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The preference and costs of sleeping under light at night in forest and urban great tits

Zeynep N. Ulgezen, Teemu Käpylä, Peter Meerlo, Kamiel Spoelstra, Marcel E. Visser and Davide M. Dominoni

Article citation details
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Review timeline

Original submission:	16 January 2019
1st revised submission:	29 April 2019
2nd revised submission:	29 May 2019
Final acceptance:	29 May 2019

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

Review History

RSPB-2019-0120.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?
Good

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
I feel that this paper provides excellent information regarding the roosting preference of Parus major to select areas under sources of dim light. I feel that this information is both valuable to the scientific community and the general public. This information may prove valuable to policy decisions as governments are beginning to implement light pollution reduction policies. This information helps better understand the differences that dim light have on behaviour, and may help inform policy moving forward.

I only have a few suggestions with regards to grammar and formatting to help the paper flow smoother, which I will outline below:

Line 160: change "that white light" to "then white light"

Line 252: Change "till" to "until"

Line 308: 1.5 diameter – what is the unit

Table 1: This is just a suggestion but the table could be adjusted so that the p values in the column line up with the rest of the values on their intended row.

Line 471: remove "even more" this isn't necessary and makes the sentence feel like it doesn't flow properly

Line 508: remove "onto"

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?
Acceptable

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?
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
The potential adverse effects that artificial light at night (ALAN) have on animals is a growing concern as humans increasingly illuminate the night. Songbirds are a developing model for examining the impact of ALAN. Under controlled lab conditions, Ulgezen et al. investigate which lighting conditions great tits prefer, and the impact that different lighting conditions have on sleep behavior, cognition, and physiology, including daily energy expenditure and blood levels of oxalic acid, a possible biomarker of sleep loss. The authors show that the birds preferred to roost under dim light, rather than darkness, and prefer green over white light. When forced to roost under the different lighting conditions, white light affected activity patterns more than green light, and white light increased daily energy expenditure. Oxalic acid and daytime performance on cognitive tests were not influenced by the lighting condition during the previous night. Differences in the response to lighting was also compared between birds from rural and urban environments.

In general, the paper is well organized and written. However, I have several concerns regarding the experimental set-up and the interpretation of the data.

Motion detection sensitivity: In experiment 2, the authors use camera traps to assess "slight movements" during sleep. As slight movements can indicate brief awakenings or REM sleep related dropping of the head in songbirds (e.g. Szymczak et al. Physiol and Behav, 1993), the authors need to be more specific. How was the threshold determined, and how were wake-related movements distinguished from sleep-related movements? Also, based on the experimental set-up, the birds could sit at varying distances from the camera (e.g. on the ground, low perch, high perch, or in the hole between the boxes). The distance to the camera could also vary depending on where on a given perch the bird sat (e.g., in the center or to the left or right). As a result, the same "slight movement" might trigger the camera only when sitting in the closest position. This is potentially problematic if the birds' position relative to the camera was influenced by light entering from the other box. Consequently, the authors need to discuss how this issue was handled.

From this data, the authors report that "ALAN affected the proportion of movements at night displayed by birds." This was most apparent under white light. In the associated figure (4A), they report "Proportion of minutes at night that birds spent without head tucked under feathers." Given that images were collected in response to motion and at 1-minute intervals, it would be useful to clarify if this was quantified exclusively based on motion detection. Also, songbirds sometimes sleep with their head facing forward (e.g. Szymczak et al. 1993) and then turn their head to the back. This postural change is usually associated with a brief awakening. How were such changes in posture handled?

As a general comment, I would strongly recommend that the authors record continuous video in future experiments. Automated methods for detecting movement or specific postures can then be applied to these images while still retaining the ability to return to the raw images for further analysis when needed.

Sensitivity to sleep loss for oxalic acid: The authors did not detect any lighting-dependent differences in oxalic acid, a putative indicator of sleep loss in mammals. This result is difficult to assess due the lack of systematic studies demonstrating that oxalic acid changes in response to sleep deprivation in birds, as it does in mammals (e.g. rats and humans). Before such validation studies are performed, I think that it is premature to use oxalic acid as a biomarker of sleep loss in birds. In this context, it would be useful for the authors to state that such studies are needed (around line 503). Also, line 438 should be adjusted accordingly.

Line 244: How was the activity level of "one or zero" calculated; e.g. how many times did the perch switch have to be triggered to count as active? Please clarify.

