nature portfolio

Peer Review File

Sterilization and contraception increase lifespan across vertebrates

Corresponding Author: Dr Michael Garratt

Any redactions in this file are there to maintain patient confidentiality, the confidentiality of unpublished data, or to remove third-party material.

This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.

Version 2:

Reviewer comments:

Referee #1

(Remarks to the Author)
This is a provocative and stunning article with broad claims - namely, that inhibition of reproduction broadly extends lifespan (and healthspan?). Potentially, this could lead to wide-ranging changes in human behavior - and thus, it is critical that the results reported here be reliable. I think there are fundamental issues that preclude from accepting the paper at the current time, but extensive revisions and the inclusion of animal data could make this an incredible important paper interesting to a wide audience of readers.

The biggest issue in my opinion is the confounding of surgical and non-surgical interventions - and, potentially, the different types of interventions. Fundamentally, hormonal inhibition of reproduction has very different effects than removal of the gonads - and yet again different effects from surgical inhibition that does not remove the gonads. In the case of hormones, the body likely "thinks" that a female is pregnant, which should not direct resources to somatic repair; in the case of gonadectomy many hormones changes and feedback loops lead to both physical and likely mental changes; and in the case of surgical interventions like vasectomy, probably the soma is completely unaware that reproduction has stopped. I think it is imperative that a revised version of this manuscript pull apart these different classes of interventions, as they are likely to have very different impacts on longevity.

As another issue, it would be wonderful to see how these interventions impact healthspan, frailty, or the hallmarks of aging. That is likely not possible given the vast number of data sources presented here, but there should be evidence at least in rodents that different classes of interventions promote healthspan. However, to my understanding the impact of castration or OVX on rodent lifespan is mixed in the literature - but regardless, a rodent system would enable in depth assays more feasible.

Finally, the grandmother hypothesis suggests that for humans, post-reproductive lifespan is important for caring of descendant children. In that case, shouldn't each successive child lead to an increase in investment in soma repair? Does that explain why OVX might shorten lifespan in females? Or is that an artifact of how humans preferentially use hormonal or physical barrier contraception?

Referee #2

(Remarks to the Author)
In this manuscript, Garratt and collaborators investigate the impact of sterilization and contraception on the lifespan of zoo animals. Their data is consistent with the notion that both surgical sterilization and hormonal/immunological contraception methods can extend the lifespan of various species, including primates, carnivores, and rodents, in both males and females. Through a comprehensive meta-analysis of existing datasets, the authors further explore the relationship between contraceptive practices and survival rates. This extended analysis aligns with the initial analysis, indicating enhanced longevity in animals subjected to permanent contraceptive measures, regardless of sex. Additionally, the authors dispel the notion that reproductive allocation significantly influences the observed differences in lifespan across sexes within the examined species.

This research sheds light on critical aspects of aging and the role of sex differences in lifespan, providing valuable insights for the field, at a critical juncture where sex differences and their origins are becoming more appreciated. However, I have a few comments regarding the scientific rigor and clarity of the study that I believe need to be addressed for the results to be appropriately measured and contextualized.

Major Comments:
1) The aging research field is increasingly raising the importance of healthspan (i.e. quality of life) vs. lifespan (time alive). Although the authors' results on lifespan are clear, it would also be interesting to discuss how sterilization/contraception impacts healthspan. For example, loss of ovarian hormones through menopause [PMID: 31955198], bilateral oophorectomy [PMID: 20226402], and chemical inhibition of the production of estrogens by use of aromatase inhibitors in breast cancer survivors [PMID: 19125005; PMID: 17898668] are known to be broadly deleterious to women's health. Thus, it is likely that the reported effects are strictly on lifespan but would have the opposite effect on healthspan, at least in females. This is a crucial point that the authors must explicitly discuss in this manuscript.

2) Gonadal hormones are known to influence behavior, including aggression [PMID: 23843821, 24685383], which can significantly affect animal survival. It is, therefore, crucial to differentiate between the behavioral and physiological effects of castration and hormonal/immunological contraception methods on overall lifespan. The current data, however, do not allow for a clear distinction between whether the observed lifespan extension after contraceptive measures is attributable to modifications in behavior or to the physiological impacts of gonadal hormones. The authors are advised to moderate their language, suggesting that the increased lifespan observed may stem from either changes in animal behavior or physiological adjustments following sterilization.

3) Additionally, examining potential differences in the causes of death between animals that have undergone sterilization or contraception and those that have not (rather than just their absolute lifespan) could provide valuable insights. Analyzing patterns in causes of death may shed light on the mechanisms behind the increased lifespan observed post-sterilization or contraception.

4) Primates experience menopause, during which post-menopausal females exhibit low estrogen levels and cease ovulation. While menopause and contraception may affect an animal's physiology differently, it would be intriguing to explore the relationship between the age at natural menopause and the lifespan of primates that have not undergone any form of alteration. This investigation could provide insightful biological distinctions between the natural cessation of reproductive ability and the effects of artificial contraceptive measures on longevity.

Minor Comments:

5) It seems like the authors are using contraception and sterilization interchangeably for female animals, which is imprecise and misleading (especially in the title). This should be amended in a revised version of the manuscript.

6) Including text labels and/or a list of species for Figures 1A and 4A would greatly help with clarity for the readers.

Referee #3

(Remarks to the Author)
This study provides a comprehensive analysis of the effects of male castration and female contraception on lifespan across a variety of zoo-housed mammalian species, taking into account different covariates including sex, species, methods of contraception/sterilization, and environmental factors. The additional meta-analysis of data from previous papers to support the findings is also a valuable component. However, I have a couple of questions that I believe need addressing to strengthen the paper further.

On "Reproductive Allocation and Sex-Differences in Survival" section

The hypotheses outlined in the first paragraph of the section need further articulation. As I understand it, the paper suggests that male-specific androgen production shortens male lifespan, whereas female-specific estrogen production extends female lifespan. Therefore, limiting reproductive investment could narrow the sex gap in lifespan by either extending male lifespan or reducing female lifespan. If my understanding is correct, I recommend clarifying this in the text, as the current explanation leaves room for alternative interpretations on how reproductive limitations might influence sex differences in lifespan. For example, restriction on reproductive allocation can increase the survival for males and females, but with stronger effect on males.

The analysis and presentation of results, particularly in the lines 249 – 264, are somewhat difficult to follow. Figure 4B shows that compared to F normal group, M contraceptive group has higher life expectancy as male contraception can increase male survival. Based on this, authors conclude that the analysis does not support the hypothesis that male gonads and reproductive investment are not the cause of sex-difference in lifespan. Why? In another word, could you clarify what specific evidence would actually support this hypothesis? No sex difference in life expectancy in the groups of F normal vs. M contraceptive?

Additionally, the conclusion drawn in lines 279 – 292, that reproductive allocation during adulthood does not significantly

influence sex-difference in lifespan, seems to disconnect from the previously discussed hypotheses for the male-only contraception and female-only contraception cases. A bit more explanation on how this conclusion integrates with the hypotheses and previous analysis would be helpful.

On the Timing of Contraception/Sterilization

Another question I have is about the timing of contraception or sterilization. The paper mentions data availability on the timing of contraception but doesn't delve deeply into how this timing might affect survival outcomes. For example, does the contraception applied at different stages of life (at birth vs. between birth and puberty vs. at puberty vs. in adulthood but before first reproduction vs. after first reproduction) have the same impact on survival?

On the maternal death for females

Is the paper using all-cause mortality in the analyses, including the specific risks associated with reproduction, such as maternal death? This clarification is crucial, especially when considering the impact of contraception on female survival. If maternal deaths are included in the overall mortality data for intact females, this could inherently increase their risk profile compared to contracepted females, who do not face the same reproductive risks.

This difference in risk exposure might partly explain why contraception appears to lead to a survival advantage for females. Therefore, this paper states that results do not support the hypothesis that estrogen production associated with gonadal activity is beneficial for female survival. Excluding maternal deaths from the analysis could provide a more direct test of the physiological effects of estrogen.

Connection to biological age

This study shows a slight decrease in survival for human females after sterilization, a notable exception among mammals. This raises interesting questions about biological age. For example, a recent paper in Cell Metabolism suggests pregnancy might reduce biological age in mice, possibly indicating similar benefits in humans. This implies reproductive benefits in slowing aging. Linking these findings could shed light on how reproductive strategies affect aging. Drawing a connection between such studies and findings in this paper could
contribute to the ongoing debate on aging, reproductive health, and longevity.

Reference: https://www.nature.com/articles/d41586-024-00843-w?WT.ec_id=NATURE-202403&sap-outbound-id=FE5DA9037F51385D30CAEBC8A0C3A082838E85ED

Some Minor Questions/Suggestions:

1. Is the analysis of zoo-housed data comparing intact and contracepted/sterilized individuals conducted within individual zoos or across different zoos for each species? The paper mentions that ”To reduce heterogeneity in husbandry practices, we only included individuals that were born or still alive after the 1st of January 1980, including records up to the 1st of February 2022.“ In addition to this, does the paper using other methods to account for the potential confounding effects of varying environmental or husbandry conditions among zoos?
2. In species where social status is influenced by physical strength, aggressiveness, or behaviors linked to testosterone, castration might decrease a male's rank or change his social role. This dynamic may diminish the positive effects of castration on survival. Do the authors observe a different impact of male castration on survival across species with such social hierarchies?
3. Line 103 mentioned that Figs S1&S2 shows survival curves for each individual species. However, I only found male surgical vs. non-contraction in S1 and female hormonal vs. non-contraception in S2. What about other comparison? E.g., female surgical vs. non-contraction? Maybe I missed anything here?
4. The interpretation of the negative value (-0.0092) for male surgical sterilization's impact on survival (lines 252 - 253) is confusing. Could you elaborate on how this negative value translates to increased survival?
5. Authors use the average difference across species when discussing the sex gap in life expectancy differences (Figure 4A). It will be very helpful to add such values for each panel of Figure 4A to enhance understanding and clarity for the reader.

