Rethinking competence in marine life cycles: ontogenetic changes in the settlement response of sand dollar larvae exposed to turbulence

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Article citation details
R. Soc. open sci. 2: 150114.
http://dx.doi.org/10.1098/rsos.150114

Review timeline
Original submission: 16 March 2015
Revised submission: 8 May 2015
Final acceptance: 26 May 2015

Note: Reports are unedited and appear as submitted by the referee. The review history appears in chronological order.

Review History
RSOS-150114.R0 (Original submission)

Review form: Reviewer 1 (Jan Pechenik)

Is the manuscript scientifically sound in its present form?
Yes

Are the interpretations and conclusions justified by the results?
No

Is the language acceptable?
Yes

Is it clear how to access all supporting data?
I believe the authors are correct in claiming that supporting data of interest are included in the manuscript

Do you have any ethical concerns with this paper?
No

Have you any concerns about statistical analyses in this paper?
No

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Recommendation?
Accept with minor revision (please list in comments)

Comments to the Author(s)
File attached (Appendix A).

Review form: Reviewer 2

Is the manuscript scientifically sound in its present form?
No

Are the interpretations and conclusions justified by the results?
No

Is the language acceptable?
Yes

Is it clear how to access all supporting data?
I think so.

Do you have any ethical concerns with this paper?
No

Have you any concerns about statistical analyses in this paper?
Yes

Recommendation?
Major revision is needed (please make suggestions in comments)

Comments to the Author(s)
This work makes a minor advance on a preceding study on the same topic. The analyses are done badly, specifically the authors do not deal with experimental runs effectively - they should use mixed models to partition run effects. The logistic regression calculation of inflection points is particularly muddled - these results cannot be trusted at all. Instead the authors must use logistic ANCOVA to determine whether the is any difference in slope or intercept rather than the indirect and inappropriate approach they have used here.

I suggest the authors consider including the work of Emlet on urchin size affecting post-metamorphic survival and I also suggest they include another paper by Swanson, showing increased sensitivity to histamine as larvae age.

Decision letter (RSOS-150114)

07-Apr-2015

Dear Dr Hodin,

The Subject Editor assigned to your paper ("Rethinking competence in marine life cycles: ontogenetic changes in the settlement response of sand dollar larvae exposed to turbulence") has now received comments from reviewers. We would like you to revise your paper in accordance with the referee and Subject Editor suggestions which can be found below (not including confidential reports to the Editor). Please note this decision does not guarantee eventual acceptance.
Please submit a copy of your revised paper within three weeks (i.e. by the 30-Apr-2015). If we do not hear from you within this time then it will be assumed that the paper has been withdrawn. In exceptional circumstances, extensions may be possible if agreed with the Editorial Office in advance. We do not allow multiple rounds of revision so we urge you to make every effort to fully address all of the comments at this stage. If deemed necessary by the Editors, your manuscript will be sent back to one or more of the original reviewers for assessment. If the original reviewers are not available we may invite new reviewers.

To revise your manuscript, log into http://mc.manuscriptcentral.com/rsos and enter your Author Centre, where you will find your manuscript title listed under “Manuscripts with Decisions.” Under “Actions,” click on “Create a Revision.” Your manuscript number has been appended to denote a revision. Revise your manuscript and upload a new version through your Author Centre.

When submitting your revised manuscript, you must respond to the comments made by the referees and upload a file “Response to Referees” in “Section 6 - File Upload”. Please use this to document how you have responded to the comments, and the adjustments you have made. In order to expedite the processing of the revised manuscript, please be as specific as possible in your response.

In addition to addressing all of the reviewers’ and editor’s comments please also ensure that your revised manuscript contains the following sections before the reference list:

• Ethics statement
If your study uses humans or animals please include details of the ethical approval received, including the name of the committee that granted approval. For human studies please also detail whether informed consent was obtained. For field studies on animals please include details of all permissions, licences and/or approvals granted to carry out the fieldwork.