As noted in section "Light spectra matters" (line 573), the authors acknowledge that sensitivity to the two light colors might be different in birds when compared to humans. Consequently, the birds might perceive the light to be of different intensities, and therefore their choice might reflect a preferred intensity, rather than frequency.
Although this is an understandable limitation of their study due to the lack of data on the avian action spectrum, they do cite some references on the topic, albeit on different species.
Nonetheless, it might be useful to estimate how different the perceived intensity might be based on the available data on a songbird (e.g., zebra finch) from ref 58.

Line 133: The behavioral decisions birds make in response to ALAN might be maladaptive given the recent advent of ALAN.

As the birds had multiple places to perch (ground, low perch, high perch, or higher hole), each with varying exposure to light from the other box, it would be interesting to know where the birds perched under the different conditions.

Line 267: incorrect reference. Check others.

Line 280: “breath samples” could be interpreted as two individual breaths. Did you mean two 10-samples of breathing?

Line 519: what is “sleep restlessness”?

Line 522: what are “abrupt light schedules”?

Line 590: The authors suggest choosing to roost under dim light might allow birds to forage at night. In this regard, the authors should state whether their birds ate at night.

Decision letter (RSPB-2019-0120.R0)

11-Mar-2019

Dear Dr Dominoni:

I am writing to inform you that your manuscript RSPB-2019-0120 entitled "The preference and costs of sleeping under light at night in forest and urban great tits" 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 substantial 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.

To upload a resubmitted 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 Resubmission." Please be sure to indicate in your cover letter that it is a resubmission, and supply the previous reference number.

Sincerely,

Proceedings B
mailto: proceedingsb@royalsociety.org

Associate Editor
Board Member: 1
Comments to Author:
Dear authors,

Your study is a valuable experimental contribution to our understanding of the effects of artificial light at night on sleep and activity patterns in birds. The experimental design is sound and the experiments appear to be well led. Both reviewers agree that overall, the paper is well organized and written – I support their opinion. I also appreciated the clear suggestions for future work made by the authors in the discussion that could further contribute to a more general understanding of ALAN on birds or wildlife in general. I also have a few comments below regarding abstract and section order (further detailed below).

At the same time, the reviewers did have contrasted views regarding the quality of the conclusions that can be derived from your study.

While reviewer 1 highlights the importance of your findings in terms of light pollution policies, reviewer 2 makes some important methodological queries – all of them ought to be addressed in detail, namely:
(1) how are wake-related movements distinguished from sleep-related movements?, (2) oxalic acid results are difficult to assess as there is a limited number of studies using this marker in birds – could you discuss this in the light of a greater range of taxa where sleep deprivation and oxalic acid concentrations were measured?
(3) differences in how birds perceive light intensity relative to a "human standard" as established on the lux scale could be developed based on the availability of zebra finch data.

Before a final assessment can be made, please address all comments from reviewers 1 and 2, as well as some comments listed below.

Abstract. Please rewrite lines 34-41 of the abstract by indicating more explicitly directional changes in trends (l 34: while light affected patters / how?; l. 36 – they did – how?). I would also suggest to delete the highly speculative (and not tested) explanation related to food intake and mate attraction, and refocus the end of the abstract on possible policy applications or other findings that naturally flow from your findings, rather than focusing on untested hypotheses.
Introduction
L105 – affects?
L128 – increasing corticosterone levels relative to what?
L139 – not clear, please rewrite
Methods
L188 – please specific how this value relates to to urban variation in light pollution
L229 – please indiciate sample size
L247 – to be consistent – shouldn't it be "total activity" and "nocturnal activity?"
L250 – "Total activity was…"

L254 – nocturnal restlessness is not introduced earlier (at least under this name) – please set this in context in the introduction.
L258-259: In cases where sleep position of the bird was not visible the data was not included – this is redundant with what is written in 261-262 and should be deleted/
L267 – are you sure?
L270 – why did you use a subsample? Is it really 11 birds or 11 birds per treatment?
L350 – not clear
L351 – do you mean light type?
Results
L370 – (Table 1), suggesting that…
L380 – Delete "birds roosting" – do you mean nocturnal activity? Please use terms defined in methods
L382 – significant difference in night time activity?
L368-388 – worth citing figure 3 here as well!
L441 – which birds were quicker (from which treatment)?
Discussion
L465-467 –this argument is ill-founded – the experiment was carried out in October, so I don't see how they could be increasing extra-pair mate attention in this context
L482 – do consider inverting discussion sections (B) with (C – line 530)
L497 – do you mean a decrease? This is not clear to me.
L500 – please detail how this could be tackled further
L509 – please discuss the relevance of 1.5 lux in an urban context
L515-516 - I understand that this part was not reported in results? Should you cite unpublished data?
L522-529 – but abrupt light schedules are not what ALAN is – could you tone down this section in this context? Could you also discuss restlessness specfically in natural studies under ALAN?
L592 – this has not been tested at all – please rephrase conclusions on what is really demonstrated in the paper, for example in a conservation setting or future research setting.