Version 4:

Reviewer comments:

Referee #1

(Remarks to the Author)
The authors have gone above and beyond and fully addressed all of my initial comments.

1) I wish the distinction between vasectomy and castration in terms of its longevity effects, was mentioned in the abstract as - while clearly presented in the text - the abstract without this info poses a risk of increasing vasectomies in male readers, especially of the many popular press articles that will cover this exciting and widely read article

2) The health benefits and risks of various types of female contraception is well known in the literature and should be discussed here - seems likely to me based on the authors statements that hormonal birth control would increase both female lifespan and - in contrast to OVX - healthspan?

Referee #2

(Remarks to the Author)
The authors have mostly addressed my previous comments. This manuscript and the analyses it contains should be illuminating for the field.

The crucial sticking point that remains in this revised version, which prevents me from a full endorsement, is the continued use of "female contraceptive methods" - especially in the title where no nuance or context is provided - to encompass both hormonal and surgical interventions. In a world where many readers or news outlets stop at a title, it is important to avoid misleading titles. Most readers - scientists included - would not realize that surgical sterilization is included in this group, which I believe is a damaging concept to include in the title. Thus, I must still recommend that sterilization (irreversible) and contraception (reversible) be included as separate concepts in a revised title for this piece.

Referee #3

(Remarks to the Author)
I thank the authors for their thorough work on the revisions. I find that my previous comments have been well addressed and the manuscript is nearly ready for publication!

Overall, this is a highly significant and original study that addresses a critical gap in the field. The methodology is sound, the data presented are of high quality and from various sources, and the statistical analyses are appropriate. As a result, the conclusions are robust and well-supported by the evidence. The manuscript is clearly written, and the revised figures effectively illustrate the key findings.

I have just a few minor points that need attention to finalize the manuscript:

In line 111, the reference to "Fig a,b" appears to be inaccurate. Given the context, "Fig 1a" would be the correct reference.

In line 350, the origin and meaning of the "11.57%" value are unclear. For the benefit of the reader, this should be clarified, as it does not seem to correspond to any of the reported effect sizes (overall or female or male).

In lines 608-609, the association between survival rates and life stages is described in a confusing way. A survival rate of $S(x) = 0.2$ (i.e., 20% survival) should correspond to "older ages," whereas the text seems to associate it with "early childhood."

Referee #4

(Remarks to the Author)
A. Summary of the key results
This important paper tests the impact of sterilization on lifespan across vertebrates (with a strong emphasis on mammals). It finds broad support for longer lifespans in sterilized individuals, but with notable heterogeneity.
B. Originality and significance: if not novel, please include reference
Yes, it's original. Nothing on this scale exists to my knowledge.
C. Data & methodology: validity of approach, quality of data, quality of presentation
Excellent, rigorous methodology.
D. Appropriate use of statistics and treatment of uncertainties
Yes, and the team includes some of the very best statisticians in the field.
E. Conclusions: robustness, validity, reliability
A wide variety of analyses provides strong support for robustness of conclusions.
F. Suggested improvements: experiments, data for possible revision
See below.
G. References: appropriate credit to previous work?
Fine.
H. Clarity and context: lucidity of abstract/summary, appropriateness of abstract, introduction and conclusions
Clear.

This important paper tests the impact of sterilization on lifespan across vertebrates (with a strong emphasis on mammals). It is well conducted and there are no methodological flaws and I recommend publication without hesitation.

As a reviewer invited into the process after previous reviews and revisions, I am both hesitant to demand further revision but also obligated to provide thorough feedback. I have identified a number of points that I think could be improved in the manuscript, almost all very simply and easily. However, let me be clear: the manuscript is already of sufficient quality and does not require any further revision to be publishable. The following comments are suggestions only, and should be

incorporated by the authors only if they feel the benefit to the manuscript is worth the effort.

Lines 52-54: It's not clear that negative interspecific correlations between lifespan and reproduction indicate a trade-off in the usual sense. They can arise simply from constraint that population growth cannot (or is not usually) too fast up or down. I believe this is touched on here:
Salguero-Gomez, et al. (2016). Fast-slow continuum and reproductive strategies structure plant life-history variation worldwide. Proceedings of the National Academy of Sciences of the United States of America, 113, 230–235. https ://doi.org/10.1073/pnas.15062 15112
And here:
Ricklefs, R. E. (2000). Density dependence, evolutionary optimization, and the diversification of avian life histories. The Condor, 102(1), 9-22.

Are sterilized individuals in zoos randomly selected? Any chance of bias? For example, I would expect only the healthiest individuals are chosen for breeding, except in the most endangered species, leading to a potential underestimation of effects. On the other hand, individuals chosen for breeding may also differ in how they are treated afterwards, independently of the reproduction.

In females, how much of the effect is explained by giving birth and raising offspring? That is, if you control for number of progeny, is the effect attenuated? How much?

Fig. 1a – confidence intervals for each species-sex should be provided, if at all possible.

Is the moderation of the effect by wild environment stronger in males? I would guess that testosterone causes risky behavior in males with accompanying mortality, and that this behavioral effect could explain much of the difference, but that this might be absent in females.

In female mammals (Fig. 1a) almost all of the effect seems to be driven by primates. Is there still a significant overall trend if primates are removed? Also, female mammals appear less heterogeneous than males in Fig. 1a (lots of males with negative effects) but this is not reflected in the confidence or prediction intervals in Fig. 1b. Why not?

Line 368 – "withholding reproduction" is probably not the right phrasing – this doesn't include animals kept in isolation and therefore unable to reproduce, for example. "sterilization" might be more accurate. Given the lack of effect of gonadectomy, it seems the unifying thread is removal or suppression of sex hormones or their production.

Discussion:
The Intro frames this study around trade-offs between survival and reproduction, but the discussion hardly mentions this. I think it interesting that the effect sizes of 10-20% arise repeatedly (though with lots of heterogeneity). This supports a non-negligible but weak-to-moderate role for trade-offs in shaping life histories. That is, getting rid of reproduction is nowhere close to doubling lifespan or explaining the whole spectrum of mammalian lifespans. On the other hand, this study supports the role of sex hormones as potential orchestrators in canalized pathways mediating these trade-offs, potentially along with IGF-1 mediation of growth/survival trade-offs. Probably worth mentioning.

It might be interesting to compare castration to other known lifespan interventions (caloric restriction, rapamycin, etc.) for magnitude of effect. [...].

Most interventions that perturb evolved homeostatic mechanisms decrease lifespan or have adverse health consequences. For example, the hygiene and old-friends hypotheses suggest that even though parasites can be harmful, their absence can also disrupt a normal balance and have negative effects on health. Sterilization is interesting in this context in that it does the opposite – improves health/longevity, despite putting the organism in a state clearly outside its homeostatic norms and for which it is clearly (by definition) not adapted. This is interesting to note in and of itself, but also suggests that the effect sizes here may be underestimates – that we are likely observing a complex mix of the benefits of not reproducing and the harms of perturbing homeostasis. This could explain much of the heterogeneity across species. Interestingly, it is analogous to the famous Van Noordwijk and De Jong 1986 paper that counterposes individual quality processes and trade-off processes, which are both present and can generate opposite effects in the data…

Version 5:

Reviewer comments:

Referee #4

(Remarks to the Author)
Authors have responded thoughtfully to all points. I recommend publication.

We thank the editors and reviewers for their careful consideration and comments on the previous version of our manuscript. They were extremely insightful and have allowed us to create a modified version with various additional data and analyses that has much improved the quality of this work. We believe that the manuscript is even more stunning and revealing, addressing key questions about how different types of contraception influence lifespan, mortality and health on a scale never tested before.

Some notable additions to the manuscript include:

1. An analysis of changes in causes of death in response to contraception across 74 mammal species. Now, in addition to providing the biggest assessment of lifespan changes with an intervention ever compiled, we provide one of the first cross-species comparative analyses of causes of death. This shows that while male and female contraception similarly effects survival across sexes, it has distinct effects on specific causes of death.

2. An analysis of changes in healthspan in response to surgical sterilization (gonadectomy) in rodents. We have conducted one of the first meta-analyses of healthspan changes, utilizing data from 194 effect sizes and 47 different studies. This allowed us to test how surgical sterilization influences healthspan on an unprecedented scale. We show that male castration causes improved healthspan across many domains of health, including cognition, physical function, muscle size, frailty and development of pathology. Ovariectomized females, by contrast, show strong reductions in the incidence of some cancers but worsened health in terms of cognition, activity and non-tumor related pathology. The differing results we show for survival versus healthspan in females undergoing ovary removal for sterilization provide insights into the survival-health paradox observed in post-menopausal women, who on average outlive men but suffer increased frailty and poorer overall health.

3. A comprehensive analysis across both datasets that shows age at castration of males (but not females) strongly influences the survival response, with castration earlier in life and prior puberty causing the greatest lifespan extension. This is consistent with the long-lasting organizational effects that sex-hormones have during this life period. Their effects over puberty are known to shape future growth and behaviour - our study indicates that these programming effects extend to subsequent ageing.

