

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

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Sorry we wrongly sent the reply for REF 1. There's the reply for you:

REVIEWER #2 Reviewer wrote: 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 pa-

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per lacks a perspective of the environmental relevance of the experimental design and modelling. Author's reply: We thank the Reviewer #2 for helping us improving the readability and the clearness of the ms. Author's changes: In doing so we applied most of the suggestion for the highlighted points, and we discussed them through the specific comments. A perspective of environmental relevance on modelling outputs has been currently added in the discussion section following what suggested by the reviewer.

Major comments Methods need much more detail (see detailed comments) Reviewer wrote: 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? Author's reply: We thank the reviewer for highlighting this point. Author's changes: We estimated the condition index through the biometric available data and compared it through the experiment, resulting in a not significant variation throughout the study period, and this supports that experimental animals were not stressed by starvation. Further, we used the locution "ad libitum" to indicate a food concentration saturating the feeding processes of animals over time. Such an experimental maintenance condition is commonly used throughout the current literature when bivalves are maintained with ad libitum condition of food in bioenergetic experiments (e.g. Sarà et al., 2013; Montalto et al., 2014; Tagliarolo et al., 2016). However, we adopted a four weeks-period to estimate the effect of OA on functional traits of mussels; such a period is judged to be enough to allow mussels to acclimate to new conditions, as showed in many experimental papers across the current literature (references added in the manuscript).

Reviewer wrote: 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 vari-

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ables 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). Author's reply: The 2006-2009 thermal series has been used as a forcing variables inside DEB models in current modelling literature (Sarà et al. 2011, 2013b; Montalto et al. 2014). The most important factors driving changes in energy budgets of ectotherms are body temperature, on which every metabolic rates depends via the Arrhenius relationships (Kooijman 2010), and food. Arrhenius temperature, that is species specific, acts as a correction factors inside DEB models to scale all rates to environmental temperature. At the same time DEB models use the 2006-2009 CHL-a series as a second forcing variables to predict LH-traits of our species. Accordingly, including a new figure of environmental data could make the ms. heavier also as the main object is not to contextualise the effect at that period, but to show that stressor's effect is simulated across a long integrated period.

Reviewer wrote: 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 or-

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ganismal functional traits, capturing variation across species to solve a very wide range of problems in ecology and evolutionary biology” (L58). Author’s reply: We did not test the effect of increasing temperature as we are pretty sure that the thermal effect is not manifested on a period so short (only 4-years); however other companion papers (e.g. Montalto et al. 2017) tested the effect of increasing temperature on mussel’s performances throughout the whole Basin. To extrapolate the effect only two stressors, we carried out simulations under two different latitudinal temperature patterns (Trieste, north Adriatic and Palermo, Southern MED). Anyway, we included some discussion lines about this issue.

Reviewer wrote: 3) Given that reproduction of this species can be quite seasonal, how does the DEB model handle this in terms of estimating reproductive output? Author’s reply: DEB manages the seasonal reproduction throughout thresholds based on the temperature-energy relationships.

Reviewer wrote: 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? Author’s reply: As we said before, we tested the effect of hypoxia as a stochastic event more than testing is in terms of frequency and timing. Thus, here we adopted a very simple scheme but there is a companion paper still under review (G. Sarà submitted PRS B) whose main aim was to test the effect of duration, frequency and timing of disturbance events on three different invertebrate species through the DEB model.

Reviewer wrote: Statistical approach needs to be justified (see detailed comments) Author’s reply: Statistical approach has been justified in the detailed comments.

Reviewer wrote: The choice of using 2500  $\mu\text{mol/kg}$  for total alkalinity (TA) based on an oceanographic study for the lab experiments is strange (L106). Especially in static

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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 pCO<sub>2</sub> parameters will be undefinable without the real TA measurement. Author's reply: We did not find any paper reporting such alteration. We tried at the same time to minimize the number of organism for each tank and to perform a sufficient weekly water change in order to maintain a stable environment for our organisms, even if in mesocosm condition. We are perfectly aware that the suggested one is without any reasonable doubt the most appropriate approach to follow, but as soon as our is not a study focused on the chemistry of the shell but it is to provide a proof to test the predictive potential of DEB model about the effect of two stressors; thus, the use of a value from oceanographic study should be considered a minor approximation due to the metabolic and mechanistic nature of the paper.

Reviewer wrote: 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. Author's reply: we didn't analyse the impact on the shell chemistry and ultrastructure in this ms. whose main objective was to predict the effect of two stressors on mussel's LHs. Author's changes: However, to accomplish the referee's suggestion, we discussed shortly shell fragility related to pH and to both combined stressor.

