   TGCGGGCGCTGGCATTGCGCTGAACGACCATTTTGTCAAGCTGGTTTCCTGGTATG-ATAAT------GAATATGGATACAG---CAACCGTGTTGTGGA
FinchAF255390   TGCGGGTGCTGGCATTGCACTGAATGACCATTTTGTCAAGCTGGTTTCCTGGTACG-ACAAC------GAATA---------------------------
RdoveAF036934   TGCGGGTGCTGGCATTGCACTGAATGACCACTTTGTCAAGCTGGTTTCCTGGTATG-ATAAT------GAGTATGGATACAG---CAACCGTGTTGTGGA
ChickNM_204305  TGCGGGTGCTGGCATTGCACTGAATGACCATTTCGTCAAGCTTGTTTCCTGGTATG-ACAAT------GAGTTTGGATACAG---CAACCGTGTTGTGGA
QuailEU035555   TGCGGGTGCTGGCATTGCACTGAATGACAACTTCGTCAAGCTTGTTTCCTGGTATG-ACAAC------GAGTTTGGATACAG---CAACCGTGTTGTGGA

                        3310      3320      3330      3340      3350      3360      3370      3380      3390      3400
                ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
GenomNC_011462  CTTGGGCGTCCTTTTGGT-TACCACTGGTGATCCAGGTGGA--TGCACAAATCTCCAGTCACTCCGTGGACTGAGATTGTTTCTTATCTGGAATTACTGT
Pmahali         CTTGGGNGTCCTTTTGNN-TNNCACTANNGATCNANGNGGN--TACNCNNACCNCNNNNCTCANNNTNNNNNGAGAGTGCTTCTTACNTGAANTTACTAT
SparrAF416452   CTTG------------------------------------------------------------------------------------------------
FinchAF255390   ----------------------------------------------------------------------------------------------------
RdoveAF036934   CTTGATGGTCCACATGGCATCCAAGGAGTGAGCCTAGCACA------CAGCCCCCCCTGCTGCCTAGGGAAGCAGGACTCTCCTTTGTTGGAGCC-----
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ChickNM_204305  CTTGATGGTCCACATGGCATCCAAGGAGTGAGCCAGGCACA------CAGCCCCCC-TGCTGCCTAGGGAAGCAGGAC---CCTTTGTTGGAGCCCCTGC
QuailEU035555   CTTGATGGTCCACATGGCATCCAAGGAGTGAGCCAGGCACAGGCACACAGCCCCCC-TGCTGCCTAGGGAAGCAGGAC---CCTTTGTTGGAGCCCCCGC

                        3410      3420      3430      3440      3450      3460      3470      3480      3490      3500
                ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
GenomNC_011462  AACTGCCTCCTTCCCTCCAGAGGGTAGATGGGAATTCAGTTGTGTGGGATGGGGCAGTGGAGCAGGAGGAATGGAAGAAGGGGTGTGAGAAATGAGTCAG
Pmahali         ANCTGCNNCNNTCNNCCTNNNNNN-ANATGGNNANTTNNNNNNNN----------------------------------------------------------
SparrAF416452   ----------------------------------------------------------------------------------------------------------
FinchAF255390   ----------------------------------------------------------------------------------------------------------
RdoveAF036934   ----------------------------------------------------------------------------------------------------------
ChickNM_204305  TCTT--CACCACCGCTCAGTTCTGCAT-CCTGCAGTGAGAGGCCAGTTCTGTTCCCTTCTGTCTCCCCCACTCCTCCAATTTCTTCCTCCACCTGGGGGA
QuailEU035555   TCTT--CACCACCACTCAGTTCTGCATGCCTGCTGTGAGAGGCCA----------------------------------------------------------
```

< Figure 1. *P. mahali* genomic sequence alignment to genomic *T. guttata* (GenomNC_011462) and cds of house sparrow (*P. domesticus*), zebra finch (*T. guttata*), rock dove (*Columba livia*) chicken (*G. gallus*) and Japanese quail (*Coturnix japonica*). Highlighted regions show primers that were tested (yellow: GAPDH_F and GAPDH_R (set 1) used in previous studies, green: GAPDH_4 and GAPDH_5 (set 2), blue: GAPDH_7 and GAPDH_8 (set 3).

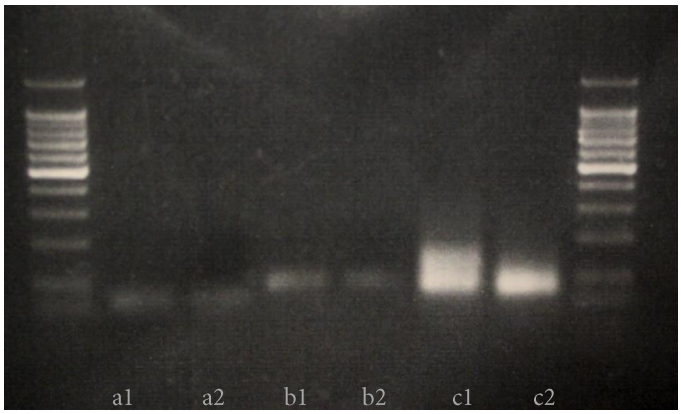


Figure 2. Gel electrophoresis of qPCR product of 1) DNA samples and 2) NTCs for a) GAPDH primer set 1, b) GAPDH primer set 3, and c) Telomere primers.

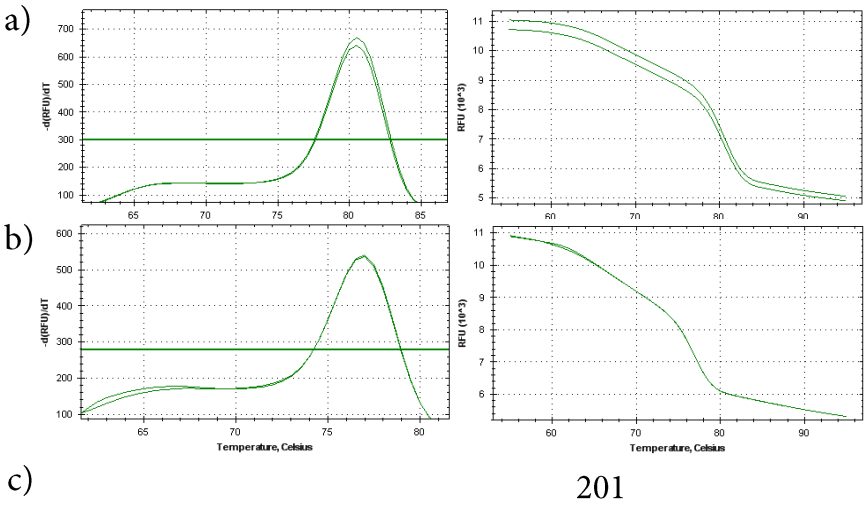
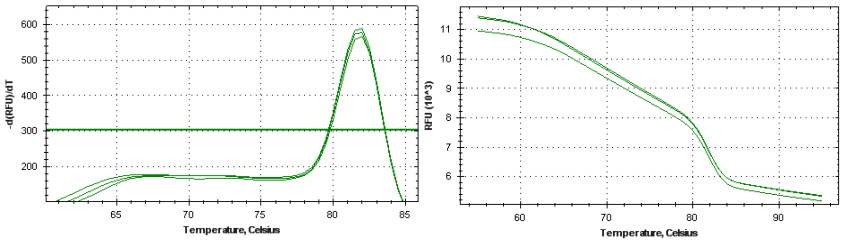
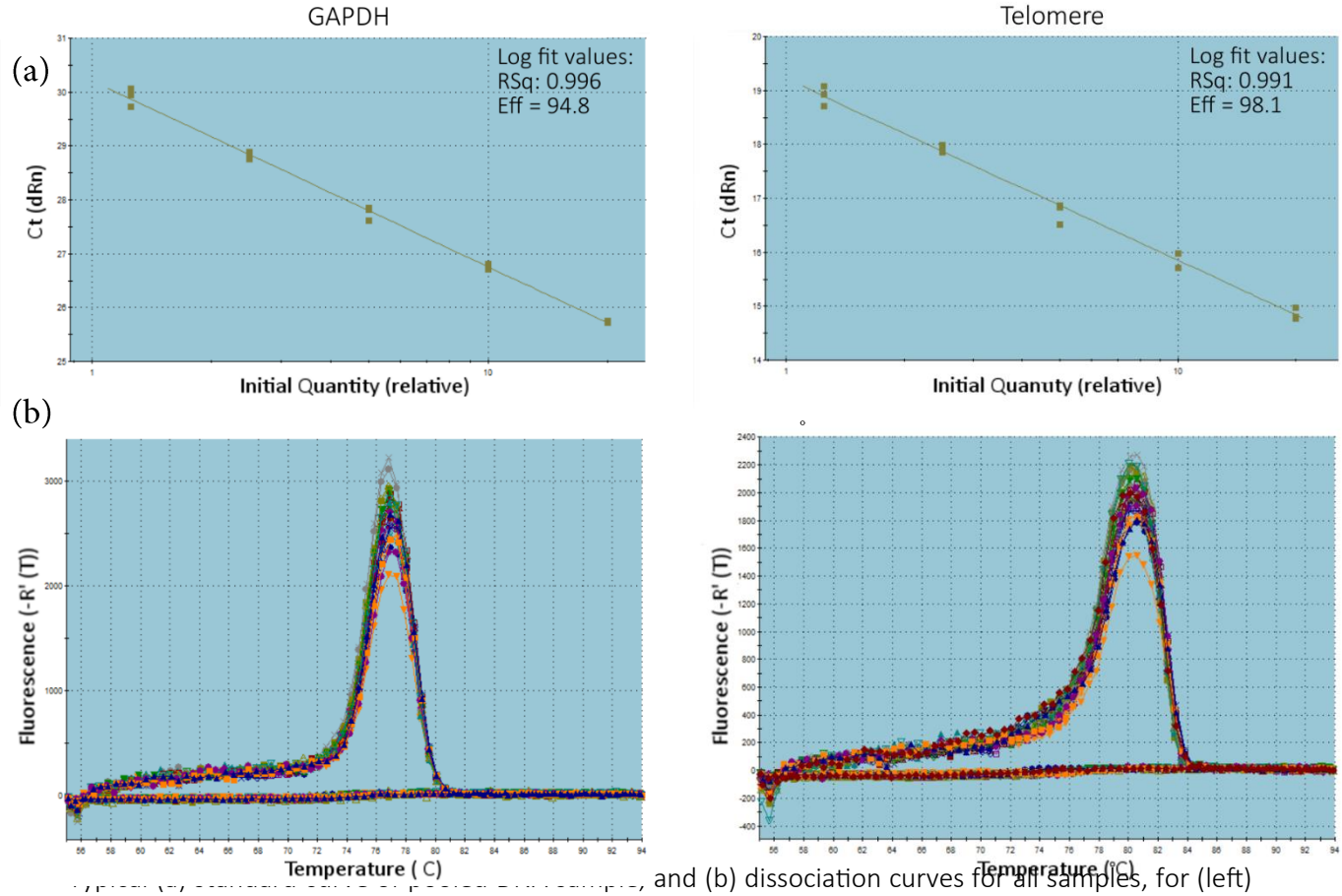


Figure 3. Melt peaks (left) and curves (right) for three sets of primers at their optimal annealing temperatures. a) telomere primers (56.8°C), b) GAPDH primer set 1 (60.7°C) and c) GAPDH primer set 3 (60.7°C).


-d(RFU)/dT
600
500
400
300
200
65
70
75
80
85
Temperature, Celsius
RFU (10^3)
11
10
9
8
7
6
60
70
80
90
Temperature, Celsius

qPCR standard curves and dissociation curves



Typical (a) standard curves of pooled DNA samples, and (b) dissociation curves for all samples, for (left) GAPDH and (right) Telomere.

Full Δ6 AICc top model set corresponding to **Table 3a in Chapter 2**, showing predictors of the probability of survival to the following season of Day 4 nestlings. Estimates are given for continuous variables. Presence of factors in the model is noted with +. Int = intercept.