As outlined below, we have thoroughly addressed every comment made by the reviewers and provide all the new analyses requested. We believe, and hope you agree, that the scale and quality of this manuscript now warrants publication in Nature.

On behalf of my coauthors,

Mike Garratt

Referees' comments:

Referee #1 (Remarks to the Author):

This is a provocative and stunning article with broad claims - namely, that inhibition of reproduction broadly extends lifespan (and healthspan?). Potentially, this could lead to wide-ranging changes in human behavior - and thus, it is critical that the results reported here be reliable. I think there are fundamental issues that preclude from accepting the paper at the current time, but extensive revisions and the inclusion of animal data could make this an incredible important paper interesting to a wide audience of readers.

The biggest issue in my opinion is the confounding of surgical and non-surgical interventions - and, potentially, the different types of interventions. Fundamentally, hormonal inhibition of reproduction has very different effects than removal of the gonads - and yet again different effects from surgical inhibition that does not remove the gonads. In the case of hormones, the body likely "thinks" that a female is pregnant, which should not direct resources to somatic repair; in the case of gonadectomy many hormones changes and feedback loops lead to both physical and likely mental changes; and in the case of surgical interventions like vasectomy, probably the soma is completely unaware that reproduction has stopped. I think it is imperative that a revised version of this manuscript pull apart these different classes of interventions, as they are likely to have very different impacts on longevity.

We appreciate the reviewer's concern about distingushing between the effects of different types of interventions. To account for this, we have now provided several different analyses that tease apart the effects of different types of interventions, which have different effects on sex-hormone signalling in males and females. In males, we demonstrate that castration is the key manipulation that leads to improved lifespan (and healthspan, see below). We have provided new evidence that the vast majority of data available from males has applied castration for surgical sterilization, but in the few species where vasectomy is applied there is a notable absence of any change in survival with sterilization. This effect, specifically of castration, on male survival is thus demonstrated in both the zoological data and in our meta-analysis from across species.

In females, our results demonstrate that a variety of different manipulations that prevent pregnancy cause improved survival. We have now compared the survival effects of treatment with progesterone-based contraceptives versus GnRH-agonists and observed remarkably similar effects on survival, with both increasing lifespan. This similar response is observed when comparing taxa that differ in the application of different contraceptives (i.e. primates versus carnivores), and also in a within species comparison of female lions. In lions we now present survival curves for those treated with progesterone-based contraceptives (that trick the body into thinking its pregnant), GnRH agonists (that shut down the HPG axis) or surgical sterilization (ovariohysterectomies, removal of the ovaries and uterus) and each manipulation increases survival to a similar degree relative to untreated animals. This complements and is in agreement with our meta-analysis, where we observe similar

improvements in survival regardless of whether the ovaries are removed (ovariectomy) or not (i.e. hysterectomy, tubal ligation etc) for sterilization.

In summary, We have now added a new figure (Figure 2) and section (lines 114-156) that demonstrates the survival effects of different types of sterilization methods using the zoo dataset. This is complemented by our meta-analysis that seperates the effects of manipulations that remove the ovary and those that leave it intact (Fig 4b and lines 221-230 and 237-259), and our new meta-analysis of the effects of castration and ovariectomy on healthspan.

As another issue, it would be wonderful to see how these interventions impact healthspan, frailty, or the hallmarks of aging. That is likely not possible given the vast number of data sources presented here, but there should be evidence at least in rodents that different classes of interventions promote healthspan. However, to my understanding the impact of castration or OVX on rodent lifespan is mixed in the literature - but regardless, a rodent system would enable in depth assays more feasible.

We have now conducted a comprehensive meta-analysis of the effects of castration and ovariectomy on healthspan in rodents during aging. This is a major new addition to the manuscript and is to our knowledge the first time that healthspan effects of an intervention have been assessed so comprehensively with such a meta-analytic approach. This analysis compares the effects of castration and ovariectomy on a variety of domains of health during ageing, including different aspects of physical function and activity, cognition, pathology, metabolism, cardiovascular function, and immune and sensory functions. These parameters were preselected and searched for as they are highlighted as important measurements of healthspan in major reviews of the field.

The results reveal remarkable sex-specific effects of gonadectomy on healthspan that contrasts with the consistent effects that these manipulations have on lifespan. In males, castration causes significant improvements in several different domains of health from middle to late age, including improved cognition, strength, decreased frailty, and increased protection from the development of tissue pathology. By contrast, the main domain of health that is improved with female ovariectomy is projection from tumor pathology of reproductive tissues. Several other important aspects of health are impaired with ovariectomy during ageing, including activity levels, cognition, muscle size, and non-tumor-related pathology. These differing results on survival versus healthspan with ovariectomy may help to explain the survival-health paradox observed in post-menopausal women that have lost ovarian estrogen production, who outlive men on average but suffer increased frailty and poorer overall health.

To summarise, we have now added a new section to the manuscript that shows the effects of castration and ovariectomy on healthspan in rodents (Fig 3b, Extended data Figure 3, lines 327-365).

Finally, the grandmother hypothesis suggests that for humans, post-reproductive lifespan is important for caring of descendant children. In that case, shouldn't each successive child lead to an increase in investment in soma repair? Does that explain why OVX might shorten

lifespan in females? Or is that an artifact of how humans preferentially use hormonal or physical barrier contraception?

The grandmother hypothesis posits that menopause may have evolved so that females reduce their own costs of reproduction by not allocating to child production, but instead gain indirect fitness benefits from allocating to their grandchildren. Our results are consistent with this in that we show that reproduction is costly and witholding this provides survival benefits.

We have now expanded our discussion to include an explanation of what the results mean for the evolution of menopause. We also connect this to the survival-health paradox as outlined in the earlier section. Please see lines 426-436 and also an earlier comment at 239-244.

Referee #2 (Remarks to the Author):

In this manuscript, Garratt and collaborators investigate the impact of sterilization and contraception on the lifespan of zoo animals. Their data is consistent with the notion that both surgical sterilization and hormonal/immunological contraception methods can extend the lifespan of various species, including primates, carnivores, and rodents, in both males and females. Through a comprehensive meta-analysis of existing datasets, the authors further explore the relationship between contraceptive practices and survival rates. This extended analysis aligns with the initial analysis, indicating enhanced longevity in animals subjected to permanent contraceptive measures, regardless of sex. Additionally, the authors dispel the notion that reproductive allocation significantly influences the observed differences in lifespan across sexes within the examined species.

This research sheds light on critical aspects of aging and the role of sex differences in lifespan, providing valuable insights for the field, at a critical juncture where sex differences and their origins are becoming more appreciated. However, I have a few comments regarding the scientific rigor and clarity of the study that I believe need to be addressed for the results to be appropriately measured and contextualized.

Major Comments:
1) The aging research field is increasingly raising the importance of healthspan (i.e. quality of life) vs. lifespan (time alive). Although the authors' results on lifespan are clear, it would also be interesting to discuss how sterilization/contraception impacts healthspan. For example, loss of ovarian hormones through menopause [PMID: 31955198], bilateral oophorectomy [PMID: 20226402], and chemical inhibition of the production of estrogens by use of aromatase inhibitors in breast cancer survivors [PMID: 19125005; PMID: 17898668] are known to be broadly deleterious to women's health. Thus, it is likely that the reported effects are strictly on lifespan but would have the opposite effect on healthspan, at least in females. This is a crucial point that the authors must explicitly discuss in this manuscript.

We thank the reviewer for raising this suggestion that has provided important new insight. We have conducted a comprehensive new healthspan analysis as explained above in

response to reviewer one. The results are largely concordant with the reviewer's suggestion – ovariectomy extends lifespan but has some negative effects on healthspan that would account for the observations in humans that the reviewer refers to with menopause and loss of estrogen production.

2) Gonadal hormones are known to influence behavior, including aggression [PMID: 23843821, 24685383], which can significantly affect animal survival. It is, therefore, crucial to differentiate between the behavioral and physiological effects of castration and hormonal/immunological contraception methods on overall lifespan. The current data, however, do not allow for a clear distinction between whether the observed lifespan extension after contraceptive measures is attributable to modifications in behavior or to the physiological impacts of gonadal hormones. The authors are advised to moderate their language, suggesting that the increased lifespan observed may stem from either changes in animal behavior or physiological adjustments following sterilization.

In response to this and the point below, we have now added a major new analysis to the manuscript that establishes how the onset of different causes of death are influenced by contraception. We were able to collate data from 74 species where data were available from contracepted and control individuals that had undergone necropsies at death. This has allowed us to test whether the major causes of death in zoo animals (infectious disease, non-infectious disease, chronic disease, mortality associated with behaviour) were influenced by contraception. To our knowledge this is the first time anyone has conducted such an analysis of causes of death in response to an intervention on a cross-species scale and we think that this may pave the way for future research.

For males, we do observe that mortality from behaviour-related deaths is reduced with castration, suggesting that behavioural changes as a consequence of castration may contribute to increased survival. However, we also find that mortality causes unrelated to behaviour are also reduced, including deaths that could not be attributed to a specific cause at necropsy. This suggests that physiological changes to the function of body systems also contribute to improved survival. Our analysis of healthspan further demonstrates that castration improves healthspan and reduces tissue pathology, illustrating that fundamental changes in physiology and dampened aging also contribute to improved survival with castration.

