

A single heat-stress bout induces rapid and prolonged heat acclimation in the California mussel, *Mytilus californianus*

Nicole E. Moyen, Rachel L. Crane, George N. Somero and Mark W. Denny

Article citation details

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Review timeline

Original submission: 13 October 2020
Revised submission: 16 November 2020
Final acceptance: 17 November 2020

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

Review History

RSPB-2020-2561.R0 (Original submission)

Review form: Reviewer 1

Recommendation

Accept with minor revision (please list in comments)

Scientific importance: Is the manuscript an original and important contribution to its field?
Excellent

General interest: Is the paper of sufficient general interest?
Excellent

Quality of the paper: Is the overall quality of the paper suitable?
Excellent

Is the length of the paper justified?
Yes

Should the paper be seen by a specialist statistical reviewer?
No

Do you have any concerns about statistical analyses in this paper? If so, please specify them explicitly in your report.

No

It is a condition of publication that authors make their supporting data, code and materials available - either as supplementary material or hosted in an external repository. Please rate, if applicable, the supporting data on the following criteria.

Is it accessible?

Yes

Is it clear?

Yes

Is it adequate?

Yes

Do you have any ethical concerns with this paper?

No

Comments to the Author

This manuscript presents a well-conducted study of heat hardening in the intertidal mussel *Mytilus* on the California coast. The results of the study are highly relevant for making accurate predictions about the effects of heat waves on mussel populations. Additionally, the study presents an important lesson for any researchers interested in heat hardening, namely that there can be significant variation in heat hardening phenology depending on initial heat exposure.

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at line 135. I suggest that the authors move the intervening paragraph (starting at line 113) to the discussion as this paragraph better fits a justification for the study design in terms of interpretation of results than a description of how the study was conducted.

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In the conclusions I'd like to challenge the authors to go a bit broader in terms of the importance of this study for considering the best ways in which heat hardening should be accounted for in experimental work and in making inferences about responses of populations to heat waves and climate change. The work, as presented, has implications more broadly than mussels alone.

Review form: Reviewer 2 (Urtzi Enriquez-Urzelai)

Recommendation

Accept with minor revision (please list in comments)

Scientific importance: Is the manuscript an original and important contribution to its field?

Good

General interest: Is the paper of sufficient general interest?

Excellent

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Overall, this is a beautiful piece of research. The general topic and research question are very timely – of interest for researchers in many fields – and clearly explained in the manuscript. The experimental design and statistical analyses are appropriate and allows the authors to answer the question being posed. However, I would like the authors to clarify a few points (all of them minor):

1) The order in which experimental groups (and sublethal temperatures) were tested should be better explained. Most experimental procedures are sufficiently explained in the text, but it is important to clarify how individual mussels were assigned to experimental groups. Apparently, the authors collected mussels from January to June. If I understand correctly, this means that they collected experimental groups of ~20 individuals gradually along those months. I am sure that the authors did not assign all the early groups to one sublethal temperature treatment (e.g. 25°C) and all late groups to another treatment (e.g. 35°C): this would have blurred results with potential seasonal acclimation from January to June. The authors should clarify how groups were assigned and the order of heat-stress bouts, to evaluate that a similar number of groups were tested in each treatment across the season.

2) To account for body size variation, the authors only collected mussels of 51-82 mm shell-length. It would be good to show that there were no differences in size (mass or shell-length) between experimental groups. Systematic, involuntary bias (e.g. assigning the biggest individuals to one treatment) could have a profound effect in results, if body size influences survival probability. Also related to this, is it a way of including body size as covariate in your analyses? Chi-square tests and Kaplan-Meier survival curves might not allow including covariates. As an alternative for Kaplan-Meier, Cox-proportional hazard models (CoxPH models) allow to test for the effects of covariates, such as body size. However, if there are no differences in size between groups, these alternative analyses might yield the same results to those presented by the authors. Thus, I would suggest the authors to test for differences in size between groups.

3) In lines 246-248 the authors state that “the rapid heat acclimation response that [they] found in [their] study is contrary to previous work in mussels that assumed heat acclimation (in the sense of improved heat tolerance) takes upwards of two weeks to be completed.” I guess that that depends on how one defines acclimation. Many traits (e.g. critical thermal limits in amphibians) take several days to weeks to reach stable values when exposed to a constant temperature, or to reach similar values (e.g. metabolic rates) between temperatures. This makes sense because animals are not constantly “acclimatizing” to their thermal surroundings, but they might rather be acclimated to the median temperature (or such a thing). In the case of the present study, the authors test for the delayed effects of an acute heat stress in heat tolerance which might involve acute responses. The authors show that these responses (e.g. cellular stress response) might last for a considerable period, and they term this acclimation. I am not completely convinced of this response being “acclimation”, but I might be wrong.

4) It would be nice to be able to view a plot of field temperature data without having to search for it in a webpage.

Decision letter (RSPB-2020-2561.R0)

11-Nov-2020

Dear Dr Moyen

I am pleased to inform you that your Review manuscript RSPB-2020-2561 entitled "A single heat stress bout induces rapid and prolonged heat acclimation in the California mussel, *Mytilus californianus*" has been accepted for publication in Proceedings B.

The referee(s) do not recommend any further changes. Therefore, please proof-read your manuscript carefully and upload your final files for publication. Because the schedule for publication is very tight, it is a condition of publication that you submit the revised version of your manuscript within 7 days. If you do not think you will be able to meet this date please let me know immediately.

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All supplementary materials accompanying an accepted article will be treated as in their final form. They will be published alongside the paper on the journal website and posted on the online figshare repository. Files on figshare will be made available approximately one week before the accompanying article so that the supplementary material can be attributed a unique DOI. Please see: <https://royalsociety.org/journals/authors/author-guidelines/>

4) Data-Sharing and data citation

It is a condition of publication that data supporting your paper are made available. Data should be made available either in the electronic supplementary material or through an appropriate repository. Details of how to access data should be included in your paper. Please see <https://royalsociety.org/journals/ethics-policies/data-sharing-mining/> for more details.

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Once again, thank you for submitting your manuscript to Proceedings B and I look forward to receiving your final version. If you have any questions at all, please do not hesitate to get in touch.

Sincerely,
Dr Daniel Costa
<mailto:proceedingsb@royalsociety.org>

Associate Editor Board Member: 1

Comments to Author:

I have now received comments from two specialists from this field. Both of them think that the study is interesting, the topic is timely, and the paper was well written. Nonetheless, they raised some minor concerns that the authors may want to fix before publication.

Reviewer(s)' Comments to Author:

Referee: 1

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You can expect to receive a proof of your article from our Production office in due course, please check your spam filter if you do not receive it. PLEASE NOTE: you will be given the exact page length of your paper which may be different from the estimation from Editorial and you may be asked to reduce your paper if it goes over the 10 page limit.

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Thank you for your fine contribution. On behalf of the Editors of the Proceedings B, we look forward to your continued contributions to the Journal.

Sincerely,

Editor, Proceedings B

mailto: proceedingsb@royalsociety.org