Sincerely,
Marta Szulkin

Reviewer(s)' Comments to Author:

Referee: 1

Comments to the Author(s)
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Line 522: what are "abrupt light schedules"?

Line 590: The authors suggest choosing to roost under dim light might allow birds to forage at night. In this regard, the authors should state whether their birds ate at night.

Author's Response to Decision Letter for (RSPB-2019-0120.R0)

See Appendix A.

RSPB-2019-0872.R0

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?
Yes

Is it clear?
Yes

Is it adequate?
Yes

Do you have any ethical concerns with this paper?
No

Comments to the Author
I am satisfied with the authors' revision and have no further comments.

Decision letter (RSPB-2019-0872.R0)

20-May-2019

Dear Dr Dominoni

I am pleased to inform you that your manuscript RSPB-2019-0872 entitled "The preference and costs of sleeping under light at night in forest and urban great tits" has been accepted for publication in Proceedings B.

The referee(s) have recommended publication, but also suggest some minor revisions to your manuscript. Therefore, I invite you to respond to the referee(s)' comments and revise your manuscript. 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 us know.

To revise your manuscript, log into https://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. You will be unable to make your revisions on the originally submitted version of the manuscript. Instead, revise your manuscript and upload a new version through your Author Centre.

When submitting your revised manuscript, you will be able to respond to the comments made by the referee(s) and upload a file "Response to Referees". You can use this to document any changes you make to the original manuscript. We require a copy of the manuscript with revisions made since the previous version marked as 'tracked changes' to be included in the 'response to referees' document.

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

1) A text file of the manuscript (doc, txt, rtf or tex), including the references, tables (including captions) and figure captions. Please remove any tracked changes from the text before submission. PDF files are not an accepted format for the "Main Document".

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. PowerPoint files are not accepted.

3) Electronic supplementary material: this should be contained in a separate file and where possible, all ESM should be combined into a single file. 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.

Online supplementary material will also carry the title and description provided during submission, so please ensure these are accurate and informative. Note that the Royal Society will not edit or typeset supplementary material and it will be hosted as provided. Please ensure that the supplementary material includes the paper details (authors, title, journal name, article DOI). Your article DOI will be 10.1098/rspb.[paper ID in form xxxx.xxxx e.g. 10.1098/rspb.2016.0049].

4) A media summary: a short non-technical summary (up to 100 words) of the key findings/importance of your manuscript.

5) Data accessibility section and data citation
It is a condition of publication that data supporting your paper are made available either in the electronic supplementary material or through an appropriate repository.

In order to ensure effective and robust dissemination and appropriate credit to authors the dataset(s) used should be fully cited. To ensure archived data are available to readers, authors should include a 'data accessibility' section immediately after the acknowledgements section. This should list the database and accession number for all data from the article that has been made publicly available, for instance:
• DNA sequences: Genbank accessions F234391-F234402
• Phylogenetic data: TreeBASE accession number S9123
• Final DNA sequence assembly uploaded as online supplemental material
• Climate data and MaxEnt input files: Dryad doi:10.5521/dryad.12311
NB. From April 1 2013, peer reviewed articles based on research funded wholly or partly by RCUK must include, if applicable, a statement on how the underlying research materials – such as data, samples or models – can be accessed. This statement should be included in the data accessibility section.

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=(Document not available) 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.
Please see https://royalsociety.org/journals/ethics-policies/data-sharing-mining/ for more details.

6) 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 revision. If you have any questions at all, please do not hesitate to get in touch.

