Thank you for the opportunity to review “Telomere length vary with sex, hatching order and year of birth in little owls”. In this study, variation in TL due to sex, hatching rank, body condition and environmental factors was investigated in a wild population of little owls. The study found females to have longer TLs compared to males. Nestlings with higher body condition also had longer TLs, independent of the sex of the individual. Finally, in large clutches, it was found that last-hatched nestlings had shorter TLs. These results present an interesting case study of how variation in biological factors such as sex and body condition influence TL in the wild. This paper is a nice addition to the burgeoning literature on how early-life conditions experienced by wild populations and how key biological variables such as sex and body condition affect TL dynamics.

My major comments are 1) the lack of information provided in the methods section relating to the decisions made during statistical modelling as well as 2) the models investigating how environmental factors influence TL where sex, body condition, year etc were not fitted as fixed effects in these models when they should have been. Re-running these models to include these biological variables as predictors would be required to infer the effects of environmental factors on TL. Some clarity in the ideas expressed in the introduction and discussion sections of the manuscript would also improve the readability of the paper.

Major Comments:

L.157 – Were models including year/cohort as a random effect tested? Not accounting for year-to-year variation could potentially have significant effects on the final results and inference. Please explain why year was not included in the models and how much variation is explained by year across the different models.

L.165 – Why does this model not include cohort as a fixed/random effect but the other models (RTL, environment) account for variation in birthyear? Please justify the exclusion of birthyear in some models but not others.

L.173 – Please specify why this model was run in the first place? Presumably, to test for environment-dependent effects on body condition. Also, in these models, were other fixed effects (such as sex, body condition, cohort) also included? Please explain why these predictors were excluded from this model. Is it because a previous model with SMI as a response variable found none of these variables to explain variation in the response. If so, then clearly state that. Re-running this model investigating how environmental variation explains variation in body mass, over and above effects due to sex, body condition, cohort etc would be great to show.

L.190 – How can it be explained that none of the biological variables (such as sex, hatching order, rank, cohort etc) explain variation in body condition/SMI but explain variation in TL? Isn’t it more obvious, biologically speaking, to expect these factors to influence body condition/SMI more than TL? Please discuss the lack of consistency in results here in the discussion. I was also wondering if body mass is used as a response variable instead of SMI, whether the results are consistent?
L.214 – I think it would only make sense to interpret any environment-related effects on TL after accounting for variation due to biological factors such as sex, hatching rank and cohort which were shown in the previous model to influence TL.

L.312 – Ideally, these models would need to be re-run after accounting for variation due to sex, hatching order and cohort and checking to see whether any environmental variables still crop up as significantly explaining variation in TL, over and above these obvious biological factors which were previously shown to affect TL in this population.

Minor Comments:

L.45 – Was the primary interest in evolutionary biologists to study TLs solely driven by stress-related effects on TL? I surmised it was also the fact that TL declines with age and therefore being involved with lifespan/the ageing process at the cellular level (Monaghan., 2010). Rephrasing the sentence to convey this would be great.

L.54 – I find this sentence confusing – is the manuscript referring to pleiotropy in the context of the antagonistic pleiotropy theory of ageing and therefore are being explicit about genetics (Williams., 1957)? Or is the paper referring to tradeoffs between life-history traits (i.e. investing resources in growth vs other traits; Stearns., 1992)? Please provide more clarity.

L.57 – Which physiological traits are being referred to as both here? I take that one of them is growth but what’s the other – cell division? It is not clear.

L.58 – I find this sentence confusing and too long, would it be more straightforward to say, “Studies have shown juveniles exposed to challenging conditions in early life to have shorter TL. This could be due to reduced investment in somatic maintenance as a consequence of low resource availability when conditions are harsh”.

L.66 – Instead of saving telomere length, would preserving TL work as a better alternative?

L.72 – What is the multivariate egg concept? Please elaborate and also provide clarity in this sentence. Is the paper intending to convey that egg-associated traits could be positively or negatively correlated with each other and may influence future offspring phenotype? If so, please rephrase sentence to reflect that.

L.85 – Please include reference to the study.

L.85 – I do not understand this sentence – is the paper trying to convey that many studies have shown negative consequences of telomere erosion during growth on future individual fitness. Please state that clearly if so.

L.89 – This sentence is very long and difficult to follow. And it is not clear to me why variability in TL within clutch is or is not an epiphenomena? Please use simple and plain language to convey the main ideas.
L.123 – How accurately does this formula capture the actual age of the bird? Please provide more information.

L.125 – What is ventral covert? Is this blood sampled from covert feathers?

L.128 – Did all 39 broods have more than 1 chick? Please specify the number of broods that were excluded/part of the final analysis.

L.138 – Was the final nest environment category assigned based on which of the groupings (buildings, meadows, crops… etc) covered the most area (in m²) within the buffer zone? Was it always clear that one category covered more area than another category? If not, what was done in such a scenario?

L.167 – Why was SMI used as a measure of individual body condition instead of the body mass of nestlings? Please justify the use of this measure instead of body mass. Were the results consistent when body mass was used instead?

L.171 – In total, how many models were compared using AICc? Please include this information.

L.172 – How many models were part of the set of top models within 2 AICc of each other? Additionally, how was model averaging performed? Were specific R packages (MuMin etc) used to obtain model-averaged estimates? Please provide these important details.

L.174 – Was the environment a single predictor consisting of six different categories? Or were they all fit as separate predictors (each environment type a different predictor)? It is not clear to me how the environment experienced by nestlings was modelled.

L.174 – How correlated were the different environment variables with each other? Was the VIF < 3? Please include this information.

L.180 – Why was RTL log-transformed? Please explain the rationale behind this modelling decision.

L.235 – What does the indication of an erosion of nestlings’ RTL over years need to be replaced with? It is not clear to me. Is the paper suggesting that the inference needs to be placed in the context of recent studies investigating physiological markers of quality in conservation biology? If so, what have these studies found – please elaborate.

L.242 – It is not clear to me how increased cell division in young, growing individuals means that they are impacted most by environmental stressors? Both of these could be simultaneously occurring as separate causes influencing TL (i.e. increased cell divisions and oxidative stress due to growth leading to TL shortening (Boonekamp et al., 2017; Reichert & Stier, 2017). Additionally, exposure to environmental stressors in early-life may affect senescence rates and by extension, TL dynamics (Lemaitre et al., 2015; Watson et al., 2015)). But there is no direct line of causality linking exposure to environmental stress and increased cell division in growing individuals.
L.247 – Is it being suggested that nestlings born prematurely may be physiologically older due to the harsh early-life conditions they may experience? If so, please state that clearly. Does premature birth constitute harsh early-life conditions? Are nestlings born earlier in the hatching order considered premature?

L.272 – I think sex and gender refer to two different things (https://orwh.od.nih.gov/sex-gender) and being consistent with what is being referred to in this context (i.e. sex) is important, even if it is repetitive.

L.275 – It is not clear to me what a consensual general pattern is?

L.279 – More information regarding general patterns of sexual dimorphism in growth rates in bird populations would be great to include.

L.295 – The suggestion that parents adaptively manipulate sex ratios or invest more in female offspring compared to male comes with some issues (see Hasselquist and Kempenaers, 2002). Please acknowledge this when providing such an adaptive explanation for the results especially when investigations linking fitness and TL have not been explored here.

L.299 – I think it is important to highlight here that the sample size of the largest clutches (n=6) is small.

L.280 – I think the paper is intending to say “even” and not “event”?

L.296 – “Benefit” instead of beneficiate may be more straightforward to understand?

References