Detailed comments: Reviewer #2 specific comment n. 1 Reviewer wrote: The title does not represent the study and reads as if the paper is a literature review. It would behove 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. Author's changes: We agreed with Reviewer's #2 point and changed the title.

Reviewer #2 specific comment n. 2 Reviewer wrote: L27-33: sentence is difficult to

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follow. Consider breaking this up. Author's changes: Sentence has been spitted up accordingly.

Reviewer #2 specific comment n. 3 Reviewer wrote: L39: this needs clarification (most functional traits? Which ones?) and references Author's changes: Although references were already present, following Reviewer #2 suggestion we specified the functional traits which we were referring to.

Reviewer #2 specific comment n. 4 Reviewer wrote: L47-48: plenty of labs conduct OA experiments for months up to at least one year Author's changes: The sentence has been deleted.

Reviewer #2 specific comment n. 5 Reviewer wrote: L48-54: long sentence, CO2 vents are unrelated to the second half of the sentence. Consider rewriting. Author's changes: Sentence has been rephrased accordingly.

Reviewer #2 specific comment n. 6 Reviewer wrote: L69: should AE be 'assimilation efficiency'? Author's changes: Sentence has been rephrased and clarified.

Reviewer #2 specific comment n. 7 Reviewer wrote: Introduction: break text up into paragraphs. Lacks introduction to functional trait-based models; I was expecting this prior to L54. Author's changes: Introduction was spitted into paragraphs following suggestion and an introduction to functional trait-based models was added.

Reviewer #2 specific comment n. 8 Reviewer wrote: 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? Author's reply: As in reply to a similar point raised by Rev#1 we used the locution "ad libitum" to indicate a food concentration saturating the feeding processes of animals over time. Such an experimental maintenance condition is commonly used throughout the current literature when bivalves are maintained with ad libitum condition of food in bioenergetic experiments (e.g. Sarà et al., 2013; Montalto et al., 2014; Tagliarolo et al., 2016).

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Reviewer #2 specific comment n. 9 Reviewer wrote: L104: dissolution threshold relates to calcium carbonate saturation state, please include the value here, rather than pH. Author's reply: Unfortunately we do not have such details on calcium carbonate saturation state. We added image details showing the effect of OA on the external shell layer, but a deep analysis on the chemical composition and alteration was out of the purpose of the present investigation.

Reviewer #2 specific comment n. 10 Reviewer wrote: L109: how was CO<sub>2</sub> dissolved? Where their pumps in all the aquaria to ensure mixing? Author's changes: Details on how CO<sub>2</sub> was dissolved and of water recirculation were added.

Reviewer #2 specific comment n. 11 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? Author's changes: Section title has been changed accordingly. Details on water changes, aeration, water recirculation and accuracy of pH measurements has been added, while details on the calculation of water chemistry were already present in section 2.1. Further details on sampling of water (8 time a day for pH), and on frequency of oxygen and temperature measurement has been added in the same section.

Reviewer #2 specific comment n. 12 Reviewer wrote: 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 imagine that flow rates could affect this behavior, but it's unclear if water motion was the same across all tanks. Author's reply: We thank the referee for his/her interest on the behavioural part of our paper. The restricted number of observation was chosen in order to make the behavioural session during as less as possible in order to not influence

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with the operator presence mussel's behaviour. Even if it has not proven sometimes mussels suddenly close when moving in front of the tanks. For this reason we decided to observe less individual but more frequently. We didn't notice any difference in the behaviour during the day as mussels were inside a temperature-controlled room, under constant flow through conditions (according to Widdows and Staff 2006 and Sarà et al. 2013) and exposed to automated artificial daylight. The food was added at the end of the day, after all observations were made.

Reviewer #2 specific comment n. 13 Reviewer wrote: L124: define [pm] Author's changes: [áŹŪM] has been here defined following suggestion.

Reviewer #2 specific comment n. 14 Reviewer wrote: L134: this equation results in units of O<sub>2</sub> 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? Author's reply: Oxygen concentration ( $\mu\text{mol l}^{-1} \text{h}^{-1}$ ) were first converted in J h<sup>-1</sup> and then in J cm<sup>-3</sup> h<sup>-1</sup> using a conversion factor (Kooijman, 2010) and following the current literature (Van der Veer et al., 2006; Ren and Schiel 2008). We never reached lethal oxygen concentration due to the short interval of the measurement as the idea was to simulate a sub-lethal effect as that reach at about 1.5-2.0 mg-l DO.

Reviewer #2 specific comment n. 15 Reviewer wrote: L140: this assumption should be justified Author's changes: As soon as the first two sentences of the section has the same reference, we moved it at the end of the second sentence.

