Dear Dr. Carvalho,

## Please find a revised version of “Environmental DNA provides quantitative estimates of Pacific hake abundance and distribution in the open ocean.” (RSPB-2021-2613) accompanying this letter. We would like to thank you and two reviewers for their thoughtful and substantive reviews to the manuscript. Most of the reviewer comments were focused on providing clarity and improving the readability of the manuscript and we have adopted many of the reviewer suggestions. Specifically, we have highlighted the link between our work and its broader fisheries implications and added relevant methodological details for the qPCR analysis to the manuscript. We provide detailed responses to individual comments below (author responses in red) and have attached a pdf showing the changes between the current and previous version of the manuscript. We believe the manuscript is much improved following revision and that it is now appropriate for publication in Proceeding of the Royal Society B.

## Sincerely,

## A.O. Shelton for the co-authors

**Reviewer 1:**

**Overview:**

The manuscript details a large-scale qPCR-based eDNA survey of hake along the U.S. west coast. The authors collected eDNA samples across the region (186 stations, 6 depths) and simultaneously gathered hake biomass data using acoustic and trawl methods. Using collected data, they separately modeled eDNA and acoustic-trawl distributions in this large area, allowing for quantitative method comparisons at management-level scales. This study represents an impressive and novel fish eDNA dataset in the ocean and draws some important conclusions regarding the use of non- invasive qPCR methods to survey fisheries-important taxa, like hake.

This manuscript should be considered for publication in Proceedings B and I think it will be of interest to a wide audience. While I do have a lot of suggestions to improve the manuscript, most of these are minor and I believe all of them can be addressed in a timely manner in a revised draft.

**General comments:**

I would consider changing the title of the manuscript to better reflect the specific target organism and scope of the study. For instance, something like “Environmental DNA provides quantitative estimates of Pacific hake abundance and distribution in the coastal ocean”. You may or may not want to include the scientific name as well.

Response: We have modified the title of the manuscript to be more specific.

There are some missing details that may strengthen the abstract. It would be nice to highlight some quantitative results. For example, is there some number or percent overlap that you can report based on the comparison of qPCR and acoustic-trawl data? What was the level of variation between methods? You could also report a correlation value (Pearson). There is also no reference of the models or eDNA index in the abstract. An important finding you touch on but may expand is that on a sample-to- sample basis, eDNA and other methods (acoustic-trawl) may not correlate due to inherent differences in the method (and in your case the differences in sampling time of day) but they do correlate nicely on large scales that are relevant for fisheries management.

Response: We think all of the points the reviewer brings up are entirely reasonable and worthy of inclusion in greater detail in the abstract. However, the abstract uses all of the 200 word limit allowed by the journal. We have made small editorial changes to the abstract to improve readability but have not added all of the detail suggested by the reviewer.

The introduction reads a bit unorganized and is redundant at times. I think you can tighten this up. I would suggest a paragraph that introduces eDNA, its benefits (e.g., non-invasive, sensitive, cost-effective), and applications to management strategies that require quantitative measurements. Important to note, is that applying eDNA to management of ocean resources is still new. Another paragraph could transition to our knowledge of traditional surveys (trawls, visual, acoustic, etc.) in the ocean vs. eDNA and include inherent differences in fish eDNA in the environment compared to a single discrete fish sample collected in a net tow. Then, you can have a paragraph or two that zoom in on your study region, taxa, and question. Somewhere in the introduction, you may also distinguish amplicon metabarcoding vs. qPCR-based eDNA approaches and how they differ in the types of information gained (e.g., metabarcoding promotes monitoring though is only qualitative vs. qPCR that is more targeted yet quantitative). This is touched on in the discussion but I would mention it earlier. Lastly, I think the introduction would benefit by zooming in on fish and fisheries earlier, establishing the potential for qPCR to inform large scale management in the ocean.

