

# Telomere dynamics in relation to experimentally increased locomotion costs and fitness in great tits

Els Atema<sup>1,2</sup>, Arie J. van Noordwijk<sup>2</sup> & Simon Verhulst<sup>1\*</sup>

1. GELIFES, University of Groningen, Groningen, the Netherlands

2. Netherlands Institute of Ecology, Wageningen, the Netherlands

\* Author for correspondence: [s.verhulst@rug.nl](mailto:s.verhulst@rug.nl), Orcid: 0000-0002-1143-6868

**Running headline:** Telomeres and great tit life-histories

## Abstract

Evidence that telomere length (TL) and dynamics can be interpreted as proxy for 'life stress' experienced by individuals stems largely from correlational studies. We tested for effects of an experimental increase of workload on telomere dynamics by equipping male great tits (*Parus major*) with a 0.9 gram backpack for a full year. In addition, we analysed associations between natural life-history variation, TL and TL dynamics. Carrying 5% extra weight for a year did not significantly accelerate telomere attrition. This agrees with our earlier finding that this experiment did not affect survival or future reproduction. Apparently, great tit males were able to compensate behaviourally or physiologically for the increase in locomotion costs we imposed. We found no cross-sectional association between reproductive success and TL, but individuals with higher reproductive success (number of recruits) lost fewer telomere base pairs in the subsequent year. We used the TRF method to measure TL, which method yields a TL distribution for each sample, and the association between reproductive success and telomere loss was more pronounced in the higher percentiles of the telomere distribution, in agreement with the higher impact of ageing on longer telomeres within individuals. Individuals with longer telomeres and less telomere shortening were more likely to survive to the next breeding season, but these patterns did not reach statistical significance. Whether successful individuals are characterized by losing fewer or more base pairs from their telomeres varies between species, and we discuss aspects of ecology and social organisation that may explain this variation.

**Keywords:** Senescence, fitness, TRF, longitudinal, birds

## Introduction

Individual variation in phenotypic quality, as evidenced for example by consistent variation in reproductive success, is ubiquitous in natural systems. Unfortunately, inferring such quality differences from phenotypic traits such as size or physiology is usually difficult, in particular in birds, where quality differences usually depend at most weakly on size. Telomeres, a repeated sequence of non-coding DNA at the ends of linear chromosomes, functioning in the protection and stabilization of the genome (Blackburn 1991), have emerged in recent years as a potential biomarker of phenotypic quality. This is based on the accumulation of studies in wild animals that find associations between telomere length and fitness components. More specifically, individuals with longer telomeres have higher survival rates in a range of species (Wilbourn et al 2018). The relation between telomere length and reproductive success has been studied less intensively, and the findings are mixed. Nevertheless, the general tendency is a positive relation between telomere length and reproductive success (Pauliny et al. 2006; Plot et al. 2012; Le Vaillant et al. 2015; Eastwood et al 2019). Also in humans a positive relation was reported between telomere length and reproductive success in indigenous rural communities in Guatemala (Barha et al. 2016). However, in common terns the opposite was found: individuals which were more successful in reproduction had shorter telomeres, which was attributed to the longitudinal finding that more successful individuals lost more base pairs with age (Bauch, Becker & Verhulst 2013, 2014). In agreement with these findings, two brood size manipulation studies found that parents raising enlarged broods lost more base pairs with age (Sudyka 2019).

The associations between telomere length and fitness components suggest that telomere studies may provide a tool (e.g. as biomarker) to study the mechanism underlying variation in phenotypic quality and the mechanisms mediating life-history trade-offs (Sudyka 2019). However, most studies of the causes and consequences of telomere length variation and dynamics are correlational, making inference about causality difficult, if not impossible. The brood size manipulation studies (op cit.) are an important exception. But also in these studies it is not clear what the brood size manipulation changes exactly, because caring parents can respond to an increase in brood demand in different ways. For example, parents can adjust their daily energy expenditure to increase provisioning rate (e.g. Tinbergen & Verhulst 2000), but alternatively, they could increase their foraging yield and provisioning without working harder, by for instance taking more risk during foraging with respect to predators and parasites (Houston, McNamara & Hutchinson 1993). These different responses could each affect telomere dynamics, but in very different ways. Hence, instead of manipulating brood size we aimed to manipulate workload more directly by letting birds carry extra mass for a whole year to examine its effect on telomere shortening rate.

In addition to the effect of carrying extra mass on telomere dynamics we investigated the correlations between TL and the two key fitness components: reproductive success and survival. We used two approaches to quantify the association between fitness components and telomeres, that address different questions: (i) cross-sectionally, correlating telomere length and reproductive output or survival in the same year. (ii) Longitudinally, relating telomere dynamics to variation in reproductive success and survival (Van de Pol & Verhulst 2006). The longitudinal analyses provide information that is complementary to the cross-sectional analysis. For example, when cross-sectional analyses reveal

that short telomeres are associated with high success, this may be due to successful individuals losing more base pairs annually than less successful individuals, which can be tested in a longitudinal analysis (see e.g. Bauch et al 2013). Likewise, only a longitudinal analysis can test whether low survival in individuals with short telomeres can be attributed to telomere length per se or to telomere attrition independent of absolute length (see e.g. Boonekamp et al 2014).