Sincerely,

Proceedings B
mailto: proceedingsb@royalsociety.org

Associate Editor
Board Member
Comments to Author:
Dear authors,

In my view, all queries made by referees have been adequately addressed in your revision. After reading your revised manuscript, I have the following remaining queries:

Abstract: please rephrase line 40 as it is misleading – I understand that this is speculation, so it is best to explicitly state so, by for example rephrasing to:
However, it is possible that negative effects of ALAN on sleep and cognition might be observed only under intensities higher than…

Methods:
L169: so its 1.5 lux the light at the perch level? Please clarify
Experimental set-up: I would say that Figure 1 is dearly missed here – I understand you moved it due to space constraints, but I feel that it is really to the detriment of a reader's understanding of how the experiment is carried out. Please consider bringing Figure S1 back to the manuscript.
L196: as a note – please rephrase
L210 – I understand you refer to sup mat figure 1b? I would still advise on bringing it back into the manuscript

L253-254: again, this feels like too little information is provided – could you squeeze a sentence or two in here?

L268: please rephrase: we ran four separate odels with the following response variables: …
L273: so there were several tasks? This is missing from the description in the "cognitive abilities" section

Results:
Figure 1: it would make more sense to me if the order of figures is: dark white / dark green / green white
L296: urban and forest birds were similarly affected by treatments – I am not sure I agree: there was a significant interaction with origin, wasn't there? Please rephrase the statement to take this into account
L317: would this suggest a habituation to light? If so, this could be mentioned in the discussion
L332-333: under which treatment? Please rephrase for increased clarity

Discussion:
L358: rephrase to: […] does not vary seasonally since these experiments were run in autumn
L385: the fact that you find opposite effects of ALAN on DEE in the wild and in the lab necessitates at least a commentary on which result (the lab or the wild one) should be more informative of biological reality, and why…

Sincerely,

Marta Szulkin

Reviewer(s)' Comments to Author:

Referee: 2

Comments to the Author(s).
I am satisfied with the authors' revision and have no further comments.

Author's Response to Decision Letter for (RSPB-2019-0872.R0)

See Appendix B.

Decision letter (RSPB-2019-0872.R1)

29-May-2019

Dear Dr Dominoni

I am pleased to inform you that your manuscript entitled "The preference and costs of sleeping under light at night in forest and urban great tits" 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.

If you are likely to be away from e-mail contact please let us know. Due to rapid publication and an extremely tight schedule, if comments are not received, we may publish the paper as it stands.

If you have any queries regarding the production of your final article or the publication date please contact procb_proofs@royalsociety.org

Your article has been estimated as being 10 pages long. Our Production Office will be able to confirm the exact length at proof stage.

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Electronic supplementary material:
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.

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,

Proceedings B
mailto: proceedingsb@royalsociety.org

Appendix A

Dear authors,

Your study is a valuable experimental contribution to our understanding of the effects of artificial light at night on sleep and activity patterns in birds. The experimental design is sound and the experiments appear to be well led. Both reviewers agree that overall, the paper is well organized and written – I support their opinion. I also appreciated the clear suggestions for future work made by the authors in the discussion that could further contribute to a more general understanding of ALAN on birds or wildlife in general. I also have a few comments below regarding abstract and section order (further detailed below).

At the same time, the reviewers did have contrasted views regarding the quality of the conclusions that can be derived from your study.

While reviewer 1 highlights the importance of your findings in terms of light pollution policies, reviewer 2 makes some important methodological queries – all of them ought to be addressed in detail, namely:
(1) how are wake-related movements distinguished from sleep-related movements?, (2) oxalic acid results are difficult to assess as there is a limited number of studies using this marker in birds – could you discuss this in the light of a greater range of taxa where sleep deprivation and oxalic acid concentrations were measured?
(3) differences in how birds perceive light intensity relative to a "human standard" as established on the lux scale could be developed based on the availability of zebra finch data.

Before a final assessment can be made, please address all comments from reviewers 1 and 2, as well as some comments listed below.
We thank the Editor and both referees for the compliments on our paper. We also thank them for the excellent feedback on our manuscript, which really helped us to improve its quality and clarity.

Please note that because the manuscript ended up being considerably longer than the accepted length, we had to move some parts to the supplementary materials. Specifically, the three following changes have been made to adhere to the length restrictions:

1. Moved the old figure 1 (the description of the design of experiment 1) to the supplements.
2. Moved the old figure 3 (results of activity traits in experiment 2) in the supplements. We don't feel these were the most important results of our paper, and we have already included a table (table 1) summarizing the statistics in the main text.
3. Shorten the methods considerably, especially the explanations of the activity recordings, DEE, oxalic acid and cognitive measurements. These are all based on previously published papers, so we briefly explained these methods, cited the relevant studies, and referred to the supplements for more detailed descriptions. We did however maintain the full description of the analysis of the camera recordings, as we felt these were somewhat novel (at least for captive studies) and needed to stand on their own in the main text.

