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March 20, 2019

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Dear PeerJ Editorial Staff,

We are pleased to resubmit to you and your editorial staff our newly revised manuscript (Article ID 25864), 'Biochemical changes throughout early- and middle-stages of embryogenesis in lobsters (*Homarus americanus*) under varying thermal regimes', for re-assessment and hope that you and the two Reviewers find our improvements acceptable for approval.

Since this time we have been able to fully address both Reviewers' follow-up comments and suggestions, and have corrected, clarified, or improved just about all these issues that are detailed in a point-by-point response. We have further consolidated Figures that were not too informative and improved one as well. We have fixed and/or clarified ambiguous writing, and qualified some of our statements as well. We are providing a marked-up version of the manuscript and a 'clean' version so that all changes that were made can be seen.

Finally, we have addressed all the mandatory corrections and formatting issues brought to our attention by the editorial staff at PeerJ – Thank You.

We hope that you will find our improved manuscript as a successful contribution to PeerJ. If you have any questions, please do not hesitate to contact me.

With Kind Regards,

Jason S. Goldstein, PhD.
Corresponding Author

Article Title: Review of “Biochemical changes throughout early- and middle-stages of embryogenesis in lobsters (*Homarus americanus*) under varying thermal regimes” by J.S. Goldstein and W.H. Watson III, submitted to PeerJ.

Reviewer-1

The authors have now provided more details on methods and findings. I have only a few last comments.

Major comments:

1. Lines 123-125. “Only lobster embryos with eye indices less than 18%... were used for this study...”. (1) This sentence has been modified in response to a comment in the first review of the manuscript. As it is now formulated, the sentence can be interpreted as meaning that the <18% eye index criterion was applied to embryos at all sampling times throughout the study rather than only on the first sampling occasion (5 Sept.). Please clarify by changing “were used for this study” to “were used at this sampling time”. (2) What were these early-sampled embryos used for? They do not appear to have been used for biochemical determinations nor for volume measurements (although another sampling for volume determinations by treatment was done in mid-September) and no information on them is reported in the results section.

Response: We agree and have rephrased this to reflect this (LINES 123-126). In regards to the second inquiry, we did this initial assessment of lobster eggs to ensure that we did not use lobsters whose embryos were too far in their development to diminish our results or initial data for obtaining baseline measurements.

2. Lines 202-207. Maybe it's worth noting that temperatures in the inshore and offshore treatments converged quickly at the outset of the experiment and diverged markedly starting only in early April (Fig. 1). Thus it is not so surprising that there was no difference in embryo development between inshore and offshore treatments, at least until the March sampling. It is perhaps more surprising that the late May sampling did not show a greater difference in biochemical content between the warmer inshore ($\approx 9-10^{\circ}\text{C}$) and colder offshore ($\approx 4^{\circ}\text{C}$) treatments after almost 2 months of divergent temperature.

Response: This is a good point, especially since temperatures over the course of the study were not significantly different. They did, however, diverge very much so in the spring which was a likely cause of differential egg development. Reviewer-2 also had a similar comment, and so we have inserted some modified text in LINES 211-214, and we also touch on this in the discussion with respect to biochemical differences.

3. Lines 330-331. “up until Stage IV (post-larval), lobsters depended upon stored capacities of lipids”. But are the larvae not also feeding exogenously, as stated in the introduction?

Response: We have clarified this to reflect the fact that the Stage IV phase in lobsters is a rather transitional one, during which they obtain nutrition from both stored reserves and active raptorial feeding.

4. Line 350. Subsection on “Female size and condition”. Neither of these variables was considered in this study, and so this subsection could be removed or substantially reduced in length.

Response: *This is a valid point so we have removed text in this sub-section to give it a more focused approach.*

Minor comments:

Line 56. “for their development”.

Response: *We have revised this whole sentence for clarity.*

L59. “Lipids comprise provide the structural”.

Response: *This has been corrected.*

L62. “proteins as are the basic”.

Response: *This has been corrected.*

L71-72. Lobster larvae do derive their nutrition from exogenous and endogenous sources, but this is not true of all crustacean larvae (some are only lecithotrophic). Can be fixed by: “because in most species they derive”.