• Data accessibility
It is a condition of publication that all supporting data are made available either as supplementary information or preferably in a suitable permanent repository. The data accessibility section should state where the article's supporting data can be accessed. This section should also include details, where possible of where to access other relevant research materials such as statistical tools, protocols, software etc can be accessed. If the data has been deposited in an external repository this section should list the database, accession number and link to the DOI for all data from the article that has been made publicly available. Data sets that have been deposited in an external repository and have a DOI should also be appropriately cited in the manuscript and included in the reference list.

• Competing interests
Please declare any financial or non-financial competing interests, or state that you have no competing interests.
• Authors’ contributions
All submissions, other than those with a single author, must include an Authors’ Contributions section which individually lists the specific contribution of each author. The list of Authors should meet all of the following criteria; 1) substantial contributions to conception and design, or acquisition of data, or analysis and interpretation of data; 2) drafting the article or revising it critically for important intellectual content; and 3) final approval of the version to be published.

All contributors who do not meet all of these criteria should be included in the acknowledgements.

We suggest the following format:
AB carried out the molecular lab work, participated in data analysis, carried out sequence alignments, participated in the design of the study and drafted the manuscript; CD carried out
the statistical analyses; EF collected field data; GH conceived of the study, designed the study, coordinated the study and helped draft the manuscript. All authors gave final approval for publication.

- Acknowledgements
  Please acknowledge anyone who contributed to the study but did not meet the authorship criteria.

- Funding statement
  Please list the source of funding for each author.

Once again, thank you for submitting your manuscript to Royal Society Open Science and I look forward to receiving your revision. If you have any questions at all, please do not hesitate to get in touch.

Yours sincerely,
Dr Kevin Padian
Senior Publishing Editor, Royal Society Open Science
openscience@royalsociety.org

Comments to Author:
Associate Editor's comments:

The expert reviewers have provided several recommendations for revisions that should be made to the manuscript. Principally these include an alternative way to perform the statistical analyses, inclusion of a wider consideration of some relevant literature, clarification of some definitions and clarification of some aspects of the experimental design (particularly with regards to a turbulence control).

Reviewers' Comments to Author:
Reviewer: 1

Comments to the Author(s)
File attached.

Reviewer: 2

Comments to the Author(s)
This work makes a minor advance on a preceding study on the same topic. The analyses are done badly, specifically the authors do not deal with experimental runs effectively -they should use mixed models to partition run effects. The logistic regression calculation of inflection points is particularly muddled - these results cannot be trusted at all. Instead the authors must use logistic ANCOVA to determine whether the is any difference in slope or intercept rather than the indirect and inappropriate approach they have used here.

I suggest the authors consider including the work of Emlet on urchin size affecting post-metamorphic survival and I also suggest they include another paper by Swanson, showing increased sensitivity to histamine as larvae age.

Author's Response to Decision Letter for (RSOS-150114)

See Appendix B.
RSOS-150114.R1 (Revision)

Review form: Reviewer 1 (Jan Pechenik)

Is the manuscript scientifically sound in its present form?
Yes

Are the interpretations and conclusions justified by the results?
Yes

Is the language acceptable?
Yes

Is it clear how to access all supporting data?
Fine

Do you have any ethical concerns with this paper?
No

Have you any concerns about statistical analyses in this paper?
I do not feel qualified to assess the statistics

Recommendation?
Accept as is

Comments to the Author(s)
The authors have followed most of my suggestions and the manuscript is now much clearer. They might want to avoid overgeneralizing in places (first sentence of Discussion, for example). It still appears to me that there is a certain degree of differentiation that must occur before these larvae can be induced to settle/metamorphose—in this case the larvae gradually become responsive to turbulence cues, which accelerates their becoming responsive to chemical cues—and so these data still seem consistent with the general idea that the onset of competence is somehow "pre-programmed" into development. But now we have a new problem to deal with: what makes these larvae suddenly responsive to turbulence? Interesting stuff.

Review form: Reviewer 2

Is the manuscript scientifically sound in its present form?
Yes

Are the interpretations and conclusions justified by the results?
Yes

Is the language acceptable?
Yes

Is it clear how to access all supporting data?
Don't know

Do you have any ethical concerns with this paper?
No

Have you any concerns about statistical analyses in this paper?
No
Recommendation?
Accept as is

Comments to the Author(s)
The manuscript has been modified adequately.