We hope these changes are ok with the editor and the referees, as we believe the paper still stands on its own.

We have uploaded a clean version of the new manuscript as well as a version with track changes. Please note that throughout this response document we cite line numbers that refer to the track changes version.

Abstract. Please rewrite lines 34-41 of the abstract by indicating more explicitly directional changes in trends (l 34: while light affected patters / how?; l. 36 – they did – how?). I would also suggest to delete the highly speculative (and not tested) explanation related to food intake and mate attraction, and refocus the end of the abstract on possible policy applications or other findings that naturally flow from your findings, rather than focusing on untested hypotheses.
Thanks for your comment and suggestions. We have rephrased the sentences between lines 34-41 to state the direction of the effects. Moreover, we have removed the speculation at the end of the abstract and focused on the relevance of the results in terms of policy applications.

Introduction
L105 – affects?
Corrected.

L128 – increasing corticosterone levels relative to what?
We specified this refers to control birds not exposed to ALAN.

L139 – not clear, please rewrite
We have rephrased this sentence as we agree with the referee this was not at all clear.

Methods
L188 – please specific how this value relates to to urban variation in light pollution
We have specified the levels of light intensity to which individual birds are exposed to in the wild when living in light polluted areas, and cited two previous studies.

L229 – please indiciate sample size
We have included the sample size of the second experiment at the end of this paragraph (N=33, as two birds died in between the two experiments due to unknown causes).

L247 – to be consistent – shouldn't it be "total activity" and "nocturnal activity?"
True, we have deleted "daily" in the new version.

L250 – "Total activity was…"
Corrected.

L254 – nocturnal restlessness is not introduced earlier (at least under this name) – please set this in context in the introduction.
Thanks for pointing this out. We have now introduced the term nocturnal restlessness in the introduction (L100).

L258-259: In cases where sleep position of the bird was not visible the data was not included – this is redundant with what is written in 261-262 and should be deleted
Deleted.

L267 – are you sure?
Thanks for pointing this out, indeed this was the wrong citation. We have cited the correct study now.

L270 – why did you use a subsample? Is it really 11 birds or 11 birds per treatment?
We used a subsample of 11 birds, and each bird was measured in every treatment period. Therefore, there was a total of 33 measurements, 11 for each treatment. We specify this in the new version of the manuscript. We used a subset of birds because it takes quite some time to conduct these

measurements, as the isotope machine needs to be “flushed” with ambient air for at least 20 minutes after each measurement. Thus the number of birds that one can measure per day is limited. We could have of course extended the measurements over several days, but we did not want to cause too much disturbance in the experimental rooms as this would have been inevitably affected activity rhythms, and thus DEE.

L350 – not clear
We have rephrased this sentence.

L351 – do you mean light type?
No this is the type of cognitive test. We have rephrased accordingly.

Results
L370 – (Table 1), suggesting that…
We are not sure what the editor suggests to do here, but we do not see any particular mistakes/lack of clarity in this sentence.

L380 – Delete “birds roosting” – do you mean nocturnal activity? Please use terms defined in methods
We have rephrased this sentence.

L382 – significant difference in night time activity?
Rephrased.

L368-388 – worth citing figure 3 here as well!
Done!

L441 – which birds were quicker (from which treatment)?
There was not treatment nor origin effect. We have now specified this in the text.

Discussion
L465-467 –this argument is ill-founded – the experiment was carried out in October, so I don’t see how they could be increasing extra-pair mate attention in this context
Well here we are trying to extrapolate to a wider context. The association between nocturnal activity and extra-pair paternity has been shown before in tits, so that’s what we are referring here. Obviously ours was first a captive experiment, and second as you correctly point out, this experiment was done in autumn. So while we cannot directly link our results to extra-pair paternity, we feel it is still worth mention that such link is possible in nature, assuming that the preference for sleeping under dim light does not vary seasonally. We mentioned this last assumption in the new version of the manuscript.

L482 – do consider inverting discussion sections (B) with (C – line 530)
We are not sure such inversion this will give justice to the logical succession of research questions, hypotheses and predictions upon which our study was based. We first wanted to assess the preference and cost of sleeping under light at night, and then assess whether these differed between urban and forest birds. So we feel that (b) should come before (c).

L497 – do you mean a decrease? This is not clear to me.
Yes this was a typo, we meant decrease, thanks for pointing out!

