Dear Dr. Senerpont Domis,

Thank you for the opportunity to respond to the reviewer's comments. We have detailed our responses (italics) to their comments (normal type) below. During the revision process, we also discovered a unit-conversion error which affected our scaling between numerical and biomass densities. When corrected, it decreased all our biomass estimates. This change did affect any of the patterns or trends that we report on, just the overall biomass levels. We have included these corrected numbers in the manuscript so that is why they differ from the reviewed version.

One of the reviewers provided a hand-annotated version of the manuscript that contained (mostly) grammatical and other minor issues. We have made changes to the manuscript based on these comments as well.

We thank you and the reviewers for your time and attention, and look forward to your response.

Sincerely,
Dr. Joseph D. Warren

This is the second time that I've been asked to review the manuscript by Urmy & Warren for Freshwater Biology. I've read this second version over a couple of times now and I feel it has improved. I'm very interested in their research because I'm working on similar pursuits. I was excited to turn each page. Most of my concerns I would characterize as minor, but there are enough of them for me to recommend revision needed. I also found some redundancy in the manuscript and some language that is not parsimonious, so I ended up making a lot of suggested edits on a mark-up copy. I don't have the time to annotate these suggestions, so I'm opting to scan the mark-up and provide it with my review. There are actual two scanned documents because my scanner has some limitations on the number of pages that can be fed without causing me headaches. I've organized my concerns into two categories: minor & major.

Minor concerns:
1)   Line 20. The authors should not report lake-wide biomass of zooplankton in Lake Tahoe because of the limited spatial coverage. It is appropriate to use the Tahoe data to explore vertical distributions of zooplankton and fish as they have done. I would change the language in the first summary point to reflect lake-wide biomass is reported for only the three smaller lakes sampled.
This language has been changed to make it clear no biomass estimate is presented for Tahoe.

2)   Line 73. I think the authors are a bit dismissive of optical plankton counters. These techniques have come a long way, but admittedly are challenging to apply with small boats on small lakes.
We have changed this sentence to make the wording less strong, although we believe our description of the tradeoffs involved with the different sampling methodologies is broadly accurate.

3)   Line 84. Another gas-filled insect that can occupy open water is Chironomids. See Kubecka et al. 2000. Aquatic Living Resources 13:361-366. I suppose they have already provided enough examples to make their point. I think these small insects also need to be masked to avoid biasing the zooplankton Sv estimates. They can be quite numerous.

Thank you for this reference; we have added it here. None of the lakes we surveyed had Chaoborid or Chironomid insects in the water column during (as shown by the net samples), so we are not concerned with their influence on our biomass estimates.

4) Line 101. I think the authors should mention dB differencing for classification in the Introduction. This is a new technique and the folks that came up with it in the marine realm deserve some intellectual credit.
We have added references to prior marine frequency-differencing work.

5) Line 155. I think the authors need to mention if the xducers were fast-multiplexed or not, and how close they were to one another when deployed. Also, how was the acoustic data georeferenced?
We have added text here clarifying that the transducers were mounted next to each other and transmitted simultaneously, with a GPS receiver on the pole mount providing position fixes.

6) Line 168. Great that they attempted to calibrate their transducers, but what was learned? Was the observed sphere TS consistent with the theoretical TS? Normally much smaller spheres are used when field calibrating high frequency xducers.
We have added the results of the calibration here.

7) Line 193. I think the authors should discuss if there are any issues with comparing 50 m high net tows versus the 30-m usable range with the 700 kHz xducer.
We have added a line to the discussion (l. 525) addressing this issue—zooplankton densities below about 10 m were uniformly low, so this is unlikely to have affected the results much.

8) Line 201. Point to clarify. Were only 30 animals measured per sampling event or were the first 30 of each taxon measured?
Reworded for clarity: all the animals were identified and counted, but only the first 30 from each sample were measured.