## **Materials and methods**

### *Study population and data collection*

We studied great tits on Vlieland, an island in the Dutch Wadden Sea, in the period 2011-2015. Breeding activity was monitored through regular nest visits, recording number of eggs and young at each visit. Parents were caught with spring traps while feeding nestlings (8-11 days old, hatching day = day 0) and subsequently nestlings were ringed. In winter, birds were caught while roosting in the nest boxes. Individual great tits were identified by ring number and unringed individuals were ringed. The overall capture probability of individuals known to be alive was 88% in our population (Atema et al. 2016), hence we were able to estimate survival based on catches of the adults in both winter and spring.

To quantify the association between telomeres and reproductive success we used data from first broods only (only 3 out of 109 males had a second brood). Furthermore, we did not investigate associations with time of breeding and clutch size, because these traits are known to be under female control with no influence of the partner in our study population (Van Noordwijk, Van Balen & Scharloo 1981; Postma & van Noordwijk 2005). We verified that this also holds in the years of our study by comparing the repeatability of clutch size and hatch date in females and males (total population, years 2010-2014), and found both to be substantially higher in females (Table S1). Based on these findings we used the number of fledglings and recruits (fledglings recaptured as breeding bird in a later year) as measures of reproductive success.

Blood samples were taken from the brachial vein and stored in 2% EDTA at 4-7 °C for up to three weeks. Subsequently, samples were snap-frozen in 40% glycerol buffer and stored at -80 °C.

### *Backpack experiment*

In order to manipulate workload, we equipped male great tits in the years 2011 – 2013 with a small backpack that they carried during 1 year, after which they were recaptured when feeding nestlings and the backpack was removed. The experiment included three treatments: i) control without a backpack (n=97), ii) 'light' with an empty backpack of 0.1 g as a control for possible effects of the harness (n=68) and iii) 'heavy' with a backpack of 0.9 g (~5% of body mass; n=80). Further details can be found in Atema et al. (2016), where we show there were no long-term fitness consequences of the manipulation, although there were short-term effects of the backpacks on male state: males carrying backpacks slightly increased their mass, and were less successful in securing a nest box as roosting site in winter (Atema et al. 2016).

#### *Telomere terminal restriction fragment analyses*

We quantified telomere length using terminal restriction fragment as described by Salomons et al. (2009) and Atema et al. (2019). Briefly, DNA was extracted in agarose plugs and subsequently DNA in half a plug was digested with a mixture of three restriction enzymes, cutting DNA sequence except for telomere sequence (5'-TTAGGG-3'). The restricted DNA and the <sup>32</sup>P end-labelled size standards were size separated with pulsed field gel electrophoreses. Subsequently gels were dried and the single-stranded overhangs of telomeres were hybridized with <sup>32</sup>P-labelled oligonucleotide (5'-CCCTAA-3')<sub>4</sub>.

An image of the distribution of telomere lengths was retrieved by phosphor imaging. Gel images were analysed using the open source software IMAGEJ v. 1.38x. We showed in Atema et al. (2019) that in adults only Class II, but not ultra-long Class III telomeres shorten with age, and hence Class II telomeres are most relevant to use as a biomarker. We therefore limited the analyses in the present study to Class II telomeres (average range 3 – 18 kb). Telomere length varies within a sample, due to TL differences between chromosomes and cells, and we previously showed that in great tits and other bird species (jackdaws *Corvus monedula*: Salomons et al 2009; common tern *Sterna hirundo*: Bauch et al 2014) the higher percentiles within the distribution lose more bp / year than the lower percentiles (Atema et al 2019). Next to average telomere length of the telomere distribution we therefore calculated the telomere length per percentile (10th – 90th) of the Class II distribution. See Atema et al. (2019) for details.

#### *Statistics*

Data were analysed using mixed-effects models in R (R Core Team 2019) and we tested the specific hypotheses explained above, i.e. the model selection procedure compared only models with and without the treatment effects. All models included random terms to correct for between gel variation and for individual identity when repeated measurements were included. All models included age as fixed effect and longitudinal models included also initial telomere length and the interaction of age with initial telomere length if significant as fixed effects.

To test for effects of the backpack experiment on telomere length, we included added mass as a continuous variable to the model, since this approach explained variation caused by the treatment best in our earlier analyses of the fitness consequences of the backpack experiment (Atema et al. 2016). Added mass was coded zero for all baseline samples, and the added mass (0, 0.1 or 0.9) for subsequent samples. We further included sampling season as factor (0 = spring; 1 = winter), age at manipulation and the time elapsed between the baseline sample and the follow-up sample (note that age at manipulation and elapsed time since manipulation add up to age at sampling). To investigate the relation between telomere length and reproduction, we included number of offspring either as continuous variable or as binomial factor (unsuccessful, 0 offspring = 0; successful, ≥1 offspring = 1) because complete and partial brood failure are likely to have different causes. To quantify the relation between telomere length and survival, we included the binomial factor survived to the model (died = 0; survived = 1). We considered an individual to have not survived when it was not seen or recaptured in

the 1-3 years after April 1st of the next year. In the analyses, we refer to the breeding season in which the backpack was added as spring 1, and the next breeding season as spring 2.

