August 05, 2021

Dear Editors,

Thank you for your thorough reevaluation of our manuscript. Your suggestions and feedback testify to your attention to detail and served to greatly improve this pioneering work.

The bulk of second major revisions involved refining the analytical methods. RDA of fish and invertebrate communities were revised to include environmental predictors. Diversity estimates were recalculated as Hill numbers, and we utilized Shannon entropy for regression analyses. Environmental predictor patterns and results were moved to supplemental materials as well as a regression pairs plot and hill number scatterplots for each community along the gradient. We removed canopy coverage as an environmental predictor because sampling took place before leaf emergence resulting in underestimation towards the humid side of the gradient. Seasonal changes in canopy coverage are better assessed through monitoring surveys through time. Although the scripts, tables, and figures were altered to reflect these methodological changes, the resulting patterns remain relatively unchanged.

There were few substantive revisions within the introduction and discussion sections. The introduction was improved by providing the context of the precipitation-flow relationship and how it relates to spatial and temporal climate gradients. The discussion of invertebrate community compositional patterns is explicitly speculative due to the lack of quantitative analysis beyond the RDA. The abstract concludes with a more specific claim. We appreciate the suggested citations and the multitude of smaller edits throughout the manuscript which serve to improve the flow and readability.

In the following, we have compiled the line edits and general comments of the editor and reviewers (Italicized and colored). We then responded directly to each (normalized font and no background color). We believe the revised manuscript is suitable for publication in PeerJ.

Sean Kinard

PhD Candidate

(804) 684-7309

Virginia Institute of Marine Science

1375 Greate Rd, Gloucester Point, VA 23062

Andrews Hall 314

[skkinard@vims.edu](mailto:skkinard@vims.edu)

*Editor 23 use "approach" instead of "substitution"*

done

*Editor 40 replace 'necessitates*

replaced 'necessitates; with 'warrants'

*Editor 51 replace 'streams' with 'stream'*

done

*Editor 52 replace "the water" with "water"*

done

*Editor 52 replace "of aquatic" with "of the aquatic"*

replaced 'habitat' with 'habitats'

*Editor 60 remove "us"*

done

*Editor 60 remove "our"*

done

*Editor 72 replace "substitution" with "approach"*

We removed 'substitution' which was followed by 'approach'.

*Editor 206 a Variance Inflation Factor (VIF) would be better to address multicollinearity*

We calculated the VIF for all ordinations to test assumptions for multicollinearity which are included in the script annotations. We present the regression table in the supplemental data since there are only 6 variables and it is more thorough.

*Editor 209 I'm not sure why you ended up doing it. It looks pretty much exploratory to me, both the figure and the discussion of it. I'd move this to the suppl mat and only leave the inferential data analysis in the main text. See also my comments on the figure below*

done

*Editor 213 remove 'statistical'*

done

*Editor 216 replace 'used linear' with 'used stepwise linear'*

We didn't use stepwise linear regression, but we tried to clarify the difference between explicit hypothesis testing using univariate regression and multiple regression to examine intermediate environmental drivers.

*Editor 221 from the tables 5, 6, 7, it seems that you made a stepwise regression and then the same models. It doesn't make sense, either use a plain multiple regression or a stepwise regression with AIC*

We used exhaustive multiple regression and evaluated significant models with a Δ AICc < 2. We revised published results to include only the sign and statistical significance of relationships between diversity and environmental variables. The full outputs are reported in the supplemental data.

*Editor 226 why only precipitation since you have other env variables? It's weird that you used all variables for the Shannon regression and only one here in the RDA The adjusted r-squared value was negative for the traditional RDA using all environmental variables, indicating poor explanatory power.*

We decided to use the "partially constrained" RDA using only precipitation because it's adjusted r-squared value was positive. A re-examination revealed that the Ezekiel's adjustment is overly conservative when the number of variables exceeds half of the sample size (which is the case in this study). So the proper gauge for fit should be an un-adjusted r-squared value. The un-adjusted r-squared value for the traditional RDA were >0.65 for both fish and invertebrates, so we revised the analysis such that the new ordinations incorporate all explanatory variables.

*Editor 228 what do you mean by that?*

This section was largely removed, with a more concise and relevant description of vector fitting and significance.

