Early-life seasonal, weather and social effects on telomere length in a wild mammal

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Abstract

Early-life environmental conditions can provide a source of individual variation in life-history strategies and senescence patterns. Conditions experienced in early life can be quantified by measuring telomere length, which can act as a biomarker of survival probability. Here, we investigate whether seasonal changes, weather conditions, and group size are associated with early-life and/or early-adulthood telomere length in a wild population of European badgers (*Meles meles*). We found substantial intra-annual changes in telomere length during the first three years of life (both between and within individuals), with shorter telomere lengths from spring to winter and longer telomere lengths over the winter torpor period. In terms of weather conditions, linked to food availability and foraging success, cubs born in warmer, wetter springs with low rainfall variability had longer early-life (<1 year old) telomere lengths. Additionally, cubs born in groups with more cubs did not have significantly shorter early-life telomeres, providing no evidence of resource constraint from cub competition. We also found that our previously documented positive association between early-life telomere length and cub survival probability remained when social and weather variables were included. Finally, after sexual maturity, in early adulthood (i.e. 12–36 months) we found no significant association between same-sex adult group size and telomere length (i.e. no effect of intra-sexual competition). Overall we show that controlling for seasonal effects is important in telomere length analyses, and that badger telomere length functions as a biomarker that reflects the physiological consequences of early-life adversity and subsequent effects on cub survival probability.

Keywords: telomere length, early-life environment, group size, weather conditions, senescence, season

1. Introduction

The early-life environment can have lasting effects on individual fitness (Lindström, 1999), with consequences for variation in life-history strategies (Metcalfe & Monaghan, 2001) and senescence patterns (Nussey, Kruuk, Morris, & Clutton-Brock, 2007). For example, it has been hypothesised that senescence, the decline in performance in older age, is faster in individuals living in adverse early-life environments due to different energy allocation trade-offs between early- and later-life in response to the environment (Kirkwood & Rose, 1991; Medawar, 1952; Williams, 1957). A more stressful early-life environment, either through a sub-optimal mean or more variable early-life environment, during this sensitive developmental period, could trigger early reproductive investment at the expense of somatic maintenance, leading to faster rates of senescence (Kirkwood & Rose, 1991; Lemaitre et al., 2015).

Empirical evidence for such detrimental effects has been found in various wild animal populations (Cooper & Kruuk, 2018; Hammers, Richardson, Burke, & Komdeur, 2013; Reed et al., 2008).

Telomere length can quantify the effects of early-life environmental conditions on senescence, functioning as a biomarker of senescence in some species (López-Otín, Blasco, Partridge, Serrano, & Kroemer, 2013; Monaghan & Haussmann, 2006), while also allowing quantification of physiological consequences of the conditions experienced (Monaghan, 2014). Telomeres are highly conserved nucleoprotein structures at the end of chromosomes consisting of a non-coding sequence (5’-TTAGGG-3’) and shelterin proteins (Blackburn, 2000; de Lange, 2005). Telomeres maintain genomic integrity by preventing chromosome degradation and fusion of chromosome ends through forming T-loops (de Lange, 2004). Generally, telomeres shorten with each cell replication due to the end-replication problem (Olovnikov, 1973), but telomere shortening can be accelerated through stressors (Epel et al., 2004; Heidinger et al., 2012) and potentially by oxidative damage (Boonekamp, Bauch, Mulder, & Verhulst, 2017; Reichert & Stier, 2017; von Zglinicki, 2002). Telomeres can, however, also elongate via the enzyme telomerase (Blackburn et al., 1989) and other telomere-elongation pathways (Cesare & Reddel, 2010; Mendez-Bermudez et al., 2012). Cells with critically short telomeres ultimately enter replicative senescence, where the accumulation of senescent cells can impair tissue function due to reduced renewal capacity (Campisi, 2005; Campisi & di Fagagna, 2007) and potentially lead to organismal senescence (Young, 2018).

In some species, variation in early-life telomere length has been linked to season with the evolved ability to tolerate winter food scarcity combined with high thermoregulatory costs. During hibernation, more frequent arousal, which increases metabolic rate and potentially increases oxidative stress, is in arctic ground squirrels (*Urocitellus parryii*) associated with shorter telomere length (Wilbur, Barnes, Kitaysky, & Williams, 2019) and in edible dormice (*Glis glis*) associated with telomere shortening (Turbill, Ruf, Smith, & Bieber, 2013). Shortening is reduced when the animals’ core temperature difference between hibernation and arousal is smaller, in both edible and garden (*Eliomys quercinus*) dormice (Nowack et al., 2019). Conversely, the use of spontaneous daily torpor in non-hibernating Djungarian hamsters (*Phodopus sungorus*) is associated with telomere lengthening due to a relatively low energy investment to return to euthermia along with the benefits of reduced metabolic rate in torpor compared to hibernation (Turbill, Smith, Deimel, & Ruf, 2012). In contrast, non-hibernating juvenile garden dormice that more frequently underwent fasting-induced torpor had telomere shortening (Giroud et al., 2014). Species that undergo facultative winter torpor may therefore conserve energy for somatic maintenance that could potentially be invested in telomere restoration/elongation.

In addition to these intra-annual changes in telomere length, extensive evidence links adverse early-life conditions to shorter telomeres (McLennan et al., 2016; Mizutani, Tomita, Niizuma, & Yoda, 2013; Watson, Bolton, & Monaghan, 2015), where shorter telomeres are associated with reduced survival probability (Wilbourn et al., 2018). Food availability, often determined by weather conditions (e.g. Campbell, Nouvellet, Newman, Macdonald, & Rosell, 2012), has been positively associated with early-life telomere length (e.g. Foley et al., 2020; Spurgin et al., 2017). Interestingly, early-life food availability may also impact life-history strategies (Bright Ross, Newman, Buesching, & Macdonald, 2020) and we consider here that this may be reflected in early-life telomere lengths. It has been hypothesised that individuals in temporally stochastic environments should modulate energy trade-offs (Erikstad, Fauchald, Tveraa, & Steen, 1998; Reid, Bignal, Bignal, McCracken, & Monaghan, 2003; Weimerskirch, Zimmermann, & Prince, 2001) and adopt a bet-hedging strategy until environmental conditions become favourable (Morris et al., 2008; Wilbur & Rudolf, 2006). Since weather variability is predicted to increase in the future (IPCC, 2018), it is important to understand the implications of variable early-life conditions for life-history strategies and early-life telomere length. The interplay between the mean of and variability in early-life environmental conditions, such as the availability and variation in food, foraging success and thermal stress for young individuals (Noonan et al., 2015; Nouvellet, Newman, Buesching, & Macdonald, 2013;

Webb & King, 1984), can thus impact developmental stress, longevity and may be reflected in early-life telomere length.

Social conditions in early-life can also shape life-history strategies and senescence due to increased competition for food and social stress. For example, female red deer (*Cervus elaphus*) that experienced high levels of resource competition in early-life showed faster rates of reproductive senescence (Nussey et al., 2007). Such competition for food may be even stronger in group living species due to a greater number of offspring competing for food and foraging space in close proximity. Additionally, there is evidence for conspecific resource competition in early-life leading to greater telomere shortening (Boonekamp, Mulder, Salomons, Dijkstra, & Verhulst, 2014; Nettle et al., 2015; Stier, Massemin, Zahn, Tissier, & Criscuolo, 2015), and shorter telomere lengths (Cram, Monaghan, Gillespie, & Clutton-Brock, 2017), where stressors (including competition) are associated with both shorter telomere lengths and higher telomere shortening (Chatelain, Drobniak, & Szulkin, 2020).

The effects of social conditions on senescence may also become apparent after sexual maturity, when individuals compete for mating opportunities (Andersson, 1994; Beirne, Delahay, & Young, 2015). In polygynous species, sex differences in senescence may be attributable to intense intra-sexual competition between males (Clutton-Brock & Isvaran, 2007; Promislow, 1992; Williams, 1957). Male investment for mating opportunities trades off with self-maintenance (Kirkwood & Rose, 1991). Intense male-male competition drives selection for shorter lifespan and faster senescence in males, compared to females (Clutton-Brock & Isvaran, 2007; Williams, 1957). While this prediction has been challenged (Bonduriansky, Maklakov, Zajitschek, & Brooks, 2008; Graves, 2007; Promislow, 2003), and sex-specific senescence may be trait-dependent with respect to the underlying physiological processes (Nussey et al., 2009), higher rates of male-biased actuarial senescence in polygynous and sexual dimorphic species exist (Clutton-Brock & Isvaran, 2007; Promislow, 1992). While social effects may also contribute to senescence in females (Sharp & Clutton-Brock, 2011; Woodroffe & Macdonald, 1995), such sex-specific social effects on senescence are expected to be greater in males (Bonduriansky et al., 2008; Clutton-Brock & Isvaran, 2007; Maklakov & Lummaa, 2013). However, whether increased intra-sexual competition (e.g. higher local densities of same-sex individuals) is associated with shorter telomere lengths remains to be tested.

To test the effects of early-life social and environmental conditions on telomere length, we use a long-term dataset from a wild population of European badgers (*Meles meles*; henceforth ‘badgers’). Badgers show reproductive senescence (Sugianto, Newman, Macdonald, & Buesching, 2020) with males having a later onset but faster rate of senescence than females (Dugdale, Pope, Newman, Macdonald, & Burke, 2011b). Additionally, early-life telomere length (<1 year old) positively correlates with first-year survival and lifespan in badgers (van Lieshout et al., 2019). Badgers are natally philopatric and form large social groups (mean group size = 11.3, range = 2–29; da Silva, Macdonald, & Evans, 1994) with latrine-marked borders (Buesching, Newman, Service, Macdonald, & Riordan, 2016; Delahay et al., 2000), although they do transgress these borders when foraging (Ellwood et al., 2017; Noonan et al., 2015) without any sex difference in foraging niche (Robertson, McDonald, Delahay, Kelly, & Bearhop, 2014).

Badgers do not hibernate (Johansson, 1957) but do undergo facultative winter torpor, reducing their body temperature by up to 8.9°C (Fowler & Racey, 1988), and thus reducing energy expenditure (Newman, Zhou, Buesching, Kaneko, & Macdonald, 2011). Badgers in Britain mainly feed on earthworms (*Lumbricus terrestris*; Johnson, Baker, Morecroft, & Macdonald, 2001; Kruuk & Parish, 1981). Earthworms are sensitive to microclimatic conditions (Edwards & Bohlen, 1996; Gerard, 1967; Newman, Buesching, & Macdonald, 2017), making their abundance and distribution highly dependent on weather conditions. High-density badger populations occur in mild areas with damp conditions where earthworms are available (Johnson, Jetz, & Macdonald, 2002; Kruuk, 1978; Macdonald, Newman, & Buesching, 2015; Newman et al., 2017). Foraging efficiency is reduced in adverse weather conditions, due to reduced availability of earthworms, thermal stress when foraging in cold and wet conditions, and/or the choice to remain in thermally-stable underground dens, termed setts (Noonan et al., 2014; Noonan et al., 2018;

Nouvellet et al., 2013). Weather conditions can therefore impact survival probability where, for example, higher annual mean daily rainfall is positively associated with adult survival probability in badgers, whereas high annual variability in temperature has detrimental consequences for cub and adult survival (Nouvellet et al., 2013).

Badger cub growth and maturation depends on the number of other cubs and adults present within the social group (Sugianto, Newman, Macdonald, & Buesching, 2019b), potentially indicating resource competition within social groups. Adult male badgers invest substantial energy into promiscuity and repeated mounting (Dugdale, Griffiths, & Macdonald, 2011a) both within and outside their social group, resulting in high rates (i.e. 48%) of extra-group paternity, with 85% of candidate fathers residing primarily in neighbouring groups (Annavi et al., 2014; Dugdale, Macdonald, Pope, & Burke, 2007). Males also exhibit substantial inter-individual variance in reproductive success (Dugdale et al., 2007; Dugdale et al., 2011b) and evidence of reproductive skew among females within a group (Dugdale, Macdonald, Pope, Johnson, & Burke, 2008; Woodroffe & Macdonald, 1995). With the polygyandrous system (Dugdale et al., 2011a), a slight sexual dimorphism and slight male-biased mortality in badgers (Bright Ross et al., 2020; Johnson & Macdonald, 2001; Sugianto, Newman, Macdonald, & Buesching, 2019a) and evidence of downstream effects of male–male competition on body mass senescence (Beirne et al., 2015), such intra-sexual competition may be reflected in telomere length in early adulthood.

Here, we investigate the relationships between early-life conditions and relative leukocyte telomere length (RLTL), by testing whether: (i) between- and within-individual variation in RLTL in early life and early adulthood can be explained by seasonal changes potentially linked to reduced energy expenditure during torpor; (ii) adverse early-life weather, as a proxy for food availability and thermal stress, is associated with shorter early-life RLTL and the social conditions that cubs are exposed to (with more cubs potentially leading to resource competition and associated with shorter early-life RLTL or more cubs reflecting more resources and thus being associated with longer early-life RLTL); (iii) the strength of the association between early-life RLTL and first-year survival probability is dependent on early-life conditions and (iv) adverse social conditions after sexual maturity (i.e. larger same-sex adult group size for females and, for males, more within-group and neighbouring-group adult (>1 year old) males), are associated with shorter RLTL in early post-maturity adulthood.

2. Methods

(a) Study population and trapping

We conducted this study in a high-density population of European badgers (hereafter 'badgers'; mean ± SE = 36.4 ± 2.55 badgers/km^2; Macdonald, Newman, Nouvellet, & Buesching, 2009) in Wytham Woods, Oxfordshire, UK (51°46’24”N, 1°20’04”W); a 424 ha mixed semi-natural woodland surrounded by mixed arable and permanent pasture (Macdonald et al., 2015). Wytham Woods had a mean annual temperature of 10.6 °C (± 5.5 SD) and mean annual precipitation of 684 (± 129 SD) mm, 1987–2010. The population consisted of 19 ± 2 (mean ± 95% CI; range = 14–26; Dugdale et al., 2008) mixed-sex social groups (Johnson et al., 2002; Newman et al., 2011) during the period that we analysed, 1987–2010, with a 50% offspring sex ratio (Dugdale, Macdonald, & Newman, 2003). The Wytham badger population is geographically discrete (Macdonald et al., 2009) with only ca. 3% annual immigration/emigration per year (Macdonald & Newman, 2002).

