**Recommender: Denis Réale**

I appreciate the great work and the amazing dataset used in this study to tackle an important ecological question about body mass plasticity an the constrains on each sex in a polygynous species showing a low sexual mass dimorphism.

**Firstly, we would like to apologise for the very long delay with the revision and re-submission of this manuscript – many thanks for your patience.**
**We thank the Recommender and Referees for their constructive evaluation of our work. We have now revised the analysis and text to account for these comments and to clarify our message. We provide a detailed point by point response below.**

I only have a couple of comments, including this important one:

1) You run mixed models with individual ID and year as random effects. However, you only show the fixed effects part of the results. I understand the goal of the study is not to look at individual variation in body mass, but I think we still need to see these results, because you models may suffer type 1 errors. Type 1 error rate for the slope x treatment interaction (in your case the sex by date interaction) increases with between-individual variation in slopes if that effect is omitted in the model (Schielzeth and Forstmeier 2012). This is because omitting random slopes in a model when you have a lot of replicates per unit of your random effect generates pseudoreplication. I assume in your case you may also have the same issue with the between-year variation in slopes. I would, thus, strongly recommend you run the models by including random slopes for both individual ID and years to make sure that you still find significant interactions between date and sex once controlling for pseudoreplication.

**We thank you for raising this important issue. In response to your comment, to eliminate any issues with potential pseudo-replication in the data sets, we attempted to fit models with random effects on both intercepts and slopes for both individual ID and year. While this proved possible for two of the five study areas (Bogesund, Grimsö), we were unable to achieve model convergence with this approach for the other three study areas due to the very high proportion of individuals that was weighed only once in a given year in these data sets, generating a low average number of repeated measures per individual (Tables 1 & S10). Therefore, for these three study areas, to verify that pseudo-replication was not a significant issue, we repeated the analysis based on a data set that included only a single observation per unique individual. For these models, as requested, we fit a random effect of year on both the intercept and the slope. Although these models successfully converged, there were residual issues of singularity. Despite this, both model selection (Tables S7-S9) and the parameter estimates derived from these models (see Fig. S1) were essentially identical to both i/ those based on models with random effects on the intercept only that were presented in the initial version of the manuscript, and ii/ those based on models with a random effect on both slope and intercept for year, plus a random effect on the intercept only for ID using the full data set. These additional analyses indicate that our results are extremely robust to the random effects structure that is employed in the fitted models.**
**As a result, we have decided to now present the latter models for these three study sites in the core of the revised manuscript, but also provide the output from models based on a single observation per individual in the Supplementary Materials for completeness (Appendix 2). In addition, we now mention in the main body of the text that using a single**

**observation per individual did not markedly alter parameter estimates or our interpretation (L. 140-145).**

Additionally, including random slopes could give you interesting biological information. You'll obtain estimates for the correlation between intercept and slope (I assume that you're scaling dates so that the intercept is estimated at the average Julian date), which will tell you whether bigger individuals at the intercept show weaker or stronger slopes (i.e., they lost/gained more/less weight with time whatever their sex). It will also tell you whether weight loss or gain varies according to the year, which you may link to climatic severity if you have weather data.

**Following on from our previous response, while among-individual differences in over-winter body mass change most likely occur, we do not have sufficient repeated measures per year to investigate this question. In contrast, as suggested, we were able to fit random effects on the slope for Year, but we did not find strong evidence for among-year variation in over-winter body mass change in three of the five study areas (see Figs appended below). There was, however, some evidence for year effects on the slope of body mass change in the two northern populations, but inspection of the data revealed that these effects were very sensitive to small sample sizes in particular years (see Table S10). As a result, we feel that investigating the drivers of among-year variation in mass is beyond the power of our dataset and, in any case, lies somewhat outside the scope of the current work. However, in response to this remark, we now mention in the final paragraph on perspectives that "Future investigations of inter-individual variation in over-winter body mass change in relation to environmental severity would be highly informative for understanding individual tactics of energy allocation to secondary sexual traits and their life history consequences." (L. 245-247).**

2) You are not testing your second hypothesis: it's only a qualitative examination. You justify the reasons why you do not pool all the populations into one single model. But you'll have to discuss that point.

