

## Responses to Editor #1's comments

First of all, thank you for all your comments that greatly helped us in improving our manuscript. Below are our responses. We have revised and re-submitted our manuscript based upon all the comments. Most of the previous figures have also been revised based on the following editor's comments we received just after submitting our previous manuscript:

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*For "Figure 2" and "Figure 3": Please ensure that the colour schemes used in your maps and charts allow readers with colour vision deficiencies to correctly interpret your findings. Please check your figures using the Coblis – Color Blindness Simulator (<https://www.color-blindness.com/coblis-color-blindness-simulator/>) and revise the colour schemes accordingly.*

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## Responses to general comments

*Fujii et al. have aimed to characterise the chemical parameters of two coastal regions important for aquaculture in Japan. They have then used this data to model future scenarios, they then use existing published research on oysters to speculate how oysters and aquaculture may be affected.*

*Overall this is a valuable piece of work because such descriptions of coastal habitats are lacking, yes these are the most productive marine aquaculture environments. This work would have been strengthened by experiments on oyster larvae replicating their modelled conditions, however, I understand that this would have included more work that might not have been feasible.*

*I raise this point because the manuscript currently relies on Waldbusser et al. to provide a critical  $\Omega_{arg}$  limit for larval survival, however, that work was completed in the USA, where local oyster genotypes are likely to differ significantly compared to those found in Japan. I think the levels of local adaptation to environments (especially when using wild oysters rather than selectively bred stock) should not be underestimated. There are many examples in the literature of physiological differences among genotypes of oysters, especially when sourced from different continents. I think the manuscript would benefit from more discussion regarding the biological relevance of the very comprehensive chemical measurements.*

→ (EC1-1) Thank you very much for all the informative comments. The authors have thoroughly discussed about the issue that our results regarding impacts of ocean acidification on Pacific oyster larvae might overestimate the reality in Japan coasts. As the editor pointed out, we used a threshold based on rearing experiments conducted in Oregon, USA, where Pacific oysters are not native and environmental conditions are considered to be different from Japan coasts. We have mentioned about this issue in Sections 2.5 (in Lines 242-246) and 4.3 (in Lines 398-402) in the revised manuscript).

## Responses to specific comments

L306-307; Salinity results are stated to have “decreased” following rainfall. It would be good to describe here the extent that salinity decreased and for how long after significant rain. Furthermore, rainfall data is included in the figures. I suggest a statistical test to show the relationship between rainfall and salinity change, perhaps the two are not even correlated?

L309; how do you know that sites H-1, H-2 and S-1 were more affected by riverine flow? Please provide a statistical justification here.

→ (EC1-2, EC1-3) Based on the editor's and Reviewer #2's comments, the authors have rewritten the entire paragraph (Lines 266-280 in the revised manuscript). We tried to find statically significant relationships between salinity and rainfall, but could not find any. In particular, the difference in the salinity between the Hinase sites was not clearly elucidated, and we have removed the statement from the manuscript. While we still kept and have added some statements about the possibility of extremely low salinity caused by heavy rainfall by referring the timing of heavy rainfall and appearance of extremely low salinity at the Hinase sites and S-1 in the Shizugawa Bay, we made sure to mention that the relationship between the salinity and rainfall was not statistically significant.

L325; replace "downward"

→ (EC1-4) The authors have modified the phrase as: "*Abrupt drawdown of estimated DIC were sometimes found,*" (in Line 300 in the revised manuscript).

L336; as mentioned above, beware of placing too much emphasis on the Waldbusser results when your own study system and organisms are likely very different. I suggest addressing this in the discussion.

→ (EC1-5) The authors have set a section in the Methods part ("*2.5 Thresholds for evaluating the impacts on Pacific oysters (C. gigas)*"), and have moved all the statements about thresholds to this section. We have clarified that the Waldbusser et al. (2015)'s results used in this study were obtained from rearing experiments performed in Oregon, USA, of which Pacific oyster species and reactions to local environments may be different from those in Japan coasts as well (in Lines 243-245 in the revised manuscript).

L337; replace "able" with "likely"

→ (EC1-6) The word "able" has been replaced with "likely" (in Line 246 in the revised manuscript).