The badger population has been trapped systematically since 1987 over three two-week periods in May–June (“spring”), August–September (“summer”) and November (“autumn”), with further trapping in January (“winter”) in focal years. Badgers were anaesthetised using an intra-muscular injection of 0.2 ml ketamine hydrochloride per kg body weight (McLaren et al., 2005). Upon first capture, badgers were assigned a unique inguinal tattoo for permanent identification. Sex, age class (cub <1 year old; adult ≥1 year old), capture date and social group were recorded. Age of badgers was defined as the number of days elapsed since the 14th of February, reflecting the averaged date of synchronised parturition, in the respective birth year (Yamaguchi, Dugdale, & Macdonald, 2006). Age of badgers first caught as adults was inferred from tooth wear, which is commonly used and highly correlated ($r^2 = 0.80$) with known age in this population (Bright Ross et al., 2020; da Silva & Macdonald, 1989; Hancox, 1988; Macdonald et al., 2009). Only badgers that didn't have an already-known age and had a tooth wear of 2 (on a 1–5 scale) were included since these typically indicate a 1-year old adult (Bright Ross et al., 2020). Whole blood samples were collected from anaesthetised badgers through jugular venipuncture into vacutainers with an EDTA anticoagulant, and stored immediately at -20°C. Badgers were released after full recovery from anaesthesia. Additionally, bait-marking (Delahay et al., 2000; Macdonald & Newman, 2002) was conducted periodically to delimit group range sizes and deduce social groups.

(b) Telomere analyses

Genomic DNA was extracted from whole blood samples (*n* = 875 samples; 562 badgers) using the DNeasy Blood & Tissue kit (Qiagen, Manchester, UK) according to the manufacturer's protocol, with changes by conducting a double elution step (2x 75 µl AE buffer) and using 125 µl of anticoagulated blood. DNA integrity was checked by running a random selection of DNA extracts (ca. 20%) on agarose gels to ensure high molecular weight. DNA concentration of all samples was quantified using the Fluostar Optima fluorometer (BMG Labtech, Ortenberg, Germany) and standardized to 20 ng/µl, after which samples were stored at -20 °C. We used monochrome multiplex quantitative PCR (MMqPCR) analysis to measure RLTL (Cawthon, 2009). This is a measure that reflects the abundance of telomeric sequence relative to a reference gene, which are both analysed in the same well, and represents the mean telomere length across cells in a sample. We used a sub-set of 875 samples from the full dataset of 1248 samples detailed in van Lieshout et al. (2019). In the full dataset, Cq-values on the qPCR plates (*n* = 34) declined in a log-linear fashion ($r^2 > 0.99$). Reaction efficiencies were (mean ± SE) 1.793 ± 0.004 for IRBP and 1.909 ± 0.004 for telomeres. Inter-plate repeatability (intraclass correlation coefficient) calculated from the reference sample was 0.82 for RLTL measurements (95% CI = 0.76–0.87; *n* = 142 samples; 34 plates), and intra-plate repeatability calculated with duplicates of the same sample on the same plate, while controlling for plate effects, was 0.90 (95%CI = 0.86–0.93; *n* = 1,248 samples; 34 plates) for IRBP, 0.84 (95%CI = 0.79–0.90; *n* = 1,248 samples; 34 plates) for telomere Cq-values and 0.87 (95% CI = 0.82–0.91; *n* = 1,248 samples; 34 plates) for RLTL measurements. A detailed description of the MMqPCR analysis can be found in van Lieshout et al. (2019).

(c) Weather conditions

Four weather metrics were calculated for each season (mean daily temperature, temperature variability, mean daily rainfall, and rainfall variability) from 1987 to 2010 to characterise the developmental stress associated with variation in earthworm food availability and thermoregulatory costs (Macdonald, Newman, Buesching, & Nouvellet, 2010; Noonan et al., 2014; Nouvellet et al., 2013). Mean daily temperature and rainfall were calculated using mean daily temperature and total daily precipitation values provided by the Radcliffe Meteorological Station, School of Geography, University of Oxford (6 km from the field site). Daily temperatures followed a sinusoidal pattern, and so seasonal temperature variability was calculated as the sum of daily squared residuals from a sinusoidal fit to the corresponding year's temperatures (i.e. cumulative unpredictability). Rainfall did not show annual trends and its seasonal variability was therefore characterised simply as the coefficient of variation (SD/mean) in daily rainfall.

(d) Group sizes

Natal group sizes were determined by the number of individuals (cubs and adults) that were present in a social group in the year of an individual's birth. Given high lifetime natal philopatry (35.8%), low permanent dispersal rates (19.1%), and high levels of short-term inter-group movements (Macdonald, Newman, Buesching, & Johnson, 2008), individuals (*n* = 1726) were assigned as a resident of a social group each year, according to published criteria (van Lieshout et al., 2020a). The number of individuals in a natal social group was then calculated as the sum of individuals present in the social group in that year.

Yearly social group size measures were then separated by age class (i.e. cub/adult) and sex (i.e. male/female) to determine sex- and age-specific group sizes per year. To measure intra-sexual competition in females, we calculated female adult group sizes, as females compete with other within-group females (Woodroffe & Macdonald, 1995). However, for males, extra-group paternity is high (48%) and affected by the number of within-group and extra-group candidate fathers (Annavi et al., 2014), so we combined both the number of within-group males and neighbouring-group males. The mean number of cubs in a social group for badgers in our dataset (n = 562 badgers) was 3.4 (± 2.4 SD; range 0–14), the mean number of female adults in a social group was 6.0 (± 3.5 SD; range 0–19) and the mean number of male adults in focal plus neighbouring social groups was 27.0 (± 11.7 SD; range 0–59).

(e) Statistical analyses

Statistical analyses were conducted in R 3.3.1 (R Development Core Team, 2020), using parametric bootstrapping (n = 5000) to estimate 95% confidence intervals and determine significance of predictors in *lme4* 1.1-14 (Bates, Machler, Bolker, & Walker, 2015). Model fit was assessed using standard residual plot techniques to ensure approximately normal distribution and constant variance, and fixed effects were ensured not to be collinear (VIF < 3). Relative leukocyte telomere length (RLTL) as response variable was first square-root and then Z-transformed (mean = 0, SD = 1) for comparability (Verhulst, 2020). Quadratic fixed effects were included if such relationships were plausible *a priori*, and removed if non-significant to test the significance of first-order effects.

In this study, we focus on early-life (<1 year old) and since badgers typically reach sexual maturity by 2 years of age (Sugianto et al., 2019b), occasionally at age 1 year (Dugdale et al., 2007) and therefore first produce offspring when they are 2–3 years of age, due to delayed implantation resulting in a full year between conception and parturition, we define early adulthood as 12–36 months old.

(i) Seasonal effects on RLTL in early-life and early adulthood

We first tested for an association between season and RLTL (≤36 months old) in early-life and early-adulthood in a Gaussian distribution model (identity link function) with RLTL as the response variable (*n* = 875 samples; 562 badgers). Threshold age (van Lieshout, 2019), age at last capture, and season were included as fixed effects, and qPCR plate, row on qPCR plate, social group, cohort, year and individual ID as random effects as these impact RLTL in badgers (van Lieshout et al., 2020b).

As we found a significant cross-sectional difference in RLTL between spring and winter, we then applied the 'within-subject centring' approach described by van de Pol and Wright (2009) to distinguish within- and between-individual effects between spring and winter. Following (Schroeder, Nakagawa, Cleasby, & Burke, 2012), we included two new fixed effects: 1) to estimate the within-individual variation component (β_W) we removed between-individual variation by subtracting the mean season value (coded as: spring = 0, winter = 1) for each individual across all years, from the season value for each RLTL measurement. So, if an individual was measured once in spring and once in winter, it was scored as -0.5 for spring and 0.5 for winter; and, 2) to estimate the between-individual variation between seasons (β_B), we included the mean season value for each individual (van de Pol & Wright, 2009). We then ran a Gaussian distribution model (identity link function) with RLTL as the response variable (*n* = 547 samples; 430 badgers) and threshold age (van Lieshout, 2019), age at last capture, within-individual season effect (β_W) and between-individual season effect (β_B) as fixed effects, and qPCR plate, row on qPCR plate, social group, cohort, year and individual ID as random effects. Subsequently, we tested whether the within-individual (β_W) and between-individual (β_B) slopes differed by including season and the between-individual effect (β_B; i.e. mean season value) in the same model (i.e. season now reflects the within-individual effect).

Lastly, to test whether telomere length decreases or increases from spring to winter we used a subset of individuals measured in their first spring or first winter, plus individuals measured consecutively in their first spring and first winter (n = 28 samples; 14 badgers). For the direction of the effect from winter to spring we used a subset of individuals measured in their first winter or second spring, plus individuals measured consecutively in their first winter and second spring (n = 12 samples; 6 badgers). In the two models (spring to winter and winter to spring) with a Gaussian distribution and RLTL as the response variable, we included age, age at last capture and season as fixed effects, and qPCR plate, row on qPCR plate, social group, cohort, year and individual ID as random effects. Subsequently, we used the within-subject centring approach again to separate within- and between-individual effects and test whether these slopes differ (van de Pol & Wright, 2009).

(ii) Weather and natal group size effects on early-life RLTL

We tested whether weather and social conditions experienced as a cub (<1 year old) were associated with early-life RLTL. We first used a GLMM to confirm the previous observation (van Lieshout et al., 2019) that early-life RLTL did not vary with age (in months), controlling for season (n = 435, β = -0.058, 95%CI = -0.222–0.360), and excluded age from subsequent analyses. The effects of first-year conditions on early-life RLTL were then modelled with early-life RLTL as the response variable in a Gaussian-distributed model (identity link function; n = 435, samples; 435 badgers). First, we determined the season in which the weather conditions (i.e. mean temperature, mean rainfall, temperature variability and rainfall variability) best explained the variation in early-life RLTL (AICc spring = 1203.8 was lowest, versus summer ΔAICc = 16.0, autumn ΔAICc = 12.4, winter ΔAICc = 9.5), with models with ΔAICc <7 from the top model being plausible (Burnham, Anderson, & Huyvaert, 2011). Spring (end of March to end of June) as the weather window reflects the strongest developmental stress on individuals. This period includes when cubs first emerge above ground from the end of February, are weaned around mid-May, and reach independence at the start of June (Dugdale, Ellwood, & Macdonald, 2010) during which time cubs exhibit high growth rates depending on food availability and social conditions (Sugianto et al., 2019a). Secondly, we determined whether the number of cubs, adults or the total number of individuals in the natal group best predicted early-life RLTL using AICc (the lowest AICc = 1203.7 was for number of cubs, versus number of adults ΔAICc = 3.3, total number of individuals ΔAICc = 3.1, number of cubs plus number of adults ΔAICc = 6.2, number of cubs plus total number of individuals ΔAICc = 6.0). Since ΔAICc <7, and VIF>3 for the other combinations in the same model, we ran five separate models with either the number of cubs, number of adults, the total number of individuals, number of cubs plus adults or number of cubs plus total number of individuals in the natal group as a fixed effect along with season, and mean daily temperature, temperature variability, mean daily rainfall and rainfall variability in spring. qPCR plate, row on qPCR plate, social group and cohort were included as random effects.

(iii) Covariation between early-life RLTL and weather conditions on cub survival probability

To understand whether the association between early-life RLTL and cub survival probability (van Lieshout et al., 2019) is due to or independent of weather effects, we tested whether the association between early-life RLTL and cub survival probability was still detected when social and weather conditions were included in the model. We first modelled survival to adulthood (≥1 year old) as a binary term in a binomially distributed model (logit link function; *n* = 435 samples; 435 badgers) with early-life RLTL as a fixed effect and qPCR plate, row on qPCR plate, social group and cohort were included as random effects. We then also included as fixed effects: number of cubs in natal group, mean daily temperature, temperature variability, mean daily rainfall and rainfall variability in a given season. We determined the season in which weather conditions best explained the variation in cub survival probability, using AICc (the lowest AICc = 447.1 was in winter, versus spring ΔAICc = 16.3, summer ΔAICc = 7.3 and autumn ΔAICc = 16.6) where models with ΔAICc <7 from the top model are plausible (Burnham et al., 2011). We then applied model selection to test whether including weather and social variables knocked early-life RLTL out of the plausible models. This would indicate that the early-life RLTL and survival probability relationship is driven by covariation between the environment and physiological state (early-life RLTL). As early-life RLTL was retained, we estimated the RLTL model-averaged parameter and 95% confidence interval using the natural averaged method (where the parameter was averaged over models in which it was present; Burnham & Anderson, 2002). This avoids the parameter estimate shrinking towards zero, from inclusion of the relatively less important models where the parameter was not retained (Nakagawa & Freckleton, 2011).

(iv) Same-sex group size effects on RLTL in early adulthood

We examined whether same-sex adult group sizes were reflected in RLTL in early adulthood (i.e. 12–36 months old). In a GLMM with RLTL in early adulthood as the response variable with one age threshold separating two periods of 12 to ≤29 months and >29 and ≤36 months (see van Lieshout et al., 2019) and season as fixed effects, we determined that RLTL did not vary with age (n = 406, 12 to ≤29 months, β = -0.087, 95%CI = -0.192–0.017; >29 and ≤36 months, β = -0.036, 95%CI = -0.163–0.091), and excluded age from the subsequent analysis. The effects of same-sex adult group size on RLTL in early adulthood were then modelled with RLTL in early adulthood as the response variable (n = 406 samples; 329 badgers). Same-sex adult group size (within-group for females and within- plus neighbouring-group for males), sex and its interaction with group size (to model differential strength in intra-sexual competition among the sexes), age at last capture (to control for selective disappearance), and season were included as fixed effects, and qPCR plate, row on qPCR plate, social group, cohort, year and individual ID as random effects.

