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Slow life-history strategies are associated with negligible actuarial senescence in Western Palearctic salamanders

Hugo Cayuela, Kurtuluş Olgun, Claudio Angelini, Nazan Üzüm, Olivier Peyronel, Claude Miaud, Aziz Avcı, Jean-François Lemaitre and Benedikt R. Schmidt

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Review timeline

Original submission:	25 April 2019
1st revised submission:	26 June 2019
2nd revised submission:	6 August 2019
Final acceptance:	6 August 2019

Note: Reports are unedited and appear as submitted by the referee. The review history appears in chronological order.

Review History

RSPB-2019-0971.R0 (Original submission)

Review form: Reviewer 1

Recommendation
Accept with minor revision (please list in comments)

Scientific importance: Is the manuscript an original and important contribution to its field?
Excellent

General interest: Is the paper of sufficient general interest?
Excellent

Quality of the paper: Is the overall quality of the paper suitable?
Good

Is the length of the paper justified?
Yes

Should the paper be seen by a specialist statistical reviewer?
No

Do you have any concerns about statistical analyses in this paper? If so, please specify them explicitly in your report.
No

It is a condition of publication that authors make their supporting data, code and materials available - either as supplementary material or hosted in an external repository. Please rate, if applicable, the supporting data on the following criteria.

Is it accessible?
Yes

Is it clear?
Yes

Is it adequate?
Yes

Do you have any ethical concerns with this paper?
No

Comments to the Author
This is a very interesting and timely paper on the senescence in salamanders, where the results show negligible actuarial senescence and an apparent lack of effect of size on survival. I believe that the study was well carried out, the manuscript is clear and concise, and, again, the results very interesting. I have only very minor comments below.

Paragraph 1 intro: It might be important to note that Kirkwood (Kirkwood, 1977) and Kirkwood & Holiday (Kirkwood & Holliday, 1979) made the additional distinction that senescence was inevitable in organisms with a clear distinction between somatic and germ lines.

Paragraph starting in line 86: It might be worth citing Finch (Finch, 1998) as the first to propose the existence of negligible senescence in indeterminate growers.

Line 135: More appropriate than "Colchero's models" I would suggest to refer to BaSTA. Also, the first reference should not be Colchero et al 2012a, but Colchero and Clark 2012.

Line 158: Here you say: "…information about individual age is updated." Did you mean, information about individual size? From what you describe, the transition probabilities correspond to s and l.

Line 213: I believe that the term "parameters" here might not be appropriate, I would instead use demographic functions. This helps distinguish the statistical definition of a parameter from the functional forms.

Discussion: I find it very interesting that size specific survival was essentially not different between small and large salamanders. It is interesting since non-ageing related advantages in size have been proposed as the mechanisms that produce the apparent lack of senescence. Here you find that this is not the case. It can also be that the two size classes you use here might include individuals of the same ages, making the distinctions in their survival hard to detect.

References:
Finch, C. E. (1998). Variations in senescence and longevity include the possibility of negligible senescence. The Journals of Gerontology Series a: Biological Sciences and Medical Sciences, 53(4), B235–B239.
Kirkwood, T. B. (1977). Evolution of ageing. Nature, 270(5635), 301–304.
Kirkwood, T., & Holliday, R. (1979). The Evolution of Ageing and Longevity. Proceedings of the Royal Society of London Series B-Biological Sciences, 205(1161), 531–546.

Review form: Reviewer 2

Recommendation
Major revision is needed (please make suggestions in comments)

Scientific importance: Is the manuscript an original and important contribution to its field?
Good

General interest: Is the paper of sufficient general interest?
Good

Quality of the paper: Is the overall quality of the paper suitable?
Acceptable

Is the length of the paper justified?
Yes

Should the paper be seen by a specialist statistical reviewer?
Yes

Do you have any concerns about statistical analyses in this paper? If so, please specify them explicitly in your report.
No

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> **Is it accessible?**
> No
>
> **Is it clear?**
> No
>
> **Is it adequate?**
> No

Do you have any ethical concerns with this paper?
No

Comments to the Author
This study investigates the pace of life history for 1 "true salamander" species and then tests for difference in mortality in 3 species (4 populations) with age and size. The authors report slow life history (in L fazilae) and negligible actuarial senescence in all species. I find it very interesting the recent comparative studies suggesting possible negligible senescence across taxa. This is an interesting case study in support of this literature.