Response: We appreciate the comments on the introduction. We are not sure what the reviewer reads as redundant. We have adopted specific suggestions from the reviewer in editing the introduction including linking the work to fisheries earlier (lines 58-60), emphasizing the importance of moving away from sample-to-sample comparisons (lines 71-76) and added additional species information to the intro (lines 77-87). We have not elaborated upon qPCR vs. metabarcoing as this distinction is not an emphasis of the research in this paper.

Regarding methods, more detail would be helpful in the main text. A lot of this detail is buried in the Supplemental file. I would pull some of this information into the manuscript and refer to the Supplement for additional details and figures. For instance, more information on your qPCR methods is needed in the main text. I know the emphasis is on the model(s) but it is important to clearly state qPCR eDNA methods, especially for reproducibility (see Langlois et al. 2021 – *Environmental DNA*). The reader may miss these key details. Some qPCR questions that arise: What samples did you use for positive hake controls? Did you use voucher specimens? What primers (and other reagents) did you use? Did you account for false positives or negatives? What about closely-relate taxa? Did you measure bulk DNA concentrations in the samples (e.g., via Qubit)? Or did you just quantify hake-specific DNA concentration? I would also pull-out information on DNA contamination and washing error and touch on these in the methods or results section (see specific points below), referencing the Supplement for more details. Again, some readers may not look at the Supplemental file.

Response: While we appreciate all of these points, the eDNA collection, extraction and qPCR methods for this study were previously published in our companion paper in great detail, and include a detailed protocol (Ramon-Laca et al. 2021, citation 25), and are otherwise summarized in the Supplement. We now make clear that all of the topics listed in the reviewers questions are discussed in the companion paper (lines 102-105). We have also added several specific sentences in response to the points above that are particularly relevant to our analyses (e.g. wash errors and contamination) as they are directly linked to the models and analyses (see additional responses below).

I would be consistent with the way you reference “acoustic-trawl” surveys throughout the manuscript. It is important to emphasize that your biomass model is integrating both acoustic and trawl data, and so, referring to this as “acoustic-trawl” seems accurate.

Response: We now use acoustic-trawl throughout the manuscript.

**More specific comments:**

Line 23: Environmental DNA does not necessarily reflect species present in a given habitat. Something like, “...and collecting this environmental DNA can provide information on species that may be present in a given habitat” may be more appropriate.

Response: While we appreciate the point of the reviewer, we think they are conflating the presence of DNA from a species in a particular location and the process of detecting that species in a particular sample or sampling regime. If a species is truly present in a habitat, its DNA will also be there. Said DNA may or may not be detected by a particular sampling regime and there are many complexities about sampling that are worthy of research and discussion. In the first line of the abstract, we are not trying to express the full complexity of the problems of detection and sampling. We only wish to note that species shed DNA and their DNA is in the environment. We disagree that adding conditional verbs indicating sampling uncertainty aids the readers understanding of our work. We have not changed this line.

Line 29: Spatially smooth signature? Sounds cryptic. Do you mean a wide spatial coverage? I would clarify here and in other parts where this is mentioned.

Response: We are uncertain what the reviewer means by “wide spatial coverage” here. However, we appreciate that the term spatially smooth may be unfamiliar to readers who are not accustomed to thinking about spatial analyses. As a result, we have removed this language and replaced it with more specific in line 30.

Line 71: You could also include information about distribution of eDNA from its source location due to movement by currents or tides. Though not a concern for your study, it is important to note that fish eDNA can also be patchy in coastal areas (see Kumar et al. 2021 – *Environmental DNA*). When you integrate over wide areas this becomes less of an issue. I would make this clear.

Response: We agree that the movement of water is an important factor in the ecology of eDNA. The citations within this paragraph (#5-10) each discuss eDNA and transportation extensively (lines 66-73). We also mention DNA transport in passing elsewhere in the introduction (line 45).

Line 75: I would be careful saying this is the most spatially extensive eDNA survey of the oceans to date. It may be more accurate to say “fish eDNA” survey. Though not sampling eDNA per se, the Tara Oceans expedition performed DNA barcoding of microbes-protists on a global scale. I am not diminishing your study; I would just clarify. You may also say “qPCR-based eDNA” survey. Both would be accurate.