*Editor 230 remove 230-233*

This section was largely removed, with a more concise and relevant description of vector fitting and significance.

*Editor 234 replace 'vegan library in …' with ' in vegan package (Oksanen et al 2019) in R.*

done

*Editor 237 remove Site-Overview section (237-247). see my comment above, this is exploratory, it doesn't make sense to have it in the main text*

Good point

*Editor 249 remove 'among the surveyed sites'*

done

*Editor 250 remove 'fish'*

done

*Editor 251 remove "Regression analysis indicates that fish'*

done

*Editor 252 replace both instances of "correlated" with "related"*

done

*Editor 253 remove 'most plausible'*

done

*Editor 254 remove 'multivariate'*

done

*Editor 254 remove fish*

done

*Editor 255 clarify what is meant by 'is 2.5 times more likely than the next-best model*

We removed this language and left the explanation for multiple regression within the methods, the Table 3 caption, and supplemental data.

*Editor 258 remove 258-260*

done

*Editor 261 remove 'the fitted'*

done

*Editor 261 remove 'vector'*

done

*Editor 261 clarify what is meant by 'significant correlation within the ordination and is closely aligned with RDA1*

Revised methods describe how the vector is calculated and significance as a p-value < 0.05. Revised results cite the supplemental data which includes the sines, cosines, correlation coefficients, and *p*-values.

*Editor 274 remove 'within the surveyed sites'*

done

*Editor 274 remove this paragraph, see comment right below*

We feel that the revisions have reduced tedious and exhaustive results into a more focused and concise presentation. Removing the first invertebrate results would neglect to address results that ground the reader as well as those results pertaining to our original hypothesis.

*Editor 277 replace 'correlate' with 'relate'*

done

*Editor 278 remove 'correlation'*

done

*Editor 279*

replace 'most plausible multivariate regression' with 'best' We revised the presentation of regression analyses to a a table and made the description within the text more concise. Now it reads "multiple regression indicates that invertebrate diversity relates negatively with LFPP".

*Editor 279 remove 'fish'*

done

*Editor 280 clarify what is meant by "is 1.6 times more likely than the bext-best model"*

We revised the presentation of regression analyses to a a table and made the description within the text more concise. Now it reads "multiple regression indicates that invertebrate diversity relates negatively with LFPP".

*Editor 281 clarify what is meant by "a fixed random effect"*

We revised the presentation of regression analyses to a a table and made the description within the text more concise. Now it reads "multiple regression indicates that invertebrate diversity relates negatively with LFPP".

*Editor 284 remove 'Redundancy Analysis" and leave "RDA"*

done

*Editor 284 why only precipitation since you have other env variables? It's weird that you used all variables for the Shannon regression and only one here in the RDA*

The adjusted r-squared value was negative for the traditional RDA using all environmental variables, indicating poor explanatory power. We decided to use the "partially constrained" RDA using only precipitation because it's adjusted r-squared value was positive. A re-examination revealed that the Ezekiel's adjustment is overly conservative when the number of variables exceeds half of the sample size (which is the case in this study). So, the proper gauge for fit should be an un-adjusted r-squared value. The un-adjusted r-squared value for the traditional RDA were >0.65 for both fish and invertebrates, so we revised the analysis such that the new ordinations incorporate all explanatory variables.

*Editor 301 remove 'Using the Texas Coastal Prairie (TCP) as a model system"*

done

*Editor 308 remove "Below, we discuss these results, place them in the context of other literature, and make suggestions for future work"*

done

*Editor 311 remove 311-334. as I said above, I don't think it is worth discussing the relationship between env variables, you should focus on the biotic element only*

done

*Editor 318 remove "(Fig.2, Table 3)"*

Figure and table citations within the discussion section were removed.

*Editor 335 remove "pattern of"*

done

*Editor 336 remove "(Fig.3)"*

Figure and table citations within the discussion section were removed.

*Editor 338 replace "correlated" with "related"*

done

*Editor 339 remove "(Table 5)"*

Figure and table citations within the discussion section were removed.

*Editor 375 remove "(Fig.3)"*

Figure and table citations within the discussion section were removed.