While the finding that L fazilae salamanders have slow life histories, exhibiting low fecundity, recruitment and long lifespans is interesting, it is not all that surprising.

From my understanding of their analysis (and I am no expert on these statistical tests), the authors compare mortality rates based on size classes (as proxy of age), find no differences in mortality between small and large (young and old). So, the assumption is that if larger (older) individuals are not dying any more than expected than smaller (younger), then they must not be aging. The analyses do support these claims.

Study Limits—The major question in these studies is if the data collection span long enough to determine senescence (or lack of in the wild). The authors analysis greatly depends on monitoring populations as long as the lifespans of taxa studied. The authors report L. fazilae live 10 years and the study involves 10 years of data. The authors do not report lifespans for the other species. The survival curves in Fig 2 questions if the study durations were long enough to encompass a complete generation.

My biggest concern is not a technical one, but the interpretation of results. The manuscript currently focuses on lifespan and negligible senesces. This may be because of interest in aging for human health implications. However, there is little discussion of the associated tradeoffs. If there is no senesce, then what are the consequences for population growth, numbers and sizes of offspring, mortality rates? If space permits, I would appreciate the authors providing what they think about the implications of their findings for fundamental questions of life history theory.

For example, The authors state in the abstract "Our results showed that salamanders have slow life histories and that they experience negligible senescence", but these species show considerable variation in offspring production; 40-65 eggs in S. perspicillata, 23 larvae per year in S salamandra, and 1 offspring per year in L fazilae and Salamandra atra (lines 311-317). This implies that although there's considerable variation in offspring production, all of these species exhibit long lifespans and negligible senescence. I would be interested in the authors discussing their thoughts on the tradeoffs associated with these life histories.

The regenerative hypothesis is certainly interesting. Again, I would encourage the authors to understand the life history consequences associated with negligible senescence. Does regenerative ability correspond with elevated metabolic rates (compared to anurans of similar size and temperature)? To provide the resources for somatic maintenance/regeneration of tissues?

Specific comments:
Abstract: Part of the reason why "small ectotherms have lifespans similar to that of large endotherms" is because of much slower and even seasonally very low metabolisms. Comparing lifespans between these lineages would require adjusting for temperature dependence and seasonal temperature models. For this same reason, it is difficult to assess if the studies are monitoring the individuals long enough to get to the senesces mortality curve since these critters should have much longer lifespans for their body size (compared to better studied endotherms).

The difference between survival and mortality is key. I would clearly define these terms and how they differ in the methods.

Line 93: what species of salamander?

Line 128-30: Are males and females same body size?

Line 341-343: The authors report lifespans for L. fazilae (10 years). Report lifespans for other species because this is essential to understand if the assumptions of the statistical test (study duration lifespan) are met.

Line 344-345: This can be interpreted as a general statement across “true salamanders” but is currently only supported by these 3 species.

Lines 354-358: The authors begin using negligible and marginal senescence interchangeably. This should be consistently one or the other or elaborate on the differences.

Decision letter (RSPB-2019-0971.R0)

13-Jun-2019

Dear Dr Cayuela:

I am writing to inform you that your manuscript RSPB-2019-0971 entitled "Slow life-history strategies are associated with negligible actuarial senescence in western Palearctic salamanders" has, in its current form, been rejected for publication in Proceedings B.

This action has been taken on the advice of referees, who have recommended that important revisions are necessary. With this in mind we would be happy to consider a resubmission, provided the comments of the referees are fully addressed. However please note that this is not a provisional acceptance.

The resubmission will be treated as a new manuscript. However, we will approach the same reviewers if they are available and it is deemed appropriate to do so by the Editor. Please note that resubmissions must be submitted within six months of the date of this email. In exceptional circumstances, extensions may be possible if agreed with the Editorial Office. Manuscripts submitted after this date will be automatically rejected.

Please find below the comments made by the referees, not including confidential reports to the Editor, which I hope you will find useful. If you do choose to resubmit your manuscript, please upload the following:

1) A ‘response to referees’ document including details of how you have responded to the comments, and the adjustments you have made.
2) A clean copy of the manuscript and one with 'tracked changes' indicating your 'response to referees' comments document.
3) Line numbers in your main document.