L347; this explanation belongs in the discussion not results.

→ (EC1-7) This explanation has been moved to Discussion section (4.3) as follows (Lines 407-409 in the revised manuscript): "*Also, considering that our current model underestimated observed sudden decreases in salinity as mentioned in 3.2, more realistic input data of freshwater from rainfall and riverine water would be necessary for better model performance.*"

L385; Considering that Oizumi et al., is not a widely available resource, the authors should include a description of how these estimates were made in the methods section, and then provide results on these estimates in the results section.

→ (EC1-8) Following the editor's comment, the authors have added a section in Methods part ("*2.5 Thresholds for evaluating the impacts on Pacific oysters (C. gigas)*"), and have explained how we applied the relation between the water temperature and spawning period of Pacific oysters obtained by Oizumi et al. (1971) in detail (Lines 236-241 in the revised manuscript). The relevant results have also been described in Results and Discussion sections (in Lines 261-265, 309-310, 323-328, and 365-372).

L400-406; This content and information belongs in the results section (not discussion) if it isn't already there.

→ (EC1-9) The authors have added the description of model results to Results section (in Lines 323-328 and 341-342 in the revised manuscript).

A figure here such as bar chart could be useful to display the mean increased/altered timing of reproduction among sites - this could replace one of the figures displaying measured parameters (these are less important in my opinion).

→ (EC1-10) The authors have replaced with a new figure (Fig. 16 in the revised manuscript).

L414; Reported by who? Please state if this is anecdotal evidence (i.e. oyster farmers) or your own observations.

→ (EC1-11) The statement was a bit exaggerated. The authors meant to say that there has been no anecdotal evidence of impacts of ocean acidification on Pacific oyster larvae found in Japan coasts to date. Therefore, we have revised the statement to: *“there has been no anecdotal evidence of impacts of ocean acidification on Pacific oyster larvae found in Japan coasts to date.”* (in Lines 430-431 in the revised manuscript).

I also think that is not sufficient to use the absence of any morphological abnormalities (as found in your study) to report no effects on oysters. Significantly reduced larval supply could still have been a result, with abnormal larvae dying before your samples are taken. I think this limitation needs to be considered. Larval supply in oysters is a very difficult (almost impossible) thing to measure, however the use of settlement plates might have given a better indication of oyster recruitment.

→ (EC1-12) The authors felt that this is the weakest point of the study, so a major revision has been done to address this issue. We have developed further the explanation and discussion in the revised manuscript, especially in newly introduced Section 4.3 (*“Thresholds for impacts of ocean acidification on Pacific oysters in Japan coasts”*) to take into consideration the editor’s helpful comments. In the two study sites, we did not use settlement plates but scallop shells to enhance oyster recruitment. We have also discussed about the possibility of larvae escaping from low salinity (and low  $\Omega_{\text{arag}}$ ) waters (in Lines 403-407 in the revised manuscript). Although it does not seem that abnormal larvae existed as many were captured by plankton nets, we have also mentioned about the possibility of our failure to collect abnormal larvae samples for the reason that they died before our samples were taken (in Lines 394-397).

L420; There is some potential for local mitigation using plants etc. see Falkenberg et al., 2021

→ (EC1-13) Thank you for the comment as well as helpful references. The authors have added further description here about local mitigation of coastal acidification by using macroalgae and seagrasses with references (in Lines 436-438 in the revised manuscript).

Some of these options should be discussed here. I know that there is seagrass restoration occurring in the oyster aquaculture regions of Japan. Perhaps these measures may have some capacity for mitigation.

→ (EC1-14) Thank you for the suggestion. The authors have added description of *“For example, eelgrass restoration, that has long been performed in the Hinase Area as mentioned in 2.1, may have some capacity for mitigation.”* in the end of this paragraph (in Lines 441-442 in the revised manuscript).

local catchment management is also another option - see Scanes et al., 2020.

→ (EC1-15) Following the comment, we have added the sentence *“Local catchment*

*management is also considered to alleviate the impacts of acidification and deoxygenation locally (e.g. Scanes et al., 2020).*” (in Lines 453-454 in the revised manuscript).