3. Results

(i) Seasonal effects on RLTL in early-life and early adulthood

We found a cross-sectional effect of season, with badgers having shorter RLTL in winter compared to spring, controlling for age (Figure 1; Table S1). We also found both a within- and between-individual effect of shorter RLTL in winter than in spring, with no significant difference between these slopes (Table S2 & S3). For spring to winter there was both a cross-sectional and within-individual decline in RLTL (Table S4 & Figure S1), whereas from winter to the following spring there was a within-individual increase in RLTL (Table S5 & Figure S1). For both spring to winter and winter to spring the slopes for within- and between-individual effects did not differ (Table S6).

(ii) Weather and natal group size effects on early-life RLTL

We found a positive association between spring temperature and early-life RLTL (Figure 2; Table 1 & S7–S10), with cubs experiencing cooler-than-average first springs having shorter early-life RLTL. We also found that cubs experiencing intermediate-to-high mean daily rainfall had longer early-life RLTL (Figure 3; Table 1 & S7–S10) than cubs developing during drier years. Cubs experiencing low rainfall variability also had longer early-life RLTL (Figure 4; Table 1 & S7–S10). We found, while controlling for weather effects, no evidence for an association between the number of cubs, adults or total number of individuals in the natal group and early-life RLTL (Table 1 & S7–S10).

(iii) Covariation between early-life RLTL and weather conditions on cub survival probability

We first replicated our published finding (van Lieshout et al., 2019) of a positive association between early-life RLTL and survival to adulthood not controlling for social and weather effects (Table S11). Then we included social and weather conditions in the model: cub survival probability exhibited a negative quadratic relationship with winter rainfall variability (Figure S2; Table S12), a negative quadratic association with mean daily temperature (Figure S3; Table S12), a positive association with mean daily rainfall (Figure S4; Table S12) but no significant effect of the number of cubs in a group (Table S12). Using model selection, early-life RLTL was present in the top 19 models and retained in 46/70 plausible models (Table S13). The naturally averaged estimate for RLTL in the plausible models was 0.313 (95% CI = 0.029 – 0.597; Table S14) and thus the 95% CIs of early-life RLTL overlapped between the models with and without (β = 0.371, 95% CI = 0.102 to 0.681, Table S11) early-life social and weather variables.

(iv) Same-sex group size effects on RLTL in early adulthood

We found no evidence of same-sex adult group size effects on RLTL in early adulthood for females or males (Table S15).

4. Discussion

Our results show both between-individual variation and within-individual changes in RLTL across seasons, where cubs had longer RLTL in their first spring, decreasing to winter then increasing again by the following spring. We also found that cubs born in conditions that were warmer and wetter, with little variation in rainfall, had longer early-life RLTL. Sociologically, the number of cubs, adults, total individuals, a combination of cubs and adults or cubs and the total number of individuals in the natal group had no effect on early-life RLTL. Our results also suggest that the link between early-life RLTL and cub survival probability is driven by conditions experienced in addition to the early-life social and weather conditions modelled. Additionally, we found no effect of the number of within-group adult females, or both within-group and extra-group adult males (i.e. intra-sexual competition) on RLTL in early adulthood.

Our finding that badgers had shorter early-life RLTL (both between- and within- individuals) in winter, compared to the preceding spring could be linked to stressful effects such as disease (Newman, Macdonald, & Anwar, 2001) and sub-optimal foraging conditions and food availability (Macdonald &

Newman, 2002; Newman et al., 2017). We then found positive within-individual changes in RLTL from the first winter to the following spring. Body temperatures in badgers fall from November to December (by a maximum of 8.9 °C compared to late-spring) and steadily rise until euthermic levels are reached by late April (Fowler & Racey, 1988; Geiser & Ruf, 1995). During harsh winter conditions, badgers use facultative torpor to reduce their core temperature and metabolic rate, conserving energy (Newman et al., 2011). This reduction of basal metabolic rates (Geiser, 2004) can reduce mitosis (Kruman, Ilyasova, Rudchenko, & Khurkhulu, 1988) and therefore potentially reduce telomere shortening. Similarly, daily torpor cycles in Djungarian hamsters had a positive effect on telomere length (Turbill et al., 2012). However, for species using torpor as a seasonal energy conservation strategy (e.g. edible dormice, garden dormice, and arctic ground squirrels; as do badgers), arousal and return to euthermia has been linked to telomere shortening; although this appears to be in proportion to the extent that body temperature must be re-warmed (Giroud et al., 2014; Hoelzl, Cornils, Smith, Moodley, & Ruf, 2016; Turbill et al., 2013; Turbill et al., 2012; Wilbur et al., 2019). We postulate that badgers use torpor and their ability to remain within thermally stable setts (Tsunoda, Newman, Buesching, Macdonald, & Kaneko, 2018) to try to mitigate RLTL shortening that would otherwise be incurred by the stresses of maintaining activity during winter, when food is scarce and thermal losses are high. We need careful analyses to explore this further, for example, comparing badgers in different regions that experience different degrees of winter severity, with a large longitudinal sample size to disentangle within- and between-individual effects. Importantly, we need to track which badgers go into torpor, for how long and how often, and then calculate how much energy is conserved. We also do not yet know to what extent torpor-arousal cycles may affect telomere shortening, where there is likely an optimal balance. In this regard predicted increases in weather variability (IPCC 2018) that may cause more frequent warm–cold winter episodes, could add to the allostatic load of badgers, causing accelerated RLTL shortening. Since positive within-individual changes in badger telomere length occur, that are greater than measurement error (van Lieshout et al., 2019), such seasonal patterns may explain some of the variability in telomere length patterns across life in badgers. However, other factors such as seasonal changes in leukocyte cell composition can also lead to apparent changes in telomere length (Beaulieu, Benoit, Abaga, Kappeler, & Charpentier, 2017), and require further investigation. For example, there is an increased proportion of neutrophils and lymphocytes that were lymphocytes from spring to autumn in badgers (van Lieshout et al., 2020a), and lymphocytes have shorter telomere lengths than neutrophils in humans and baboons (Baerlocher, Rice, Vulto, & Lansdorp, 2007; Kimura et al., 2010). Nonetheless, our findings also highlight the importance of controlling for seasonal effects when analysing telomere dynamics.

Cubs born into more energetically favourable springs (warm, rainy, and low rainfall variability) had longer early-life RLTL. These weather conditions present optimal soil conditions for earthworm surfacing, enhancing food supply (Kruuk, 1978; Newman et al., 2017). Dry conditions in spring have negative consequences for badger foraging success (Macdonald & Newman, 2002). However, while we found no effect of spring temperature variability on early-life RLTL, cubs experiencing lower daily rainfall variability in spring had longer early-life RLTL. Greater rainfall variability can reduce the predictability of food availability and impact foraging activity (Noonan et al., 2014). Higher levels of variability in rainfall may require individuals to modulate their energy trade-offs (Erikstad et al., 1998; Reid et al., 2003; Weimerskirch et al., 2001) and adopt a bet-hedging strategy until environmental conditions are favourable (Morris et al., 2008; Wilbur & Rudolf, 2006). The variability in spring rainfall and thus early-life conditions experienced shape life-history trade-offs, and since variability is likely to increase under current climate change (IPCC, 2018), this can impact ecological and individual resilience.

Our estimate of post-dependence social effects was positive but only marginally non-significant, suggesting that in groups with more independent cubs there may potentially be more food available per capita which permits faster growth and cell replication without inducing stress, hence facilitating longer early-life telomere length. This is in contrast with studies reporting that competition for food within litters and juvenile cohorts can cause telomere shortening (Boonekamp et al., 2014; Cram et al., 2017; Nettle et al., 2015). However, these studies were able to measure telomere length within the first month of life. In contrast, we were unable to sample individuals until at least 3 months of age, due to welfare legislation (Protection of Badgers Act, 1992), when the weakest cubs could have already succumbed, reducing group sizes. We therefore do not have a measure of the number of dependent cubs in a group and could only measure RLTL in the first year from three to twelve months of age; thus, we cannot test for social effects during the dependent period, including selective disappearance which may also lead to similar positive associations between the number of cubs and early-life RLTL.

We found that the association between early-life RLTL and cub survival probability was retained in the top 19 most plausible models and 46/70 plausible models when including early-life weather and social variables. This indicates that, in badgers, the association between early-life RLTL and survival is not solely driven by covariation between the early-life environment and early-life RLTL (i.e. physiological state). Early-life RLTL therefore reflects the physiological consequences of conditions experienced independent of the weather and social variables included in the models. Thus, telomere length can be used as a comprehensive measure of the environmental consequences for physiology and survival probability.

There was no significant association between same-sex adult group size and RLTL in early adulthood. While female–female reproductive competition occurs in badgers (Sharp & Clutton-Brock, 2011; Woodroffe & Macdonald, 1995), in polygynous species, theory predicts intra-sexual competition for mating opportunities to be stronger among males than females. In Wytham badgers, there is slight sexual dimorphism (Johnson & Macdonald, 2001) and slight male-biased mortality (Bright Ross et al., 2020). Reproductive skew is higher in sexually-mature males than females (Dugdale et al., 2008) and males with a higher body-condition index attain more reproductive success (Dugdale et al., 2011a). High levels of polygynandrous and repeated mounting behaviour may however reduce male–male aggression and infanticide from males (Dugdale et al., 2011a; Wolff & Macdonald, 2004). Secondly, cryptic female choice (i.e. delayed implantation, superfecundation and superfetation) may promote sperm competition and mask paternity, reduce pre-copulatory male–male competition (Birkhead & Pizzari, 2002). Finally, group size and/or density could be a poor metric for competition due to foraging niches and variation in sex-ratio. In line with this, we found no evidence that variation in telomere length is due to intra-sexual competition in early adulthood. Early-life telomere length may reflect the consequences of the weather conditions experienced, thus with little impact of social conditions in early adulthood on badgers. However, in bad quality years only females in good condition breed, whereas in good quality years breeding success is related to status (Woodroffe & Macdonald, 1995). We can therefore not exclude that there may only be female–female competition in good years. Additionally, early-adulthood male–male competition impacts on body mass senescence in a badger population at Woodchester Park (Beirne et al., 2015). While we detected no significant evidence of direct effects of early-adulthood intra-sexual competition on telomere length, there may be downstream effects on senescence.

In conclusion, we demonstrate the importance of accounting for seasonal variation when analysing telomere dynamics. We also evidence that early-life adversity is reflected in shorter early-life telomere lengths in badgers, where the physical environment (weather) but not the social environment predicts early-life telomere length. When accounting for these environmental effects, the positive association between early-life telomere length and survival probability remains. We conclude that telomere length in badgers is a biomarker that allows quantification of the physiological consequences of early-life environmental conditions and subsequent effects on cub survival probability.

Ethics

All work was approved by the University of Oxford's Animal Welfare and Ethical Review Board, ratified by the University of Leeds, and carried out under Natural England Licenses, currently 2017-27589-SCI-SCI and Home Office Licence (Animals, Scientific Procedures, Act, 1986) PPL: 30/3379.

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Author contributions

This study was conceived by S.H.J.v.L, A.B., H.L.D; Samples were collected by S.H.J.v.L., C.N., C.D.B., D.W.M. and H.L.D.; S.H.J.v.L. conducted laboratory work with input from T.B., environmental metrics were calculated by S.H.J.v.L, E.P.B, J.G.B. and statistical analyses were conducted by S.H.J.v.L with input from E.P.B and H.L.D; The paper was written by S.H.J.v.L and H.L.D. with extensive input from all authors. All authors gave final approval for publication.

Data accessibility

Data will be deposited in the Dryad Digital Repository upon acceptance.

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Tables and figures

Table 1: Parameter estimates and 95% confidence intervals of fixed effects from a mixed model and parametric bootstrap tests of the number of cubs in natal group, season and weather effects in spring on early-life (< 1 year old) relative leukocyte telomere length (Z-score) in European badgers (full model and with non-significant 2nd order effects removed). β = parameter estimate, S.E. = standard error, 95% CI = 95% confidence intervals; reference terms in brackets = reference level for factors. Significant parameter estimates (95% CI does not overlap zero) are in bold.