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Sincerely,

Professor Hans Heesterbeek
mailto: proceedingsb@royalsociety.org

Associate Editor
Comments to Author:
Both reviewers and I agree that this is a very interesting and timely study, it is well written, and the analyses have been rigorously executed. I agree with reviewer two that the manuscript would be improved by discussing potential trade-offs with lifespan, in the context of life history theory and what is known about these species.

Reviewer(s)' Comments to Author:

Referee: 1

Comments to the Author(s)
This is a very interesting and timely paper on the senescence in salamanders, where the results show negligible actuarial senescence and an apparent lack of effect of size on survival. I believe that the study was well carried out, the manuscript is clear and concise, and, again, the results very interesting. I have only very minor comments below.

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Paragraph starting in line 86: It might be worth citing Finch (Finch, 1998) as the first to propose the existence of negligible senescence in indeterminate growers.

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Line 213: I believe that the term “parameters” here might not be appropriate, I would instead use demographic functions. This helps distinguish the statistical definition of a parameter from the functional forms.

Discussion: I find it very interesting that size specific survival was essentially not different between small and large salamanders. It is interesting since non-ageing related advantages in size have been proposed as the mechanisms that produce the apparent lack of senescence. Here you find that this is not the case. It can also be that the two size classes you use here might include individuals of the same ages, making the distinctions in their survival hard to detect.

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Referee: 2

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Study Limits—The major question in these studies is if the data collection span long enough to determine senescence (or lack of in the wild). The authors analysis greatly depends on monitoring populations as long as the lifespans of taxa studied. The authors report L. fazilae live 10 years and the study involves 10 years of data. The authors do not report lifespans for the other species. The survival curves in Fig 2 questions if the study durations were long enough to encompass a complete generation.

My biggest concern is not a technical one, but the interpretation of results. The manuscript currently focuses on lifespan and negligible seneses. This may be because of interest in aging for human health implications. However, there is little discussion of the associated tradeoffs. If there is no senesce, then what are the consequences for population growth, numbers and sizes of offspring, mortality rates? If space permits, I would appreciate the authors providing what they think about the implications of their findings for fundamental questions of life history theory.

For example, The authors state in the abstract "Our results showed that salamanders have slow life histories and that they experience negligible senescence", but these species show considerable variation in offspring production; 40-65 eggs in S. perspicillata, 23 larvae per year in S salamandra, and 1 offspring per year in L fazilae and Salamandra atra (lines 311-317). This implies that although there's considerable variation in offspring production, all of these species exhibit long lifespans and negligible senescence. I would be interested in the authors discussing their thoughts on the tradeoffs associated with these life histories.

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Specific comments:
Abstract: Part of the reason why "small ectotherms have lifespans similar to that of large endotherms" is because of much slower and even seasonally very low metabolisms. Comparing lifespans between these lineages would require adjusting for temperature dependence and seasonal temperature models. For this same reason, it is difficult to assess if the studies are monitoring the individuals long enough to get to the senesces mortality curve since these critters should have much longer lifespans for their body size (compared to better studied endotherms).

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Line 344-345: This can be interpreted as a general statement across "true salamanders" but is currently only supported by these 3 species.

Lines 354-358: The authors begin using negligible and marginal senescence interchangeably. This should be consistently one or the other or elaborate on the differences.

Author's Response to Decision Letter for (RSPB-2019-0971.R0)

See Appendix A.

RSPB-2019-1498.R0

Review form: Reviewer 1

Recommendation
Accept as is

Scientific importance: Is the manuscript an original and important contribution to its field?
Excellent

General interest: Is the paper of sufficient general interest?
Excellent

Quality of the paper: Is the overall quality of the paper suitable?
Excellent

Is the length of the paper justified?
Yes

Should the paper be seen by a specialist statistical reviewer?
No

Do you have any concerns about statistical analyses in this paper? If so, please specify them explicitly in your report.
No

It is a condition of publication that authors make their supporting data, code and materials available - either as supplementary material or hosted in an external repository. Please rate, if applicable, the supporting data on the following criteria.

