Environmental DNA Metabarcoding Reveals Winners and Losers of Global Change in Coastal Waters

Response to reviewers

We are thankful for the feedback, comments and suggestions Our manuscript RSPB-2019-2901 "Environmental DNA Metabarcoding Reveals Winners and Losers of Global Change in Coastal Waters" has received. Find below our response to their suggestions, and how we implement them in our manuscript.

Reviewer 1:

- 1. The manuscript needs to be restructured so that it follows a more conventional Intro, Methods, Results, Discussion format. In its current format, the results are difficult to follow as some of the methods that are crucial in interpreting the data are not detailed until the end of the MS. Please revise so that Methods come before Results.

  Response:

  We have now reorganized the manuscript sections following a standard structure. As a result, a few sentences in the results section that introduced the procedures follow to model and project the likelihood of taxon presence have now been merged into the Methods section:

  line 107: Within a geographic area and season, temperature and pH were most strongly associated with community-level changes. We therefore developed a hierarchical logistical regression model relating the presence of each taxon to temperature and pH (as fixed effects) -salinity is predicted to remain largely unchanged in future scenarios [?].

- 2. This is certainly not the first eDNA metabarcoding paper to track shifts in community composition across environmental gradients, yet there are almost no references to other studies that have used this approach, nor how eDNA metabarcoding is being applied more broadly as a monitoring tool. Pleas incorporate this literature into the Introduction.

  Response:

  We now include in the Introduction references to previous efforts to use eDNA metabarcoding for community composition changes assessment linked to environmental gradients, and biomonitoring of marine fauna in general

  Line 78: A growing body of evidence backs up the efficacy of eDNA metabarcoding for monitoring biodiversity (see a review in [?]), and this approach has been successfully used to detect community composition variation across environmental changes in aquatic [?], estuarine [? ?], and marine ecosystems [?]

- 3. The manuscript suffers from a lack of transparent results pertaining to the sequencing and bioinformatic methods. How many samples failed to pass QC? How many reads per sample were the analyses based on? How many samples did you collect from each site? I think this manuscript will benefit from including a table in the main text that summarizes some of these data.

  Response:

  It was the intent of the authors to have raw data and scripts available for the reviewers and the readership when published. We have now created an open github repository with all the code and the raw fastq files are in a google drive link.

- 4. The title needs to be more specific as this study only focusses on plankton communities. Please consider including Plankton in the title to give the readers a better sense of what the paper is actually about.

- 5. The figures do a good job at displaying the study site and data, however they are very busy and figure legends/axes are very difficult to read in print. Please consider revising figures to make them more legible and digestible. For example, perhaps Figure 3 only needs to include taxa that show significant shifts?

Reviewer 2:

- Figures: the colors red and blue are used both to distinguish time periods (2019/2095) and locality (San Juan/Hood Canal) in different figures. Figure 2 legend doesn?t say which color is which (presumably they are the same as in Figure 1). Use consistent colors for categories across figures and use different colors for time period and locality, if possible.

  Response: We realize that the shared color scales can lead to confusion. This has now been fixed

- Figure 2 is presented as for Hood Canal in summer but differs from the image in the supplemental information for that same place and time period. The points and axes in the CAP plot are the same, but the plots in sections B and C differ. What?s the difference between Fig 2 and the (ostensibly same) figure in the supplement? If these differ, how do we interpret the others in the supplement? The legend doesn?t explain the colors in Fig 2C.

  Response:

  Figure 2 should be exactly the same as the first part of the Supplemental figure - A thorough review of the code that generates the supplemental figures revealed a small difference in the input dataset. We thank the reviewer for noticing this, and now the code that generates each of the figures is consistent.

- As Figure 3 is divided into sections A, B, and C, these ought to be referred to in the text (see below). The text is very small and hard to read in the review version I have.

  Response:

  In an effort to declutter Figure 3, and following suggestions from reviewer 1, we have focused the figure in those species showing a more dramatic change in suitability. The new version of the figure has two panels (A and B) and we refer to them specifically in the text

- Lines 74-76 ?with different points along the environmental gradient simultaneously showing differences equivalent to those predicted between present-day and future oceans?: This seems like an important point that perhaps should be included in the Introduction.

- Line 96: Specify Figure 2A

- Line 99: The value of predicting which cluster (?community?) a given set of environmental parameters will fall within is not entirely clear to me. Is there a consistent set of taxa that make of up these clusters across time and space? Can specific groups of taxa be deduced from the ?community? number? Do the environmental variables accurately predict which set of taxa will be present? This section needs a bit more clarity.

- Line 102-104: According to my interpretation of Fig. 2C, it seems like the colder water communities actually had *lower* values of the given taxa, as the differences are all negative relative to other communities. It may help to specify in the text which characteristics are specific to which communities (communities 2 and 3 both span the lower temperature area of Fig. 2B).

- Line 112-117: The authors base much of their calculation on a set of simulated temperature and pH values for different regions in the Salish Sea in 2095. The underlying data was not available so it?s not clear to me exactly how they subdivide the regional variation in the model predictions from Khangaonkar et al. (2019) (see comments on the Methods section as well).

- Line 118-120: Please use figure subsections (A-C) when referring to Figure 3. Line 188 should reference Fig. 3A, line 119 should refernce Fig. 3C, and line 120 should reference Fig. 3B (the text erroneously refers to middle and bottom panels, when it ought to be bottom and middle panels, respectively.

- Line 121: Refer to the relevant section of Figure 3 (A, B, or C)

- Line 121-133: When referring to specific taxa as winners or losers, I was expecting to see them in Figure 3B, but not all of them were there. Or if it did appear, it was only at one site, but it was mentioned as being an overall winner or loser.

- Line 122-123: According to Figure 3B, Thalassionema, Navicula, and Nitzschia appear to refer specifically to San Juan. If so, please state this.

- Line 123: Coscinodiscus and Ditylum seem to refer to Hood Canal. This ought to specified as before.

- Line 129: Write out Emiliania. The ?sp.? after Alexandrium should not be italicized. This sentence might read better if written: ?The coccolithophore Emilinania huxleyi and the dinoflagellate Alexandrium sp. both find more suitable habitat? (rather than ?each finds?)

- Line 136-137: This is minor, but this section (or methods) might benefit from a brief mention of why ANOVA was used in one case and Wilcoxon in another.

- Line 138-141: It seems like this paragraph could be moved to the discussion section.

- Line 147-148: Need a citation for Coscinodiscus as key fish food.

- Line 178: Spell out harmful algal bloom the first time it?s used.

- Line 192: Might be helpful to specifically mention carbon sequestration as an important aspect of sinking rates.

- Line 204-205: Citation needed for fish mortality. Eutrophication seems like a cause of blooms, not a result of them. What are the potential cascade effects on benthic-pelagic coupling? The term here on its own isn?t really saying anything.

- Line 208-214: This is a big aspect of why this study is valuable! I think it?s worth discussing this in the introduction.

- Line 237: Should be Scripps Institution of Oceanography

- Line 241: Should be Figure 1C

- Line 267-268: Which index of abundance from Kelly et al. (2019) was used? That paper presents several and though it declares one to be more useful, I think it would be helpful in this manuscript to state which one was used.

- Line 273-282: It?s hard to discern based on the limited code and data provided exactly how the authors subsampled the different subregions of Puget Sound, since the paper they base their data on has overall values for the whole area. I think this section could use a bit of clarification, and all the code and raw data should be given (the provided github link is broken).