Parameter (reference level)	β	S.E.	95% CI
Intercept[†]	-0.010	0.093	-0.199 to 0.182
Number of cubs in natal group	0.091	0.048	-0.006 to 0.183
Season (Spring)			
Summer	0.105	0.106	-0.096 to 0.314
Autumn	0.003	0.193	-0.371 to 0.380
Winter	**-0.918**	**0.266**	**-1.441 to -0.391**
Mean temperature	-2.983	2.857	-8.515 to 2.730
Mean temperature^2	3.428	2.889	-2.307 to 9.025
Daily temperature variability	0.101	1.325	-2.513 to 2.652
Daily temperature variability^2	0.031	1.358	-2.564 to 2.719
Mean daily rainfall	**-1.496**	**0.645**	**-2.737 to -0.265**
Mean daily rainfall^2	**1.655**	**0.650**	**0.399 to 2.914**
Daily rainfall variability	**-3.406**	**1.351**	**-6.056 to -0.756**
Daily rainfall variability^2	**3.256**	**1.367**	**0.580 to 5.923**
Without non-significant 2nd order effects			
Intercept[††]	-0.002	0.088	-0.177 to 0.178
Number of cubs in natal group	0.087	0.047	-0.009 to 0.177
Season (Spring)			
Summer	0.103	0.105	-0.094 to 0.312
Autumn	-0.023	0.191	-0.397 to 0.347
Winter	**-0.939**	**0.264**	**-1.457 to -0.417**
Mean temperature	**0.409**	**0.073**	**0.269 to 0.554**
Daily temperature variability	0.144	0.078	-0.013 to 0.292
Mean daily rainfall	**-1.051**	**0.497**	**-2.020 to -0.093**
Mean daily rainfall^2	**1.190**	**0.491**	**0.252 to 2.148**
Daily rainfall variability	**-3.102**	**1.116**	**-5.288 to -0.857**
Daily rainfall variability^2	**2.992**	**1.110**	**0.762 to 5.169**

Random effect estimates (variance): [†]qPCR plate ($3.747*10^{-2}$), Row on qPCR plate ($3.882*10^{-3}$), Social group ($1.366*10^{-2}$), Cohort ($3.792*10^{-2}$), Residual ($7.752*10^{-1}$)

[††]qPCR plate ($3.916*10^{-2}$), Row on qPCR plate ($4.007*10^{-3}$), Social group ($1.487*10^{-2}$), Cohort ($5.046*10^{-2}$), Residual ($7.713*10^{-1}$)

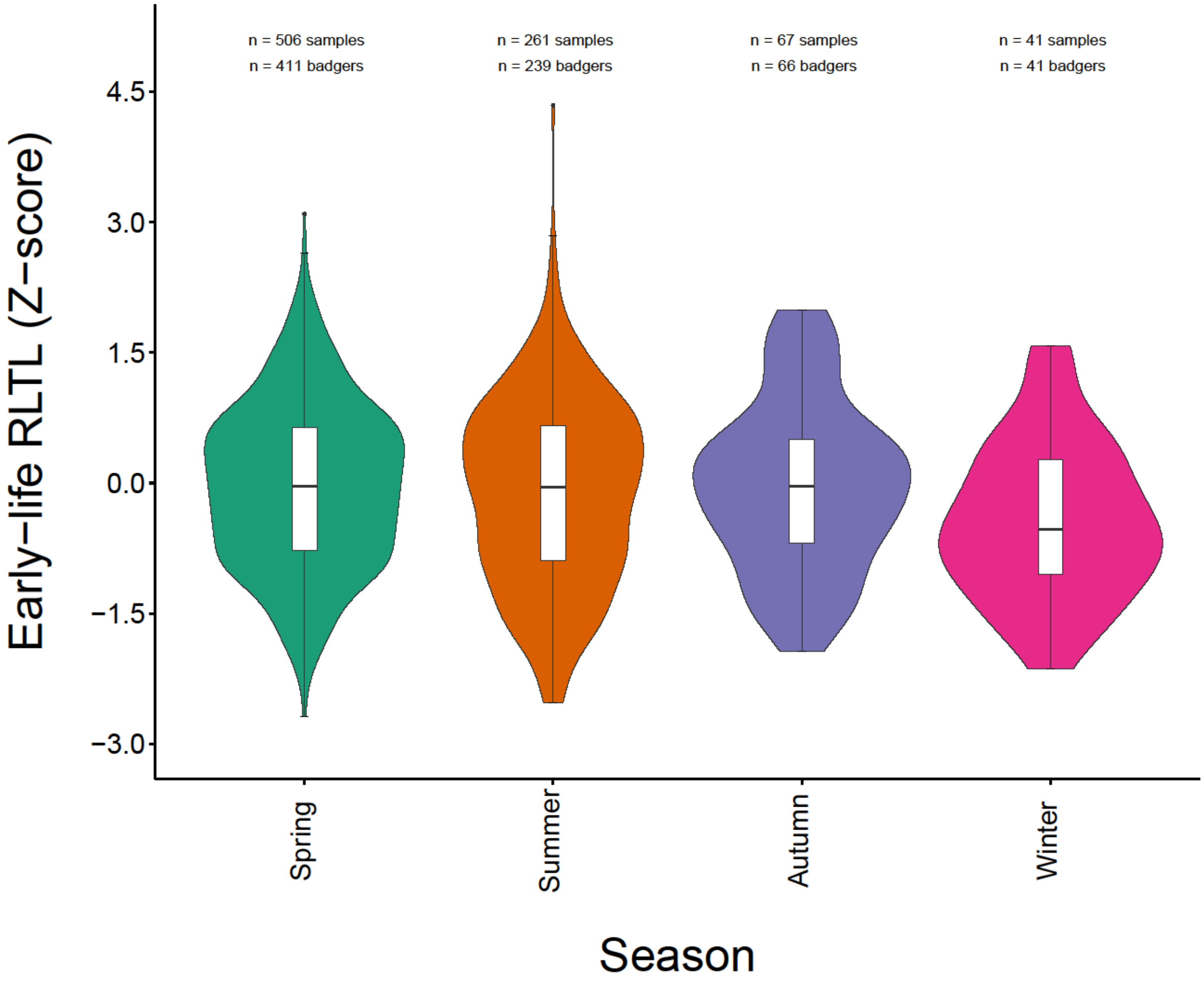

Figure 1: Variation in early-life relative leukocyte telomere length (RLTL) among seasons in European badgers. The data distributions and probability densities are shown (*n* = 875 samples; 562 badgers - the sum of badgers in the plot is >562 due to repeated measures). The line in the boxplot represents the median, with first and third quartiles, and whiskers represent 1.57 times the inter-quartile range.

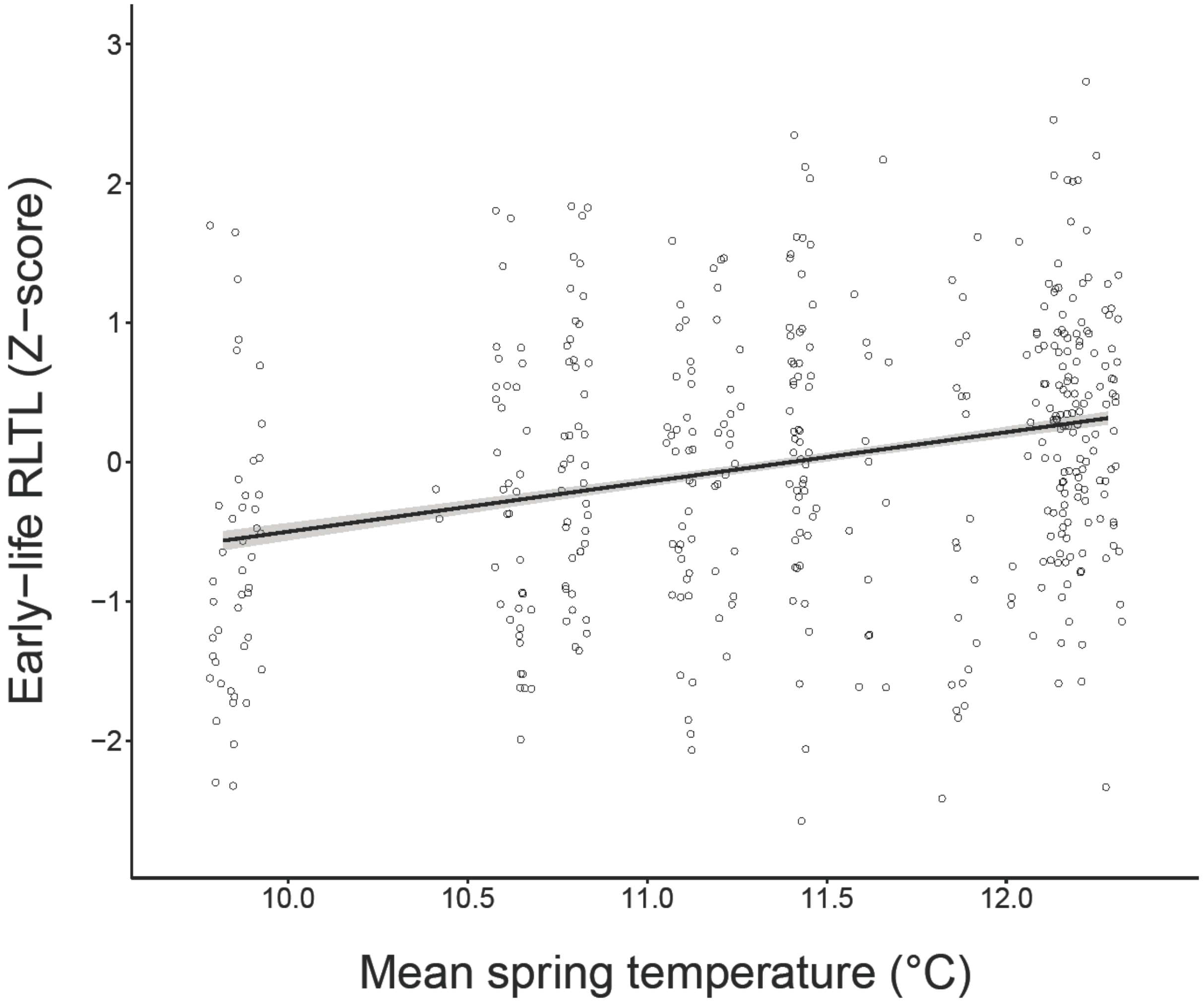

Figure 2: The association between mean spring temperature and early-life relative leukocyte telomere length (RLTL). Raw data points (n = 435 samples; 435 badgers) are shown, and jittered for clarity on the amount of data. The fitted line represents the regression from the mixed model, and the 95% confidence intervals as shaded areas.

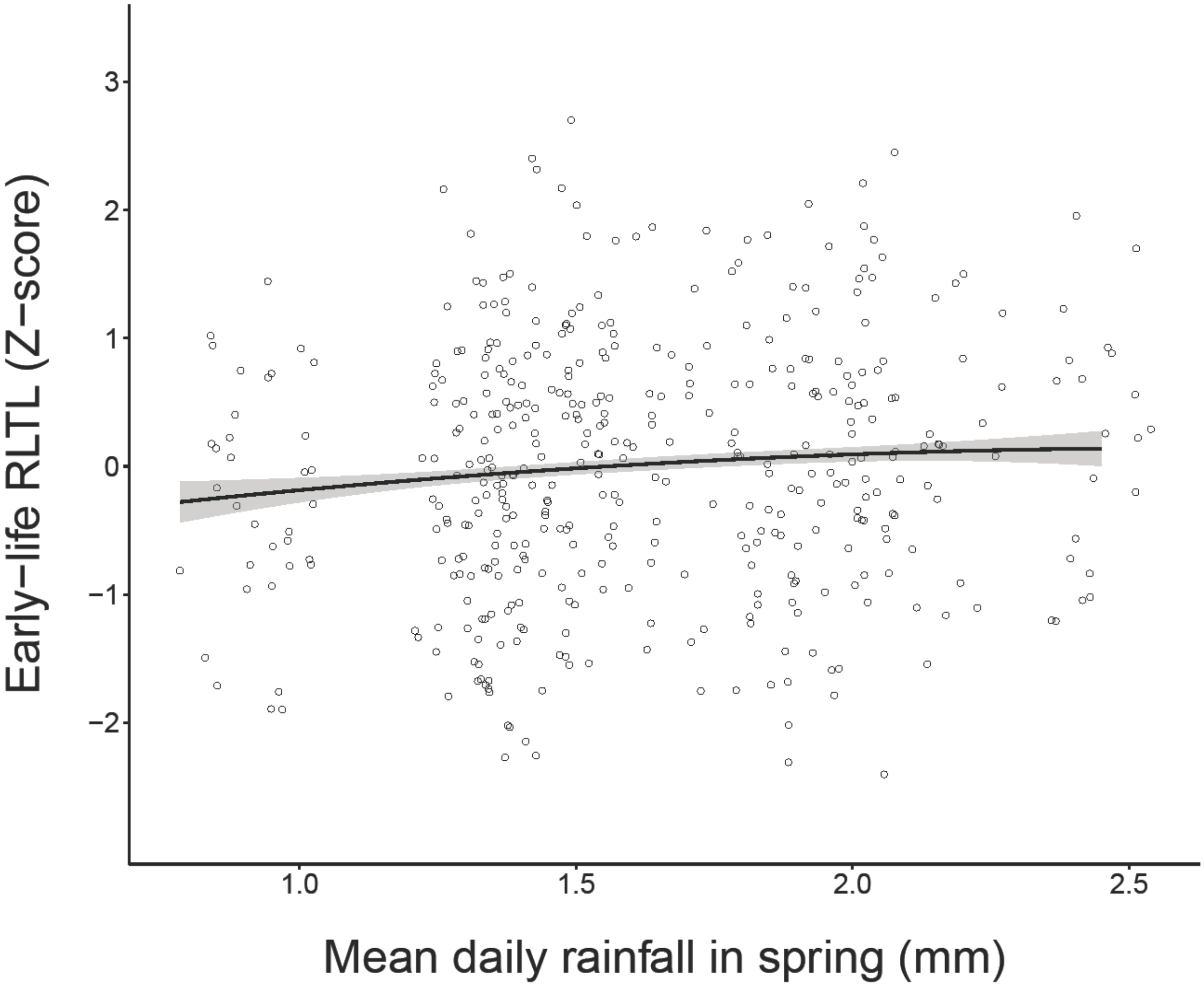

Figure 3: The association between mean daily rainfall in spring and early-life relative leukocyte telomere length (RLTL). Raw data points (n = 435 samples; 435 badgers) are shown, and jittered for clarity on the amount of data. The fitted line represents the quadratic regression from the mixed model, and the 95% confidence intervals as shaded areas.