L424-440; Here there is no mention of the adaptive capacity of oysters themselves? Or the selective breeding work that is being undertaken to improve their capacity to withstand warming and acidification. I think that could also be raised here.

→ (EC1-16) In this manuscript, the adaptive capacity of oysters themselves was not mentioned because the authors do not have any evidence. Considering the editor’s comment, we have added description about selective breeding work in the revised manuscript (in Lines 461-462).

L433; replace “good”. This paragraph also has the opportunity to expand on the hatchery rearing of oysters and it’s potential the alleviate issues with recruitment.

→ (EC1-17) The term “*good*” has been replaced with “*suitable*”. The enhancement of hatchery rearing of Pacific oyster larvae has also been described in this sentence (in Lines 457-459 in the revised manuscript).

Figure legends – replace “pints” with “points” throughout.

→ (EC1-18) Corrected.

Figure legends – please use the full figure captions for all figures not “as figure xx”.

→ (EC1-19) All the figure captions have been fully described in the revised manuscript.

Figure 1 – Eel grass is a type of seagrass but is labelled in addition to seagrass. Please clarify whether the “seagrass” labelled are also eelgrass or another species?

→ (EC1-20) The authors used the term “*seagrass*” several times in the previous manuscript. Most of them actually specifies “*eelgrass*”, so the authors have replaced these with “*eelgrass*” in the revised manuscript.

References cited:

Scanes, E., Scanes, P., Ross, P.M., 2020. Climate change warms and acidifies Australian estuaries. *Nature Communications* 11(1), 1803

Falkenberg, L.J., Scanes, E., Ducker, J. and Ross, P.M., 2021. Biotic habitats as refugia under ocean acidification. *Conservation Physiology*, 9(1), p.coab077.

→ (EC1-21) The two references are informative for our study and have been cited in the revised manuscript. Thank you for the suggestion.

## Responses to Reviewer #1's comments

First of all, thank you for all your comments that greatly helped us in improving our manuscript. Below are our responses. We have revised and re-submitted our manuscript based upon all the comments. Most of the previous figures have also been revised following your and the editor's comments.

### Responses to general comments

*This manuscript by Fujii et al. submitted to Biogeoscience deals with current and future habitat for Pacific oyster (*Crassostrea gigas*) in two coastal sites in Japan. Recent reports that acidification has already negatively impacted on oyster growth along the West Coast of the United States are widely known among researchers. Also in Japan, the economic impact should be very large since oysters are a representative marine product. As such, the suggestion that the reduction of anthropogenic CO<sub>2</sub> can largely alter future habitat for oyster will be impressive for not only scientific community, but also for the general public.*

*Another commendable point of this paper is a successful long-term monitoring in coastal sites with several sensors. The figures presented in the manuscript suggest that the quality of data obtained by the sensors was good. As far as I know, there are not so much cases of such successful long-term monitoring in coastal sites. These observations should be maintained in the future.*

*My largest concern about this manuscript is the absence of long-term warming under RCP8.5 at Hinase. It is too unrealistic that future warming at Hinase is almost negligible (Figure 13a). There was no mention about future physical environment in the Seto Inland Sea in Nishikawa et al. [2021], which cited in the text. So, I could not verify whether negligible warming in this region is true or not. However, air temperature will likely increase over the long term under RCP8.5. It is difficult to believe that rising in air temperature will not affect water temperatures in the shallow Seto Inland Sea. I strongly urge the authors to check water temperature projections in the Seto Inland Sea.*

*Negligible warming in Hinase has resulted in much of the discussion being focused on whether or not there is an increase in water temperature. The differences in expected spawning period between Hinase and Shizuagawa appear to be due to the presence or absence of long-term warming. As this manuscript covered two sites, I want the authors to discuss the relationship between regional characteristics and expected changes in habitat for oysters. For example, Hinase is more enclosed area than Shizukawa. Do these differences in characteristics have any effect on acidification in the future? Unfortunately, there is little discussion about the impact of factors other than water temperature on environmental change at current manuscript.*

→ (RC1-1) After receiving the reviewer's very helpful comments, we checked the boundary conditions for the future simulations for both Hinase and Shizugawa. And we found that we had not been able to account for future increases in air temperature for the Hinase simulations. Using air temperature data from FORP historical and future scenario runs, we were able to correct the previous atmospheric forcings we used. By simulating with the improved atmospheric conditions, the future projected results became more reasonable, with higher water temperature compared to the present (Figure 14 in the revised manuscript). However, it seems that the climate model outputs used as boundary conditions in our model

underestimate rise in water temperatures in the Seto Inland Sea. Therefore, the authors could not compare directly the projected environmental change, especially temperature and subsequent  $\Omega_{\text{arag}}$  values between Hinase and Shizugawa.

