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When does early-life telomere length predict survival? A case study and meta-analysis

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Abstract

Suboptimal conditions during development can shorten telomeres, the protective DNA caps on the end of chromosomes. Shorter early-life telomere length (TL) can indicate reduced somatic maintenance, leading to lower survival and shorter lifespan. However, despite some clear evidence, not all studies show a relationship between early-life TL and survival or lifespan, which may be due to differences in biology or study design (e.g., survival period measured). In superb fairy-wrens (*Malurus cyaneus*), we assessed whether early-life TL predicts mortality across different life-history stages (fledgling, juvenile, adult). However, in contrast to a similar study on a congener, early-life TL did not predict mortality across any life stage in this species. We then performed a meta-analysis including 32 effect sizes from 23 studies (15 birds and three mammals) to quantify the effect of early-life TL on mortality whilst taking into consideration potential sources of biological and methodological variation. Overall, the effect of early-life TL on mortality was significant, corresponding to a 15% reduction in mortality risk with each standard deviation increase in TL. However, the effect became weaker when correcting for publication bias. Contrary to our predictions, there was no evidence that effects of early-life TL on mortality varied with species lifespan or the period over which survival was measured. However, negative effects of early-life TL on mortality risk were pervasive throughout life. These results imply that effects of early-life TL on mortality are more likely to be context-dependent than age-dependent, although substantial power and publication bias issues highlight the need for more research.

KEYWORDS
fitness, juvenile, lifespan, longevity, mortality, review, superb fairy-wren, young

1 | INTRODUCTION

Early-life conditions can have long-lasting impacts on individual form, function and fitness (Eyck et al., 2019; Metcalfe & Monaghan, 2001). These impacts may arise when physiological dysregulation or damage during development permanently reduces health and increases the risk of mortality (Metcalfe & Monaghan, 2001). Alternatively, developing organisms may trade-off resources between growth,

somatic maintenance and survival at the expense of future fitness when conditions are suboptimal (Ricklefs & Wikelski, 2002; Stearns, 1992). The mechanisms connecting early-life conditions to fitness are not well understood because of the practical limitations in performing the necessary long-term studies and the difficulty in assessing physiological state. It has been suggested that telomere length (TL) might represent such a mechanism, since TL can reflect an individual's somatic state during development and connect early-life conditions to future fitness (Monaghan & Haussmann, 2006).

Telomeres are DNA–protein complexes on the end of linear eukaryotic chromosomes which contain the highly conserved repeating 5′-TTAGGG-3′ sequence (Blackburn, 1991). They function as a protective cap involved in maintaining chromosome integrity and cellular processes (de Lange, 2004). Telomeres also perform a vital role in cellular replication by acting as a buffer against the "end replication problem," so that after each replication cycle a portion of the terminal end of the telomere is lost (Olovnikov, 1973). In addition, oxidative stress can damage telomeres and contribute to their shortening rate (Armstrong & Boonekamp, 2023); this has been shown to be modulated by nutritional constraints, environmental conditions, sibling competition, infection and immunocompetence (Chatelain et al., 2019). Consequently, in the absence of telomere lengthening mechanisms such as telomerase, telomeres shorten over time (Remot et al., 2021). TL shortening may be greatest during development because of the high levels of cellular replication and high oxidative stress (Hall et al., 2004; Monaghan & Ozanne, 2018; Sheldon et al., 2022) (but see Remot et al., 2021). Once cellular TL reaches below a threshold, the cell initiates apoptosis or remains in an inactive senescent state, and the build-up of such cells with short telomeres is thought to contribute to the ageing process (Aubert & Lansdorp, 2008). Thus, TL is considered a biomarker of somatic state and remaining lifespan.

Previous meta-analytical evidence (Wilbourn et al., 2018) has shown that shorter telomeres predict a higher mortality risk in adults (>1 year) and juveniles (<1 year), although their study did not explicitly consider early life. When TL has been measured during growth, evidence for a relationship with mortality has been found in both birds and mammals (e.g., Cram et al., 2017; Eastwood et al., 2019; Heidinger et al., 2012), although there are also studies that find no relationship (Caprioli et al., 2013), or alternatively a relationship with telomere attrition (Boonekamp et al., 2014). It is far less common that longer telomeres are associated with reduced survival, but a few examples exist (McLennan et al., 2017; Wood & Young, 2019). Thus, the relationship between early-life TL and mortality risk, fitness or lifespan may vary between species. Even within species, the predictive capacity of early-life TL can change over time (Eastwood et al., 2019). Explanations for these within- and between-species inconsistencies are rarely considered but are important for understanding the causal mechanisms underlying the relationship between early-life somatic state and fitness.

There are several plausible reasons why the effect of early-life TL on mortality risk might vary between studies. The period over which mortality is measured is often determined by what is practically feasible and is typically close to when TL was measured with relatively short follow-up times (e.g., fledging mortality). However, studies measuring mortality early in life may be less likely to detect early-life TL effects on mortality due to high levels of stochastic mortality events in very young individuals, diluting TL effects. In this way, the strength of the relationship between early-life TL and mortality risk may be low initially and increase later in life. Such a pattern is predicted by the proposed mechanistic link between accumulations of short telomeres, cell death, and the progression of ageing and age-related increase in mortality (Boonekamp et al., 2013). Indeed, there is evidence for a stronger association between early-life TL and later life mortality in a study explicitly addressing this in a bird (purple-crowned fairy-wren, Eastwood et al., 2019). The relationship between early-life TL and survival may also be context-dependent and relate to environmental perturbations causing variation in individual condition. For example, in groups with experimentally increased sibling competition, and therefore reduced resources, the strength of the effect of TL on survival to fledging tends to be higher (e.g., Boonekamp et al., 2014; Costanzo et al., 2017; Nettle et al., 2013). This suggests that TL effects may only be present under suboptimal conditions when resources are limited. Both age- and context-dependent variables explaining the variation in TL-related mortality patterns may also be overlain by broader evolutionary or life-history factors such as lifespan or sex. Lastly, the sources of variation may be methodological and relate to differences in the technique used to measure TL, low statistical power or publication bias. Ultimately, resolving where between-study heterogeneity in TL effects on mortality arises, and whether it is dependent on age, environment, or methodological factors, is important for understanding how early-life conditions affect fitness.