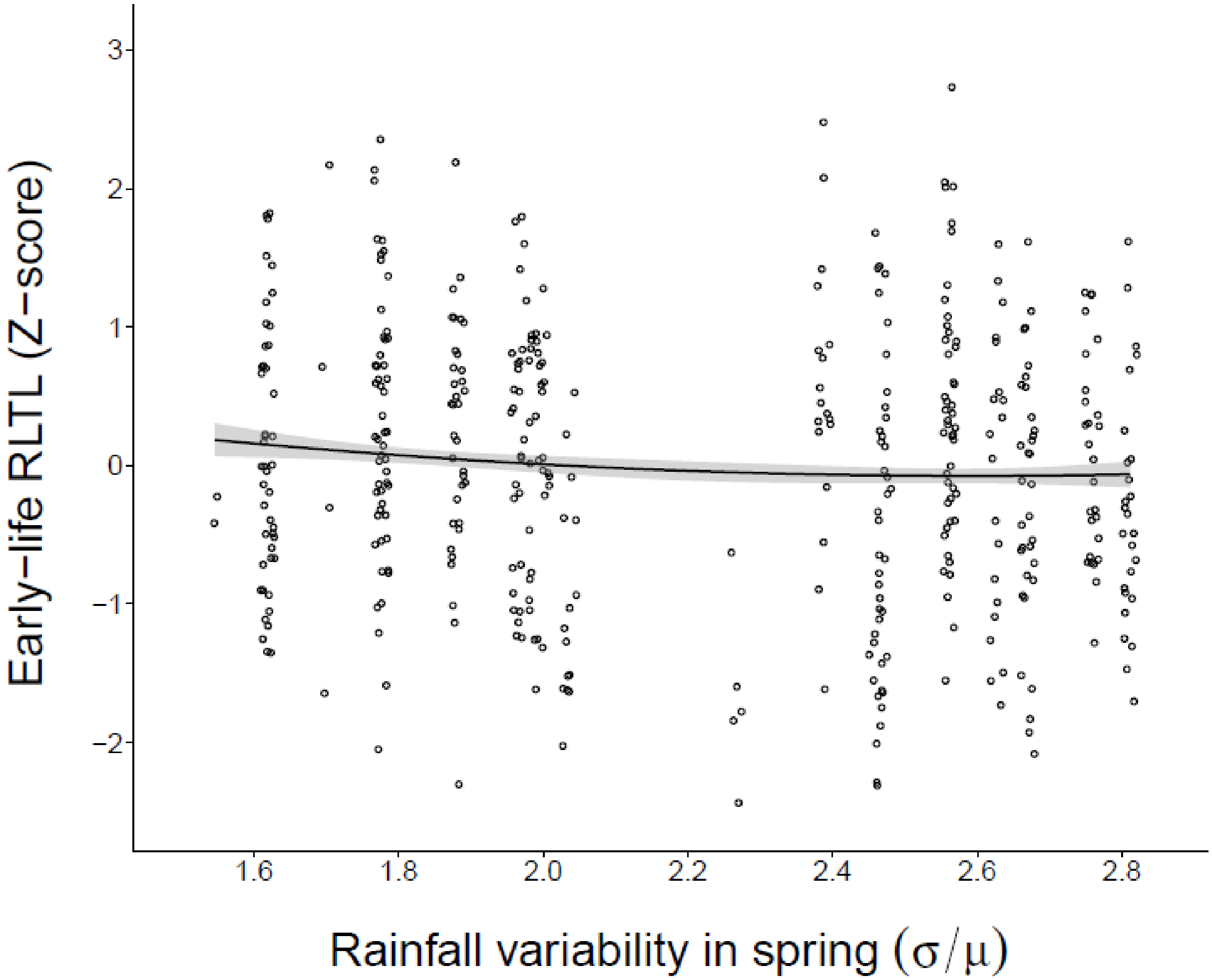

Figure 4: The association between the rainfall variability in spring and early-life relative leukocyte telomere length (RLTL). Raw data points (n = 435 samples; 435 badgers) are shown, and jittered for clarity on the amount of data. The fitted line represents the quadratic regression from the mixed model, and the 95% confidence intervals as shaded areas.

Supporting information

Early-life seasonal, weather and social effects on telomere length in a wild mammal

Sil H.J. van Lieshout, Elisa P. Badás, Julius G. Bright Ross, Amanda Bretman, Chris Newman, Christina D. Bueschi ng, Terry Burke, David W. Macdonald & Hannah L. Dugdale

This supplementary materials document includes supplementary tables and figures.

Table S1: Parameter estimates and 95% confidence intervals (from parametric bootstrapping) of fixed effects from a mixed model of age and seasonal effects on relative leukocyte telomere length (Z-score) in European badgers. β = parameter estimate, S.E. = standard error, 95% CI = 95% confidence intervals; reference terms in brackets = reference level for factors. A threshold age of 29 months was modelled, such that two parameter estimates are generated for ≤29 months and >29 to ≤36 months of age. Significant parameter estimates (95% CI does not overlap zero) are in bold.

Parameter (reference level)	β	S.E.	95% CI
Intercept	-0.018	0.149	-0.466 to 0.121
Age ≤29 months	-0.013	0.042	-0.096 to 0.065
Age >29 and ≤ 36 months	-0.002	0.035	-0.072 to 0.068
Age at last capture	0.050	0.035	-0.018 to 0.112
Season (Spring)			
Summer	0.041	0.076	-0.109 to 0.189
Autumn	0.105	0.125	-0.140 to 0.355
Winter	**-0.437**	**0.162**	**-0.764 to -0.117**

Random effect estimates (variance): qPCR plate ($5.502*10^{-2}$), Row on qPCR plate ($3.018*10^{-3}$), Social group ($7.384*10^{-3}$), Cohort ($4.959*10^{-2}$), Year ($3.249*10^{-1}$), Individual ID ($<1.000*10^{-12}$), Residual ($7.803*10^{-1}$)

Table S2: Parameter estimates and 95% confidence intervals (from parametric bootstrapping) of fixed effects from a mixed model of age effects and both within-individual ($\beta_{W;}$ the mean season value for each individual subtracted from the season value for each RLTL measurement) and between-individual (β_B; the mean season value for each individual) seasonal effects on relative leukocyte telomere length (Z-score) in European badgers. β = parameter estimate, S.E. = standard error, 95% CI = 95% confidence intervals. A threshold age of 29 months was modelled, such that two parameter estimates are generated for ≤29 months and >29 to ≤36 months of age. Significant parameter estimates (95% CI does not overlap zero) are in bold.

Parameter (reference level)	**β**	**S.E.**	**95% CI**
Intercept	-0.112	0.100	-0.308 to 0.081
Age ≤29 months	0.055	0.050	-0.041 to 0.147
Age >29 and ≤36 months	0.032	0.045	-0.054 to 0.118
Age at last capture	0.077	0.044	-0.009 to 0.160
Season (β_W)	**-0.103**	**0.040**	**-0.182 to -0.024**
Season (β_B)	**-0.109**	**0.045**	**-0.198 to -0.020**

Random effect estimates (variance): qPCR plate ($1.383*10^{-2}$), Row on qPCR plate ($1.450*10^{-3}$), Social group ($<1.000*10^{-12}$), Cohort ($7.398*10^{-2}$), Year ($6.471*10^{-2}$), Individual ID ($<1.000*10^{-12}$), Residual ($7.998*10^{-1}$)

Table S3: Parameter estimates and 95% confidence intervals of fixed effects from a mixed model and parametric bootstrap tests of age effects and within-individual ($\beta_{W;}$ the mean season value for each individual subtracted from the season value for each RLTL measurement) and the difference in between- and within-individual (β_B - β_W) seasonal effects on relative leukocyte telomere length (Z-score) in European badgers. β = parameter estimate, S.E. = standard error, 95% CI = 95% confidence intervals. A threshold age of 29 months was modelled, such that two parameter estimates are generated for ≤ 29 months and >29 to ≤36 months of age. Significant parameter estimates (95% CI does not overlap zero) are in bold.

Parameter (reference level)	β	S.E.	95% CI
Intercept	-0.058	0.103	-0.256 to 0.143
Age ≤29 months	0.055	0.050	-0.041 to 0.147
Age >29 and ≤36 months	0.032	0.045	-0.054 to 0.118
Age at last capture	0.077	0.044	-0.009 to 0.160
Season (β_W)	**-0.718**	**0.277**	**-1.265 to -0.169**
Season (β_B - β_W)	0.049	0.073	-0.094 to 0.194

Random effect estimates (variance): qPCR plate ($1.383*10^{-2}$), Row on qPCR plate ($1.450*10^{-3}$), Social group ($<1.000*10^{-12}$), Cohort ($7.398*10^{-2}$), Year ($6.471*10^{-2}$), Individual ID ($<1.000*10^{-12}$), Residual ($7.998*10^{-1}$)

Table S4: Parameter estimates and 95% confidence intervals of fixed effects from a mixed model and parametric bootstrap tests of the direction of the effect on relative leukocyte telomere length (Z-score) from a cross-sectional model and when separating within- from between-individual effects from spring to winter in European badgers. β = parameter estimate, S.E. = standard error, 95% CI = 95% confidence intervals. Significant parameter estimates (95% CI does not overlap zero) are in bold.

Parameter (reference level)	**β**	**S.E.**	**95% CI**
Intercept[†]	-0.041	0.129	-0.289 to 0.214
Age ≤29 months	0.289	0.122	0.050 to 0.524
Age at last capture	0.100	0.067	-0.027 to 0.234
Season (Spring)			
Winter	**-1.187**	**0.381**	**-1.929 to -0.426**

Intercept[††]	-0.185	0.120	-0.413 to 0.055
Age ≤29 months	0.296	0.124	0.055 to 0.534
Age at last capture	0.092	0.067	-0.036 to 0.226
Season (β_W)	**-0.267**	**0.086**	**-0.436 to -0.097**
Season (β_B)	**-0.294**	**0.109**	**-0.509 to -0.080**

Random effect estimates (variance):

[†]qPCR plate ($<1.000*10^{-12}$), Row on qPCR plate ($8.360*10^{-3}$), Social group ($3.580*10^{-2}$), Cohort ($3.326*10^{-4}$), Year ($1.259*10^{-1}$), Individual ID ($<1.000*10^{-12}$), Residual ($7.518*10^{-1}$)

[††]qPCR plate ($<1.000*10^{-12}$), Row on qPCR plate ($7.587*10^{-3}$), Social group ($3.547*10^{-2}$), Cohort ($1.596*10^{-3}$), Year ($1.251*10^{-1}$), Individual ID ($<1.000*10^{-12}$), Residual ($7.513*10^{-1}$)

Table S5: Parameter estimates and 95% confidence intervals of fixed effects from a mixed model and parametric bootstrap tests of the direction of the effect on relative leukocyte telomere length (Z-score) from a cross-sectional model and when separating within- from between-individual effects from winter to spring in European badgers. β = parameter estimate, S.E. = standard error, 95% CI = 95% confidence intervals. Significant parameter estimates (95% CI does not overlap zero) are in bold.

Parameter (reference level)	**β**	**S.E.**	**95% CI**
Intercept†	-0.021	0.152	-0.338 to 0.264
Age ≤29 months	0.160	0.146	-0.126 to 0.455
Age at last capture	-0.127	0.111	-0.342 to 0.083
Season (Spring)			
Winter	-0.391	0.328	-1.002 to 0.254
Intercept††	-0.113	0.127	-0.388 to 0.133
Age ≤29 months	-0.103	0.211	-0.510 to 0.323
Age at last capture	-0.102	0.112	-0.320 to 0.110
Season (β_W)	**-0.391**	**0.197**	**-0.785 to -0.014**
Season (β_B)	-0.181	0.125	-0.413 to 0.060

Random effect estimates (variance):

†qPCR plate ($8.295*10^{-2}$), Row on qPCR plate ($<1.000*10^{-12}$), Social group ($<1.000*10^{-12}$), Cohort ($3.434*10^{-2}$), Year ($<1.000*10^{-12}$), Individual ID ($<1.000*10^{-12}$), Residual ($7.408*10^{-1}$)

††qPCR plate ($5.646*10^{-2}$), Row on qPCR plate ($<1.000*10^{-12}$), Social group ($<1.000*10^{-12}$), Cohort ($3.569*10^{-2}$), Year ($<1.000*10^{-12}$), Individual ID ($<1.000*10^{-12}$), Residual ($7.384*10^{-1}$)

Table S6: Parameter estimates and 95% confidence intervals of fixed effects from a mixed model and parametric bootstrap tests of the direction of the effect on relative leukocyte telomere length (Z-score) to test whether slopes differ between within- and between-individual slopes from spring to winter and winter to spring in European badgers. β = parameter estimate, S.E. = standard error, 95% CI = 95% confidence intervals. Significant parameter estimates (95% CI does not overlap zero) are in bold.

Parameter (reference level)	**β**	**S.E.**	**95% CI**
Intercept[†]	-0.001	0.134	-0.258 to 0.264
Age ≤29 months	0.296	0.124	0.054 to 0.533
Age at last capture	0.092	0.067	-0.036 to 0.226
Season (β_W)	**-1.535**	**0.496**	**-2.510 to -0.561**
Season (β_B - β_W)	0.129	0.116	-0.098 to 0.353
Intercept[††]	0.387	0.284	-0.191 to 0.943
Age ≤29 months	-0.112	0.210	-0.509 to 0.303
Age at last capture	-0.101	0.111	-0.318 to 0.110
Season (β_W)	**-2.095**	**1.039**	**-4.137 to -0.012**
Season (β_B - β_W)	0.622	0.363	-0.102 to 1.345

Random effect estimates (variance):

[†]qPCR plate (<1.000*10^{-12}), Row on qPCR plate (7.587*10^{-3}), Social group (3.547*10^{-2}), Cohort (1.596*10^{-3}), Year (1.251*10^{-1}), Individual ID (<1.000*10^{-12}), Residual (7.513*10^{-1})

[††]qPCR plate (5.646*10^{-2}), Row on qPCR plate (<1.000*10^{-12}), Social group (<1.000*10^{-12}), Cohort (3.569*10^{-2}), Year (<1.000*10^{-12}), Individual ID (<1.000*10^{-12}), Residual (7.384*10^{-1})

Table S7: Parameter estimates and 95% confidence intervals of fixed effects from a mixed model and parametric bootstrap tests of the number of adults in natal group, season and weather effects in spring on early-life (< 1 year old) relative leukocyte telomere length (Z-score) in European badgers (full model and with non-significant 2nd order effects removed). β = parameter estimate, S.E. = standard error, 95% CI = 95% confidence intervals; reference terms in brackets = reference level for factors. Significant parameter estimates (95% CI does not overlap zero) are in bold.

