**Response to Reviewer Comments**

We want to thank both reviewers and the editor for the thoughtful comments on the manuscript. In response to the comments, and given the time during the review process, we are happy to re-submit the paper with an additional 7 months of data, including a second culvert replacement. We believe the addition of the new data strengthens the analyses and importantly, though we found a minimal effect of the first culvert (in the original submission), we found that the second culver was in fact a blockage and therefore the inclusion of these data strengthen the message of the manuscript. Additionally, we have moved from using the auto-regressive time series model to a linear mixed effect model. We hope the reviewers and editors find the new version of this manuscript to satisfy the original comments.

*I have reviewed the manuscript by Allan et al. The authors tried to quantify the impact of environmental alteration (a culvert removal) on salmonid fish by using eDNA analysis. Quantitative PCR and metabarcoding was applied and the results showed that eDNA methods can be an effective and efficient approach to monitor the impacts. Overall, the manuscript is well-written, and the quality of the experiments is high. However, the organization of the paper is not excellent and needs to be reconstructed.*

We thank the reviewer for their comments and taking the time to improve the organization of the paper.  *First, Introduction is a bit long (12 paragraphs!) and not well constructed. Please consider reconstruction of Introduction to five to six paragraphs. Also, some previous studies reported the methods for quantitative metabarcoding, such as adding known concentration of standard DNA to the samples and using random sequence tags. I suggest authors to cite the literature and compare pros and cons with the method used in this manuscript.*

We have shortened and restructured the introduction. We note that there is quite a lot to cover from environmental impact assessments, eDNA (including metabarcoding, relative abundance, quantification), culverts, and how eDNA has been used to monitor culvert replacements in the past.  *Second, the Methods are overly descriptive. For example, "Site and Species Selection" consists of six paragraphs, much of which should be written in the Introduction. It could be shortened to 3-4 paragraphs and the volume could be halved. Also, the Results section contains information that should be written in the Methods section. For example, information on Line 420-425 should appear in Methods.*

We have moved some of the text in the Methods to the supplemental and have rearranged things between the Results and Methods.

*Finally, Discussion should also be reconstructed. I think the sections "Decoupling of eDNA from fish abundance" and "Accounting for flow with eDNA concentrations" should be in one section, "Appropriateness of methods used" etc. Also, the volume of discussion on this should be reduced.  
  
More minor points are as follows:  
  
Throughout the manuscript: Scientific names and common names are mixed and confusing, so please unify them into one after indicating both at the beginning.+*

We thank the reviewer for the suggestion to unify common and scientific names. We have gone throughout the manuscript and unified so that when referring to a species, it is common name and then scientific name in parentheses. At some points, we just use the scientific name as described in the text there are different haplotypes of species that we cannot distinguish genetically with eDNA metabarcoding. Therefore, there are a few select points in the text where we just use scientific names.

*L 76: From my understanding, quantitative PCR includes realtime PCR and digital PCR. Also, there are non-droplet digital PCR. So, this should be “… such as realtime PCR, digital PCR, or traditional PCR…”*

We thank the reviewer for this suggestion but have chosen to keep the original wording to be as explicit as possible. As we expect the audience of *Ecological Applications* to have varying levels of molecular expertise, we think that explicitly listing both quantitative PCR and digital droplet PCR rather than “realtime PCR” is helpful for readers.

*L 209 (Sup Fig 4): I think this information is presented by a supplemental table, not figure.*

*L 228: The effectiveness of the correction with "correction factor" should be demonstrated by comparison with available measured data.*

*L 231: "Though" should be "Through".*

This really is “though”. We are saying, the range of discharge was high throughout the whole year (0-23 m3/s), but on the days that we actually sampled, the maximum discharge was only 1.3 m3/s. Therefore, we did not change this.

*L 248 :"DNEasy" should be "DNeasy".*

We have changed this accordingly.

*L 293 and else: Name of the program is better to be Italicized.*

We have italicized these.

*L 302:  Only ~2% of ASVs were annotated to species level, it is too low. What is the reason for this?*

*L 350: Terminology should be unified between text and Figure 3.*

*Reference: Fish, W. D. and Wildlife 2019ab seems odd.*

*Fig 1. The legend of Fig.1 is insufficient. Please provide the meaning of triangles and circles. Also, indicate which is the treatment creek.*

*Supplementary tables: Please provide titles and legends for Supplemental Tables.*

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Reviewer #2 Comments to the Author:  
  
Review of Andruszkiewica Allan et al., “Quantifying impacts of an environmental intervention using environmental DNA”  
  