This study investigates the relationship between early-life TL and lifetime mortality across different life stages using both a case study and a meta-analysis. Specifically, (i) we use a large data set to test for an association between nestling TL and survival across different life-history stages in superb fairy-wrens (*Malurus cyaneus*). Investigating TL-related survival in superb fairy-wrens is of interest because it is an ecological model species with detailed life-history information available (e.g., Cooper et al., 2021), with which we can compare findings of a similar study in a sister species, the purple-crowned fairy-wren (Eastwood et al., 2019). Additionally, we conducted a meta-analysis to (ii) test whether there is an overall significant relationship between early-life TL and mortality, and (iii) investigate plausible sources of heterogeneity between studies including age-dependent effects, differences between short- and long-lived species, phylogenetic effects, and methodological factors.

2 | MATERIALS AND METHODS

2.1 | Study species and fieldwork

Superb fairy-wrens are a small (8–11 g) cooperatively breeding passerine found in southeastern Australia. Breeding occurs during the spring and summer months (September–February) and a

dominant breeding pair can have multiple nesting attempts (one to four) with one to four nestlings each. While socially monogamous, they have high extrapair paternity rates with more than 60% of nestlings sired by males outside the social group (Hajduk et al., 2021; Mulder et al., 1994). The breeding pair often breed alone, but they can be supported by one to five subordinate "helpers" which assist in territorial defence against predators and in nestling provisioning. The presence of helpers increases survival of female breeders and is associated with greater breeding output (Cockburn, Sims, et al., 2008). Superb fairy-wrens are ideal for this study because of the detailed insights from 30 years of study into the complexity of their reproductive life history, including sex-biased differences in social behaviour, reproductive strategy and dispersal, and variability in mortality rates over lifetime. This allows us to relate early-life TL to individual survival using a sophisticated analytical design.

Here, we used data from a long-term study of an individually marked population of superb fairy-wrens at the Australian National Botanic Gardens in Canberra, Australia (35°16′S, 149°06′E). Detailed life-history data and DNA samples were available from six cohorts (1996–2001) totalling $n = 294$ nestlings. During each breeding season, territories were searched for nesting activity every 3 days so that accurate hatch dates could be determined (±1 day). At approximately 7 days (6–8) after hatching, nestlings were banded with a unique numbered band (Australian Bird and Bat Banding Scheme) and a combination of colour bands used for identification. Upon capture, blood was collected from the brachial vein using a heparinized capillary tube and frozen at −20°C. Sex was determined by observing sexually dimorphic plumage characteristics (breeding males moult a blue plumage; McQueen et al., 2017; Peters et al., 2000) or PCR (polymerase chain reaction) for nestlings that died prior to moulting into adult plumage (Griffiths et al., 1998). Individuals were followed from hatching throughout life until death or dispersal from the study area.

2.2 | Measuring survival in superb fairy-wrens

In superb fairy-wrens, survival decreases linearly with age and at similar rates in males and females, with a mean adult lifespan of 3.4 years in males and 3 years in females, and maximum lifespan between 10 and 12 years (Cooper et al., 2021). Nestlings fledge ~12 days after hatching and while they start to forage after about 1 week, they can receive part of their food via provisioning from adults for several weeks. Although occasional adult provisioning can persist for at least 6 weeks, we know that juvenile females can successfully disperse long distances in the fifth week after fledging, so we define independence as being achieved 28 days after fledging. Males remain highly philopatric throughout their lifetime, generally attaining their first breeding vacancy on their natal territory, or moving to a vacant immediately neighbouring territory when all males on that territory have died (Cockburn, Osmond, et al., 2008). All female juveniles disperse but in two distinct windows determined by the time of fledging (early or late fledglings) (Mulder, 1995). Early fledglings (before about December 15) disperse voluntarily from the natal group. However, later fledglings (fledged after December 15 up to the end of the breeding season in February) remain on their natal territory until the following breeding season, presumably because they are too young to disperse before winter. Population immigration (15%–20%) occurs over the same period as the emigration of juvenile female dispersers (Mulder, 1995). Females must establish a breeding position within their first year (individuals reach maturity at ~1 year; defined here as the following August), where most remain until death (80%; Cockburn et al., 2003). Adult dispersal is rare in both males and females and is usually limited to very short distances within the study population and is traceable (Cockburn et al., 2003; Double et al., 2005). Females can only gain a position by replacing a dominant female that has died or by splitting a territory, whereas dominant males are replaced by the next oldest male within the territory (Cockburn, Osmond, et al., 2008).

To identify whether the relationship between early-life TL and mortality risk changes with life stage, we compared three biologically relevant mortality measurement periods (Figure 1), survival from: (i) fledging to independence, (ii) independence to maturity and (iii) maturity to death. Population surveys were conducted throughout the year with the aim of seeing each bird at least every 2 or 3 days during the breeding season, and at least once per week during the nonbreeding season. If a bird is missed during a weekly census, the group is visited repeatedly until its disappearance is confirmed. Social status (i.e., breeder position or subordinate helper) was also confirmed by repeat censusing. Because males remain on (72%) or near their natal territory (95% of dispersers) (Cockburn, Osmond, et al., 2008), and observation probability is high, lifespan can be accurately determined across all life stages. However, to achieve the same survival accuracy for females, we excluded individuals where the survey could not distinguish between dispersal and death. All birds could be included in the fledging to independence mortality measurement period because this precedes dispersal. However, for the independence to maturity measurement mortality period, females fledging prior to December 15 were excluded, because most of these early-fledged females dispersed to a subordinate position in a neighbouring group, and often over long distances (Cockburn et al., 2003). Likewise, very few breeding individuals of both sexes disperse and, therefore, we have accurate lifespan information for these birds (Mulder, 1995). However, subordinate females were excluded from post-maturity analyses because their fate is less certain (Mulder, 1995). Whether an individual gains a breeding position or not is defined here as having at least one offspring.

2.3 | Telomere measurement

DNA was extracted from whole blood using an ammonium acetate protocol within a few months of collecting the sample (Bruford et al., 1998). For this study, we used archived DNA samples stored