Decision letter (RSOS-150114.R1)

26-May-2015

Dear Dr Hodin,

I am pleased to inform you that your manuscript entitled "Rethinking competence in marine life cycles: ontogenetic changes in the settlement response of sand dollar larvae exposed to turbulence" is now accepted for publication in Royal Society Open Science.

You can expect to receive a proof of your article within approximately 10 working days. Please contact the production office (openscience_proofs@royalsociety.org) to let us know if you are likely to be away from e-mail contact during that period. Due to rapid publication and an extremely tight schedule, if comments are not received, your paper may experience a delay in publication.

Royal Society Open Science operates under a continuous publication model (http://bit.ly/cpFAQ). Your article will be published straight into the next open issue and this will be the final version of the paper. As such, it can be cited immediately by other researchers. As the issue version of your paper will be the only version to be published I would advise you to check your proofs thoroughly as changes cannot be made once the paper is published.

In order to raise the profile of your paper once it is published, we can send through a PDF of your paper to selected colleagues. If you wish to take advantage of this, please reply to this email with the name and email addresses of up to 10 people who you feel would wish to read your article.

On behalf of the Editors of Royal Society Open Science, we look forward to your continued contributions to the Journal.

Best wishes,
Ms Emilie Aime
emilie.aime@royalsociety.org
http://rsos.royalsocietypublishing.org/
This was an interesting and well-written paper. The findings seem novel, although perhaps not creating quite the revolution in our thinking about competence as the authors suggest. What the work adds is the interesting idea that larvae can become competent to respond not just to ambient chemical cues, but to physical cues as well, and that once competent to respond to them, the physical cues can accelerate responsiveness to chemical cues. Indeed, their data support that idea very nicely. In Figure 5, for example, we see young larvae becoming more and more responsive to the effects of turbulence as they age. But the larvae are still metamorphosing in response to chemical cues, not to turbulence; it seems to me that the onset of competence to respond to chemical cues has been speeded up by exposure to a short period (only 3 minutes!) of turbulence. Similarly, a number of previous studies have shown that larvae become more responsive to chemical cues as they age.

So here is the way I see the story here. In the absence of turbulence the larvae eventually become responsive to certain chemical cues. But turbulence can speed up that process, so that larvae become responsive (“competent to metamorphose”) to the chemical cues earlier. So in the absence of turbulence larvae will disperse farther and be more developed at metamorphosis, while in the presence of turbulence—if they are far enough along in development—they will disperse less and metamorphose sooner if the right chemical cues are present. If metamorphosis is under inhibitory control, as a number of studies suggest for a number of marine species, then turbulence may simply be shutting down the inhibition faster.

The authors should define exactly what they mean by the terms competence, pre-competence, settlement, and metamorphosis. They describe “settlement” on pages 11 and 12 as the withdrawal of epithelial tissue from the tips of the larval arms. But isn’t this the start of “metamorphosis”? I suppose they could define “settlement” as the initiation of metamorphosis, but why not just talk about metamorphosis? To me, “settlement” just means that the larvae have stopped swimming and settled to the bottom of the container. Larvae will “settle” if you add formalin to the water! Moreover, “settlement” is reversible, while “metamorphosis” is not in these organisms, and that is an important distinction. I say more about the terms competence and pre-competence below.

Did the authors include a treatment in which the larvae were exposed only to the turbulence without being subsequently exposed to natural or artificial inducer? I didn’t see that, but it seems like an important control to include. It’s important to establish that the larvae are not metamorphosing in response to turbulence directly. The only controls I saw described were exposing larvae to cues directly in the absence of turbulence (p 10, line 42-43), and putting larvae in the turbulence machine for 3 minutes without turning it on (p 10, lines 50-53). I would like to see some assurance that turbulence alone does not stimulate metamorphosis.

Page 5 bottom (H1). “Increased responsiveness to turbulence” might be a little misleading, since it seems that the larvae are not metamorphosing in response to turbulence. Rather, turbulence is speeding up the onset of metamorphic competence. On p 12 (lines 28-34), were none of these larvae
set aside for documenting the effects of turbulence alone? That seems an essential part of the experiment. Also, do we know that the turbulence itself is not causing physical damage to the larvae? That is also important to know, and should be made clear.