9) Line 231. The De Robertis and Higginbottom (2007) noise removal works best if there is a lot of data collected below the actual bottom. Was that the case? There is no way to judge given methods provided.
Data were collected to a range of at least 100 m in all lakes. In practice, noise was only an issue at 710 kHz, and the low SNR of that instrument at ranges beyond 30 m gave a perfectly adequate estimate of the background noise.

10) Line 227. Using a model to estimate sigma for scaling sv to calculate volume density is a cool approach. The authors make admissions about assumptions they had to make which was a good thing to do. I'm wondering if they could discuss what could be done in the future by the next researcher, say a sensitivity analysis, to propel us boldly into the future.
We do address this issue somewhat in the Discussion (ll. 531-541)--given the inherent uncertainties in each step from TS model to biomass estimate, the concept of an "error budget" is probably more useful in this context than a sensitivity analysis, which may convey a false sense of precision. We provide several citations to studies which have estimated such error budgets.

11) Line 247. Fish TS is largely invariant across frequencies, while copepod TS frequency response increases at higher frequency (Lavery et al. 2007; J. Acoust. Soc. Am 122:3304-3326). The authors are harnessing these differences & using dB differencing for excluding fish. They should be including some other citations here that demonstrate the approach. It is not a new idea.

We have added several references for this standard acoustical method.

12) Line 336. There are some #s > -111 dB in Table 2.
Thank you for noticing, this was either an oversight on our part or left over from an earlier version of the analysis. It has been corrected

13) Line 351. The figures should appear in chronological order in the text. They need to renumber their Figures 3 & 4 accordingly.
The order of the figures has been corrected.

14) Line 411. Seven of the nine surveys have different signs of the coefficients.
After reviewing the table, neither our original text nor the reviewer is correct—seven of the nine coefficients are the same (compare columns "inlet distance" and "shore distance"). The paper's text has been corrected here.

15) Line 439. The opening paragraph of the Discussion is weak in my opinion.
We have re-written this paragraph, hopefully putting more emphasis on the main findings of the paper and the hypotheses laid out in the introduction

16) Line 447. A range of biomass densities of 4-6 g/m3 does not comport with acoustic results in Figure 2. Reconcile.
Thank you for catching this. The 4-6 g m^3 figure appears to have been left over from an earlier version of the paper (possibly referring to the October 2013 zooplankton densities), and has been corrected.

17) Line 475. They talk a lot about the conditions near and around the lakes that may have had bearing on the results. The paragraph is fine, but I'm wondering if it would be better to present a lot of this information in the Study Lakes section.
This is a good suggestion. While we still believe that the fire and drought are important to mention, even if we cannot directly evaluate their effects, we have moved their first mention to the Study Lakes section. This allowed us to streamline this paragraph, hopefully making it less of a digression from the main Discussion.

18) Line 507. I recommend the authors close this paragraph with some ideas on how best we can move forward. They tried something and got promising results. They had to make some assumptions. What do they recommend as the next steps to move the method they used forward in freshwater systems?
We have added a few lines here suggesting how the uncertainties discussed in this paragraph could be clarified in future studies.

19) Line 601 – Ecospace models are being developed. The approach they describe could be used to fuel this type of modelling and I think they should mention this.
We have added text here mentioning these types of models.

20) Table 1. Is it Euyclops or Eurycyclops?
This was a typo—it should be "Eurycyclops," and has been corrected.

21) Table 3. I would add estimated on top of L & W in the table and would report fish lengths in cm. The current version of the table reads mm.

We have added the word "estimated" to the table as suggested. The "mm" column was mis-labeled: the lengths are actually in cm, and the column heading has been changed accordingly.

22)   Table 4. I didn't like this Table caption much because it does not define abbreviations used. Also, I encourage striking the October 2013 estimate of lake-wide biomass of zooplankton in Tahoe. *It is not clear to which abbreviations the reviewer refers here—the only ones in the caption are standard SI units (g m^{-3}, kg). We have removed the estimate of zooplankton biomass for Lake Tahoe and updated the caption to reflect this.*

23)   Table 5. I don't understand why only one set of R2s are provided. I assume the models being reported here are linear regression and not multiple linear regression. Better definition of the model type is needed in the Methods.
The models reported here are multiple regression, i.e. log(biomass) ~ 1 + inlet distance + shore distance. The methods have been changed to make this clear.