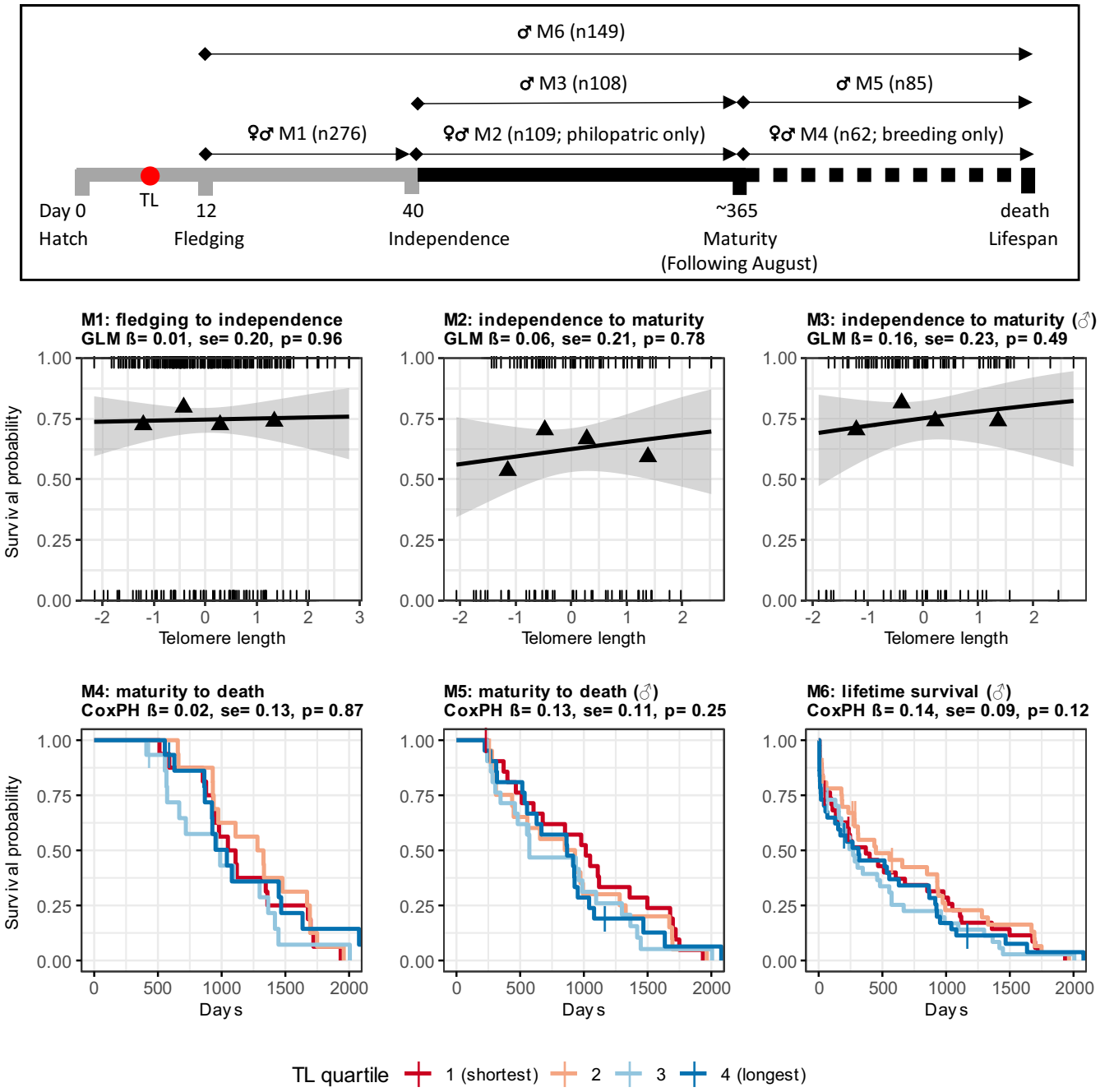

FIGURE 1 Early-life telomere length does not predict superb fairy-wren survival across different developmental, reproductive and life-history periods (M1–M6). The top panel shows a breakdown of the survival analysis structure according to superb fairy-wren life-stages. Arrow length represents the start and end to the survival period in days (e.g., M1 survival from fledging to independence; not to scale). M2 includes philopatric birds that fledged after December 15, to include females with known survival between independence and maturity. M4 includes breeding birds which rarely disperse and have known survival. M3 and M5 include similar or larger sample sizes compared to M2 and M4 due to the inclusion of males fledging early (prior to December 15) or helper (nonbreeding) males with known survival, respectively. The red circle labelled TL denotes approximately day 7 when a blood sample was collected for telomere measurement. Grey bars represent life stages when nestlings or juveniles are dependent on parental care and provisioning, whilst black refers to independent life stages. The dotted line represents adulthood and when they were followed until death. For survival models M1–M3 we used general linear models (GLMs; middle panels; Tables S1–S3) and M4–M6 Cox proportional hazards models (CoxPH; bottom panels; Tables S4–S6). The middle panels show raw data (circles) and quartile mean telomere length vs. mean survival (triangles). To graphically present M4–M6 we partitioned telomere length into quartiles and plotted the relationship with survival probability (Kaplan–Meier): the first quartile represents the shortest (red) and the fourth quartile the longest telomeres (dark blue). Sex was included in M1, M2 and M4 and breeding status in M5 and M6 as controlling variables.

in Low Tris-EDTA buffer (10 mM Tris–HCl, 0.1 mM EDTA; pH 7.5–8.0) at −20°C between the years 1996 and 2001. DNA concentration and signs of contamination (residual DNA extraction reagents or proteins) were assessed using a NanoDrop (ND-1000) with only samples with a 260/280 ratio between 1.6 and 2 and 260/230 ratio between 1.8 and 3.0 deemed suitable for telomere quantitative (q) PCR. Likewise, we assessed DNA quality on an 1.5% agarose gel and excluded samples based on the clear presence of a smear indicating sample degradation.

To measure TL, we optimized a qPCR method based on Criscuolo et al. (2009), which has been used in a closely related species, *Malurus coronatus* (Eastwood et al., 2018). The telomere and normalizing control gene (glyceraldehyde-3-phoshate dehydrogenase; GAPDH) assays were run on separate plates. The pipetting of each 96-well plate was automated the using an EpMotion 5075 (Eppendorf). Each 15-µl reaction contained: 7.5 µL of SYBR Green I (Roche), 18–20 ng of DNA, 300 nM of both GAPDH primers (Integrated DNA Technologies; GT2-GAPDH-forward 5′-CCATCACAGCCACACAGAAG-3′ and GT2-GAPDH-reverse 5′-TTTTCCCACAGCCTTAGCAG-3′; Atema et al., 2013) or 500 nM of both telomere primers (Tel1b 5′-CGGTTTGTTTGGGTTTGGGTTTGGGTTTGGGTTT-3′ and Tel2b 5′-GGCTTGCCTTACCCTTACCCTTACCCTTACCCTTACCCT-3′). For each run, the samples and a no-template control (nuclease-free water; Ambion) were run in duplicate, whilst an interplate control (gold standard containing multiple individuals of different ages) was run in quadruplicate. The reaction was performed using a LightCycler 480 (Roche) machine under the following conditions: 95°C for 15 min, followed by 25 cycles of 15 s at 95°C, 30 s at 56°C, 30 s at 72°C for the telomere assay, and 95°C for 15 min, followed by 40 cycles of 15 s at 95°C, 30 s at 60°C, 30 s at 72°C for GAPDH. If within-plate repeats differed by more than 0.5 the sample was excluded. A melt-curve analysis followed both reactions to ensure the correct product was amplified. Relative telomere length (rTL) was calculated following Eastwood et al. (2018), which accounts for variations in well efficiency (mean [SD] efficiency telomere 94.13 [3.37]%, GAPDH 97.34 [2.72]%), DNA concentration, and the between-plate control. To calculate assay repeatability, we ran extracted DNA of $n = 39$ samples two to three times on separate plates ($n = 110$ rTL measures). Using the RPTR package (Stoffel et al., 2017), we confirmed that the repeatability of rTL was high (repeatability = 0.85, SE = 0.07).