In females, we have also now been able to establish that contraception reduces the occurrence of several different aspects of mortality, including deaths from infectious and non-infectious diseases. We have therefore established that physiological adjustments underlie improved survival, and provide a discussion about whether these are initiated by the contraceptive methods themselves, or whether they are indirectly generated by the fact that the initiation of pregnancy is inhibited in females that are contracepted.

The new additions to the manuscript are found in Fig 1c&d and lines 158-192.

3) Additionally, examining potential differences in the causes of death between animals that have undergone sterilization or contraception and those that have not (rather than just their absolute lifespan) could provide valuable insights. Analyzing patterns in causes of

death may shed light on the mechanisms behind the increased lifespan observed post-sterilization or contraception.

We thank the reviewer for this suggestion. As outlined in our response to the previous point, this has provided some major insight into how sterilization and contraption are impacting lifespan.

4) Primates experience menopause, during which post-menopausal females exhibit low estrogen levels and cease ovulation. While menopause and contraception may affect an animal's physiology differently, it would be intriguing to explore the relationship between the age at natural menopause and the lifespan of primates that have not undergone any form of alteration. This investigation could provide insightful biological distinctions between the natural cessation of reproductive ability and the effects of artificial contraceptive measures on longevity.

We appreciate the reviewer's comment and see the potential of carrying out an analysis as suggested. Unfortunately though, menopause has only been conclusively demonstrated in a handful of species, such as certain toothed whales (Ellis et al. 2018, 2024). Among primates, strong evidence of menopause is limited to humans and possibly chimpanzees, with the latter documented only in the Ngogo population (Wood et al. 2023). While extended post-reproductive lifespans have been suggested to occur in zoo populations (Winkler and Goncalves 2023), these findings are controversial and likely the result of misinterpretation of data and methodological issues, as highlighted in Chapman et al. (2024). Moreover, analysis of survival of animals that continue to live past their last age of reproduction would be difficult due to extremely small sample sizes, limiting the feasibility and robustness of any comparative analysis. Thus, we have not been able to conduct such an analysis due to the lack of data and controversy over species classifications, but we do provide a more thorough discussion of our results in relation to menopause and its potential benefits.

Minor Comments:

5) It seems like the authors are using contraception and sterilization interchangeably for female animals, which is imprecise and misleading (especially in the title). This should be amended in a revised version of the manuscript.

We apologise for this and have carefully checked the manuscript for the correct use of each term throughout the text. We have also adjusted the title so that it that it refers to "female contraceptive methods", a term that is often used to capture both permanent sterilization and ongoing hormonal contraception.

6) Including text labels and/or a list of species for Figures 1A and 4A would greatly help with clarity for the readers.

We have now provided a corresponding data table (Extended Data Table 1) for Figure 1a so that readers can quickly match the results to specific species. Figure 4A has been removed from the manuscript in response to Referee 3's comments (see below).

Referee #3 (Remarks to the Author):

This study provides a comprehensive analysis of the effects of male castration and female contraception on lifespan across a variety of zoo-housed mammalian species, taking into account different covariates including sex, species, methods of contraception/sterilization, and environmental factors. The additional meta-analysis of data from previous papers to support the findings is also a valuable component. However, I have a couple of questions that I believe need addressing to strengthen the paper further.

On "Reproductive Allocation and Sex-Differences in Survival" section

The hypotheses outlined in the first paragraph of the section need further articulation. As I understand it, the paper suggests that male-specific androgen production shortens male lifespan, whereas female-specific estrogen production extends female lifespan. Therefore, limiting reproductive investment could narrow the sex gap in lifespan by either extending male lifespan or reducing female lifespan. If my understanding is correct, I recommend clarifying this in the text, as the current explanation leaves room for alternative interpretations on how reproductive limitations might influence sex differences in lifespan. For example, restriction on reproductive allocation can increase the survival for males and females, but with stronger effect on males.

We thank the reviewer for their detailed response and thoughts in relation to the sex-difference analysis. Given the major expansion of the revised manscript with new data, we have reduced the focus on sex-differences in ageing and moved this to supplementary information. We have, however, taken on the reviewer's points and thought carefully about how we present this analysis in the supplementary section so it is easier to follow.

Our initial analysis was complicated because we were trying to test multiple hypotheses about how male and female gonadal hormones influence sex-differences in lifespan. The key hypothesis in relation to females is that ovariectomy and removal of female ovarian hormones should reduce female lifespan and thereby reduce sex-differences in lifespan. We have actually disproved this earlier in the manuscript where we show that removal of female ovaries increases female lifespan and has no impact on the degree of lifespan extension when females are sterilized. Therefore, to make the later sex-differences analysis easier to follow, we now just focus on the response in males, because here the degree of lifespan extension with castration is hypothesized to be linked to sex-differences in lifespan.

The analysis and presentation of results, particularly in the lines 249 – 264, are somewhat difficult to follow. Figure 4B shows that compared to F normal group, M contraceptive group has higher life expectancy as male contraception can increase male survival. Based on this, authors conclude that the analysis does not support the hypothesis that male gonads and reproductive investment are not the cause of sex-difference in lifespan. Why? In another

word, could you clarify what specific evidence would actually support this hypothesis? No sex difference in life expectancy in the groups of F normal vs. M contraceptive?

We have now been more cautious about this analysis and explained the results more simply. The hypothesis is that castration should have the biggest effects on lifespan in species where females live much longer than males. Thus, where males have a much shorter lifespan than females due to intense sexual competition, we expect that castration and removal of this male-specific investment will have the strongest beneficial effects on survival.

We note first that it is not possible to simply correlate the sex difference in lifespan with the degree of lifespan extension with male castration: both calculations share the same demoninator (i.e. the intact male lifespan values are used to calculate both the sex difference in lifespan value and the degree of lifespan extension with castration value), and effects observed with such a correlation may represent a regression to the mean where extreme lifespan effects observed in intact males of some species may regress to more normal values (i.e. like females) when a second population of males (i.e. castrated males) is assessed. Thus, the appropriate way to determine whether there is a true relationship is to determine whether the variation in lifespan is smaller when comparing sterilized males to females than when comparing intact males to females. This analysis is now justifed within the methods section (lines 842-860), citing a statistical method paper dealing with this issue and also citing a previous meta-analysis where we have used this approach to correlate relative changes in lifespan extension with manipulations in a different context.

To make this analysis easier to follow in the supplementary information we have now created a simplified three part figure (Extended Data Figure 4). Panel A shows the hypothetical change that would be expected if male castration reduces sex differences in ageing, which is that variation in lifespan should be reduced. Panel B then shows the test of this hypothesis with the data from zoos, focusing on the most important test which is whether the sex gap in longevity is reduced. Alongside, in Panel C, we show the same analysis using the data from the meta-analysis. Panels B and C clearly show no reduction in the sex gap in longevity with male castration, and thus demonstrate that the degree of improvement in survival with castration is unrelated to the sex difference in lifespan found in a given species. We have removed the phylogenic tree figure that was included in the earlier version because it did not test the hypothesis and was distracting.

Additionally, the conclusion drawn in lines 279 – 292, that reproductive allocation during adulthood does not significantly influence sex-difference in lifespan, seems to disconnect from the previously discussed hypotheses for the male-only contraception and female-only contraception cases. A bit more explanation on how this conclusion integrates with the hypotheses and previous analysis would be helpful.

We have now added this section to the discussion and used this analysis to test whether variation in the lifespan response to castration is linked to sex differences in lifespan. In this section we provide more background on the specific hypotheses being tested, and we link the sex-difference test to the previous analysis to meet the reviewer's requests.

On the Timing of Contraception/Sterilization

Another question I have is about the timing of contraception or sterilization. The paper mentions data availability on the timing of contraception but doesn't delve deeply into how this timing might affect survival outcomes. For example, does the contraception applied at different stages of life (at birth vs. between birth and puberty vs. at puberty vs. in adulthood but before first reproduction vs. after first reproduction) have the same impact on survival?

We thank the reviewer for suggesting this insightful analysis. We have now provided two comprehensive analyses on the timing of sterilization and its effects on lifespan using data from the zoo dataset and the published literature.

We first conducted an analysis within the meta-analysis of published data to test whether the age at which sterilization occurred influenced the lifespan response. We split data according to whether sterilization occurred at birth, before puberty, at puberty or during adulthood, facilitating a cross-species comparison. We find a sex-specific effect of age at sterilization on the lifespan response, with males showing a greater improvement in survival when they undergo castration early in life, before puberty. By contrast, effects in females are not dependent on the life stage when sterilization is initiated.

We were then able to further validate this finding using the zoo dataset. We were able to conduct an analysis across 43 species, comparing the lifespan response in males who had been sterilized either pre- or post- their typical age at puberty. We found a greater increase in life expectancy when the manipulation is conducted pre-puberty, although the degree of this change is dependent on phylogeny. In primates, life expectancy is increased irrespective of whether sterilization occurs pre- or post- sexual maturity. However, across other taxa, life extension is greater when sterilization occurs prior to sexual maturity.

This major new analysis is reported in Figure 5 and lines 288-325. In the discussion we also highlight that these effects could be linked to the effects of androgen exposure during puberty, leading to long-lasting changes in pathways that regulate aging and improved survival later in life.

On the maternal death for females

Is the paper using all-cause mortality in the analyses, including the specific risks associated with reproduction, such as maternal death? This clarification is crucial, especially when considering the impact of contraception on female survival. If maternal deaths are included in the overall mortality data for intact females, this could inherently increase their risk profile compared to contracepted females, who do not face the same reproductive risks.

