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Interactive comment on "Functional spatial contextualisation of the effects of multiple stressors in marine bivalves" *by* Antonio Giacoletti and Gianluca Sarà

Anonymous Referee #2

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This study integrates laboratory-derived parameters of mussel metabolism and assimilation efficiency to run DEB models testing the effect of pH and hypoxia, using environmental data input (temperature, food) from two sites within the mussel's biogeographic range. I appreciate the approach of introducing hypoxia events (although I have a comment on the design these events) as a means of incorporating environmental variability in the model. This literature is sparse with such perspectives, especially in the context of multiple stressors. However, the paper lacks a perspective of the environmental relevance of the experimental design and modeling.

Major comments

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- Methods need much more detail (see detailed comments)

- Physiological condition of experimental mussels during the experiment is not quantified. Feeding was ad libitum and it was not assessed if mussels were being fed at conditions of either site used for the modeling. This is problematic as, for example, if the mussels are starving relative to their natural food supply, the derived experimental parameters for the DEB model may be inappropriate. The authors also do not explain the experimental design. Why was the experiment 4 weeks?

- DEB models: As a reader of Biogeosciences, but not an expert on DEB models, it would be helpful if the authors reviewed this approach more clearly (perhaps using a schematic, what program is used to run models, table of input variables etc.). After reading the paper, I am unclear about the exact implementation and conclusions that can be drawn from this simulation based on the following 4 sources of confusion:

1) From what I understand, temperature from 2006-2009 is used as one of the model inputs. However, all the biological parameters taken from the experiments come from 21 degr. C (although this is not stated explicitly for respiration, but I assume it's 21 C). Since environmental data would vary in terms of temperature across the four years, I don't understand how biological performance is scaled across this temperature regime. It would be good to include a figure of the environmental data (means are not great time-series descriptors, especially for biological processes that are seasonal, such as reproduction), as well as a figure on how the biological parameters were scaled for temperature effects over the years. This same argument applies to food concentration (which I assume varies by time of year as well).

2) The authors use hypoxia and acidification, two future stressors, with temperature data from a few years ago. This design ignores the fact that warming is currently the dominant stressors for this species in the Mediterranean Sea and is expected to continue in the future. As the environmental relevance of the study design is not discussed, as written, the results do not match any realistic environmental situation. This counters

the original intent of using DEB models to better “predict organismal functional traits, capturing variation across species to solve a very wide range of problems in ecology and evolutionary biology” (L58).

3) Given that reproduction of this species can be quite seasonal, how does the DEB model handle this in terms of estimating reproductive output?

4) L188-189: Why is the hypoxia event randomized by month of the year? Hypoxia would most likely occur during summer warming and stratification. It seems that varying the duration of summertime hypoxia is a more environmentally relevant exercise rather than randomizing what month the hypoxia event occurred in. How long was each hypoxia event?

- Statistical approach needs to be justified (see detailed comments)

- The choice of using 2500 umol/kg for total alkalinity (TA) based on an oceanographic study for the lab experiments is strange (L106). Especially in static cultures, mussels can alter the TA of a small body of water. I assume the authors did not measure TA during the experiment. In such a case, it would be best to simulate the experiment again, and measure TA so the authors have some idea of the TA variation in their experimental conditions could have been. Either way, the calculated pCO2 parameters will be undefinable without the real TA measurement.

- In addition to lacking an environmental context, the Discussion lacks comments on the non-DEB model functional traits (shell strength, dissolution patterns), their relevance to the study, and by what mechanism hypoxia and pH would differentially or synergistically impact the periostracum and shell quality.

Detailed comments:

The title does not represent the study and reads as if the paper is a literature review. It would behoove the authors include more detail in the title (DEB model, hypoxia, OA). Use of “marine bivalves” is inappropriate given that only one species was assessed.

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L27-33: sentence is difficult to follow. Consider breaking this up.

L39: this needs clarification (most functional traits? Which ones?) and references

L47-48: plenty of labs conduct OA experiments for months up to at least one year

L48-54: long sentence, CO2 vents are unrelated to the second half of the sentence. Consider rewriting.

L69: should AE be 'assimilation efficiency'?

Introduction: break text up into paragraphs. Lacks introduction to functional trait-based models; I was expecting this prior to L54.

L92: Mussels were fed ad libitum, but this is an energetics study. So how do the authors know the condition of the mussels used to get model parameters?

L104: dissolution threshold relates to calcium carbonate saturation state, please include the value here, rather than pH.

L109: how was CO2 dissolved? Where their pumps in all the aquaria to ensure mixing?

Section 2.1: Sampling of what (section title)? How often was water replaced in the treatment tanks? Methods need a better description of how carbonate chemistry was calculated. What was the accuracy of the pH measurements? How often was the water sampled for each of the four parameters? How was oxygen maintained and measured in the treatments?

Section 2.2 (L112-120): if there are 25 mussels per tank, and there are 3 tanks, why are only five mussels observed for valve opening and closure? Why were observations made 6 times per day every week rather than fewer times per day but more frequently throughout the experiment? Does time of day matter for this behavior? What about time that food was added? I image that flow rates could affect this behavior, but it's unclear if water motion was the same across all tanks.

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L124: define [pm]

L134: this equation results in units of O2 concentration x volume per unit time, oxygen units are not defined and there is no explanation as to how this is converted to [pm], which is in J per cubic cm per hour. What level of oxygen undersaturation was reached by the end of the incubation?

L140: this assumption should be justified

Section 2.3: explain that the same individual was used for the respiration rate followed by AE. It's unclear until the end of section 2.4. Given that the respiration methods continue in the end of Section 2.4, merge Section 2.3 and 2.4.

L141-145: Please explain why AE experiment was not done in treatment water, and justify how AE can then be related to experimental treatments.

L162: Again, if food availability is important at the field sites, food availability during the experiment should be known. Is it closer to that of Trieste or Palermo?

Section 2.7: How are simulations performed (what code or computer program?)?

L180: State what DEB parameters these are.

L181: Is AE the same as [pM]? AE was already defined in the Introduction

L185-186: Was this data from week 1 or 4? How is a 4-week acclimation period determined sufficient enough to extrapolate to 4 years?

Section 2.9: The assumption of normally distributed residuals is not tested for the ANOVA. This needs to be done before moving forward with ANOVAs. A sample size of 16 is not large. The statistical analyses for valve closure does not match the data collection. By using ANOVAs, I assume all the data are pooled across the 4 weeks. This is not appropriate because it does not account for acclimation and it is a repeated measure since there are only 25 mussels in each tank which were observed over 4 weeks. ANOVAs also don't control for the tank replicate per treatment. The authors

need to clarify how the data was pooled (and which behavior was analyzed – open or closed). Since this is binary data, reporting both in the bar graph is duplication the data (Section 3.2), report one, or as a stacked bar graph where each bar graph fills 100%.

Section 3.1: Analysis comparing experimental treatments seems unnecessary, especially given the uncertainty of the calculated parameters using a poor assumption of TA.

L350-352: is this to be expected?

Figures: What is the error bar?

L259: capital I

L261: replace M&M with Section #

Table 1: Include temperature

Table 5: include input parameters

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