Response: We have modified this line and refer to the survey as simply very large (line 77).

Line 76: I would remove the statement “an area of ocean approximately equivalent to Portugal’s land area”. This seems extraneous.

Response: We wrote this to provide people who are not familiar with this part of the world a rough mental reference for scale, but we have removed it in the new version (see line 77).

Line 81: You mention there are rich datasets of hake biomass, presumably from trawls. Have there been many studies targeting hake with qPCR-based eDNA methods? Have others conducted trawl vs. eDNA of important fisheries taxa? What have they found?

Response: There have been no comparable large-scale studies of hake or any other fisheries species using qPCR to our knowledge. We provide reference to the fisheries relevant literature (citations 10-12) but these are nearshore or riverine applications. We have added a sentence making the uniqueness of our application to fisheries more explicit (see lines 58-60)

Line 87: Has this type of model been used in terrestrial applications? Any papers you could cite?

Response: Spatial models of various flavors and structures have an enormous literature with many types of applications in all sorts of habitats - terrestrial, oceanic, riverine, etc. We now provide a few citations to some of the more general introductions to spatial models in ecological and management settings (see line 91-3)

Lines 96-99: I would move this material to the introduction.

Response: We have moved this information to the introduction. (see lines 77-87)

Line 101: You reference a related paper with more details. Still, I think it would be nice to point out lat-long range here or geographic sites that cover the study area.

Response: We have added relevant geographical coordinates to this section of the methods (line 102)

Line 114: Here, you could briefly mention that you found some DNA in the negative   
controls but it was mostly detected below xxx copies L . Then reference the Supplemental file for more information.

Response: We have added this information to the methods (see lines 122).

Lines 116-117: Is this the filtering protocol used for controls and discrete seawater samples? I would specify. How long were the filters stored before extraction? Response: We have clarified that we filtered both field samples and controls using the same methodology (line 124-129). Filter storage time before extraction ranged from 6 to 14 months. We have added this information to the supplement (page 1 of the ESM).

Lines 119-120: Citation associated with the extraction method and the increase in yield?

Response: We have added the relevant citation (line 125)

Lines 120-123: Expand on qPCR details. Pull out from Supplemental file and reference additional details and figures.

Response: See the response to the general comments above.

Line 126: Confused by this sentence. What is the “process”?

Response: The notion of separating a model for a biological process and a model for the observations of that process are the foundation of the world of state-space models. In our case the process is the true, but unobserved distribution of hake DNA in space and with depth. The observation model is how we deal with sample stochasticity, inhibition, wash errors, etc. We have added a sentence to elaborate on what we mean by a process model and provide several references to the general literature of state-space models (lines 133-137)

Line 134: I would refer to specific figures in the Supplemental file that are associated with model optimization mentioned in text.

Response: We don’t show alternate model formulations in the supplement, we only describe our exploration in words (see page 8 of the ESM).

Line 140: I would briefly expand on the wash error and refer to the Supplemental material for relevant figures. Otherwise, the reader will have no idea why you included this in your model.

Response: We have added language about wash error earlier in the methods (see lines 129-131).

Line 147: Could say “indicates” instead of “indexes”.  
Line 153: Could say “simultaneously” instead of “contemporaneously”.

Lines 159-161: This is a key aspect of the study. The reader should be aware of this somewhere in the introduction. Not necessarily the day vs. night differences between methods that preclude direct site level comparisons but rather that fact that you are focusing on broad-scale and not sample-to-sample comparisons.