Parameter (reference level)	**β**	**S.E.**	**95% CI**
Intercept[†]	-0.023	0.092	-0.207 to 0.167
Number of adults in natal group	-0.011	0.048	-0.106 to 0.085
Season (Spring)			
Summer	0.128	0.106	-0.072 to 0.336
Autumn	0.007	0.194	-0.373 to 0.385
Winter	**-0.963**	**0.266**	**-1.485 to -0.430**
Mean temperature	-2.578	2.890	-8.209 to 3.227
Mean temperature^2	3.003	2.921	-2.809 to 8.679
Daily temperature variability	-0.303	1.326	-2.900 to 2.259
Daily temperature variability^2	0.444	1.360	-2.185 to 3.079
Mean daily rainfall	**-1.360**	**0.650**	**-2.616 to -0.125**
Mean daily rainfall^2	**1.515**	**0.654**	**0.271 to 2.769**
Daily rainfall variability	**-3.323**	**1.373**	**-6.031 to -0.630**
Daily rainfall variability^2	**3.159**	**1.388**	**0.459 to 5.874**
Without non-significant 2nd order effects			
Intercept[††]	-0.015	0.085	-0.185 to 0.159
Number of adults in natal group	-0.014	0.048	-0.108 to 0.082
Season (Spring)			
Summer	0.124	0.105	-0.074 to 0.331
Autumn	-0.024	0.192	-0.402 to 0.347
Winter	**-0.982**	**0.264**	**-1.499 to -0.458**
Mean temperature	**0.405**	**0.073**	**0.265 to 0.549**
Daily temperature variability	0.141	0.077	-0.014 to 0.289
Mean daily rainfall	**-0.968**	**0.494**	**-1.927 to -0.007**
Mean daily rainfall^2	**1.107**	**0.487**	**0.157 to 2.051**
Daily rainfall variability	**-3.228**	**1.118**	**-5.450 to -0.971**
Daily rainfall variability^2	**3.118**	**1.112**	**0.885 to 5.325**

Random effect estimates (variance):

[†] qPCR plate ($3.692*10^{-2}$), Row on qPCR plate ($3.468*10^{-3}$), Social group ($8.673*10^{-3}$), Cohort ($5.277*10^{-2}$), Residual ($7.835*10^{-1}$)

[††] qPCR plate ($3.534*10^{-2}$), Row on qPCR plate ($3.233*10^{-3}$), Social group ($7.534*10^{-3}$), Cohort ($3.669*10^{-2}$), Residual ($7.880*10^{-1}$)

Table S8: Parameter estimates and 95% confidence intervals of fixed effects from a mixed model and parametric bootstrap tests of the total number of individuals in natal group, season and weather effects in spring on early-life (< 1 year old) relative leukocyte telomere length (Z-score) in European badgers (full model and with non-significant 2nd order effects removed). β = parameter estimate, S.E. = standard error, 95% CI = 95% confidence intervals; reference terms in brackets = reference level for factors. Significant parameter estimates (95% CI does not overlap zero) are in bold.

Parameter (reference level)	**β**	**S.E.**	**95% CI**
Intercept[†]	-0.017	0.092	-0.203 to 0.175
Number of individuals in natal group	0.025	0.049	-0.071 to 0.121
Season (Spring)			
Summer	0.124	0.106	-0.077 to 0.333
Autumn	0.012	0.194	-0.365 to 0.392
Winter	**-0.959**	**0.266**	**-1.481 to -0.429**
Mean temperature	-2.674	2.879	-8.247 to 3.094
Mean temperature^2	3.103	2.910	-2.700 to 8.777
Daily temperature variability	-0.309	1.320	-2.880 to 2.248
Daily temperature variability^2	0.448	1.353	-2.191 to 3.049
Mean daily rainfall	**-1.360**	**0.646**	**-2.599 to -0.126**
Mean daily rainfall^2	**1.518**	**0.651**	**0.272 to 2.771**
Daily rainfall variability	**-3.207**	**1.363**	**-5.899 to -0.518**
Daily rainfall variabilityl^2	**3.041**	**1.378**	**0.326 to 5.741**
Without non-significant 2nd order effects			
Intercept[††]	-0.010	0.086	-0.183 to 0.167
Number of individuals in natal group	0.021	0.049	-0.074 to 0.117
Season (Spring)			
Summer	0.119	0.105	-0.080 to 0.326
Autumn	-0.019	0.192	-0.397 to 0.351
Winter	**-0.979**	**0.264**	**-1.498 to -0.456**
Mean temperature	**0.405**	**0.073**	**0.265 to 0.548**
Daily temperature variability	0.140	0.078	-0.015 to 0.287
Mean daily rainfall	-0.956	0.493	-1.914 to 0.004
Mean daily rainfall^2	**1.097**	**0.486**	**0.148 to 2.044**
Daily rainfall variability	**-3.107**	**1.118**	**-5.319 to -0.831**
Daily rainfall variability^2	**2.997**	**1.112**	**0.755 to 5.228**

Random effect estimates (variance):

[†] qPCR plate ($3.700*10^{-2}$), Row on qPCR plate ($2.938*10^{-3}$), Social group ($1.374*10^{-2}$), Cohort ($5.190*10^{-2}$), Residual ($7.800*10^{-1}$)

[††] qPCR plate ($3.549*10^{-2}$), Row on qPCR plate ($2.713*10^{-3}$), Social group ($1.249*10^{-2}$), Cohort ($3.684*10^{-2}$), Residual ($7.843*10^{-1}$)

Table S9: Parameter estimates and 95% confidence intervals of fixed effects from a mixed model and parametric bootstrap tests of the number of cubs and number of adults in the natal group, season and weather effects in spring on early-life (< 1 year old) relative leukocyte telomere length (Z-score) in European badgers (full model and with non-significant 2nd order effects removed). β = parameter estimate, S.E. = standard error, 95% CI = 95% confidence intervals; reference terms in brackets = reference level for factors. Significant parameter estimates (95% CI does not overlap zero) are in bold.

Parameter (reference level)	**β**	**S.E.**	**95% CI**
Intercept[†]	-0.012	0.093	-0.200 to 0.180
Number of cubs in natal group	0.094	0.048	-0.004 to 0.188
Number of adults in natal group	-0.021	0.050	-0.120 to 0.076
Season (Spring)			
Summer	0.105	0.106	-0.097 to 0.315
Autumn	0.001	0.193	-0.377 to 0.377
Winter	**-0.916**	**0.266**	**-1.440 to -0.389**
Mean temperature	-2.968	2.868	-8.541 to 2.756
Mean temperature^2	3.414	2.900	-2.331 to 9.069
Daily temperature variability	0.138	1.332	-2.507 to 2.693
Daily temperature variability^2	-0.005	1.365	-2.619 to 2.695
Mean daily rainfall	**-1.511**	**0.649**	**-2.759 to -0.275**
Mean daily rainfall^2	**1.668**	**0.654**	**0.424 to 2.929**
Daily rainfall variability	**-3.486**	**1.367**	**-6.179 to -0.819**
Daily rainfall variability^2	**3.338**	**1.383**	**0.637 to 6.053**
Without non-significant 2nd order effects			
Intercept[††]	-0.004	0.088	-0.179 to 0.175
Number of cubs in natal group	0.089	0.048	-0.007 to 0.180
Number of adults in natal group	-0.022	0.049	-0.120 to 0.076
Season (Spring)			
Summer	0.104	0.106	-0.093 to 0.313
Autumn	-0.025	0.191	-0.399 to 0.344
Winter	**-0.938**	**0.264**	**-1.455 to -0.415**
Mean temperature	**0.409**	**0.073**	**0.268 to 0.554**
Daily temperature variability	0.146	0.078	-0.013 to 0.295
Mean daily rainfall	**-1.068**	**0.500**	**-2.039 to -0.108**
Mean daily rainfall^2	**1.206**	**0.494**	**0.257 to 2.156**
Daily rainfall variability	**-3.175**	**1.127**	**-5.408 to -0.914**
Daily rainfall variability^2	**3.066**	**1.121**	**0.807 to 5.298**

Random effect estimates (variance):

[†] qPCR plate ($3.937*10^{-2}$), Row on qPCR plate ($4.485*10^{-3}$), Social group ($1.320*10^{-2}$), Cohort ($5.110*10^{-2}$), Residual ($7.735*10^{-1}$)

[††] qPCR plate ($3.762*10^{-2}$), Row on qPCR plate ($4.395*10^{-3}$), Social group ($1.168*10^{-2}$), Cohort ($3.834*10^{-2}$), Residual ($7.777*10^{-1}$)

Table S10: Parameter estimates and 95% confidence intervals of fixed effects from a mixed model and parametric bootstrap tests of the number of cubs and total number of individuals in the natal group, season and weather effects in spring on early-life (< 1 year old) relative leukocyte telomere length (Z-score) in European badgers (full model and with non-significant 2nd order effects removed). β = parameter estimate, S.E. = standard error, 95% CI = 95% confidence intervals; reference terms in brackets = reference level for factors. Significant parameter estimates (95% CI does not overlap zero) are in bold.

Parameter (reference level)	**β**	**S.E.**	**95% CI**
Intercept[†]	-0.012	0.093	-0.200 to 0.180
Number of cubs in natal group	0.101	0.054	-0.007 to 0.205
Number of individuals in natal group	-0.022	0.055	-0.131 to 0.086
Season (Spring)			
Summer	0.105	0.106	-0.096 to 0.315
Autumn	0.001	0.193	-0.376 to 0.377
Winter	**-0.916**	**0.266**	**-1.440 to -0.389**
Mean temperature	-2.956	2.866	-8.532 to 2.773
Mean temperature^2	3.401	2.897	-2.359 to 9.068
Daily temperature variability	0.135	1.331	-2.501 to 2.688
Daily temperature variability^2	-0.002	1.364	-2.614 to 2.688
Mean daily rainfall	**-1.506**	**0.648**	**-2.747 to -0.268**
Mean daily rainfall^2	**1.663**	**0.652**	**0.414 to 2.928**
Daily rainfall variability	**-3.482**	**1.366**	**-6.174 to -0.814**
Daily rainfall variability^2	**3.334**	**1.382**	**0.653 to 6.037**
Without non-significant 2nd order effects			
Intercept[††]	-0.004	0.088	-0.179 to 0.176
Number of cubs in natal group	0.097	0.053	-0.008 to 0.199
Number of individuals in natal group	-0.025	0.054	-0.132 to 0.084
Season (Spring)			
Summer	0.104	0.105	-0.093 to 0.313
Autumn	-0.025	0.191	-0.399 to 0.344
Winter	**-0.938**	**0.264**	**-1.456 to -0.417**
Mean temperature	**0.409**	**0.073**	**0.268 to 0.554**
Daily temperature variability	0.146	0.078	-0.013 to 0.295
Mean daily rainfall	**-1.065**	**0.499**	**-2.034 to -0.108**
Mean daily rainfall^2	**1.202**	**0.492**	**0.257 to 2.151**
Daily rainfall variability	**-3.176**	**1.126**	**-5.415 to -0.911**
Daily rainfall variability^2	**3.066**	**1.120**	**0.815 to 5.290**

Random effect estimates (variance):

[†] qPCR plate ($3.933*10^{-2}$), Row on qPCR plate ($4.435*10^{-3}$), Social group ($1.345*10^{-2}$), Cohort ($5.093*10^{-2}$), Residual ($7.734*10^{-1}$)

[††] qPCR plate ($3.758*10^{-2}$), Row on qPCR plate ($4.360*10^{-3}$), Social group ($1.184*10^{-2}$), Cohort ($3.810*10^{-2}$), Residual ($7.777*10^{-1}$)

Table S11: Parameter estimates and 95% confidence intervals of fixed effects from a mixed model and parametric bootstrap tests of the association between relative leukocyte telomere length and survival to adulthood (>1 year old) in European badgers. β = parameter estimate, S.E. = standard error, 95% CI = 95% confidence interval. Significant parameter estimates (95% CI does not overlap zero) are in bold.

Parameter (reference level)	**β**	**S.E.**	**95% CI**
Intercept	1.381	0.296	0.824 to 1.986
Relative leukocyte telomere length	**0.371**	**0.146**	**0.102 to 0.681**

Random effect estimates (variance): qPCR plate ($<1.000*10^{-12}$), Row on qPCR plate ($1.550*10^{-1}$), Social group ($2.274*10^{-1}$), Cohort ($5.930*10^{-1}$)

Table S12: Parameter estimates and 95% confidence intervals of fixed effects from a mixed model and parametric bootstrap tests of the association between relative leukocyte telomere length, winter weather conditions and survival to adulthood (>1 year old) in European badgers (full model and with non-significant 2nd order effects removed). β = parameter estimate, S.E. = standard error, 95% CI = 95% confidence interval. Significant parameter estimates (95% CI does not overlap zero) are in bold.