When measuring telomere length using qPCR, this yields a combined measurement of terminal telomeres and interstitial telomeric sequences (ITS). To assess the effect of ITS on TL estimates we compared TL measurements using telomere restriction fragment (TRF) between nondenatured and denatured DNA. On average, telomeres were 1130 ± 360 bp (SD) longer when using a denatured gel compared to a nondenatured gel (nondenatured: 10,450 ± 1053 bp; denatured: 11,580 ± 1165 bp), and these values were highly correlated ($r = .96$, $n = 24$). Thus, while qPCR-based telomere measurements will overestimate TL due to inclusion of ITS, this does not affect our results which are based on relative values.

2.4 | Statistical analysis

Statistical analyses were conducted in R version 4.0.5 (R Core Team, 2016), and results presented using the GGPLOT2 package (Wickham, 2016). TL measurement using qPCR is prone to methodological sources of error which could reduce the power to detect a relationship between survival and TL (Lindrose et al., 2021). Here, we address the issue by calculating the TL residuals from a linear regression controlling for nestling age (factor: 6–8 days) and the number of days between blood sample collection and TL qPCR (mean 7289.21, SD 676.53 days standardized). qPCR plate ID was included as a random term in a linear mixed effects model but was excluded in favour of the simpler regression above as it explained zero variance. In this model, 7-day-old (estimate [SE] = −0.41 [0.15], $t = -2.81$, $p = .005$) and 8-day-old (−0.40 [0.18], $t = -2.24$, $p = 0.03$) nestlings had shorter telomeres on average compared to day 6 nestlings whilst the effect of storage time (days between sampling and telomere measurement) was not significant (−0.01 [0.06], $t = -0.16$, $p = .88$). Prior to analysis, complete nest failures were excluded because they did not fledge and probably died due to stochastic, nontelomere-related, mortality (e.g., predation or extreme weather events). Model residuals were standardized to have a mean of zero and a standard deviation of one (Verhulst, 2020) for use in all subsequent analyses. We performed six models which tested the relationship between early-life TL (independent variable) and mortality (dependent variable: lived = 1, died = 0) across different mortality measurement periods and only included birds with highly accurate mortality information (i.e., excluding juvenile and nonbreeding females; Figure 1): survival from fledging to independence (model 1), survival from independence to maturity including late-fledged (>December 15) birds only (model 2), survival from independence to maturity including males only regardless of hatch date (model 3), survival from adulthood until death within breeding birds only (model 4), survival from adulthood until death with males only but including breeding and nonbreeding individuals (model 5), and survival over the entire lifespan of males (model 6). In models 1–3, we first fitted a generalized linear mixed effects model (GLMM) with logit link function and optimizer "bobyqa" using the function glmer (LME4 package, version 1.1–28; Bates et al., 2015). Cohort was included as a random effect but explained zero variance in models 1 and 3. In which case, we applied a generalized linear model (GLM) using the R function glm. For models 4–6, we fitted Cox proportional hazard (PH) models using the coxph function in the SURVIVAL package (Therneau, 2022). These models were used to test for the relationship between rTL and adult lifetime survival using the lifespan data with individuals with unknown fates censored (i.e., unable to distinguish between death or dispersal).

The relationship between TL and survival may differ between male and female fairy-wrens due to sex-specific differences in physiology or behaviour. For example, males moult annually into a bright blue plumage which makes them more conspicuous and wary of predators (McQueen et al., 2017), females are driven to disperse whilst males remain philopatric, and males have higher levels of testosterone which can be immunosuppressive (Peters, 2000).

Therefore, we included as a fixed factor sex and an interaction term between sex and rTL in all models. However, in all cases the interaction was nonsignificant and removed from the final models. Similarly, in models 5 and 6, we included final breeding status (helpers [that produce zero offspring] = 0, breeders [that produce at least one offspring] = 1) as a fixed factor to account for differences in social and reproductive effort. An interaction between breeding status and rTL was not significant and subsequently removed from the final model. In each survival period, we excluded nestlings that had died in the preceding survival period (e.g., model 2 does not contain individuals that died in model 1).

2.5 | Meta-analysis and meta-regression

To assess the overall effect of early-life TL, which we define as a measure during the growth period prior to independence, on the risk of mortality and the sources of variance across studies, we adopted a meta-analytical approach. First, we conducted a comprehensive literature search following the preferred reporting items for systematic reviews and meta-analyses (PRISMA; O'Dea et al., 2021—for PRISMA flowchart see Figure S1). Our search was conducted in the *SCOPUS* and *ISI Web of Knowledge* databases, using the following search terms: "telom* AND (surviv* OR longevity OR lifespan OR life span OR life expectancy OR mortality OR fitness OR reproductive success) AND (early* OR juvenile OR young OR offspring OR nestling OR hatchling OR pup OR calf)". In each search, we applied the broadest possible criteria to ensure that all relevant articles were captured. Additional searching by backward and forward screening of reference and citation lists did not reveal any additional papers, suggesting our search terms and screening strategy were sufficient. Between the initial search and publication, we monitored the field for new papers using Google alerts using the broadest possible term "telomere." We are not aware of any additional relevant papers published during this period. The total number of studies identified through our search was $n = 4937$ (Figure S1).

After a preliminary screen for relevance, we assessed each study's eligibility based on the following criteria: (i) empirical research, (ii) nonhuman vertebrate organisms, (iii) nongenetically modified organisms (i.e., excluding domesticated species, transgenic laboratory animals), (iv) observational or experimental (including captive animals), (v) measures survival, lifespan or reproductive success, and (vi) measures TL early in life. Early-life TL was defined as a TL measure from any tissue that was collected during an early developmental stage that included growth or some level of parental care (e.g., food provisioning). Justifications for study inclusion or exclusion can be found in Figure S1, but see published literature search data. From each study we collected information on the species, TL measurement method (qPCR or TRF analysis), maximum and average lifespan, and the mortality period measured for each effect (beginning and end). In order of preference, we obtained maximum and average lifespan (years) as recorded within the literature, publicly available data, or the AnAge database if based on banding records in wild populations (http://genomics.senescence.info/species/).