## Results

### *Backpack experiment*

Initial telomere length did not differ between the three experimental groups ( $F_{2,71.8} = 0.33$ ,  $P = 0.72$ ). Because the aim of the study was to evaluate long-term effects of carrying a backpack, the analyses below are limited to individuals that were resampled in the subsequent winter and / or in spring 2. We found no effect of the added mass on telomere loss during the treatment period ( $F_{1,113.5} = 0.01$ ,  $P = 0.9$ , Fig. 1) when pooled over the recaptures in winter and spring 2, and neither was there a significant interaction between treatment and time elapsed since the start of the treatment ( $F_{1,111.5} = 0.04$ ,  $P = 0.8$ ). Testing for an effect of added mass on telomere shortening separately for recaptures in winter or spring 2 did not change this result (both  $P > 0.4$ ). Thus, we concluded that the added mass did not significantly accelerate telomere dynamics.

The rate of telomere shortening rate appeared slightly higher in the winter subset of samples than in the spring subset (model estimates respectively:  $55 \pm 44$  bp/year and  $76 \pm 40$  bp/year), but not significantly so ( $F_{1,57.7} = 0.16$ ,  $P = 0.69$ ). Likewise, among individuals which were successfully sampled on all three occasions there was no difference in telomere shortening rate from spring 1 to winter and winter to spring 2 ( $F_{1,33.0} = 0.06$ ,  $P = 0.80$ ).

### *Reproduction*

Neither the number of fledglings, nor the number of recruits was affected by the backpack experiment (Atema et al. 2016), and hence we ignored the experiment in the following analyses. Telomere length was not associated with reproductive success in that same year in terms of number of fledglings or recruits (Table 1a and b). When, for example, more successful individuals lose fewer base pairs per year than less successful individuals, the association between telomere length and reproductive success may become stronger with age. We therefore analysed the correlation between telomere length and number of recruits separately for males of 1 year and males older than 1 year. We did this for recruit production only, because in this stage of the breeding cycle the effects of ageing were found to be strongest in great tits in our study population (Bouwhuis et al. 2010). However, there was no correlation between telomere length and the number of recruits in either one year old males (slope:  $36.07 \pm 180.39$ ;  $F_{1,60.9} = 0.040$ ,  $P = 0.84$ ) or in males older than one year (slope:  $-22.64 \pm 33.13$ ;  $F_{1,30.6} = 0.47$ ,  $P = 0.50$ ).

In the longitudinal analyses, we investigated telomere dynamics in the year following reproduction (including winter captures). We found no association between the number of fledglings and subsequent telomere dynamics (Table 1c), but individuals who produced more recruits lost fewer base pairs in that year (Table 1d). Based on the model estimates, individuals that were unsuccessful in producing recruits lost on average  $177.6 \pm 36.4$  bp/year, whereas successful individuals lost on average only  $4.1 \pm 39.6$  bp/year (Fig. 2).

The difference in telomere dynamics between individuals producing recruits or not, was more pronounced in the longer telomeres of the telomere distribution (Fig.3; interaction recruits x percentile:  $F_{1,1045} = 37.82$ ,  $P < 0.0001$ ; including sample identity as random effect).

### *Survival*

We found no difference in telomere length between individuals that survived to the next breeding season and individuals that did not (Table 2a). The interaction between survival and year of sampling was close to significance ( $F_{2,60.03} = 2.80$ ,  $P = 0.069$ ), but none of the years analysed separately yielded significant differences in telomere length between surviving and non-surviving individuals (Fig. 4; all  $P > 0.05$ ).

To investigate the link between telomere attrition and survival we compared telomere attrition from spring 1 to spring 2 between survivors and non-survivors to year three, taking all individuals observed any time after April first of year three as survivors. We restricted the dataset to samples taken in spring of year 1 and 2 (i.e. ignored samples taken in winter), and found that surviving individuals lost  $32.2 \pm 76.5$  bp/year, whereas non-surviving individuals lost  $137.3 \pm 42.5$  bp/year, but also this fourfold difference did not reach statistical significance (Table 2b). Including the samples from winter did not change the conclusions (survived:  $P = 0.29$ ). In common terns the differences between survivors and non-survivors were largest at the higher percentiles of the distribution (Bauch et al. 2014), and we tested for a similar effect in our study. However, we found no interaction between survival and percentiles of the telomere distribution ( $F_{1,517.4} = 0.12$ ,  $P = 0.73$ ).