24)   Figure 5. What might be going on with the peaks in zooplankton biomass at depth in Cherry (October 2013 & September 2014). I think this anomaly needs to be mentioned in the results.
We have added a mention of these peaks in the results, but don't want to read too much into them because their absolute densities are quite low (note variable x-axis scales).

Major concern:
I.   Line 386. I don't think the authors should be reporting lake-wide biomass of either zooplankton or fish in Lake Tahoe given the spatial extent of their sampling there. This must be remedied before publication.
As requested, we have removed these numbers from Table 4 and the Results section.

II.   Line 447. The authors added hypotheses at the end of their new Introduction. Now I encourage them to circle back to those hypotheses in the Discussion to let the reader know if their results support or refute said hypotheses. There was an effort made to do this, but I think it can still be improved. Help the reader along by being more explicit about whether the data support the hypotheses.
In our rewrite of the opening paragraph of the Discussion section, we now reference the hypotheses more explicitly. We hope this will help remind readers of the big picture and lead them into the more detailed discussion that follows.

Sincerely yours,

Daniel L. Yule, Lake Superior Biological Station, 2/1/2019.

Referee: 2

Comments to the Author
The authors have responded to many of my suggestions, including adding temperature profile data, lake depth, and other sampling information. The simultaneous measurement of fish and zooplankton biomass is a powerful tool, although somewhat limited in this test.

A few questions- Was the acoustic zooplankton biomass measured to the same depth as the net depth (e.g. 50 m in general case).

The maximum depth for the acoustic zooplankton measurements was determined by the signal-to-noise ratio achieved with the 710 kHz echosounder, which depends on the attenuation of the transmitted signal and the level of background noise, and was not known exactly before the surveys began. The net depth was chosen to match the depths of the CTD casts, which were set at 50 m because that was the reported maximum depth of the lakes. Once we actually surveyed them for the first time, we determined that the 710 kHz sounder could integrate zooplankton to about 30 m depth, while the 120 kHz echosounder showed that the max depth of Cherry and Eleanor was closer to 75 m. At that point, however, it seemed to us better to maintain the same net and CTD protocol for all four sets of surveys. In any event, zooplankton densities were always low below the thermocline, so any discrepancy between the 30 m acoustic profiles and the 50 m net hauls should be minor.

I am somewhat worried about the three nets used (two with different ring size and one Van Dorn) particularly since the biovolume was indetectable from the Van Dorn. But you did seem to get enough material for taxonomy counts. Also the low zooplankton net biomass collected with high acoustic backscatter for Eleanor in April 2014 seems a bit worrisome.   You do try to explain this discrepancy, as you did for several other reviewer misgivings.

These concerns are entirely valid, and we share them. The zooplankton in Cherry and Eleanor were small enough that we are not terribly worried about them avoiding the improvised Van Dorn net, and we did get enough to describe the community makeup with confidence. We wish we had a clearer explanation for the April 2014 outlier in Lake Eleanor, but after checking all the processing steps we are currently able to (splitting volumes, counting, data analysis), we have been unable to find any processing errors. Given that situation, we have tried to be transparent in our treatment of the data, and present the results both with and without that data point.

Suggest delete lines 475-495 about fire. Doesn't really add much info to it.

The original impetus for this study was to examine the effects of the fire on the lake ecosystems. Unfortunately a Federal Government shutdown delayed our initial sampling field season so the fire effects could not be completely studied. However given their importance in altering alpine lake ecosystems, we think that this section is still reasonable to include in the manuscript.

Minor point Table 1 still has two misspellings Eurytemora and Diaphanasoma

We thank the reviewer for noticing this. Both have been corrected.