*Editor Fig.1 it doesn't make sense to plot precipitation as a continuous variable to color the sites and use the same variable in the ordination diagram. The results are obvious, the value of precipitation will be higher towards the direction arrow is pointing to*

Sites are no longer colored by precipitation which is plotted as a vector in the PCA. The PCA was also moved to supplemental figure 1.

*Editor Fig.3 I'm not sure what is this plot, with RDA1 and PC1.... it doesn't make sense to have it this way*

The former RDA was partially constrained (only constrained to precipitation on one axis). The revised ordinations utilized all environmental predictors.

*Editor Fig.4a what is the alpha?*

Figure 2 contains a regression with the associated correlation coefficient and p value printed in the lower right corner of the panel. The inverterbate scatterplot in figure 3 did not produce a statistically significant regression, so we did not plot it at all. The confidence level for all subsequent regressions was a p-value < 0.05 or an Δ AICc < 2.

*Editor Fig.4c same comment as above... I really don't understand what's the point of having RDA plotted against PC1. When you conduct a constrained ordination analysis, you usually plot the first and 2nd constrained axes, not the unconstrained ones*

The former RDA was partially constrained (only constrained to precipitation on one axis). The revised ordinations utilized all environmental predictors.

*Editor Fig.4d precipitation is already one of the variables used to build the ordination diagram and already appears as an arrow... it doesn't make sense to have sites colored by this variable*

The arrow indicates the loading of the environmental predictor while the site-specific colors indicate the variation between sites. We included both to aid the reader in visualizing the gradient within the ordination. If the impropriety of this method warrants disqualification, we could remove the arrow and place a comment in the caption stipulating that precipitation was used in the ordination, was significant, and was represented by rainfall-coded site points for ease of interpretation rather than a loading vector.

*Editor G.comment I'm not sure what you mean by "RDA constrained by precipitation". Did you mean you model Hellinger-transformed abundance data as a function of precipitation?*

The adjusted r-squared value was negative for the traditional RDA using all environmental variables, indicating poor explanatory power. We decided to use the "partially constrained" RDA using only precipitation because it's adjusted r-squared value was positive. A re-examination revealed that the Ezekiel's adjustment is overly conservative when the number of variables exceeds half of the sample size (which is the case in this study). So the proper gauge for fit should be an un-adjusted r-squared value. The un-adjusted r-squared value for the traditional RDA were >0.65 for both fish and invertebrates, so we revised the analysis such that the new ordinations incorporate all explanatory variables.

*Editor G.comment Also, consider his/her comments on the use of Shannon entropy. Questions about diversity indices are better framed in terms of Hill numbers, since they allow you to use diversity profiles increasingly weighting rare species (see several papers by Anne Chao and the R package iNExt), differently from using a single diversity measure.*

We recalculated our diversity metrics using iNEXT (see supplemental graphs and tables) and found no major differences in the overall results. We used Shannon entropy in our regression and multiple regression.

*Editor G.comment All raw data displayed in tables should be moved to the supplemental material, as well as background information on test results.*

Thank you for specifying what needed to be supplemental.

*Editor Table 1 move to suppl mat*

Moved mean and interquartile range information for environmental metrics to supplemental data.

*Editor Table 2 move to suppl mat*

Moved selected environmental predictors for each sampling location to supplemental table 2.

*Editor Table 3 consider replacing this by the plots of GGally::ggpairs or corrplot packages* [*https://ggobi.github.io/ggally/articles/ggpairs.html*](https://ggobi.github.io/ggally/articles/ggpairs.html)[*https://cran.r-project.org/web/packages/corrplot/vignettes/corrplot-intro.html*](https://cran.r-project.org/web/packages/corrplot/vignettes/corrplot-intro.html) *It would give readers a much nicer view of the relationships among variables. And since it's exploratory, you don't need to report all the summary statistics. But it should also go to the suppl mat*

A pairs plot is included in Supplemental Figure 2.

*Editor Table 4 move to suppl mat*

Table 4 is included in the supplemental materials

*Editor Table 6 what are those numbers? please, only show the variables included in the models*

Multiple regression outputs are listed as tables within the supplemental materials. The results are summarized in the revised Table 1 with the sign of the relationship and '\*' denoting a Δ AICc < 2.