### **Discussion**

Carrying 5% additional mass during one whole year seems a severe challenge, but we found no effect of this manipulation on telomere dynamics. On the one hand, this was surprising, because of the nature of the manipulation and because our earlier analysis of the experiment on other traits revealed that males responded to the manipulation by changing their mass, and males with heavy backpacks appeared less successful in competition over roosting sites (Atema et al. 2016). On the other hand, it agrees with our finding that these effects did not translate into long-term consequences for reproduction or survival (Atema et al. 2016). Apparently great tits have a way to avoid or compensate for costs of the additional mass. Our backpack treatment was comparable to the weight of transmitters used in great tits (e.g. Snijders et al. 2014). Altogether we showed that equivalent devices to the backpacks can be used in small songbirds without long-term consequences on fitness or telomere shortening and presumably ageing. We are aware of one comparable handicap experiment, in adult Adélie penguins, that also found no effect on telomere attrition (Beaulieu et al. 2011). Indeed, a recent meta-analysis did not find a significant overall effect of diverse stressors on TL in wild populations (Chatelain, Drobniak, & Szulkin, 2019), and our findings fit this pattern.

Previous cross-sectional comparisons of the association between telomere length and reproductive success have yielded mixed results (review: Sudyka 2019), sometimes even contrasting between the sexes within a species (Bauch et al. 2020). In our study, neither the number of fledglings nor the number of recruits was correlated with telomere length. However, a longitudinal comparison

revealed that successful males (with recruits) lost fewer base pairs compared to unsuccessful individuals. The positive relationship in great tits could be due to the male being of better quality and/or having a better territory, and agrees with the finding that individuals that are more successful at reproduction also have higher survival rates than less successful individuals (e.g. Olsson, Shine & Wapstra 2001; Lane et al. 2010) also in great tits (Bouwhuis et al. 2009). Alternatively, more successful males may have been mated with females that provided more parental care, resulting into the high reproductive success and leaving the male with more resources for somatic maintenance.

When analysing the association between reproductive success and telomere dynamics in more detail, by dividing the telomere distribution in percentiles, it appeared that the positive correlation between recruit production and telomere shortening was strongest in the longer telomeres within individuals. A similar pattern was found in common terns (Bauch et al. 2014), which is to our best knowledge the only other species in which this was investigated. This finding agrees with our expectation, because the longest telomeres also shorten at the highest rate in great tits and other species (Kimura et al. 2007; Salomons et al. 2009; Bauch et al. 2014; Atema et al. 2019), and it confirms our interpretation that the longer telomeres within individuals are more sensitive to environmental conditions and hence more informative read-out parameters of experienced life-stress. The non-significant correlation between reproductive success and telomere length in the cross-sectional dataset, while finding a significant effect in the longitudinal dataset, are superficially inconsistent with each other. If reproductive output is a permanent characteristic of an individual, a cumulative effect on telomere length could be expected. A similar hypothesis was proposed in common terns, in which individuals show consistent individual differences in reproductive success (Bauch et al. 2014). In this species, also cross-sectional analyses yielded significant relations between reproduction and telomeres. However, great tits have low survival rates and hence their lifespan may be too short to detect such cumulative effects. Moreover, individual variation in reproductive output may be less consistent in which case the accumulation of effects would be more difficult to detect.

Individuals that were most likely to survive to the next year had longer telomeres and also lost fewer telomere base pairs, in line with findings in other species (Wilbourn et al 2018), but none of these correlations reached significance in our study. Our population of great tits on Vlieland is a relatively closed island system, and once males settle in the population as breeding birds they are not known to leave the island (Verhulst & van Eck 1996). Therefore, selective emigration is unlikely to have biased our findings. Moreover, our sample size was relatively large, leading us to conclude that the correlation between survival and telomeres in great tits is apparently weak when compared to other species. Salmón et al (2017) also found no association between TL and survival in adult great tits, but in their telomere measurements they pooled all telomere classes (i.e. interstitial telomeric sequences and the ultralong class III telomeres that do not shorten with age in addition to class II telomeres, and hence their results are only superficially similar to our results that refer to class II telomeres up to 18kb only; Atema et al. 2019).

Contrary to what we observed in great tits, in some other species, including common terns, the individuals with higher reproductive output lost more telomere base pairs than less successful individuals (Bauch et al. 2013, 2014; Sudyka 2019). This contrast raises an interesting question, which

has received little attention, namely how the factors that determine who attains higher fitness varies between species. To some extent, these factors will be shared between species (e.g. 'health', although what constitutes 'health' may vary between species), but species differences in social and ecological factors are likely to result in different selection pressures in different species. Indeed, such differences are part of the causes of the evolution of species differences, but general patterns explaining variation between species in associations with individual variation in fitness remain to be identified. Since telomeres are evolutionary well conserved, developing an explanation for interspecific variation in fitness / telomere dynamics association may be a fruitful starting point for this endeavour.

