

Dear Editor,

We, the authors of BG-2013-150, appreciate the two anonymous reviewers' comments and one additional interactive comment, which helped us to revise the manuscript. Most comments seemed to be fair and reasonable, so we paid heed to most of their advice and suggestions, and the manuscript has been massively revised. We believe that the contents and the clarity of our paper are much improved in the revised version.

However, it is unfortunate that we needed to remove the element analysis section due to the insufficient information to reach any firm conclusions as Reviewer #2 suggested. After this reduction it might not be sufficiently long to be a regular paper. In this case, we don't mind if the Editor assigns our manuscript as a 'short note' instead of regular article. Correction of grammatical errors and English improvement were carried out by a native English-speaking editor as suggested. Below are point-by-point responses for the three reviewers' comments. Finally, MS files of revised manuscripts were attached in two forms: 'Track Changed' and our 'final version'.

Reviewer #1

General comments:

Basically, Reviewer #1 pointed out four items as general comments: (1) improvement of the English, (2) clarification of the water chemistry in the experiments, (3) explanation of statistical analyses, and (4) elaboration of the discussion section.

Improvement of English:

Response: The manuscript has been edited by an English-speaking native, so we hope it now matches the journal standard.

Clarification of water chemistry:

Q: Unfortunately, the presentation and analysis of this data lacks depth and scrutiny. There are several problems with the methods section. Specifically, the authors assume the water chemistry results from the July experiment can be applied to the May and June experiments despite the fact that no data for those experiments is provided (and may not have been measured). This is a glaring error and in my opinion calls the validity of those experimental data into question since we do not know what the actual treatments were.

Response: Unfortunately, we only measured the Total Alkalinity (TA) value in July, and there were no measurements of that parameter during the May and June experiments. However, monitoring of water temperature, dissolved oxygen, pH in rearing water, and CO₂ concentration of mixed air was carried out during the entire experiment from May through July. Water temperatures, dissolved oxygen and CO₂ concentration of mixed air were very stable. A newly added Figure 2 shows that our pH values of different rearing water tanks were clearly different from each other depending on pCO₂ during the experiment. The pH values measured in May, June and July indicated the lower pH in higher concentrations of pCO₂, and vice versa: i.e., 7.91±0.08 (574 ppm), 7.84±0.05 (988 ppm), and 7.68±0.10 (1297 ppm CO₂ concentration). Therefore we believe we can assume that water chemistry such as the CO₂ concentrations in May and June experiments were not much different from that in July.

Statistical analysis:

Q: There is also a complete lack of a description of statistical analysis, and questionable use of linear regression on a non-linear growth process.

Response: At the reviewer's suggestion, we have replaced the linear regression with a nonlinear curve for larval growth. Also, we introduced some statistical analysis (ANCOVA, Minitab 16, Minitab INC.) to test growth differences among different rearing tanks.

Elaboration of discussion:

Q: The discussion is incomplete, does not clearly present the authors' conclusions, and make some broad generalizations that are not based upon the data presented.

Response: We have revised the 'Discussion' section by adding and deleting some sentences and paragraphs.

SPECIFIC COMMENTS:

Although the results of this series of experiments appear to be intriguing, and may represent an important contribution to this field of study, they need to be analyzed and presented in a more clear fashion before that conclusion can be made. I have prepared comments on each section of the paper below.

ABSTRACT:

Q: A nice and concise summary of the experimental results. Second to last sentence is confusing.

Response: Thanks. Because both reviewers felt uncomfortable to the results of the chemical analysis without replicate measurements on element concentrations of fish tissue, and because there is no alternative way to improve this difficulty, we decided to remove this section from our paper.

INTRODUCTION:

Q: The introduction is in need of substantial editing for grammatical errors and use of unclear terminology (i.e. 'trouble in marine ecosystems', line 24 on page 7414). This section should be re-written to provide a much more clear introduction to the phenomena of ocean acidification and its impacts on marine organisms. Paragraph 1 provides a vague description of ocean acidification (OA) and needs to be written more precisely. It is lacking any citation of literature for many of the initial statements about OA, its history, progress, and projected future scenarios. Paragraph 2 presents detailed information on several studies on non-fish study species. This is unnecessary information for this manuscript, and should be limited to summary statements about the effects of OA on those broad organismal groups (i.e. phytoplankton and invertebrates).