L500 – please detail how this could be tackled further
We have now expanded this paragraph as suggested.

L509 – please discuss the relevance of 1.5 lux in an urban context
Done.

L515-516 - I understand that this part was not reported in results? Should you cite unpublished data?
In the old version we had reported the analysis of sleep movements in the results section, the lines 435-437 and figure 4A. For clarity, in the new version we added in the estimates for each treatment (L427-433). Also, birds only showed more movements under white light, and not green light, compared to darkness. We corrected this mistake by specifically stating that white light had a clear effect on the proportion of movements during sleep.

L522-529 – but abrupt light schedules are not what ALAN is – could you tone down this section in this context? Could you also discuss restlessness specifically in natural studies under ALAN?
In our previous version we did write "*However, in our experiment birds were still exposed to natural LD cycles, and thus they might not experience the same degree of circadian disruption*". So we think we have recognized that our experiment is not directly comparable to previous studies that have exposed birds to constant, high intensity light throughout the night. Nevertheless, we have slightly rephrased this section to highlight differences with these previous studies (L529-538). We have also discussed restlessness in the light of studies of the effects of ALAN in natural conditions (L99-106).

L592 – this has not been tested at all – please rephrase conclusions on what is really demonstrated in the paper, for example in a conservation setting or future research setting.
While it's true that we have not tested this hypothesis, we believe that science and scientific papers should also offer some vision into the future, and propose testable hypotheses that future work can explore. We started this sentence with "*We propose*", which should make it clear that we are offering our own view on the results obtained from this work. We wrote this in the very last sentence of the discussion, after a thorough discussion of our results on the basis of the available data. So we feel that a short speculation should not undermine everything else we have written. We have nevertheless expanded this conclusion paragraph by discussing our results in a conservation setting, as suggested by you also for the abstract.

Reviewer(s)' Comments to Author:

Referee: 1

Comments to the Author(s)
I feel that this paper provides excellent information regarding the roosting preference of Parus major to select areas under sources of dim light. I feel that this information is both valuable to the scientific community and the general public. This information may prove valuable to policy decisions as governments are beginning to implement light pollution reduction policies. This information helps better understand the differences that dim light have on behaviour, and may help inform policy moving forward.

I only have a few suggestions with regards to grammar and formatting to help the paper flow smoother, which I will outline below:
Thanks for praising the organization and writing of the paper. We also thank you for the very useful comments, to which we respond individually below.

Line 160: change "that white light" to "then white light"
We have rephrased accordingly.

Line 252: Change “till” to “until”
Corrected.

Line 308: 1.5 diameter – what is the unit
We have now specified the unit is cm.

Table 1: This is just a suggestion but the table could be adjusted so that the p values in the column line up with the rest of the values on their intended row.
Good suggestion. We have now formatted the table accordingly.
Line 471: remove “even more” this isn’t necessary and makes the sentence feel like it doesn’t flow properly
Removed.

Line 508: remove “onto”
Removed.

Referee: 2

Comments to the Author(s)
The potential adverse effects that artificial light at night (ALAN) have on animals is a growing concern as humans increasingly illuminate the night. Songbirds are a developing model for examining the impact of ALAN. Under controlled lab conditions, Ulgezen et al. investigate which lighting conditions great tits prefer, and the impact that different lighting conditions have on sleep behavior, cognition, and physiology, including daily energy expenditure and blood levels of oxalic acid, a possible biomarker of sleep loss. The authors show that the birds preferred to roost under dim light, rather than darkness, and prefer green over white light. When forced to roost under the different lighting conditions, white light affected activity patterns more than green light, and white light increased daily energy expenditure. Oxalic acid and daytime performance on cognitive tests were not influenced by the lighting condition during the previous night. Differences in the response to lighting was also compared between birds from rural and urban environments.

In general, the paper is well organized and written. However, I have several concerns regarding the experimental set-up and the interpretation of the data.
Thanks for praising the organization and writing of the paper. We also thank you for the very useful comments, to which we respond individually below.

Motion detection sensitivity: In experiment 2, the authors use camera traps to assess “slight movements” during sleep. As slight movements can indicate brief awakenings or REM sleep related dropping of the head in songbirds (e.g. Szymczak et al. Physiol and Behav, 1993), the authors need to be more specific. How was the threshold determined, and how were wake-related movements distinguished from sleep-related movements? Also, based on the experimental set-up, the birds could sit at varying distances from the camera (e.g. on the ground, low perch, high perch, or in the hole between the boxes). The distance to the camera could also vary depending on where on a given perch the bird sat (e.g., in the center or to the left or right). As a result, the same “slight movement” might trigger the camera only when sitting in the closest position. This is potentially problematic if the birds’ position relative to the camera was influenced by light entering from the other box. Consequently, the authors need to discuss how this issue was handled.