Parameter (reference level)	**β**	**S.E.**	**95% CI**
Intercept[†]	1.627	0.275	1.131 to 2.189
Relative leukocyte telomere length	**0.364**	**0.155**	**0.074 to 0.683**
Number of cubs in natal group	0.105	0.147	-0.179 to 0.397
Mean temperature	**5.183**	**1.353**	**2.597 to 7.926**
Mean temperature^2	**-4.842**	**1.294**	**-7.465 to -2.335**
Daily temperature variability	-3.497	2.120	-8.126 to 0.315
Daily temperature variability^2	3.897	2.195	-0.005 to 8.693
Mean daily rainfall	-0.804	1.031	-2.922 to 1.204
Mean daily rainfall^2	1.853	1.199	-0.370 to 4.424
Daily rainfall variability	3.910	2.134	-0.167 to 8.199
Daily rainfall variability^2	**-4.607**	**2.072**	**-8.828 to -0.665**
Without non-significant 2nd order effects			
Intercept[††]	1.548	0.247	1.095 to 2.047
Relative leukocyte telomere length	**0.304**	**0.149**	**0.014 to 0.611**
Number of cubs in natal group	0.089	0.144	-0.186 to 0.371
Mean temperature	**5.192**	**1.333**	**2.627 to 7.855**
Mean temperature^2	**-4.918**	**1.283**	**-7.478 to -2.424**
Daily temperature variability	0.247	0.141	-0.032 to 0.534
Mean daily rainfall	**0.919**	**0.359**	**0.258 to 1.658**
Daily rainfall variability	**3.700**	**1.805**	**0.220 to 7.389**
Daily rainfall variability^2	**-4.199**	**1.741**	**-7.722 to -0.890**

Random effect estimates (variance):

[†] qPCR plate (2.672*10^{-2}), Row on qPCR plate (1.933*10^{-1}), Social group (2.682*10^{-1}), Cohort (<1.000*10^{-12})

[††] qPCR plate (2.092*10^{-2}), Row on qPCR plate (1.570*10^{-1}), Social group (2.228*10^{-1}), Cohort (<1.000*10^{-12})

Table S13: Comparison of models describing the relationship between early-life relative leukocyte telomere length (RLTL) and cub survival probability with qPCR plate, cohort, social group, and row on qPCR plate as random effects. Models were ordered and numbered by AICc, and the difference from the top model (lowest AICc) is stated in the column termed ‘ΔAICc’, with only models within ΔAICc <7 (omitting 254 models). CV_R = Daily rainfall variability, μ_R = mean daily rainfall, μ_T = Mean temperature, σ_T = Daily temperature variability.

Model	Intercept	CV_R	$(CV_R)^2$	μ_R	$(\mu_R)^2$	μ_T	$(\mu_T)^2$	σ_T	$(\sigma_T)^2$	N cubs in natal group	RLTL	Degrees of freedom	AICc	ΔAICc
1	1.530405	3.804	-4.447	-0.736	1.749	4.985	-4.672	-3.051	3.405		0.349	14	444.508	0.000
2	1.502905	2.583	-3.195	0.740		4.963	-4.701	-2.452	2.755		0.333	13	445.460	0.951
3	1.478782	3.603	-4.079	0.901		5.069	-4.809	0.225			0.297	12	445.470	0.961
4	1.494113	4.652	-5.132	-0.238	1.364	5.067	-4.772	0.250			0.305	13	445.528	1.019
5	1.48041	-0.780				3.479	-3.392	-3.529	3.865		0.317	11	445.860	1.351
6	1.55029	3.695	-4.355	-0.719	1.706	4.985	-4.670	-3.230	3.602	0.102	0.343	15	445.966	1.458
7	1.484775	3.407	-3.832	0.883		5.304	-5.094				0.294	11	446.327	1.819
8	1.502782	-0.772		0.142		3.787	-3.688	-3.590	3.907		0.298	12	446.754	2.246
9	1.525709	2.485	-3.118	0.719		4.964	-4.697	-2.666	2.989	0.115	0.325	14	446.765	2.257
10	1.5052	-0.795				3.509	-3.413	-3.719	4.077	0.133	0.306	12	446.907	2.399
11	1.477048	-0.559				3.387	-3.462				0.300	9	446.992	2.484
12	1.492618	3.573	-4.057	0.891		5.067	-4.805	0.235		0.086	0.289	13	447.064	2.556
13	1.498554	4.187	-4.614	-0.046	1.100	5.295	-5.060				0.298	12	447.076	2.567
14	1.504825	4.596	-5.081	-0.212	1.321	5.063	-4.768	0.258		0.071	0.298	14	447.254	2.745
15	1.466726	-0.618				3.167	-3.198	0.192			0.287	10	447.689	3.181
16	1.486358	-0.975	0.188			3.487	-3.407	-3.611	3.943		0.310	12	447.778	3.270
17	1.526841	-0.788		0.135		3.812	-3.705	-3.778	4.115	0.125	0.290	13	447.919	3.410
18	1.510884	-0.804		-0.602	0.753	3.581	-3.495	-3.988	4.323		0.299	13	448.042	3.533
19	1.494856	3.379	-3.809	0.876		5.312	-5.103			0.060	0.287	12	448.129	3.621
20	1.45436	2.785	-3.295	0.792		4.970	-4.774	0.210				11	448.216	3.708
21	1.471589	3.771	-4.285	-0.279	1.282	4.977	-4.748	0.236				12	448.478	3.970
22	1.488154	-0.559		0.127		3.580	-3.634				0.279	10	448.514	4.005
23	1.489415	-0.562				3.412	-3.487			0.074	0.291	10	448.707	4.199
24	1.495764	3.081	-3.716	-0.665	1.574	4.912	-4.677	-2.206	2.518			13	448.715	4.207
25	1.457918	2.647	-3.110	0.790		5.219	-5.057					10	448.768	4.259
26	1.510055	-0.959	0.158			3.517	-3.426	-3.787	4.141	0.131	0.301	13	448.850	4.342
27	1.473984	-0.400	-0.154			3.409	-3.479				0.306	10	448.962	4.453
28	1.505383	4.143	-4.573	-0.022	1.066	5.299	-5.066			0.045	0.293	13	448.968	4.460
29	1.409293	-0.398									0.343	7	448.996	4.488
30	1.468109	2.021	-2.625	0.671		4.891	-4.699	-1.663	1.925			12	449.159	4.651
31	1.476085	-0.616		0.117		3.364	-3.374	0.184			0.263	11	449.198	4.689
32	1.480993	-0.626				3.180	-3.206	0.205		0.094	0.274	11	449.224	4.715
33	1.533084	-0.818		-0.582	0.726	3.606	-3.511	-4.155	4.510	0.121	0.291	14	449.260	4.752
34	1.44201	-0.748				3.538	-3.496	-2.564	2.864			10	449.330	4.822
35	1.474	2.777	-3.295	0.782		4.974	-4.773	0.223		0.115		12	449.474	4.966
36	1.412073	-0.489						0.237			0.336	8	449.487	4.979
37	1.474211	-0.741		0.179		3.920	-3.860	-2.688	2.964			11	449.517	5.009
38	1.462442	-0.390	-0.221			3.189	-3.212	0.198			0.296	11	449.615	5.107
39	1.471163	3.348	-3.812	-0.045	0.989	5.214	-5.032					11	449.669	5.160
40	1.435607	-0.574				3.524	-3.599					8	449.669	5.161
41	1.519961	2.946	-3.602	-0.641	1.516	4.909	-4.669	-2.441	2.774	0.127		14	449.846	5.337
42	1.486554	3.707	-4.227	-0.235	1.216	4.969	-4.739	0.247		0.100		13	449.918	5.409
43	1.474649	-0.768				3.586	-3.530	-2.828	3.154	0.158		11	449.937	5.428
44	1.42699	-0.635				3.273	-3.300	0.202				9	449.975	5.467

45	1.496639	1.916	-2.544	0.647		4.898	-4.699	-1.940	2.227	0.139		13	450.110	5.601
46	1.418713	-0.590						-2.478	2.799		0.354	9	450.171	5.663
47	1.499513	-0.562		0.123		3.599	-3.652			0.070	0.270	11	450.253	5.745
48	1.475098	2.642	-3.110	0.786		5.247	-5.081			0.089		11	450.312	5.804
49	1.503315	-0.760		0.170		3.952	-3.880	-2.938	3.238	0.147		12	450.332	5.823
50	1.45851	-0.576		0.186		3.823	-3.864					9	450.341	5.832
51	1.494359	-0.566		-0.190	0.318	3.467	-3.531				0.279	11	450.424	5.916
52	1.44712	-0.633		0.168		3.574	-3.570	0.188				10	450.525	6.016
53	1.486285	-0.396	-0.161			3.435	-3.505			0.074	0.296	11	450.674	6.165
54	1.420825	-0.412				-0.099					0.343	8	450.731	6.222
55	1.48905	-0.623		0.112		3.369	-3.376	0.196		0.090	0.252	12	450.768	6.260
56	1.419524	-0.400								0.061	0.335	8	450.806	6.298
57	1.484231	-0.775		-0.550	0.738	3.723	-3.674	-3.096	3.393			12	450.833	6.324
58	1.457583	-1.194	0.427			3.550	-3.522	-2.782	3.074			11	450.894	6.386
59	1.360303										0.362	6	450.906	6.398
60	1.406343	-0.228	-0.166								0.347	8	450.968	6.459
61	1.412133	-0.395		0.034							0.340	8	450.969	6.461
62	1.456803	-0.578				3.558	-3.632			0.108		9	451.021	6.512
63	1.481892	-0.624		-0.241	0.359	3.233	-3.255	0.188			0.265	12	451.068	6.560
64	1.449464	-0.643				3.288	-3.308	0.217		0.127		10	451.069	6.561
65	1.476645	-0.380	-0.239			3.203	-3.220	0.212		0.095	0.284	12	451.136	6.627
66	1.425873	-0.496						0.249		0.082	0.325	9	451.145	6.636
67	1.484417	3.301	-3.769	0.001	0.929	5.235	-5.052			0.075		12	451.353	6.845
68	1.407911	-0.244	-0.240					0.242			0.342	9	451.419	6.910
69	1.417156	-0.492				-0.047		0.229			0.336	9	451.421	6.912
70	1.413554	-0.487		0.018				0.236			0.334	9	451.478	6.970

Table S14: Parameter estimates and 95% confidence intervals of fixed effects from a model averaging approach (natural average) between relative leukocyte telomere length, social and weather variables and survival to adulthood (>1 year old) in European badgers. β = parameter estimate, S.E. = standard error, 95% CI = 95% confidence interval. Significant parameter estimates (95% CI does not overlap zero) are in bold.

Parameter (reference level)	**β**	**S.E.**	**95% CI**
Intercept	1.488	0.244	1.010 to 1.967
Relative leukocyte telomere length	**0.313**	**0.144**	**0.029 to 0.597**
Number of cubs in natal group	0.101	0.139	-0.172 to 0.375
Mean temperature	**4.352**	**1.555**	**1.299 to 7.405**
Mean temperature^2	**-4.262**	**1.373**	**-6.958 to -1.565**
Daily temperature variability	-1.819	2.234	-6.203 to 2.564
Daily temperature variability^2	3.437	2.018	-0.528 to 7.402
Mean daily rainfall	0.102	0.910	-1.684 to 1.889
Mean daily rainfall^2	1.367	1.080	-0.754 to 3.489
Daily rainfall variability	1.639	2.558	-3.379 to 6.657
Daily rainfall variability^2	-3.556	2.250	-7.974 to 0.862

Table S15: Parameter estimates and 95% confidence intervals of fixed effects from a mixed model and parametric bootstrap tests of same-sex group size effects on early-adulthood relative leukocyte telomere length (Z-score) in European badgers. β = parameter estimate, S.E. = standard error, 95% CI = 95% confidence interval; reference terms in brackets = reference level for factors; * = interaction. Significant parameter estimates (95% CI does not overlap zero) are in bold.

Parameter (reference level)	**β**	**S.E.**	**95% CI**
Intercept†	-0.097	0.284	-0.655 to 0.444
Number of same-sex adults in group	-0.086	0.293	-0.658 to 0.480
Sex (Female)	0.189	0.280	-0.352 to 0.723
Season (Spring)			
Summer	-0.056	0.111	-0.274 to 0.169
Autumn	0.120	0.199	-0.287 to 0.513
Winter	-0.043	0.310	-0.660 to 0.573
Age at last capture	0.042	0.052	-0.056 to 0.148
Number of same-sex adults in group * Sex (Female)	0.040	0.301	-0.535 to 0.624
Without non-significant 2nd order effects			
Intercept††	-0.067	0.162	-0.389 to 0.273
Number of same-sex adults in group	-0.049	0.089	-0.221 to 0.129
Sex (Female)	0.160	0.177	-0.194 to 0.509
Season (Spring)			
Summer	-0.056	0.111	-0.269 to 0.161
Autumn	0.120	0.198	-0.277 to 0.513
Winter	-0.044	0.310	-0.667 to 0.582
Age at last capture	0.041	0.052	-0.059 to 0.142

Random effect estimates (variance):

†qPCR plate ($4.315*10^{-2}$), Row on qPCR plate ($<1.000*10^{-12}$), Social group ($8.914*10^{-9}$), Cohort ($2.297*10^{-2}$), Year ($1.721*10^{-1}$), Individual ID ($2.359*10^{-8}$), Residual ($8.397*10^{-1}$)

††qPCR plate ($4.322*10^{-2}$), Row on qPCR plate ($<1.000*10^{-12}$), Social group ($<1.000*10^{-12}$), Cohort ($2.328*10^{-2}$), Year ($1.725*10^{-1}$), Individual ID ($1.619*10^{-7}$), Residual ($8.372*10^{-1}$)

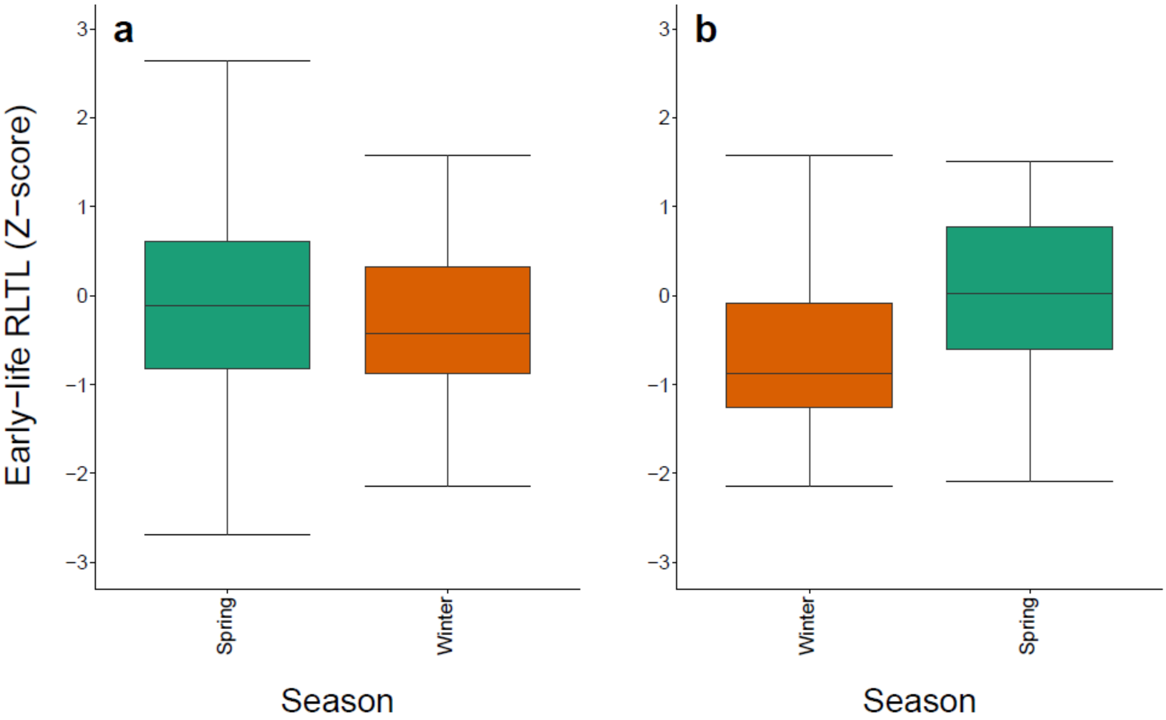

Figure S1: The effect of longitudinal measures from spring to winter (a) and winter to spring (b) on relative leukocyte telomere length (RLTL). Raw data for spring to winter (a; *n* = 28 samples; 14 badger) and winter to spring (b; *n* = 12 samples; 6 badgers) are shown.