As for the comparison of Pacific oysters between Hinase and Shizugawa, it is quite difficult to compare them directly, mainly because of the following two reasons: first, the oysters hatch and spawn eggs locally and have long been accustomed to the local environments in each site; second, relations between environmental characteristics and oysters' responses to environmental change have not yet been clarified well, except for those due to temperature, acidity and dissolved oxygen. Therefore, we focused only on discussing the difference between Hinase and Shizugawa in terms of the impacts of these parameters on oyster farming in our previous manuscript. However, referring the editor's comment, although the impacts of many factors on Pacific oysters are still unclear, the authors have added further discussion about the impacts of low salinity on Pacific oyster larvae (in Lines 403-409).

*Also, I think long-term oligotrophication in the Seto Inland Sea is a hot topic. I recommend that the authors mention regarding oligotrophication. If it is impossible, the author should mention as limitation of the projection in the text.*

→ (RC1-2) The authors have mentioned regarding oligotrophication in Lines 127-129 in the revised manuscript. We have also added explanation about observed nutrients (in Lines 281-287 in the revised manuscript). However, there are no thresholds of nutrient concentrations to express oligotrophication, so instead, we referred to the half-saturation constants of nutrients used in the model, and suggested that  $\text{NO}_3$  and  $\text{PO}_4$  are considered to be depleted, which is regarded as an oligotrophic condition in some seasons in the surface water in both sites.

*I agree with the posted comment (by Dr. Ishizu) that discussion is too little in the current manuscript. The authors have competent observational data and computational methods. Further discussions utilizing these resources are needed for the acceptance.*

→ (RC1-3) We have developed the Discussion section following your and Dr. Ishizu's comments in the revised manuscript. Firstly, future projection results have been discussed in Section 4.1 (in Lines 345-362 in the revised manuscript). Anticipated change in Pacific oyster's spawning period in the future has been described as a projected combined impact of coastal warming and acidification on Pacific oysters (in Lines 365-372). Also, following editor's comments, the authors have added a new section ("4.3 Thresholds for impacts of ocean acidification on Pacific oysters in Japan coasts") and have discussed about thresholds used in this study to express the impacts of coastal warming, acidification and deoxygenation and their limitations (in Lines 390-409).

## **Responses to specific comments**

*(Line 164) The reference about oligotrophication (i.e., an overcome of eutrophication) is needed. I think that Abo and Yamamoto [2019], which was already cited in the text is suitable.*

→ (RC1-4) Based on the comment, we have referred to Abo and Yamamoto (2019) and Yamamoto et al. (2021) here, and have added a relevant statement ("Eutrophication has been overcome in many surface waters of the Seto Inland Sea through measures to control excessive inflow of nutrients from land over the last few decades, and the surface waters are even oligotrophic nowadays (e.g. Abo and Yamamoto, 2019; Yamamoto et al., 2021), but exchange of seawater with the open sea is weak, and the bottom layer is hypoxic.") in Lines 127-129 in the revised manuscript.

(Line 207) *Information about the calibration of DO sensor is needed. I suppose that it was done by two-point calibration at 0% and 100%.*

→ (RC1-5) As the reviewer points out, the calibration of DO sensor was done by two-point calibration at 0% and 100%. We have added the following sentence to the Method section of the revised manuscript (in Lines 159-160): *“Calibration of the DO sensor was carried out by two-point (zero and span) calibration using 0 and 100% (saturated) oxygen waters (Fujii et al., 2021).”*

(Line 237) *“The maximum error ~ is about 10  $\mu\text{mol kg}^{-1}$ ” Is this true? In Figure 2, some TA data appear to be deviated by more than 10  $\mu\text{mol kg}^{-1}$  from possible regression line.*

→ (RC1-6) Following the reviewer’s comment, we have checked the values again, and as the reviewer pointed out they were found to be underestimated. Therefore, we have revised the maximum error of TA and  $\Omega_{\text{arag}}$  to 30  $\mu\text{mol/kg}$  and 0.06, respectively (in Line 191). Thank you for the helpful comment.