General:  
This is a well written paper that provides interesting results on a topic that could be very valuable in assessing the effects of culvert replacement on stream communities. Given minor/moderate revision, the paper should be accepted and will provide value to the growing eDNA literature as the science moves from research to practice. The authors use existing eDNA techniques that have proven successful for other comparable studies. The results focus mostly on short-term effects of the construction itself (i.e., construction effects that could possibly occur to impact downstream communities) instead of longer-term upstream effects after fish passage was returned. The authors make a confident claim that the technique could be widely used in assessing the (presumably short-term) impacts of culvert replacement projects from the actual construction/engineering impacts of “working in the wet”, but also presumably to the longer term intended impact of restoring connectivity for migratory fish populations. Given this goal, I have two concerns with the existing study that the authors should address in their revisions.*

We thank the reviewer for the positive feedback on the manuscript.  *-One of the strengths of BACI approaches is that it allows comparisons of a main factor (the impact, in this case culvert replacement) on a variable of interest (fish community diversity/eDNA abundance) while accounting for natural differences due to some other factor(s). Without getting into the thick weeds of past controversies of BACI approach (i.e., Hurlbert v. Stewart-Oaten; Underwood), two issues seem to be at play in the current paper. (1) The differences in the streams in the control group, in terms of things that might affect any upstream-downstream differences (e.g., passable, maybe passable) and (2) the duration of the before/after monitoring.*

*I think that the issue in #1 is accounted for in the time series modeling approach used, but the authors should provide some additional explanation in their description of the time-series modeling approach.*

We have added additional text in the description of the time series model (new Lines X-X) to explain how the model accounts for differences in the streams in the control group that might affect upstream and downstream differences. Essentially, we separate out the term specifically for the difference between upstream and downstream (in Equation 1, the eta term). The intercept (alpha) varies by time, creek, and species to allow for deviations between the creeks at different time points, while the species-specific slope (beta) is where information is shared across creeks and time.

*Issue #2 (the duration of the before and after monitoring), however, is a concern, especially in the context of how confident the authors frame the utility of eDNA metabarcoding for estimating environmental impacts of culvert replacement (e.g., lines 436 – 444, but especially lines 602-603). Smokorowski and Randall (2017;*[*https://urldefense.com/v3/\_\_https://doi.org/10.1139/facets-2016-0058\_\_;!!K-Hz7m0Vt54!mN\_SEOLYNXNnOr6TlJ186HeixWIasV3g5veTzOUBFBo5N0T8WFa3cpODnOeu3iLbvjbUNnkTKHL3I4y\_3zbDBAOqsQ$*](https://urldefense.com/v3/__https:/doi.org/10.1139/facets-2016-0058__;!!K-Hz7m0Vt54!mN_SEOLYNXNnOr6TlJ186HeixWIasV3g5veTzOUBFBo5N0T8WFa3cpODnOeu3iLbvjbUNnkTKHL3I4y_3zbDBAOqsQ$)*) argue that multiple years are needed to properly account for interannual variability when assessing impacts to fish populations. The authors of the current paper need to address or acknowledge the limitations of inference due to the relatively short time frame of the study.*

We thank the reviewer for the additional reference and for acknowledging the need for the manuscript to emphasize the short duration of this particular study.

In (previous) lines 436-444,

In (previous) lines 602-603,  *-Another factor worth mentioning is that this study is of a single stream where culvert replacement has been conducted. I’m assuming that a population of culvert replacements was not available for the authors during the conduct of the study…but some mention of the limitations of replication in the discussion seems warranted. There are very few citations of culvert replacement results, outside of a few in the introduction. How do the results of the current paper compare to previous studies of culvert replacement and is there any additional context that they might provide for making policy recommendations (i.e., using eDNA metabarcoding as a methodology to assess culvert replacement impacts)?*

We have added X.  *Specific:  
Line 26-27. Is the use of “intervention” here a typo? BACI designs are universally referred to as Before-After-Control-Impact. While your “environmental intervention” is a specific type of impact, it still is an impact and I don’t think we need to muddy the water by calling it something else. Suggest change to Before-After-Control-Impact.*

We thank the reviewer for catching this typo and have changed “intervention” to “impact”.  *Line 222. Consider providing USGS stream gage station numbers (e.g., Chuckanut = 12201700). Also the citation for streamflow data by USGS is:  USGS. (2022). USGS Water data for the nation. Retrieved from*[*https://urldefense.com/v3/\_\_https://waterdata.usgs.gov/nwis\_\_;!!K-Hz7m0Vt54!mN\_SEOLYNXNnOr6TlJ186HeixWIasV3g5veTzOUBFBo5N0T8WFa3cpODnOeu3iLbvjbUNnkTKHL3I4y\_3zYcWRJOYw$*](https://urldefense.com/v3/__https:/waterdata.usgs.gov/nwis__;!!K-Hz7m0Vt54!mN_SEOLYNXNnOr6TlJ186HeixWIasV3g5veTzOUBFBo5N0T8WFa3cpODnOeu3iLbvjbUNnkTKHL3I4y_3zYcWRJOYw$)*<accessed on date>.*