Int	Sex	Age	Age next ssn	D5 Body Mass	Group size	post-lay rain	pre lay rain	RTL	df	logLik	AICc	delta	weight
-0.073			-0.595					-1.063	6	-48.56	110.24	0.00	0.105
-0.060			-0.684			-0.304		-1.111	7	-47.83	111.16	0.92	0.066
-0.069			-0.528		-0.231			-1.043	7	-48.16	111.83	1.59	0.048
-0.075			-0.806				-0.300	-1.026	7	-48.26	112.04	1.80	0.043
-0.211	+		-0.571					-1.067	7	-48.41	112.33	2.09	0.037
-0.073			-0.566	0.074				-1.051	7	-48.52	112.55	2.31	0.033
-0.071		-0.047	-0.611					-1.058	7	-48.55	112.60	2.36	0.032
-0.063			-0.944			-0.330	-0.357	-1.072	8	-47.42	112.82	2.58	0.029
-0.072		0.354	-0.644			-0.529		-1.206	8	-47.43	112.83	2.59	0.029
-0.059			-0.623		-0.212	-0.291		-1.092	8	-47.50	112.98	2.74	0.027
-0.242	+		-0.657			-0.327		-1.119	8	-47.58	113.12	2.88	0.025
-0.078								-0.843	5	-51.20	113.19	2.95	0.024
-0.083					-0.367			-0.851	6	-50.05	113.22	2.98	0.024
-0.061			-0.641	0.133		-0.328		-1.098	8	-47.71	113.39	3.15	0.022
-0.081		0.558	-1.017			-0.689	-0.587	-1.160	9	-46.52	113.55	3.31	0.020
-0.069			-0.722		-0.213		-0.266	-1.011	8	-47.93	113.84	3.60	0.017
-0.077				0.313	-0.405			-0.853	7	-49.24	114.00	3.76	0.016
-0.068			-0.463	0.137	-0.263			-1.018	8	-48.03	114.03	3.79	0.016
-0.172	+		-0.515		-0.213			-1.047	8	-48.08	114.13	3.89	0.015
-0.077							0.304	-0.966	6	-50.55	114.22	3.98	0.014
-0.208	+		-0.778				-0.295	-1.030	8	-48.13	114.23	3.99	0.014
-0.070		0.008	-0.524		-0.233			-1.044	8	-48.16	114.29	4.05	0.014
-0.077				0.273				-0.856	6	-50.60	114.31	4.07	0.014
-0.076			-0.781	0.107			-0.325	-1.005	8	-48.18	114.34	4.10	0.014
-0.071		0.428	-0.536		-0.272	-0.547		-1.182	9	-46.93	114.36	4.12	0.013
-0.075		0.003	-0.806				-0.301	-1.026	8	-48.26	114.50	4.26	0.013
-0.079					-0.339		0.255	-0.947	7	-49.59	114.70	4.46	0.011
-0.215	+	-0.064	-0.592					-1.060	8	-48.38	114.73	4.50	0.011
-0.205	+		-0.549	0.058				-1.058	8	-48.38	114.74	4.50	0.011
-0.287	+							-0.863	6	-50.83	114.78	4.54	0.011
-0.094		0.622			-0.433	-0.554		-1.011	8	-48.44	114.86	4.62	0.010
-0.244	+		-0.913			-0.354	-0.355	-1.080	9	-47.19	114.87	4.63	0.010
-0.059			-0.867		-0.185	-0.316	-0.323	-1.058	9	-47.19	114.87	4.64	0.010

Int	Sex	Age	Age next ssn	D5 Body Mass	Group size	post-lay rain	pre lay rain	RTL	df	logLik	AICc	delta	weight
-0.064			-0.906	0.173		-0.357	-0.399	-1.038	9	-47.22	114.95	4.71	0.010
-0.239	+	0.326	-0.611			-0.526		-1.195	9	-47.22	114.95	4.71	0.010
-0.070		-0.063	-0.582	0.085				-1.042	8	-48.49	114.96	4.72	0.010
-0.084		0.202			-0.390			-0.892	7	-49.74	114.99	4.75	0.010
-0.059			-0.537	0.190	-0.256	-0.319		-1.057	9	-47.27	115.04	4.80	0.010
-0.077						-0.154		-0.848	6	-50.98	115.09	4.85	0.009
-0.083		0.151						-0.880	6	-51.02	115.17	4.93	0.009
-0.073		0.343	-0.608	0.115		-0.543		-1.193	9	-47.34	115.19	4.95	0.009
-0.206	+		-0.608		-0.181	-0.311		-1.100	9	-47.35	115.20	4.96	0.009
-0.085					-0.366	-0.146		-0.856	7	-49.87	115.24	5.00	0.009
-0.226	+				-0.339			-0.864	7	-49.88	115.27	5.03	0.009
-0.093		0.554				-0.530		-1.004	7	-49.88	115.27	5.03	0.009
-0.081		0.631	-0.912		-0.254	-0.711	-0.573	-1.146	10	-46.10	115.30	5.06	0.008
-0.229	+		-0.616	0.109		-0.341		-1.101	9	-47.49	115.49	5.25	0.008
-0.081				0.379	-0.414	-0.243		-0.860	8	-48.78	115.53	5.29	0.007
-0.077				0.340		-0.243		-0.869	7	-50.13	115.78	5.54	0.007
-0.080		0.554	-0.987	0.160		-0.713	-0.626	-1.131	10	-46.36	115.81	5.57	0.006
-0.232	+	0.531	-0.982			-0.689	-0.572	-1.160	10	-46.36	115.83	5.59	0.006
-0.089		0.552		0.321	-0.464	-0.588		-1.005	9	-47.72	115.94	5.70	0.006
-0.269	+						0.288	-0.975	7	-50.24	115.99	5.75	0.006
-0.075						-0.188	0.329	-0.985	7	-50.25	116.02	5.78	0.006
-0.069			-0.667	0.160	-0.246		-0.294	-0.977	9	-47.76	116.02	5.78	0.006
-0.077				0.201			0.234	-0.948	7	-50.27	116.04	5.80	0.006
-0.076				0.262	-0.383		0.151	-0.910	8	-49.10	116.18	5.94	0.005
-0.172	+		-0.708		-0.195		-0.267	-1.015	9	-47.85	116.21	5.97	0.005
-0.250	+			0.244				-0.868	7	-50.35	116.22	5.98	0.005

Full Δ 6 AICc top model set corresponding to **Table 3b in Chapter 2**, showing predictors of the probability of survival to the following season of day 12 nestlings. Estimates are given for continuous variables. Presence of factors in the model is noted with +. Int = intercept.

Int	Sex	Age	Age next season	D13 Body mass	Group size	post-lay rain	pre lay rain	RTL	df	logLik	AICc	delta	weight
0.60			-2.16	0.70		-0.60	-1.70		8.00	-84.68	186.41	0.00	0.08
1.08	+		-2.47	0.85		-0.67	-1.97		9.00	-83.60	186.53	0.12	0.08
0.93	+		-1.93	0.93			-1.72		8.00	-84.98	187.00	0.60	0.06
0.60			-1.73	0.64			-1.40		7.00	-86.14	187.10	0.69	0.06
0.43			-2.05	0.72	0.50		-1.60		8.00	-85.06	187.16	0.76	0.06
0.92	+		-2.40	0.89	0.53		-1.91		9.00	-83.95	187.22	0.82	0.06
0.42			-2.29	0.69	0.43	-0.56	-1.77		9.00	-84.05	187.42	1.01	0.05
0.85	+		-2.61	0.85	0.42	-0.62	-2.06		10.00	-83.12	187.87	1.47	0.04
0.59			-2.16	0.68		-0.61	-1.70	-0.17	9.00	-84.42	188.17	1.76	0.03
1.06	+		-2.45	0.83		-0.68	-1.96	-0.20	10.00	-83.31	188.25	1.85	0.03
0.61		0.02	-2.17	0.70		-0.60	-1.70		9.00	-84.67	188.67	2.26	0.03
0.92	+		-1.94	0.90			-1.71	-0.20	9.00	-84.72	188.77	2.36	0.03
1.10	+	0.03	-2.49	0.86		-0.67	-1.98		10.00	-83.59	188.82	2.41	0.03
0.57			-1.72	0.64			-1.39	-0.14	8.00	-85.97	189.00	2.59	0.02
0.43			-2.05	0.70	0.48		-1.59	-0.14	9.00	-84.91	189.15	2.74	0.02
0.91	+		-2.37	0.87	0.50		-1.88	-0.15	10.00	-83.81	189.24	2.83	0.02
0.99	+	0.06	-2.04	0.99			-1.80		9.00	-84.97	189.27	2.86	0.02
0.61		0.04	-1.75	0.65			-1.40		8.00	-86.14	189.32	2.91	0.02
0.42			-2.28	0.68	0.41	-0.58	-1.76	-0.15	10.00	-83.85	189.33	2.92	0.02
0.44		0.07	-2.09	0.73	0.52		-1.61		9.00	-85.02	189.37	2.96	0.02
0.95	+	0.09	-2.48	0.93	0.56		-1.95		10.00	-83.91	189.45	3.04	0.02
0.42		0.01	-2.29	0.69	0.44	-0.57	-1.77		10.00	-84.05	189.72	3.32	0.02
0.85	+		-2.58	0.83	0.39	-0.64	-2.03	-0.18	11.00	-82.90	189.77	3.36	0.02
0.78			-2.14				-1.42		6.00	-88.63	189.86	3.45	0.01
0.75			-2.47			-0.47	-1.65		7.00	-87.69	190.19	3.79	0.01
0.86	+	0.03	-2.64	0.86	0.43	-0.62	-2.07		11.00	-83.12	190.21	3.80	0.01
0.59		0.01	-2.16	0.69		-0.61	-1.70	-0.17	10.00	-84.42	190.47	4.06	0.01
0.46			-2.25		0.51		-1.53		7.00	-87.85	190.52	4.11	0.01
1.07	+	0.01	-2.45	0.83		-0.69	-1.96	-0.20	11.00	-83.31	190.59	4.18	0.01
0.48			-2.56		0.47	-0.46	-1.72		8.00	-87.00	191.05	4.64	0.01
0.92	+	0.03	-1.95	0.91			-1.71	-0.19	10.00	-84.72	191.07	4.66	0.01
1.10	+		-2.34				-1.56		7.00	-88.16	191.14	4.73	0.01
0.46				0.78					5.00	-90.36	191.15	4.75	0.01
0.58		0.02	-1.72	0.64			-1.39	-0.14	9.00	-85.97	191.26	4.85	0.01
0.43		0.06	-2.08	0.72	0.49		-1.60	-0.13	10.00	-84.89	191.41	5.01	0.01
1.08	+		-2.71			-0.50	-1.81		8.00	-87.19	191.43	5.02	0.01
0.93	+	0.07	-2.44	0.90	0.52		-1.92	-0.15	11.00	-83.78	191.52	5.12	0.01

Int	Sex	Age	Age next season	D13 Body mass	Group size	post-lay rain	pre lay rain	RTL	df	logLik	AICc	delta	weight
0.42		0.00	-2.28	0.68	0.41	-0.58	-1.76	-0.15	11.00	-83.85	191.67	5.26	0.01
0.76			-2.12				-1.41	-0.15	7.00	-88.45	191.70	5.30	0.01
0.73			-2.46			-0.49	-1.65	-0.18	8.00	-87.42	191.89	5.48	0.01
0.55			-0.43	0.66					6.00	-89.68	191.96	5.55	0.01
0.73	+		-2.45		0.53		-1.68		8.00	-87.50	192.04	5.63	0.01
0.79		0.02	-2.15				-1.42		7.00	-88.63	192.06	5.65	0.00
0.86	+	0.01	-2.58	0.83	0.39	-0.64	-2.03	-0.18	12.00	-82.90	192.14	5.74	0.00
0.77	+			0.89					6.00	-89.84	192.27	5.87	0.00

Full Δ 6 AICc top model set corresponding to **Table 4a in Chapter 2**, showing predictors of the probability of survival to the fledging of all nestlings. Estimates are given for continuous variables. Presence of factors in the model is noted with +. Int = intercept.

Int	Sex	Age	D5 Body mass	Group size	post lay rain	pre lay rain	RTL	df	logLik	AICc	delta	weight
0.35			0.58	-0.42			-0.82	7	-47.30	110.11	0.00	0.073
0.36			0.70	-0.44	-0.41		-0.85	8	-46.09	110.15	0.04	0.072
0.38			0.69		-0.43		-0.89	7	-47.42	110.35	0.24	0.065
0.37			0.56				-0.86	6	-48.66	110.44	0.33	0.062
-0.02	+		0.61		-0.46		-0.91	8	-46.39	110.75	0.64	0.053
0.03	+		0.50				-0.89	7	-47.87	111.26	1.15	0.041
0.06	+		0.65	-0.36	-0.44		-0.89	9	-45.45	111.40	1.30	0.038
0.10	+		0.54	-0.37			-0.86	8	-46.82	111.61	1.50	0.034
0.35			0.66	-0.46		-0.22	-0.75	8	-46.99	111.96	1.85	0.029
0.35		-0.18	0.63	-0.41			-0.80	8	-47.08	112.13	2.02	0.027
0.35			0.77	-0.46	-0.40	-0.21	-0.78	9	-45.82	112.15	2.04	0.026
0.37		-0.23	0.62				-0.82	7	-48.34	112.20	2.09	0.026
-0.06	+						-0.84	6	-49.62	112.35	2.24	0.024
0.33							-0.80	5	-50.81	112.41	2.30	0.023
0.36		0.17	0.68	-0.45	-0.52		-0.89	9	-45.98	112.46	2.35	0.023
0.38			0.73		-0.43	-0.13	-0.84	8	-47.33	112.62	2.52	0.021
0.36			0.60			-0.13	-0.82	7	-48.57	112.65	2.54	0.021
0.38		0.13	0.67		-0.51		-0.91	8	-47.37	112.71	2.60	0.020
0.32				-0.34			-0.79	6	-49.81	112.73	2.62	0.020
0.00	+	-0.26	0.56				-0.84	8	-47.41	112.80	2.69	0.019
-0.03	+		0.66		-0.46	-0.15	-0.86	9	-46.25	113.01	2.90	0.017
-0.11	+				-0.31		-0.86	7	-48.82	113.15	3.04	0.016
-0.02	+	0.08	0.60		-0.51		-0.93	9	-46.37	113.23	3.12	0.015
0.00	+			-0.29			-0.83	7	-48.91	113.33	3.22	0.015
0.05	+		0.73	-0.39	-0.44	-0.22	-0.82	10	-45.18	113.45	3.35	0.014
0.02	+		0.55			-0.14	-0.85	8	-47.76	113.49	3.38	0.013
0.10	+		0.63	-0.41		-0.23	-0.79	9	-46.52	113.53	3.42	0.013
0.08	+	-0.21	0.60	-0.35			-0.83	9	-46.52	113.55	3.44	0.013
0.33					-0.24		-0.81	6	-50.32	113.76	3.65	0.012
0.07	+	0.14	0.63	-0.37	-0.52		-0.91	10	-45.38	113.86	3.75	0.011
0.35		0.37	0.77	-0.51	-0.63	-0.35	-0.79	10	-45.42	113.94	3.83	0.011
0.32				-0.34	-0.22		-0.80	7	-49.38	114.28	4.17	0.009
0.35		-0.12	0.68	-0.44		-0.18	-0.75	9	-46.92	114.33	4.22	0.009
-0.06	+			-0.27	-0.29		-0.85	8	-48.24	114.45	4.34	0.008
-0.07	+	-0.10					-0.83	7	-49.53	114.58	4.47	0.008
0.36		-0.21	0.63			-0.06	-0.81	8	-48.33	114.62	4.52	0.008
0.33						0.08	-0.83	6	-50.76	114.64	4.53	0.008

-0.05	+					0.05	-0.86	7	-49.59	114.70	4.59	0.007
0.33		-0.04					-0.80	6	-50.80	114.72	4.61	0.007
0.38		0.24	0.73		-0.58	-0.21	-0.86	9	-47.17	114.85	4.74	0.007
0.32				-0.34		0.03	-0.80	7	-49.80	115.11	5.00	0.006
0.32		0.00		-0.34			-0.79	7	-49.81	115.12	5.02	0.006
-0.10	+	0.20			-0.45		-0.90	8	-48.64	115.25	5.14	0.006
0.00	+	-0.25	0.58			-0.06	-0.82	9	-47.40	115.29	5.18	0.005
-0.03	+	0.19	0.66		-0.57	-0.21	-0.88	10	-46.15	115.40	5.29	0.005
0.07	+	0.33	0.73	-0.44	-0.64	-0.34	-0.83	11	-44.87	115.50	5.39	0.005
-0.10	+				-0.32	0.08	-0.89	8	-48.77	115.52	5.41	0.005
0.34		0.27			-0.42		-0.87	7	-50.04	115.60	5.49	0.005
-0.01	+	-0.05		-0.28			-0.83	8	-48.89	115.75	5.64	0.004
0.00	+			-0.29		0.01	-0.84	8	-48.91	115.79	5.68	0.004
0.09	+	-0.15	0.65	-0.38		-0.17	-0.79	10	-46.38	115.87	5.76	0.004
0.33		0.32		-0.37	-0.43		-0.86	8	-48.97	115.92	5.81	0.004
0.34					-0.25	0.11	-0.85	7	-50.24	115.98	5.88	0.004

Full Δ 6 AICc top model set corresponding to **Table 4b in Chapter 2**, showing predictors of the probability of survival to the fledging. 'predated' nestlings removed. Estimates are given for continuous variables. Presence of factors in the model is noted with +. Int = intercept.