Page 6, Line 54. Give a reference for “our prior work”.

Page 17, lines 13-23. Is it really “increased responsiveness to turbulence exposure,” or rather a speeding up of the onset of competence (i.e., responsiveness) to chemical cues?

P 21 top paragr. This just repeats material from the Introduction.

P 21 middle paragr. Discuss the ecological implications of this batch variability, particularly in terms of dispersal consequences.

P 22, lines 30-45. Do larvae “have the ability to adjust their turbulence responses during ontogeny,” or does turbulence just set something else in motion (competence to respond to chemical cues), once the larvae become responsive to turbulence?

P 23 top. If the larvae metamorphosed in response to inducer after being exposed to turbulence, then the larvae were not “precompetent.” They were precompetent before the turbulence exposure, but then apparently became competent to respond to chemical cues (at a smaller size) through the turbulent experience. And they were “competent” to respond to the turbulence. This is an important point. Figure 6 shows that only about 20% of turbulence-treated larvae responded to chemical inducers, which presumably means that 80% of the larvae were not yet competent to respond to the turbulence by becoming competent to respond to the chemical cues.

Conclusions (p 27). I don’t see how this research clarifies “a mechanistic understanding of what it means for larvae to be competent.” Lines 1-56: “precompetent larvae” OF A CERTAIN AGE; the larvae were not competent to respond to chemical cues before they experience turbulence, but they were competent to respond to the turbulence (by becoming competent to respond to chemical cues). This gets back to the importance of defining precisely what is meant by competence. As a number of authors (e.g., Pechenik and Gee) have pointed out previously, it’s important to define competence with respect to the particular cue of interest, since larvae apparently become competent to respond to different cues at different ages. So here in this paper we have two cues really, one physical and one chemical; it’s just that the larvae don’t metamorphose in response to the physical cue, but instead become capable of metamorphosing in response to the chemical cue.

Figure 3. Are all larvae from a single culture? That should be made clear.

I had a lot of trouble reading Figure 4, in particular figuring out what the different symbols represented.
RESPONSE TO REVIEWERS, "RETHINKING COMPETENCE IN MARINE LIFE CYCLES: ONTOGENETIC CHANGES IN THE SETTLEMENT RESPONSE OF SAND DOLLAR LARVAE EXPOSED TO TURBULENCE" BY HODIN ET AL., RSOS-150114

Associate Editor's comments:

The expert reviewers have provided several recommendations for revisions that should be made to the manuscript. Principally these include an alternative way to perform the statistical analyses, inclusion of a wider consideration of some relevant literature, clarification of some definitions and clarification of some aspects of the experimental design (particularly with regards to a turbulence control).

We appreciate the opportunity to respond to the reviewers' comments, and we concur with Dr. Ferrier that these are the main points that the reviewers focused on in their comments and suggestions. We reply to each of these points in the course of our replies to the comments of Reviewers 1 and 2, below.

Reviewer 1's comments:

Introductory comments

We thank the reviewer for the kind words and the very detailed and constructive review that (s)he provided. We do not repeat the entire text of the reviewer's comments below, but instead list the points where the reviewer has specifically asked for a clarification or a change to the manuscript, or otherwise has written something that we felt necessitated a reply.

Page and line numbers below refer to those in the revised version of the manuscript. Indented italicized text are quotes from the reviewer's comments, followed by our replies in unindented, non-italicized text.

...a number of previous studies have shown that larvae become more responsive to chemical cues as they age.

Yes, and we agree that these previous studies are directly relevant to our findings. We discuss these issues in the discussion starting on Page 24 Line 596 and on Page 27 Line 651 and provide several relevant references to this literature (refs. 62-65 in our reference list). We also have here added an additional reference (ref. 42) on this topic by Swanson and colleagues at the urging of Reviewer #2 (see below).

So here is the way I see the story here. In the absence of turbulence the larvae eventually become responsive to certain chemical cues. But turbulence can speed up that process, so that larvae become responsive (“competent to metamorphose”) to the chemical cues earlier. So in the absence of turbulence larvae will disperse farther and be more developed at metamorphosis, while in the presence of turbulence—if they are far enough along in development—they will
We thank the reviewer for this succinct and accurate summary, which comports well with the way that we frame and discuss our experiments in the sections starting on Page 4 Line 98, Page 21 Line 502 and Page 24 Line 581. We discuss the issue of inhibition starting on on Page 26 Line 645.