One way to interpret individual variation in telomere dynamics is that it reflects variation in reproductive effort, with a larger effort accelerating telomere attrition. The small number of studies in which brood size was manipulated tend to support this interpretation (Sudyka 2019). Interpreted in this way, our findings suggest that in great tits the more successful individuals made a smaller effort than the less successful individuals, while the opposite pattern emerged in common terns. For the contrast between great tits and common terns this interpretation is supported by information on the association between reproductive success and corticosterone, a hormone of which concentrations in plasma increase with increasing energy expenditure (e.g. Jimeno et al 2017, 2018). In great tits, the individuals with high baseline corticosterone achieved the lowest reproductive success (Ouyang et al. 2013), while in common terns the individuals with high baseline corticosterone achieved the highest success (Bauch et al. 2016). An ecological explanation of this contrast may be that great tits are territorial, and hence each pair has their own resource pool, and individuals with a rich territory can provide more food with less effort compared to individuals on poor territories. Indeed, great tit energy expenditure during brood rearing is known to be lower on territories with higher food availability (Tinbergen & Dietz, 1994), while such territories yield a higher reproductive success (Verboven, Tinbergen & Verhulst, 2001). In contrast, common terns are colonial, and thus have a shared resource pool, suggesting that reproductive success is likely to increase with increasing foraging effort, resulting in higher rates of provisioning. Thus, the social organisation as it determines resource access may determine whether successful individuals lose more or fewer base pairs than less successful individuals, but more (longitudinal) studies are required to test this hypothesis and the underlying assumptions.

### Acknowledgements

We thank the State Forestry Commission for their permission to work on their property. We are grateful to J. den Ouden, E.A de Ruijter, N. Weites, A. Sibma, M. Rousselle, J. Zhang and C. Vinke for their great help in the field and to E. Mulder for her invaluable advice about the TRF analyses. Sample collection and experimental procedures were approved by the Animal Experimental Committee of the Royal Netherlands Academy of Arts and Sciences (DEC-KNAW, NIOO 11.03). E.A. was supported by NWO open competition grant 821.01.003 to AJvN and SV.



## References

- Atema, E., van Noordwijk, A.J., Boonekamp, J.J. & Verhulst, S. (2016) Costs of long-term carrying of extra mass in a songbird. *Behavioral Ecology*, 27, 1087–1096.
- Atema, E., Mulder, E., van Noordwijk, A.J. & Verhulst, S. (2019). Ultra-long telomeres shorten with age in nestling great tits but are static in adults and mask attrition of short telomeres. *Molecular Ecology Resources*, 19, 648–658.
- Barha, C.K., Hanna, C.W., Salvante, K.G., Wilson, S.L., Robinson, W.P., Altman, R.M. & Nepomnaschy, P.A. (2016) Number of children and telomere length in women: A prospective, longitudinal evaluation. *PLoS ONE*, 11, 1–12.
- Bauch, C., Becker, P.H. & Verhulst, S. (2013) Telomere length reflects phenotypic quality and costs of reproduction in a long-lived seabird. *Proceedings of the Royal Society B: Biological Sciences*, 280, 20122540.
- Bauch, C., Becker, P.H. & Verhulst, S. (2014) Within the genome, long telomeres are more informative than short telomeres with respect to fitness components in a long-lived seabird. *Molecular Ecology*, 23, 300–310.
- Bauch, C., Riechert, J., Verhulst, S. & Becker, P. (2016) Telomere length reflects reproductive effort indicated by corticosterone levels in a long-lived seabird. *Molecular Ecology*, 25, 5785–5794.
- Bauch, C., Gatt, M. C. , Granadeiro, J. P. , Verhulst, S., & Catry, P. (2020) Sex-specific telomere length and dynamics in relation to age and reproductive success in Cory's shearwaters. *Molecular Ecology*, 29, 1344–1357.
- 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.
- Bize, P., Criscuolo, F., Metcalfe, N.B., Nasir, L. & Monaghan, P. (2009) Telomere dynamics rather than age predict life expectancy in the wild. *Proceedings of the royal society B biological sciences*, 276, 1679–83.
- Blackburn, E.H. (1991) Telomeres. *Trends in Biochemical Sciences*, 16, 378–381.
- Bouwhuis, S., Sheldon, B.C., Verhulst, S. & Charmantier, A. (2009) Great tits growing old: selective disappearance and the partitioning of senescence to stages within the breeding cycle. *Proceedings of the Royal Society B: Biological Sciences*, 276, 2769–2777.
- Bouwhuis, S., Van Noordwijk, A.J., Sheldon, B.C., Verhulst, S. & Visser, M.E. (2010) Similar patterns of age-specific reproduction in an island and mainland population of great tits *Parus major*. *Journal of Avian Biology*, 41, 615–620.
- Chatelain, M., Drobniak, S. M., & Szulkin, M. (2019) The association between stressors and telomeres in non-human vertebrates: a meta-analysis. *Ecology Letters*, 99, 21–18.
- Eastwood JR, Hall ML, Teunissen N, Kingma SA, Hidalgo Aranzamendi H, Fan M, Roast M, Verhulst S & Peters A. (2019). Early-life telomere length predicts lifespan and lifetime reproductive success in a wild bird. *Molecular Ecology*, 28, 1127–1137.
- Houston, A.I., McNamara, J.M. & Hutchinson, J.M.C. (1993) General results concerning the trade-off between gaining energy and avoiding predation. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 341, 375–397.
- Jimeno, B., Hau, M. & Verhulst, S. (2017) Strong association between corticosterone and temperature dependent metabolic rate in individual zebra finches. *Journal of Experimental Biology*, 220, 3280–3289.
- Jimeno, B., Hau, M. & Verhulst, S. (2018) Corticosterone levels reflect variation in metabolic rate independent of 'stress'. *Scientific Reports* 8: 13020.
- Kimura, M., Barbieri, M., Gardner, J.P., Skurnick, J., Cao, X., van Riel, N., Rizzo, M.R., Paoliso, G. & Aviv, A. (2007) Leukocytes of exceptionally old persons display ultra-short telomeres. *American Journal of Physiology- Regulatory, Integrative and Comparative Physiology*, 293, R2210–2217.
- Lane, J.E., Boutin, S., Speakman, J.R. & Humphries, M.M. (2010) Energetic costs of male reproduction in a scramble competition mating system. *Journal of Animal Ecology*, 79, 27–34.
- Van Noordwijk, A.J., Van Balen, J.H. & Scharloo, W. (1981) Genetic and environmental variation in clutch size of the Great tit *Parus major*. *Netherlands Journal of Zoology*, 31, 342–372.
- Olsson, M., Pauliny, A., Wapstra, E., Uller, T., Schwartz, T., Miller, E. & Blomqvist, D. (2011) Sexual differences in telomere selection in the wild. *Molecular Ecology*, 20, 2085–2099.
- Olsson, M., Shine, R. & Wapstra, E. (2001) Costs of reproduction in a lizard species: a comparison of observational and experimental data. *Oikos*, 93, 121–125.
- Ouyang, J.Q., Sharp, P., Quetting, M. & Hau, M. (2013) Endocrine phenotype, reproductive success and survival in the great tit, *Parus major*. *Journal of Evolutionary Biology*, 26, 1988–1998.