**We agree with the Recommender and the Referees that we could not quantitatively test our second hypothesis from a statistical point of view, given that we have only five independent data points (populations). In response to this comment, we have now highlighted this limitation in the text, as follows "Note that, as a consequence of this choice, it was not possible to formally test our second hypothesis with just five independent data points (populations)." (L. 124-125) and referred to an alternative hypothesis outlined by Referee 2 (see below) (L. 215-220).**

**<u>Review 1: Patrick Bergeron</u>**

The authors are presenting a study about indirect sex-specific costs of reproduction in the moderately dimorphic roe deer. Males tend to lose more, or gain less, body mass than females during the winter months. This analysis is based on a robust long-term dataset conducted on five populations distributed along a north south gradient. I find that this manuscript is well written, the analyses sounded, and the topic is also relevant to non dimorphic species where body mass is associated with fitness.

**We thank the referee for their positive and constructive review which we have used to revise the text in line with their comments.**

_Comments:_

The populations cannot be statistically compared because the analyses were conducted on each population separately. I believe this somewhat reduces the quantitative comparison of the populations along a latitudinal gradient (and the scope of the second objective), but still allows to qualitatively evaluate over-winter body mass variations between populations. This aspect could be mentioned in the discussion.

**As the referee rightly points out, we did not quantitatively test our second hypothesis from a statistical point of view, given that we have only five independent data points (populations). In response to this remark, we have now highlighted this limitation in the text, as follows "Note that, as a consequence of this choice, it was not possible to formally test our second hypothesis with just five independent data points (populations)." (L. 124-125).**

I think it could be important to mention how many times an individual was measured per year. Given that the variance in body mass is extremely high, it could be an aspect you comment on with respect to the expected within-individual seasonal change in body mass (i.e. mass specific change in body mass during winter).

**We fully agree with the referee that this information was missing from the original text. In response to this comment and the editor's comments on the question of random slope effects (see above), we now provide a full break down per population and per year of the total number of capture events with the number of unique individuals in the Supplementary Materials (Table S10). As pointed out above in response to the Recommender's comments, the very low number of repeated measures for a given individual in a given year prevented us fitting models that included a random effect of individual ID on the slope in the three French populations (see L. 138-140).**

I think it could be relevant to also provide the AIC and weights for the main effects of Sex and Julian date alone in your models?

**In response to this remark, we have now added this information to the relevant Tables (Tables S1-9).**

## Review 2: Philip McLoughlin

This short paper presents a concise analysis documenting a consistency in over-winter change in mass of male roe deer relative to females, with magnitude apparently modulated by winter severity from one study area to the next. The analysis is appropriate and uses an impressive dataset. I only have a few comments for the authors to consider, which could result in a major revision or even possibly a recasting of the paper (if the second comment is followed-up on).

**We thank the referee for their positive and thought-provoking review which we have used to revise the text.**

1) As we are talking about a trait that is likely under sexual selection, I am curious about the adult (if not operational) sex ratio in each of the populations (averaged among years?) and whether study-area specific differences existed, that could also account for the observed results. Adult sex ratios can vary for many reasons, including selective harvesting and with increasing density, if males suffer more than females from overcrowding (e.g., Clutton-Brock et al. 1997. Density-relate changes in sexual selection red deer. Proc. R. Soc. Lond. B 264: 1509-1516). Right now, the paper is built around winter severity as the driving explanation at a macro-ecological scale, but the analysis does not directly incorporate any of environmental variables except as a study-area factor in the discussion. This raises some questions about the study areas, e.g., does the sensitivity of over-winter change in mass of roe deer depend on the sex ratio at breeding more or less than the winter severity measure, or is there an interaction that could be informative? We are talking about a sexually selected trait that should be subject to selective pressure exerted by the sex ratio. It is possible that these data do not exist, but I would suspect adult sex ratio would factor into the results somewhere.

**We agree with the Referee that winter severity is likely not the only factor causing the observed differences in over-winter body mass change between the Swedish and French populations. However, with only five independent data points (populations), it is not possible to investigate the potential environmental drivers of this variation (see above). Furthermore, we think it very unlikely that adult sex ratio (ASR) might be the driver of observed differences in over-winter body mass change for the following reasons: first, the intensity of sexual selection in roe deer is moderate at best: new-born males and females have similar birth mass and growth rates (Gaillard et al. 1993 Oecologia), while sexual size dimorphism is around 10%, i.e. below the threshold usually for considering a species as sexually dimorphic (1.12, Perez-Barberia et al. 2002 Evolution); second, based on raw capture data, the ASR at capture did not vary markedly across the five populations of our study (number of males/(number of males + females) = Bogesund: 0.43; Grimsö: 0.41; Aurignac: 0.41; Chizé: 0.44; Trois Fontaines: 0.47), with no obvious contrast between the French and Swedish populations. Moreover, the highest value of ASR actually occurred in Trois-Fontaines i.e. opposite to the expectation under this hypothesis of lower ASR where males fare best (i.e. lowest difference in the sex-specific slope of body mass vs. date).**
**To address the reviewer's point, we now i/ briefly discuss the hypothesis that, conceptually speaking, site-specific variation in the operational sex ratio could potentially influence the allocation priority to secondary sexual characters, such as antlers, driven by variation in the intensity of male-male competition but ii/ reject this hypothesis in our specific case based on the roe deer's weakly polygynous mating system and population-specific information on ASR (L. 215-220).**