Int	Sex	Age	D5 Body mass	Group size	post lay rain	pre lay rain	RTL	df	logLik	AICc	delta	weight
0.86			0.57					5	-39.86	90.69	0.00	0.122
0.89			0.58				-0.33	6	-39.17	91.72	1.03	0.073
0.64	+		0.54					6	-39.56	92.49	1.80	0.049
0.86			0.61		-0.20			6	-39.60	92.58	1.89	0.047
0.81								4	-42.03	92.70	2.01	0.044
0.86		-0.07	0.59					6	-39.83	93.05	2.36	0.037
0.86			0.58			-0.02		6	-39.86	93.10	2.41	0.037
0.86			0.57	-0.01				6	-39.86	93.10	2.41	0.036
0.62	+		0.54				-0.38	7	-38.71	93.28	2.59	0.033
0.90			0.63		-0.23		-0.35	7	-38.83	93.54	2.85	0.029
0.82							-0.31	5	-41.35	93.67	2.98	0.027
0.51	+							5	-41.41	93.80	3.11	0.026
0.89			0.56			0.08	-0.35	7	-39.14	94.15	3.46	0.022
0.89			0.59	-0.05			-0.34	7	-39.15	94.18	3.49	0.021
0.89		-0.03	0.59				-0.32	7	-39.17	94.20	3.51	0.021
0.62	+		0.58		-0.22			7	-39.24	94.36	3.67	0.019
0.48	+						-0.37	6	-40.52	94.41	3.72	0.019
0.81						0.16		5	-41.85	94.67	3.98	0.017

Int	Sex	Age	D5 Body mass	Group size	post lay rain	pre lay rain	RTL	df	logLik	AICc	delta	weight
0.62	+	-0.11	0.57					7	-39.49	94.84	4.15	0.015
0.81		0.10						5	-41.96	94.89	4.20	0.015
0.62	+		0.53	0.06				7	-39.54	94.94	4.25	0.015
0.63	+		0.56			-0.06		7	-39.54	94.94	4.25	0.015
0.81					-0.07			5	-42.00	94.96	4.27	0.014
0.87		0.12	0.60		-0.27			7	-39.56	94.98	4.29	0.014
0.60	+		0.59		-0.26		-0.41	8	-38.29	95.01	4.32	0.014
0.81				0.04				5	-42.03	95.02	4.33	0.014
0.86			0.62		-0.20	-0.02		7	-39.60	95.07	4.38	0.014
0.86			0.61	-0.02	-0.20			7	-39.60	95.07	4.38	0.014
0.84						0.26	-0.38	6	-40.96	95.29	4.60	0.012
0.86		-0.07	0.59	-0.01				7	-39.83	95.53	4.84	0.011
0.86		-0.07	0.59			0.01		7	-39.83	95.54	4.85	0.011
0.86			0.58	-0.02		-0.02		7	-39.86	95.58	4.89	0.011
0.90		0.23	0.60		-0.37		-0.39	8	-38.67	95.77	5.08	0.010
0.60	+	-0.08	0.56				-0.37	8	-38.67	95.79	5.10	0.010
0.83		0.14					-0.33	6	-41.23	95.83	5.14	0.009
0.62	+		0.53			0.03	-0.38	8	-38.70	95.84	5.15	0.009
0.61	+		0.54	0.02			-0.37	8	-38.70	95.85	5.16	0.009
0.83					-0.10		-0.32	6	-41.28	95.94	5.25	0.009
0.49	+				-0.12			6	-41.31	96.01	5.32	0.009
0.48	+			0.12				6	-41.31	96.01	5.32	0.009
0.90			0.64	-0.07	-0.23		-0.36	8	-38.80	96.05	5.36	0.008
0.90			0.61		-0.23	0.08	-0.37	8	-38.80	96.05	5.36	0.008
0.54	+					0.11		6	-41.34	96.06	5.37	0.008
0.82				0.00			-0.31	6	-41.35	96.08	5.39	0.008
0.52	+	0.04						6	-41.40	96.19	5.50	0.008
0.52	+					0.20	-0.42	7	-40.30	96.46	5.77	0.007
0.46	+				-0.16		-0.39	7	-40.35	96.57	5.88	0.006
0.89		-0.06	0.57			0.10	-0.35	8	-39.12	96.69	6.00	0.006

Full Δ 6 AICc top model set corresponding to **Table 4c in Chapter 2**, showing predictors of the probability of survival to the fledging. 'expired' nestlings removed. Estimates are given for continuous variables. Presence of factors in the model is noted with +. Int = intercept.

Int	Sex	Age	D5 Body mass	Group size	post rain	pre rain	RTL	df	logLik	AICc	delta	Weight
0.94					-0.67		-1.23	6	-40.21	93.67	0.00	0.071
0.83				-0.55	-0.57		-1.10	7	-38.99	93.68	0.00	0.071
8.24								4	-42.59	93.75	0.08	0.068
0.81				-0.56			-1.04	6	-40.50	94.25	0.58	0.053
0.90							-1.15	5	-41.92	94.72	1.05	0.042
0.55	+				-0.65		-1.18	7	-39.87	95.43	1.76	0.029
9.21	+							5	-42.31	95.50	1.83	0.028
0.83			0.27	-0.59	-0.62		-1.08	8	-38.66	95.53	1.86	0.028
7.90					-0.79			5	-42.38	95.64	1.96	0.026
0.96			0.22		-0.72		-1.23	7	-40.02	95.74	2.06	0.025
0.56	+			-0.49	-0.56		-1.07	8	-38.81	95.83	2.16	0.024
0.81		-0.29		-0.54			-1.01	7	-40.09	95.89	2.21	0.023
8.33			-0.34					5	-42.51	95.90	2.23	0.023
8.23						-0.17		5	-42.57	96.03	2.35	0.022
0.94		0.13			-0.76		-1.25	7	-40.17	96.04	2.37	0.022
0.89		-0.35					-1.10	6	-41.40	96.05	2.37	0.022
0.82		0.15		-0.55	-0.67		-1.13	8	-38.93	96.08	2.41	0.021
0.94					-0.67	-0.02	-1.22	7	-40.21	96.11	2.44	0.021
0.82				-0.55	-0.56	-0.07	-1.07	8	-38.97	96.15	2.48	0.020
0.81			0.17	-0.59			-1.02	7	-40.36	96.41	2.74	0.018
0.65	+			-0.53			-1.03	7	-40.41	96.52	2.84	0.017
0.80				-0.57		-0.12	-1.00	7	-40.44	96.58	2.90	0.017
0.62	+						-1.13	6	-41.70	96.66	2.99	0.016
8.38		1.43			-1.95			6	-41.73	96.71	3.04	0.015
0.90			0.10				-1.15	6	-41.88	97.01	3.33	0.013
0.89						-0.06	-1.13	6	-41.91	97.07	3.39	0.013
5.55				-1.62	-3.19			6	-42.03	97.31	3.63	0.011
0.58	+		0.18		-0.68		-1.18	8	-39.73	97.67	4.00	0.010
9.16	+		-0.18					6	-42.29	97.83	4.16	0.009
0.56	+	-0.37					-1.07	7	-41.09	97.88	4.21	0.009
0.81		-0.34	0.23	-0.57			-0.98	8	-39.83	97.88	4.21	0.009
0.61	+		0.23	-0.53	-0.60		-1.05	9	-38.54	97.89	4.22	0.009
0.56	+	0.09			-0.71		-1.20	8	-39.85	97.91	4.24	0.008
0.81			0.30	-0.59	-0.60	-0.16	-1.01	9	-38.56	97.93	4.26	0.008
0.54	+				-0.64	-0.04	-1.17	8	-39.86	97.93	4.26	0.008
7.91					-0.76	-0.14		6	-42.37	97.99	4.32	0.008
7.99			-0.24		-0.63			6	-42.37	97.99	4.32	0.008

Int	Sex	Age	D5 Body mass	Group size	post rain	pre rain	RTL	df	logLik	AICc	delta	Weight
0.83		0.10	0.26	-0.59	-0.68		-1.10	9	-38.63	98.08	4.41	0.008
0.60	+	-0.31		-0.50			-0.99	8	-39.95	98.12	4.45	0.008
0.95			0.24		-0.71	-0.09	-1.19	8	-40.00	98.21	4.53	0.007
0.89		-0.39	0.18				-1.09	7	-41.26	98.21	4.54	0.007
0.96		0.09	0.21		-0.78		-1.24	8	-40.00	98.22	4.55	0.007
0.57	+	0.12		-0.49	-0.64		-1.09	9	-38.77	98.35	4.68	0.007
0.54	+			-0.49	-0.55	-0.09	-1.03	9	-38.77	98.36	4.69	0.007
5.73	+				-4.78			6	-42.57	98.39	4.71	0.007
0.81		-0.30		-0.54		0.02	-1.01	8	-40.09	98.40	4.73	0.007
0.90		-0.40				0.11	-1.13	7	-41.36	98.41	4.74	0.007
7.95		1.47		-1.24	-2.41			7	-41.37	98.43	4.76	0.007
0.79		0.26		-0.56	-0.71	-0.18	-1.07	9	-38.84	98.49	4.82	0.006
0.93		0.19			-0.79	-0.10	-1.23	8	-40.15	98.52	4.85	0.006
9.31		2.02	-0.83		-2.06			7	-41.46	98.62	4.95	0.006
0.80			0.22	-0.60		-0.18	-0.95	8	-40.22	98.66	4.99	0.006
7.66		3.01		-4.75	-5.03	-3.91		8	-40.26	98.73	5.06	0.006
0.67	+		0.15	-0.56			-1.01	8	-40.29	98.80	5.13	0.005
0.63	+			-0.54		-0.13	-0.99	8	-40.34	98.90	5.23	0.005
8.40		1.53			-1.90	-0.60		7	-41.61	98.92	5.24	0.005
6.75	+	1.92			-6.89			7	-41.64	98.99	5.31	0.005
12.04	+	5.38		-7.53	-7.33	-5.59		9	-39.09	98.99	5.32	0.005
0.63	+		0.08				-1.13	7	-41.68	99.05	5.38	0.005
0.62	+					-0.07	-1.11	7	-41.68	99.07	5.39	0.005
6.25	+			-1.88	-3.70			7	-41.77	99.24	5.56	0.004
0.90			0.12			-0.09	-1.12	7	-41.85	99.39	5.72	0.004
7.16				-3.99	-2.86	-1.62		7	-41.91	99.52	5.85	0.004

Full Δ 6 AICc top model set corresponding to **Table 5a in Chapter 2**, showing predictors of the probability of survival of day 12 nestlings to the following season. All data included Estimates are given for continuous variables. Presence of factors in the model is noted with +. Int = intercept.