Response: We agree with the reviewer. The 'Introduction' has been re-written by combining the first and the second paragraphs, and we reduced the statements with unnecessary information. .

Q: Paragraph 3 again presents detailed information about OA studies on fish, which are more relevant to the topic, but do not provide a broad base of information which a reader can then use to assess the importance of the following data. This section should include less details (less specific numbers, etc.) and a more basic introduction to the current understanding of OA impacts of larval fishes.

Response: We reduced the inclusion of specific data and added more results from recent works on larval fish.

Q: Paragraph 4 presents some useful information about the physiological effects of OA on fishes, and mentions the potential problems with rapid OA and the ability for species to adapt. However, this paragraph is very vague (i.e. 'The results for various groups of scientists', lines 4 and 5 on pg 7416) and the ultimate point that the author is making is not clear.

Response: We have revised this paragraph explaining species-specific differences in life history parameters.

Q: Paragraph 5 introduces the study species well, but then provides very vague mention of projected OA scenarios.

Response: We have improved this section.

MATERIALS AND METHODS:

Q: This section is in need of substantial editing for grammatical errors. In general, the authors provide a somewhat clear description of what was done. However, someone who is unfamiliar with this type of research would have difficulty determining the precise methods. There is a major problem with the reporting of chemical water parameters and the assumption made to apply results from one experiment to all experiments. There is no mention of methods for determining growth rate (until it is presented in the Results section). There is no presentation of the statistical methods used for analysis.

Response: Grammatical errors have been checked. We now discuss how we express the non-linear growth pattern of larvae. Also, we now clarify the statistical analyses we applied.

Q: Pg. 7417, line 14-15: unclear whether the statement of light being detrimental to survival is based on personal experience or literature: : please include citation.

Response: Scientists in the olive flounder hatchery have a lot of experience with hatching eggs and rearing larvae. They believe that direct light seems to be detrimental to fish larvae. We added the citation: 'Standard manual of olive flounder culture' (NFRDI, 2006).

Q: Pg. 7418, line 10: author should describe how 'regularly' pH was monitored

Response: We measured pH of the rearing tanks 3 times a day (09:00, 13:00 and 20:00).

Q: Pg. 7418, lines 16-18: the authors assume that pCO₂ calculated in one experiment could be applied to the other experiments. This is incorrect and it should be made clear that the measurements/calculations are only valid for the July experiment. This is a serious problem regarding the results of the May and June experiments, because experimental systems often experience technical problems that may go undetected without measurements. If the measurements for May and June are available, they should be reported, otherwise this is a SERIOUS problem because there is no way to determine the actual treatments experienced by the fish, in which case those results are only anecdotal evidence of a possible treatment effect.

Response: This is discussed above.

Q: Last paragraph: It is not made clear why the concentration of chemical elements were measured. The method is only described as 'hot plate digestion' (pg. 7419, line 7) and lacks a citation. There is no mention of how many larvae were tested, although it is mentioned in the abstract that statistics were not possible due to single measurements: : was only 1 fish measured???

Response: Hot plate (= Acid) digestion method is the preconditioning process of ICP (Inductively Coupled Plasma) analysis. In order to see the accumulation of some chemical elements in the larval tissue, concentrations of elements were measured. Unfortunately, however, we only had a single measurement for each experiment because we used about 100 larval fish for one measurement. (The minimum quantity for ICP analysis requires 1 gram/sample, but total dry weight of one flounder larva was quite small.) Therefore we decided to remove this part from our manuscript.