2) It also possible that natural selection for heavier-bodied animals in colder climates is perhaps an important factor to consider, noting the heaviest males (25 kg and higher) are only found in the populations with a January temp less than 4 degrees. I bring this up because, for the Swedish populations, it becomes harder to argue that body size is a sexually selected trait, as both males and females are large to begin with and sexual size dimorphism is weakest anywhere on the timeline from January 1 through March 21st. When I look at Fig. 1, what stands out for me (if I were to formulate a hypothesis from reading the paper) is that body size appears to be a strong intrasexually selected trait, leading to sexual dimorphism, but only where the climate allows; and is overshadowed by natural selection for large body size (both sexes) at higher latitudes (which thus weakens dimorphism). In the north, the changes in body mass become a seasonal phenomenon divorced from sexual selection; but in the south there is something else going on. Try this…sort Fig. 1 top to bottom from warmest to coldest January: Chizé-Aurignac VCG-Trois-Fontaines-Bogesund-Grimsö. The gradual change in slopes is interesting (TF is the transition area), and what looks like a sexual-size dimorphism story in response to intrasexual selection among males is replaced by lack of sexual-size dimorphism and a story of natural selection for body size in colder climates. I suspect that in a larger statistical framework study area or winter severity would stand out as a slope-influencing variable that can be quantified. Reduction in the strength of sexually selected body-size related traits and the antlers that go along with them is corroborated by the smaller antler sizes in Bogesund noted during the period of data collection (Vanpé et al., the Am Nat paper), if I recall this paper correctly. This all reminds me of horses, where due to their social system and length of breeding season, maintaining anything but a little sexual dimorphism becomes a losing game for males, and so intrasexual selection involves behaviour, intimidation, and weapons that must be yielded with agility (hoof kicks, tusks to bite ankles), not body size contests paired with big horns or antlers.

**We agree with the Referee that, as detailed on L. 158-164, sexual dimorphism is, indeed, more pronounced in the French populations than in the Swedish populations, at least at the onset of winter. While, as the referee suggests, this is plausibly the result of natural selection for larger body size at higher latitudes, as predicted by Bergmann's rule (Bergmann 1847), we respectfully disagree that this implies that sexual selection on secondary sexual characteristics, such as antlers, is superseded. While the referee is correct that we previously showed that relative antler size was lower *for a given date* in one of the Swedish populations (Bogesund) compared to two of the French populations (Chizé, Trois Fontaines), this actually reflected the marked difference in antler phenology between countries, as antler growth occurs substantially later at higher latitudes (Vanpé et al. 2007). That is, average absolute clean antler size was similar across populations, largely unaffected by annual variation in environmental conditions and strongly correlated with body mass at the individual level (Vanpé et al. 2007), suggesting similar priority of energy allocation to this secondary sexual trait. Indeed, following on from our previous response, the ASR is similar among study sites (see above), suggesting that the opportunity for sexual selection is of a similar magnitude across the five populations (see Vanpé et al. 2008). Although the slope of the body mass-date relationship does, indeed, decrease in strength from north to south, this is the case for both sexes. However, across all five populations, males do worse than females, indicating that the costs of allocation to antler growth during winter are universal. In response to this comment, we have added a brief consideration of the possible latitudinal effects of natural selection on body mass *per se* to the body of the text, as follows: "Larger**

**body size has often been reported at higher latitudes within species of mammals (Ashton et al. 2000), in line with Bergmann's rule, and is thought to reflect natural selection for greater thermoregulatory buffering in endotherms (He et al. 2023). Our data are also in line with this general pattern, but indicate that sexual selection is the ultimate driver of between-sex differences in over-winter body mass change, suggesting similar priority of energy allocation to this secondary sexual trait across hugely contrasted environments" (L. 220-225), but have emphasised that the question of environmental drivers of variation in over-winter body mass change lies outside the scope of the present work (L. 245-247), see above).**

***Other minor comments:***

Abstract: In "most" polygynous vertebrates…not all polygynous vertebrates show this, even among large mammals (e.g., zebras, horses, notwithstanding canines).