*Editor Table 7 what are those numbers? please, only show the variables included in the models*

Multiple regression outputs are listed in tabular form within the supplemental materials. The results are summarized in the revised Table 1 with the sign of the relationship and '\*' denoting a Δ AICc < 2.

*Editor Table 8 move to suppl mat*

All analytical outputs were moved to supplemental materials

*Editor Table 9 move to suppl mat*

All analytical outputs were moved to supplemental materials

*R: V. Saito 25 change USGS gauged to ‘monitored streams’*

Although these streams are part of a nation-wide monitoring system, none of these streams are biologically monitored. To avoid misleading readers that there may be pre-existing biological data, we elect to remain specific in our language here.

*R: V. Saito 29 remove ‘significantly’*

done

*R: V. Saito 34 ‘a variety of omnivores and piscivores fish’*

replaced with "a variety of predatory fish"

*R: V. Saito 37 why consider the changes abrupt?*

Removed 'abrupt'

*R: V. Saito 47 ‘in general’*

Included

*R: V. Saito 68 Perhaps complement with 'as long as species can disperse to suitable habitats'*

In the previous paragraph, we included more details on how hydrologic flow regime dictates instream connectivity and how that may change with alterations to precipitation regime.

*R: V. Saito 114 ‘characterization of fish and invertebrate communities and quantification of environmental variables.’*

done

*R: V. Saito 121 ‘The link between stable conditions and habitat heterogeneity is not very clear.’*

We removed 'habitat heterogeneity'.

*R: V. Saito 147 Ok, so the sampling effort was proportional to stream width, but not equal among sites. Am I correct? How do you compare abundances among sites? Using individuals per square meter?*

In our study, the wetted stream widths approximated 5 meters. These study sites are characterized by low variation in geomorphology and overall habitat heterogeneity. Shocking intensity is high due to narrow stream widths and homogenous habitat profiles, so we elected to survey a representative 125-meter reach for each site. Since the area sampled was similar across sites, we assumed fish abundances to correspond to the same catch effort and did not report them in terms of area sampled. The revision includes "Low variability in stream withs (4.9 ± .6 m) resulted in comparable catch effort across sites, so fish abundances were reported in terms of catch per sample event. "

*R: V. Saito 169 You need to describe this type of information for fish as well. Could you be more specific?*

Capture, storage, and identification methods for fish are detailed in the previous paragraph.

*R: V. Saito 186 gathered?*

We replaced 'mined' with 'gathered'. Thanks!

*R: V. Saito 216 Because of this property, diversity indices are commonly criticized because the changes you are seeing cannot be interpreted as pure changes in richness or equitability (e.g. Magurran and McGill's book - Measuring Biological Diversity). I would recommend you have also a pure metric of evenness, even if only to show on the Sup. Material. This would allow us to understand the changes in Shannon Diversity.*

We recalculated diversity Hill numbers using the R Package iNEXT. We explored trends in richness, Shannon entropy and Simpson-Gini diversity for each community and included figures in the supplemental figures. We detected minor differences in diversity trends by Hill number order, but we elected to use Shannon entropy because it is not overly sensitive to rare or common taxa.

*R: V. Saito 233 And which function for the constrained RDA? Also, I think the specific method for constraining an RDA is new (I did not find the information on the two references provided), so a reference would be nice.*

We included the function, citation, and reference for the statistical guidance provided in Numerical Ecology Using R.

*R: V. Saito 250 How correlated they are? Some of the Shannon diversity changes could be due to changes in evenness and this would be nice to show.*

We detected minor differences in diversity trends by Hill number order and have included several figures in the supplemental materials.

*R: V. Saito 261 This is because it is constrained before the RDA, right?*

Indeed, the former RDA was constrained. The new RDA uses all environmental variables, and this section now reads "Sites are stratified in ordination space horizontally which aligns with fitted environmental vectors of precipitation, conductivity, and NH4+."