Below, we respond directly to the issue of “metamorphosis” per sé and definitions thereof.

The authors should define exactly what they mean by the terms competence, pre-competence, settlement, and metamorphosis. They describe “settlement” on pages 11 and 12 as the withdrawal of epithelial tissue from the tips of the larval arms. But isn’t this the start of “metamorphosis”? I suppose they could define “settlement” as the initiation of metamorphosis, but why not just talk about metamorphosis? To me, “settlement” just means that the larvae have stopped swimming and settled to the bottom of the container. Larvae will “settle” if you add formalin to the water! Moreover, “settlement” is reversible, while “metamorphosis” is not in these organisms, and that is an important distinction. I say more about the terms competence and pre-competence below.

We agree with the reviewer that these terms should be more precisely defined. We have now added a section to the Methods (see paragraph starting on Page 7 Line 159) where we offer precise definitions of the four terms listed above.

On the issue of metamorphosis, the definition of that term is not anywhere near universally agreed upon, as the paper by Bishop et al. 2006 (“What is Metamorphosis?” ICB 46: 655) –with its more than a dozen different definitions by as many authors– clearly demonstrates. One of us (Hodin) is a co-author on this paper. Hodin also has provided a more complete analysis of metamorphosis in general and in sea urchins specifically in a single-authored 2006 paper, which we have now also cited in this context in the manuscript (see new Ref. 25).

We note that while defining, as we do, metamorphosis in sea urchins as a longer term process (beginning with the first formation of juvenile structures) –and settlement as a stage of metamorphosis– is somewhat unorthodox, it does have precedent in the literature. We point specifically to the similar definitions of these terms (emphasis added) by the eminent invertebrate and larval biologist Fu Shiang Chia in his own article (pages 283-5) within his co-edited 1978 classic volume Settlement and Metamorphosis of Marine Invertebrate Larvae:

“Settlement, including attachment in some cases, refers to general behavioral and habitat changes, whereas metamorphosis denotes morphological and physiological changes. Settlement and metamorphosis usually occur in a relatively short period of time and neither is reversible. Many invertebrate larvae settle first and then metamorphose, others metamorphose first and then settle, and in others the two processes take place simultaneously.”

We would only note that there are some rare examples where settlement is reversible (e.g., see Reitzel et al. 2006, ICB 46: 827), but in broad terms we agree with Chia here, and, thus, respectfully disagree with the reviewer that formalin treated larvae are properly “settled” when they fall to the bottom of the dish. Furthermore, we consider sea urchin larvae an example of those that “metamorphose first and then settle” (see also Hodin 2006; new ref 25).
One difficulty in providing definitions for *precompetence* and *competence* is that we feel that our results suggest a rethinking of these terms, and we discuss this at some length on Pages 24-27. Therefore, what we have done is to outline what we consider the general consensus view of these terms for the purposes of the bulk of the manuscript (now specifically defined starting on Page 7 Line 166), and then offer our own definitions in the aforementioned Discussion section towards the end.

*Did the authors include a treatment in which the larvae were exposed only to the turbulence without being subsequently exposed to natural or artificial inducer? I didn’t see that, but it seems like an important control to include.*

We thank the reviewer for noticing this oversight. Yes, we have indeed confirmed that turbulence alone does not induce settlement in sand dollar larvae. As we have already published such data for purple sea urchins (see Ref. 16), we have now decided to add a note to this effect, and cite the identical control result for sand dollar larvae that the author seeks as “data not shown” (see Page 16 Line 392). We hope that this will suffice.

*Page 5 bottom (H1). “Increased responsiveness to turbulence” might be a little misleading, since it seems that the larvae are not metamorphosing in response to turbulence. Rather, turbulence is speeding up the onset of metamorphic competence.*

*Page 17, lines 13-23. Is it really “increased responsiveness to turbulence exposure,” or rather a speeding up of the onset of competence (i.e., responsiveness) to chemical cues?*

We again thank the reviewer for noticing this point which might indeed have aroused confusion in readers. We have now gone through the manuscript carefully and ensured that in each place that we mentioned this wording for H1, we made clear that turbulence is promoting competence, rather than settlement per sé. See, for example, our revised wording on Page 5 Lines 114 and 123, Page 14 Lines 332, 336 and 340, and Page 17 Line 415.