Int	Sex	Age	Delta TL rate	Delta TL rate^2	Group size	post lay rain	RTL D4	Mass D12	df	logLik	AICc	delta	weight
1.15			1.05	-1.17				1.02	6	-18.32	51.35	0.00	0.102
1.80	+		0.78	-1.21				1.27	7	-17.32	52.38	1.03	0.061
1.37			0.75	-1.35					5	-20.26	52.39	1.04	0.061
1.09	+							1.28	5	-20.35	52.58	1.24	0.055
1.42		-0.65	1.06	-1.49					6	-19.31	53.33	1.98	0.038
0.45								0.99	4	-22.10	53.42	2.07	0.036
0.35			0.73					1.27	5	-20.88	53.63	2.28	0.033
1.25	+						-0.61	1.45	6	-19.49	53.68	2.33	0.032
0.99	+		0.65					1.45	6	-19.51	53.74	2.39	0.031
1.17		-0.37	1.15	-1.24				0.91	7	-18.02	53.78	2.44	0.030
1.83	+		0.60	-1.42					6	-19.64	53.99	2.64	0.027
1.12			1.12	-1.16		-0.23		1.17	7	-18.21	54.15	2.80	0.025
1.14			1.05	-1.15	-0.06			1.02	7	-18.31	54.35	3.00	0.023
1.15			1.04	-1.17			-0.01	1.02	7	-18.32	54.37	3.02	0.023
1.27	+					-0.46		1.54	6	-19.89	54.49	3.14	0.021
0.44							-0.49	1.00	5	-21.42	54.72	3.38	0.019
1.97	+	-0.77	1.01	-1.63					7	-18.51	54.75	3.41	0.019
1.37			0.75	-1.36		0.21			6	-20.12	54.94	3.60	0.017
1.81	+	-0.41	0.94	-1.31				1.14	8	-17.00	54.97	3.62	0.017
1.35			0.56	-1.32			-0.24		6	-20.14	54.99	3.64	0.017
1.13	+				-0.21			1.25	6	-20.21	55.12	3.78	0.015
1.79	+		0.85	-1.17		-0.31		1.43	8	-17.11	55.19	3.84	0.015
1.50	+					-0.68	-0.77	1.86	7	-18.73	55.20	3.85	0.015
1.37			0.75	-1.35	0.01				6	-20.26	55.22	3.87	0.015
1.08	+	-0.13						1.25	6	-20.30	55.32	3.97	0.014
1.85	+		0.56	-1.19			-0.22	1.25	8	-17.25	55.46	4.12	0.013
1.82	+		0.80	-1.18	-0.16			1.25	8	-17.26	55.48	4.14	0.013
1.46		-0.87	1.15	-1.62	0.37				7	-18.98	55.70	4.36	0.012
1.40		-0.73	0.77	-1.42			-0.39		7	-19.02	55.78	4.43	0.011
0.47						-0.23		1.09	5	-21.95	55.78	4.43	0.011
1.09	+		0.68			-0.45		1.72	7	-19.06	55.86	4.52	0.011
1.40		-0.66	1.06	-1.50		0.28			7	-19.09	55.91	4.56	0.010
0.46					-0.12			1.00	5	-22.05	55.98	4.63	0.010
0.44		-0.11						0.98	5	-22.07	56.01	4.66	0.010
0.34			0.78			-0.30		1.44	6	-20.66	56.02	4.68	0.010
1.24	+	-0.38					-0.74	1.38	7	-19.20	56.13	4.78	0.009
0.36			0.74		-0.17			1.28	6	-20.78	56.27	4.92	0.009

Int	Sex	Age	Delta TL rate	Delta TL rate^2	Group size	post lay rain	RTL D4	Mass D12	df	logLik	AICc	delta	weight
0.33		-0.18	0.75					1.26	6	-20.78	56.27	4.92	0.009
0.36			0.64				-0.17	1.24	6	-20.82	56.35	5.01	0.008
1.11	+		0.40				-0.36	1.47	7	-19.33	56.39	5.04	0.008
1.29	+				-0.25		-0.63	1.42	7	-19.33	56.40	5.06	0.008
1.02	+		0.65		-0.23			1.44	7	-19.34	56.42	5.07	0.008
1.88	+		0.25	-1.37			-0.39		7	-19.37	56.46	5.12	0.008
0.96	+	-0.20	0.68					1.43	7	-19.39	56.52	5.17	0.008
1.19		-0.52	1.19	-1.34	0.22			0.87	8	-17.94	56.84	5.50	0.007
1.81	+		0.62	-1.43		0.13			7	-19.59	56.91	5.57	0.006
1.17		-0.41	1.03	-1.22			-0.14	0.86	8	-17.99	56.95	5.60	0.006
1.85	+		0.60	-1.40	-0.10				7	-19.61	56.95	5.61	0.006
1.16		-0.33	1.17	-1.23		-0.10		0.98	8	-18.01	56.98	5.63	0.006
2.11	+	-0.91	0.56	-1.61			-0.60		8	-18.01	56.98	5.64	0.006
0.46						-0.34	-0.55	1.17	6	-21.16	57.04	5.69	0.006
0.42		-0.28					-0.56	0.93	6	-21.22	57.15	5.81	0.006
1.32	+				-0.21	-0.46		1.52	7	-19.77	57.28	5.94	0.005

Full Δ 6 AICc top model set corresponding to **Table 5b in Chapter 2**, showing predictors of the probability of survival of day 12 nestlings to the following season. One outlier removed. Estimates are given for continuous variables. Presence of factors in the model is noted with +. Int = intercept.

Int	Sex	Age next season	Delta RTL rate	Delta RTL rate^2	Group size	post rain	D4 RTL	D12 Body Mass	df	logLik	AICc	delta	weight
1.15			1.05	-1.17				1.02	6	-18.32	51.35	0.00	0.102
1.80	+		0.78	-1.21				1.27	7	-17.32	52.38	1.03	0.061
1.37			0.75	-1.35					5	-20.26	52.39	1.04	0.061
1.09	+							1.28	5	-20.35	52.58	1.24	0.055
1.42		-0.65	1.06	-1.49					6	-19.31	53.33	1.98	0.038
0.45								0.99	4	-22.10	53.42	2.07	0.036
0.35			0.73					1.27	5	-20.88	53.63	2.28	0.033
1.25	+						-0.61	1.45	6	-19.49	53.68	2.33	0.032
0.99	+		0.65					1.45	6	-19.51	53.74	2.39	0.031
1.17		-0.37	1.15	-1.24				0.91	7	-18.02	53.78	2.44	0.030
1.83	+		0.60	-1.42					6	-19.64	53.99	2.64	0.027
1.12			1.12	-1.16		-0.23		1.17	7	-18.21	54.15	2.80	0.025
1.14			1.05	-1.15	-0.06			1.02	7	-18.31	54.35	3.00	0.023
1.15			1.04	-1.17			-0.01	1.02	7	-18.32	54.37	3.02	0.023
1.27	+					-0.46		1.54	6	-19.89	54.49	3.14	0.021
0.44							-0.49	1.00	5	-21.42	54.72	3.38	0.019
1.97	+	-0.77	1.01	-1.63					7	-18.51	54.75	3.41	0.019
1.37			0.75	-1.36		0.21			6	-20.12	54.94	3.60	0.017
1.81	+	-0.41	0.94	-1.31				1.14	8	-17.00	54.97	3.62	0.017
1.35			0.56	-1.32			-0.24		6	-20.14	54.99	3.64	0.017
1.13	+				-0.21			1.25	6	-20.21	55.12	3.78	0.015
1.79	+		0.85	-1.17		-0.31		1.43	8	-17.11	55.19	3.84	0.015
1.50	+					-0.68	-0.77	1.86	7	-18.73	55.20	3.85	0.015
1.37			0.75	-1.35	0.01				6	-20.26	55.22	3.87	0.015
1.08	+	-0.13						1.25	6	-20.30	55.32	3.97	0.014
1.85	+		0.56	-1.19			-0.22	1.25	8	-17.25	55.46	4.12	0.013
1.82	+		0.80	-1.18	-0.16			1.25	8	-17.26	55.48	4.14	0.013
1.46		-0.87	1.15	-1.62	0.37				7	-18.98	55.70	4.36	0.012
1.40		-0.73	0.77	-1.42			-0.39		7	-19.02	55.78	4.43	0.011
0.47						-0.23		1.09	5	-21.95	55.78	4.43	0.011
1.09	+		0.68			-0.45		1.72	7	-19.06	55.86	4.52	0.011
1.40		-0.66	1.06	-1.50		0.28			7	-19.09	55.91	4.56	0.010

Int	Sex	Age next season	Delta RTL rate	Delta RTL rate^2	Group size	post rain	D4 RTL	D12 Body Mass	df	logLik	AICc	delta	weight
0.46					-0.12			1.00	5	-22.05	55.98	4.63	0.010
0.44		-0.11						0.98	5	-22.07	56.01	4.66	0.010
0.34			0.78			-0.30		1.44	6	-20.66	56.02	4.68	0.010
1.24	+	-0.38					-0.74	1.38	7	-19.20	56.13	4.78	0.009
0.36			0.74		-0.17			1.28	6	-20.78	56.27	4.92	0.009
0.33		-0.18	0.75					1.26	6	-20.78	56.27	4.92	0.009
0.36			0.64				-0.17	1.24	6	-20.82	56.35	5.01	0.008
1.11	+		0.40				-0.36	1.47	7	-19.33	56.39	5.04	0.008
1.29	+				-0.25		-0.63	1.42	7	-19.33	56.40	5.06	0.008
1.02	+		0.65		-0.23			1.44	7	-19.34	56.42	5.07	0.008
1.88	+		0.25	-1.37			-0.39		7	-19.37	56.46	5.12	0.008
0.96	+	-0.20	0.68					1.43	7	-19.39	56.52	5.17	0.008
1.19		-0.52	1.19	-1.34	0.22			0.87	8	-17.94	56.84	5.50	0.007
1.81	+		0.62	-1.43		0.13			7	-19.59	56.91	5.57	0.006
1.17		-0.41	1.03	-1.22			-0.14	0.86	8	-17.99	56.95	5.60	0.006
1.85	+		0.60	-1.40	-0.10				7	-19.61	56.95	5.61	0.006
1.16		-0.33	1.17	-1.23		-0.10		0.98	8	-18.01	56.98	5.63	0.006
2.11	+	-0.91	0.56	-1.61			-0.60		8	-18.01	56.98	5.64	0.006
0.46						-0.34	-0.55	1.17	6	-21.16	57.04	5.69	0.006
0.42		-0.28					-0.56	0.93	6	-21.22	57.15	5.81	0.006
1.32	+				-0.21	-0.46		1.52	7	-19.77	57.28	5.94	0.005

Appendix C: Chapter 3 unabridged top model sets

Full Δ 6 AICc top model set corresponding to **Table 1a in Chapter 3**. Response is day 4 nestling RTL with the influential point *retained*. Estimates are given for scaled and centred continuous variables. Presence of factors in the model is noted with +. Int = intercept.

Int	Sex	Bird age	D4 Body mass	mean egg mass	log Group size	Clutch size	pre-egg rain	Clutch size: rain	df	logLik	AICc	Δ AICc	weight
0.007							0.040		7	20.76	-26.09	0.00	0.053
0.008									6	19.39	-25.74	0.35	0.044
0.008		-0.035					0.055		8	21.75	-25.66	0.43	0.043
-0.026	+								7	20.48	-25.54	0.55	0.040
-0.022	+						0.037		8	21.61	-25.37	0.72	0.037
-0.022	+	-0.036					0.052		9	22.73	-25.12	0.98	0.033
0.007				-0.023			0.039		8	21.23	-24.62	1.48	0.025
0.008			-0.020				0.046		8	21.19	-24.54	1.56	0.024
0.009				-0.026					7	19.98	-24.53	1.56	0.024
-0.027	+			-0.029					8	21.17	-24.50	1.59	0.024
-0.027	+					0.025			8	20.98	-24.11	1.98	0.020
-0.024	+			-0.025			0.035		9	22.18	-24.03	2.07	0.019
0.010						0.018			7	19.67	-23.93	2.16	0.018
0.008		-0.033		-0.021			0.053		9	22.12	-23.91	2.19	0.018
0.008						0.010	0.038		8	20.84	-23.83	2.27	0.017
0.009		-0.016							7	19.62	-23.82	2.27	0.017
-0.021	+		-0.020				0.043		9	22.08	-23.82	2.28	0.017
-0.027	+	-0.020							8	20.82	-23.80	2.29	0.017
0.007					0.006		0.042		8	20.79	-23.74	2.35	0.016
0.009			-0.010						7	19.51	-23.59	2.50	0.015
0.008		-0.039			0.016		0.060		9	21.96	-23.59	2.50	0.015
0.009		-0.032	-0.013				0.057		9	21.95	-23.56	2.53	0.015
-0.027	+	-0.042			0.025		0.058		10	23.22	-23.54	2.56	0.015
-0.024	+	-0.034		-0.023			0.049		10	23.17	-23.45	2.64	0.014
-0.026	+		-0.011						8	20.62	-23.39	2.71	0.014
0.008					0.000				7	19.39	-23.37	2.72	0.014
-0.024	+					0.016	0.033		9	21.83	-23.33	2.77	0.013
-0.027	+				0.008				8	20.54	-23.23	2.87	0.013
0.008		-0.034				0.006	0.054		9	21.78	-23.22	2.87	0.013
-0.025	+				0.014		0.039		9	21.77	-23.20	2.89	0.012

Int	Sex	Bird age	D4 Body mass	mean egg mass	log Group size	Clutch size	pre-egg rain	Clutch size: rain	df	logLik	AICc	Δ AICc	weight
-0.022	+	-0.033	-0.014				0.054		10	22.94	-22.98	3.11	0.011
-0.023	+	-0.035				0.012	0.048		10	22.84	-22.79	3.31	0.010
0.008			-0.016	-0.019			0.044		9	21.51	-22.67	3.42	0.010
-0.028	+	-0.018		-0.028					9	21.47	-22.60	3.49	0.009
-0.028	+			-0.024		0.019			9	21.45	-22.56	3.53	0.009
0.009		-0.015		-0.026					8	20.16	-22.47	3.62	0.009
0.010				-0.023		0.013			8	20.10	-22.35	3.74	0.008
0.000						-0.010	0.037	0.026	9	21.31	-22.28	3.82	0.008
-0.027	+	-0.019				0.024			9	21.29	-22.24	3.85	0.008
0.007				-0.024	0.008		0.040		9	21.29	-22.24	3.85	0.008
-0.029	+			-0.030	0.011				9	21.27	-22.20	3.89	0.008
0.009			-0.006	-0.025					8	20.02	-22.19	3.90	0.008
0.009			-0.019			0.008	0.044		9	21.25	-22.16	3.93	0.007
0.008			-0.020		0.008		0.047		9	21.25	-22.16	3.94	0.007
0.007				-0.022		0.005	0.038		9	21.25	-22.16	3.94	0.007
-0.027	+		-0.007	-0.027					9	21.23	-22.13	3.97	0.007
0.009				-0.026	0.002				8	19.98	-22.11	3.98	0.007
-0.023	+		-0.016	-0.021			0.040		10	22.47	-22.05	4.05	0.007
-0.027	+		-0.011			0.025			9	21.13	-21.92	4.17	0.007
-0.028	+	-0.040		-0.024	0.026		0.055		11	23.71	-21.91	4.19	0.007
0.011		-0.015				0.018			8	19.87	-21.90	4.19	0.007
-0.027	+			-0.027	0.016		0.037		10	22.40	-21.90	4.19	0.007
0.008		-0.037		-0.021	0.017		0.057		10	22.36	-21.82	4.27	0.006
-0.028	+				0.008	0.025			9	21.04	-21.74	4.35	0.006
0.010			-0.010			0.019			8	19.79	-21.74	4.36	0.006
0.000		-0.035				-0.016	0.053	0.028	10	22.28	-21.67	4.42	0.006
-0.025	+			-0.023		0.011	0.032		10	22.28	-21.66	4.43	0.006
-0.025	+		-0.021		0.015		0.046		10	22.27	-21.65	4.44	0.006
-0.023	+		-0.020			0.015	0.039		10	22.26	-21.63	4.46	0.006
0.008		-0.031	-0.010	-0.018			0.055		10	22.24	-21.58	4.52	0.006
-0.029	+	-0.022			0.012				9	20.94	-21.55	4.55	0.005
0.010					0.000	0.018			8	19.67	-21.50	4.59	0.005
-0.029	+					-0.001	0.033	0.023	10	22.20	-21.50	4.59	0.005
0.009		-0.014	-0.006						8	19.66	-21.48	4.62	0.005
0.009		-0.036	-0.014		0.016		0.062		10	22.17	-21.44	4.65	0.005
0.009		-0.017			0.002				8	19.62	-21.40	4.69	0.005
-0.026	+	-0.018	-0.006						9	20.87	-21.40	4.69	0.005
0.008					0.006	0.010	0.039		9	20.87	-21.40	4.69	0.005
-0.027	+	-0.039	-0.014		0.025		0.061		11	23.44	-21.36	4.74	0.005
0.008		-0.033		-0.020		0.001	0.053		10	22.12	-21.35	4.74	0.005
0.009			-0.010		0.000				8	19.51	-21.17	4.93	0.005

Int	Sex	Bird age	D4 Body mass	mean egg mass	log Group size	Clutch size	pre-egg rain	Clutch size: rain	df	logLik	AICc	Δ AICc	weight
-0.028	+	-0.041			0.024	0.011	0.055		11	23.31	-21.09	5.00	0.004
-0.026	+				0.013	0.016	0.035		10	21.98	-21.07	5.02	0.004
-0.023	+	-0.032	-0.010	-0.020			0.051		11	23.29	-21.07	5.03	0.004
0.008		-0.038			0.016	0.004	0.058		10	21.98	-21.06	5.03	0.004
0.009		-0.031	-0.013			0.005	0.056		10	21.97	-21.05	5.05	0.004
-0.027	+		-0.011		0.009				9	20.68	-21.02	5.07	0.004
-0.029	+	-0.036				-0.007	0.048	0.025	11	23.25	-20.98	5.12	0.004
-0.024	+	-0.033		-0.021		0.007	0.047		11	23.21	-20.91	5.19	0.004
-0.023	+	-0.031	-0.013			0.011	0.051		11	23.04	-20.57	5.53	0.003
-0.028	+	-0.018		-0.024		0.019			10	21.73	-20.57	5.53	0.003
0.000				-0.021		-0.014	0.037	0.025	10	21.69	-20.49	5.61	0.003
-0.031	+	-0.020		-0.029	0.014				10	21.63	-20.36	5.73	0.003
0.002			-0.016			-0.009	0.042	0.023	10	21.60	-20.30	5.79	0.003
0.008			-0.016	-0.020	0.009		0.045		10	21.58	-20.27	5.82	0.003
0.010		-0.014		-0.023		0.012			9	20.27	-20.21	5.88	0.003
-0.030	+			-0.025	0.011	0.019			10	21.55	-20.20	5.89	0.003
-0.028	+		-0.008	-0.022		0.019			10	21.52	-20.15	5.94	0.003
0.008			-0.016	-0.018		0.004	0.043		10	21.52	-20.15	5.95	0.003

Full Δ 6 AICc top model set corresponding to **Table 1b in Chapter 3**, showing predictors of day 4 nestling RTL with the influential point *removed*. Estimates are given for scaled and centred continuous variables. Presence of factors in the model is noted with +. Int = intercept.