(Line 305) *“Although no significant differences were observed among the sites in Hinase, salinity was generally higher at H-4 than at the other three sites throughout the year.” What do these sentences mean? Was the difference in salinity statistically insignificant? The author should clarify whether this difference is important in this study or not.*

→ (RC1-7) All the statements in this paragraph were not clear and complete. Therefore, the authors have rewritten the entire paragraph (Lines 266-280 in the revised manuscript). Especially, we have paid an attention to mention that extremely low salinity seems to be related to heavy rainfall at some sites, but the relationship between the salinity and rainfall was not statistically significant.

(Line 313) *Does the upper limit of optimal DO range (269  $\mu\text{mol kg}^{-1}$ ) have any biological meaning? Most of observed DO exceeded this value (Fig. 4).*

→ (RC1-8) As the upper limit is much less important than the lower limit with regard to biological implications and might also cause confusion, we have removed the upper limit from this sentence (in Lines 247-248), Figure 4 and the caption in the revised manuscript.

(Line 341) *“In Shizugawa, the  $\Omega_{\text{arag}}$  value was below the threshold ~. However, no morphological abnormalities were observed ~.” Then, what does the threshold mean?*

→ (RC1-9) The two facts contradict each other. To address the issue, a major revision has been done. As mentioned above, the authors have further developed the explanation and discussion in the revised manuscript, especially in 4.3 (*“Thresholds for impacts of ocean acidification on Pacific oysters in Japan coasts”*). We have also discussed about the possibility of larvae escaping from low salinity (and low  $\Omega_{\text{arag}}$ ) waters (in Lines 403-409 in the revised manuscript). Although it does not seem that abnormal larvae existed as many larvae were captured by plankton nets, we have also mentioned the possibility of our failure to collect abnormal larvae samples for the reason that they died before our samples were taken (in Lines 391-397).

(Line 438) *“Extreme events such as severe storms are anticipated to occur more frequently and intensely in the future.” References are essential.*

→ (RC1-10) The authors have added a reference (e.g. Kimoto et al., 2005; IPCC, 2022) (in Line 439 in the revised manuscript).

## Responses to Reviewer #2's comments

First of all, thank you for all your comments that greatly helped us in improving our manuscript. Below are our responses. We have revised and re-submitted our manuscript based upon all the comments. Most of the previous figures have also been revised following your and the editor's comments.

### Responses to major comments

*Reading through this manuscript, I thought that the volumes of each section are unbalanced. Especially, the volume for the result section is too short, compared to the volumes of introductions and methods.*

→ (RC2-1) The authors have revised the manuscript based on the comment, especially by adding further description to results section, including horizontal distribution of modeling results (as mentioned below) (in Lines 317-319 in the revised manuscript) and have also developed the discussion, including oligotrophication (following the other reviewer's comment; in Lines 281-287), observed low salinity (in Lines 266-280) and Pacific oyster spawning period (in Lines 261-265 and 323-328). On the other hand, the introduction and methods sections have been shortened appropriately, as mentioned below.

*I believe that one of the highlights in this study is to develop the model for the specific coastal area. Therefore it would be better to add more analysis by using the model outputs to show how their model is reproduced well in this target coastal area. At that time, seasonal horizontal distributions of each variable could be useful with comparison of the other observational data. Probably DIC and ALK are probably difficult to be got horizontally, but temperature and salinity, oxygen data could be available if you try to find in Japan. These improvements could make you deepen for your understanding of the model, which will make useful for your future study.*

→ (RC2-2) Following the comments, the authors have added figures and descriptions of horizontal distribution of model results for temperature, salinity and dissolved oxygen (in Lines 317-319 and Figs. 8 and 9 in the revised manuscript). Unfortunately, we could not obtain sufficient observational data in our study sites. Therefore, the authors performed comparison of model results with observational data, both of which were obtained in our study (in Figs. 10-13 in the revised manuscript).