*R: V. Saito 277 One site with very low precipitation looks like an outlier here. You could try a robust regression that accounts for extreme values because overall the scatterplot shows a decreasing trend in diversity with increasing precipitation.*

We revised the discussion to touch on this topic. It reads "Alternatively, the driest site (Tranquitas Creek) displayed uncharacteristically low diversity compared to other semi-arid sites and may constitute an outlier. When removed, invertebrate diversity correlated negatively with precipitation (R2 = 0.43, *p*-value = 0.06). "

*R: V. Saito 401 This paragraph is mostly based on non-gathered results. You do not have data on basal resources, competitive specialization, competition among species, and predation pressures. Altogether, this logical construction is too fragile. Moreover, it is not well explained why these changes could be considered as abrupt ecosystem shifts. Abrupt shifts have breaking points and your RDA shows a continuous change in composition.*

We reorganized this paragraph into two sections. The first describes the lack of relationship between precipitation and invertebrate community diversity. The second paragraph describes the three patterns that we observed within the compositional shifts in invertebrate communities along the gradient. We present them as speculative topics that require follow up with more robust sampling design that is described in the following paragraph. We removed the topic of abrupt ecosystem shift because it was not apparent in our results as you pointed out.

*R: V. Saito G.comment Methods are well described, but I miss a reference or more information for the constrained RDA.*

"To discern compositional shifts in fish and invertebrates across the precipitation gradient, we used Redundancy Analysis (RDA) on Hellinger-transformed community data, constrained to the six environmental variables in Table 1 (Legendre and Gallagher 2001; Legendre and Legendre 2012)."

*R: V. Saito G.comment the correlation between precipitation and diversity seen to be impacted by an outlier that could be tackled by robust regression.*

Thanks for noticing this. We included a supplemental figure and added the following to the discussion of invertebrate diversity "Alternatively, the driest site (Tranquitas Creek) displayed uncharacteristically low diversity compared to other semi-arid sites and may constitute an outlier. When removed, invertebrate diversity correlated negatively with precipitation (R2 = 0.43, *p*-value = 0.06). Regardless, the relation between precipitation and invertebrate diversity remains unclear."

*R: V. Saito G.comment The findings are sound in general, but I have one point of concern. The discussion around invertebrate community changes is too speculative and not based on proper results.*

In contrast to the quantitative discussions of diversity, the community composition discussion is speculative. To maintain transparency to this effect, we changed the topic sentence for this paragraph to openly state " compositional shifts with rising precipitation invite continued assessment of the following speculative premises within the region". The Following sentences maintain uncertainty with language such as "alludes towards", " possible shift in" and "may have been". Unfortunately, this preliminary survey did not permit a robust functional trait analysis which limited our ability to present a firmer discussion of the matter.

*R: V. Saito G.comment The idea of an abrupt change is also not well based or explained.*

We removed mentions of abrupt ecosystem shifts which detracted from the observed gradual effects along the gradient.

*R: V. Saito G.comment Only the discussion of invertebrates' abrupt changes are too speculative.*

We removed mentions of abrupt ecosystem shifts which detracted from the observed gradual effects along the gradient.

*R: V. Saito G.comment Speculative about invertebrates are taken too far supporting the conclusion.*

In contrast to the quantitative discussions of diversity, the community composition discussion is speculative. To maintain transparency to this effect, we changed the topic sentence for this paragraph to openly state " compositional shifts with rising precipitation invite continued assessment of the following speculative premises within the region". The Following sentences maintain uncertainty with language such as "alludes towards", " possible shift in" and "may have been". Unfortunately, this preliminary survey did not permit a robust functional trait analysis which limited our ability to present a firmer discussion of the matter*.*

*Reviewer 3 36 But what would these new communities look like? Modify something? I think here authors have to make it clear that the modification would not be very good as it would increase/decrease diversity? Or change competition? Function on community?*

We revised the concluding sentence of the abstract to better characterize our discussion points in the following way: "These results warn that aridification coincides with the loss of competitive and environmentally sensitive taxa which could yield less desirable community states."