Response: We agree that this is an important part of our study and that is why we devote a paragraph in the introduction describing how eDNA observation methods are likely to differ from other traditional sampling approaches (see lines 61-76). Like the reviewer, we think that day-night comparisons is an example of a much broader class of comparisons that we bring up in the introduction. As suggested by the reviewer, we have added language about sample-to-sample comparisons in the introduction to clarify our general point about comparisons among sampling methods. (see line 73-4)

Lines 216-217: Remove “genetic signatures of hake” and replace with “hake eDNA”. When you mention detected hake eDNA, are you implying this is above your qPCR detection limit (e.g., 20 copies L-1). I would mention this detection limit somewhere in the qPCR methods section. May be nice to report the range (and mean) of hake DNA concentrations you observed in the study.

Response: We have clarified our language in this section and specified that any signal of DNA is included in our detection of hake. We have added a reference to our detection thresholds as well as the range of hake concentrations estimated by our models (see lines 235)

Line 218: Spatial smoothing sounds odd. Maybe, spatial coverage?

Response: We have removed spatial smoothing and replaced it with language about the relative rates of detection for the two survey types (lines 230-232)

Lines 220-222: Give a range of values and average.

Response: We have added values describing the observed variation in hake DNA among individual water samples as well as among stations by depth (sampled with multiple individual water samples; lines 234-240).

Line 223: Results are vague. What is generally low? <50 copies L-1? What is meant by higher and more heterogenous? Give us some values (average and range).

Response: See previous two comments.

Lines 226-228: Confusing and seems like a discussion topic. Why do you think there was more variability between replicates at the surface? Just a more environmentally heterogeneous sample compared to deeper waters. Hake would be more abundant at the surface when you sampled eDNA at night, so this may reflect feeding behaviors on patchy prey. I wonder if including environmental variables (via CTD) would support your data or provide more context. Did you explore this?

Response: We agree that this is confusing. We think it is especially worth mentioning because we do not have a good explanation for why this pattern would occur. To improve readability, we have broken our writing into two short paragraphs, the first on mean DNA concentrations (233-239) and the second on variability in DNA concentrations (line 240-245). We have no data on hake abundance or distribution at night but there is literature that hake do vertically migrate. It certainly does motivate further examination and we hope to look into it in future work.

Line 229: Still not liking the sound of “spatially smooth”. Lines 231-232. Move to introduction.

Response: We have removed lines 231-232 and added comparable language to the introduction (lines 91-92)

Line 233: What is meant by strong spatial patterning? Line 235: Move some of this to discussion.

Response: Strong spatial patterning is simply substantial variation in hake biomass in the acoustic-trawl survey in space. We have left this sentence in the results as it is describing what we found.

Line 237: You present Pearson correlation data between the two indices but do not explain this in the methods. You should also report significance of the correlations (p- values).

Response: We have added a sentence describing the correlations in the methods (lines 225-227). We provide uncertainty intervals on the bounds of the correlation coefficient which clearly support the estimates being very far from and not including zero. As we work within the Bayesian paradigm for the entire paper, we generally disagree with the use of p-values in any context. We believe our points are made clearly without the use of a p-value.

Lines 247-248: This statement seems redundant after you provide strong Pearson correlation data.

Response: We have cut this sentence from the manuscript.

Lines 249-251: Redundant with information in methods.

Response: We agree that this information is mentioned in two places separately in the methods, but we think it is relevant to draw attention to the different efforts expended between the acoustic-trawl and the eDNA surveys (lines 259-262)

Lines 256-257: Move to discussion. You may consider combining the last two paragraphs of the results.

Response: We think the last two paragraphs are about different measures of spatial and depth distribution. We have retained the current structure.

Lines 259-263: I may be a bit more direct here in terms of what you did and the major findings. For instance, “ Our study assessed the efficacy of eDNA sampling to quantitatively estimate hake concentration and distribution along the US west coast, a well-established fisheries habitat for this species. We sampled eDNA at 186 stations, with depth and latitude, and used a model to estimate and compare with traditional acoustic-trawl data. We found these methods were comparable across wide geographic scales (Pearson of xx), despite less eDNA samples being collected compared to acoustic transects.”

Response: We appreciate the suggestion and have made modest changes to this paragraph (starting line 278).

Lines 271-272: Okay, so this type of model has been used elsewhere?