Int	Sex	Bird age	D4 Body mass	mean egg mass	log Group size	Clutch size	pre-lay rain	Clutch size: rain	df	logLik	AICc	Delta AICc	Weight
-0.014	+						0.073		8	32.65	-47.42	0.000	0.108
0.017							0.076		7	31.09	-46.73	0.688	0.076
-0.013	+			-0.025			0.071		9	33.32	-46.27	1.146	0.061
-0.014	+		-0.020				0.081		9	33.28	-46.19	1.234	0.058
-0.013	+	-0.021					0.081		9	33.25	-46.13	1.294	0.056
0.018				-0.024			0.075		8	31.69	-45.50	1.923	0.041
0.018		-0.021					0.085		8	31.66	-45.44	1.980	0.040
0.018			-0.019				0.084		8	31.64	-45.40	2.019	0.039
-0.013	+				-0.005		0.072		9	32.67	-44.98	2.444	0.032
-0.014	+					0.005	0.071		9	32.67	-44.97	2.455	0.032
-0.013	+		-0.018	-0.023			0.078		10	33.84	-44.75	2.675	0.028
-0.013	+	-0.019		-0.023			0.079		10	33.81	-44.68	2.746	0.027
0.017					-0.011		0.075		8	31.22	-44.57	2.850	0.026
-0.013	+	-0.018	-0.017				0.087		10	33.73	-44.52	2.906	0.025
0.017						0.000	0.076		8	31.09	-44.30	3.122	0.023
0.019		-0.019		-0.022			0.083		9	32.15	-43.92	3.500	0.019
0.019			-0.017	-0.021			0.082		9	32.12	-43.88	3.545	0.018
-0.013	+			-0.025	-0.004		0.071		10	33.34	-43.75	3.668	0.017
0.018		-0.018	-0.016				0.091		9	32.06	-43.75	3.673	0.017
-0.013	+			-0.025		0.000	0.071		10	33.32	-43.71	3.711	0.017
-0.014	+		-0.020			0.004	0.079		10	33.30	-43.66	3.758	0.016
-0.014	+		-0.020		-0.001		0.080		10	33.28	-43.63	3.794	0.016
-0.013	+	-0.021				0.003	0.080		10	33.26	-43.59	3.838	0.016
-0.014	+	-0.022			0.003		0.082		10	33.26	-43.58	3.841	0.016
0.018				-0.024	-0.011		0.074		9	31.81	-43.26	4.166	0.013
0.018				-0.025		-0.006	0.076		9	31.72	-43.06	4.359	0.012
0.018			-0.018		-0.008		0.083		9	31.71	-43.06	4.367	0.012
0.018		-0.019			-0.004		0.085		9	31.68	-42.98	4.441	0.012
0.018		-0.021				-0.002	0.086		9	31.66	-42.95	4.471	0.012
0.018			-0.019			-0.001	0.084		9	31.64	-42.91	4.515	0.011
-0.013	+	-0.016	-0.016	-0.021			0.085		11	34.21	-42.84	4.579	0.011
-0.013	+				-0.006	0.005	0.071		10	32.70	-42.47	4.952	0.009
-0.013	+					0.008	0.071	-0.004	10	32.69	-42.44	4.985	0.009
-0.013	+		-0.018	-0.023	-0.001		0.078		11	33.84	-42.12	5.305	0.008
-0.013	+		-0.018	-0.023		0.000	0.078		11	33.84	-42.11	5.309	0.008
0.017					-0.011	0.001	0.075		9	31.22	-42.08	5.345	0.007
-0.013	+	-0.019		-0.023	0.003		0.079		11	33.81	-42.06	5.366	0.007

Int	Sex	Bird age	D4 Body mass	mean egg mass	log Group size	Clutch size	pre-lay rain	Clutch size: rain	df	logLik	AICc	Delta AICc	Weight
-0.013	+	-0.019		-0.023		-0.001	0.079		11	33.81	-42.05	5.376	0.007
0.019		-0.016	-0.015	-0.020			0.088		10	32.47	-42.01	5.410	0.007
-0.014	+	-0.019	-0.018		0.005		0.088		11	33.75	-41.94	5.485	0.007
-0.013	+	-0.018	-0.017			0.003	0.086		11	33.74	-41.90	5.519	0.007
0.017						-0.001	0.076	0.000	9	31.09	-41.80	5.620	0.006
0.019			-0.016	-0.021	-0.008		0.081		10	32.20	-41.46	5.960	0.005
0.018		-0.019		-0.023		-0.007	0.085		10	32.19	-41.44	5.982	0.005

Full Δ 6 AICc top model set corresponding to **Table 2 in Chapter 3**, showing predictors of the rate of change in relative telomere length during early development (between day 4 and day 12). Estimates are given for scaled and centred continuous variables. Presence of factors in the model is noted with +. Int = intercept.

Int	Comp	Group type	Sex	Group size	pre-lay Rain	Post lay rain	Day 4 RTL	day 12 Body mass	df	logLik	AICc	delta AICc	weight
0.001							-0.005		6	109.42	-204.29	0.000	0.071
0.002							-0.006	-0.004	7	110.84	-204.18	0.104	0.067
0.003		+					-0.006	-0.004	8	111.85	-203.05	1.237	0.038
0.004			+				-0.006	-0.004	8	111.84	-203.04	1.250	0.038
0.003		+					-0.005		7	110.26	-203.03	1.259	0.038
0.001									5	107.35	-202.94	1.345	0.036
0.004			+				-0.005		7	110.22	-202.93	1.352	0.036
0.005			+					-0.005	7	110.21	-202.93	1.358	0.036
0.001								-0.005	6	108.63	-202.72	1.572	0.032
0.005			+						6	108.60	-202.66	1.624	0.031
0.003		+							6	108.38	-202.22	2.069	0.025
0.001						-0.002	-0.005		7	109.72	-201.93	2.354	0.022
0.001					-0.002		-0.005		7	109.61	-201.72	2.570	0.020
0.001				0.001			-0.005		7	109.58	-201.67	2.621	0.019
0.001				0.002			-0.006	-0.004	8	111.11	-201.58	2.709	0.018
0.003	+						-0.005		7	109.47	-201.45	2.839	0.017
0.003		+						-0.003	7	109.46	-201.43	2.858	0.017
0.003	+						-0.006	-0.004	8	110.98	-201.31	2.979	0.016
0.002						-0.001	-0.006	-0.004	8	110.91	-201.18	3.104	0.015
0.005			+		0.003			-0.005	8	110.86	-201.07	3.214	0.014
0.002					0.000		-0.006	-0.004	8	110.84	-201.04	3.247	0.014
0.005		+	+				-0.006	-0.004	9	112.51	-201.02	3.269	0.014
0.001						-0.002			6	107.74	-200.93	3.354	0.013
0.004		+	+				-0.005		8	110.76	-200.87	3.420	0.013
0.001					0.003			-0.006	7	109.07	-200.65	3.641	0.011
0.004			+			-0.002	-0.005		8	110.61	-200.57	3.718	0.011
0.005			+			-0.002			7	109.01	-200.51	3.776	0.011
0.001				0.002					6	107.53	-200.51	3.776	0.011
0.004		+			0.003		-0.005	-0.005	9	112.25	-200.51	3.779	0.011
0.004	+							-0.005	7	108.97	-200.43	3.856	0.010
0.003	+								6	107.48	-200.41	3.878	0.010
0.005		+	+						7	108.90	-200.30	3.988	0.010
0.001					-0.001				6	107.39	-200.24	4.049	0.009
0.003		+				-0.001	-0.005		8	110.40	-200.15	4.134	0.009
0.007	+		+					-0.005	8	110.40	-200.15	4.141	0.009
0.005			+			-0.001		-0.004	8	110.36	-200.07	4.221	0.009
0.006		+	+					-0.005	8	110.34	-200.03	4.258	0.008

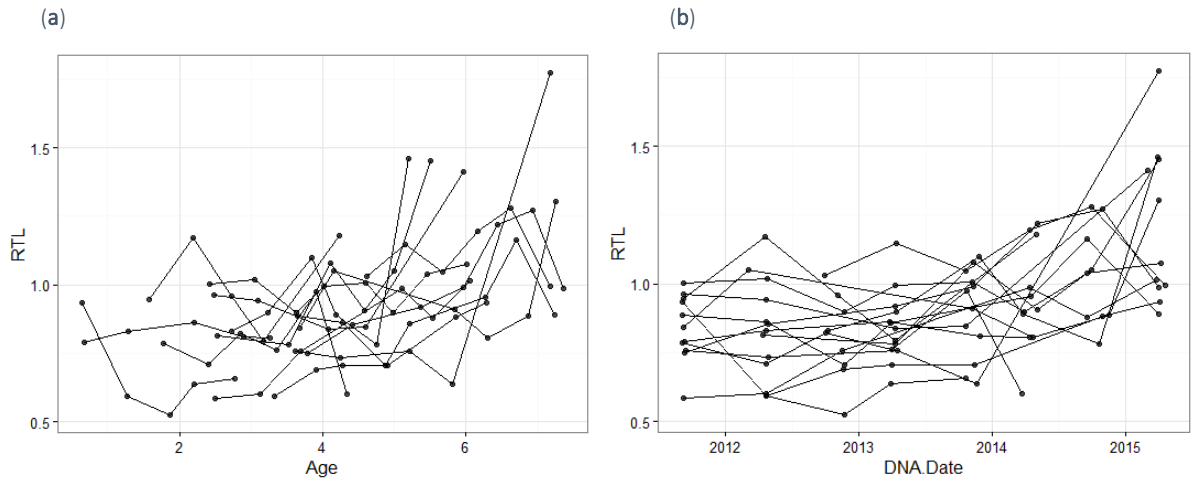
Int	Comp	Group type	Sex	Group size	pre-lay Rain	Post lay rain	Day 4 RTL	day 12 Body mass	df	logLik	AICc	delta AICc	weight
0.001	+	+					-0.005		8	110.32	-200.00	4.285	0.008
0.005			+		0.002				7	108.72	-199.94	4.348	0.008
0.005			+			-0.001	-0.006	-0.004	9	111.96	-199.93	4.358	0.008
0.004			+		-0.001		-0.005		8	110.27	-199.90	4.387	0.008
0.003		+			0.000		-0.005		8	110.26	-199.88	4.404	0.008
0.001						-0.001		-0.004	7	108.69	-199.87	4.412	0.008
0.001				0.001				-0.004	7	108.69	-199.87	4.416	0.008
0.005			+		0.002		-0.005	-0.005	9	111.93	-199.87	4.417	0.008
0.004			+	0.001			-0.005		8	110.24	-199.84	4.446	0.008
0.005			+	-0.001				-0.005	8	110.24	-199.83	4.455	0.008
0.005	+		+				-0.005		8	110.23	-199.82	4.463	0.008
0.004			+	0.001			-0.006	-0.004	9	111.90	-199.81	4.478	0.008
0.005	+		+				-0.006	-0.004	9	111.90	-199.80	4.486	0.008
0.006	+		+						7	108.64	-199.78	4.503	0.007
0.005			+	-0.001					7	108.63	-199.76	4.526	0.007
0.003		+				-0.002			7	108.62	-199.75	4.539	0.007
0.002	+	+					-0.006	-0.004	9	111.87	-199.73	4.553	0.007
0.003		+				0.000	-0.005	-0.004	9	111.85	-199.70	4.590	0.007
0.004		+			0.005			-0.005	8	110.12	-199.60	4.686	0.007
0.007		+	+		0.006			-0.006	9	111.67	-199.34	4.943	0.006
0.003		+			0.001				7	108.42	-199.34	4.947	0.006
0.002	+	+							7	108.41	-199.31	4.976	0.006
0.001					-0.001	-0.002	-0.005		8	109.83	-199.01	5.275	0.005
0.001				0.001		-0.002	-0.005		8	109.80	-198.96	5.324	0.005
0.003	+					-0.002	-0.005		8	109.80	-198.95	5.338	0.005
0.007		+	+		0.004		-0.005	-0.005	10	113.14	-198.70	5.584	0.004
0.001				0.001	-0.001		-0.005		8	109.65	-198.66	5.626	0.004
0.002	+				-0.002		-0.005		8	109.63	-198.62	5.665	0.004
0.002	+			0.001			-0.005		8	109.61	-198.57	5.717	0.004
0.002				0.002	0.002		-0.006	-0.005	9	111.22	-198.44	5.846	0.004
0.003		+				-0.001		-0.003	8	109.52	-198.39	5.895	0.004
0.003	+			0.002			-0.006	-0.004	9	111.18	-198.36	5.928	0.004
0.003	+					-0.002			7	107.91	-198.32	5.968	0.004

Full Δ 6 AICc top model set corresponding to **Table 3 in Chapter 3**, showing predictors of the rate of change in relative telomere length during late development (between day 12 and day 30). Estimates are given for scaled and centred continuous variables. Presence of factors in the model is noted with +. Int = intercept. The interaction between mass and change in mass in the global model but is not present in the top model set.