Response: *This is a good point and we have reworked this sentence.*

L120. “to the propus”.

Response: *This has been corrected.*

L158. “a fine powder”.

Response: *Thank you for seeing this; this has been corrected.*

L183-184. “All calculations eggs were measured to the nearest 0.1 mm”.

Response: *This has been corrected.*

L334. In line with a comment in the review of the first version of the manuscript, please consider changing “The increase of water in the eggs (egg volume) as seen in this study and others is directly related to water uptake and has been noted to increase by more than 50% over the course of development (Pandian, 1970) ” to “The increase in egg volume as seen in this study and others is directly related to water uptake and may reach more than 50% over the course of development (Pandian, 1970)”.

Response: *This is a good suggestion and we have changed this accordingly (LINES 339-340).*

L359. “these factors in more depth. Since, since female size”.

Response: *This has been corrected.*

Reviewer-2 (Kiran Liversage)

Basic Reporting

1. Line 58, 59: words “both” and “alike” maybe not needed

Response: This has been corrected.

2. Line 62: change “as” to “are”

Response: Grammatically speaking, this is correct as is.

3. Line 66: remove word “also”

Response: This has been corrected.

4. Line 88: I would say the word “lab” may be too colloquial

Response: Agreed, and this has been modified.

5. Line 102: change “we sought to test” to “we tested”

Response: This has been corrected.

6. Line 150: maybe to increase succinctness remove “and placed in labeled plastic sample trays”.

Response: Good point; this has been corrected.

7. Line 211: maybe remove “very”

Response: This has been corrected.

8. Figure 2: please add standard error bars to the sampling points on the line graphs

Response: This has been amended to Figure 2.

9. Line 217: change “and these results are summarized in Tables 1 & 2” to “(Tables 1 & 2)”

Response: This has been corrected.

10. Table 1: the means in this table appear to be exactly duplicating the information on Fig 2. I think the standard errors and post-hoc tests should be presented on Fig. 2 and Table 1 removed.

Response: Yes, this could be considered redundant. We have eliminated Table 1 and integrated some of the caption material into the Figure 2 caption description for clarity (see Figure captions). Accordingly, we have also re-numbered the Tables in the text.

11. Table 2 legend: please mention in the 1st sentence that the values shown are P values.

Response: This has been corrected.

12. Line 236: change “The main goal of this study was” to “Our main goal was”

Response: This has been corrected.

13. Line 267: change “couple” to “two”

Response: This has been corrected.

14. Line 272: The use of the word “real” here is a bit confusing, I would revise.

Response: Agreed. We removed this word and changed the sentence a bit (LINE 278).

15. Line 305-309: this long sentence could be split in two.

Response: A good point and we have changed this (LINES 310-314).

16. Line 384: Change “dramatically” to “significantly”

Response: This has been corrected.

17. Figure 2, 3: It is confusing for the reader that bar graphs were used Fig. 3 and line graphs Fig. 2, when they both show very similar time series. I would recommend for consistency to change all time series as line graphs, unless there is some specific reason its like this.

Response: Although we can appreciate the consideration being made here, we feel that the bar graph for egg volume is effective as is and we are inclined to keep this way.

Experimental design

18. I think it would be useful for some of the discussion about pseudo-replication in the response to reviewers document to be briefly mentioned somewhere in the manuscript.

Response: We have provided a discussion of this (LINES 153-163), as suggested.

Validity of the findings

19. Line 102-104: The hypotheses should directly match the tests done (i.e. the actual statistical comparisons). The only factors tested here were temperature and time; no measurements of migration were made. Also, the temperatures were not changed according to any migrations, they just tested how eggs would develop from lobsters staying either inshore or offshore. So the hypotheses should state something like “It was hypothesized that different water temperatures, simulated in the laboratory to be similar to temperatures experienced by lobsters in varying coastal zones (inshore vs offshore), will affect egg biochemistry. This hypothesis was tested to gain information useful for understanding how egg development may be affected by lobsters migrating between those zones”

Response: This is a fair and valid point and we have re-worked our hypothesis based on your suggestion, which is much more clear and straight-forward (LINES 103-107).