- Pauliny, A., Wagner, R.H., Augustin, J., Szép, T. & Blomqvist, D. (2006) Age-independent telomere length predicts fitness in two bird species. *Molecular ecology*, 15, 1681–1687.
- 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*, 167, 766–773.
- Postma, E. & van Noordwijk, A.J. (2005) Gene flow maintains a large genetic difference in clutch size at a small spatial scale. *Nature*, 433, 65–68.
- R Core and Team (2019). R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing.
- Reichert, S., Criscuolo, F., Zahn, S., Arrive, M., Bize, P. & Massemin, S. (2014) Immediate and delayed effects of growth conditions on ageing parameters in nestling zebra finches. *Journal of Experimental Biology*, 218, 491–499.
- Salmón, P., Nilsson, J. F., Watson, H. , Bensch, S., & Isaksson, C. (2017) Selective disappearance of great tits with short telomeres in urban areas. *Proceedings of the Royal Society B: Biological Sciences*, 284, 20171349–8.
- Salomons, H.M., Mulder, G.A., van de Zande, L., Haussmann, M.F., Linskens, M.H.K. & Verhulst, S. (2009) Telomere shortening and survival in free-living corvids. *Proceedings of the Royal Society B: Biological Sciences*, 276, 3157–3165.
- Snijders, L., van Rooij, E.P., Burt, J.M., Hinde, C. a., van Oers, K. & Naguib, M. (2014) Social networking in territorial great tits: slow explorers have the least central social network positions. *Animal Behaviour*, 98, 95–102.
- Sudyka, J., (2019) Does Reproduction Shorten Telomeres? Towards Integrating Individual Quality with Life-History Strategies in Telomere Biology. *BioEssays*, 32, 1900095–12.
- Tinbergen, J.M., & Dietz, M.W. (1994) Parental energy expenditure during brood rearing in the great tit (*Parus major*) in relation to body mass, temperature, food availability and clutch size. *Functional Ecology*, 8, 563–572.
- Tinbergen, J.M. & Verhulst, S. (2000) A fixed energetic ceiling to parental effort in the great tit? *Journal of Animal Ecology*, 69, 323–334.
- Le Vaillant, M., Viblanc, V.A., Saraux, C., Le Bohec, C., Le Maho, Y., Kato, A., Criscuolo, F. & Ropert-Coudert, Y. (2015) Telomere length reflects individual quality in free-living adult king penguins. *Polar Biology*, 2059–2067.
- Verboven, N., Tinbergen, J. M., & Verhulst, S. (2001) Food, reproductive success and multiple breeding in the Great Tit *Parus major*,” *Ardea*, 89, 387–406.
- Verhulst, S. & Van Eck, H.M. (1996) Gene flow and immigration rate in an island population of great tits. *Journal of Evolutionary Biology*, 9, 771–782.
- Wilbourn, R.V., Moatt, J. P., Froy, H., Walling, C. A. , Nussey, D. H., & Boonekamp, J. J. (2018) The relationship between telomere length and mortality risk in non-model vertebrate systems: a meta-analysis. *Philosophical Transactions of the Royal Society B: Biological Science*, 373, pp. 20160447–9.

#### Data Accessibility

The data used in these analyses will be deposited in the DRYAD repository upon acceptance of the paper.