From each study we obtained empirical data including individual early-life TL, sex and mortality information (binary or lifespan). Because the methodology of these studies is too diverse and complex for a meta-analysis and regression, we re-analysed published data sets using standardized statistical methods to calculate effect sizes, following the methods of Wilbourn et al. (2018), enabling us to directly compare findings and integrate their standardized estimates ($n = 10$) into our data set. We found 13 additional studies with publicly available data. Prior to obtaining each effect size, the data for each study were appropriately formatted and TL was scaled to have a mean of zero and standard deviation of one for the purpose of standardizing the effect sizes among studies (Verhulst, 2020). For each study period, we calculated the effect size of standardized TL on mortality (natural logarithm of the hazard ratio, lnHR) using a Cox PH regression. A negative lnHR estimate indicates that on average individuals with a longer TL value have a lower mortality risk in comparison to those with shorter telomeres. For each study, the mortality measurement period could be binary (survived = 0, died = 1) or measured as mortality over the lifetime of individuals (lifespan in years). In the first instance, the start time was at TL sampling and end time was the end of the survival period. When lifespan or time of last observation data were available, individuals still alive or missing at the study conclusion were censored. Where possible, we adopted the same a priori data partitioning of mortality as the authors. For example, if the authors tested the relationship between early-life TL from two different time points and mortality, we obtained both lnHR estimates (e.g., Quque et al., 2021; Wood & Young, 2019). Also, we replicated the analyses of authors who published early TL effects for multiple mortality measurement periods (e.g., this study). However, we did not include instances of any *post hoc* exploratory analyses.

The meta-analysis and regression were performed using the METAFOR package (Viechtbauer, 2010). We adopted a multilevel random-effects design using restricted maximum likelihood estimation to account for the nonindependence of multiple effects within studies and the replication of species across studies. In the null model, we included study ID, effect ID and species ID as variance components in an intercept-only model. In a separate model we also accounted for phylogenetic nonindependence (Hadfield & Nakagawa, 2010; Nakagawa et al., 2017) using the relatedness estimates derived from a species-level phylogeny obtained using the ROTL package (Michonneau et al., 2016). Using ROTL, we assembled taxonomic information stored on the Open Tree of Life database (for details see: Michonneau et al., 2016) and calculated branch length ("grafen" method) from a phylogenetic tree using the APE package (Paradis et al., 2004). The direction and strength of the TL–survival relationship may be due to differences in selection pressures between the sexes which can potentially influence TL-related mortality (Heidinger et al., 2021; Wilbourn et al., 2018). Therefore, we partitioned the meta-analysis into males and females separately and obtained overall estimates and heterogeneity statistics for each subgroup. Between-study heterogeneity was tested

using a Cochran's Q test statistic and by calculating I^2 following Nakagawa and Santos (2012). Cook's *d* was calculated to determine whether effect sizes disproportionately influenced overall effect (no evidence as indicated by all Cook's $d < 0.4$). Publication bias was investigated by visually inspecting a funnel plot to identify asymmetry and by including variance in the null model above (Egger's test equivalent).

The full model had the same random structure as the null model but included moderator variables predicted to influence the study effects. To test whether the period over which mortality was investigated influenced the effect of early-life TL on mortality, we categorized each mortality period into one of four categories: growth to independence, growth/independence to adulthood, post-maturity and lifetime. For example, some studies test whether early-life TL predicts survival from a time point during growth until adulthood (growth/independence to adulthood), whilst others test whether early-life TL predicts survival throughout adulthood excluding those that died prior to adulthood (post-maturity). Whether early-life TL predicts mortality may relate to species longevity with TL senescence evolving differently between long- vs. short-lived species. Average adult lifespan data were available for 74% of species but adult lifespan was highly correlated with maximum lifespan (Pearson's $r = .79$, $n = 13$, $p = .001$). Therefore, only maximum lifespan (mean centred) was included in order to use the complete data set. Furthermore, we tested for an association between year of publication (mean centred) and mortality risk to account for the phenomenon whereby positive or confirmative results are published earlier. Interpretation of the continuous scaled moderators is based on the change in lnHR with each unit change in the moderator variable holding all other variables constant. TL measurement method (fixed factor with two levels: qPCR or TRF) was included because of differences in accuracy and precision of the methodology and previous work has demonstrated its importance (Wilbourn et al., 2018). To ensure that collinearity between the moderator variables did not influence the results we calculated the variance inflation factor and ran the model with each variable separately.

3 | RESULTS

3.1 | No relationship between early-life TL and mortality in superb fairy-wrens

Across all periods, we found no evidence to suggest that early-life TL predicts mortality in superb fairy-wrens (Figure 1). We hypothesized that the strength of the relationship between TL and mortality might change depending on the mortality period measured and its associated biology. However, there was no effect detected across early (Tables S1–S3) or late life stages (Tables S4–S6). Neither sex with nor without the interaction with TL had a significant effect on mortality. Within adult males only, those with a breeding position had a higher probability of survival compared to subordinate helpers (Tables S5 and S6).

3.2 | Negative effect of early-life TL on mortality risk across species

Including the present study, our literature review identified $n = 24$ studies matching the inclusion criteria (PRISMA diagram Figure S1). Because only a single reptile study was found (Ujvari & Madsen, 2009) and reptiles may have different telomere dynamics, we excluded this study from the meta-analysis and focused on endotherm species. The final sample size included 23 studies with 32 effect sizes from 18 species (15 birds and three mammals, see Figure 2). We found 11 negative effect sizes with confidence intervals excluding zero, 20 effect sizes close to zero and, notably, a single study (two effect sizes) identifying a positive relationship (Wood & Young, 2019) between early-life TL and mortality risk (Figure 2). By re-analysing data sets as described above, we are potentially oversimplifying each model by failing to account for nonindependence or methodological factors. However, in all comparable cases (24 out of 32), we found that the Cox PH regression effect sizes were in the same direction 88% of the time (21 out of 24). Those three effect sizes that differed in the direction of the effect were near zero and not significant (i.e., showed consistent null results). A further eight effect sizes were not comparable to the original study estimates because they included interactions, the study investigated nonlinear effects or the data set included TL measures from different ages.