This difference in risk exposure might partly explain why contraception appears to lead to a survival advantage for females. Therefore, this paper states that results do not support the hypothesis that estrogen production associated with gonadal activity is beneficial for female survival. Excluding maternal deaths from the analysis could provide a more direct test of the physiological effects of estrogen.

We thank the reviewer for raising this possible alternative explanation. We have now provided a comprehensive analysis of different causes of death, and this included collection of information on maternal deaths at birth. Maternal deaths at birth only contribute to a very small amount of female mortality in zoos and across most species we did not find any

maternal mortality at birth for the data included in our study. Furthermore, we have firmly established that contraception delays death in females from several other causes of death that are more prevalent.

We now raise this point in the discussion of the manuscript (lines 414-418) and point the reader to a supplementary table that shows in the majority of species included in this analysis death at birth did not occur, and where it did it was at very low levels so cannot explain the observed results.

Connection to biological age

This study shows a slight decrease in survival for human females after sterilization, a notable exception among mammals. This raises interesting questions about biological age. For example, a recent paper in Cell Metabolism suggests pregnancy might reduce biological age in mice, possibly indicating similar benefits in humans. This implies reproductive benefits in slowing aging. Linking these findings could shed light on how reproductive strategies affect aging. Drawing a connection between such studies and findings in this paper couldcontribute to the ongoing debate on aging, reproductive health, and longevity.

Reference: https://www.nature.com/articles/d41586-024-00843-w?WT.ec_id=NATURE-202403&sap-outbound-id=FE5DA9037F51385D30CAEBC8A0C3A082838E85ED

We are aware of the interesting paper that the reviewer suggests. The complication with it is that it shows that pregnancy hastens biological aging but lactation reverses this effect. As lactation is often considered to be one the most costly components of reproduction for mammals (at least energetically, Clutton-Brock et al. 1989) this effect is at odds with what we know about trade-offs between reproduction and survival. Nonetheless, we have now mentioned the importance of studies investigating the biology of aging and epigenetic changes (lines 405-412), particularly since it has also been shown that castration reduces epigenetic aging in sheep. We note that future research to understand the biological underpinnings of how reproductive processes trade-off against survival could provide a major insight into the causes of ageing.

Some Minor Questions/Suggestions:

1. Is the analysis of zoo-housed data comparing intact and contracepted/sterilized individuals conducted within individual zoos or across different zoos for each species? The paper mentions that ”To reduce heterogeneity in husbandry practices, we only included individuals that were born or still alive after the 1st of January 1980, including records up to the 1st of February 2022.“ In addition to this, does the paper using other methods to account for the potential confounding effects of varying environmental or husbandry conditions among zoos?

We agree with the reviewer that differences in husbandry between zoos can produce unwanted heterogeneity. Husbandy standards maintained by zoos over the past 100 years have increased dramatically and so has the lifespan of animals housed in these. Since 1990 onwards, in particular, the European (European Association of Zoos and Aquariums, EAZA) and USA (Association of Zoos and Aquariums, AZA) zoo associations have developed strong veterinary, enrichment and husbandry guidelines for their associated zoos. It is these zoos

where the vast majority of the data used in this analysis has come from. This has contributed to increased life expectancy compared to previous periods and reduced heterogeneity across zoos. To minimize any heterogeneity arising from animals being housed in different zoos with varying environmental and husbandry conditions, we have now restricted our analysis to survival for animals born or still alive from 1st January 2005 to 1st Febuary 2023. Over this more recent period animals in EAZA, AZA and other zoo associations have been maintained under strict guidelines for animals under their care and mortality due to husbandy practises has been reduced (Roller et al 2021; Tidière et al 2023).

Using this dataset we have also conducted an analysis of life expectancy across different zoo associations, for which there were sufficient records of surgical contraception between zoos belonging to ASA, EAZA, and all “other” zoos that contributed to the database. It is important to note that most of the data we used for this study comes from AZA and EAZA, but we decided to include any other zoos under the “other” category for completeness. Note that we were unable to account for differences in specific zoos given that zoos are anonymized, but, given the strict guidelines within associations, we considered that comparing differences in results by association should be sufficient to establish whether there is potential for zoo heterogeneity. As we show in the figure below, all the estimated life expectancies for surgically contracepted males between the three regions share 95% credible intervals with only one exception for *Antilope cervicapra*. As the reviewer can see, in general, there are no major differences, which shows that our findings are not affected by differences between zoos or regions.

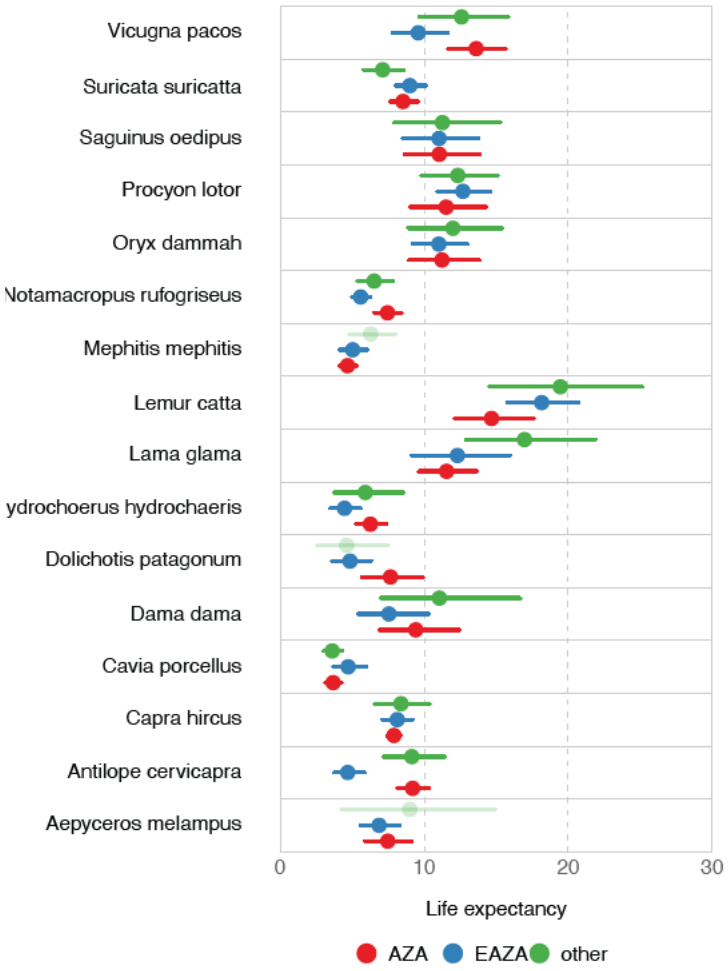

Figure R1 (of reviewer response). Estimated life expectancy and 95% credible intervals of surgically contracepted males in different associations (AZA, EAZA and any other). Faded points and lines correspond to datapoints with less than 20 individuals for inference.

2. In species where social status is influenced by physical strength, aggressiveness, or behaviors linked to testosterone, castration might decrease a male's rank or change his social role. This dynamic may diminish the positive effects of castration on survival. Do the authors observe a different impact of male castration on survival across species with such social hierarchies?

We have conducted an analysis to establish whether there are differences in the degree of lifespan response when comparing group-living species to socially monogamous and solitary species. We did not find any difference in the lifespan response when comparing these groups.

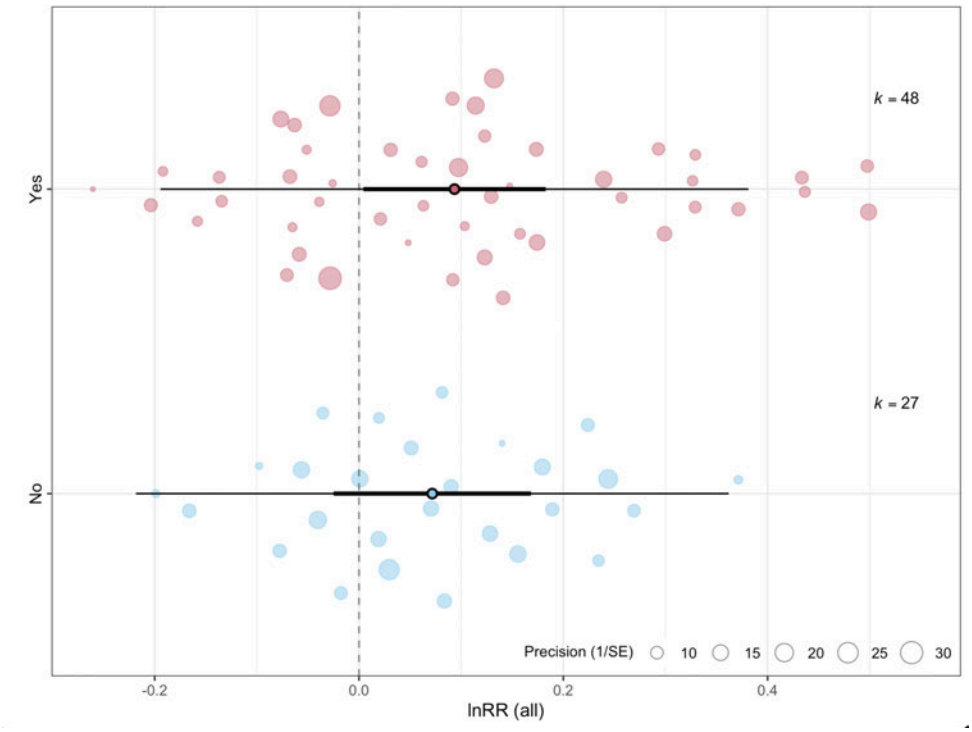

Figure R2 (of reviewer response). Species were split according to whether they were group-living (Yes) or solitary (No). Data on group-living or solitary lifestyles for species was extracted from the following reference:

Lukas D and, Clutton-Brock TH. The evolution of social monogamy in mammals. Science. 2013 2;341(6145):526-30

and matched to data available on the effects of surgical sterilization on changes in life expectancy in the zoo data. Bars show 95% confidence intervals (thick lines) and 95% prediction intervals (thin lines). *k* is the number of effect sizes within each sub-group.