## Tables

*Table 1.* Models testing for an association between telomeres (bp) and reproductive success while controlling for age (years). Telomere length was the dependent variable in all models, and all models included gel identity as random effect; the longitudinal models in addition included male identity as random effect. Models (a) and (b) are cross-sectional, with respectively number of fledglings and recruits as estimates of reproductive success. Models (b) - (f) are longitudinal, with reproductive success coded as zero in spring 1, and as the number of fledglings or recruits produced in spring 1 at all later captures. This coding yields estimates of the effect of the number of offspring produced while controlling for age. Effects were tested for both number of offspring as continuous variable (c, d) and as binary factor for reproductive output (e, f). As random effect gel identity was included in all models and individual identity in longitudinal models. N = 103 in tables a and b), and N=222 in tables .

Model	Fixed effect	Estimate (s.e.)	F ratio	P-value
<i>Cross-sectional</i>				
(a)	age # fledglings	-301.0 (118.8) 60.9 (49.6)	6.42 1.51	0.013 0.22
(b)	age # recruits	-269.0 (118.9) -16.4 (113.8)	5.12 0.02	0.026 0.89
<i>Longitudinal</i>				
(c)	age # fledglings	137.2 (54.9) 3.83 (7.5)	6.25 0.26	0.013 0.61
(d)	age # recruits	162.3 (38.6) 59.1 (25.9)	17.68 5.21	<0.0001 0.024

*Table 2.* Models testing for an association between telomeres and male survival while controlling for age. (a) Cross-sectional model fitted, while controlling for year differences, (b) longitudinal model, where telomere loss was modelled by having follow-up telomere length as dependent variable and including initial telomere length as covariate. The variable 'survived' was 1 for survivors and 0 for non-survivors.

Model	Fixed effect	Estimate (s.e.)	F ratio	P-value
<i>Cross-sectional</i>				
(a)	age year [2012] [2013] survived	2.91 (116.48) -137.87 (186.33) 167.65 (216.32) 172.88 (324.42)	0.0006 0.39 0.28	0.98 0.68 0.60
<i>Longitudinal</i>				
(b)	initial telomere length age survived	0.92 (0.040) -8.66 (40.05) 105.05 (97.32)	522.76 0.047 1.17	<0.0001 0.83 0.29

# Figures

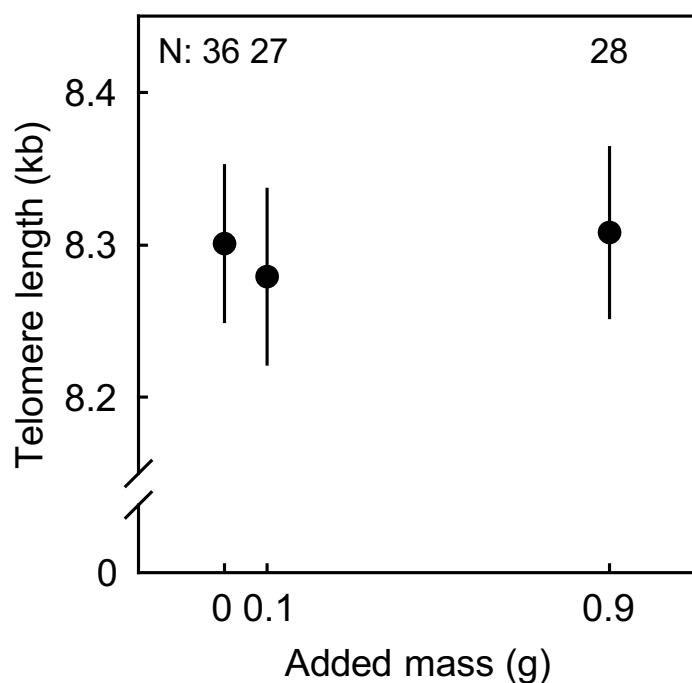


Figure 1. Telomere length of males after carrying additional mass (X-axis) for one year. Plotted values are estimated means  $\pm$  standard error and numbers in the graph indicate the number of males.

