Please see our responses below and the highlighted revisions to the manuscript.

Reviewer: 1

Overview: This is timely and well-written. The authors present a very sobering summary of the lack of inclusion in studies examining circadian rhythms in molecular function. I have several minor concerns and suggestions for this work.

Thank you for the constructive feedback.

1. The authors should note that in the meta-analyses for sex bias in neuroscience (mouse, rats), circadian research was one of the fields where their results were impacted by sex.

We were unable to find a specific mention of circadian research in the two meta-analyses for sex bias in neuroscience (Beery and Zucker, 2011 and Becker et al., 2016). We have included citations to the two meta-analyses of sex bias in circadian research with which we're familiar.

2. Figure – Females could be yellow (blue+yellow = green). This would make it a bit easier to distinguish sex, especially in panel 1B.

Thanks for the suggestion. We have revised Fig. 1 to use the colorblind-safe Okabe-Ito color palette, and increased the relative width of the plot for mouse studies.

3. Did the authors include papers where both sexes were used as wildtype controls? There is 1 paper with male and female mouse liver that may not be included
(PMID: 26938655).

We included as many studies as we could find that met our inclusion criteria. That particular study does not include transcriptome data, only qRT-PCR data of selected genes.

4. Page 4,: It would benefit the reader if the authors include citations for the 5 papers in which male and female mice were used (line 7-8) and the 2 papers that reported results by sex (lines 25-27). It is currently difficult to extract this information.

We have added the relevant citations. We leave the decision of whether to include citations for all the studies to the editor.

5. The authors should comment at greater depth on the publishing trends they have detected (Figure 1D).

We have elaborated on the results in Fig. 1D, still focusing on the clearest trends. We wish to avoid overinterpreting year-to-year fluctuations.

a. It can be pointed out that there have been 2 spikes in number of studies with both sexes (2013, 2017), with one pre-dating the NIH mandate.

See response above.

b. Over the last 6 years since the NIH mandate, there has been an equal chance of seeing an increase in studies with both sexes (2017-2018) as an increase/doubling of “unspecified” (2019-2020).

See response above.

c. Early gain in 2017-2018 has not been matched or maintained in 2019-22.

See response above.

d. Last year, the disparity in publishing was not improved, what is the ratio of Males:both?

See response above.

e. Of the “both” papers published in the last 3 years, is there any reason to be encouraged in these papers?

See response above.

6. The authors could clarify or expand on several points.

a. The authors should consider expanding commentary and discussion of Table S10. The 3 points listed are very interesting and could be unpacked. In studies where lack of inclusion was concordant across experiments, were there any trends in what else had been studied or techniques that had been used?

We examined these studies more closely, but did not notice any other clear trends.

b. Page 5: some additional discussion required to unpack statement “some differences may be subtle, others less so” (lines 4-5) and “in order to avoid misinterpretation” (lines 10-12).

We have revised this sentence to add a bit more context while staying concise.

c. The authors could point out the overwhelming focus on mice. This is to be expected given the genetic advantages of mice, but this may not continue going forward.

We have revised the text to address this point.

d. The authors may discuss practices or standards that lead to less bias in human studies for number of studies (Figure 1B) and number of samples (Figure 1C).

We have revised the text to speculate on this point.

7. The authors could discuss at greater length how circadian transcriptional programs may or may not differ by sex. What factors could be at play and what considerations may be relevant for work going forward?

We feel a lengthy discussion of this issue is out of scope of the current paper, but have mentioned a couple examples and cite other studies.

a. Circadian reproductive programs clearly differ, so transcriptional programs across levels/tissues/systems probably also differ.

See response above.

b. How about for other systems? Studies should not be limited to reproductive function. It is notable the only head-to-head studies with transcriptional profiling in both sexes is mouse liver
c. Hormonal environments may drive the need for distinct transcriptional programs to maintain optimal timing. There is need to examine similarities and differences in genetic programs regardless of how the molecular clock oscillates in each sex.

See response above.

d. The authors may wish to point out that if the sexes differ in phase/amplitude/waveform of genetic rhythms, we will lack accurate information for either sex if studies are underpowered and average across sexes. How this is considered during experimental design and analysis is important, perhaps especially when not powering for sex differences.

We have revised the text to briefly touch on this point in the third-to-last paragraph.

Reviewer: 2

This is a beautifully written and explained study that was a pleasure to review. I particularly appreciate that the authors distinguish in their findings between papers that use and report data from both sexes (male and female) and studies that use both but do not report the results by sex (mixed).

Thank you so much for the kind words.

This may be an example where the data speak for themselves, but I'm struck by the lack of any statistical analyses. I presume that was a conscious choice based on the size of different sub-components of the data set, but at least a few analyses on the full data set would be of

interest (for example underscoring that male-only studies are more common than expected by chance, or giving an effect size of the difference between numbers of males and numbers of females in studies that used both).

We agree that the data largely speak for themselves, and that statistical tests could distract from the obvious trends. Statistical analyses are often used to estimate the extent to which the findings from a limited sample set would generalize to the entire (unobserved) population. To a first approximation however, our collection already includes the population of circadian transcriptome datasets. That said, given the few studies that include samples from both males and females, we have now added the relevant means and standard deviations to accompany Fig. 1C.

Where you say: “Third, of the nine studies that included males and females for any experiments, two reported results by sex.” It would be great to elaborate on this point a little more. This is representative of a key gap in the literature, where inclusion of females does not imply reporting by sex (as discussed in the Woitowich and Mamlouk papers you cite), so you could underscore that you reiterate this finding in the genomic circadian literature. You later point out that sex-based analyses will have to wait for better data “Given the few studies and tissues having sufficient data from males and females” and here it might be worth emphasizing that “mixed” studies as you call them can’t address this. I know your “male and female” category implies separate reporting, but this point may be lost on a fast reader.

We have emphasized in the penultimate paragraph the importance of reporting results by sex. We have also emphasized in the final paragraph the dearth of sex-based reporting. The third-to-last paragraph states that “mixed” studies do not enable a sex-based analysis.

Reviewer: 3

This brief report summarizes the sex inclusion characteristics of published circadian transcriptomics datasets. While it is not especially groundbreaking in either its approach or conclusions, the paper is very clear, and highlights a known gap with actual data. I have only one suggestion: the original papers used in this review should also be cited. I realize that this will make the references longer than the paper itself, but this manuscript gives important context for those original papers, and I think it is crucial that *this* paper pops up when people search for articles that refer to any of those original works. The only way that pubmed, google scholar, &c will link this paper to those is if they are part of the bibliography.

Thank you for the suggestion. We discussed this amongst ourselves prior to submission as well. We will defer to the editor here, since it would make the reference list extremely long.