**Done (L. 21).**

Also remove the word "the" in front of secondary, first sentence.

**Done (L. 21).**

**<u>Review 3: Achaz von Hardenberg</u>**

I read this manuscript with great interest. It is well written, and analyses an impressive dataset (more than 7000 individuals) to explore overwinter variation in body mass of male and female roe deer in 5 European populations on a latitudinal gradient. My comments are mostly minor as can be seen in the attached pdf, and therefore I would recommend publication after minor revisions.

**We thank the referee for a positive and constructive review which we have used to revise the text in line with their comments.**

Add scientific name of species in title, and the first time you mention the species in abstract and main text.

**Done (L. 25, L.58).**

Can you provide citations to papers from these study areas with details on capture and marking techniques used?

**In response to this comment, we have added the appropriate references for each study site which provide more details on the capture and marking techniques employed (L. 97-98).**

If decreasing should there not be a minus sign for your estimates as you did for the males in Chize?

**Done (L. 153).**

Please provide standard error around these average estimates.

**Done (L. 157-163).**

Please provide scientific names of species the first time you mention them.

**Done (L. 186-187).**

Can you provide a reference for this statement?

**In response to this comment, we have added a reference which provides this information for the first half of the winter (Beyes et al. 2017) (L. 198).**

You mean the pattern of changes in sexual mass dimorphism? It's a bit unclear here as before you talk about foetal growth patterns… can you rewrite this making it more explicit what you mean?

**In response to this comment, we have re-written the sentence to be more explicit, as: "We suggest that this over-winter decrease in sexual dimorphism of body mass is likely due to sex differences in the schedule of reproductive effort…" (L. 198-199).**

Please provide also measures of variation around these estimates.

**We have not done this, as these are predicted values.**

It is actually also highlighted in grey?

**Corrected to "the selected model is shaded grey" (Tables S1-9).**

Use Greek letter delta as in the caption.

**Done (Tables S1-9).**

Here you used the R. Notation to indicate the interaction term + the fixed terms. I think, that it may be clearer if you write it down explicitly as Sex + Julian date + Sex:Julian date.

**Done (Tables S1-9).**

References?

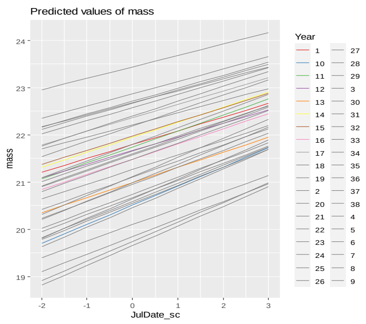
**In response to this comment, we now refer to the relevant package (L. 409-412).**

In previous tables you have provided delta AIC instead of the actual AIC values. I think it would make the comparison easier here as well.

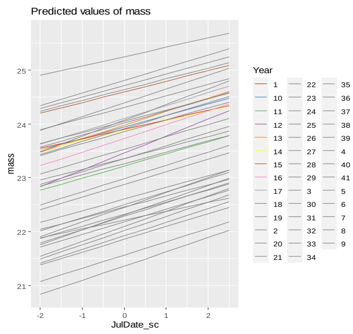
**We think this complicates the presentation, so have left it as is.**

**Fig. L1:** Yearly variation in the slope describing over-winter body mass change of roe deer in five intensively monitored populations of roe deer, obtained from the model that included year as a random effect on the date-mass slope:

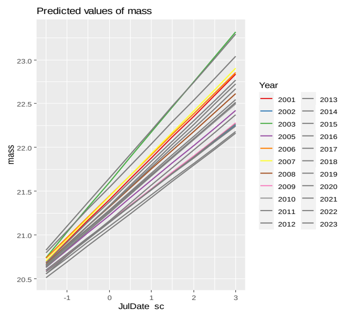
**Chizé**



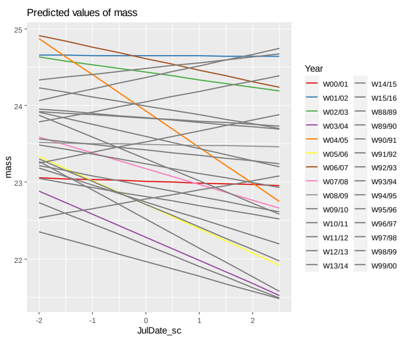

**Trois Fontaines**




**Aurignac**




**Bogesund**




**Grimsö**