Reviewer #2 specific comment n. 16 Reviewer wrote: 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. Author's reply: As answered to Reviewer #1 we do not agree in merging both sections because they represent two different part of metabolic stuff (feeding and respiration). However, we now clear specified that specimens were

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the same between both measure following Reviewer's #2 suggestion.

Reviewer #2 specific comment n. 17 Reviewer wrote: L141-145: Please explain why AE experiment was not done in treatment water, and justify how AE can then be related to experimental treatments. Author's changes: We now clearly specified that the experiment was conducted with water specifically treated for each treatment.

Reviewer #2 specific comment n. 18 Reviewer wrote: 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? Author's reply: As in reply to a similar point raised by Rev#1 we used the locution "ad libitum" to indicate a food concentration saturating the feeding processes of animals over time. Such an experimental maintenance condition is commonly used throughout the current literature when bivalves are maintained with ad libitum condition of food in bioenergetic experiments (e.g. Sarà et al., 2013; Montalto et al., 2014; Tagliarolo et al., 2016).

Reviewer #2 specific comment n. 19 Reviewer wrote: Section 2.7: How are simulations performed (what code or computer program?)? Author's reply: Our simulation were performed using R routine, and we specified it in the m accordingly.

Reviewer #2 specific comment n. 20 Reviewer wrote: L180: State what DEB parameters these are. Author's changes:: DEB parameters are now reported in Table 1.

Reviewer #2 specific comment n. 21 Reviewer wrote: L181: Is AE the same as [pM]? AE was already defined in the Introduction Author's changes: According to Reviewer's #1 point we checked and fixed both assimilation efficiency and the somatic maintenance costs definition.

Reviewer #2 specific comment n. 22 Reviewer wrote: 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? Author's reply: The [áZÜM] parameter used inside our simulations was that calculated at week #4. All the species specific parameters derived from

one species and freely available online on the Add my pet collection were previously determined either by the covariation method through data present on literature or experimentally by short experimental sessions. Even without considering the effect of a stressor, a parameter estimated for a well-fed organism is then used inside simulation making predictions up to 4, 10 or even 50 years, as parameters are assumed to be specific for each species (Kooijman, 2010). We did not account any possible evolutionary effect whose effect is still far to be assessed in the DEB theory.

Reviewer #2 specific comment n. 23 Reviewer wrote: 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%. Author's reply: The assumption of normal distribution has been tested through the Anderson–Darling test using Past<sup>®</sup> software. We are aware that the sample size is not large, but pooling the six observation per week we obtain a sample of 24, and we believe it is sufficient for the purpose of the present paper. Author's changes: We repeated the data analysis and accordingly to the suggestion we compared week1 and week4 using two levels of the factor time and 4 levels of the factor treatment. We analysed the open valve behaviour and following suggestion we decided to use only one category in the graph. The paragraph, the table and the figure has been modified accordingly.

Reviewer #2 specific comment n. 24 Reviewer wrote: Section 3.1: Analysis comparing experimental treatments seems unnecessary, especially given the uncertainty of

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the calculated parameters using a poor assumption of TA. Author's changes: Analysis comparing experimental treatments were removed accordingly to both referee's suggestions.

Reviewer #2 specific comment n. 25 Reviewer wrote: L350-352: is this to be expected? Author's reply: We thank the referee for the question and we have already answered to this point being highlighted by Referee#1. *M. galloprovincialis* in Sicily is observed to be limited by oligo-trophic conditions although it grows in highly trophic-enriched areas such as harbours or under Integrated Multi-Trophic Aquaculture (IMTA) conditions (Sarà et al 2012; 2013b, Giacoletti et al. 2018 in press JEMA) which supports what we gathered in the present ms. through the DEB simulations.

Reviewer #2 specific comment n. 26 Reviewer wrote: Figures: What is the error bar? Author's reply: The error bar indicated standard errors for means. Author's changes: Details were added in each figure following Reviewer's suggestions.

Reviewer #2 specific comment n. 27 Reviewer wrote: L259: capital I Author's changes:: Replaced.

Reviewer #2 specific comment n. 28 Reviewer wrote: L261: replace M&M with Section # Author's changes:: Replaced accordingly.

Reviewer #2 specific comment n. 29 Reviewer wrote: Table 1: Include temperature Author's changes: Temperature included inside Table 1.

Reviewer #2 specific comment n. 30 Reviewer wrote: Table 5: include input parameters Author's changes: DEB parameters were included in Table 1.

Please also note the supplement to this comment:

<https://www.biogeosciences-discuss.net/bg-2018-13/bg-2018-13-AC3-supplement.pdf>

Interactive comment on Biogeosciences Discuss., <https://doi.org/10.5194/bg-2018-13>, 2018.

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