We have also conducted an additional analysis looking at whether the degree of promiscuity found within a species predicts the lifespan response to sterilization in males. Here we also did not detect any association between promiscuity and the increase in survival after sterilization. We have now added this second analysis to the supplementary information (Extended data Figure 5) and highlight in the revised manuscript that these factors do not explain any substantial variation in the survival response.

3. Line 103 mentioned that Figs S1&S2 shows survival curves for each individual species. However, I only found male surgical vs. non-contraction in S1 and female hormonal vs. non-contraception in S2. What about other comparison? E.g., female surgical vs. non-contraction? Maybe I missed anything here?

We have now checked that all survival curves for individual species are included in the supplementary figures.

4. The interpretation of the negative value (-0.0092) for male surgical sterilization's impact on survival (lines 252 - 253) is confusing. Could you elaborate on how this negative value translates to increased survival?

We sincerely apologize, the negative value was a mistake. This statement has now been removed from the manuscript during the restructuring.

5. Authors use the average difference across species when discussing the sex gap in life expectancy differences (Figure 4A). It will be very helpful to add such values for each panel of Figure 4A to enhance understanding and clarity for the reader.

We have now removed this panel as explained above so that this analysis is easier to understand for the reader.

References

Chapman, S. N., S. Ellis, M. Lahdenperä, D. P. Croft, and V. Lummaa. 2024. Menopause has not evolved as a general trait in mammals: A response to 'Do mammals have menopause?' bioRxiv, doi: 10.1101/2024.02.29.582687.

Clutton-Brock, T. H., Albon, S. D., & Guinness, E. F. (1989). Fitness costs of gestation and lactation in wild mammals. *Nature*, *337*(6204), 260-262.

Ellis, S., D. W. Franks, S. Nattrass, M. A. Cant, D. L. Bradley, D. Giles, K. C. Balcomb, and D. P. Croft. 2018. Postreproductive lifespans are rare in mammals. Ecol Evol 8:2482–2494.

Ellis, S., D. W. Franks, M. L. K. Nielsen, M. N. Weiss, and D. P. Croft. 2024. The evolution of menopause in toothed whales. Nature 627:579–585.

Roller M, Müller DWH, Bertelsen MF, Bingaman Lackey L, Hatt JM, Clauss M. The historical development of juvenile mortality and adult longevity in zoo-kept carnivores. Zoo Biol. 2021 Nov;40(6):588-595. doi: 10.1002/zoo.21639.

Tidière M, Colchero F, Staerk J, Adkesson MJ, Andersen DH, Bland L, Böye M, Brando S, Clegg I, Cubaynes S, Cutting A, De Man D, Derocher AE, Dorsey C, Elgar W, Gaglione E, Anderson Hansen K, Jungheim A, Kok J, Laule G, Goya AL, Miller L, Monreal-Pawlowsky T, Mucha K, Owen MA, Petersen SD, Pilfold N, Richardson D, Richardson ES, Sabo D, Sato N, Shellabarger W, Skovlund CR, Tomisawa K, Trautwein SE, Bonn WV, Elk CV, Von Fersen L, Wahlberg M, Zhang P, Zhang X, Conde DA. Survival improvements of marine mammals in zoological institutions mirror historical advances in human longevity. Proc Biol Sci. 2023 Oct 25;290(2009):20231895. doi: 10.1098/rspb.2023.1895.

Winkler, I., and A. Goncalves. 2023. Do mammals have menopause? Cell 186:4729–4733.

Wood, B. M., J. D. Negrey, J. L. Brown, T. Deschner, M. E. Thompson, S. Gunter, J. C. Mitani, D. P. Watts, and K. E. Langergraber. 2023. Demographic and hormonal evidence for menopause in wild chimpanzees. Science (1979) 382.

Dear Dr Garratt, dear Mike

Your manuscript, "Male castration and female contraception increase lifespan across vertebrates", has now been seen by 4 referees. You will see from their comments below that while they find your work of interest, some important points are raised. We are very interested in the possibility of publishing your study in Nature, but would like to consider your response to these concerns in the form of a revised manuscript before we make a final decision on publication.

We would like to receive a comprehensive response to the concerns raised by reviewer #4, including the provision of additional data wherever possible. We consider the textual changes suggested by reviewers #1 and #3 to be important and essential.

Dear Editorial Team

Thank you for the opportunity to address these points. We have responded to each comment made by the reviewers, adjusting the figures, adding additional data/analysis where required, and ensuring that all types of sterilization/contraception are accurately referred to throughout. This has further enhanced the manuscript. In particular, our changes in response to reviewer four's comments have allowed us to clarify how our results fit within theoretical and mechanistic frameworks for ageing, while in response to reviewer one's suggestion we have also tied our findings on healthspan, cautiously, to knowledge of the health benefits/costs of oophorectomy and birth control in humans.

We have uploaded a track-changes copy of the manuscript that shows all changes made to the manuscript since the last version, in addition to a clean copy and high quality figures. The manuscript is currently slightly longer that the word limit guidelines for a typical Nature manuscript in Biological Sciences (5042 words), although as it is stated this at the editors discretion we have left it at this length for now. We would of course be happy to make edits to the manuscript to comply with space limits as required.

We hope you find the revised manuscript acceptable for publication. Please let us know if you require any additional information.

On behalf of all authors,

Mike Garratt

Referees' comments:

Referee #1 (Remarks to the Author):

The authors have gone above and beyond and fully addressed all of my initial comments.

1) I wish the distinction between vasectomy and castration in terms of its longevity effects,

was mentioned in the abstract as - while clearly presented in the text - the abstract without this info poses a risk of increasing vasectomies in male readers, especially of the many popular press articles that will cover this exciting and widely read article

We have now highlighted in the abstract that the longevity effects of sterilization in males occur specifically in response to castration.

2) The health benefits and risks of various types of female contraception is well known in the literature and should be discussed here - seems likely to me based on the authors statements that hormonal birth control would increase both female lifespan and - in contrast to OVX - healthspan?

We appreciate this being pointed this out. We have now connected the healthspan effects of ovariectomy to the health consequences of surgical menopause in humans, and highlighted that these effects are less dramatic in women who undergo hysterectomy with ovarian conservation. Regarding hormonal birth control, we are cautious about making direct connections between our results and the health/survival effects of ongoing hormonal contraception in contemporary humans. However, we mention that data on the long-term health effects of ongoing hormonal contraception in humans is mixed, since studies have reported a range of different relationships, and it remains to be seen whether this treatment would influence long-term healthspan in controlled settings.

These additions are in the healthspan section of the manuscript, lines 368-375.

Referee #2 (Remarks to the Author):

The authors have mostly addressed my previous comments. This manuscript and the analyses it contains should be illuminating for the field.

The crucial sticking point that remains in this revised version, which prevents me from a full endorsement, is the continued use of "female contraceptive methods" - especially in the title where no nuance or context is provided - to encompass both hormonal and surgical interventions. In a world where many readers or news outlets stop at a title, it is important to avoid misleading titles. Most readers - scientists included - would not realize that surgical sterilization is included in this group, which I believe is a damaging concept to include in the title. Thus, I must still recommend that sterilization (irreversible) and contraception (reversible) be included as separate concepts in a revised title for this piece.

We appreciate this being pointed out and have now referred to these as separate concepts in the title. We have also been through the manuscript carefully and have been more specific and consistent about the use ongoing hormonal contraception and permanent sterilization when each reference to these methods arises.

Referee #3 (Remarks to the Author):

I thank the authors for their thorough work on the revisions. I find that my previous

comments have been well addressed and the manuscript is nearly ready for publication!

Overall, this is a highly significant and original study that addresses a critical gap in the field. The methodology is sound, the data presented are of high quality and from various sources, and the statistical analyses are appropriate. As a result, the conclusions are robust and well-supported by the evidence. The manuscript is clearly written, and the revised figures effectively illustrate the key findings.

I have just a few minor points that need attention to finalize the manuscript:

In line 111, the reference to "Fig a,b" appears to be inaccurate. Given the context, "Fig 1a" would be the correct reference.

Apologies, we missed the "1" in this reference, which is now corrected. Fig 1a and Fig 1b are both relevant to this point. Figure 1a shows the lifespan changes across the mammalian tree for male surgical manipulations and female hormonal manipulations. However, Fig 1b shows the estimated overall effect size and confidence intervals for each manipulation in each sex, which is relevant to the point that the effects of the interventions are similar in males and females.

In line 350, the origin and meaning of the "11.57%" value are unclear. For the benefit of the reader, this should be clarified, as it does not seem to correspond to any of the reported effect sizes (overall or female or male).

We apologize, this value was not originally clarified with additional text. For simplicity we have deleted this from manuscript and just show the effect sizes as originally described.

In lines 608-609, the association between survival rates and life stages is described in a confusing way. A survival rate of S(x) = 0.2 (i.e., 20% survival) should correspond to "older ages," whereas the text seems to associate it with "early childhood."