Int	Comp	Group type	Group size	Day 12 body mass	pre-lay rain	post-lay rain	Day 12 RTL	Delta mass	df	logLik	AICc	delta AICc	weight
-0.0012		+							7	165.21	-313.61	0.00	0.12
-0.0029			0.0022						7	164.78	-312.76	0.85	0.08
-0.0029									6	163.05	-312.06	1.55	0.05
-0.0015		+						-0.0012	8	165.71	-311.74	1.87	0.05
-0.0029								-0.0021	7	164.22	-311.65	1.96	0.04
-0.0010		+					0.0010		8	165.57	-311.45	2.16	0.04
-0.0010		+				0.0009			8	165.51	-311.32	2.29	0.04
-0.0029			0.0029				0.0015		8	165.43	-311.17	2.44	0.03
-0.0011		+		0.0007					8	165.38	-311.06	2.55	0.03
-0.0005	+	+							8	165.31	-310.92	2.69	0.03
-0.0013		+			-0.0003				8	165.25	-310.80	2.81	0.03
-0.0029			0.0018					-0.0012	8	165.24	-310.79	2.82	0.03
-0.0029			0.0025			0.0009			8	165.07	-310.44	3.17	0.02
-0.0020	+		0.0021						8	164.93	-310.17	3.44	0.02
-0.0029			0.0022	0.0005					8	164.86	-310.02	3.59	0.02
-0.0029			0.0023		0.0002				8	164.79	-309.90	3.72	0.02
-0.0016	+								7	163.31	-309.83	3.78	0.02
-0.0029					-0.0008				7	163.25	-309.71	3.90	0.02
-0.0012		+				0.0011		-0.0013	9	166.12	-309.51	4.10	0.01
-0.0029				0.0004					7	163.12	-309.44	4.17	0.01
-0.0029							0.0001		7	163.06	-309.32	4.29	0.01
-0.0029						0.0001			7	163.06	-309.31	4.30	0.01
-0.0013		+					0.0009	-0.0011	9	165.99	-309.25	4.36	0.01
-0.0030				-0.0010				-0.0025	8	164.42	-309.15	4.46	0.01
-0.0029					-0.0007			-0.0020	8	164.37	-309.04	4.57	0.01
-0.0028						0.0005		-0.0023	8	164.32	-308.94	4.67	0.01
-0.0023	+							-0.0019	8	164.27	-308.84	4.77	0.01
-0.0008		+				0.0008	0.0009		9	165.77	-308.80	4.81	0.01
-0.0015		+			-0.0004			-0.0012	9	165.76	-308.78	4.83	0.01
-0.0029							0.0001	-0.0021	8	164.23	-308.76	4.85	0.01
-0.0011	+	+						-0.0012	9	165.74	-308.75	4.86	0.01
-0.0011		+			-0.0007		0.0012		9	165.73	-308.72	4.89	0.01
-0.0015		+		-0.0001				-0.0013	9	165.72	-308.70	4.91	0.01
-0.0030			0.0025				0.0013	-0.0009	9	165.70	-308.67	4.94	0.01
-0.0001	+	+				0.0010			9	165.65	-308.57	5.04	0.01
-0.0009		+		0.0006		0.0009			9	165.65	-308.56	5.05	0.01

-0.0010		+		0.0005			0.0009		9	165.64	-308.55	5.06	0.01
-0.0005	+	+					0.0010		9	165.62	-308.49	5.12	0.01
-0.0029			0.0031			0.0007	0.0014		9	165.61	-308.48	5.13	0.01
-0.0029			0.0021			0.0010		-0.0013	9	165.59	-308.44	5.17	0.01
-0.0010		+			-0.0003	0.0009			9	165.54	-308.35	5.26	0.01
-0.0023	+		0.0028				0.0014		9	165.50	-308.26	5.36	0.01
-0.0005	+	+		0.0007					9	165.46	-308.19	5.42	0.01
-0.0012		+		0.0007	-0.0004				9	165.44	-308.15	5.46	0.01
-0.0029			0.0029	0.0001			0.0015		9	165.43	-308.13	5.48	0.01
-0.0029			0.0029		0.0000		0.0015		9	165.43	-308.13	5.48	0.01
-0.0006	+	+			-0.0003				9	165.34	-307.95	5.66	0.01
-0.0023	+		0.0018					-0.0011	9	165.30	-307.87	5.74	0.01
-0.0017	+		0.0025			0.0011			9	165.30	-307.86	5.75	0.01
-0.0029			0.0017	-0.0004				-0.0015	9	165.28	-307.83	5.79	0.01
-0.0029			0.0018		0.0001			-0.0012	9	165.24	-307.75	5.86	0.01

While initial plots of the patterns of telomere length with age for the 15 most sampled individuals (n = 89 samples; range 5-8 per individual) suggest a trend for within-individual increases in telomere length with age (Figure 1a), replotting this same data by sampling date reveals a clear increase in telomere length measures from samples collected in 2014/2015. That the increases we see in adult RTL in 2015 are not also apparent in nestlings suggest that this is not a storage effect.



Raw data plots for the 15 most sampled individuals, showing the patterns of relative telomere length with a) age, and b) date of sampling.

Appendix E: Chapter 4 unabridged top model sets and extra model

Full Δ 6 AICc top model set corresponding to **Table 1 in Chapter 4**, showing predictors of adult relative telomere length. Models are of the effects of population level age (age was not partitioned). Estimates are given for scaled and centred continuous variables. Presence of factors in the model is noted with +. Int = intercept.

Intercept	Dominance status	Sex	Bird age	Bird age^2	df	logLik	AICc	Delta AICc	weight
-0.023		+	-0.041		8	104.09	-191.68	0.000	0.240
-0.032		+			7	102.87	-191.36	0.319	0.205
-0.039		+	-0.044	0.013	9	104.75	-190.88	0.796	0.161
-0.009	+	+	-0.052		9	104.27	-189.93	1.754	0.100
-0.038	+	+			8	102.94	-189.38	2.296	0.076
-0.023	+	+	-0.058	0.014	10	105.04	-189.32	2.359	0.074
-0.073					6	100.57	-188.85	2.832	0.058
-0.071			-0.022		7	100.93	-187.47	4.215	0.029
-0.088			-0.024	0.014	8	101.73	-186.96	4.722	0.023
-0.072	+				7	100.57	-186.75	4.928	0.020
-0.055	+		-0.034		8	101.14	-185.79	5.887	0.013

Full Δ 6 AICc top model set corresponding to **Table 2 in Chapter 4**, showing predictors of adult relative telomere length. Showing the effects of partitioned age. Estimates are given for scaled and centred continuous variables. Presence of factors in the model is noted with +. Int = intercept

Int	Status	Sex	Delta Age	Delta Age^2	Mean Age	Mean Age^2	Status : Sex	Status: Delta Age	Status: Mean Age	Sex : Delta Age	Sex : Mean Age	Delta Age : Mean Age	df	logLik	AICc	Delta AICc	weight
-0.029		+			-0.028								8	104.08	-191.66	0.00	0.054
0.008	+	+			-0.060								9	105.02	-191.42	0.24	0.048
-0.032		+											7	102.87	-191.36	0.30	0.046
-0.021	+	+			-0.026				+				10	105.76	-190.76	0.90	0.034
-0.046		+			-0.031	0.015							9	104.40	-190.18	1.48	0.026
-0.009	+	+			-0.064	0.017							10	105.41	-190.05	1.61	0.024
-0.025		+	-0.010		-0.037								9	104.23	-189.84	1.82	0.022
-0.026		+	0.001		-0.038					+			10	105.25	-189.73	1.93	0.021
-0.029		+			-0.034						+		9	104.13	-189.64	2.02	0.020

Int	Status	Sex	Delta Age	Delta Age^2	Mean Age	Mean Age^2	Status : Sex	Status: Delta Age	Status: Mean Age	Sex : Delta Age	Sex : Mean Age	Delta Age : Mean Age	df	logLik	AICc	Delta AICc	weight
-0.035		+	0.008										8	103.05	-189.61	2.05	0.019
0.009	+	+	-0.007		-0.064								10	105.08	-189.40	2.26	0.017
0.008	+	+			-0.065						+		10	105.08	-189.39	2.27	0.017
-0.038	+	+											8	102.94	-189.38	2.28	0.017
0.007	+	+	0.003		-0.064			+					11	106.13	-189.34	2.32	0.017
-0.036		+	0.019							+			9	103.96	-189.29	2.37	0.016
0.007	+	+			-0.060		+						10	105.02	-189.28	2.38	0.016
0.006	+	+	0.003		-0.063					+			11	105.97	-189.03	2.64	0.014
-0.023	+	+			-0.032				+		+		11	105.91	-188.91	2.75	0.014
-0.073													6	100.57	-188.85	2.81	0.013
-0.022	+	+	0.004		-0.029			+	+				12	106.91	-188.72	2.94	0.012
-0.021	+	+			-0.006	-0.013			+				11	105.82	-188.72	2.95	0.012
-0.019	+	+	-0.006		-0.030				+				11	105.81	-188.71	2.96	0.012
-0.023	+	+			-0.025		+		+				11	105.77	-188.61	3.05	0.012
-0.023	+	+	0.005		-0.027				+	+			12	106.77	-188.46	3.21	0.011
-0.044		+	0.001		-0.041	0.017				+			11	105.65	-188.37	3.29	0.010
-0.042		+	-0.010		-0.039	0.015							10	104.56	-188.36	3.30	0.010
-0.048		+			-0.039	0.017					+		10	104.50	-188.24	3.42	0.010
-0.027		+	-0.013		-0.036							0.006	10	104.46	-188.16	3.50	0.009
-0.011	+	+			-0.072	0.018					+		11	105.52	-188.12	3.54	0.009
-0.011	+	+	0.003		-0.068	0.018		+					12	106.58	-188.08	3.59	0.009
-0.008	+	+	-0.007		-0.068	0.017							11	105.48	-188.04	3.63	0.009
0.006	+	+	0.008		-0.063			+		+			12	106.52	-187.96	3.71	0.008
-0.011	+	+			-0.064	0.017	+						11	105.41	-187.91	3.75	0.008
-0.074					-0.019								7	101.11	-187.84	3.82	0.008
-0.033	+				-0.053								8	102.17	-187.84	3.82	0.008
-0.025		+	-0.010		-0.042						+		10	104.29	-187.81	3.85	0.008
-0.012	+	+	0.003		-0.067	0.018				+			12	106.44	-187.79	3.87	0.008
0.009	+	+	-0.009		-0.064							0.007	11	105.36	-187.79	3.87	0.008
-0.027		+	-0.009	0.002	-0.037								10	104.27	-187.78	3.88	0.008
-0.026		+	0.001		-0.044					+	+		11	105.32	-187.71	3.95	0.007
-0.026		+	-0.001		-0.038					+		0.003	11	105.30	-187.67	3.99	0.007
-0.027		+	0.002	0.002	-0.038					+			11	105.28	-187.65	4.02	0.007
0.003	+	+	0.008	0.005	-0.065			+					12	106.36	-187.64	4.03	0.007
-0.063	+				-0.014				+				9	103.11	-187.60	4.06	0.007
-0.037		+	0.010	0.002									9	103.08	-187.54	4.13	0.007
-0.035	+	+	0.008										9	103.05	-187.48	4.18	0.007
-0.037	+	+	0.019					+					10	104.10	-187.44	4.23	0.007
-0.024	+	+	0.009		-0.027			+	+	+			13	107.34	-187.40	4.26	0.006
0.007	+	+	0.008		-0.064			+				-0.006	12	106.24	-187.38	4.28	0.006

Int	Status	Sex	Delta Age	Delta Age^2	Mean Age	Mean Age^2	Status : Sex	Status: Delta Age	Status: Mean Age	Sex : Delta Age	Sex : Mean Age	Delta Age : Mean Age	df	logLik	AICc	Delta AICc	weight
