Villacorta-Rath et al. have produced an excellent manuscript which describes the application of eDNA to identify a well-studied amphibian population. The in-depth knowledge including downstream limits and population abundances of *L. lorica* and *L. nannotis* have provided a unique system to test multiple eDNA collection methods for downstream detection of amphibians.

The introduction provided key information and was written to a high standard. The importance and novelty of the work was made very clear by the authors. The research is relevant to a variety of scientists across a number of fields including; eDNA, population genetics and herpetology. The research question and reason for undertaking the study was made clear. The methods included a number of site and technical replicates, there was also thoughtful consideration made to DNA inhibitors. It is an excellent use of eDNA methodologies and has been a delight to review.

General comments

My only concern about the manuscript (and it is a very minor one) is the clarity and explanation of how many in-field replicates were collected and how many laboratory replicates were analysed. The manuscript would benefit by having simple table indicating the number of samples, taken at which sites, how many on-site replications AND how many technical PCR replicates. Its currently unclear about the samples successfully worked and were analysed. Some of this information is provided in Table 1 however it is hard to understand. I suggest reconstructing the table, including better headings for columns, columns for numbers of replicates (field and technical) and making it clear within the table (not the caption) the total amount of water tested.

I also think the manuscript could be improved with more in-depth comparison about the detection rates and where this study sits with regards to similar literature. It is currently unclear if the detection rates presented here are average, better or worse than what would be expected, especially between replicate comparisons.

Specific comments

My most significant comments are focused on the figures, see below. This is a very novel study which I think will be of interest to many in the eDNA/herpetology field, my comments on the figures are therefore suggestions of how to make the very interesting results more accessible for readers.

Figures

Figure 1

- Suggest using something other than a star for the downstream limits, maybe a solid horizontal line and then have an additional tear shape for sampling of site 2, similar to all other sampling sites. When first looking at the figure it is unclear why one star is labelled and the other isn't, it's also not intuitive that a start is representing the end of a something. Additionally, it is unclear the star is an additional sampling point, even though it is numbered. Suggest editing the figure slightly to make the downstream limits and sampling sights more clear.

- Why does the figure not show filtering results also? This makes it hard to interpret the results from the whole study. I would suggest somehow representing the filtering on the figure OR explain clearly why it is not included in the manuscript.

- I suggest 3 figures could be generated; one per method showing positive and negative detections to make it clear between methods what was identified. Otherwise generate a table to clearly define detections.

Figure 2

- I do not think calling the on-site filtration a 'replicate'. Instead, maybe use 'n' to represent samples collected. This would look like
    - 15mL samples n=5,
    - 375mL samples n=2-4 and
    - On-site filtering n=1

Figure 4

- Would benefit by making the text larger on the axis labels and the legend
- Edit change in text in the title of (b), the 'and' looks different to the other text style

Intro

Line 87- Suggest changing 'however' when beginning this sentence, you are not providing an alternative view, more of a supporting argument.

Line 212- Species names not fully italicised

Methods

Line 414- were detection frequencies calculated per method also? If so, please state this.

Results

When discussing the percentage of detections, it would be good to express this quantitatively. For example- "species were detected 30% (30/100) of the time"

Discussion

Line 614 (and throughout)- The Hoskin & Puschendorf (2014) estimate of population size was published quite a few years ago. Do the authors think the population is the same size today, if so why? If not, why? I suggest the authors at the least caveat that the population size will have changed since the last assessment.