### Responses to minor comments

*Abstract: The sentence of the abstract should be blushed up. The current abstract was not a self-contained summary of your work. Method, how to examine and what you found should be included. The sentence "Coastal warming, acidification, ... to facilitate mitigation measures" can be shorten. The sentence "To minimize... oyster farming practiced locally might also be required" is not necessary.*

→ (RC2-3) Following the reviewer's comment, the authors have revised the entire abstract. The last sentence has been removed following your comment. The sentence "Coastal warming, acidification, ... to facilitate mitigation measures." has been shortened as: "Moreover, there is concern regarding the combined impacts of coastal warming, acidification, and deoxygenation on Pacific oysters." (in Lines 21-22 in the revised manuscript)



*Title: After revising the manuscript, I suggest you reconsider the title. The current title does not reflect the content of the current manuscript.*

→ (RC2-4) The authors have revised the title to: “Assessing impacts of coastal warming, acidification, and deoxygenation on Pacific oyster (*Crassostrea gigas*) farming: A case study in the Hinase Area, Okayama Prefecture and Shizugawa Bay, Miyagi Prefecture, Japan”.

1. *Introduction: The sentences are too long. The sentence can be shorter. The current manuscript is divided into 5 sections (1.1, ~ 1.5), but I think it would be better to write it all in one. When you improve the manuscript, you also think about the balance of the volumes in the section. The volume of the introduction is heavy compared to the results, discussion and conclusion sections.*

→ (RC2-5) Based on the comment, the authors have combined all the sections together. Also, the section has been shortened by cutting descriptions that are too detailed and will be repeated in following sections.

*Study sites: This section is also too heavy, compared to the results, discussion and conclusion sections.*

→ (RC2-6) This section has also been shortened by cutting out overly detailed descriptions and summarizing the information about model boundary conditions using Table 1.

*Observed results: The current version has been divided the section into 3.1.1~3.1.7, but you don't need to divide individually. Please reconsider this part.*

→ (RC2-7) The authors have combined all the sections together.

*Modeling results: The results of the numerical models are necessary. Please put additional analysis such as horizontal distributions and so on to show how the model reproduces in this target area horizontally and timely.*

→ (RC2-8) Thank you for the helpful comments. Based on the reviewer's comments, the authors have added horizontal distributions of modeled temperature, salinity and dissolved oxygen (in Lines 317-319 and Figs. 8 and 9 in the revised manuscript). These results and comparison of time series of modeling results of individual parameters with the observed (in Figs. 10-13 in the revised manuscript) have been developed in Section 3.2 (“Modeling results”) in the revised manuscript.

*Future projection: This section is a discussion matter. This part can be moved to the discussion section if you add analyses of the model reproducibility.*

→ (RC2-9) This section has been moved to discussion section (Section 4.1) in the revised manuscript.

*Section 4.2: The section 4.2, 4.2.1 and 4.2.2 can be moved to the conclusion section. Please reconsider your construction.*

→ (RC2-10) Those sections have been moved to Conclusion part (“5.1 Alleviation of impacts on Pacific oyster farming”), following the reviewer's comment.

*Please add references after the sentence (Extreme events such as severe...intensely in the future) in page 16.*

→ (RC2-11) The authors have added references (e.g. Kimoto et al., 2005; IPCC, 2022) (in Line 439 in the revised manuscript).

*5. Conclusion: The conclusion should be improved. Basic conclusion includes the purpose, the summary of this study and self-evaluation/prospects.*

→ (RC2-12) The authors have revised the conclusion part (“5. Conclusion and Remarks”), mainly following the reviewer’s valuable comments. Suggestions on mitigation and adaptation measures based on our study have mentioned in this part (“5.1 Alleviation of impacts on Pacific oyster farming”).

*Fig 1: The information of latitude and longitudes are necessary. Right figures are not appropriate in scientific papers. I think that making an original map by yourself is necessary. In that case, Japanese character should not be included in your map. Right figure is relatively too small.*

→ (RC2-13) Based on the comment, maps in Fig. 1 have been regenerated to more clearly show the most relevant information.