Is it accessible?
N/A

Is it clear?
N/A

Is it adequate?
N/A

Do you have any ethical concerns with this paper?
No

Comments to the Author
The authors have fully addressed all my comments. I have no further comments. I still believe that this is going to be a very important contribution

Review form: Reviewer 2

Recommendation
Accept as is

Scientific importance: Is the manuscript an original and important contribution to its field?
Good

General interest: Is the paper of sufficient general interest?
Good

Quality of the paper: Is the overall quality of the paper suitable?
Good

Is the length of the paper justified?
Yes

Should the paper be seen by a specialist statistical reviewer?
No

Do you have any concerns about statistical analyses in this paper? If so, please specify them explicitly in your report.
No

It is a condition of publication that authors make their supporting data, code and materials available - either as supplementary material or hosted in an external repository. Please rate, if applicable, the supporting data on the following criteria.

Is it accessible?
No

Is it clear?
No

Is it adequate?
Yes

Do you have any ethical concerns with this paper?
No

Comments to the Author
The authors addressed all of my previous concerns sufficiently.

Decision letter (RSPB-2019-1498.R0)

26-Jul-2019

Dear Dr Cayuela

I am pleased to inform you that your Review manuscript RSPB-2019-1498 entitled "Slow life-history strategies are associated with negligible actuarial senescence in western Palearctic salamanders" has been accepted for publication in Proceedings B.

The referees do not recommend any further changes. Therefore, please proof-read your manuscript carefully and upload your final files for publication. Because the schedule for publication is very tight, it is a condition of publication that you submit the revised version of your manuscript within 7 days. If you do not think you will be able to meet this date please let me know immediately.

To upload your manuscript, log into http://mc.manuscriptcentral.com/prsb and enter your Author Centre, where you will find your manuscript title listed under "Manuscripts with Decisions." Under "Actions," click on "Create a Revision." Your manuscript number has been appended to denote a revision.

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Before uploading your revised files please make sure that you have:

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2) A separate electronic file of each figure (tiff, EPS or print-quality PDF preferred). The format should be produced directly from original creation package, or original software format. Please note that PowerPoint files are not accepted.

3) Electronic supplementary material: this should be contained in a separate file from the main text and the file name should contain the author's name and journal name, e.g authorname_procb_ESM_figures.pdf
All supplementary materials accompanying an accepted article will be treated as in their final form. They will be published alongside the paper on the journal website and posted on the online figshare repository. Files on figshare will be made available approximately one week before the accompanying article so that the supplementary material can be attributed a unique DOI. Please see: https://royalsociety.org/journals/authors/author-guidelines/

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It is a condition of publication that data supporting your paper are made available. Data should be made available either in the electronic supplementary material or through an appropriate repository. Details of how to access data should be included in your paper. Please see https://royalsociety.org/journals/ethics-policies/data-sharing-mining/ for more details.

If you wish to submit your data to Dryad (http://datadryad.org/) and have not already done so you can submit your data via this link http://datadryad.org/submit?journalID=RSPB&manu=RSPB-2019-1498 which will take you to your unique entry in the Dryad repository.

If you have already submitted your data to dryad you can make any necessary revisions to your dataset by following the above link.

5) For more information on our Licence to Publish, Open Access, Cover images and Media summaries, please visit https://royalsociety.org/journals/authors/author-guidelines/.

Once again, thank you for submitting your manuscript to Proceedings B and I look forward to receiving your final version. If you have any questions at all, please do not hesitate to get in touch.

Sincerely,
Professor Hans Heesterbeek
mailto:proceedingsb@royalsociety.org

Reviewer(s)' Comments to Author:

Referee: 1

Comments to the Author(s).
The authors have fully addressed all my comments. I have no further comments. I still believe that this is going to be a very important contribution.

Referee: 2

Comments to the Author(s).
The authors addressed all of my previous concerns sufficiently.

Decision letter (RSPB-2019-1498.R1)

06-Aug-2019

Dear Dr Cayuela

I am pleased to inform you that your manuscript entitled "Slow life-history strategies are associated with negligible actuarial senescence in western Palearctic salamanders" has been accepted for publication in Proceedings B.

You can expect to receive a proof of your article from our Production office in due course, please check your spam filter if you do not receive it. PLEASE NOTE: you will be given the exact page length of your paper which may be different from the estimation from Editorial and you may be asked to reduce your paper if it goes over the 10 page limit.