RESULTS:

Q: Experimental results are presented in a vague manner, with many references to means of 'about: : 'a certain magnitude. This does not provide a clear description of the results and should be changed to include specific means and variances/ errors. This section is missing references to statistical analyses, except for a couple occasions. The ANCOVA results of growth rate seem to be driven by a couple data points when referencing figure 3. I am not convinced of the validity of fitting a linear regression to growth data that is clearly not linear and in general is known to be non-linear for fishes. Very unclear why no statistics were performed on the chemical analysis results!

Response: We now have described larval growth as a non-linear pattern. We have removed the chemical analysis

section from the paper, as we mentioned above.

DISCUSSION:

Q: The discussion needs to be presented in a more clear manner, presenting a summary of results and their relation to published literature. The authors do not clearly present their conclusions and more text should be dedicated to this section.

Response: We changed this section as advised.

Q: Paragraph 1 - The authors begin this section with discussion of invertebrates, instead of discussion of their results. This is not very relevant to this experiment.

Response: This paragraph now begins with fish.

Q: Paragraph 2 - The discussion of possible reasons for increased size and growth includes some important points (possible increased appetite or gustatory sensation), but lacks discussion or any reference to other papers that also report increased size with OA (Munday 2009, Proc R Soc B).

Response: We re-wrote manuscript about the reasons of enhanced growth as advised.

Q: Paragraph 3 - The discussion of chemical elements is very confusing. There is not citation for the precipitation of CaCO₃ in the fish gut (pg. 7422, line 10). It is very unclear what the authors mean by 'caused by these processes' (pg. 7422, line 12-13).

Response: The chemical analysis part was deleted.

Q: Paragraph 4 - Discussion of ecological consequences of increased size/growth is satisfactory. However, the statement 'temperature and acidification with ultimately determine the consequences' (Pg. 7422, lines 25-26) should not be stated as fact, but as a possibility. The last three sentences seem out of place and provide a weak finish to the discussion.

Response: We have changed this paragraph.

Q: TABLE 1: this table does not provide any SE ranges for these measurements, so it is unclear how much the treatments varied over the course of the experiment.

Response: agreed. We have revised Table 1.

Q: Figure 2: graphs are clear, but no statistical results are presented.

Response: We added the statistical results.

Q: Figure 3: Why are there no error bars on these data points? Unclear which line is associated with which data series.

Response: In this figure, we believe it might be better presented without error bars.

Q: Figure 4: graph is clearly presented.

Referee #2

Reviewer #2 also pointed out similar critical flaws in our original manuscript as Reviewer #1 did: (1) improvement of English, (2) clarification of carbonate chemistry in the experiments, (3) explanation of statistical analysis, (4) pattern of larval flounder growth, and (5) CO₂ influence on metamorphosis. Our responses to Reviewer #1 answer many of the questions (1)–(4) of Reviewer #2. For the metamorphosis issue,

we are preparing another paper considering morphological differences and swimming capabilities from another experiment. Therefore we excluded the theme of metamorphosis from this paper. Below are our responses to Reviewer #2

General comments:

Q: The conclusion that CO₂ enhanced growth of flounder larvae was based on data with only two replicates in each month, and with some unknown statistical analysis. The authors reached the same conclusion by applying linear regression to the data in Fig. 3, which shows non-linear growth. I do not think that the conclusions are supported by the data.

Response: In revision, we described the larval growth with an exponential curve from the hatching to metamorphosis instead of the linear pattern used in original manuscript.

Q: The assumption of same carbonate chemistry in three experiments (in May, June and July) is unacceptable. This is an important flaw of the study. How can the authors deny the possibility of system failure without monitoring it? Did not the authors measure even seawater pH in May or June?

Response : This question is addressed above in response to the General Question of Reviewer #1.

Q: What I miss much in this manuscript is about possible influences of CO₂ on metamorphosis. Early development of flounders is a most unique example of fish metamorphosis, and I wonder why the authors did not analyze/observe metamorphosis rate, abnormal morphology or success/failure of settling at the termination of metamorphosis. Or maybe the authors are preparing another manuscript on these parameters?