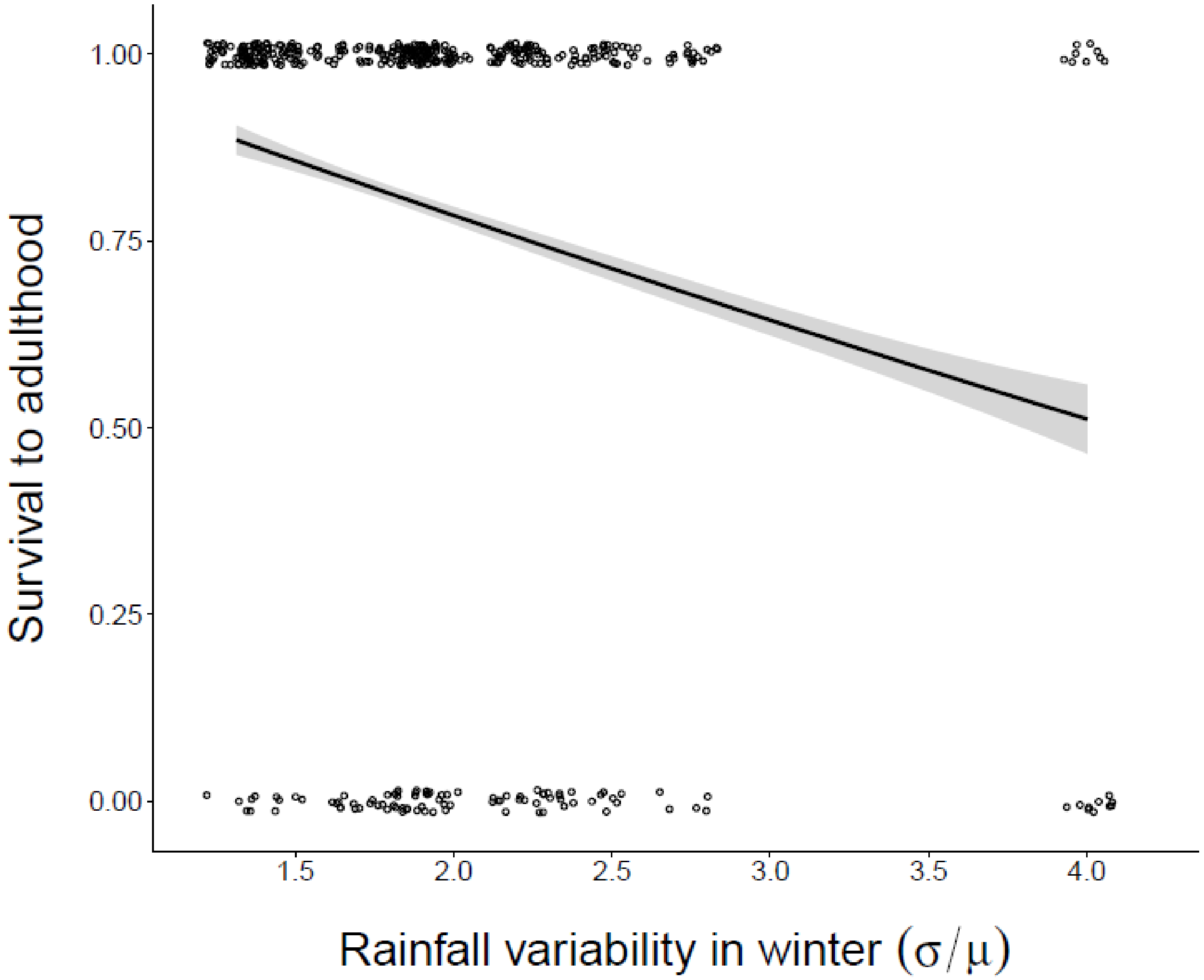

Figure S2: The association between the rainfall variability in winter and survival to adulthood. Raw data points (*n* = 435 samples; 435 badgers) are shown, and jittered for clarity on the amount of data. The fitted line represents the regression from the mixed model, and the 95% confidence intervals as shaded areas.

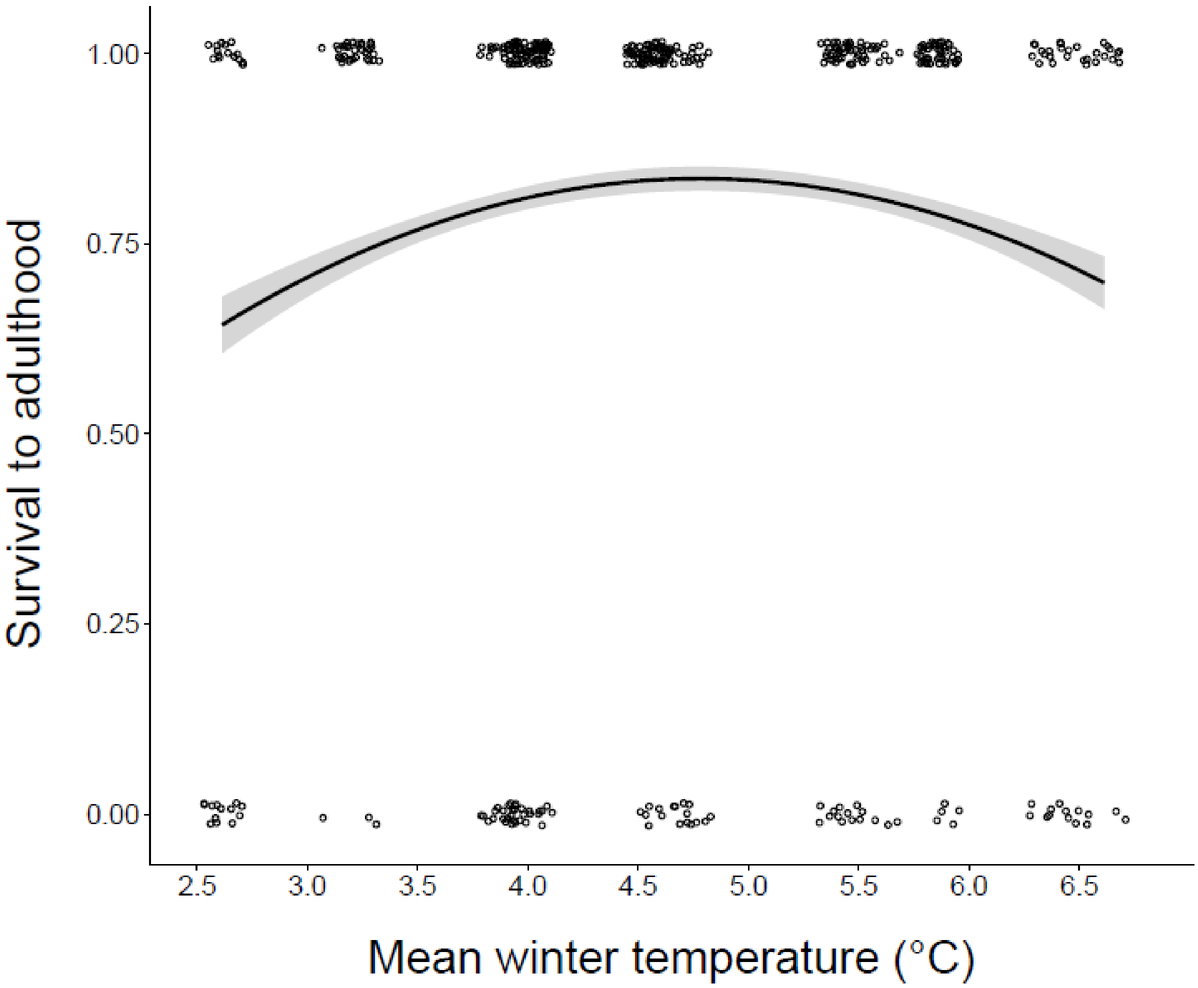

Figure S3: The association between mean winter temperature and survival to adulthood. Raw data points (*n* = 435 samples; 435 badgers) are shown, and jittered for clarity on the amount of data. The fitted line represents the quadratic regression from the mixed model, and the 95% confidence intervals as shaded areas.

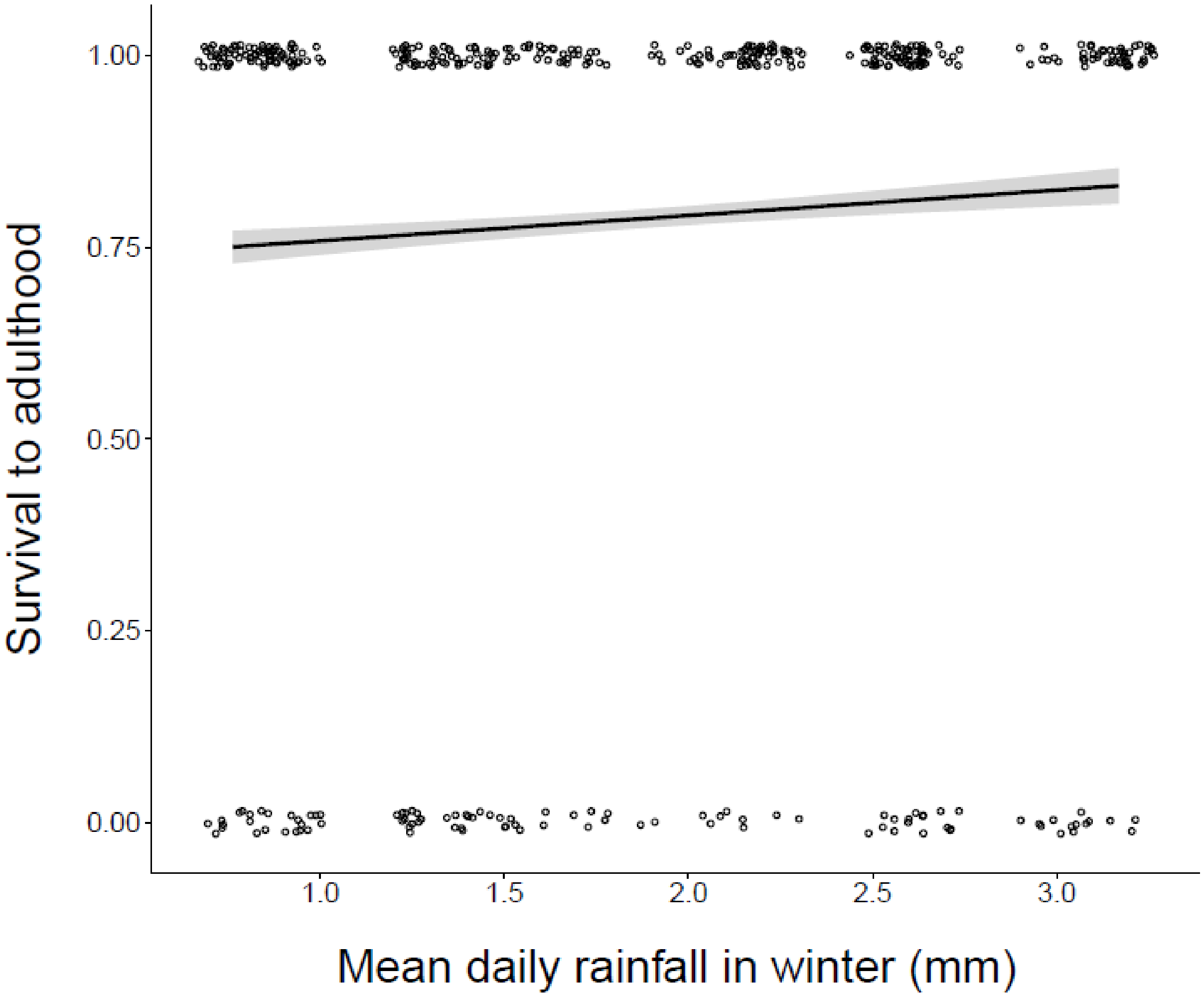

Figure S4: The association between the mean daily rainfall in winter and survival to adulthood. Raw data points (*n* = 435 samples; 435 badgers) are shown, and jittered for clarity on the amount of data. The fitted line represents the regression from the mixed model, and the 95% confidence intervals as shaded areas.