Date:	Dec 11 2023 10:01AM
To:	"Laura H Spencer" laura.spencer@noaa.gov;lhs3@uw.edu
cc:	rr_editorassignment@plos.org, promila.bishnoi@newgen.co, nc.james@saiab.nrf.ac.za
From:	"PLOS Climate" climate@plos.org
Subject:	PLOS Climate Decision: Revision required [PCLM-D-23-00215]

PCLM-D-23-00215
Narrowed gene functions and enhanced transposon activity are associated with high tolerance to ocean acidification in a juvenile subarctic crustacean
PLOS Climate

Dear Dr. Spencer,

Thank you for submitting your manuscript to PLOS Climate. After careful consideration, we feel that it has merit but does not fully meet PLOS Climate's publication criteria as it currently stands. Therefore, we invite you to submit a revised version of the manuscript that addresses the points raised during the review process.

Please submit your revised manuscript by Jan 10 2024 11:59PM. If you will need more time than this to complete your revisions, please reply to this message or contact the journal office at climate@plos.org. When you're ready to submit your revision, log on to https://www.editorialmanager.com/pclm/ and select the 'Submissions Needing Revision' folder to locate your manuscript file.

Please include the following items when submitting your revised manuscript:

- A rebuttal letter that responds to each point raised by the editor and reviewer(s). You should upload this letter as a separate file labeled 'Response to Reviewers'.
- A marked-up copy of your manuscript that highlights changes made to the original version. You should upload this as a separate file labeled 'Revised Manuscript with Track Changes'.
- An unmarked version of your revised paper without tracked changes. You should upload this as a separate file labeled 'Manuscript'.

Guidelines for resubmitting your figure files are available below the reviewer comments at the end of this letter.

We look forward to receiving your revised manuscript.

Kind regards,

Nicola Caroline James, Ph.D
Academic Editor
PLOS Climate

Journal Requirements:

1. Please review your reference list to ensure that it is complete and correct. If you have cited papers that have been retracted, please include the rationale for doing so in the manuscript text, or remove these references and replace them with relevant current references. Any changes to the reference list should be mentioned in the rebuttal letter that accompanies your revised manuscript. If you need to cite a retracted article, indicate the article's retracted status in the References list and also include a citation and full reference for the retraction notice.

2. In your Methods section, please provide additional information regarding the permits you obtained for the work. Please ensure you have included the full name of the authority that approved the field site access and, if no permits were required, a brief statement explaining why."

3. Please note that PLOS CLIMATE has specific guidelines on code sharing for submissions in which author-generated code underpins the findings in the manuscript. In these cases, all author-generated code must be made available without restrictions upon publication of the work. Please review our guidelines at https://journals.plos.org/climate/s/materials-and-software-sharing#loc-sharing-code and ensure that your code is shared in a way that follows best practice and facilitates reproducibility and reuse.

Additional Editor Comments (if provided):

Your manuscript was reviewed by two reviewers who both indicated that the manuscript is well written and will be of interest to physiologists and molecular biologists. The reviewers suggested minor edits, mostly around writing (Reviewer 1) and framing and incorporation of additional literature on OA and crustaceans, particularly studies that have a molecular focus (Reviewer 2).

[Note: HTML markup is below. Please do not edit.]

Reviewers' comments:

Reviewer's Responses to Questions

Comments to the Author

1. Does this manuscript meet PLOS Climate's publication criteria? Is the manuscript technically sound, and do the data support the conclusions? The manuscript must describe methodologically and ethically rigorous research with conclusions that are appropriately drawn based on the data presented.

Reviewer #1: Yes

Reviewer #2: Yes

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2. Has the statistical analysis been performed appropriately and rigorously?

Reviewer #1: I don't know

Reviewer #2: Yes

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3. Have the authors made all data underlying the findings in their manuscript fully available (please refer to the Data Availability Statement at the start of the manuscript PDF file)?

The PLOS Data policy requires authors to make all data underlying the findings described in their manuscript fully available without restriction, with rare exception. The data should be provided as part of the manuscript or its supporting information, or deposited to a public repository. For example, in addition to summary statistics, the data points behind means, medians and variance measures should be available. If there are restrictions on publicly sharing data—e.g. participant privacy or use of data from a third party—those must be specified.

Reviewer #1: Yes

Reviewer #2: Yes

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4. Is the manuscript presented in an intelligible fashion and written in standard English?