Response: We didn't perform an investigation about metamorphosis in this study. Our next paper assesses the effect of ocean acidification on metamorphosis.

Q: Statistical method is totally lacking. Please state in detail what statistical methods were used for analyses in Materials and methods section, and their results in Results section.

Response: We added some details on statistical methods in the 'Materials and Methods' section..

Q: The data in Figure 4 are from only single determination (P7414 L16), and should therefore be omitted. This is too preliminary.

Response: Yes. We deleted this section.

Q: English usage in this manuscript must be substantially improved. There are many grammatical errors and vague descriptions.

Response: The English should now be improved.

Specific comments

Abstract:

Q: L5: 'atm' is not a unit for concentration, but is for partial pressure. There seems to be confusion about this point throughout the manuscript.

Response: We agree, and have changed 'atm' to 'ppm'.

Introduction:

Q: P7415 L4-13: This paragraph on the data for selected invertebrates is somewhat distracting, and should be deleted.

Response: We re-wrote 'Introduction' based on the Reviewers' comments.

Q: P7416 L10-15: I am not sure what the authors mean by 'adaptive mechanism'. Please give more explicit description on what the authors intend to argue about. For your information, the definition of relevant terms and their appropriate use in 'Environmental Physiology of Animals' by Willmer et al. Blackwell may be useful.

Response: Thank you for recommending this reference. For avoiding confusion, however, we have removed the word 'adaptive mechanism'.

Q: P7416 L21: What is 'round fish'? Is this a common name for some particular species?

Response: We deleted this unclear word.

Q: P7416 L23-25: This is a very vague statement. What do the authors mean by 'Marine ecosystem will be modified'?

Response: This sentence was deleted.

Materials and Methods

Q: P7417 L 7: 'KOFLOC' not 'KOFIOC'.

Response: We corrected it.

Q: P7417 L21: How did the authors measure the concentration of dissolved oxygen?

Response: We used DO meter (5 Star-pH meter with DO sensor, Thermo Scientific Orion, USA) twice a week. This detail is now included

Q: P7417 L24: Why did the authors add chlorella? This is not a feed for the fish? The genus name (C.) should be given in full.

Response: 'Chlorella' is prey for rotifer in the rearing tank. Chlorella keeps the rotifers in the tanks alive. Also, we believe that Chlorella blocks the direct and harmful light entering rearing tank.

Q: P7417 L25: What is 'the practice of feeding'?

Response: Fish larvae find prey organism such as rotifer by sight. Therefore, larvae need to 'practice' feeding. But we have deleted those words from this sentence.

Q: P7418 L1-2: 'it was gradually increased' Please state more specifically. Was there no period during which both rotifers and Artemia were given?

Response: ok, we have included that information.

Q: P7418 L14-15: What are the 'other carbonate chemistry factors'?

Response: The sentence will be changed to 'TA and pH with temperature and salinity were used for calculations'.

Q: P7418 L16-18: The authors should have determined the necessary parameters for the experiments in May and June as well. You cannot assume this since a trouble in the setup might have gone undetected, which affected carbonate chemistry of the experimental seawater. Did not the authors measure even seawater pH in May or June?

Response: this has been fixed.

Q: P7418 L19: 'all live larvae' Does this mean there was some mortality? If yes, mortality or survival rate of each group must be given in Results.

Response: Regrettably, we don't have mortality and survival data because we lost some larvae during experiments (sampling and handling larvae). So, we can't use the data of final alive larvae for estimating survival and mortality rates.

Q: P7419 L7: What is 'hot plate digestion method'?

Response: Hot plate digestion method is the preconditioning process of ICP, and it can be changed to 'Acid digestion method'. However, this paragraph was deleted.

Q: P7419 L10: iHORIVA should be iHORIBA

Response: This sentence is deleted.

Q: P7419 L8: Why did the authors analyze for 13 elements but only report data for 5 elements? The statement in P7420 L18-19 (because their proportions... to others) cannot be the reason for the selection of 5 out of 13.

Response: This section was deleted.