Thanks for your very detailed comment. In short, we could not really distinguish between sleep- and wake-related movements. It was simply impossible to do so with our set-up, without having a corresponding and detailed EEG. We needed to rely on the assumption that every time the bird had the head tucked into the feather, this represented a sleep bout, and whenever this was not the case, the birds was awake, even though this was for a brief period of time and could have potentially represented a REM sleep related movement. We have slightly rephrased this passage to recognize this assumption of our study, including a citation to the work you refer to (L280-283).
As for the sleeping position of the birds, we need to point out that in experiment 2 the birds were held in single cages, so there was no "light entering from the other box", and thus no potential bias. However, whether a bird was visible or not in the video frame could have been influenced by the light coming from the same cage, as birds might have tried to hide more behind feeders, for instance. We have tested for this statistically and found no evidence (P>0.1). We have now included a sentence in the manuscript to explain this (L274-276).

From this data, the authors report that "ALAN affected the proportion of movements at night displayed by birds." This was most apparent under white light. In the associated figure (4A), they report "Proportion of minutes at night that birds spent without head tucked under feathers." Given that images were collected in response to motion and at 1-minute intervals, it would be useful to clarify if this was quantified exclusively based on motion detection. Also, songbirds sometimes sleep with their head facing forward (e.g. Szymczak et al. 1993) and then turn their head to the back. This postural change is usually associated with a brief awakening. How were such changes in posture handled?
Thanks for your comment. As we wrote in the manuscript, we only assign a sleep bout to birds that had their head tucked under feathers. Any other postural change was assumed to represent a brief awakening. We are aware of the fact, as the referees correctly pointed out, that sometimes birds might also sleep with other postures. But since 1) there is uncertainty on how often such alternative sleeping postures really occur and 2) there is widespread acceptance that when birds tucked the head into the feather they are likely sleeping, we decided to assign sleeping bouts only to pictures where the head was tucked under the feathers. Obviously the best way to approach such uncertainties would be to do EEG recordings, but that was not possible on our birds.

As a general comment, I would strongly recommend that the authors record continuous video in future experiments. Automated methods for detecting movement or specific postures can then be applied to these images while still retaining the ability to return to the raw images for further analysis when needed.
We fully agree with you on this. It was not possible in our experiment to record all birds simultaneously with continuous video-recordings, but it's definitely something we will consider for future studies.

Sensitivity to sleep loss for oxalic acid: The authors did not detect any lighting-dependent differences in oxalic acid, a putative indicator of sleep loss in mammals. This result is difficult to assess due the lack of systematic studies demonstrating that oxalic acid changes in response to sleep deprivation in birds, as it does in mammals (e.g. rats and humans). Before such validation studies are performed, I think that it is premature to use oxalic acid as a biomarker of sleep loss in birds. In this context, it would be useful for the authors to state that such studies are needed (around line 503). Also, line 438 should be adjusted accordingly.
We agree that oxalate might not be the best marker of sleep disruption in birds, at least not based on the available data from this and previous studies. We have therefore added a note on this issue around the old line 503 (now L504-514). Moreover, we followed your suggestion and deleted the second part of the sentence ("suggesting that birds did not suffer from chronic sleep disruption") at line 438 (now L434).

Line 244: How was the activity level of “one or zero” calculated; e.g. how many times did the perch switch have to be triggered to count as active? Please clarify.
As soon as the perch switch was triggered once within the two-minute bin, the bird was considered active within that bin. This has now been explained in detailed in the supplements.