The intercept-only model, including the variance components study ID, effect ID and species ID, showed a statistically significant overall negative effect (estimate [SE] = −0.16 [0.07]; Table S7), with longer early-life TL associated with a decrease in mortality risk (overall estimate in Figure 2). The hazard ratio can be interpreted biologically as the risk of mortality decreasing by 15.1% for each standard deviation increase in TL. However, across studies there was a considerable level of heterogeneity in the relationship between TL-related mortality ($Q_{(df = 31)} = 133.87$, $p < .001$). Overall, variance among studies was high ($I^2 = 88.6\%$) which was mostly explained by species identity ($I^2 = 57\%$), followed by study ID ($I^2 = 26.6\%$) and effect ID ($I^2 = 4.99\%$). Phylogeny explained little variation ($I^2 = 6.5\%$) when included in the null model (Table S8) and did not improve the model fit (Akaike Information Criterion corrected difference) $\Delta AIC_c = 2.81$ compared to the null model). Both with and without phylogeny, the term species ID explained the most variance (>50%) suggesting a nonphylogenetic component of the term species is important (Tables S7 and S8).

3.3 | Evidence of publication bias

Visual inspection of the funnel plot shows asymmetry towards a negative lnHR effect size (Figure 3) which is supported by Egger's test (intercept = -0.34 ± 0.09, $z = -3.9$, $p < .001$; performed by including the inverse standard error as a moderator in the null model). To explore the impact of publication bias on the overall effect, we performed a Bayesian mixed-effects meta-analysis (Table S11), using a trim-and-fill method to correct for the publication bias

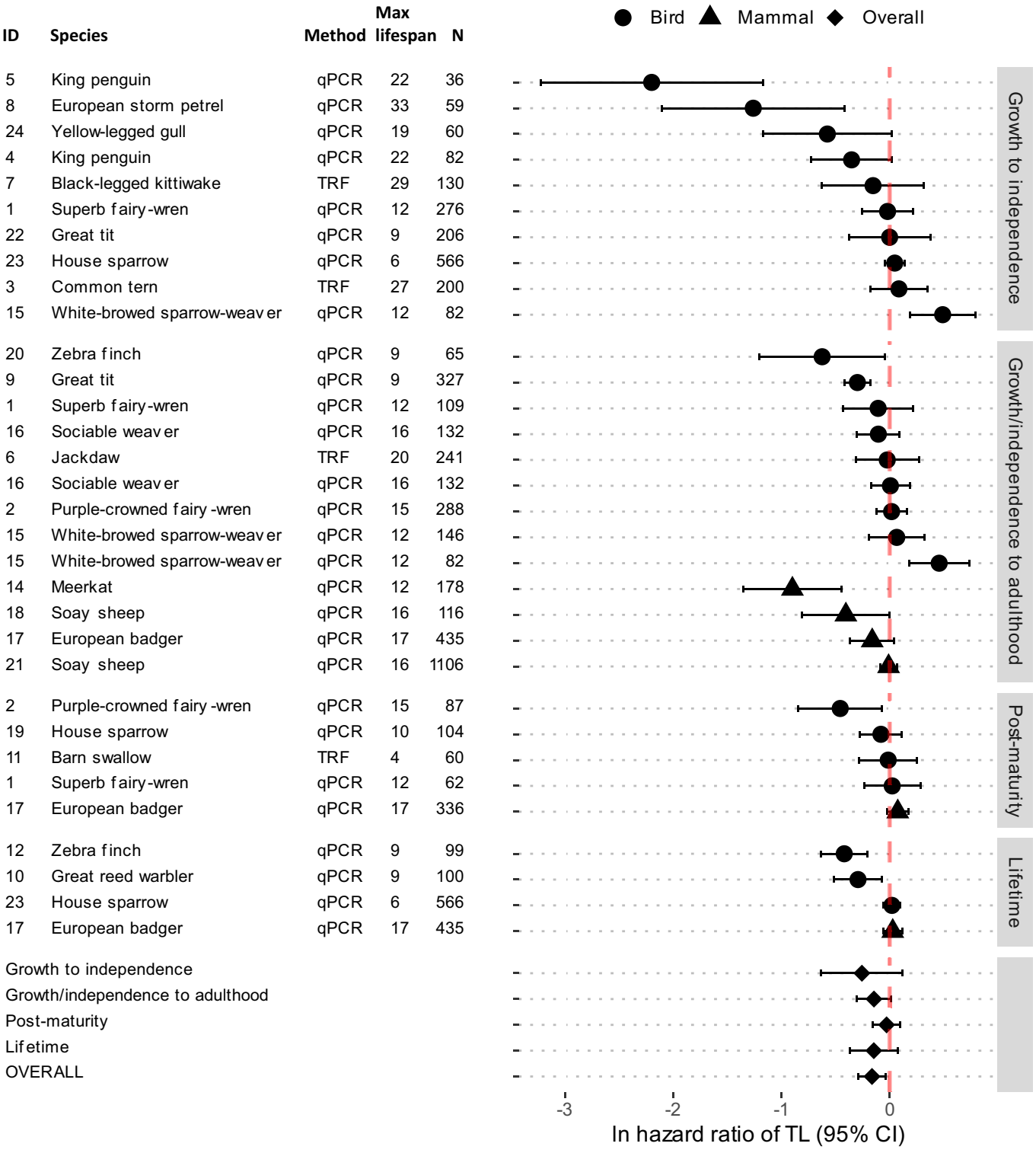

FIGURE 2 Overall, mortality risk (lnHR) is slightly lower for individuals with longer early-life telomeres. The side bar refers to the species-specific life stage representing the period over which survival was measured (Growth to independence = early life survival between growth and independence, Growth/independence-adulthood = survival spanning early life into adulthood, Post-maturity = later life survival after maturity, and Lifetime = survival across the entire lifespan from growth until death). Method indicates the telomere measurement technique (qPCR: quantitative real-time PCR, or TRF: telomere restriction fragment length analysis) and *N* denotes the sample size. ID references for each study, in no particular order: 1, this study; 2, Eastwood et al. (2019); 3, Vedder et al. (2017); 4, Stier et al. (2014); 5, Geiger et al. (2012); 6, Boonekamp et al. (2014); 7, Young et al. (2017); 8, Watson et al. (2015); 9, Salmón et al. (2017); 10, Asghar et al. (2015); 11, Caprioli et al. (2013); 12, Heidinger et al. (2012); 14, Cram et al. (2017); 15, Wood and Young (2019); 16, Quque et al. (2021); 17, van Lieshout et al. (2019); 18, Fairlie et al. (2016); 19, Heidinger et al. (2021); 20, Reichert et al. (2015); 21, Froy et al. (2021); 22, Grunst et al. (2019); 23, Pepke et al. (2022); 24, Noguera et al. (2020). Dashed red line highlights an lnHR estimate of zero. Symbols refer to taxon and summary estimates (see key).