*Reviewer 3 112 I understand that this is a pioneering work. But it does not justify the lack of some information. Throughout the introduction, the authors talk about changes in the biota of fish and macroinvertebrates. I think the authors can be more specific on this issue. for example: diversity increases with rainy and decreases in the dry region ... Or decreases the richness of fish species in a given place. I want to make it clear that the authors have already put references on this, I just think that adding specific information, like the one I mentioned above, would help to understand the objectives and predictions of the manuscript. In my opinion, using results from work done in drought and wetter regions would only improve the introduction of the manuscript.*

We revised the second paragraph to relate the physical relationship between climate and hydrology with the effects of climate change and observed biological consequences. Specifically, to address the comment, we included new citations from a fish community survey along climate gradients as well as a temporal study of macroinvertebrate responses to aridification: " Aridification increases the prevalence of droughts and flash floods which disturb local communities by imposing intolerable conditions or physically displacing individuals. Lengthening dry periods cause changes in macroinvertebrate communities where drought sensitive taxa (Ephemeroptera, Plecoptera, and Trichoptera) are replaced by drought tolerant species (Storey 2016). In contrast, humid precipitation regimes have low interannual variability and frequent bank flooding that promotes hydrological connectivity and resource exchange between aquatic and terrestrial systems. Fish communities become increasingly diverse with precipitation along continental climate gradients (Griffiths et al. 2014). "

*Reviewer 3 158 Isn't it a short time? is there any reference that can help saying that this time is enough for the collection?*

A reviewer requested a time, which is usually system dependent. In low-gradient sandy streams, 15 seconds is sufficient to adequately disturb and sweep within the sample space. Longer sweeps risked losing our catch in the slack water. We included our guiding reference for kicknet sampling (Southerland et al. 2007).

*Reviewer 3 162 see this sentence with a different font.*

Thanks, we corrected the font.

*Reviewer 3 235 In the end of the “Analyses” section authors can add that all scripts were available in the supplementary material.*

"Statistics and analytical R scripts for analyses described above are reported in the supplemental data."

*Reviewer 3 339 The result of the regression between canopy cover and shannonfish remained to be discussed further. I suggest that it is not an expected result (Dala-Corte et al. 2020, Journal of Applied Ecology), but the authors need to discuss more about this because this result brings new information that is the influence of the precipitation gradient associated with canopy cover. Are precipitation and canopy cover correlated variables? It would be relevant information. Until maybe authors use the factor as a covariable. So authors can see if the canopy cover can influence the fish divesity however it depends on precipitation gradient. Also, this result “fish shannon negatively with canopy” is in the abstract ... I think that the authors can remove from abstract and discuss more about it in the discussion section.*

Indeed, this didn't make sense until we realized that peak leaf development in the deciduous trees within this region doesn't occur until May. We underestimated the evergreen coverage in semi-arid sites and overestimated coverage at many of our sub-humid streams. We stated, "The negative relationship between fish diversity and canopy coverage was attributed to incomplete leaf-emergence in deciduous canopies prior to May."

*Reviewer 3 347 I think this is speculation, maybe I would remove that sentence or identify.*

Good catch, we removed this observation and speculation which may be interesting but do not discuss the results of the manuscript.

*Reviewer 3 371 I suggest modifying the word "curiously". Example: “Contrary to expectations Red shiners are absent….” I think the language gets more scientific.*

We incorporated this recommendation to improve scientific syntax.

*Reviewer 3 374 I suggest removing "Rosgen" leaving only "stream morphology", since the authors have already explained that it is a proxy in the material and methods.*

We retained stream morphology and omitted the mention of the Rosgen index within the discussion.

*Reviewer 3 445 This part need to be summarized on the abstract it is a very important conclusion of the research.*

The abstract was revised to improve the compositional descriptions which preclude this conclusion: "Semi-arid fish and invertebrate communities were dominated by euryhaline and live-bearing taxa. Sub-humid communities contained environmentally sensitive trichopterans and ephemeropterans as well as piscivores which may impose top-down controls on primary consumers. These results warn that aridification coincides with the loss of competitive, environmentally sensitive taxa which could yield less desirable community states."

*Reviewer 3 Fig. 3 the graphs could be enlarged. They were very good, but small.*

We removed the scatterplots beyond Shannon entropy vs precipitation. So, the revised figure has two large panels.

*Reviewer 3 G.comment The manuscript needs a revision of the references because they are not standardized with the journal's norms. For example: names are different abbreviated in the first and write out in the second.*

Each reference was edited to be consistent with the publisher guidelines. Specifically, we converted first names to abbreviations that follow each corresponding last name.