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Thank you for your fine contribution. On behalf of the Editors of the Proceedings B, we look forward to your continued contributions to the Journal.

Sincerely,

Editor, Proceedings B
mailto: proceedingsb@royalsociety.org

Appendix A

June 25, 2019

Dear editor,

We thank the two reviewers for their interesting and constructive comments. We are glad to read that they found our study "very interesting". The reviewer 1 only had minor comments (adding references, replacing some terms by others) that were readily addressed below – the sections of the text that have been modified are highlighted in yellow in the main manuscript. The reviewer 2 had two main recommendations: first, he proposed to show that the length of the capture-recapture survey was equal or longer than the mean lifespan in each population of salamanders. This was done by completing the Table S1 in Supplementary material. The survey length was equal to the mean lifespan of salamanders in two populations, and longer than the mean lifespan in the two others. We are therefore confident in our ability to detect senescence using our capture-recapture data. Second, reviewer 2 recommended to deepen our discussion about the absence of trade-offs between senescence and fecundity in true salamanders, and about the link between negligible senescence and body size temperature and metabolism. We address these points by modifying some paragraphs in the discussion section.

We hope that you and the two reviewers will enjoy the revised version of the MS. Thank you for your time and effort.

Best regards
Hugo Cayuela,
on the behalf of the authors

Hugo Cayuela, PhD
Postdoctoral researcher
Département de Biologie
Institut de Biologie Intégrative et des Systèmes (IBIS)
Pavillon Charles-Eugène Marchand 1030 rue de la Médecine
Université Laval
Québec (Québec)
G1V 0A6
Canada

Referee: 1

Comments to the Author(s)
This is a very interesting and timely paper on the senescence in salamanders, where the results show negligible actuarial senescence and an apparent lack of effect of size on survival. I believe that the study was well carried out, the manuscript is clear and concise, and, again, the results very interesting. I have only very minor comments below.
*****Author response: We thank the reviewer for this positive evaluation of the MS. We address her/his minor comments below.**

Paragraph 1 intro: It might be important to note that Kirkwood (Kirkwood, 1977) and Kirkwood & Holiday (Kirkwood & Holliday, 1979) made the additional distinction that senescence was inevitable in organisms with a clear distinction between somatic and germ lines.
*****Author response: We agree with the reviewer and modified the sentence at lines 58-61 It now reads: "The senescence process was expected to be ubiquitous among age-structured populations (Hamilton 1966), which led to the view that senescence was an unavoidable process in organisms with a clear distinction between somatic and germ lines (Kirkwood 1977, Ackermann et al. 2003, Nussey et al. 2013)."We added the following reference to the reference list: Kirkwood, T. B. (1977). Evolution of ageing. Nature, 270, 301.**

Paragraph starting in line 86: It might be worth citing Finch (Finch, 1998) as the first to propose the existence of negligible senescence in indeterminate growers.
*****Author response: We agree with the reviewer. The following reference is now cited at line 76: Finch, C. E. (1998). Variations in senescence and longevity include the possibility of negligible senescence. The Journals of Gerontology Series A: Biological Sciences and Medical Sciences, 53, B235-B239.**

Line 135: More appropriate than "Colchero's models" I would suggest to refer to BaSTA. Also, the first reference should not be Colchero et al 2012a, but Colchero and Clark 2012.
*****Author response: We agree with the reviewer, we now refer to "BaSTA models" here and throughout the MS. We also corrected the reference of Colchero and Clark 2012 throughout the paper.**

Line 158: Here you say: "…information about individual age is updated." Did you mean, information about individual size? From what you describe, the transition probabilities correspond to s and l.
*****Author response: Indeed, that's a mistake. We corrected the following sentence at line 162: "In the second modeling step, information about individual size is updated."**

Line 213: I believe that the term "parameters" here might not be appropriate, I would instead use demographic functions. This helps distinguish the statistical definition of a parameter from the functional forms.
*****Author response: We fully agree with reviewer. The sentence at lines 220-221 has been modified as following: "It allows estimating two demographic functions: cumulative probability survival until a given age and mortality rate (i.e., hazard rate) at a given age."**