As noted in section “Light spectra matters” (line 573), the authors acknowledge that sensitivity to the two light colors might be different in birds when compared to humans. Consequently, the birds might perceive the light to be of different intensities, and therefore their choice might reflect a preferred intensity, rather than frequency. Although this is an understandable limitation of their study due to the lack of data on the avian action spectrum, they do cite some references on the topic, albeit on different species. Nonetheless, it might be useful to estimate how different the perceived intensity might be based on the available data on a songbird (e.g., zebra finch) from ref 58.
We thank the referee for this comment. First, we want to stress that we used these human-orientated light measurements in lux because lux will be the real currency when implementing light in real situations (for instance, new light installations by city councils). Second, we recognized, as the referee correctly points out, that the birds might perceive the white and green light as of different intensities. However, there are significant differences in sensitivity to light between avian species, so that a comparison between zebra finches and great tits is probably not very meaningful. Indeed, published studies have shown that finches are much less sensitive to light at night than great tits, and even less than thrushes such as blackbirds and robins (see Kempenaers et al 2010, for instance, which is cited in our paper). Third, the spectral characteristics of the visual system are a limited predictor of how intense birds perceive light. For instance, a very nice study by Prayitno and Philips (British Poultry Science 38, 136–141) shows that the difference in perceived colour-dependent light intensity (in a discrimination test) can be difficult to predict from the known spectral sensitivity of the eye. We now cite this study and expanded this section according to our answer.

Line 133: The behavioral decisions birds make in response to ALAN might be maladaptive given the recent advent of ALAN. As the birds had multiple places to perch (ground, low perch, high perch, or higher hole), each with varying exposure to light from the other box, it would be interesting to know where the birds perched under the different conditions.
The birds basically perched everywhere, on the perches themselves, but also on the feeders, behind the feeders, on the ground etc. The quantification of differences in perching behaviour would be extremely difficult, but we have included a sentence explaining such variation in the new version of the manuscript (L229-232).

Line 267: incorrect reference. Check others.
Correct, thanks for pointing this out. We have now included a more relevant reference.

Line 280: “breath samples” could be interpreted as two individual breaths. Did you mean two 10-samples of breathing?
We have reformulated this sentence as we agree with you that it was highly confusing.

Line 519: what is “sleep restlessness”?
We have changed this to *sleep disruption* as we agree that *sleep restlessness* was ambiguous.

Line 522: what are “abrupt light schedules”?
Again, thanks for pointing out such ambiguous statement. We have reformulated this entire passage (now L529-538) and deleted the expression abrupt light schedules. We hope that now what we mean is clearer and will help differentiate between our results and those of previous studies.

Line 590: The authors suggest choosing to roost under dim light might allow birds to forage at night. In this regard, the authors should state whether their birds ate at night.

We have now included a statement about this and also references that support this claim from other studies (L589-590).

Appendix B

Abstract: please rephrase line 40 as it is misleading – I understand that this is speculation, so it is best to explicitly state so, by for example rephrasing to:
However, it is possible that negative effects of ALAN on sleep and cognition might be observed only under intensities higher than…
Rephrased.

Methods:
L169: so its 1.5 lux the light at the perch level? Please clarify Experimental set-up: I would say that Figure 1 is dearly missed here – I understand you moved it due to space constraints, but I feel that it is really to the detriment of a reader's understanding of how the experiment is carried out. Please consider bringing Figure S1 back to the manuscript.
We specific these measurements were done at the perch level. We would love to bring figure S1 back to the ms but there is really no more space. We already trimmed several sections to the bare minimum, and we feel that trimming even more would considerably hamper the interpretation of our manuscript.

L196: as a note – please rephrase
Rephrased.

L210 – I understand you refer to sup mat figure 1b? I would still advise on bringing it back into the manuscript
We corrected the typo.

L253-254: again, this feels like too little information is provided – could you squeeze a sentence or two in here?
We have added two sentences were we explain these tests more in detail.

L268: please rephrase: we ran four separate odels with the following response variables: …
Done

L273: so there were several tasks? This is missing from the description in the "cognitive abilities" section
We have now revised the cognitive abilities section to make clear that different types of tasks were tested.

Results:
Figure 1: it would make more sense to me if the order of figures is: dark white / dark green / green white
Good point. We have revised the figure accordingly.

L296: urban and forest birds were similarly affected by treatments – I am not sure I agree: there was a significant interaction with origin, wasn't there? Please rephrase the statement to take this into account
Thanks for pointing out this imprecision. We have now rephrased this part.

L317: would this suggest a habituation to light? If so, this could be mentioned in the discussion
Done, now at lines 377-8.

L332-333: under which treatment? Please rephrase for increased clarity.
Rephrased.

Discussion:
L358: rephrase to: […] does not vary seasonally since these experiments were run in autumn
Rephrased.

L385: the fact that you find opposite effects of ALAN on DEE in the wild and in the lab necessitates at least a commentary on which result (the lab or the wild one) should be more informative of biological reality, and why…
We now discussed this in lines 389-393.