Response: Yes. Spatial models are used in many ecological contexts and applications including fisheries.

Lines 275-279: You hint at this but I would emphasize that with eDNA you are non- invasively collecting seawater and applying more sensitive methods to target hake. This can be applied to other fisheries-important species within the same samples and supplement existing fisheries operations and stock assessment data.

Response: We appreciate this suggestion and have presented this information in the introduction (lines 53-55) and at several points in the discussion (line 329-32).

Line 291: Do you report environmental metadata collected with the water samples (CTD data)? This may be nice to include, if not for the standard practice of reporting metadata. Including extra variables in the models is likely beyond the scope of this study, which was aimed at comparing sampling methods, but it may be useful to interpret spatial trends in hake eDNA.

Response: There is an enormous amount of data associated with this survey. All of that information is contained and made available elsewhere [citation 28]. This topic is beyond the scope of the manuscript.

Line 292: Typical to reference figures in the discussion for this journal?

Line 307: I agree it is worth mentioning barcoding here. I would expand a bit. Incorporating both approaches would be useful, allowing for biomonitoring of the food web (barcoding), as well as more targeted approaches that inform fisheries (qPCR). Both methods can be exploited from the same collected sample and DNA samples can be archived for long periods of time, preserved or frozen. There is thus a lot of potential and upside to collecting water samples in conjunction with traditional surveys and would not add too much more effort (e.g., filtering water and storing it).

Response: We agree and mention these positive aspects of eDNA sampling in both the introduction and discussion (lines 296, 325-329).

Lines 318-321: Add more detail here about what this information can provide to fisheries management and stakeholders. Expand or wrap this into a Conclusions section. What is the next step? Do you need to complete more eDNA surveys over different years/seasons and compare to trawl data? Are there other types of surveys we could compare with in this region, like camera-based monitoring? How do we translate a strong correlation between eDNA index and fish biomass to an actual fisheries-reliable metric (akin to landings or stock assessments)? Can we use this to model future populations? Add more variables to the model? Mentioning next steps would be helpful.

Response: Some of these questions are already answered in the discussion – e.g. the important role of time-series for making an index of abundance fisheries management is discussed in lines 316-318. One of the major points of the paper is that eDNA is not fundamentally all that different from existing survey methodologies. Logically eDNA can do similar things to existing methods. We have added a sentence to the final paragraph about future links to oceanography and estimates of distribution (paragraph starting line 341).

Lines 322-324: All files are on the GitHub page! I would think about adding more to the description (readme) to indicate what types of data files and R scripts are in the zip file. It will make it easier for those trying to access the data in the future.

Response: We have added information to the readme and archived this data to a permanent Dryad repository associated with this manuscript.

Figure 1: Overall, the figures look good. The bars in panel G are a bit hard to interpret.

Response: We have slightly modified the caption to figure 1

Figure 2: Panel letters are on the inside and smaller compared to Figure 1. I would try to be consistent. I would reference that latitudinal bins are denoted by dotted lines.

Response: We have added a reference about latitudinal bins to the figure caption.

Figure 4: Panel C is hard to interpret. The colors are faint in the graph and everything is blended. You may try faceting panel C based on bottom depth. This figure or parts of it could also go into the Supplemental section. I think Figures 1-3 are most central to the story.

Response: We think Figure 4 is important as it reports several aspects of distribution that are commonly used in the species distribution literature. We have retained it but adjusted the figure size to enhance readability and make it more comparable to the other figures in the manuscript.

**Supplemental Information:**

Standards: Include information on qPCR standards and controls in the main text.

Response: We have mentioned the range of information included in the accompanying supplement.

Investigation of contamination: Some of this information (sentence 2) is redundant with main text. I would briefly mention this in the methods/results section and refer to the Supplemental figures or additional details. Any speculation as to the sources of the contamination? I would move some of your explanation regarding the contamination to the main text. This is important to report.

Response: We have move reference to contamination to the main text (see responses above). We are uncertain about the source of contamination.