Discussion: I find it very interesting that size specific survival was essentially not different between small and large salamanders. It is interesting since non-ageing related advantages in size have been proposed as the mechanisms that produce the apparent lack of senescence. Here you find that this is not the case. It can also be that the two size classes you use here might include individuals of the same ages, making the distinctions in their survival hard to detect.
*****Author response: Yes, that's an interesting result. First, we do not think that the two size classes may include individuals of the same age because age and size are highly correlated in *L. fazilae* (see Olgun et al. 2001, Figure 4 in this paper). Our results strongly suggest that indeterminate growth would not be the main driver of the negligible senescence reported in our study. By contrast, we postulate that the exceptional regenerative abilities of salamanders could be an important driver. We modified the sentences at lines 365-373 to better emphasize that point: "The negligible senescence of salamanders likely relies on their high regenerative capacities. Contrary to other amniotes, salamanders are able to retain near perfect regeneration of most organs and appendages (e.g., spinal cord, heart, brain, skin, digit, and lens) well into adulthood (Seifert & Voss 2013). Although almost no studies have tested these abilities in old animals (Seifert & Voss 2013), their great potential for tissue repair and regeneration likely probably allow true salamanders to escape actuarial senescence. In parallel, although we did not detect size-dependent survival in *L. fazilae*, an indeterminate growth (Jones & Vaupel 2017) could also contribute to the negligible senescence reported in our study. Furthermore, a low body temperature and a low metabolic rate (Hulbert et al. 2007, Flouris & Piantoni 2015) might also limit actuarial senescence."**

References:
Finch, C. E. (1998). Variations in senescence and longevity include the possibility of negligible senescence. The Journals of Gerontology Series a: Biological Sciences and Medical Sciences, 53(4), B235–B239.
Kirkwood, T. B. (1977). Evolution of ageing. Nature, 270(5635), 301–304.
Kirkwood, T., & Holliday, R. (1979). The Evolution of Ageing and Longevity. Proceedings of the Royal Society of London Series B-Biological Sciences, 205(1161), 531–546.

Referee: 2

Comments to the Author(s)
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*****Author response: We thank the reviewer for her/his positive evaluation. We address her/his comments below.**

While the finding that L fazilae salamanders have slow life histories, exhibiting low fecundity, recruitment and long lifespans is interesting, it is not all that surprising.
*****Author response: Indeed, we expected a slow life history. Here we present the data support the expectation and, importantly, we describe senescence. While actuarial senescence has been heavily documented in wild populations of birds and mammals (with evidence that slow species show a lower rate of senescence, Jones et al. 2008), such studies are lacking in other taxa displaying different life history strategies (e.g. hibernating species or species displaying repeated moult events). Moreover, while we made the hypothesis that *L fazilae* salamanders should show slower rate of senescence, our finding that this species show negligible senescence remains surprising and particularly relevant to the current debate on the inevitability of senescence (Jones & Vaupel 2017).**

Jones, O. R., Gaillard, J. M., Tuljapurkar, S., Alho, J. S., Armitage, K. B., Becker, P. H., ... & Clutton- Brock, T. (2008). Senescence rates are determined by ranking on the fast–slow life- history continuum. Ecology Letters, 11(7), 664-673.
Jones, O. R., & Vaupel, J. W. (2017). Senescence is not inevitable. Biogerontology, 18(6), 965-971.

From my understanding of their analysis (and I am no expert on these statistical tests), the authors compare mortality rates based on size classes (as proxy of age), find no differences in mortality between small and large (young and old). So, the assumption is that if larger (older) individuals are not dying any more than expected than smaller (younger), then they must not be aging. The analyses do support these claims.
*****Author response: We respectfully disagree with the referee. In fact, our conclusions are based on BaSTA models where mortality rate is directly modeled as a function of individual age rather than size (specified at lines 216-237; please also see Table S1 in the Supplementary material). But, indeed, we also used multievent models with size classes that especially allowed estimating the probability of recruiting small-sized individuals and examining size dependent survival (specified at lines 144-153).**

Study Limits—The major question in these studies is if the data collection span long enough to determine senescence (or lack of in the wild). The authors analysis greatly depends on monitoring populations as long as the lifespans of taxa studied. The authors report L. fazilae live 10 years and the study involves 10 years of data. The authors do not report lifespans for the other species. The survival curves in Fig 2 questions if the study durations were long enough to encompass a complete generation.