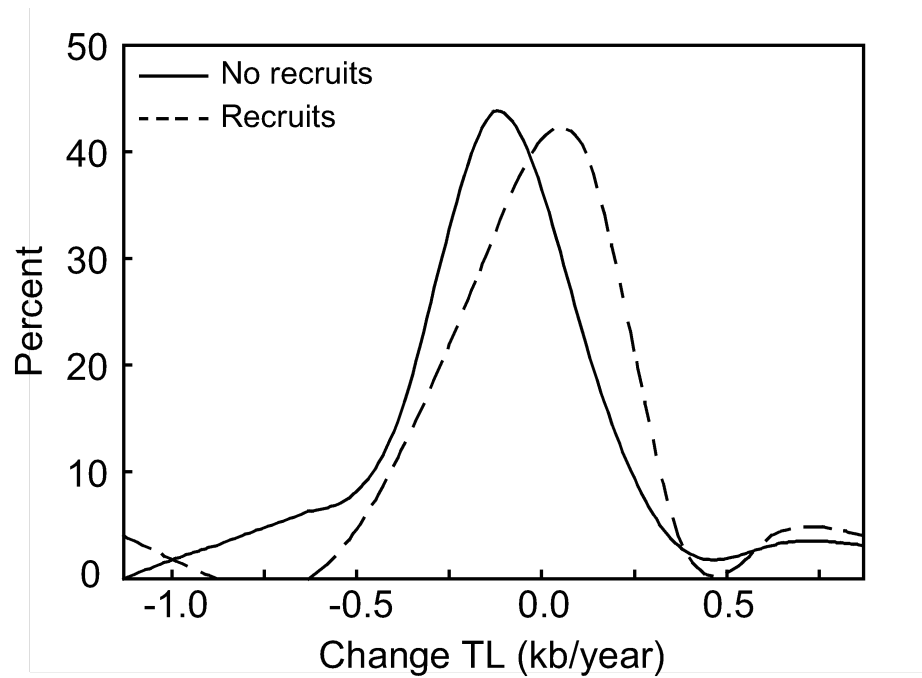


Figure 2. Frequency distributions of the change in telomere length from spring 1 to spring 2 for individuals that did (N = 25, dashed line) or did not produce recruits (N = 32, solid line). The curves are fitted through the 0.1 kb bins of the frequency distributions. See table 1 for statistics.

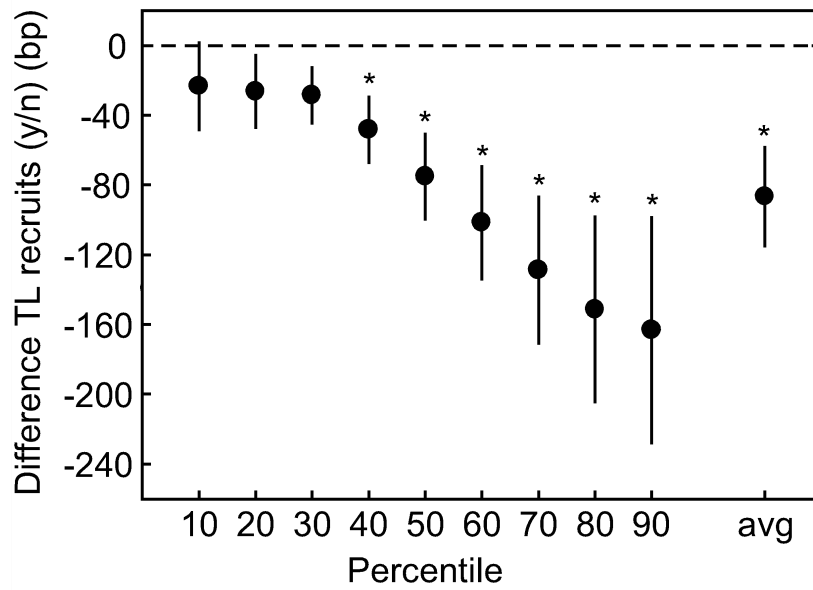


Figure 3. Difference in telomere length ( $\pm$  s.e.) between individuals that did and did not produce recruits across the percentiles in the telomere distribution. Negative values indicate individuals without recruits lost more base pairs. Plotted values are estimates for the binary term recruits from the mixed model of telomere length in year two, with telomere length in year one as covariate (Table S2). avg = average telomere length. Significant differences marked with \*.

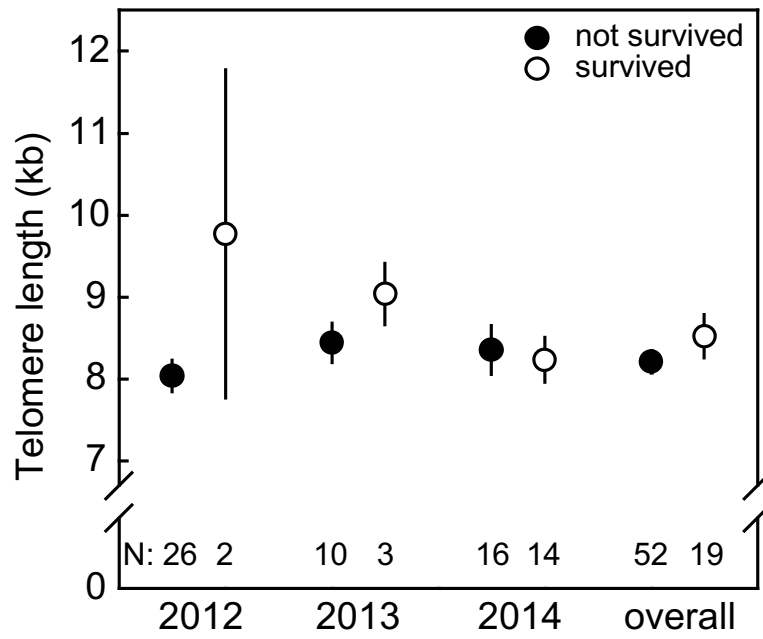


Figure 4. Telomere length in spring 2 per study cohort and the overall estimates for survivors and non-survivors to year 3 or later. Plotted values are calculated means  $\pm$  standard error. Numbers in the graph indicate the number of males. "Not survived" implies that the individual was not seen or recaptured in the 1-3 years after spring 2.