We appreciate the reviewer noticing the lack of clarity here. We have now reversed the order of the survival rates so that the order corresponds to the description for each age point. This now reads:

"For each species and contraception type, we calculated the cumulative incidence for a given cause of death at three ages, namely when $S(x) \in \{0.8, 0.5, 0.2\}$, this is early adulthood, median adulthood, and older ages". Lines 799-801.

Referee #4 (Remarks to the Author):

A. Summary of the key results
This important paper tests the impact of sterilization on lifespan across vertebrates (with a strong emphasis on mammals). It finds broad support for longer lifespans in sterilized individuals, but with notable heterogeneity.
B. Originality and significance: if not novel, please include reference
Yes, it's original. Nothing on this scale exists to my knowledge.

C. Data & methodology: validity of approach, quality of data, quality of presentation
Excellent, rigorous methodology.
D. Appropriate use of statistics and treatment of uncertainties
Yes, and the team includes some of the very best statisticians in the field.
E. Conclusions: robustness, validity, reliability
A wide variety of analyses provides strong support for robustness of conclusions.
F. Suggested improvements: experiments, data for possible revision
See below.
G. References: appropriate credit to previous work?
Fine.
H. Clarity and context: lucidity of abstract/summary, appropriateness of abstract, introduction and conclusions
Clear.

This important paper tests the impact of sterilization on lifespan across vertebrates (with a strong emphasis on mammals). It is well conducted and there are no methodological flaws and I recommend publication without hesitation.

As a reviewer invited into the process after previous reviews and revisions, I am both hesitant to demand further revision but also obligated to provide thorough feedback. I have identified a number of points that I think could be improved in the manuscript, almost all very simply and easily. However, let me be clear: the manuscript is already of sufficient quality and does not require any further revision to be publishable. The following comments are suggestions only, and should be incorporated by the authors only if they feel the benefit to the manuscript is worth the effort.

We are extremely grateful to the reviewer for their support, ideas and considered comments presented as suggestions as part of a second round of review. We have thought about all of these carefully and used them to improve the manuscript and tie up important loose ends that were left after the last round of revisions.

Lines 52-54: It's not clear that negative interspecific correlations between lifespan and reproduction indicate a trade-off in the usual sense. They can arise simply from constraint that population growth cannot (or is not usually) too fast up or down. I believe this is touched on here:
Salguero-Gomez, et al. (2016). Fast-slow continuum and reproductive strategies structure plant life-history variation worldwide. Proceedings of the National Academy of Sciences of the United States of America, 113, 230–235. https ://doi.org/10.1073/pnas.15062 15112
And here:
Ricklefs, R. E. (2000). Density dependence, evolutionary optimization, and the diversification of avian life histories. The Condor, 102(1), 9-22.

We have now adjusted this sentence to make it clear that negative interspecific correlations between lifespan and reproduction *maybe* caused by trade-offs, rather than the previous implication that these interspecific negative correlations are direct evidence that there is a trade-off (lines 52-55). As the manuscript is running long in both words and number of

references cited we are unable to go into greater detail on other potential causes for these negative relationships, but we appreciate this being higlighted for us.

Are sterilized individuals in zoos randomly selected? Any chance of bias? For example, I would expect only the healthiest individuals are chosen for breeding, except in the most endangered species, leading to a potential underestimation of effects. On the other hand, individuals chosen for breeding may also differ in how they are treated afterwards, independently of the reproduction.

There are a variety of different reasons that individuals are sterilized in zoos, depending on the individual, species and institution, including stopping the production of unwanted surplus animals, maintaining genetic diversity, managing behaviour and for health reasons. Animals are not necessarily selected randomly, but mostly we expect this to occur in an unbiased manner over time in relation to the specific needs of zoos at specific times. In a subset it is possible that there is specific selection for sterilization or contraception of certain animals; For example, if an animal is not genetically compatible with other mates etc.

It is possible that within some species the effects of sterilization could be under or over-estimated due to the reasons for why sterilization is conducted, and this might explain some of the heterogeneity that we observe. However, the scale of species sampled means it is very unlikely to explain the overall effect, particularly considering we observe no effects of vasectomy on survival, while we see a difference in response to castration depending on whether it is conducted pre- or post- puberty.

Moreover, the meta-analysis of the published data conclusively demonstrates that sterilization increases survival even when only considering “controlled studies” where animals have been randomly allocated to sterilized and unsterilized groups and maintained in equivalent conditions across the remaining lifespan/survival assessment. One of the strengths of our paper is that we are able to draw evidence from a variety of different sources to show the taxanomic breadth of the response across species, but also quantify the effects that can be observed in controlled, experimental studies (where animals are randomly allocated to experimental groups and maintained in equivalent environments across the study period).

Action: We have now made clear the main reasons for sterilization and contraception in zoos at the start of the article (lines 92-94), and within the text we have rephrased the sentence about “non-controlled” studies so that it is obvious this apples to the zoo data and the possible limitations of this type of data are clearly acknowledged (267-270). We have also added an opening paragraph to the discussion that draws together the different pieces of evidence and their consistency of effects, highlighting the insight derived from each type of analysis and the conclusions that can be drawn about trade-offs when considered together (lines 378-390; also see the comments below relating to the new discussion paragraph).

In females, how much of the effect is explained by giving birth and raising offspring? That is, if you control for number of progeny, is the effect attenuated? How much?

As we understand this, the reviewer is suggesting that if we control for the number of offspring that each animal has had at an individual level in our survival analysis, then the effect of contraception would be lessened if it is due to diminishing the costs of offspring production, i.e. control animals that produce none or few offspring would have a similar lifespan to contracepted animals, because they have not been exposed to these costs, while there would be a big difference when comparing the lifespan of control animals that have had lots of offspring to contracepted animals.

We appreciate the reviewer raising this interesting idea, but the premise relies on there being a negative relationship between lifespan and number of offspring produced within control animals. However, when looking within cohorts of animals, a positive relationship between number of offspring and life-expectancy is often observed. This is because animals that live longer have more opportunity to reproduce across their life, and importantly because differences in individual quality across a group of individuals mask trade-offs and generate positive relationships. The important point about individual quality masking trade-offs is raised by the reviewer in their last point in relation to the Van Noordwijk and De Jong 1986 paper.

We have tested whether producing or not producing offspring had an effect on survival and life expectancy in non-contracepted females using the zoo database. However, we found that, for most species, the life expectancy of those that produced offspring was longer than those that did not (see Table 1 pasted at the bottom of the response). However, this effect is seriously confounded by the fact that those animals that did not produce offspring died early in life before they had sufficient chance to be able to reproduce. As shown in Fig. 1 (pasted at the bottom of the letter), early mortality was disproportionately higher in those that did not produce offspring, and their advantage in life expectancy (i.e., life expectancy difference) is strongly driven by this steep initial mortality (almost 50% in the variance in life expectancy differences is explained by first year mortality differences). Thus animals that do not reproduce are the ones that had higher early life mortality, so never got opportunity to reproduce because they were already dead.

A previous analysis of a limited number of zoo species has looked at the relationship between early-life reproductive effort and subsequent lifespan, so controlling for this factor but excluding mortality costs arising during reproduction (i.e. consequences of diminished immunity during pregnancy etc). However, they also largely failed to see a relationship (Ricklefs and Cadena 2007, doi: 10.1111/j.1461-0248.2007.01085.x.), likely in part because of the masking of trade-offs by individual quality. This is why manipulations of reproductive effort are so important for detecting trade-offs because they remove/mitigate this individual quality component. This is nicely reviewed in a recent modelling article about these positive relationships (Zuk et al. 2025, doi;10.1093/jeb/voaf094).

Action: We have now modified the introduction of the paper to highlight the complexity of studying within individual trade-offs between reproduction and lifespan, making the explicit

point that positive relationships can arise because of variability in individual quality, and citing the Van Noordwijk and De Jong 1986 that the reviewer refers to below (lines 62-66). Currently, we have refrained from adding the data on positive relationships between lifespan and offspring production to the manuscript because it is confounded by animals living longer having more opportunity to reproduce. It would be possible to add this as supplementary data, or conduct a more exhaustive assessment of reproductive investment early in life and its relationship to subsequent survival, but given previous analysis of zoo data we strongly suspect no relationship would be detected. Given the option of how to address points by the referee, and the current length of the paper, we have instead cited the previous study that largely observed no relationship between reproduction and lifespan within zoo animals, and highlight the requirement for manipulations that inhibit reproduction as studied in this paper (within lines 62-66).

Fig. 1a – confidence intervals for each species-sex should be provided, if at all possible.

This is a very good point. We have now added these to Figure 1, which makes the individual level species effects much easier to understand, and also Figure 5C.

Is the moderation of the effect by wild environment stronger in males? I would guess that testosterone causes risky behavior in males with accompanying mortality, and that this behavioral effect could explain much of the difference, but that this might be absent in females.

We have now tested whether there is an interaction between the type of environment (wild/semi wild or not) animals are studied in and sex on the change in survival. For this analysis the data mainly comes from studies of female sterilization, so we cannot make strong inferences about whether effects are stronger in one sex or the other, but they are clearly present in females.

Action: In response to the reviewer's point, we have now included sex as a variable in the moderator tests for the meta-analysis, including moderating effects of environment, whether the study is controlled or not, and if a sham manipulation was conducted (see the new Extended Data Figure 2). For all of these tests it appears that the response is similar between sexes. For the important tests of whether the study was controlled and if a sham manipulation was conducted, we also have good representation in both sexes and can thus be confident that the effects (or lack of) are similar in both sexes.