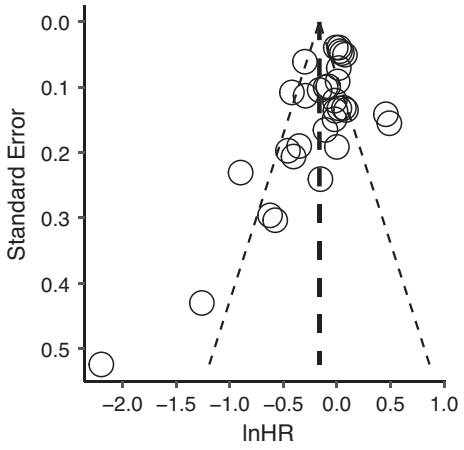

FIGURE 3 Funnel plot of asymmetry towards the over-reporting of negative lnHR results, indicating the presence of publication bias. The plot shows several studies with large negative effect sizes and high standard errors, which appear to skew the overall estimate. This bias was confirmed using Egger's test and the overall estimate was not robust to a trim-and-fill method (Figure S2). Dashed lines represent the mean estimate from the null model (bold) with the 95% confidence intervals.

(Table S11). This analysis was implemented using the MCMCGLMM package (Hadfield, 2010) as the trim-and-fill method was not available for multilevel random effects in the METAFOR package. We found that the overall effect of TL estimated using the Bayesian model was similar but with wider confidence intervals (intercept = −0.20, lower CI = −0.55, upper CI = 0.15, p = .15). To determine publication bias we extracted residuals from this model, which include the effects of random factors and applied Egger's test. This test also showed significant evidence for funnel asymmetry (intercept = −0.84, SD error = 0.30, t = −2.77, p = .01). Trim-and-fill revealed six effects missing on the right side of the funnel, and when these were 'filled in,' the trim-and-fill corrected meta-analytical mean was closer to zero (corrected meta-analytical mean = −0.17, 95% CI −0.52 to 0.18; Figure S2). This suggests that if publication bias is the cause of the funnel asymmetry observed, then the overall effect of TL on the risk of mortality is closer to zero than initially estimated. Under this Bayesian framework, the global model (Table S11) also showed similar effects compared to the global model using METAFOR (Table S7). However, phylogeny appeared to explain more study heterogeneity (I^2 = 29.4 vs. 6.5%).

3.4 | Early-life TL predicts mortality throughout life but is not age-dependent

We included the following moderators which we hypothesized to influence the strength and direction of lnHR effect size: telomere measurement method (qPCR or TRF), period over which mortality was measured (growth to independence, growth/independence to adulthood, post-maturity and lifetime), species maximum lifespan and year of publication (Table S9; Figure S3). When including moderators in the model, the random term species explained zero variance and was subsequently removed from the model. Residual heterogeneity of the full model was still high ($Q_{(df = 25)}$ = 92.86, p < .001; Table S9) and explained some of the variance ($Q_{M(df = 6)}$ = 15.61, p = .02; Table S9). The decrease in between-study heterogeneity was negligible (I^2 = 82.4%). The variance inflation factors for each moderator were low (1.2–1.7), indicating negligible multicollinearity impacts on moderator effect sizes.

We expected that lnHR estimates would be more negative when mortality was measured later in life because the impact of shorter TL might be associated with remaining lifespan. In the global model, the period over which mortality was measured had no statistically significant effect on the relationship between TL and mortality risk (Table S9). The largest effects were observed when mortality was measured from growth to independence, but overall these differences were negligible, and the confidence intervals overlapped when plotting overall effects within each mortality period category separately (Figure 2). Negative effects were observed across all mortality period categories, which implies that effects of early-life TL on mortality risk are persistent throughout life (Figure 2). Maximum lifespan was not a significant moderator of effects (Figure S3), suggesting the strength of the association does not vary between short- or long-lived species.

Telomere measurement method was a significant moderator of effect sizes, with studies using TRF having estimates closer to zero compared to those using qPCR (Table S9; Figure S3). However, the significance of TL measurement method should be viewed with caution due to the paucity of studies using the TRF (four TRF vs. 19 qPCR studies). Year of publication was positively associated with effect size, indicating that the magnitude of mortality risk effects became closer to zero with time since first publication (Table S9; Figure S3). Apparently, earlier studies tended to be those with clear and significant relationships between TL and survival.

To test for potential sex differences in the relationship between TL and mortality, we calculated an estimate and confidence intervals for each sex (male or female) separately using the null model. The overall effect tended to be higher in females (−0.11 ± 0.06, p = .06) compared to males (−0.07 ± 0.04, p = .11). However, this difference was small and confidence intervals overlapped (Table S10).

4 | DISCUSSION

Despite predictions to the contrary, we found that early-life TL does not predict mortality in superb fairy-wrens. We hypothesized, based on earlier findings in a sister species (purple-crowned fairy-wrens), that the strength of the association would increase with age (Eastwood et al., 2019), but no such pattern was evident in the superb fairy-wren (Figure 1). Nonetheless, across the literature, our meta-analysis showed a significant overall effect of early-life TL on mortality risk, corresponding to a 15% reduction in mortality risk with each standard deviation increase in early-life TL. This is consistent with previous findings that longer TL is associated with a

lower mortality risk regardless of when TL is measured (Wilbourn et al., 2018). However, the overall estimate in our study was not robust when adjusting for the biased publishing of confirmative results. Also against our predictions, the heterogeneity of between-study effects was not explained by the period over which mortality was investigated, but instead we observed that early-life TL predicted mortality throughout all measurement periods including over the entire lifetime. The reason why TL may predict mortality in some species but not others is still unknown, although our study offers some insight into several alternative hypotheses.

The relationship between early-life TL and mortality risk might increase with advancing age for two reasons. One hypothesis is that weaker effects of early-life TL on early-life mortality arise due to differences in stochastic mortality events unrelated to TL. For instance, in purple-crowned fairy-wrens, early-life TL did not predict mortality in the first year of life, when extrinsic deaths are at their highest, but negatively predicted mortality in adulthood, when we assume mortality to be less stochastic (Eastwood et al., 2019). However, this pattern was not apparent in superb fairy-wrens, despite mortality levels depending similarly on age (juvenile and adult) and breeding status. An alternative hypothesis is that early-life TL could indicate remaining lifespan because TL is mechanistically involved in organismal ageing. In this scenario, individuals with shorter TL early in life reach a critical TL sooner relative to those with long early-life TL. Such an effect of TL due to declining somatic redundancy would be less important for juvenile or young adult survival and it would result in stronger effects of TL on mortality later in life (Boonekamp et al., 2013; Monaghan & Ozanne, 2018). Our data do not support this hypothesis, but instead suggest that early-life TL predicts mortality equally at any stage of life. In fact, contrary to our predictions, the strongest effects identified in the meta-analysis occurred prior to independence and not in the post-maturity mortality period. Overall, our findings indicate that early-life TL can predict mortality at any life stage but that the strength of the association does not necessarily change with age, and this can vary between closely related species.