Inhibition: Why so much inhibition in surface samples?

Response: We suspect that the inhibition is related to phytoplankton collected in the samples, but we are uncertain the ultimate source of inhibition.

Ethanol wash error: Some of this should be in main text to provide context for the model.

Response: We have made these additions to the main text (see responses above).

eDNA model – Measures of fit and uncertainty: Regarding the 3m samples. Was the ships intake line properly sanitized or flushed? Was there a visual difference in the flow rate that may have caused DNA to shear?

Response: The intake line for surface waters is not sanitized. The entire system is continuously flushed with surface sea waters when in operation, and the local access point is flushed for 5-10 minutes prior to sample collection.

Referee: 2  
  
Comments to the Author(s)  
The paper is extremely relevant for conservation. It stems from the fact that eDNA has become an efficient method to assess species diversity and changes in community with the potential to greatly improve our understanding of natural communities while it remains unclear whether eDNA signals can provide quantitative metrics of abundance to support management. The study is based on the results of a large ocean survey (spanning 86,000 km2 to depths of 500m) and is focused on the abundance and distribution of Pacific hake (Merluccius productus) along the west coast of the United States. The knowledge available for hake provides an opportunity to rigorously compare available information from traditional surveys with eDNA assessment. The paper is well written and suitable for the journal and it could be accepted on its present form. My only questions and suggested revisions are the following:

- among the most significant results there is the assessment of hake DNA variability in the study area which varied substantially with depth, with the highest concentrations between 100m and 300m depth (which I believe is consistent with the species preferred habitat) and concentrations lower and more homogeneous at depth than near the surface. I was wondering whether the fact that the most of water collection for eDNA occurred at night may have also an influence in this respect. Perhaps the authors may want to add this in their discussion;

Response: The reviewer presents and interesting point. Unfortunately we have no information to evaluate the effect of night vs. day sampling on eDNA. We have added a short note (line 339) suggesting that diel migration patterns may play a role in the observations.  
  
- about the e DNA index that was created for the purpose of the spatial analysis, the authors explain that they have generated a depth-integrated index of hake DNA summing the values across all depths and not integrating values across the entire water column or multiplying by the total water volume within each grid cell so that the absolute value of the index depends upon the number of discrete depths at each location. I have two questions in this respect (same questions that I would expect also the readers may have): why the authors decided to use this index instead of the posterior predictions at each depth provided at 200, 250, 350, 400, and 450m for each 5km grid cell, and secondly, given that some locations spatial locations had depths lower than 500m, why they did not standardise the index to a depth common to all the locations?

Response: The reviewer raises several interesting points. As far as this question :

“why the authors decided to use this index instead of the posterior predictions at each depth provided 200, 250, 350, 400, and 450m” We did this because we have no observations at those depths, and therefore no estimated model or posterior predictions for those depth. We think of the model as providing a predicted spatial surface at the depths we have observations (3m, 50m, 100m, 150m, 300m, 500m). An independent spatial (latitude-longitude) smooth is estimated for each depth which allows for different depths to have different spatial patterns and the flexibility to follow the observations at each depth. This can be observed in the spatial maps presented in Fig. 1. However, this statistical form does mean that there is no direct connection between the predictions at a particular spatial location outside of the covariates that are shared due to location (in this model, the bottom depth covariate effect is shared among water depths). As a result, there are no posterior predictions at other depths to sum. We have added a sentence to the section about creating an eDNA index to emphasize that we do not have predictions to other, unobserved depths (line 212).

As far as why we do not sum to deeper depths, we have no information from eDNA below 500m even if the bottom depth is more than 500m. Additionally, the acoustic survey only includes information between 50m and 500m. So we developed an index for between 50m and 500m to make a legitimate comparison between the two methods.

Finally, we do not standardize the index to a common depth, because the intention of the survey is to provide an estimate of total abundance not an estimate at a particular depth. Thus we are interested in an index of the sum across depths, not an index associated with a particular depth.