In female mammals (Fig. 1a) almost all of the effect seems to be driven by primates. Is there still a significant overall trend if primates are removed? Also, female mammals appear less heterogeneous than males in Fig. 1a (lots of males with negative effects) but this is not reflected in the confidence or prediction intervals in Fig. 1b. Why not?

For Fig 1a, primates do make up the majority of the species where we were able to assess the effects of hormonal contraception on life-expectancy. When we break down the female data into different families we find a significant overall effect in primates (estimate: 0.133, CIs: 0.095-0.171) and carnivores (estimate: 0.076, CIs: 0.0169-0.134) but in the other groups

we do not have sufficient replication for statistical analysis although the trends are qualitatively similar.

The addition of confidence intervals to Fig 1a makes it easier to see the patterns in different taxa. We have also provided the separate estimates for primates and non-primates within in the supplementary information file and refer to this on lines 111-112.

Regarding the differences in heterogeneity between the sexes, qualitatively there does seem to be slightly more variation in the response in Fig1A, although not a dramatic difference. However, there is also double the sample size in the male surgical analysis so this explains why the confidence and prediction intervals are similar between the two groups.

Line 368 – "withholding reproduction" is probably not the right phrasing – this doesn't include animals kept in isolation and therefore unable to reproduce, for example. "sterilization" might be more accurate. Given the lack of effect of gonadectomy, it seems the unifying thread is removal or suppression of sex hormones or their production.

We appreciate this being pointed out and have now specifically referred to the types of manipulation that lead to increased life-expectancy.

Discussion:
The Intro frames this study around trade-offs between survival and reproduction, but the discussion hardly mentions this. I think it interesting that the effect sizes of 10-20% arise repeatedly (though with lots of heterogeneity). This supports a non-negligible but weak-to-moderate role for trade-offs in shaping life histories. That is, getting rid of reproduction is nowhere close to doubling lifespan or explaining the whole spectrum of mammalian lifespans. On the other hand, this study supports the role of sex hormones as potential orchestrators in canalized pathways mediating these trade-offs, potentially along with IGF-1 mediation of growth/survival trade-offs. Probably worth mentioning.

This is a very good suggestion. Within the new first paragraph of the discussion we now reflect on the evidence that trade-offs are important in vertebrate life-history while making the point that the magnitude of lifespan change is smaller than that which occurs with gonadal manipulations in invertebrates (lines 378-390).

It might be interesting to compare castration to other known lifespan interventions (caloric restriction, rapamycin, etc.) for magnitude of effect.

[Redacted text]

We have now highlighted this point in our discussion (lines 427-428) about how sex-hormones may interact with IGF1 and mTOR signalling pathways, which these lifespan interventions are assumed to interact with. We cite several meta-analyses that have estimated the average lifespan response to these manipulations and show changes in lifespan of a similar magnitude as we report here.

Most interventions that perturb evolved homeostatic mechanisms decrease lifespan or have adverse health consequences. For example, the hygiene and old-friends hypotheses suggest that even though parasites can be harmful, their absence can also disrupt a normal balance and have negative effects on health. Sterilization is interesting in this context in that it does the opposite – improves health/longevity, despite putting the organism in a state clearly outside its homeostatic norms and for which it is clearly (by definition) not adapted. This is interesting to note in and of itself, but also suggests that the effect sizes here may be underestimates – that we are likely observing a complex mix of the benefits of not reproducing and the harms of perturbing homeostasis. This could explain much of the heterogeneity across species. Interestingly, it is analogous to the famous Van Noordwijk and De Jong 1986 paper that counterposes individual quality processes and trade-off processes, which are both present and can generate opposite effects in the data…

This is a very interesting and thought-provoking point. We could speculate that, in the case of sterilization and contraception, this is not necessarily putting animals outside of their evolved homeostatic range because manipulations like gonadectomy effectively induce a state that animals are exposed to pre-puberty (castration and ovariectomy) or during pregnancy (hormonal contraception). In particular, castration prior to puberty effectively leaves animals in a state where an animal simply does not undergo the major homeostatic alterations that occur with adulthood. In the case of progesterone-based birth control, the body is tricked into thinking it is pregnant, which is also a normal physiological response. We would love to have space to go into detail with this, but the discussion is already long for a Nature paper and this would probably require an additional paragraph, so we have opted to leave this alteration for now.

Thank you for pointing out the seminal Van Noordwijk and De Jong 1986 paper. We now cite this important paper in the introduction to explain why trade-offs between fecundity and lifespan are often not observed when looking across individuals within a population. Indeed, this highlights the need for manipulations of reproductive investment in order to observe trade-offs with lifespan, as we focus on in this study.

[Redacted table]

[Redacted table]

[Redacted table]

[Redacted table]

[Redacted table]

[Redacted table]

[Redacted table]

[Redacted text and figure]

Dear Editorial Team

Many thanks for the comments on our manuscript. Please see below responses to all the questions that were found in the Accept In Principle letter and associated attachments. The Reporting summary has also been filled and modified as requested.

Please let me know of any other modifications that are required.

Best wishes

Mike

Main Text Word Count: 5047. Figure Count: 5. Requested to reduce the manuscript to nine total pages in length.

We have reduced the manuscript length from 5047 words to 4590 words. Following the guideline that 4300 words and five modest size figures would be eight pages in length, 4590 words plus five figures (one of which is relatively large) should fit into nine pages.

The shortening of the manuscript has consisted of removing any repetition of key points that were made in the results and discussion, shortening of the introduction to remove the sentences about sex-differences in lifespan (which are explained as it arises later in the manuscript), removing some methodological information that was provided in the main manuscript but could be presented in the methods, and general editing to ensure a concise message throughout. A track changes version of the manuscript has also been uploaded.

1. Please submit a revised title within 75 characters (including spaces) that is free of any punctuation marks like colons, exclamation marks, full stops or speech marks.

The title has now been reduced in size and punctuation marks removed.

2. The number of main text references should be 60 in total or less - currently there are 88.

The main text references have now been split from the method references and with text deletions there are now only 52 references in the main text.

3. Flagging that there are no methods references - please create a separate reference list for the methods with continuous numbering.

A separate list of references for the methods has now been created.

4. Please reduce subheadings to 40 characters (with spaces) or less.

These have now been reduced to less than 40 characters.

5. X

6. Please provide a supplementary information guide.

We have now provided a supplementary information guide.

7. Flagging that there are potential third party rights issues in the figures - please check sources or if permissions are needed for the mammal silhouettes, gender symbols illustrations in the figures.

All symbols and mammalian silhouettes have either been created by us (those in Figure 3) or have been downloaded from PhyloPic under CC0 1.0 Universal Public Domain Dedication which allows unrestricted use anywhere, in anyway, without attribution. We have checked each silhouette to ensure they were from this domain.

8. Flagging that there are potential third party rights issues in the figures and the TPR table has not been provided on eJP.

There are no issues so we have filled the form in with NA.

9. Please re-supply Extended data Figure 9 and Extended data table 1 individually in EPS, JPEG or TIF format.

These have been converted into the requested format. Note that Extended data Table 1 was too long to fit into an individual image page so this has now been moved to the supplementary information.

10. Please ensure that the text size in all figures is at least 5 pt Arial.

We have checked that the text in the figures is of sufficient size.

Reporting summary queries

Information on code availability has been provided under the data and materials availability section of the manuscript. A request has been made in the reporting summary, for provision of a separate ‘Code availability’ statement in the manuscript.

A code availability statement has now been made in the manuscript.

Please note that the authors have stated in the data availability statement that ‘The extracted data from primary sources used in the meta-analysis are included in the Supplementary Information and they will be archived in Zendo once the manuscript is accepted for publication''. Hence, a request has been made to the authors for the provision of valid and appropriate accession identifier/web-link in the manuscript under the ‘data availability' section as well as in the ‘data’ section of the reporting summary along with public release of the same

These have now been added to the manuscript and reporting summary.

Please note that the authors have marked and filled ‘Animals and other research organisms’ module in the reporting summary. There is no direct involvement of any animals observed in the manuscript and only animal data is used in the current study. Kindly look into this.

We have highlighted that data from animals is from previously published studies or already collected data. We have filled in that sex is always clearly defined as that is important irrespective of whether we conducted the experiment or not.

Attached editorial requests

Legends

Information missing in Fig 1a

We are not sure what information is missing here but have rephrased the legend so it is clear what the centre of the error bars and error bars show, and have added the exact sample size for number of species.

Precise value of n in Figure 5b

We have changed this so that the N shown in the figure legend represents the sample size for number of species. This is consistent with how the sample size is represented within all the other legends and figures of the manuscript (i.e. the number of effect sizes (k values) for the meta-analysis plots).

Measure of centre for error bars in 3a, b, 5b, extended data figures 3 and 4

We have made it clear what the centre of the error bars represents in each of these figures.

Definition of box plots for 1b, c 4a-e, extended data figures 2a-c, 5b,c

We have made sure that the definition of these is easier to understand and all centre dots and bars are defined.

Please ensure that datasets deposited in public repositories are now publicly accessible, and that accession codes or DOI are provided in the "Data Availability" section. As long as these datasets are not public, we cannot proceed with the acceptance of your paper. For data that have been obtained from publicly available sources, please provide a URL and the specific data product name in the data availability statement. Data with a DOI should be further cited in the methods reference section

All datasets are now publicly available at Github (https://github.com/itchyshin/lifespan_contraception), and also archived at Zenodo (DOI: 10.5281/zenodo.17333443).