An alternative explanation for variation in effects of early-life TL on mortality risk is that the association is context-dependent. For example, the association between early-life TL and mortality might vary between the sexes due to differences in physiology, behaviour or life history. Such sex-specific variation is present in house sparrows (*Passer domesticus*), where females, but not males, with longer TL had higher fitness (Heidinger et al., 2021). Although we found a suggestion of a stronger relationship between early-life TL and mortality in females, this sex difference was small, so sex differences are unlikely to be pervasive. Alternatively, the relationship between TL and fitness may also vary according to the environmental conditions experienced. It is known that early-life TL indicates somatic state and health, which can be diminished by experiencing oxidative stress (Reichert & Stier, 2017), nutritional constraints (Cram et al., 2017), immune function (Roast et al., 2022), pathogen infection (Asghar et al., 2015), competition (e.g., Boonekamp et al., 2014; Costanzo et al., 2017; Nettle et al., 2013), predation threat (Kärkkäinen et al., 2019) or environmental perturbations (Eastwood et al., 2022). An example of environmental conditions affecting the relationship between early-life TL and mortality is found in king penguin (*Aptenodytes patagonicus*) chicks. Here, late-hatched chicks, which experienced poor conditions and higher physiological strain, showed a strong relationship between shorter early-life TL and increased mortality, but in early-hatched chicks, which experience less severe conditions, no such effect was visible (Stier et al., 2014). In addition to environmental conditions, the relationship between early-life TL and mortality may depend on intrinsic individual life history, such as inbreeding (Bebbington et al., 2017), sex (Watson et al., 2017), growth (van de Crommenacker et al., 2022) or the extent to which TL is canalized. For example, it was suggested that in common tern (*Sterna hirundo*) nestlings (Vedder et al., 2017) resources were prioritized for TL maintenance, resulting in a low variance in TL compared to growth. Subsequently, the prioritization of telomere maintenance could reduce covariation between TL and survival. It is then conceivable that extreme conditions may promote the breakdown of prioritization of telomere maintenance, increasing TL variance and its covariation with survival. Whether studies detect a survival effect of TL would, therefore, depend on the variation in resources available for telomere maintenance. In other words, TL and mortality may covary more when individuals within the population experience poor conditions but the effect is close to zero when conditions are adequate. This conjecture is possibly supported by differing results in the two fairy-wren species, since the purple-crowned fairy-wren is threatened due to habitat degradation and superb fairy-wrens are not (although both study populations are relatively stable). Further research into telomere dynamics, intrinsic individual qualities (e.g., growth and inbreeding) and how they covary with survival under different conditions could provide insight into the importance of environmental conditions and condition-dependent or canalized trait effects. However, these factors are unlikely to be the only important considerations.

Species-level variables account for a large proportion of the heterogeneity between studies we identified: over half of the variation was attributed to species in the null model and appeared to be independent of phylogenetic effects (although phylogenetic effects were more prominent in the Bayesian meta-analyses). One obvious difference between species is their lifespan, which might relate to telomere dynamics (Tricola et al., 2018). However, we found no differences between short- and long-lived species in the effect of TL on mortality (similar to Wilbourn et al., 2018). However, it is important to note the disproportionate number of short-lived compared to long-lived species in our data set (only five of 19 species with maximum lifespan >20 years). Our study might underestimate the importance of species or phylogeny, since only a single study investigated early-life TL effects on mortality in reptiles (Ujvari et al., 2017; Ujvari & Madsen, 2009) and none in aquatic species, despite evidence that TL dynamics in these taxa may differ from those in mammals and birds. While limited sampling of species, even within birds, may prevent the detection of an evolutionary component, these species-level differences might also be a result of methodological differences between studies.

Low sample sizes and methodological limitations could increase the risk of both type I and type II errors, with the former more likely to be published. Indeed, studies with the largest lnHR effect sizes tended to have larger confidence intervals (Figure 2). In addition, high levels of stochastic mortality or variation in adult TL dynamics (e.g., due to variation in oxidative damage) may reduce statistical power and mask TL-related mortality effects. This is especially the case when mortality is measured over long time periods (e.g., growth to adulthood or lifetime) in wild populations (but see Eastwood et al., 2019). In addition, statistical power may be reduced by the common use of qPCR to measure telomeres which are known to be susceptible to both pre-analytical and precision error (Lindrose et al., 2021). Similar to a previous meta-analysis (Wilbourn et al., 2018), we found TL–mortality effect sizes tended to be larger in studies using qPCR compared to TRF (Table S9; Figure S3), although this contrast is based on only four TRF studies. It is also evident that earlier studies tended to have stronger TL effects on mortality risk, but that more recent publications suggest the estimate is closer to zero (Figure S3), confirming a commonly observed prior publication of positive results (Koricheva & Kulinskaya, 2019; Nakagawa et al., 2022). Importantly, the overall effect of TL on mortality risk was close to zero after adjusting for publication bias.

In conclusion, the present study demonstrates that the effect of early-life TL on mortality risk is likely to be negative, pervasive throughout lifespan but small. Definitive inference on the causes of variation in the ubiquity of early-life TL as a biomarker of fitness prospects is difficult given publication bias, methodological constraints, and the limited and heterogeneous data set. This limits development of greater understanding of potential common mechanisms determining early-life TL and subsequently fitness prospects. To resolve these issues, more studies are required incorporating survival across multiple life stages and across different species varying in life history and social and environmental conditions. Understanding the heterogeneity of TL-related mortality effects will be important for understanding the relationship between somatic state and fitness.

AUTHOR CONTRIBUTIONS

J.E, S.V., A.C. and A.P. were involved in study design; A.C. conducted the fieldwork and collected samples; A.D. performed the laboratory work; J.E. performed the statistical analysis with input from A.C., S.V. and A.P; J.E. and K.D. performed the meta-analysis; all authors contributed to writing the manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors declare no competing interests.

DATA AVAILABILITY STATEMENT

All data presented in this paper are available from the DRYAD repository: https://doi.org/10.5061/dryad.hmgqnk9mw.

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