*****Author response: We agree with the reviewer. It is important to specify that length of the survey is equal or higher than the mean lifespan of the studied population because inference is better if this condition is fulfilled. We therefore calculated mean lifespan of adults for the *L. fazilae, S. perspicillata,* and *S. salamandra* using the following classical formulae: *a*+(*s*/(1+*s*)) where *s* is the survival at adult stage and *a* is the age at sexual maturity. The mean adult lifespan was 6 years in *L. fazilae,* 9.5 years in *S. perspicillata*, and 8.5 years in *S. salamandra*. We conclude that the length of survey period was equal or longer than the mean lifespan of salamander in the four studied populations. We are therefore confident in the robustness of our results and conclusions. We added this information at lines 207-210 in the material and methods section: "In all cases, the survey length was equal or longer than the mean lifespan of adults in the four populations (Supplementary material, Table S1). Given the simulations of Colchero & Clark 2012), we are therefore confident in our ability to detect senescence if it actually occurred."**

In addition, we included the information about lifespan and survey length in the table S1 provided in Supplementary material:

Table S1. Information about age-dependent capture-recapture data in four populations of *Lyciasalamandra fazilae, Salamandrina perspecillata*, and *Salamandra salamandra* (two populations). The mean adult lifespan was calculated using the following formulae: *a*+(*s*/(1+*s*)) where *s* is the survival at adult stage and *a* is the age at sexual maturity. Age at sexual maturity were obtained from the following references: *L. fazilae*, Olgun et al. 2001; *S. perspecillata,* Angelini et al. 2010; and *S. Salamandra*, Reinhard et al. 2015 (in *Salamandra algira*).

Parameter	***L. fazilae***	***S. perspecillata***	***S. salamandra 1***	***S. salamandra 2***
Number of individuals	**133**	**911**	**304**	**376**
Number with known birth year	**67**	**55**	**0**	**0**
Number with known death year	**5**	**0**	**0**	**0**
Total number of captures	**179**	**1629**	**609**	**1474**
Earliest detection time	**1999**	**1998**	**2008**	**1965**
Latest detection time	**2009**	**2006**	**2015**	**1985**
Earliest recorded birth year	**1990**	**1994**	**0**	**0**
Latest recorded birth year	**1997**	**2002**	**0**	**0**
Earliest recorded death year	**2000**	**0**	**0**	**0**
Latest recorded death year	**2003**	**0**	**0**	**0**
Total survey length (years)	**11**	**9**	**8**	**21**
Mean adult lifespan	**6**	**9.5**	**8.5**	**8.5**

My biggest concern is not a technical one, but the interpretation of results. The manuscript currently focuses on lifespan and negligible senesces. This may be because of interest in aging for human health implications. However, there is little discussion of the associated tradeoffs. If there is no senesce, then what are the consequences for population growth, numbers and sizes of offspring, mortality rates? If space permits, I would appreciate the authors providing what they think about the implications of their findings for fundamental questions of life history theory. For example, The authors state in the abstract "Our results showed that salamanders have slow life histories and that they experience negligible senescence", but these species show considerable

variation in offspring production; 40-65 eggs in S. perspicillata, 23 larvae per year in S salamandra, and 1 offspring per year in L fazilae and Salamandra atra (lines 311-317). This implies that although there's considerable variation in offspring production, all of these species exhibit long lifespans and negligible senescence. I would be interested in the authors discussing their thoughts on the tradeoffs associated with these life histories.
*****Author response: We thank the referee for its very interesting comment. We now address this point in the discussion section at lines 364-372: "Interestingly, our results suggest an absence of trade-off between senescence and offspring production in true salamanders. Although they display large variation in annual offspring numbers, the three species experience negligible senescence. This seems to indicate a partial decoupling of senescence and reproductive effort whose the variation is rather associated with species reproductive mode (oviparity, lecithotrophic viviparity, and matrotrophic viviparity). However, our study focused on a limited number of salamander species and further investigations are required to examine in details covariation between senescence (speed and age at onset of senescence) and life history traits at the clade level."**

