Biogeosciences
Discussions

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Interactive comment on "Ocean acidification effects in the early life-stages of summer flounder, *Paralichthys dentatus*" *by* R. C. Chambers et al.

R. C. Chambers et al.

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[Correction: The online version or our original Interactive / Final Authors' Comments omitted several lines of text from our uploaded content. Specifically, part of our response regarding the first major comment by Referee 2 was truncated (response to Referee 2, paragraph 2, text after '80'). The complete text appears in this correction.]

We appreciate the comments and suggestions by A. Schreiber. Regarding his first comment, we agree with him that because it is CO_2 that we manipulated, it would be more parsimonious to cast the paper –and revise the title – to reflect that fact. We have changed the paper title as well as text where OA was too flippantly used. Regarding our approach with respect to achieving more acidic seawater, we chose to infuse our treatment seawater with CO_2 to lower acidities indirectly rather than lower acidities by

augmentation with acids. Our protocol more closely simulates the process of ocean acidification in nature and conforms to recommended best practices in ocean acidification studies. As Schreiber correctly points out, without further experimentation we cannot comment on the degree to which the Root and/or the Bohr effects are operative.

Regarding Schreiber's comment on the possible role of population density on the growth and development of larvae from different CO_2 levels, there did not seem to be density dependence in the larval portion of the experiment. Recall that the first experiment – which subjected embryos to different CO_2 levels – did show reduced survival to hatching at higher CO_2 levels. The second, larval stage experiment was started with a constant number of larvae that successfully hatched from a common source environment. Those larvae did not show significant differences in survival to experiment termination at 28 d post-hatching. This terminal count was our only measure of density after the start of the experiment. Our weekly photographs of N = 10 fish per treatment replicate were the source of data on size and developmental state of experimental larvae. By choice and statistical principle, these weekly observations on size and shape were analyzed as replicate means (actually the vector of weekly means). The sample size within replicates (N = 10) is marginally small to detect skewing of the population size structure if it indeed existed. We are inspecting the terminal sample for evidence of skewing of body sizes.

Schreiber's editorial suggestions were implemented.

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We greatly appreciate the comments and detailed suggestions by the anonymous referee. The referee provided structural suggestions for manuscript improvement, four major comments, and several pages of suggestions for clarification of experimental details and editorial recommendations.

Regarding this referee's most broad comment – that the experimental work reported is only a part of a larger research plan, that results from this larger plan are not included

here, and that this reality is not stated as obviously as could be in the current formulation of the Abstract and Introduction – has been reconciled in the revision. We clearly state the scope of the reported work first and later present a broader experimental research paradigm as a suggested way forward for future work by others and us.

Regarding this referee's first major comment – justification of experimental CO2 levels – we clarify the heterogeneity of CO2 levels that could be encountered by the early life-stages of summer flounder within the species geographic range. Although data are unfortunately quite sparse for the most regions worldwide and our region (the Mid-Atlantic Bight) is no exception, there is evidence from historic surveys (MARMAP 1976-1984) and more recent OA NOAA Northeast Fisheries Science Center surveys (2008-2010) of seasonally and spatial heterogeneous CO2 levels (Wieczorek et al., 2013, Oceans in High CO2 World). The marine shelf habitat of the Mid-Atlantic Bight has spatial (nearshore to offshore) and seasonal variation with nearshore habitats in autumn exhibiting mean bottom water pCO2 values as high as 722 ppm. Some summer flounder spawn at this time and their early life-stages occupy these habitats. Moreover, the Mid-Atlantic Bight as a whole is a net sink for atmospheric CO2, with the annual cycle of heating and cooling combined with high winds during the period of undersaturation (winter) accounting for a significant portion of the uptake of CO2 (DeGranpre et al., 2002. Deep Sea Research II 49:4355-4367). Although the shelf waters of the Mid-Atlantic Bight are stable relative to estuarine habitats, summer flounder early life-stages could be exposed to CO2 levels as high as the our lowest experimental treatment of 775 ppm. Our mid-level treatment of 1,860 ppm CO2 (variances to be given in revised manuscript table) represents a 2.4-fold increase above our lowest level (again, using an inshore autumn benchmark of 775 ppm). This intermediate value is also just under an 80 percent increase above the IPCC prediction for 2100, and close to the IPCC projects for 2300. Our highest level (4700 ppm), as described in the manuscript, was intentionally set high in order to establish whether an effect could be elicited in our various response variables at extreme conditions. We have reworded the revision to clarify the intent and logic of our initial one-way (CO2) experimental design. We have

also applied appropriate conditions to the interpretation of the responses to the highest CO2 treatment level.

Regarding this referee's second major comment – replication and genetic diversity – we expand on the rationale of our experimental and spawning design. First, in the embryo period sub-experiment, we used 3 females with each mated with up to 5 different males not used for the other females. We chose to keep the female sibgroups as separate entities (space restricted replicating within females). We chose to use the three available containers per treatment level for the separate females. Although we could test for female effects by using the interaction term (CO2 $\times$ female) as the F-test denominator, we have not done that. Rather, we intentionally confounded female with replicate and used the among-female variance as our F-test denominator. This is akin to replicating over time wherein the investigator chooses or the logistics forces a design wherein replicates are initiated at different times. Whether confounding of replicates is done with female or time or a spatial variable (e.g., blocking), the resulting test is legitimate and is in fact a conservative test of the treatment effect. This follows from the fact that if the blocking variable accounts for some component of the response variance it is captured by the F-test error term, making it harder to reject the null hypothesis. Regarding the number of adults used – 3 females and a total of 9 to 15 males for this experiment – this is not a trivially small number and by most calculations would result in a substantial fraction of the genetic variance in the population assuming that the adults used were unrelated, which is a fair assumption with summer flounder.

Regarding this referee's third major comment – description and presentation of the histopathology results – we have 1) reworked and expanded the description of the protocol including our bias-prevention methods, 2) presented a more full description of the results (including a multivariate test on cranio-facial data analogous to the test on whole-body morphometrics), and 3) included a brief series of example images of normal vs abnormal tissues / organs.

Regarding this referee's fourth major comment – water chemistry – the original

manuscript was submitted concurrently with a manuscript that describes our experimental system. This associated manuscript was not accepted to Biogeoscience, hence the gap in information as correctly pointed out by this referee. To remedy this, we have greatly expanded our description of the CO_2 infusion system including its monitoring and performance. We have added a table (to be included within text or as a supplement) that summarizes water chemistry parameters (means, SDs, ranges, and temporal variability) for pH, pCO_2, HCO_3, CO_3^{2+}, DIC, total alkalinity, and aragonite saturation state.

Regarding the numerous minor comments and edits suggested by this referee, we have either accepted most (e.g., edits, suggested clarifications) or have revised the manuscript to preempt the problem or source(s) of confusion. Again, we appreciate the referee's efforts in flushing out our oversights or improving on our poor wording.

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