We have added the gage numbers and updated the citations for the USGS streamflow data.  *Line 314; 347-357-Quantitative PCR testing. It is unclear (in the main text) why you didn’t do qPCR on all four salmon species given that qPCR primers exist for all 4 species. Because of life history differences, the fact that not all streams contain all species, and seasonal differences, variable PCR efficiencies among the assays, could there be an effect of using C\_cutthroat DNA as a surrogate for the other 3 species concentrations from the metabarcoding? Was using just cutthroat qPCR along just a matter of cost? Would using all 4 species, or selecting a different species as the reference (e.g., rainbow trout) appreciably change your results? Including a bit more description of this topic in the main text would help others considering the approach assess why and how to pick a reference species for such an analysis. Although covered in part in the discussion, a bit more exploration of this topic could be helpful.*

We thank the reviewer for this question! In fact, we could have done 4 qPCR assays as opposed to metabarcoding and using a single qPCR assay to expand from proportions to quantities. Here, we are trying to not only answer the question about culverts and salmonid passage, but also demonstrate the ability to use a single qPCR assay in combination with metabarcoding results for many species. It is true that in this case -- with only four species of interest, which all have published qPCR assays – quantifying all four using qPCR would be appropriate. However, in other studies with either many species (>6 say) or species where qPCR assays are not developed, this is a very cost and time effective approach.

As for the question on selecting a different species as the reference, the main difference is that we would lose information on many samples – or would have to use multiple assays to get from proportions to absolute concentrations. In our case, we chose cutthroat trout because it was found in almost every single environmental sample. If we had used rainbow trout, there were several samples where rainbow trout were not found in the metabarcoding results and therefore we could not obtain quantitative information about cutthroat and coho in those samples.

In theory, using quantitative PCR for all species in a sample to obtain the total quantifiable DNA in a sample should give the same results regardless of which the reference species was. We suspect this will not work when there are very, very low percentages of a species in the metabarcoding results, resulting in very small denominators when expanding.  *Line 404-407. This seems a strange case. If the culvert is indeed a total barrier and there are no resident populations upstream, then you should get something close to the patterns you describe. But what accounts for those positive detections in 25% of the sampling months. Both clarkii and mykiss could be resident upstream, but if so then you would expect to see them in other months as well. If a “partial barrier” then you wouldn’t necessarily expect detections during the lowest flow time period in August/September. I think it appropriate to exclude Barnes because of the wonky results upstream, but what could be some explanations? Contamination, exogenous sources of eDNA?*

*Line 447: Elwha not Elwah. Also, suggest changing to “…after a large dam removal project (Elwha River near Port Angeles, Washington)  since there were two dams removed as part of this project.*

We thank the reviewer for catching this typo. We have changed this sentence accordingly.  *Line 477 to 480: I’d say that it is likely to be the case that they are overwintering juveniles. Also, with both snorkel and electrofishing surveys it is possible to identify young of year individuals to species.*

*Line 568-573. “…we find that culverts designated as barriers were likely not blocking fish passage.” I can’t understand how you can make this statement when all of the species except Coho can have both anadromous and resident forms. Those resident forms could have been present upstream and downstream of the culvert before one was ever installed, and then the remained as two separate populations upstream and downstream. Using eDNA (or any other method) to show that there are fish upstream of the barrier does not by itself demonstrate the blocking of fish passage. Obligate anadromous species like Pink, Chum, Chinook salmon would have been much better species to use for addressing the question because if a culvert is indeed a barrier, then you shouldn’t find any signal upstream of total barriers. In the case where species can be both anadromous and resident, other methods (e.g. radio telemetry, pit tagging) would have to be used to show that fish are migrating past the barrier.*

We thank the reviewer for pointing out the challenges associated with designating barriers when a species has both a resident and migratory form. Unfortunately, we did not have signals from pink, chum, and chinook salmon in these creeks for information on obligate anadromous species.  *Line 596-97. “…Here we found very minimal effects of both culverts in general and construction,…” Something seems to be missing from this sentence.*

We have changed the sentence to clarify the confusion to: “Here we found very minimal effects of both culverts in general and of construction during culvert replacement…”

*Figures:  
Figure 2. Cite source as USGS gage data somewhere in figure or in caption.*

*Figure 4. An indication of when the intervention occurred on Padden Creek would be helpful on this graph.*

*Figure 6. It is really hard to distinguish between the light and dark colored symbols because of the overlapping in many of the time periods.*

*Figure 8. Unclear why you’ve included the other creeks in a graph about Padden…the overlapping just serves to wash out the view of symbols (clearly shown in the O. nerka graph, which is labeled incorrectly – should be blue symbol not gray).*