PLOS Climate does not copyedit accepted manuscripts, so the language in submitted articles must be clear, correct, and unambiguous. Any typographical or grammatical errors should be corrected at revision, so please note any specific errors here.

Reviewer #1: Yes

Reviewer #2: Yes

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5. Review Comments to the Author

Please use the space provided to explain your answers to the questions above. You may also include additional comments for the author, including concerns about dual publication, research ethics, or publication ethics. (Please upload your review as an attachment if it exceeds 20,000 characters)

Reviewer #1: Overview
I appreciate the opportunity to review this manuscript on Red King Crab where the authors demonstrate differential gene expression in RKC reared from larval to juvenile stages among OA treatments. The manuscript is clearly written, results interpretable and discussed in detail. I must declare upfront that I am not an expert on transcriptomics or functional genomics – two key words in the manuscript. I can therefore comment on the scientific framing/interpretation rather than methodological and more technical concerns. I therefore have only minor comments and based on my non-expert opinion in genetics endorse its publication.

Major
No major comments

Minor
Line 78 – 81: are these studies showing deleterious effects on short term exposure? Is that why results differ to long exposure I Long et al? Please indicate.
Line 86: remove "Here, we"
Line 101-106: rephrase, it comes across as methods.
Line 141-145 = results
Table 1: Is this data pooled for each treatment among the replicates? It would be good to see this data broken down by tank replicate in the supplementary material to see if something untoward may have occurred in a replicate.
Line 138, Supplementrat figure S1: you mention elsewhere that it was a three-month exposure trial but you only have two months of time series data in the supplemental material?

Sample size? For analyses?
Line 5: what were the phenotypic results?

Reviewer #2: The authors present a functional genomics study examining molecular pathways that may contribute to juvenile red king crab (RKC) tolerance to ocean acidification. I believe the authors did a thorough job exploring the various molecular datasets and their relationship to the phenotypes observed in a previous study. Their interrogation of transposable element activity in response to OA is an interesting use of genomics data that will be of interest to physiologists and molecular biologists alike. While the authors focus on connections with previously published (RKC) studies, I think the manuscript would be strengthened by incorporating OA studies in other crustaceans, particularly those that evaluate OA impacts on molecular pathways. Additionally, I have minor suggestions for framing in the introduction section and questions surrounding the enrichment methods.

Overarching Comments:

Connections to other crustacean literature: The focus of the manuscript currently is RKC-centric. While connecting this study with previous RKC studies is important, I think the manuscript would be of interest to a wider audience if the authors discussed their findings in the context of other crustacean OA studies. A handful of studies have examined molecular mechanisms involved in crustacean responses to OA. I've included some suggested references below (one physiology study, two molecular studies) that may be useful for the authors.

Functional Analyses: It was unclear which enrichment analyses were completed, and which datasets were used for those analyses, when reading the methods section alone. Consider restructuring the methods section and adding subheadings to match the results section for increased clarity. In lines 276-279, the authors state that two analyses were performed for each pairwise contrast. What were these analyses?

Specific Comments:

Introduction:

Line 57: When does the Bering Sea experience lower aragonite saturation, and which part of the crab life cycle does it coincide with?

Line 61: Replace first "]" with ","

Lines 73-75: What have existing mechanistic crustacean OA studies demonstrated?

Line 86: Delete "Here, we"

Lines 88-89: Consider replacing "genes and functions" with "molecular mechanisms and pathways" to encompass DEG and TE information.

Lines 89-106: Consider restructuring this section of the introduction for improved flow and clarity. Move lines 101-106 after lines 87-89 to introduce the experimental design in the previous study. Then, modify lines 89-91 to explicitly define the molecular approaches used by the study/the different ways RNA-Seq data was leveraged.

Line 94-96: Consider moving to line 75, as that paragraph discusses previous crustacean OA literature.
Lines 100-101: Leveraged the genome to do what?

Methods:

Lines 141-149: Consider moving this information to an Experimental Design section in the results.

Lines 218-220: Consider modifying Table 2 to include how many samples were used for each analysis (DEG, SNPs, etc.)

Lines 220-222: Why not use an RDA, or something similar, to understand how genetic variation constrains gene expression variation?

