

Interactive comment on “Effects of ocean acidification on the larval growth of olive flounder (*Paralichthys olivaceus*)” by K.-S. Kim et al.

Anonymous Referee #2

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General comments This study examined how growth and element content was affected during early development of *P. olivaceus*, one of the most important aquaculture species in several Asian countries. I agree with the authors' opinion that responses of commercially important fishes need to be urgently investigated. However, it is unfortunate that the authors did not pay enough consideration to experimental design and analyses in this study to make data solid and convincing.

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.

C2941

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?

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?

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.

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

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

Specific comments Abstract L5 “ μatm ” is not a unit for concentration, but is for partial pressure. There seems to be confusion about this point throughout the manuscript.

Introduction P7415 L4-13 This paragraph on the data for selected invertebrates is somewhat distracting, and should be deleted. 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. P7416 L21 What is “round fish”? Is this a common name for some particular species? P7416 L23-25 This is a very vague statement. What do the authors mean by “Marine ecosystem will be modified”?

C2942

Materials and methods P7417 L 7 “KOFLOC” not “KOFIOC”. P7417 L21 How did the authors measure the concentration of dissolved oxygen? 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. P7417 L25 What is “the practice of feeding”? P7418 L1-2 “it was gradually increased” Please state more specifically. Was there no period during which both rotifers and Artemia were given? P7418 L14-15 What are the “other carbonate chemistry factors”? 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? 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. P7419 L7 What is “hot plate digestion method”? P7419 L10 “HORIVA” should be “HORIBA”. 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. P7419 L11 Why did the author use data only from the lowest and highest CO₂ groups, and ignored the mid-CO₂ group?

Results L7419 L15 pCO₂ is not “dissolved carbon dioxide concentration”. P7420 L2-3 What statistical method was used here? Give more details about stat results too. P7420 L7 Same as above. 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? 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? P7421 L10 “Frommel et al. 2011” is not a proper reference here.

Discussion P7421 L9 “Pörtner” not “Pötner”. 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. P7421 L21-23

C2943

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? P7422 L9 “the resulting enrichment of Ca²⁺ in the gut fluid” Do the author have data? P7422 L12-13 “by these processes” Which processes do the authors mean? P7422 L27-28 “The Korean waters...the industrial complexes” Please provide evidence for this statement.

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

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”.

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

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