0.021	+	+			-0.076		+				+		11	105.15	-187.38	4.28	0.006
0.009	+	+	-0.007		-0.069						+		11	105.14	-187.36	4.30	0.006
0.007	+	+	-0.005	0.002	-0.064								11	105.13	-187.35	4.32	0.006
0.007	+	+	0.003		-0.071			+			+		12	106.21	-187.33	4.33	0.006
-0.041	+	+					+						9	102.95	-187.28	4.38	0.006
0.008	+	+	-0.007		-0.064		+						11	105.09	-187.26	4.41	0.006
0.004	+	+	0.003		-0.064		+	+					12	106.15	-187.21	4.46	0.006
-0.037		+	0.021	0.002						+			10	103.98	-187.19	4.47	0.006
-0.038	+	+	0.018							+			10	103.97	-187.17	4.49	0.006
-0.005	+	+			-0.046		+		+		+		12	106.12	-187.15	4.51	0.006
-0.075			0.009										7	100.76	-187.14	4.52	0.006
-0.020	+	+	-0.009		-0.029				+			0.007	12	106.10	-187.12	4.54	0.006
0.006	+	+	0.001		-0.063					+		0.004	12	106.05	-187.01	4.65	0.005
0.006	+	+	0.003		-0.069					+	+		12	106.05	-187.00	4.66	0.005
0.004	+	+	0.005	0.002	-0.063					+			12	106.02	-186.94	4.72	0.005
-0.024	+	+	0.004		-0.037			+	+		+		13	107.10	-186.93	4.73	0.005
-0.026	+	+	0.009	0.005	-0.031			+	+				13	107.10	-186.92	4.75	0.005
0.003	+	+	0.003		-0.063		+			+			12	105.99	-186.88	4.78	0.005
-0.022	+	+	-0.006		-0.036				+		+		12	105.97	-186.85	4.81	0.005
-0.024	+	+			-0.014	-0.012			+		+		12	105.97	-186.84	4.82	0.005
-0.013	+	+	0.008		-0.068	0.019		+		+			13	107.02	-186.76	4.90	0.005
-0.072	+												7	100.57	-186.75	4.91	0.005
-0.022	+	+	0.009		-0.029			+	+			-0.006	13	107.01	-186.74	4.92	0.005
-0.044		+	-0.013		-0.039	0.016						0.007	11	104.82	-186.72	4.94	0.005
-0.051	+				-0.058	0.020							9	102.66	-186.70	4.96	0.005
-0.026	+	+	0.004		-0.035				+	+	+		13	106.96	-186.65	5.01	0.004
-0.020	+	+	-0.006		-0.012	-0.012			+				12	105.86	-186.63	5.03	0.004
-0.021	+	+	-0.005	0.002	-0.030				+				12	105.84	-186.60	5.07	0.004
-0.022	+	+	0.004		-0.015	-0.009		+	+				13	106.94	-186.59	5.07	0.004
-0.026	+	+	0.004		-0.029		+	+	+				13	106.93	-186.59	5.08	0.004
-0.092					-0.022	0.018							8	101.52	-186.55	5.11	0.004
-0.022	+	+	-0.006		-0.029		+		+				12	105.82	-186.55	5.11	0.004
-0.023	+	+			-0.006	-0.013	+		+				12	105.82	-186.55	5.11	0.004
-0.009	+	+	-0.010		-0.068	0.018						0.007	12	105.79	-186.48	5.18	0.004
-0.046		+	0.001		-0.050	0.018				+	+		12	105.78	-186.48	5.19	0.004
-0.024	+	+	0.002		-0.027				+	+		0.004	13	106.86	-186.43	5.23	0.004
-0.043		+	-0.011		-0.047	0.017					+		11	104.67	-186.41	5.25	0.004
-0.024	+	+	0.005		-0.013	-0.009			+	+			13	106.80	-186.33	5.33	0.004
-0.045		+	-0.001		-0.041	0.017				+		0.003	12	105.71	-186.32	5.34	0.004

Int	Status	Sex	Delta Age	Delta Age^2	Mean Age	Mean Age^2	Status : Sex	Status: Delta Age	Status: Mean Age	Sex : Delta Age	Sex : Mean Age	Delta Age : Mean Age	df	logLik	AICc	Delta AICc	weight
-0.024	+	+	0.006	0.002	-0.028				+	+			13	106.80	-186.32	5.34	0.004
-0.031		+	-0.010	0.005	-0.037							0.008	11	104.62	-186.32	5.35	0.004
-0.027	+	+	0.004		-0.027		+		+	+			13	106.79	-186.31	5.35	0.004
-0.015	+	+	0.008	0.005	-0.069	0.017		+					13	106.78	-186.29	5.38	0.004
-0.043		+	-0.009	0.002	-0.040	0.015							11	104.59	-186.26	5.40	0.004
-0.045		+	0.002	0.002	-0.041	0.017				+			12	105.67	-186.25	5.41	0.004
-0.013	+	+	0.003		-0.078	0.020		+			+		13	106.74	-186.21	5.46	0.004
0.004	+	+			-0.086	0.020	+				+		12	105.64	-186.20	5.47	0.004
0.001	+	+	0.012	0.005	-0.064			+		+			13	106.70	-186.12	5.54	0.003
-0.027		+	-0.013		-0.042						+	0.006	11	104.52	-186.12	5.54	0.003
-0.039	+	+	0.023					+		+			11	104.51	-186.11	5.56	0.003
-0.009	+	+	-0.007		-0.077	0.018					+		12	105.60	-186.11	5.56	0.003
-0.010	+	+	0.008		-0.068	0.018		+				-0.006	13	106.68	-186.07	5.59	0.003
0.006	+	+	0.014		-0.063			+		+		-0.006	13	106.64	-186.00	5.66	0.003
0.004	+	+	-0.006	0.005	-0.065							0.009	12	105.54	-186.00	5.67	0.003
-0.016	+	+	0.002		-0.068	0.018	+	+					13	106.62	-185.96	5.71	0.003
0.005	+	+	0.008		-0.070			+		+	+		13	106.61	-185.94	5.72	0.003
-0.009	+	+	-0.006	0.002	-0.068	0.017							12	105.51	-185.93	5.73	0.003
-0.014	+	+	0.003		-0.077	0.020				+	+		13	106.59	-185.91	5.75	0.003
-0.011	+	+	-0.007		-0.068	0.017	+						12	105.49	-185.89	5.77	0.003
0.002	+	+	0.007		-0.063		+	+		+			13	106.55	-185.82	5.85	0.003
-0.013	+	+	0.001		-0.068	0.019				+		0.004	13	106.53	-185.79	5.87	0.003
-0.034	+		0.014		-0.051			+					10	103.27	-185.78	5.88	0.003
-0.027		+	-0.009	0.002	-0.043						+		11	104.33	-185.75	5.91	0.003
0.009	+	+	-0.009		-0.070						+	0.007	12	105.42	-185.74	5.92	0.003
-0.033	+		0.003		-0.052								9	102.18	-185.74	5.92	0.003
-0.074			-0.001		-0.019								8	101.12	-185.73	5.93	0.003
-0.042	+	+	0.025	0.005				+					11	104.30	-185.69	5.98	0.003
-0.030		+	0.000	0.003	-0.038					+		0.005	12	105.38	-185.67	5.99	0.003

Full Δ 6 AICc top model set corresponding to Table 3 in Chapter 4, showing predictors of adult rate of change in relative telomere length over the course of a breeding or non-breeding period (season). Estimates are given for scaled and centred continuous variables. Presence of factors in the model is noted with +. Int = intercept, Dom = dominance.

Int	Dom Status	Season	Sex	log Group size	start RTL	Annual rain	Mass change	start mass	Status: sex	Status : log group size	Status: start RTL	Status: annual rain	Season: annual rain	Start mass: Mass change	df	logLik	AICc	delta AICc	weight
0.006	+	+			-0.008	-0.011					+	+	+		13	451.1	-874.0	0.000	0.127
0.005	+	+			-0.009	-0.011	0.003				+	+	+		14	452.0	-873.6	0.383	0.105
0.006	+	+			-0.008	-0.015					+		+		12	449.3	-872.9	1.146	0.072
0.005	+	+			-0.008	-0.015	0.003				+		+		13	450.1	-872.1	1.934	0.048
0.005	+	+		-0.003	-0.009	-0.011				+	+	+	+		15	452.4	-872.0	2.030	0.046
0.006	+	+		-0.001	-0.008	-0.011					+	+	+		14	451.2	-871.9	2.090	0.045
0.006	+	+			-0.008	-0.011		0.000			+	+	+		14	451.1	-871.7	2.261	0.041
0.006	+	+	+		-0.008	-0.011					+	+	+		14	451.1	-871.7	2.290	0.040
0.005	+	+			-0.009	-0.011	0.003	0.001			+	+	+		15	452.1	-871.4	2.656	0.034
0.005	+	+		0.000	-0.009	-0.011	0.003				+	+	+		15	452.1	-871.3	2.700	0.033
0.005	+	+	+		-0.009	-0.011	0.003				+	+	+		15	452.0	-871.3	2.747	0.032
0.004	+	+		-0.003	-0.009	-0.011	0.003			+	+	+	+		16	453.2	-871.2	2.828	0.031
0.006	+	+		-0.001	-0.008	-0.015					+		+		13	449.4	-870.7	3.286	0.025
0.006	+	+	+		-0.008	-0.015					+		+		13	449.4	-870.6	3.365	0.024
0.006	+	+			-0.008	-0.015		0.000			+		+		13	449.3	-870.6	3.455	0.023
0.005	+	+		-0.003	-0.008	-0.015				+	+		+		14	450.4	-870.4	3.623	0.021
0.005	+	+			-0.008	-0.015	0.003	0.001			+		+		14	450.2	-870.0	4.030	0.017
0.005	+	+		0.000	-0.008	-0.015	0.003				+		+		14	450.1	-869.8	4.245	0.015
0.005	+	+	+		-0.008	-0.015	0.003				+		+		14	450.1	-869.8	4.252	0.015
0.005	+	+		-0.003	-0.009	-0.011		-0.001		+	+	+	+		16	452.5	-869.7	4.264	0.015
0.005	+	+	+	-0.003	-0.009	-0.011				+	+	+	+		16	452.4	-869.7	4.326	0.015
0.006	+	+		-0.001	-0.008	-0.011		0.000			+	+	+		15	451.2	-869.6	4.393	0.014
0.006	+	+	+	-0.001	-0.008	-0.011					+	+	+		15	451.2	-869.6	4.441	0.014
0.006	+	+	+		-0.008	-0.011		-0.001			+	+	+		15	451.1	-869.5	4.519	0.013
0.006	+	+	+		-0.008	-0.011			+		+	+	+		15	451.1	-869.4	4.651	0.012
0.004	+	+		-0.003	-0.008	-0.015	0.003			+	+		+		15	451.0	-869.3	4.753	0.012
0.005	+	+			-0.009	-0.011	0.004	0.001			+	+	+	-0.00065	16	452.2	-869.1	4.861	0.011

0.005	+	+		0.000	-0.009	-0.011	0.003	0.001			+	+	+		16	452.1	-869.0	5.005	0.010
0.005	+	+	+		-0.009	-0.011	0.004	0.001			+	+	+		16	452.1	-869.0	5.036	0.010
0.005	+	+	+	0.000	-0.009	-0.011	0.003				+	+	+		16	452.1	-868.9	5.093	0.010
0.005	+	+	+		-0.009	-0.011	0.003		+		+	+	+		16	452.0	-868.9	5.137	0.010
0.004	+	+	+	-0.003	-0.009	-0.011	0.003			+	+	+	+		17	453.2	-868.8	5.218	0.009
0.004	+	+		-0.003	-0.009	-0.011	0.003	0.000		+	+	+	+		17	453.2	-868.8	5.236	0.009
0.005	+	+	+	-0.001	-0.008	-0.015					+		+		14	449.4	-868.4	5.571	0.008
0.006	+	+		-0.001	-0.008	-0.015		0.000			+		+		14	449.4	-868.4	5.623	0.008
0.005	+	+	+		-0.008	-0.015		0.000			+		+		14	449.4	-868.3	5.678	0.007
0.006	+	+	+		-0.008	-0.015			+		+		+		14	449.4	-868.3	5.700	0.007
0.004	+	+	+	-0.003	-0.008	-0.015				+	+		+		15	450.5	-868.2	5.816	0.007
0.005	+	+		-0.003	-0.008	-0.015		0.000		+	+		+		15	450.4	-868.0	5.970	0.006

Full Δ 6 AICc top model set corresponding to **Table 4 in Chapter 4**, showing predictors of adult rate of change in relative telomere length over the course of a breeding period. Estimates are given for continuous variables. Presence of factors in the model is noted with +. Int = intercept, Dom = dominance.

