

Interactive comment on “Effect of ocean acidification on early life stages of Atlantic herring (*Clupea harengus* L.)” by A. Franke and C. Clemmesen

Anonymous Referee #2

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In the present work the authors studied the effect of effect of ocean acidification on early life stages of Atlantic herring (*Clupea harengus* L.). It is a nice experimental study and based on the published data I can say that this work provide new laboratory evidences regarding the effects of ocean acidification on the on early life stages of fish. I have two main concerns that it could be nice to be adressed by the authors before considering the manuscript for publication:

1) The choise of the proper biochemical or physiological indicator(s) which may indicate the thresholds of pCO₂ impacting the physiological processes. I agree that the ratio RNA/DNA is a useful biochemical indicator of protein synthesis. Some authors,

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however, reported that this ratio is depended on the protein synthesis and breakdown and except of RNA/DNA ratio they use other index as well as DNA:dry weight and RNA:protein (I think this reference should be cited: Maria Alexandra Chícharo and Luis Chícharo (2008). RNA:DNA Ratio and Other Nucleic Acid Derived Indices in Marine Ecology. *Int. J. Mol. Sci.* 2008, 9, 1453-1471; DOI: 10.3390/ijms9081453). Moreover, for metabolic patterns and impacts (e.g. negative as characterized by the authors), transcriptomic and proteomic methods may provide a more realistic picture regarding the rate of protein synthesis and even more the changes in the metabolic patterns. For further discussion see and methods see:

Gracey, A. Y. and Cossins, A. R. (2003). *Annu. Rev. Physiol.* 65, 231-259. Tomanek L. and Zuzow J.M. (2010). *J. Exp. Biol.* 213: 3559-3574. Tomanek L., Zuzow M.J., Ivanina A.V., Beniash E. and Sokolova M.I. (2011). *J. Exp. Biol.* 214: 1836-1844. Hofmann E.G. and Place P.S. (2007). *Mar. Ecol. Prog. Ser.* 332: 249–255.

Besides, the discrepancy between growth and RNA/DNA ratio after incubation at different CO₂ atmospheres raises the question whether this biochemical indicator may indicate metabolic changes.

2) In the nature the stressful factors may act in a synergistic manner. Consequently, the challenge of predicting the outcomes of climate change on marine organisms in Regional or Global Scale is made difficult when the combined effects of two or more variables cannot be predicted from the individual effect of each. Non-independent effects are common in nature, and may arise in one of two principle ways: (a) the impact of one factor is either strengthened or weakened by variation in another factor and (b) the combined influence of two stressors pushes an individual or population beyond a critical threshold that would not be reached via variation in either forcing variable operating in isolation. On the other hand, changes in species physiological performance and fitness occur not only as the result of “acute” extremes of a stressor, which kill organisms over periods of days and weeks, but also as the result of slower, more chronic, cumulative stressors (longer duration, but typically of smaller magnitude) that reduce

growth, somatic condition and reproduction. Accordingly, the authors need to be more cautious when interpreting their data (even more when the ecological significance of them is concerned) and statements as that in page 7111, par 10 (The present study has shown that herring eggs can cope with increase in pCO₂, exceeding future predictions of CO₂-driven ocean acidification. . .) should be avoided. Besides, correctly the authors state below that the synergistic effects of stressful factors are needed further examination.

I encourage the authors to discuss my concerns in a revision.

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