20. Line 207: the lack of significant difference in temperature between inshore and offshore water was surprising and may influence the validity of the conclusions, as this study is meant to inform about lobsters migrating between these areas to expose their eggs to different temperatures. Please provide some text about the implications of the non-significant

temperature result in the discussion.

*Response: Yes, the **average** inshore vs. offshore temperature over the course of the study was not different. However, **the rate of change in temperature** between these two locations (Fig. 1) is substantial and enough to then modulate disparate changes in development and subsequent hatch as noted in Goldstein and Watson (2015a,b). In the revised Discussion we spend more time on this point.*

21. Line 324-326: I think this text needs to be clarified/corrected. I guess the “constant” temperature being referred to here is the one used in this experiment, which according to line 248 appears more optimal for growth, but here it is stated as “sub-optimal”. Also, if more protein is used for energy as stated here, then there should be less rather than more protein in the “constant” treatment which is not the case (Fig. 2).

Response: We dealt with this in the Discussion a bit and removed the phrase ‘sub-optimal’ which is misleading.

22. To allow the reader to understand the conclusions better, please somewhere early in the discussion provide a clear statement about whether or not the results supported the specific hypotheses from the introduction.

Response: We have addressed this in the Discussion section in various places.

23. Line 384-385: Please mention here why “there is still the potential”, rather than just saying that the potential exists. It seems the results may indicate that no evidence could be found to support the hypothesis that “variations in the energetics of embryogenesis influenced by the seasonal movements of some lobsters to and from these two disparate locations”, especially since these disparate locations did not even have significantly different temperatures according to my understanding (line 207). Also I think the word “are” is needed between “embryogenesis” and “influenced”.

Response: We have also reconciled this in the Discussion.

Comments for the Author

24. I think a concluding sentence in the abstract stating the relevance of the results would be useful.

Response: We have revised the Conclusions section accordingly.

25. Line 39: add “potentially” between “thereby” and “exposing”, especially since this study did not appear to find any significant different (Line 207) between the two areas where migrations occur between.

Response: This has been corrected.

26. Line 130: Here it says the “constant” treatment was 12 degrees, but in figure 1 it seems this treatment had 16 degrees. Please correct one or the other.

Response: Thank you for catching that mistake in the text. Should be 16 C and we have changed this.

27. Line 211-217: According to how I understood this, here all the results are from the 2 factor analyses. Firstly the text here details the main effects, then results of the interactions. The interactions are all significant, which makes it redundant to mention the main effects as was done first (i.e. effects of the factors in isolation are not informative). If this understanding is correct, then the text from line 211-215 may not be needed.

Response: This was a bit redundant and diluted the main thrust of the summary statistics, so we revised this section and remove much of the text in the original lines 211-215 (now LINE-216).

28. Line 300-302: a reference needs to be provided for this statement.

Response: This has been added in the text (LINE-307) and in the References section.

29. Line 350-378: These paragraphs are on a different topic to the study and are sufficiently unrelated that they confuse the story a bit and I would say they are probably not needed, or could be reduced to a few sentences.

Response: This same issue was also brought up by Reviewer-1 and we have addressed this by substantially revising and shorting this section.

Editorial office

Funding Statement:

At the next revision, please use full names, instead of initials, for the author names in the Funding Statement. Edit here https://peerj.com/manuscripts/25864/declarations/#question_18.

Response: We will do this.

Tables:

Tables should not be an image pasted into the Word document. At the next revision, please revise Table 2 so that the text is editable and upload it as a separate Word document (one file per table), including a title and any necessary legends in the text fields using the Edit button to the right of the file name here <https://peerj.com/manuscripts/25864/files>.

Response: Yes, we will take care of this.

Figures:

A) Figure 2 has multiple parts. Each part needs to be labeled alphabetically to use (A, B, C, D, etc) instead of directions (left, right, upper, lower, etc). At the next revision, please provide a replacement figure measuring minimum 900 pixels and maximum 3000 pixels on all sides, saved as PNG, EPS, or PDF (vector images) file format without excess white space around the images.

Response: This has been revised as indicated above by the editorial office.