The regenerative hypothesis is certainly interesting. Again, I would encourage the authors to understand the life history consequences associated with negligible senescence. Does regenerative ability correspond with elevated metabolic rates (compared to anurans of similar size and temperature)? To provide the resources for somatic maintenance/regeneration of tissues?
*****Author response: Following reviewer suggestion, the revised version of the MS includes a new paragraph where the potential absence of trade-off between senescence and young production is discussed (see lines 357-364). However, it seems difficult to address the question about the link between regenerative capacity and metabolic rate in amphibians – and in true salamanders in particular. A full discussion of this topic would be well beyond the scope of this paper. Although the topic is exiting and promising, no comparative study has already examined this relationship. Yet, we specified at lines 370-373 that an indeterminate growth, a body temperature, and a low metabolic rate could also contribute to negligible senescence: "In parallel, although we did not detect size-dependent survival in *L. fazilae*, an indeterminate growth (Jones & Vaupel 2017) could also contribute to the negligible senescence reported in our study. Furthermore, a low body temperature and a low metabolic rate (Hulbert et al. 2007, Flouris & Piantoni 2015) might also limit actuarial senescence."**

Specific comments:
Abstract: Part of the reason why "small ectotherms have lifespans similar to that of large endotherms" is because of much slower and even seasonally very low metabolisms. Comparing lifespans between these lineages would require adjusting for temperature dependence and seasonal temperature models. For this same reason, it is difficult to assess if the studies are monitoring the individuals long enough to get to the senesces mortality curve since these critters should have much longer lifespans for their body size (compared to better studied endotherms).
*****Author response: We fully agree with the reviewer. For this reason, we modified the sentence at lines 41-44 as following: "The regenerative capacities of salamanders, in combination with other physiological and developmental features such as an indeterminate growth and a low metabolic rate, likely explain why these small ectotherms have lifespans similar to that of large endotherms and, in contrast to most amniotes, undergo negligible senescence."**

The difference between survival and mortality is key. I would clearly define these terms and how they differ in the methods.
*****Author response: We agree with the reviewer. We better present the difference between the two parameters at lines 218-221: "BaSTA allowed us to account for imperfect detection, left-truncated (i.e., unknown birth date) and right-censored (i.e., unknown death date) capture-recapture data in our analysis. It allows estimation of two demographic functions: cumulative probability survival until a given age and mortality rate (i.e., hazard rate) at a given age."**

Line 93: what species of salamander?
*****Author response: the species considered in the study of Colchero et al. (2019) was *Salamandra salamandra*. Yet, only one population of *S. salamandra* was considered in this previous study whereas two are analyzed in our paper. The name of the species is now given at line 95: "Interestingly, senescence was negligible in a salamander (*Salamandra Salamandra*), an organism with an indeterminate (i.e., continuous) growth (Bouzid et al. 2017) that is well known for its regenerative capacity at the adult stage (Yokoyama 2008, Poss 2010, Seifert & Voss 2013)."**

Line 128-30: Are males and females same body size?
*****Author response: males are a bit smaller than females (Olgun et al. 2001), as usual in amphibians. However, we assumed that this should not affect our inferences or conclusions. We stated this point at lines 131-133: "We assumed that sex should have a little influence on adult survival as males and females have the same age structure in the population (Olgun et al. 2001)."**

Line 341-343: The authors report lifespans for L. fazilae (10 years). Report lifespans for other species because this is essential to understand if the assumptions of the statistical test (study duration lifespan) are met.
*****Author response: Ten years is the maximum longevity in *L. fazilae*. The mean lifespan is 6 years according to our capture-recapture data. As specified in our response to the reviewer's general comments, the survey length was equal or longer than the mean lifespan in the four studied populations. Information on lifespans is now available in the Supplementary material, Table S1.**

Line 344-345: This can be interpreted as a general statement across "true salamanders" but is currently only supported by these 3 species.
*****Author response: We agree with the reviewer and we therefore modified the sentence as following at lines 350-352: "A long lifespan, a high level of iteroparity, and a low reproductive effort appear to be closely associated with negligible actuarial senescence in the three species of true salamanders considered in our study."**

Lines 354-358: The authors begin using negligible and marginal senescence interchangeably. This should be consistently one or the other or elaborate on the differences.
*****Author response: Corrected. We kept the term "negligible senescence" throughout the MS.**