Int	Dom Status	Sex	log Group size	num BAs	start RTL	Annual rain	Status: Sex	Status: num BAs	Status: start RTL	Status: Rain	Sex: num BAs	log group size: num Bas	Status: Sex: num Bas	df	logLik	AICc	delta AICc	weight
0.005	+				-0.006	-0.010			+					10	285.8	-549.7	0.00	0.136
0.004	+					-0.009								8	282.9	-548.5	1.22	0.074
0.001						-0.010								7	281.7	-548.4	1.23	0.074
0.004	+			0.002	-0.006	-0.010			+					11	286.1	-547.7	2.02	0.050
0.004	+		-0.001		-0.006	-0.010			+					11	286.0	-547.6	2.11	0.047
0.003	+	+			-0.006	-0.010			+					11	286.0	-547.5	2.19	0.046
0.005	+				-0.006	-0.010			+	+				11	285.8	-547.3	2.43	0.040
0.001			-0.002			-0.010								8	282.0	-546.7	2.97	0.031
0.001				0.002		-0.011								8	282.0	-546.6	3.06	0.029
0.004	+			0.002		-0.010								9	283.1	-546.6	3.07	0.029
0.004	+					-0.010				+				9	282.9	-546.2	3.44	0.024
0.004	+				0.001	-0.009								9	282.9	-546.2	3.47	0.024
0.001					0.000	-0.010								8	281.7	-546.2	3.49	0.024
0.004	+	+				-0.009								9	282.9	-546.2	3.50	0.024
0.004	+		0.000			-0.009								9	282.9	-546.2	3.51	0.024
0.001		+				-0.010								8	281.7	-546.2	3.53	0.023
0.004	+			0.003	-0.005	-0.010		+	+					12	286.3	-545.8	3.91	0.019
0.004	+		-0.001	0.002	-0.006	-0.011			+					12	286.2	-545.5	4.18	0.017
0.003	+	+		0.002	-0.006	-0.010			+					12	286.2	-545.4	4.27	0.016

0.003	+	+	-0.001		-0.006	-0.010			+				12	286.1	-545.2	4.44	0.015
0.002	+	+			-0.006	-0.010	+		+				12	286.1	-545.2	4.45	0.015
0.004	+			0.002	-0.006	-0.010			+	+			12	286.1	-545.2	4.49	0.014
0.004	+		-0.001		-0.006	-0.010			+	+			12	286.0	-545.1	4.58	0.014
0.003	+	+			-0.006	-0.010			+	+			12	286.0	-545.0	4.66	0.013
0.004	+			0.003		-0.010		+					10	283.5	-544.9	4.79	0.012
0.001			-0.002	0.002		-0.011							9	282.2	-544.8	4.86	0.012
0.001				0.002	0.001	-0.011							9	282.0	-544.4	5.25	0.010
0.001			-0.002		0.000	-0.010							9	282.0	-544.4	5.30	0.010
0.001		+	-0.002			-0.010							9	282.0	-544.4	5.30	0.010
0.004	+			0.002	0.001	-0.010							10	283.2	-544.3	5.33	0.009
0.004	+			0.002		-0.011				+			10	283.2	-544.3	5.36	0.009
0.001		+		0.002		-0.011							9	282.0	-544.3	5.40	0.009
0.004	+	+		0.002		-0.010							10	283.1	-544.3	5.42	0.009
0.004	+		0.000	0.002		-0.010							10	283.1	-544.3	5.42	0.009
0.003	+		-0.002	0.001	-0.006	-0.011			+			-0.002246297	13	286.8	-544.2	5.52	0.009
0.002	+	+				-0.009	+						10	283.1	-544.1	5.56	0.008
0.001			-0.002	0.001		-0.011						-0.002660383	10	283.1	-544.1	5.58	0.008
0.004	+				0.001	-0.010				+			10	283.0	-543.9	5.74	0.008
0.004	+	+				-0.010				+			10	283.0	-543.9	5.76	0.008
0.004	+		0.000			-0.010				+			10	283.0	-543.9	5.78	0.008
0.004	+	+			0.001	-0.009							10	283.0	-543.9	5.79	0.008
0.004	+		0.000		0.001	-0.009							10	282.9	-543.9	5.82	0.007
0.001		+			0.001	-0.010							9	281.7	-543.9	5.83	0.007
0.004	+	+	0.000			-0.009							10	282.9	-543.8	5.86	0.007

Δ 6 AICc top model set after implementation of the model nestling rule (Richard 2011), showing the **equivalent of Table 3 Chapter 4, but with only known age birds included**. The model was subsequently run with all samples including minimum age birsds as age was in the global model but does not appear in the top model set here.

Int	Social status	Season	Start RTL	Annual rainfall	Status: start RTL	Status: annual ran	Season: Annual rain	df	LogLik	AICc	Delta AICc	Weight
0.010	+	+	-0.011	-0.010	+	+	+	13	396.85	-765.30	0.00	0.74
0.009	+	+	-0.010	-0.015	+		+	12	394.26	-762.49	2.81	0.18
0.009	+		-0.011	0.00	+	+		11	392.33	-760.95	4.36	0.08

Appendix F: Chapter 5 unabridged top model sets

Full Δ 6 AICc top model set corresponding to **Table 1 in Chapter 5**, showing oxidative stress predictors of adult rate of change in telomere length. Estimates are given for continuous variables. Presence of factors in the model is noted with +. Int = intercept

Int	Social status	Sex	MDA	start RTL	SOD	rTAC	Status: MDA	Status: start RTL	Status: SOD	Status: TAC	Sex: MDA	Sex: SOD	Sex: TAC	df	logLik	AICc	delta	weight
0.002		+			-0.005							+		7	182.0	-348.0	0.00	0.066
-0.001	+	+			-0.004							+		8	183.0	-347.5	0.46	0.052
-0.001	+	+			-0.003	-0.005				+		+		10	185.7	-347.3	0.67	0.047
-0.001	+	+				-0.006				+				8	182.7	-346.9	1.10	0.038
0.000	+	+				-0.011				+			+	9	184.0	-346.8	1.16	0.037
-0.005	+													5	178.8	-346.6	1.38	0.033
-0.001														4	177.5	-346.2	1.74	0.027
-0.002	+	+												6	179.8	-346.1	1.88	0.026
0.002		+												5	178.4	-345.8	2.15	0.022
0.000	+	+			-0.003	-0.009				+		+	+	11	186.4	-345.8	2.16	0.022
-0.005	+					-0.005				+				7	180.9	-345.7	2.23	0.021
0.002		+		0.001	-0.005							+		8	182.1	-345.6	2.33	0.020
-0.001	+	+	-0.002		-0.003	-0.005				+		+		11	186.3	-345.6	2.39	0.020
0.002		+			-0.005	0.001						+		8	182.1	-345.6	2.39	0.020
0.002		+	-0.001		-0.005							+		8	182.0	-345.5	2.48	0.019
-0.001	+	+	-0.001		-0.004							+		9	183.2	-345.2	2.80	0.016
-0.001	+	+			-0.005	0.001						+		9	183.2	-345.1	2.85	0.016
-0.001	+	+		0.000	-0.005							+		9	183.1	-344.9	3.11	0.014
-0.001	+	+			-0.004				+			+		9	183.0	-344.8	3.16	0.014
-0.002	+	+	-0.001			-0.006				+				9	183.0	-344.8	3.17	0.013
-0.005	+		-0.001											6	179.1	-344.7	3.29	0.013
0.002		+			-0.005	-0.003						+	+	9	183.0	-344.6	3.33	0.012
-0.001	+	+		0.001	-0.003	-0.005				+		+		11	185.7	-344.5	3.49	0.011
-0.002	+	+	-0.005			-0.006				+	+			10	184.3	-344.5	3.51	0.011
-0.001	+	+			-0.003	-0.005			+	+		+		11	185.7	-344.4	3.57	0.011
-0.001	+	+	-0.001			-0.011				+			+	10	184.2	-344.3	3.69	0.010
-0.001	+	+			0.000	-0.006				+				9	182.7	-344.2	3.75	0.010
-0.005	+					0.000								6	178.8	-344.2	3.76	0.010
-0.005	+			0.000										6	178.8	-344.2	3.77	0.010
-0.005	+				0.000									6	178.8	-344.2	3.78	0.010
-0.002	+	+	-0.004		-0.002	-0.006				+	+	+		12	187.1	-344.2	3.80	0.010
-0.001			-0.001											5	177.6	-344.2	3.80	0.010

Int	Social status	Sex	MDA	start RTL	SOD	rTAC	Status: MDA	Status: start RTL	Status: SOD	Status: TAC	Sex: MDA	Sex: SOD	Sex: TAC	df	logLik	AICc	delta	weight
-0.001	+	+		0.000		-0.006				+				9	182.7	-344.2	3.80	0.010
-0.001					-0.001									5	177.6	-344.2	3.81	0.010
-0.001				0.001										5	177.6	-344.2	3.83	0.010
-0.005	+		-0.002			-0.005				+				8	181.3	-344.1	3.89	0.009
0.000	+	+			0.000	-0.011				+			+	10	184.1	-344.1	3.90	0.009
0.000	+	+		0.000		-0.011				+			+	10	184.1	-344.1	3.90	0.009
0.001		+				-0.005							+	7	180.0	-344.0	3.97	0.009
-0.001						0.000								5	177.5	-343.9	4.08	0.009
-0.002	+	+	-0.001											7	179.9	-343.9	4.09	0.008
0.002		+			-0.001									6	178.6	-343.8	4.17	0.008
-0.002	+	+			-0.001									7	179.8	-343.7	4.31	0.008
-0.002	+	+				0.001								7	179.8	-343.7	4.32	0.008
-0.002	+	+		-0.001										7	179.8	-343.6	4.34	0.007
-0.005	+		-0.005				+							7	179.8	-343.6	4.39	0.007
-0.001	+	+	-0.002		-0.002	-0.008				+		+	+	12	186.8	-343.6	4.40	0.007
0.002		+	-0.001											6	178.5	-343.6	4.42	0.007
0.002		+				0.000								6	178.4	-343.4	4.56	0.007
0.002		+		0.000										6	178.4	-343.4	4.57	0.007
-0.005	+			0.001		-0.005				+				8	181.0	-343.4	4.58	0.007
0.002		+	-0.002		-0.005						+	+		9	182.3	-343.3	4.68	0.006
-0.001	+	+			-0.004	-0.002						+	+	10	183.7	-343.3	4.71	0.006
0.002		+		0.001	-0.006	0.001						+		9	182.3	-343.2	4.74	0.006
-0.005	+			-0.002				+						7	179.6	-343.2	4.75	0.006
-0.002	+	+	-0.004		-0.003		+					+		10	183.6	-343.2	4.76	0.006
-0.005	+				0.000	-0.005				+				8	180.9	-343.2	4.78	0.006
-0.002	+	+	-0.004		-0.002	-0.005	+			+		+		12	186.6	-343.1	4.83	0.006
0.000	+	+		0.001	-0.003	-0.009				+		+	+	12	186.5	-343.1	4.91	0.006
0.002		+	-0.001		-0.005	0.001						+		9	182.2	-343.1	4.92	0.006
0.002		+	-0.001	0.001	-0.005							+		9	182.2	-343.0	4.94	0.006
-0.001	+	+	-0.004			-0.010				+	+		+	11	185.0	-343.0	4.97	0.005
-0.002	+	+	-0.003		-0.004						+	+		10	183.5	-343.0	5.01	0.005
-0.001	+	+				-0.004							+	8	180.8	-343.0	5.02	0.005
-0.001	+	+	-0.001		-0.004	0.001						+		10	183.5	-342.8	5.14	0.005
0.000	+	+			-0.003	-0.009			+	+		+	+	12	186.4	-342.8	5.17	0.005
-0.001	+	+	-0.002		-0.003	-0.005			+	+		+		12	186.3	-342.6	5.38	0.004
-0.001	+	+	-0.002	0.000	-0.003	-0.005				+		+		12	186.3	-342.6	5.38	0.004
-0.002	+	+	-0.004								+			8	180.6	-342.5	5.43	0.004
-0.002	+	+	-0.003			-0.006	+			+				10	183.2	-342.4	5.55	0.004
-0.001	+	+		0.001	-0.005	0.001						+		10	183.2	-342.4	5.57	0.004
-0.001	+	+	-0.001	0.000	-0.004							+		10	183.2	-342.4	5.58	0.004
-0.001	+	+	-0.001		-0.004				+			+		10	183.2	-342.4	5.60	0.004

Int	Social status	Sex	MDA	start RTL	SOD	rTAC	Status: MDA	Status: start RTL	Status: SOD	Status: TAC	Sex: MDA	Sex: SOD	Sex: TAC	df	logLik	AICc	delta	weight
-0.006	+		-0.004			-0.005	+			+				9	181.8	-342.3	5.65	0.004
-0.001	+	+			-0.005	0.001			+			+		10	183.2	-342.3	5.65	0.004
0.001		+		0.001	-0.005	-0.003						+	+	10	183.2	-342.3	5.65	0.004
-0.003	+	+	-0.007			-0.006	+			+	+			11	184.6	-342.3	5.66	0.004
-0.005	+		-0.001			0.001								7	179.1	-342.3	5.68	0.004
-0.003	+	+	-0.004				+							8	180.4	-342.3	5.70	0.004
-0.002	+	+	-0.002		0.001	-0.006				+				10	183.1	-342.2	5.77	0.004
-0.005	+		-0.001	0.000										7	179.1	-342.2	5.79	0.004
-0.005	+		-0.001		0.000									7	179.1	-342.2	5.81	0.004
-0.006	+			-0.002		-0.004		+		+				9	181.7	-342.1	5.83	0.004
-0.001	+	+		0.001	-0.005			+				+		10	183.1	-342.1	5.85	0.004
0.001		+	-0.003								+			7	179.0	-342.1	5.85	0.004
-0.001	+	+		0.000	-0.005				+			+		10	183.1	-342.1	5.91	0.003
-0.001				0.001	-0.001									6	177.7	-342.0	5.94	0.003
-0.001	+	+	-0.001	0.000		-0.006				+				10	183.1	-342.0	5.95	0.003
-0.003	+	+	-0.007		-0.002	-0.005	+			+	+	+		13	187.6	-342.0	5.98	0.003

Full Δ 6 AICc top model set corresponding to **Table 3 in Chapter** 5, showing models of the effects of change in oxidative state on adult rate of change in telomere length. Estimates are given for scaled and centred continuous variables. Presence of factors in the model is noted with +. Int = intercept

Int	Sex	delta MDA	delta SOD	delta TAC	SOD	Sex: SOD	df	logLik	AICc	delta	weight
0.0045	+				-0.0073	+	7	118.94	-220.48	0.00	0.39
0.0046	+		-0.0026		-0.0081	+	8	119.52	-218.53	1.95	0.15
0.0009							4	113.32	-217.54	2.94	0.09
0.0046	+	-0.0006			-0.0075	+	8	118.97	-217.44	3.03	0.09
0.0045	+			0.0004	-0.0072	+	8	118.95	-217.40	3.08	0.08
0.0008					-0.0018		5	113.63	-215.54	4.93	0.03
0.0047	+	-0.0011	-0.0028		-0.0086	+	9	119.64	-215.47	5.01	0.03
0.0027	+						5	113.55	-215.39	5.09	0.03
0.0009			-0.0018				5	113.54	-215.37	5.11	0.03
0.0045	+		-0.0026	0.0006	-0.0081	+	9	119.55	-215.29	5.19	0.03
0.0009		0.0011					5	113.45	-215.18	5.30	0.03
0.0009				0.0005			5	113.34	-214.97	5.51	0.02