*Page 6, Line 54. Give a reference for “our prior work”.*

Done (now Page 6 Line 147) – ref. 16.

*P 21 top paragr. This just repeats material from the Introduction.*

This 8-line paragraph (which now begins on Page 21 Line 516) is indeed a summary of a much longer section in the intro (see Section 2.3 Pages 5-6), but we felt it important to remind the readers what H0, H1 and H2 entail in order to make the manuscript easier to read. Therefore, we would like to retain this paragraph as is.

*P 21 middle paragr. Discuss the ecological implications of this batch variability, particularly in terms of dispersal consequences.*

We welcome this suggestion, and provide a very brief discussion of this point on Page 22 beginning on Line 532.
P 22, lines 30-45. Do larvae “have the ability to adjust their turbulence responses during ontogeny,” or does turbulence just set something else in motion (competence to respond to chemical cues), once the larvae become responsive to turbulence?

We hold that the finding that responses change across ontogeny is an example of developmental phenotypic plasticity, which can be defined as an adjustment of responses across ontogeny as a result of environmental exposure. Therefore we would like to retain this wording, though we now provide a brief additional justification for it in the text (see Page 23 Line 562).

P 23 top. If the larvae metamorphosed in response to inducer after being exposed to turbulence, then the larvae were not “precompetent.” They were precompetent before the turbulence exposure, but then apparently became competent to respond to chemical cues (at a smaller size) through the turbulent experience. And they were “competent” to respond to the turbulence. This is an important point.

We thank the reviewer for identifying our confusing wording here, which we have now rectified (see Page 23 Line 574 – Page 24 Line 576). In our revised wording, we indicate that the younger larvae were precompetent before exposure to turbulence, whereas the older larvae tested had become competent in the absence of turbulence exposure. Furthermore, we looked carefully through the entire manuscript with this comment in mind to ensure that we avoided any such confusion throughout.

Conclusions (p 27). I don’t see how this research clarifies “a mechanistic understanding of what it means for larvae to be competent.”

We agree that our experiments do not fully clarify this issue, but we also note that we do not make such a claim for our study. We believe that the new definitions we provide (Pages 26-27) offer a more accurate and clear (and testable) conception of competence, which we believe will pave the way for a clearer mechanistic understanding of competence, an understanding that we feel has been impeded by a somewhat limited (and overly operational) definition of competence in the literature to date.

Lines 1-56: “precompetent larvae” OF A CERTAIN AGE; the larvae were not competent to respond to chemical cues before they experience turbulence, but they were competent to respond to the turbulence (by becoming competent to respond to chemical cues). This gets back to the importance of defining precisely what is meant by competence.

We appreciate the reviewer’s perspective here, but we feel that it would be far too confusing to use the term “competence” to refer both to the ability to settle AND to the ability to respond to turbulence to become competent to settle! This is why we advocate a more precise definition of “precompetence” as this latter stage: namely, the stage when larvae are able to respond to turbulence and become competent (see paragraph beginning on Page 26 Line 644).

We would also like to point out that although -in our study- the broader habitat scale cue is physical (turbulence) and the settlement cue itself is chemical (KCl or sand extract), there are also examples of localized settlement cues being physical in nature (light, surface roughness, sound, etc.) and even
perhaps of the broader, habitat scale cues being chemical (see Ref. 86).

Figure 3. Are all larvae from a single culture? That should be made clear.

Yes, all larvae in this experiment are from a single culture (batch), and we have now clarified this in the legend to Figure 3.

I had a lot of trouble reading Figure 4, in particular figuring out what the different symbols represented.

We apologize for the confusion here and have now provided a revised Figure 4 which we hope will be easier to interpret. We have adjusted the size of the circle symbols in all panels, and made the dashed lines/solid lines consistent across panels.

Reviewer 2's comments:

Page and line numbers below refer to those in the revised version of the manuscript. Indented italicized text are quotes from the reviewer’s comments, followed by our replies in unindented, non-italicized text.