Q: P7419 L11: Why did the author use data only from the lowest and highest CO₂ groups, and ignored the mid-CO₂ group?

Response: This section was deleted.

Results:

Q: L7419 L15: pCO₂ is not 'dissolved carbon dioxide concentration'.

Response: We deleted 'dissolved carbon dioxide'.

Q: P7420 L2-3: What statistical method was used here? Give more details about stat results too. P7420 L7: Same as above.

Response: Results of statistical methods have been included in the revision.

Q: P7420 L9-10: This is not stated in Materials and methods. Please describe in some details in M&M section. How did the authors anesthetize the fish? Were the fish returned to experimental tanks after determinations or sacrificed?

Response: We added growth pattern analysis in 'Materials and Methods', and used ice for anesthesia of larvae, which was mentioned in P7418 L20.

Q: P7420 L13: The data in Fig. 3 show that the growth was not linear. Based on what criteria, did the authors decide to use linear regression?

Response: We changed it to non-linear regression.

Q: P7421 L10: iFrommel et al. 2011 is not a proper reference here.

Response: Agreed, we deleted this reference here.

Discussion:

Q: P7421 L9: 'Pörtner' not 'P Pötner'.

Response: We fixed it.

Q: P7421 L20-21: 'Increased flux of ions is....for marine fishes' Why? Marine fishes face continuous loading of ions from surrounding water and spend substantial energy to regulate body fluid ionic status.

Response: We changed this sentence 'Elevated CO₂ and low pH stimulated appetite and dietary intake by larvae, because they must actively excrete excess ions to maintain their osmotic balance. Consequently, we surmise that the excess energy was used for growth.'

Q: P7421 L21-23: In order to enhance growth, energy intake must exceed additional energy demand for osmoregulation. Do the authors know any example from recent publications of ocean acidification research?

Response: We could not find any proper references about increased growth at high-CO₂ group. However, we referenced this paper: Ishimatsu, A., Hayashi, M. and Kikkawa, T.: Fishes in high-CO₂ acidified oceans, Mar. Ecol. Prog. Ser., 373, 295-302, 2008.

Q: P7422 L9: the resulting enrichment of Ca²⁺ in the gut fluid Do the author have data?

Response: This paragraph was deleted.

Q: P7422 L12-13 'by these processes' Which processes do the authors mean?

Response: This paragraph was deleted.

Q: P7422 L27-28: 'The Korean waters...the industrial complexes' Please provide evidence for this statement.

Response: We referenced KORDI and NFRDI reports.

Q: Table 1: Please add SE's and the number of determination.

Response: Agreed. We revised the table.

Q: Figure 2: Why was body weight so different between the fish in May and the other two month (almost double) when the difference in body length was not that much? These are all at the end of 4th week after hatching (P7418 L4-5)? Were they at similar developmental stages at the completion of the experiment?

Response: We believe that the differences in weight and length of larvae among experimental months resulted from egg quality and size. Usually the early spawned eggs tend to have bigger sizes. There is no big difference in developmental stages at the completion of the experiment in May, June and July.

Q: Figure 3: It is stated that 'we measured the 5-8 individual larval lengths' (P7420 L9-10). But there were two tanks for each CO₂ treatment. Do the authors mean 5-8 larvae from each tank or a total of 5-8 from two tanks? Was there no difference between mean length from two tanks? If so, how did the author confirm? Label for X-axis must be 'Days after hatching'.

Response: We sampled 5~8 ind. larvae from each tank. We measured lengths of all larvae. Larval length of each experimental group was averaged (i.e., control, medium and high concentrations). Results of length measurement indicated no difference between mean lengths from the two tanks. Mean larval length in each tank had no significant difference.

Q: Figure 4: This figure must be omitted because of single determinations (P7414 L16).

Response: We agree your opinion. Deleted.

Interactive comment provided by H. Baumann:

Dr. Baumann suggested that we needed to include recent works on negative growth and survival effects in a larval marine fish in response to elevated CO₂ levels. Therefore, we used some references he recommended us to add.