Lines 249-254: Can be hard to follow methods for gene expression variation calculations when DEG analysis is not yet described. Consider incorporating the relevant gene-wise variation analyses into the global gene expression and DEG analysis sections.

Lines 278-279: Specify L2FC thresholds in the "Differential gene expression analysis" section, as I assume the same thresholds listed here were used with DESeq2.

Lines 288-289: I would argue that searching for transposable elements wouldn't constitute an enrichment analysis, but a separate functional analysis. Consider changing the wording and adding a descriptive subheading to match this.

Line 291: Restate the gene sets used for clarity.

Lines 290-294: Why not use RepeatMasker to search for transposable elements in addition to the manual curation already performed?

Results:

Lines 327-329: …mapped to non-coding regions (22.3%), or were assigned…

Line 335: The high number of genes that were removed? That had reads mapped to them?

Line 336: Add "...repeat elements characteristic of crustacean genomes" to add context for readers who are unfamiliar with crustacean genomics.

Line 338: Replace "and ranged" with "ranging"

Figure 4: Consider changing the green-purple color scheme to a single-color gradient-based color scheme to facilitate better interpretation, especially for color-blind individuals. Move A/B/C and plot titles above the volcano plot panels so they are easier to read.

Line 386: Consider "darker shades" instead of "darker colors"

Lines 387-388: Are the DEG those with darker shades in the volcano plots? If so, add that information for clarity.

Lines 414-415: Are these GOterm names or user-defined categories? If user-defined, how were they defined?

Figure 6: Consider adding a pathway diagram that summarizes important information from these figures, as these pathways are a cornerstone of the discussion section.

Lines 448-449: What is meant by a "more active" biological process? Increased expression of these genes?

Lines 451-466: Was this a separate enrichment test conducted in DAVID? What was the gene background used for the enrichment?

Line 467: Was there a statistical test associated with examining differences in TE activity/composition between the different OA treatments?

Figure 7: Did the authors consider making a version of this figure where TE activity/composition is examined in relation to OA treatment?

Discussion:

Lines 675-677: Is there another crustacean study that could be cited here instead of the Olympia oyster study?

References:

Noisette, F., Calosi, P., Madeira, D., Chemel, M., Menu-Courey, K., Piedalue, S., Gurney-Smith, H., Daoud, D., & Azetsu-Scott, K. (2021). Tolerant Larvae and Sensitive Juveniles: Integrating Metabolomics and Whole-Organism Responses to Define Life-Stage Specific Sensitivity to Ocean Acidification in the American Lobster. Metabolites, 11(9). https://doi.org/10.3390/metabo11090584

Trigg, S. A., McElhany, P., Maher, M., Perez, D., Busch, D. S., & Nichols, K. M. (2019). Uncovering mechanisms of global ocean change effects on the Dungeness crab (Cancer magister) through metabolomics analysis. Scientific Reports, 9(1), 10717. https://doi.org/10.1038/s41598-019-46947-6

Glandon, H. L., Paynter, K. T., Rowe, C. L., & Miller, T. J. (2019). Resilience of Oxygen Consumption Rates in the Juvenile Blue Crab Callinectes sapidus to Future Predicted Increases in Environmental Temperature and pCo2 in the Mesohaline Chesapeake Bay. Journal of Shellfish Research, 38(3), 711–723. https://doi.org/10.2983/035.038.0323

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6. PLOS authors have the option to publish the peer review history of their article (what does this mean?). If published, this will include your full peer review and any attached files.

Do you want your identity to be public for this peer review? If you choose "no", your identity will remain anonymous but your review may still be made public.

For information about this choice, including consent withdrawal, please see our Privacy Policy.

Reviewer #1: No

Reviewer #2: No

[NOTE: If reviewer comments were submitted as an attachment file, they will be attached to this email and accessible via the submission site. Please log into your account, locate the manuscript record, and check for the action link "View Attachments". If this link does not appear, there are no attachment files.]

While revising your submission, please upload your figure files to the Preflight Analysis and Conversion Engine (PACE) digital diagnostic tool, https://pacev2.apexcovantage.com/. PACE helps ensure that figures meet PLOS requirements. To use PACE,

you must first register as a user. Registration is free. Then, login and navigate to the UPLOAD tab, where you will find detailed instructions on how to use the tool. If you encounter any issues or have any questions when using PACE, please email PLOS at figures@plos.org. Please note that Supporting Information files do not need this step.