The authors do not deal with experimental runs effectively—they should use mixed models to partition run effects.

The reviewer is correct that in the analyses of the data shown in Figure 4 we did not run a mixed model (with batch and runs nested within batch as random effects), and we have now done so in the data shown in Figure 5 and the new Figure 6. With respect to Figure 4 itself, we have decided to limit our analysis to the specific tests of H1 (Figure 5) and H2 (Figure 6), since those are the issues of interest in our data set, and not attempt any additional analyses of the data in Figure 4 per sé. Furthermore, to avoid confusion (and because these data are now shown more clearly and accurately in the new Figure 6; see below), we removed panel D in Figure 4 as originally submitted.

With respect to H1, we have now run a mixed model on the regression analysis (as described in the Methods on Page 13 Lines 331-345) and report the results in section 4.2.1 (starting on Page 17 Line 410) and Figure 5. The new analysis did not qualitatively affect our conclusions here.

With respect to H2, to address the reviewer’s concerns, we have decided to completely re-do this analysis of infection points using a generalized linear mixed model. As a result, we have removed what were previously Table 1 and the Supplemental figure, and replaced them with a new Figure 6.

Our first efforts in the re-analysis of the data to test hypothesis H2 (now Figure 6) involved trying to find the best-supported model among a series of possible parameters. We employed a “forward selection” methodology here, adding parameters (including the random effects listed above) and checking the Akaike information criterion (AIC) score each time to find the model with lowest score, and hence the best-fit to the data (Akaike 1978, now referenced in our manuscript as Ref. 31). We
outline our model selection process and the various AIC scores of the models that we tested in the Methods on Page 15 Line 347 – Page 16 Line 356.

The resulting best-supported model was a logistic model with random effects, as well as a quadratic term for turbulence intensity to approximate the drop off in settlement that we see at high turbulence intensities (see Figures 4 and 6), and which substantially improved the AIC score (465.7 with the quadratic term versus 481.8 without). Adding in interaction terms did not improve AIC scores, so we left them out of the final model. All of this is now outlined in the Methods as noted above.

Using this best-supported model, we generated best-fit curves for day 9 and day 11 across our range of tested turbulence intensities, along with 95% confidence interval (CI) curves surrounding the best-fit curves. We then ran a non-parametric bootstrap analysis for 10,000 iterations (with the random effects included), resulting in 10,000 inflection point estimates for day 9 and day 11. We used these data to estimate inflection points with 95% CI for our day 9 and day 11 data, and estimated the P value based on the maximum range (% CI) of non-overlapping inflection point estimates (see our revised Methods, Page 15 Lines 362-368). The outcome of this more thorough analysis (when compared to our previously submitted version of the manuscript) is a result that is qualitatively similar to what we previously reported: borderline support for H2.

We describe this new analysis in section 4.2.2 of the Results (beginning on Page 17 Line 421), in the Discussion (Page 22 Lines 527-550) and in the legend to the new Figure 6, where we present the results of this new analysis.

We note that the data shown in Figure 3 and what are now Figures 7 and 8 (formerly Figures 6 and 7, respectively) were all done on a single batch (with replication) and so mixed models would not be appropriate for these data.

As we note above, in the revised manuscript, we no longer report any analyses on the data shown in Figure 4 per sé, which we feel has been superseded by our now more complete analyses on the data shown in Figures 5 and 6.

The logistic regression calculation of inflection points is particularly muddled - these results cannot be trusted at all. Instead the authors must use logistic ANCOVA to determine whether there is any difference in slope or intercept rather than the indirect and inappropriate approach they have used here.

We agree with the reviewer’s comment here and apologize for the confusing nature of our previous analysis. As detailed above, we have addressed this concern by completely re-working our analysis of H2, shown now in Figure 6 and described in the text.

I suggest the authors consider including the work of Emlet on urchin size affecting post-metamorphic survival and I also suggest they include another paper by Swanson, showing increased sensitivity to histamine as larvae age.

We thank the reviewer for this suggestion and have added the suggested additional references as reference numbers 42 and 58-59, cited now on pages 20, 23 and 24.